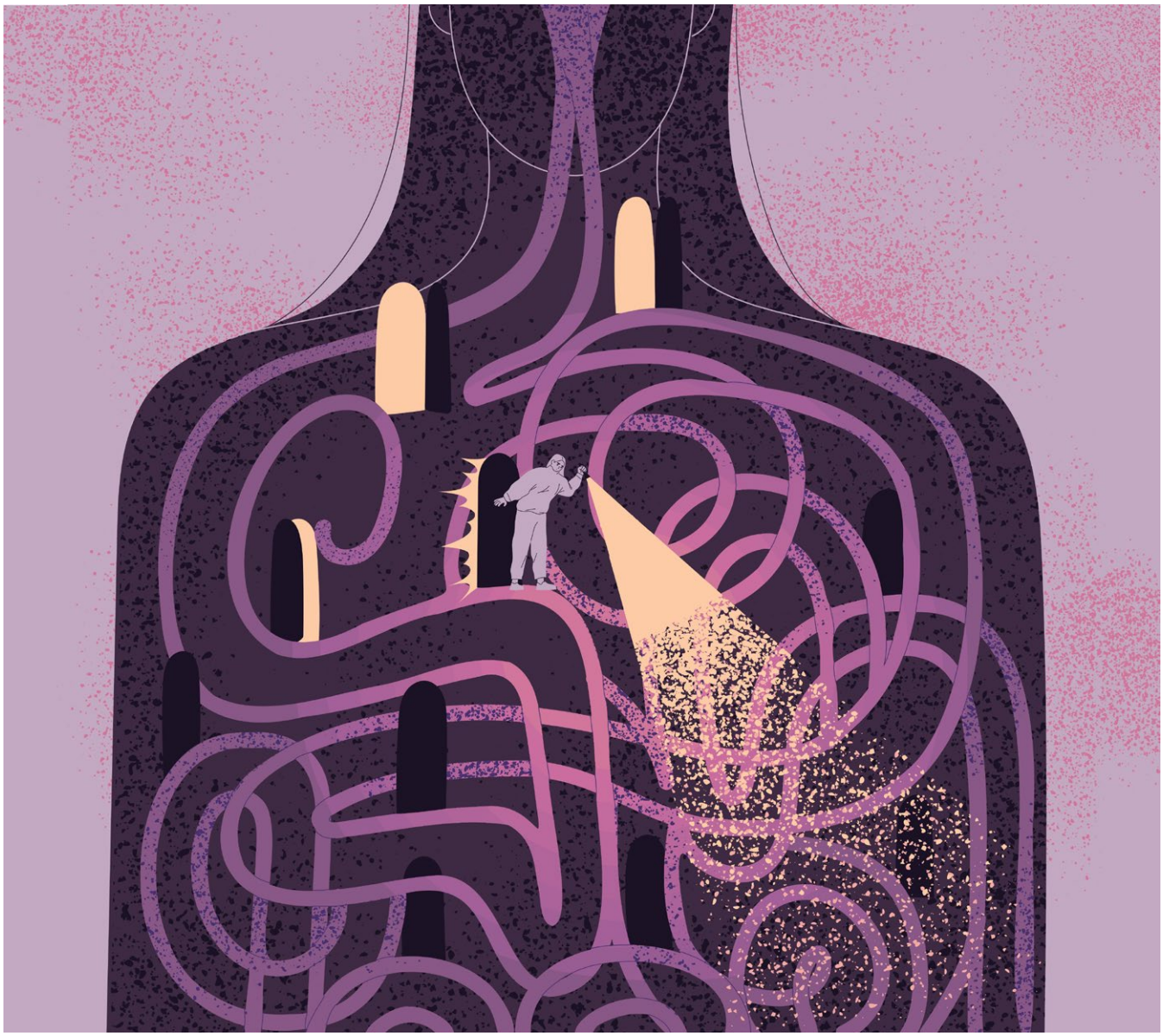

Regional Physiology Meeting 2025

Ljubljana, Slovenia
24–26 September

Advancing Physiology:
Bridging Education, Research and Practice

Abstract Book with Programme

Editors

Jernej Jorgačevski, *University of Ljubljana (SI)*

Nina Vardjan, *University of Ljubljana (SI)*

Anemari Horvat, *University of Ljubljana (SI)*

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Sponsors and Acknowledgements

Special Thanks to our Sponsors for their Generous Support of the Meeting.

Welcome to the Regional Physiology Meeting 2025

On behalf of the Programme and Organizing Committees, we warmly welcome you to the *Regional Physiology Meeting 2025*, entitled *"Advancing Physiology: Bridging Education, Research and Practice"*, taking place in Ljubljana, Slovenia, from 24-26 September 2025.

The meeting is jointly organized by the *Slovenian Physiological Society* (with representatives from the Faculty of Medicine and Biotechnical Faculty, University of Ljubljana, and Faculty of Medicine, University of Maribor, and Jožef Stefan Institute, Slovenia) and the *Croatian Physiological Society*, the *Hungarian Physiological Society* and the *Italian Society of Physiology*. It brings together experts and students from across Europe and beyond, including Austria, Czech Republic, Croatia, Finland, Hungary, Italy, Poland, Portugal, Slovenia, Sweden, Turkey, and the USA.

The programme features a wide range of topics addressing current challenges and advances in physiology in the context of education, research, and practice, including:

- Cardiovascular physiology,
- Cancer physiology,
- Neurophysiology,
- Metabolic/Endocrine physiology,
- Microcirculation,
- Sports physiology,
- Space physiology,
- Cellular and molecular physiology,
- Structural biology and physiology,
- Invertebrate physiology, and more.

In addition to an educational workshop for students, teachers, and the general public, the meeting includes twelve thematic symposia, five keynote lectures, two short "blitz" presentation and two poster sessions. One of the symposia is dedicated to the memory of Academician Prof. Dr. Andrej O. Župančič, founder of the Slovenian Physiological Society, which celebrates its 70th anniversary this year.

We believe that the meeting will provide an excellent platform for networking, knowledge exchange, and fostering collaboration in the field of (patho)physiological research, both basic (preclinical) and clinical. We very much look forward to your participation.

Sincerely,

Nina Vardjan and Jernej Jorgačevski

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Zaloška cesta 4
1000 Ljubljana
Slovenia
E-mail: neuroendo@mf.uni-lj.si

Keynote Speakers

Ákos Koller is a distinguished professor and leading expert in microvascular physiology, with an MD from Semmelweis University (1975) and a Ph.D. from the Hungarian Academy of Sciences (1992). His groundbreaking research has significantly advanced the understanding of wall shear stress-induced vascular responses, endothelial function, and cerebral blood flow autoregulation, shaping the field of microcirculation. With around 200 publications, he has mentored numerous researchers and fostered international collaborations across Europe, the U.S., and Asia. His exceptional contributions have been recognized with prestigious awards, including the Gabor Kaley Award (2015), Malpighi Award (2019), Albert Szent-Györgyi Award (2021), Eugen M. Landis Award (2023), and Carl J. Wiggers Award (2025).

Michela Matteoli is Full Professor of Pharmacology at Humanitas University and Director of the Neuroscience program at Humanitas Research Hospital, Milano, Italy. She is a member of the European Molecular Biology Organization (EMBO), of the Academia Europaea and of the Accademia Nazionale dei Lincei, the oldest European academy. Her research activity focuses on the role of the immune system and inflammation in the formation and function of brain circuits. Michela serves/served in several international scientific committees, including the European Research Council (ERC), the Swiss National Science Foundation (SNSF), and the Harvard-Armenise Foundation. She received various prizes and in 2022 has been awarded with an ERC Advanced Grant.

Viacheslav O. Nikolaev is a leading expert in experimental cardiovascular research and a Full Professor at the University Medical Centre Hamburg-Eppendorf, where he heads the Department of Experimental Cardiovascular Research. His groundbreaking work on cyclic nucleotide signalling in heart function is advancing new therapeutic strategies for cardiovascular diseases and has earned him two patents, the Albert-Fraenkel Prize, the Scientific Prize of Lower Saxony, and other prestigious awards. A dedicated mentor, he has supervised over 40 BSc, MSc, PhD, MD students, and postdocs. He serves on the editorial board of Circulation Research and has held key roles in various international research committees.

Lydie Plecitá-Hlavata is the Head of the Laboratory of Pancreatic Islet Research at the Institute of Physiology in Prague. Her research focuses on mitochondrial function and redox signalling in diseases such as type 2 diabetes and cancer. She earned her PhD in Microbiology from Charles University and the Institute of Microbiology in Prague, where her work on oxidative stress and aging in yeast was highly recognized. Her pioneering research utilizes advanced microscopy techniques to study mitochondrial morphology and glucose-stimulated insulin secretion. Awarded the Otto Wichterle Medal and the L'Oréal Prize for Women in Science, she has published 87 high-impact papers and mentored several PhD students.

Thomas McWilliams is Associate Professor of Mitochondrial Medicine at the University of Helsinki, Finland. He studied biochemistry in Ireland before moving to the UK to complete the Wellcome Trust Four-Year PhD Programme in neuroscience with Alun M. Davies FMedSci FRS and Stephen B. Dunnett FMedSci. Subsequently, he was recruited to the MRC Protein Phosphorylation and Ubiquitylation Unit, where he made groundbreaking advances in the study of physiological mitophagy and Parkinson's disease with Ian Ganley FRSE and Miratul Muqit FMedSci. In 2021, he was elected a Scholar of the FENS-Kavli Network of Excellence. His team has been generously funded by numerous sources, including the Novo Nordisk Foundation, the Jane and Aatos Erkko Foundation, and the Research Council of Finland. A major focus of the lab is on understanding tissue-specific autophagy mechanisms, metabolic signalling, and neurodegeneration, with particular interest in identifying early inflection points and mechanisms that drive disease progression.

Session Speakers

Helena Haque Chowdhury
Anna Csiszar
Marta Cvijić
Natasa Djalinac
Ines Drenjančević
Aleksandra Dugandžić
Eszter Mária Horváth
Eszter Farkas
Bruno Grassi
Senka Holzer
Anemari Horvat
László Hricisák
Martin Hultman
Eva Jarc Jovičić
Nina Kaludercic
Monika Kos
Hrvoje Lalic
Domen Lazar
Giampiero Leanza
Nataša Lindič
Francesco Lodola
Tomaž Martini
Igor Mekjavić
Francesco Moccia
Mitsuhiro Morita
Marco Narici
Akinori Nishi
Nadine Ortner
Éva Pál
Morten Gram Pedersen
Rado Pišot
Maja Potokar
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Marko Šestan
Dušan Turk
Zoltan Varga
Viktoria Venglovecz
Nunzio Vicario
Dora Višnjić
Bayram Yilmaz
Robert Zorec

Scientific Programme

WEDNESDAY, SEPTEMBER 24

09:00-13:00 **PRE-CONFERENCE EDUCATIONAL WORKSHOP #**
Fiziologija brez meja: Delovanje organizmov v ekstremnih okoljih
(Lecture Room 2)

Chairs: Anemari Horvat, Urška Černe, Andraž Stožer

Rok Kostanjšek: Prilagoditve človeške ribice na podzemno okolje

Damjana Rozman: Fiziološki izzivi pomanjkanja teme

Leja Dolenc Grošelj: Spanje v ekstremnih okoljih

0DMOR

Lidija Križančić Bombek: Prilagoditve telesa na višjo nadmorsko višino

Boštjan Rituper: (Pato)fiziološke prilagoditve delovanja kardiovaskularnega sistema na bivanje v mikrogravitaciji

13:00-19:00 **REGISTRATION DESK OPEN** *(Hall)*

14:00-14:15 **WELCOME AND OPENING** *(Lecture Room 1)*

14:15-15:00 **KEYNOTE LECTURE 1** *(Lecture Room 1)*

Chair: Robert Zorec (SI)

Michela Matteoli (IT): *Microglial and TREM2 Dialogues in the Developing Brain*

15:00-16:30 **S01: Innovations in Cardiovascular Physiology: From Mechanisms to Therapeutics** *(Lecture Room 1)*

Chairs: Francesco Moccia (IT), Marta Cvijić (SI)

Francesco Moccia (IT): The Role of Endothelial Ca^{2+} Signalling in Ca^{2+}-Dependent Nitric Oxide Production at the Neurovascular Unit

Francesco Lodola (IT): Light-Controlled Modulation of Cardiac Excitable Cell Activity via a Membrane-Targeted Photoswitch

Carmine Rocca (IT): Key Implications of the FcγRIIA Receptor in Fc Region-Dependent Cardiotoxicity of Trastuzumab

Marta Cvijić (SI): RV Hemodynamics and Understanding RV-PA (un)Coupling in Clinical Practice

15:00-16:30 **S02: Bed Rest Studies as a Model for Disuse and Microgravity** *(Lecture Room 2)*

Chairs: Bruno Grassi (IT), Marco Narici (IT)

Bruno Grassi (IT): Oxidative Metabolism and Mitochondria in Disuse and Microgravity: Functional Impairments, Bottlenecks, Basic Science, Consequences on Health

Igor Mekjavić (SI): The Slovene Bedrest Research Programme: Past, Present and Future

Rado Pišot (SI): The Effects of Prolonged Bed Rest: Influence of Age, Duration and Countermeasures - Experience of Bedrest Center Koper

Marco Narici (IT): Neuromuscular Impairment with Chronic Inactivity

16:30-17:00 **REFRESHMENT BREAK & Poster Viewing** *(Hall)*

17:00-17:15 **POSTER BLITZ 1** *(Lecture Room 1)*

Chairs: Justina Mihaljević (CR), Anemari Horvat (SI)

17:15-19:15 **S03: Neurophysiology in Health and Disease & Andrej O. Župančič Symposium** *(Lecture Room 1)*

Chairs: Robert Zorec (SI), Evelin Szabó (HU)

Giampiero Leanza (IT): Acetylcholine, Noradrenaline and their Peculiar Involvement in Alzheimer's Disease

Akinori Nishi (JP): p11 in Cholinergic Interneurons in the NAc is Essential for Dopamine Response to Rewards: Insight into the Dentate Gyrus-NAc Circuit

Mitsuhiro Morita (JP): AQP4-Dependent ATP/Adenosine Release and Psychiatric Disorders

Bayram Yilmaz (TU)**:** Neuronal Circuits of the Arcuate Nucleus in Obesity: Classical and Novel Perspectives

Evelin Szabó (HU): The Effect of Prolactin-releasing Peptide (PrRP) on Depression-like Behaviour in Rats

19:15-22:00 **WELCOME RECEPTION** *& 70 years of Slovenian Physiological Society (Lecture Room 1/Hall)*

THURSDAY, SEPTEMBER 25

08:00-12:00 **REGISTRATION DESK OPEN** *(Hall)*

09:00-10:30 **S04: Cerebrovascular Impairment in Aging and Disease** *(Lecture Room 1)*

Chairs: Eszter Farkas (HU), Anna Csiszár (HU)

Éva Pál (HU): Disruption of Vitamin D Signalling Impairs Cerebrovascular Adaptation to Carotid Artery Occlusion

Eszter Farkas (HU): Impaired Neurovascular Coupling after Acute Ischemic Stroke in the APP/PS1 Transgenic Mouse Model of Alzheimer's Disease

Anna Csiszár (US/HU): Age-related Vascular Cognitive Impairment: Role of Neurovascular Senescence

László Hricisák (HU)**:** Roles of NOS Isoforms in the Adaptation of the Cerebrocortical Circulation to Unilateral Carotid Artery Occlusion

09:00-10:30 **S05: Organelle Dynamics and Metabolism in Health and Disease** *(Lecture Room 2)*

Chairs: Toni Petan (SI), Eva Jarc Jovičić (SI)

Tomaž Martini (SI/CH): A Sexually Dimorphic Hepatic Cycle of Periportal VLDL Generation and Subsequent Pericentral VLDLR-mediated Re-uptake

Marko Šestan (CR): Neuro-endocrino-immune Regulation of Metabolic Homeostasis

Eva Jarc Jovičić (SI): Lipid Droplets Control Membrane Integrity and Mitochondrial Homeostasis in Starvation and Ferroptosis

Short Talk **Ivo Kosmačin** (SI): Chronic 5-HT_2A Receptor Activation Differentially Affects Mitochondrial Biogenesis and Stress Tolerance in Neonatal and Adult Rat Cortical Astrocytes

Short Talk **Lara Batičić (CR):** Dynamic Changes of Interleukin-18 Concentration in Peripheral and Coronary Circulation Following Coronary Artery Bypass Surgery

10:30-10:45 **POSTER BLITZ 2** *(Lecture Room 1)*

Chairs: Simona D'Aprile (IT), Jurij Dolenšek (SI)

10:45-12:00 **REFRESHMENT BREAK with POSTER SESSION 1** (*Hall*)

12:00-12:45 **KEYNOTE LECTURE 2** *(Lecture Room 1)*

Chair: Andraž Stožer (SI)

Lydie Plecita-Hlavata (CZ): *Redox Signalling in Pancreatic β-Cells: Balancing Health and Dysfunction*

12:45-13:45 **LUNCH** *(Hall)*

13:45-15:15 **S06: Impact of Nutrients and Lifestyle on Microvascular Function** *(Lecture Room 1)*

Chairs: Helena Lenasi (SI), Ines Drenjančević (CR)

Ines Drenjančević (CR): High Salt Diet Affects the Microvascular Reactivity – is there a Salvage Way? – Comparative Studies in Animals and Humans
Lana Kralj (SI): Time-dependent Microvascular and Autonomic Nervous System Alterations after Oral Glucose Loading in Young Healthy Individuals
Eszter Mária Horváth (HU): Ivabradine Induced Vasorelaxation in a Hyperandrogenic Rat Model of Polycystic Ovarian Syndrome, Effect of Vitamin D Supplementation
Henrique Silva (PT): The Impact of Acute Mental Stress on Microvascular Reactivity
Martin Hultman (SE): Towards a Standardized Framework for Microvascular Flowmotion Analysis

13:45-15:15 **S07: Insect Neurobiology and Neuroethology** *(Lecture Room 2)*

Chairs: Gregor Belušič (SI), Anemari Horvat (SI)

Nataša Stritih Peljhan (SI): Beyond Spectral Overlap: an Integrative Study Reveals a Novel Mechanism of Vibrational Masking in Insects
Monika Kos (SI): Connectome of the Early Visual Pathway of a Putterfly Elucidates the Neural Toolkit for the Green-red Colour Opponent Axis
Domen Lazar (IT): The Role of Vision and Visual Cues in Host Plant Location by the Meadow Spittlebug Philaenus Spumarius
Anemari Horvat (SI): Age-Related Dysregulation of Octopaminergic Ca^{2+} Signalling and Metabolism in *Drosophila* Brain

15:15-16:30 **REFRESHMENT BREAK with POSTER SESSION 2** *(Hall)*

16:30-18:00 **S08: New Frontiers in Cardiovascular Research** *(Lecture Room 1)*

Chairs: Simon Sedej (AT), Nina Kaludercic (IT)

Nina Kaludercic (IT): Mitochondrial ROS Formation in Cardiac Disease

Zoltán Varga (HU): Immune Checkpoint Signalling and Heart Failure

Natasa Djalinac (IT): Human Microtissue Models for Studying Cardiolaminopathies
Senka Holzer (AT): Calcium Signalling in Early and Late Hypertensive Cardiac Remodelling

16:30-18:00 **S09: Endocrine Physiology: From Mechanisms to Phenotypes** *(Lecture Room 2)*

Chairs: Andraž Stožer (SI), Viktoria Venglovecz (HU)

Viktoria Venglovecz (HU): Endocrine-Exocrine Interactions in the Pancreas: Impact of Diabetes on Ductal Function

Morten Gram Pedersen (IT): Dynamical Time-to-event Analysis Provides Insight into the Role of Syntaxin in Docking of Insulin Granules

Nadine Ortner (AT): Preclinical Models for a Rare Cav1.3 Channelopathy: Insights into the Pathophysiology and Therapeutic Option

Short talk **Nastja Murko** (AT/SI): Effects of Arginine Vasopressin on Pancreatic α and β Cells: Glucose-Dependent Modulation and Receptor-Specific Responses

Short talk **Marko Gosak** (SI): Integrating Imaging and Network Science to Decode Capillary Architecture in Health and Disease

18:00-18:45 **KEYNOTE LECTURE 3** *(Lecture Room 1)*

Chairs: Eva Jarc Jovičič (SI), Toni Petan (SI)

Thomas McWilliams (FN): *Physiological Mitophagy Dynamics in Healthy Brain Aging*

19:00-22:00 **NETWORKING EVENT with Boat Tour and Social Dinner at Livada** *(meet in the Hall at 19:00 for a boat tour or 19:30 at Livada, Hladnikova cesta 15)*

FRIDAY, SEPTEMBER 26

08:30-11:30 **REGISTRATION DESK OPEN** *(Hall)*

09:00-09:45 **KEYNOTE LECTURE 4** *(Lecture Room 1)* *****

Chair: Nina Vardjan (SI)

Viacheslav O. Nikolaev (DE): *Illuminating Cardiac cAMP Microdomains for Better Heart Failure Therapies*

09:45-10:55 **S10: Workshop on Structural Biology and Physiology** *(Lecture Room 1)* *****

Chairs: Nina Vardjan (SI), Robert Zorec (SI)

Dušan Turk (SI): All Roads Lead to Cathepsins

Nataša Lindič (SI): Engineering Endogenous Cathepsin Inhibitor for Subcellular Targeting and Antiviral Intervention

Robert Zorec (SI): Searching for a New Lactate Receptor Mediating Metabolic Excitability in Astrocytes

10:55-11:30 **REFRESHMENT BREAK & Poster Viewing** *(Hall)*

11:30-13:00 **S11: Pathways in Physiology: From Wellness to Illness** *(Lecture Room 1)*

Chairs: Aleksanda Dugandzić (CR), Lana Ružić Švegl (CR)

Aleksanda Dugandzić (CR): The Role of Uroguanylin in Genetic Predisposition and the Development of Severe Forms of Diabetes Type 2

Dora Višnjić (CR): The Physiology of Differentiation under Stress: Insights from Nucleotide Metabolism

Hrvoje Lalić (CR): Monocyte-Driven Pathways in Chronic Graft-versus-Host Disease

Lana Ružić Švegl (CR): Are Widespread Hypoxic Chambers Undermining the Use of Hypobaric Altitude Training and Its Associated Adaptations?

11:30-13:00 **S12: Cellular Strategies in Cancer and Neural Pathologies** *(Lecture Room 2)*

Chairs: Jernej Jorgačevski (SI), Maja Potokar (SI)

Helena H. Chowdhury (SI): Autologous Immunohybridoma Therapy: A Platform for Solid Tumour Treatment

Maja Potokar (SI): Insights into Plectin's Potential as a Glioblastoma Biomarker

	Nunzio Vicario (IT): Connexin 43-based Intercellular Communication and Its role in Neuropathic Pain
Short Talk	**Luka Lapajne** (SI): Neuropsin, TRPV4 and Intracellular Calcium Mediate Intrinsic Photosensitivity in Corneal Epithelial Cells
13:00-13:45	**KEYNOTE LECTURE 5** *(Lecture Room 1)*
	Chair: Zoltan Benyo (HU)
	Akos Koller (HU): *The Brain Flow: Regulation of Fluids in and out of the Brain to Maintain Intracranial Pressure*
13:45-14:00	**POSTER AWARDS AND CLOSING** *(Lecture Room 1)*
14:00-15:00	**LUNCH** *(Hall)*

 # Slovenian language
**In collaboration with Instruct-ERIC Consortium*

Abstracts

Keynote speakers

Microglial and TREM2 Dialogues in the Developing Brain

Michela Matteoli[1,2]

[1]Humanitas Clinical and Research Center - IRCCS, Rozzano, Milan, Italy; CNR Institute of Neuroscience, Milano, Italy.
[2]Humanitas Clinical and Research Center - IRCCS, Rozzano, Milan, Italy; Department of Biomedical Sciences, Humanitas University, Pieve Emanuele, Milan, Italy.

Microglia are key players in brain development, orchestrating a range of spatially and temporally regulated processes critical for proper brain formation. During neurogenesis, microglia interact closely with neural precursor cells, modulating their proliferation, differentiation, and migration. They also regulate neuronal apoptosis, eliminating excess or improperly formed neurons, and contribute to the correct positioning of neurons within the developing cortex. As development advances, microglia continue to shape neural circuits through synaptic remodelling. By constantly surveying the brain environment, they promote the elimination of weak or unnecessary synapses while preserving functional ones, thereby refining the architecture of neuronal networks.
This functional versatility is driven by the extraordinary plasticity of microglia, which enables them to respond dynamically to evolving signals from surrounding brain cells. Their ability to adopt different phenotypes is tightly regulated through a complex interplay of transcriptional, epigenetic, translational, and metabolic mechanisms. Among the molecular regulators of microglial states, TREM2 (Triggering Receptor Expressed on Myeloid Cells 2) stands out. This microglia-specific receptor, known for its association with Alzheimer's disease, also plays a crucial role during early brain development. Our research has shown that TREM2 facilitates the pruning of excess synapses—a process essential for proper neural circuit refinement—and is also necessary for regulating neuronal metabolism. Disruptions in TREM2 function during these critical developmental windows can impair microglial activity, potentially leading to long-term consequences that increase the brain's vulnerability to damage later in life.

Redox Signalling in Pancreatic β-Cells: Balancing Health and Dysfunction

Blanka Holendová[1], Štěpánka Benáková[1], Monika Křivonosková[1], Linda Stokičová[1], Lydie Plecitá-Hlavatá[1]

[1]Laboratory of Pancreatic Islet Research, Institute of Physiology, Czech Academy of Sciences, Prague, Czech Republic

Pancreatic β-cells are essential for maintaining systemic glucose homeostasis by regulating insulin secretion. Their function is tightly linked to metabolite fluctuations, allowing them to act as the precise sensors of blood glucose levels. Reactive oxygen species (ROS) and hydrogen peroxide in particular, produced during metabolic activity, serve as critical signalling molecules under physiological conditions. However, excessive ROS production during pathological states can lead to oxidative stress. Our research demonstrates that hydrogen peroxide generated by NADPH oxidase 4 (NOX4) plays a key role in modulating metabolic proteins and acts as a crucial coupling factor for insulin secretion upon glucose stimulation. However, sustained NOX4 activity under conditions of nutritional excess disrupts this balance, leading to pro-oxidative signalling and local inflammation, which can escalate into systemic inflammation—a hallmark of type 2 diabetes. Interestingly, long-term NOX4 inactivation does not protect β-cells, despite having no impact on lifespan in mice. These findings underscore the importance of understanding redox signalling, oxidative stress, and β-cell dysfunction to develop targeted therapies that preserve β-cell health and function in type 2 diabetes.

Physiological mitophagy dynamics in healthy brain aging

Thomas McWilliams[1]

[1]University of Helsinki, Finland

Mitophagy safeguards neural integrity by eliminating damaged mitochondria, thereby alleviating metabolic stress. Although age-related decreases in mitophagy are well established in short-lived species, the regulation of physiological mitophagy in the mammalian brain and its relationship to other autophagy pathways remains unclear. I will present new insights into the spatiotemporal dynamics of mitophagy in vivo. Using genetically-encoded optical reporter systems, we reveal significant cell-type-specific variation in basal mitophagy, challenging the notion of a uniform homeostatic process in the mammalian brain. We identify neural populations in which mitophagy is sustained into old age, alongside others where it declines, revealing mechanisms that may underlie selective resilience and vulnerability. Our work pinpoints midlife as a critical turning point marked by emerging lysosomal dysfunction that may predispose specific neurons to later degeneration. Integrating these findings with human data and unconventional model systems, our work reframes mitophagy as a dynamic, circuit-defined process with therapeutic implications for preserving mitochondrial integrity in the aging brain.

Illuminating Cardiac cAMP Microdomains for Better Heart Failure Therapies

Viacheslav O. Nikolaev[1]

[1]University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Heart failure is a very frequent devastating cardiovascular disease with poor prognosis and five-year survival rates not much exceeding 50%, despite modern therapies. Current medications are aimed at ameliorating neuro-hormonal overactivation mechanisms with beta blockers as one of the prime drug classes used to halt disease progression. However, these drugs also inhibit cardiac contractility and relaxation, limiting their use in patients with late-stage and diastolic heart failure. Recently, we developed a live cell imaging technique to directly monitor cAMP responses engaged beta1-adrenergic receptors (b_1-AR) in various subcellular microdomains of healthy and failing cardiac myocytes. This approach has allowed identification of mechanisms driving heart failure at the subcellular, microdomain specific level, including redistribution of specific phosphodiesterase isoforms, receptors and effector proteins. We also gained deeper insights into how distinct b_1-AR effects on cardiac remodelling vs. contractility are regulated by the membrane structure environment and could develop a new generation of beta blockers to prevent detrimental receptor signalling, leaving contractility intact. In summary, our live cell imaging approach provides a detailed landscape of cardiac microdomains and opens up a new way of modulation for better heart failure therapies.

The Brain Flow: Regulation of Fluids in and out of the Brain to Maintain Intracranial Pressure

Akos Koller[1,2,3,4,5]

[1]Institute of Translational Medicine, HUN-REN-SE, Cerebrovascular and Neurocognitive
[2]Disease Research Group, Semmelweis University, Budapest, Hungary
[3]Department of Morphology and Physiology, Semmelweis University, Budapest, Hungary
[4]Research Center for Sport Physiology, Hungarian University of Sports Science, Budapest, Hungary
[5]Department of Physiology, New York Medical College, Valhalla, NY, USA

It was realized centuries ago that the relative constancy of intracranial pressure and volume is utmost important for the healthy functioning of the brain. In this overview the roles of cerebrospinal fluid (CSF), brain lymphatics/glymphatic (BLS) and cerebral blood flow (CBF), are described in maintaining relatively constant pressure and volume in the intracranial environment. There are several jointly functioning, passive and active autoregulatory mechanisms (AR), which ensure the constancy of intracranial pressure and volume.
The brain is suspended and supported by CSF within the skull, effectively making it float, thereby substantially reducing its weight (Archimedes' principle). CSF provides - among others - cushioning, protection from injury and contributes to the maintenance of intracranial pressure/volume. CSF is produced by the choroid plexus and circulates in the cerebral ventricular system, a series of interconnected, fluid-filled cavities connected also to the spinal cord, subarachnoid space and perivascular space. CSF also removes waste, and its reduced production can allow an increase in CBF in ischemia.
CSF is connected to the brain lymphatic/glymphatic systems, which represent a new biological concept proposing that it is likely to participate in many physiological and pathological processes in the central nervous system.
CBF ranges ~50 mL/100g/min in the brain supplying oxygen, glucose and other nutritional molecules and removes CO_2 and metabolic end products. CBF is actively autoregulated, meaning that increases in perfusion pressure (PP) do not increase linearly CBF because the arterial vessels constrict, increasing thereby vascular resistance and maiming CBF close to constant between 60-150 mmHg systemic blood pressure. The two main vascular mechanisms eliciting constrictions are pressure sensitive myogenic and the flow sensitive shear stress mechanisms. The myogenic response is initiated by an increase in intracellular Ca^{2+} in the smooth muscle leading to constriction, whereas the flow sensitive mechanism is achieved by endothelial arachidonic acid metabolites, such as 20-hydroxyeicosatetraeonic acid (20-HETE), produced by cytochrome P450 4A, and acting on thromboxane A_2 (TP) receptors. The constrictor effect is modulated by the dilator nitric oxide mechanism. Protein and gene expressions also confirmed the importance and regional specificity of these enzymes in the intracranial and extracranial cerebral arteries corresponding to their vasomotor function. Cerebrovascular resistance is further modulated by metabolic, hormonal, and neural mechanisms to serve local needs.
The interaction of these mechanisms not only maintains an appropriate intracranial environment but also ensures the appropriate function of the brain in normal and challenging conditions.

Support: TKP2020-NKA-17, TKP2021-EGA-37, OTKA132596, K-19 (Ministry of Innovation and Technology of Hungary from the National Research, Development and Innovation Office of Hungary.

Symposia Abstracts

S01: Innovations in Cardiovascular Physiology: From Mechanisms to Therapeutics

The Role of Endothelial Ca^{2+} Signalling in Ca^{2+}-Dependent Nitric Oxide Production at the Neurovascular Unit

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Recent studies demonstrated that mouse brain microvascular endothelial cells may integrate physical-chemical signals to selectively increase cerebral blood flow (CBF) towards the firing neurons through the production of nitric oxide (NO). Additionally, endothelial-derived NO could modulate neuronal excitability and long-term plasticity, thereby adding a novel layer of complexity to the vascular-to-neuronal communication. Here, I discuss that human brain microvascular endothelial cells are also able to release NO in response to neural and physical cues. We illustrate that human brain microvascular endothelial cells express group 1 metabotropic glutamate receptors (mGluRs), i.e., mGluR1 and mGluR5, which elicit a concerted interplay between inositol-1,4,5-trisphosphate (InsP3)-dependent Ca2+ release from the endoplasmic reticulum (ER), nicotinic acid adenine dinucleotide phosphate (NAADP)-dependent Ca2+ release from the lysosomes, and store-operated Ca2+ entry (SOCE). Furthermore, these cells express N-methyl-D-aspartate receptors (NMDARs) that signal in a flux-independent manner by functionally interacting with group 1 mGluRs to produce NO in a Ca2+-dependent manner. Finally, I present evidence that Ca2+ entry and NO release can occur in response to the activation of the mechanosensitive Piezo1 channel, which is the primary target of blood flow-mediated shear stress.
These findings lend support to the notion that human cerebrovascular endothelial cells may directly sense synaptically released neurotransmitters. The following increase in local CBF may further boost NO signalling upon the activation of Piezo1 channels, which might contribute to prolonging the hemodynamic response during a sustained neuronal activity.

Light-Controlled Modulation of Cardiac Excitable Cell Activity via a Membrane-Targeted Photoswitch

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Light-based strategies for modulating cellular bioelectricity are gaining interest due to their high spatial and temporal precision combined with minimal invasiveness. Non-genetic (geneless) optostimulation is emerging as a multidisciplinary field that exploits photoactive materials to confer light sensitivity to cells without the need of genetic modification. In this context, we investigate the application of Ziapin2, an amphiphilic azobenzene-based photoswitch that targets the plasma membrane and modulates cellular excitability through light-induced changes in membrane capacitance. By integrating electrophysiological recordings, optical imaging, and motion analysis, we demonstrate that Ziapin2 modulates cardiac excitability through an optomechanical mechanism, allowing precise and reversible control of the entire excitation–contraction coupling process, both in isolated cells and in engineered anisotropic aligned cardiac microtissues. Altogether, these findings highlight the potential of Ziapin2 as a geneless photostimulation tool for cardiac research and encourage further exploration of light-driven methods for cardiac modulation.

Key Implications of the FcγRIIA Receptor in Fc Region-Dependent Cardiotoxicity of Trastuzumab

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Fcγ receptors (FcγRs) are implicated in various cardiovascular responses. They also participate in the mechanism of action of trastuzumab (TRZ), a monoclonal antibody that exerts anticancer activity by targeting the HER2 receptor, but its blockade in cardiac cells leads to cardiotoxicity, particularly in elderly patients. However, the involvement of FcγRs in TRZ cardiotoxicity is not completely established. In this study, we investigated the impact of the Fc region on TRZ-induced cardiomyocyte toxicity by evaluating whether a recombinant Fab fragment of TRZ (rFab-HER2) could result in a lower toxicity profile while maintaining TRZ's ability to inhibit HER2 in human cells. Our results showed that cardiomyocytes were more vulnerable to cytotoxicity and cell death upon TRZ treatment than with rFab-HER2. Both TRZ and rFab-HER2 downregulated HER2 expression and the downstream AKT/ERK pathways, confirming target engagement. However, unlike TRZ, rFab-HER2 did not affect mitochondrial dynamics, and did not trigger oxidative stress, inflammation, or apoptosis. Moreover, TRZ, but not its Fab fragment, significantly upregulated the expression levels of FCγRIIA, an FcγR expressed in cardiomyocytes and markedly implicated in TRZ-induced antibody-dependent cellular cytotoxicity (ADCC). These findings suggest that the Fc region of TRZ can play a critical role in mediating, at least in part, TRZ cardiotoxicity, and identify FcγRIIA as a novel physiological agent whose targeting may help mitigate the cardiomyocyte side effects of TRZ.

RV Hemodynamics and Understanding RV-PA (un)Coupling in Clinical Practice

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Assessment of right ventricular (RV) haemodynamic is very challenging in everyday clinical practice. All the imaging parameters that reflect RV function, such as ejection fraction, fractional area change or Doppler parameters, obtained either by echocardiography or cardiac magnetic resonance, are influenced by loading conditions. When the RV faces pathological increases in preload and afterload, the accuracy of these imaging parameters is very limited. This is especially expressed in patients with tricuspid regurgitation (TR). Sometimes RV dysfunction in patients with TR is too subtle to see, escaping detection because it is hidden behind the volume overload. However, a careful assessment of right heart function is particularly relevant for disease such as TR because RV function significantly influences outcome. Some of the above-mentioned limitations can be overcome with the recently introduced new parameter of ventricular-arterial coupling, which enables us to determine whether RV function is adequately compensated for specific loading condition. RV-pulmonary arterial (PA) coupling index was first defined in invasive hemodynamics using the pressure-volume analysis, and refers to the relationship between RV contractility (Ees) and effective arterial elastance (Ea), a measure of total RV arterial load. RV-PA coupling (Eas/Ea) describes a hemodynamic state where mechanical stroke work is most efficiently transferred to the pulmonary circulation, whereas uncoupling suggests that RV contractility can no longer compensate for the increase in afterload. It has been shown that this parameter is useful in identifying early stages of RV systolic dysfunction that would otherwise be unrecognized by traditional imaging modalities. There might be several potential applications of using this parameter in clinics. RV-PA coupling in patients with TR can be used to assess disease progression as well as evaluate RV compensation to abnormal RV loading. In compensated states, RV contractile function increases in step with the rise in afterload to maintain steady RV-PA coupling ratios. In contrast, in decompensated states, RV contractile function no longer rises relative to afterload, thereby resulting in lower RV-PA coupling ratios. Due to the invasive nature of the method, the clinical application of pressure-volume assessment is limited. Therefore, some non-invasive surrogates for RV-PA coupling have been proposed. Most of these non-invasive coupling indices consist of a parameter of RV function and one parameter of RV afterload or resistance, usually represented by estimated PA pressure. TAPSE/sPAP is one of the most often used non-invasive surrogates for RV-PA coupling. Importantly, these non-invasive indexes remain load dependent, and absolute and relative changes should be interpreted accordingly. However, these non-invasive indices proved their prognostic impact in the presence of severe TR, but while prognostic, their accurate representation of coupling is less certain. In conclusion, RV-PA coupling is a promising parameter to determine whether RV function is adequately compensated for specific loading conditions. However, which measures and what cut-offs of RV-PA coupling should be used in clinics remains unknown.

S02: Bed Rest Studies as a Model for Disuse and Microgravity

Oxidative Metabolism and Mitochondria in Disuse and Microgravity: Functional Impairments, Bottlenecks, Basic Science, Consequences on Health

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Skeletal muscle oxidative metabolism and mitochondrial function represent “the last step” in the long pathway for O_2, from ambient air to oxidative phosphorylation. This metabolism is often neglected in studies dealing with the effects of microgravity/disuse on skeletal muscle, which mainly take into consideration muscle mass and muscle force. This is unfortunate, considering that all activities lasting longer than 1-2 minutes substantially rely on oxidative metabolism for ATP turnover. Apart from its direct effects on exercise tolerance, a decreased “cardiorespiratory fitness” determined by microgravity/disuse, as identified by the a decreased maximal O_2 uptake ($\dot{V}O_2max$), is associated with profound negative consequences on the general health status of the subjects, such as decreased insulin sensitivity, “pro-inflammatory” condition, impaired endothelial function, mitochondrial dysfunction, altered function of the neuromuscular junction, altered redox status, increased oxidative stress. A $\dot{V}O_2max$ decrease during microgravity/disuse has been described in “bed rest” studies, ranging from hours to a few months. These studies have allowed us to identify “bottlenecks” along the O_2 transport and O_2 utilization pathway, mainly related to impaired cardiac function, reduced blood volume, impaired microvascular/endothelial function. These impairments would occur relatively early during exposure, whereas peripheral O_2 conductance and mitochondrial respiration would be significantly affected during longer exposures. There are, however, areas in which bed rest studies appear to be relatively weak in terms of the inferences on real spaceflight conditions, in relation to the significantly longer durations of space missions, to the role of in-flight countermeasures (e.g. exercise, artificial gravity), to the very relevant role played by space radiations.
According to recent work from our group short periods of microgravity/disuse could have an interesting effect on skeletal muscle and whole body oxidative metabolism in resting conditions, that is a substantial decrease in resting muscle $\dot{V}O_2$ and whole-body resting energy expenditure (REE). Even by being far less pronounced compared to the typical reduction of REE observed in obligate hibernating animals, the observed inactivity-related decrease in resting muscle $\dot{V}O_2$ and REE, possibly aimed at preventing ATP accumulation or excessive ROS production, could mitigate numerous biological and logistic challenges of prolonged spaceflights by lowering rates of crewmember consumable use (food, water, O_2) and CO_2 production. On the other hand, the inactivity-related decrease in REE would have negative consequences on the health status of subjects, by altering body mass homeostasis and increasing the risk of metabolic diseases.

Fundings
PRIN Projects 2020EM9A, Italian Ministry of Research; Departmental Strategic Plan (PSD), University of Udine, Interdepartmental Project on Healthy Ageing (2020-25)

The Slovene Bed Rest Programme: Past, Present and Future

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In 1972, Genin and Pestov reported that the *»reactions of cosmonauts on return to terrestrial gravitation are reproduced to a considerable degree by the laboratory simulation of weightlessness. For example, they are observed after prolonged immersion of human subjects in water or multiday confinement to bed with restriction of mobility«*. Since then, the methodologies of »dry immersion« and »bed rest« are used by Space agencies to study the adaptation of physiological systems to weightlessness, and to assess the efficacy of measures mitigating these adaptations, to facilitate the return of astronauts to Earth (so-called »countermeasures«).

PAST: Effect of bed rest on the adaptation of physiological systems to inactivity and unloading

The Slovene Bed Rest programme was initiated by Eiken and Mekjavic in 2011 to investigate the effect of a 35-d bed rest on vascular distensibility. This study conducted at the Valdoltra Orthopaedic Hospital revealed a significant effect of inactivity/unloading on increasing the distensibility of arteries and veins in the lower limbs. In addition, the pattern of muscle atrophy in muscles of the hip, thigh and calf was reported for the first time. The Valdoltra study also initiated programmes of research investigating the effect of inactivity and unloading on human temperature regulation and the ophthalmic system.

PRESENT: The effect of hypoxia on the adaptation of physiological systems to bed rest

In 2007 our team was tasked to develop and install a hypoxic system in the Olympic Sport Cenre Planica. The focus of research initially focused on hypoxic training of athletes, altitude acclimatisation of alpinists and Slovene Army personnel, and exploring the aetiology of altitude anorexia. In 2011, the European Space Agency initiated a programme of research in Planica to assess the effect of hypoxia on the process of adaptation of physiological systems to inactivity and unloading. This programme was initiated due to the likelihood that future environments in space vehicles and habitats will be hypoxic.

FUTURE: Countermeaures, body fluid shifts and thromoembolytic events

The European Space Agency accredited the Jozef Stefan Institute laboratory in Planica as a ground based research facility in 2021. With the installation of ESA's short arm human human centrifuge, we have embarked on a new phase of research, that of assessing the efficacy of countermeasures, the most recent one being exercise with artificial gravity, to mitigate the loss of muscle and bone mass during bed rest. We are now preparing for projects that will investigate sex differences in the adaptation to inactivity/unloading and also the degree to which body fluid shifts contribute to Spaceflight Associated Neuro-ocular Syndrome (SANS). This latter study will also focus on markers for thromboemolytic events, as this, together with SANS, are phenomena that need to be resolved prior to future deep space missions, such as the planned mission to Mars.

Of note is also the initiation of the Erasmus Mundus Joint M.Sc. programme on »The Physiology and Medicine of Humans in Space and in Extreme Environments«. Together with the University of Caen (Caen, France) and Charite University (Berlin Germany), the International Postgraduate School Jožef Stefan (with partner Faculty of Medicine, University of Ljubljana) will train students from around the world in the field of Space life sciences and involve them in ongoing research in Slovenia.

Acknowledgments: We are indebted to the Ministry of the Economy, Tourism and Sport (Slovenia), European Space Agency, European Commission, Swedish Space Board, Italian Space Agency, Ministry of Defence (Slovenia) and Slovene Research and Innovation Agency for support.

The Effects of Prolonged Bed Rest: Influence of Age, Duration and Countermeasures - Experience of Bedrest Center Koper

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Physical activity (PA) has played a fundamental role in human evolution, with gravity shaping the structure and function of the human body. However, contemporary lifestyles have disrupted this intrinsic relationship, shifting from active opposition to gravity toward passive compliance. As a result, the evolutionary imperative for movement is increasingly undermined, making physical activity more crucial than ever for maintaining health in terrestrial environments.
PA serves as a key determinant of physical fitness, work capacity, immune resilience, and psychophysical homeostasis. Conversely, the global rise in sedentarism and physical inactivity represents a serious public health challenge. Even brief periods of inactivity can exacerbate physiological decline, particularly in older adults, whose health is already compromised by the aging process. The interaction between inactivity and aging is a well-established contributor to the development of chronic diseases and increased mortality risk.
The experimental model of Bed Rest is presently regarded as the best ground-based model of human spaceflight as it affords to study the detrimental effects of chronic inactivity on most physiological systems of the human body, mimicking changes occurring with spaceflight. Microgravity imposes many negative health consequences that need to be counteracted by physical exercise modalities for astronauts' health.
Over the last 20 years, at the Bedrest Centre Koper, Slovenia, we conducted a total of six Bedrest studies, together with many international partners and through an excellent collaboration with the Izola General Hospital and the Valdoltra Orthopaedic Hospital. Some of the fundamental observations and specific differences between the results of the changes in the subjects of the individual studies (lasting between 10 and 35 days) and the differences between younger (20 to 30 years) and older (60+) subjects will be presented in this lecture.
Over the past two decades, the Bed Rest Centre in Koper, Slovenia, has conducted seven bed rest studies in collaboration with numerous international partners, the Izola General Hospital, and the Valdoltra Orthopaedic Hospital. These studies, lasting between 10 and 35 days, have provided critical insights into the physiological consequences of prolonged inactivity across different age groups. This lecture will highlight key findings, with particular emphasis on age-related differences in physiological responses—comparing younger (20–30 years) and older (60+) participants—as well as the effectiveness of the implemented countermeasures.

Neuromuscular Impairment with Chronic Inactivity

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Chronic exposure to actual and simulated spaceflight has a profound impact on the structural and functional integrity of the neuromuscular system. Studies have shown that even few days of inactivity induced by bed rest can lead to significant losses in muscle mass, strength, and gene expression, all of which are associated with substantial neuromuscular alterations.
For instance, Demangel et al. reported that just three days of unloading via dry immersion resulted in marked atrophy and force loss in the knee extensors, accompanied by muscle fiber denervation. Within ten days, additional signs of neuromuscular impairment emerge, including neuromuscular junction (NMJ) instability, altered NMJ morphology, extensive acetylcholine receptor remodelling, denervation, and axonal damage. These alterations disrupt motor unit recruitment thresholds, reduce firing frequency, reduce conduction velocity, and diminish neuromodulatory input from monoaminergic neurotransmitters—which are essential for regulating the excitability of spinal motor neurons. Notably, associated with these changes, is an impairment of NMJ transmission, leading to a decline in muscle strength exceeding what predicted by muscle atrophy alone.
Recent findings suggest that mitochondrial dysfunction—both in skeletal muscle fibers near the NMJ and within motor neuron terminals—plays a central role in NMJ instability and muscle denervation. In a 10-day bed rest study, Motanova et al. observed NMJ remodelling marked by reduced overlap between pre- and postsynaptic terminals, which likely impairs synaptic transmission and signal propagation. Our findings suggest that these changes are driven by oxidative stress, increased mitochondrial fission, and reduced mitochondrial volume density. Collectively, these factors likely contribute to denervation and the associated decline in muscle strength observed with chronic inactivity.

Funding: "This study was carried out within the *Space-It-Up* project funded by the Italian Space Agency, ASI, and the Ministry of University and Research, MUR, under contract n. 2024-5-E.0 - CUP n. I53D24000060005."

S03: Neurophysiology in Health and Disease & Andrej O. Župančič Symposium

Acetylcholine, Noradrenaline and Their Peculiar Involvement in Alzheimer's Disease

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Loss of cholinergic neurons in the basal forebrain nuclei and of noradrenergic neurons in the pontine Locus Coeruleus have been seen for decades as major contributors to the complex spectrum of cognitive and histopathological alterations seen in Alzheimer's Disease (AD). Notably, given the multifactorial nature of AD, modelling these changes in experimental animals has proven difficult, thus hampering the quest for sound therapies. The talk will deal with recent evidence, obtained in rats with selective immunolesions, substantiating the primary role of acetylcholine and noradrenaline in the regulation of specific aspects of cognitive function and their possible involvement in the regional expression of pathological proteins and in adult hippocampal neurogenesis.

p11 in Cholinergic Interneurons in the NAc is Essential for Dopamine Response to Rewards: Insight into the Dentate Gyrus-NAc Circuit

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Dopaminergic neurons projecting to the nucleus accumbens (NAc) regulate reward-related behaviours, and their function is impaired in depression. Cholinergic interneurons (CINs) in the NAc play critical roles in modulating behavioral responses to psychostimulants and natural rewards. The multifunctional protein p11 (S100A10) is highly expressed in NAc CINs, and p11 in CINs is implicated in depression-like behaviors. We previously reported that p11 in CINs is required to activate CINs and induce acetylcholine release in response to rewarding stimuli, enhancing dopamine release (Hanada, eNeuro 2018). This effect of p11 is mediated by HCN2 gene expression in CINs, which is crucial for CIN tonic activity.
In this study, we investigated how rewarding stimuli activate CINs in the NAc. CINs receive excitatory inputs from the thalamus, cortex, and hippocampus. Given the critical role of the hippocampus-NAc circuit in dopamine neurotransmission, we focused on the hippocampus-NAc circuit. Activation of the hippocampal dorsal dentate gyrus (dDG) with NMDA infusion induced dopamine release in the NAc, as measured by *in vivo* microdialysis. This dopamine release was abolished by selective inhibition of CINs using AAV-DIO-rM4D(Gi) in ChAT-Cre mice after deschloroclozapine (DCZ) administration. The dopamine response was also reduced in the NAc of ChAT-Cre p11 conditional KO mice, and the reduced response was rescued by overexpression of p11 in NAc CINs. Furthermore, dopamine responses to natural rewards such as exposure to palatable food and a female mouse, but not cocaine infusion, were abolished by blocking the neural activity of the dDG through lidocaine infusion. These findings highlight the critical role of the dDG-NAc circuit in p11-dependent activation of CINs, which induces dopamine release in response to natural rewards. Future studies should explore the involvement of the hippocampus-NAc circuit in depressive behaviours.

AQP4-Dependent ATP/adenosine Release and Psychiatric Disorders

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The brain is equipped with unique water systems consisting of the blood-brain barrier, cerebrospinal fluid, and astrocyte AQP4. The water environment in the brain is supposed to be essential for the highly developed information processing by neurons, but the crosstalk between water and neuron is still to be elucidated. We had reported that water influx mediated by AQP4 induces ATP release and subsequent increase of extracellular adenosine, which modulate neuronal activities, as well as blood flow and immune cells. Furthermore, we are finding that adenosine derived from astrocytes affects dopamine neurotransmission and is associated with psychiatric disorders. Depressive behaviour is affected in AQP4 knockout mice, which shows altered adenosine release, adenosine receptor expression and adenosine-dependent modulations of dopamine neurotransmission. Since AQP4 localizes to the astrocytic endfeet along blood vessels, these data suggest that the flow of water from the bloodstream to brain tissue influences neural activity. And this water flow is likely accelerated by the nutrient uptake during neuronal activities, because astrocyte active transport is the exclusive gate to the brain parenchyma. Thus, the water system is an emerging clue for understanding brain function.

Neuronal Circuits of the Arcuate Nucleus in Obesity: Classical and Novel Perspectives

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Obesity is a multifactorial disorder involving the disruption of central circuits that regulate feeding and metabolism. The arcuate nucleus (ARC) of the hypothalamus is a key hub in this regulation, with Agouti-Related Peptide (AgRP) and Proopiomelanocortin (POMC) neurons being established players in hunger and satiety control. AgRP neurons have recently been shown to encode circadian feeding time, while POMC neurons display impaired leptin responsiveness under chronic high-fat diet conditions.
Beyond these populations, ARC Tyrosine Hydroxylase (TH) neurons have been described as mediators of hypoglycemic hunger via medial hypothalamic pathways. However, their role in obesity remains largely unexplored. Our laboratory has recently undertaken a systematic investigation of ARC TH neurons in the context of diet-induced obesity. By combining chemogenetic and electrophysiological approaches with analyses of metabolic parameters and projections to other brain regions, we aim to define how ARC TH neurons contribute to the integrative function of the ARC hub.
As a result of these investigations, ARC TH neurons appear to be functionally engaged in obesity and may be considered an integral part of the hypothalamic feeding hub. By linking metabolic, behavioural, and autonomic processes, these neurons represent novel components of hypothalamic regulation and potential targets for future therapeutic strategies.

Acknowledgement: This work was supported by TÜBİTAK (Projects #118S245 and #122S382).

The Effect of Prolactin-Releasing Peptide (PrRP) on Depression-Like Behavior in Rats

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Several data suggest that the RFamide peptides - especially the prolactin-releasing peptide (PrRP) – play a role in the regulation of stress responses, the disturbances of which can cause many diseases, including depression. PrRP was identified as an endogenous ligand of the GPR10 receptor but can also bind to the NPFF2 receptor. Here we would like to reveal the association between depressive-like behaviour and changes in the PrRP regulation.
Fifteen minutes forced swimming test (FST) was used to induce depression-like symptoms in male Wistar rats. Based on immobility time, resilient and vulnerable subgroups were identified, and 10 brain regions were studied using qPCR to measure mRNA expression levels of PrRP and its receptors. Stress-vulnerable animals exhibited depression-like behaviours and showed altered mRNA expression levels in the medullary A1 region, the habenula, and the arcuate nucleus. Additionally, we identified corticotropin-releasing hormone and vesicular glutamate transporter 2 positive neurons in the A1 medullary region that contained Prrp, suggesting a modulatory role of PrRP in these excitatory neurons involved in stress regulation. Our findings reinforce the hypothesis that PrRP plays a role in depression, highlighting its brain region-specific effects.
This research is supported by This study was supported by the PTE-AOK-KA-2022 project of the University of Pécs, the National Research Development and Innovation Office of Hungary (grant numbers K141934, K138763, and K146086); the ÚNKP-23-2-III-PTE-2068 National Excellence Program of the Ministry for Innovation and Technology; the National Brain Research Program (NAP 3.0) of the Hungarian Academy of Sciences; and the Thematic Excellence Program 2021 Health Subprogram of the Ministry for Innovation and Technology in Hungary within the framework of the TKP2021-EGA-16 project of Pécs University and TKP2021-EGA-25 project of Semmelweis University. V.K. was supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences (BO/00750/22/5).

S04: Cerebrovascular Impairment in Aging and Disease

Disruption of vitamin D signalling impairs cerebrovascular adaptation to carotid artery occlusion

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Deficiency in vitamin D, a lipid-soluble steroid hormone, affects approximately 24% to 40% of the population in the Western world. In recent decades, it has been increasingly linked to the pathogenesis and progression of cerebrovascular diseases. Our aim was to investigate the impact of impaired vitamin D signalling on cerebral microcirculation, considering sex hormone status as it may also influence the prevalence of cerebrovascular disorders. We analysed the anatomical and functional aspects of cerebrovascular adaptation to unilateral carotid artery occlusion (CAO) in intact male and female, as well as in ovariectomized and hyperandrogenic female mice, with either normal or functionally inactive vitamin D receptor (VDR). Cerebrovascular adaptation was assessed by analysing changes in cerebrocortical blood flow using laser-speckle imaging following CAO. In addition, the morphology of leptomeningeal anastomoses was evaluated. Cerebrocortical blood flow showed a significantly increased drop and delayed recovery after CAO in male mice with a functionally inactive VDR. This was associated with a reduced number and increased tortuosity of pial anastomoses. In contrast, in female mice, ablation of VDR alone did not impair cerebrovascular adaptation to CAO despite the reduced number of pial collaterals. Surprisingly, ovariectomy did not exacerbate the effects of disrupted VDR signalling. However, androgen excess combined with VDR inactivity resulted in prolonged hypoperfusion in the cerebral cortex ipsilateral to the occlusion. In conclusion, our findings suggest that the cerebrovascular consequences of disrupted VDR signalling are less pronounced in females than in males, indicating a level of protection in females even after ovariectomy. Conversely, even short-term androgen excess in the absence of VDR signalling may lead to vasoregulatory dysfunction, potentially contributing to the increased risk and severity of cerebrovascular diseases.

Grant support: NRDIO K-125174, K-139230, K-151053, and PD-143327, RRF-2.1.2.-12-2022-0001, TKP2021-EGA-25, R01AG055395, R01AG068295, R01AG070915, R01NS100782, R01CA255840.

Impaired Neurovascular Coupling after Acute Ischemic Stroke in the APP/PS1 Transgenic Mouse Model of Alzheimer's Disease

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Age is the most significant independent risk factor for both Alzheimer's disease (AD) and acute ischemic stroke, which share distinct microvascular pathologies. Additionally, cerebral amyloid angiopathy impairs microvascular function in AD. Although the incidence of acute ischemic stroke is as high in AD patients as in non-demented elderly individuals, stroke occurrence accelerates dementia progression in AD. Here, we sought to determine whether neurovascular dysfunction associated with acute ischemic stroke is exacerbated in AD, potentially accounting for the accelerated progression of dementia.
Aged (19–23 months) female and male APP/PS1 (AD, n = 7) and wild-type control (WT, n = 8) mice were used in this study. Acute ischemic stroke was induced under isoflurane anesthesia (0.8–1%) by transient (60-minute) microfilament-guided occlusion of the unilateral middle cerebral artery (MCAO). Complete reperfusion was achieved by removing the microfilament. Sensorimotor deficits were assessed daily over a 72-hour survival period using the Composite Garcia Neuroscore Scale (GNS; maximum score indicating intact function: 21 points). After three days, T2 and DWI MRI sequences were acquired to assess infarct volume, and functional ultrasound imaging was used to characterize neurovascular coupling (NVC) in response to mechanical whisker stimulation (2–5 Hz).
No significant difference was observed in stroke-related neurological deficits between groups (GNS: 13 ± 2 vs. 12 ± 4 points; AD vs. WT). However, infarcts in AD mice were more diffuse, in contrast to the compact lesions observed in WT mice. Unexpectedly, somatosensory stimulation to test NVC often elicited spreading depolarizations (SDs) instead of the typical neuronal activity associated with functional hyperemia (SD score: 13 vs. 3; AD vs. WT), reflecting increased neuronal hyperexcitability in AD mice. Furthermore, when functional hyperemia did occur, the cerebrovascular response amplitude was significantly reduced in the AD group compared to WT (NVC: 9.3 ± 5 vs. 15.5 ± 3.1%; AD vs. WT), indicating impaired NVC in AD.
SD is known to exacerbate cerebral ischemic injury. Moreover, somatosensory stimulation in the context of acute ischemic stroke has been shown to cause a metabolic supply-demand mismatch, triggering SD. The frequent occurrence of SD in AD mice suggests a more severe metabolic imbalance during cerebral ischemia in the AD brain. Additionally, neuronal hyperexcitability in AD has recently been supported by the detection of epileptiform discharges in AD patients. While epileptiform activity and SD are interlinked, the evolution of SD has not previously been observed in AD. Taken together, our results suggest that SD may represent a novel and intriguing mechanism of neurodegeneration in AD.

Age-Related Vascular Cognitive Impairment: Role of Endothelial Senescence

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Age-related phenotypic changes in cerebromicrovascular endothelial cells contribute to the dysregulation of cerebral blood flow and disruption of the blood-brain barrier, thereby promoting the development of vascular cognitive impairment (VCI). In recent years, there has been growing recognition of endothelial cell senescence as a potential underlying mechanism driving microvascular abnormalities. This realization has paved the way for exploring the potential of senolytic drugs in preclinical research to improve cerebromicrovascular function and structure and positively impact cognition.
In order to identify senescent endothelial cells in the preclinical mouse model of aging, we used single cell RNA sequencing to meticulously examine cells derived from enriched fractions of cerebromicrovascular endothelial cells and other components of the neurovascular unit. We characterized and delineated 13 distinct transcriptomic cell types. Our investigation revealed a notable elevation in the proportion of senescent endothelial cells within the cerebral microcirculation of aged mice. This phenomenon was further validated using spatial transcriptomics, which confirmed the heightened presence of senescent endothelial cells within key regions such as the hippocampus and cortex of the aged mouse brain.
To investigate the impact of senescence on the cerebromicrovascular endothelial phenotype and function, including NVC and maintenance of BBB and microcirculatory network architecture we tested the efficacy of a clinically relevant senolytic treatment regimen, and administered the BCL-2/BCL-xL inhibitor senolytic drug ABT263/Navitoclax to aged mice. Encouragingly, our findings indicate that Navitoclax intervention leads to an enhancement in neurovascular coupling and decreased blood brain barrier permeability among aged mice, which in turn correlates with significant improvements in cognitive performance. These findings collectively underscore the potential therapeutic utility of senolytic drugs across various age-related cerebrovascular conditions, as underscored by our preclinical investigations. By shedding light on the role of endothelial cell senescence and its modulation, our results open the avenue to the therapeutic exploitation of senolytic drugs in multiple age-related cerebrovascular pathologies in preclinical studies.

Roles of NOS Isoforms in the Adaptation of the Cerebrocortical Circulation to Unilateral Carotid Artery Occlusion

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The investigation of cerebral autoregulation mechanisms has gained significant importance due to the rising prevalence of carotid artery stenosis globally. Our study aimed to examine the changes in cerebrocortical blood flow (CoBF) patterns and explore the involvement of pial collaterals in the adaptive response following unilateral carotid artery occlusion (CAO). Additionally, we assessed the role of nitric oxide (NO), with a particular focus on the different isoforms of nitric oxide synthase (NOS).
Experiments were performed on wild-type (WT), NOS1 KO, NOS3 KO, NOS1/3 DKO (NOS1 and NOS3 double knockout), and WT mice treated with the non-selective NO synthase inhibitor: L-NAME. The adaptational capability of cerebrovascular autoregulation was determined by analysing the regional changes in cerebrocortical blood flow (CoBF) using laser-speckle imaging after unilateral CAO. The morphology of the pial collaterals, connecting the frontoparietal and temporal regions, was also analysed.
Our results show that the Willis circle alone is insufficient to compensate immediately for the loss of one carotid artery, as a significant CoBF decrease can be seen in the ipsilateral temporal cortex in the acute phase of the adaptation. However, pial collaterals attenuate the ischemia of the temporal cortex at the expense of the blood supply of the frontoparietal region. Regarding the role of NOS isoforms, NOS1 KO and NOS3 KO animals show a partially preserved ability for adaptation, whereas in animals lacking both NOS1 and NOS3 isoforms, an impaired cerebrocortical blood flow adaptation to CAO can be determined, mainly in the subacute phase, which alteration can be attributed partly to the higher level of tortuosity in pial collateral vessels. The pharmacological inhibition of NOS isoforms with L-NAME results in severe decrease of CoBF values in the acute phase, in both hemispheres.
In conclusion, our experiments provide evidence for the major role of NO in cerebrovascular adaptation, involving both morphological and functional changes of the cerebrocortical microcirculation.

Grant support: NRDIO K-125174, K-139230, K-151053, and PD-143327, as well as ÚNKP-22-4-II-SE-17, RRF-2.1.2.-12-2022-0001, TKP2021-EGA-25, R01AG055395, R01AG068295, R01AG070915, R01NS100782, R01CA255840.

S05: Organelle Dynamics and Metabolism in Health and Disease

A Sexually Dimorphic Hepatic Cycle of Periportal VLDL Generation and Subsequent Pericentral VLDLR-mediated Re-uptake

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We recently performed a scRNA-seq analysis of the liver from female and male mice, using hepatic cells collected at distinct time-points within a day, and developed a new method of analyzing scRNA-seq data. This allowed us to digitally re-position the dissociated hepatocytes back onto the hepatic pericentral to periportal axis and, for the first time, obtain a comprehensive overview of how lobular position, time of day, and sex shape the lobular transcriptome. These data revealed that the very low density lipoprotein (VLDL) receptor (*Vldlr*), crucial for VLDL uptake, and previously reported to be very lowly expressed in the liver, is restricted to the hepatic pericentral zone, with significantly higher mRNA levels in female mice, which was confirmed on the protein level with immunostaining. Additionally, we demonstrated a periportal bias in VLDL assembly, both with transcriptomics and electron microscopy, revealing a previously unknown sexually dimorphic hepatic cycle of periportal formation and pericentral uptake of VLDL. This spatial separation of VLDL production and lipoprotein re-uptake is additionally enhanced in time, as lipoprotein production is boosted postprandially, enabling systemic circulation of triglyceride-rich lipoproteins during the fasting phase in mammals. Conversely, VLDLR-mediated lipoprotein uptake occurs at the fasting-feeding transition, in anticipation of feeding, leading to rhythmic changes in the hepatic lipidome. VLDLR's sexual dimorphism is conserved in humans, with significantly higher VLDLR expression in premenopausal women than in age-matched men. We also found a strong association between low hepatic VLDLR and an incidence of progressive atherosclerotic lesions and a medical history of myocardial infarction. These findings suggest that sex-specific VLDLR regulation may underlie observed differences in coronary heart disease risk between young men and women. Our current research builds on these insights to refine diagnostic tools and therapeutic strategies in humans, while also probing the molecular mechanisms governing hepatic lipoprotein homeostasis in mouse models.

Neuro-Endocrino-Immune Regulation of Metabolic Homeostasis

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Immune cell signals contribute to the regulation of metabolism and have been implicated in several metabolic diseases. Neuronal and immune cell interactions have been recently shown to play key roles in host physiology and defense. Nevertheless, whether neuronal and immune cells establish inter-organ communication axes to orchestrate endocrine function remains elusive. Here we report a neuroimmune circuit that controls glucagon production and blood glucose levels via a gut-pancreatic axis. We found that group 2 innate lymphoid cells (ILC2) control glucose homeostasis via the regulation of the pancreatic hormone glucagon. Fasting led to a selective increase of pancreatic ILC2, glucagon production and gluconeogenesis. ILC2 regulate alpha cell-derived glucagon production in a type 2 cytokine-dependent manner. Strikingly, fasting induced intestinal ILC2 migration and accumulation in the pancreas, via the beta-2 adrenergic receptor. Accordingly, fasting and adrenergic receptor signals decreased expression of gut postcode receptors by migratory ILC2. Retrograde tracing, chemical, genetic and chemogenetic manipulations showed that intestinal sympathetic neurons control gut-pancreatic ILC2 migration and that local neurons connect to high-order brain areas. Our work identifies a neuroimmune hub that responds to body energy levels, setting an inter-organ communication route that controls endocrine function and metabolism.

Lipid Droplets Control Membrane Integrity and Mitochondrial Homeostasis in Starvation and Ferroptosis

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Lipid droplets (LDs) are fat storage organelles with emerging roles in the cellular adaptation to various stress conditions. LDs release free fatty acids through gradual lipolysis, catalysed by surface-bound lipases, or through lipophagy, a selective form of macroautophagy leading to complete breakdown of the organelle within lysosomes. Both lipolysis and lipophagy contribute to cellular homeostasis during stress; yet, it remains unclear whether and how these processes direct lipid fluxes to specific subcellular sites to support cancer cell adaptation. Here, we investigated the roles of lipolysis and lipophagy in cancer cell adaptation to nutrient deprivation and to redox imbalances that promote membrane peroxidation characteristic of ferroptotic cell death. Our results reveal that both pathways are continuously active in starved breast cancer cells but exert distinct effects on mitochondrial function and integrity depending on the degree of starvation. When key anti-ferroptotic mechanisms are impaired in these invasive cells, LDs dynamically regulate phospholipid acyl-chain composition, thereby modulating membrane susceptibility to lipid peroxidation and cellular ferroptosis sensitivity. Furthermore, under pro-ferroptotic conditions, LDs contribute to the maintenance of mitochondrial redox homeostasis by buffering excess dietary (exogenous) polyunsaturated fatty acids. Together, our findings establish LDs as central hubs that integrate multiple sources of fatty acids and coordinate their trafficking to mitochondria and membranes, enabling cancer cells to adapt to nutrient stress and redox imbalance.

S06: Impact of nutrients and lifestyle on microvascular function

High Salt Diet Affects the Microvascular Reactivity – is there a Salvage Way? - Comparative Studies in Animals and Humans

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Deleterious effect of high table salt (NaCl, HS) diet on endothelium-dependent vascular reactivity, both in microcirculation and microcirculation have been well known. HS diet physiologically suppresses the renin-angiotensin system (RAS). Our results demonstrated significantly impaired vasodilator responses to acetylcholine (AChID) and flow-induced dilation (FID) in middle cerebral arteries (MCA) of Sprague-Dawley rats on a HS diet due to oxidative-antioxidative disbalance. There was increased vascular and systemic oxidative stress and decreased vascular production of nitric oxide (NO), together with altered balance between vasoactive prostaglandins and eicosanoids, such as metabolites of COX-1,2 and epoxygenases, together with decreased expression of antioxidative defense systems. Scavenging of reactive oxygen species with TEMPOL restored NO production and vascular reactivity. Importantly, chronic low-dose ANG II supplementation in HS-fed rats restored FID of MCAs. ANG II changed the protein/gene expression of COXs, HIF-1α and VEGF and significantly decreased systemic oxidative stress, decreased superoxide/ROS levels and increased GPx4 and EC-SOD antioxidative enzyme expression, and increased NO bioavailability in the vascular wall. Nuclear factor erythroid 2-related factor 2 (NRF2) upregulates antioxidant response elements (AREs)-mediated expression of antioxidant enzymes and cytoprotective proteins. Our results showed that the NRF2 signalling pathway contributed to endothelium-dependent vasodilation and was suppressed/inhibited by HS diet. All NRF2 activators restored FID in HS groups to values similar to LS group. Interestingly, carnosine (a beta-alanine+histidine dipeptide) exerts antioxidative effects in various diseases. Carnosine consumption restored previously attenuated FID in rats on a HS diet, the effect that was annulated by NRF2 antagonist. Carnosine significantly increased NRF2 and NQO1 gene expression.
Similar was observed in our human studies; HS diet led to impaired peripheral microvascular and macrovascular reactivity in response to ACh and post-occlusive hyperemia. Plasma renin activity (PRA) and serum aldosterone level were significantly suppressed after HS diet and markers of oxidative stress were increased. Impaired AChID and increased salt intake, as well as impaired AChID and suppressed RAS were significantly positively correlated. Furthermore, in healthy young humans on HS diet, cerebral blood flow in response to orthostatic test was preserved due to altered vascular reactivity of MCA, with increased cerebrovascular resistance and blunted baroreceptor sensitivity and sympathetic activity. Concomitant intake of antioxidants together with HS diet prevented adverse effects of HS diet on vasorelaxation and oxidative status.
Taken altogether, our results support conclusion that there are several salvage pathways for reduction of oxidative stress and restoration of microvascular reactivity in HS diet: physiological levels of circulating ANG II are crucial to maintain the HIF-1α dependent mechanisms of endothelium-dependent vasodilation and vascular oxidative balance without affecting mean arterial pressure and carnosine which could act a salvage compound for FID in HS diet exhibiting its effects via NRF2 signalling pathway.

Funding: IP1-MEFOS-2022, IP26-MEFOS-2023, IP18-MEFOS-2024 and IP20-MEFOS-2025 grants (PI-Ines Drenjančević); IP16-MEFOS-2022 and IP22-MEFOS-2023 (PI-Zrinka Mihaljević); HRZZ IP-2016-06-8744 (RAS-AdrenOX) (PI-Ana Stupin).

Time-Dependent Microvascular and Autonomic Nervous System Alterations after Oral Glucose Loading in Young Healthy Individuals

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Understanding how acute hyperglycemia modulates microvascular and autonomic nervous system (ANS) reactivity may reveal mechanisms contributing to endothelial dysfunction, a precursor to various cardiometabolic diseases. We investigated the physiological effects of oral glucose tolerance test (OGTT)-induced hyperglycemia on skin microvascular and ANS reactivity in healthy young adults.

We measured microvascular and ANS responses before and 45 and 120 minutes after ingestion of 75 g of glucose or water in 28 participants (16 females, 12 males). Laser Doppler flowmetry assessed baseline perfusion and responses to post-occlusive reactive hyperemia, acetylcholine (ACh; evaluating endothelium-dependent vasodilation), and sodium nitroprusside (SNP; evaluating endothelium-independent vasodilation) iontophoresis. Spectral components reflecting endothelial nitric oxide-independent (NOi), endothelial nitric oxide-dependent (NOd), and myogenic activity of vascular smooth muscle cells (vSMC) were quantified using wavelet analysis with the implemented cone of influence, enabling a more accurate analysis of transient changes that characterize PORH and iontophoretic LDF responses. ANS reactivity was assessed non-invasively via heart rate variability (HRV), derived from electrocardiogram recordings using time- (root mean square of successive RR interval differences (RMSSD) and the standard deviation of normal-to-normal RR intervals (SDNN)) and frequency-domain (low-frequency (LF: 0.04–0.15 Hz), high-frequency (HF: 0.15–0.4 Hz) and the LF/HF ratio) indices. A two-way repeated measures ANOVA was conducted to assess the effects of glucose and water loading on wavelet spectral components and HRV indices across the three time points (0, 45, and 120 minutes).

Our results revealed that glucose ingestion altered specific microvascular and HRV parameters in a time-dependent manner. Significant interactions were found in SNP-induced endothelial NOi ($p = 0.014$) and myogenic ($p = 0.029$; two-way repeated measures ANOVA) spectral components, but not in endothelial NOd, indicating a selective impairment of NOi endothelial pathways and vSMC responsiveness. This interpretation is further supported by the absence of significant changes in ACh- and PORH-induced LDF responses.

Time-domain HRV parameters (RMSSD: $p = 0.009$, SDNN: $p = 0.008$; two-way repeated measures ANOVA) showed continuous decrease over the two-hour post-glucose loading, suggesting that glucose loading modulates overall ANS reactivity. This modulation likely reflects reduced parasympathetic activity and a shift toward sympathetic dominance, possibly as an adaptive response to glucose metabolism-induced physiological stress; however, the absence of significant interactions in frequency-domain HRV measures suggests that glucose's effects cannot be exclusively attributed to alterations in either sympathetic or parasympathetic activity.

Our findings support a mechanistic link between acute hyperglycemia, impaired microvascular reactivity, and ANS imbalance. Unlike previous studies, which have primarily focused on NOd mechanisms, our results reveal selective and transient impairments in endothelial NOi and myogenic responses following glucose ingestion. Our multi-layered, integrated approach offers a sensitive method for detecting microvascular dysfunction associated with acute glycemic load.

Ivabradine Induced Vasorelaxation in a Hyperandrogenic Rat Model of Polycystic Ovarian Syndrome, Effect of Vitamin D Supplementation

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Polycystic ovary syndrome (PCOS) is the most common endocrine disorder among women of reproductive age. It is characterized by hyperandrogenism and is frequently associated with Vitamin D deficiency, observed in approximately 80% of cases. PCOS is also linked to an increased risk of cardiovascular disease. In the thoracic aorta of rats, all four isotypes of hyperpolarization-activated cyclic nucleotide-gated (HCN) channels have been identified, and the non-selective HCN channel inhibitor ivabradine has been shown to induce vasorelaxation. Altered expression of HCN1–4 has also been reported in diabetes mellitus. This study aimed to investigate changes in HCN-dependent vascular responses in a rat model of hyperandrogenism and to assess the potential modulatory effect of Vitamin D supplementation.
Forty-four female Wistar rats (70–90 g) were randomly assigned to one of four groups: Control (C), Vitamin D-treated (D), testosterone-treated (T), and a combined treatment group (T+D) receiving both testosterone and Vitamin D for 8 weeks. Thoracic aortas were excised and cut into 3–4 mm segments for wire myography. Following pre-contraction with phenylephrine, ivabradine dose-response curves were obtained. Additional segments were processed for immunohistochemical labeling of HCN1–4.
Vascular relaxation was expressed as a percentage of the maximal contraction induced by phenylephrine (10^{-6} M). Vitamin D supplementation increased ivabradine-induced relaxation (interaction terms ivabradine concentration × Vitamin D: $p < 0.05$, 3-way RM ANOVA). Immunostaining showed that the double treated group had significantly higher density of HCN1 compared to all other groups. HCN2 and HCN4 expression was significantly increased by testosterone treatment, whereas HCN2 and HCN3 density was decreased by Vitamin D supplementation.
Vitamin D supplementation enhanced ivabradine-induced vasorelaxation, potentially through modulation of HCN2 and HCN3 expression. Hyperandrogenism was associated with increased HCN1, HCN2 and HCN4 expression. These results suggest a role for HCN channels in the vascular dysfunction associated with PCOS and support the potential of cardiovascular interaction of ivabradine in this condition.

The Impact of Acute Mental Stress on Microvascular Reactivity

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Acute stress has been recognized as a risk factor for the increased incidence of cardiovascular events. Recent evidence suggests that endothelial dysfunction plays a key role in the pathophysiological mechanisms linking stress to cardiovascular disease, a process largely mediated by the sympathetic nervous system (SNS). The present study aimed to evaluate the impact of acute stress on microvascular reactivity using a mental stress test and to characterize the vascular response to stress through post-occlusive reactive hyperemia (PORH) of the upper limb. Twenty-five healthy young adults of both sexes (21.8 ± 2.0 years) were enrolled. Each participant underwent two PORH protocols: one at rest and another during exposure to mental stress induced by the Stroop color test. Local blood flow and pulse were recorded by photoplethysmography (PPG) on the fingers of both hands, along with skin temperature and electrodermal activity (EDA). PPG signals were further analyzed using wavelet transform to decompose them into distinct spectral components associated with cardiac, respiratory, myogenic, sympathetic, and endothelial activities. Our results showed that, during the mental stress test, skin blood flow and temperature significantly decreased in both limbs, whereas EDA and pulse increased. The magnitude of reactive hyperemia was significantly attenuated under stress compared with rest. Moreover, significant alterations in spectral activity across physiological frequency bands were observed between protocols. These findings indicate that acute stress impairs microvascular reactivity, with perfusion reduction mediated by sympathetic vasoconstriction. This vascular response may counteract the vasodilatory mechanisms of reactive hyperemia, suggesting a potential pathophysiological pathway through which stress contributes to microvascular dysfunction.

Towards a Standardized Framework for Microvascular Flowmotion Analysis

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Flowmotion analysis of microvascular blood flow signals has long been a promising technique for accessing the physiology behind the temporal dynamics often seen in the measurements. The technique involves separating the blood flow signal, e.g. from laser Doppler flowmetry, into five (sometimes six) distinct frequency intervals which have been shown to originate from cardiac, respiratory, myogenic, neurogenic, and endothelial regulatory activity, respectively. Changes in flowmotion activity has been linked to several chronic conditions and diseases, such as type-2 diabetes, chronic smoking, and arterial hypertension, to name just a few examples. Despite these promising results, comparisons between different studies remain difficult due to a lack of standards for how to perform the analysis in a robust manner. The research community would greatly benefit from a validated methodology incorporating agreed upon best practices.
This talk will present an attempt at building an open standardized library for flowmotion analysis, which we have published open-source for other researchers to use. The project currently includes two main components. First, conventional analysis of resting (steady-state) flowmotion and the methods we have developed to ensure good signal quality by correcting for motion artifacts. Second, analysis of transient signals during provocations such as post-occlusive reactive hyperemia, local heating, or topical application of methyl nicotinate, which requires more advanced analysis to ensure robust results. Additionally, this talk will present recent research on extending flowmotion analysis to imaging modalities, e.g. using laser speckle contrast imaging, in order to capture the spatial variation in flowmotion activity.

S07: Insect Neurobiology and Neuroethology

Beyond Spectral Overlap: an Integrative Study Reveals a Novel Mechanism of Vibrational Masking in Insects

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Noise poses a major challenge to signal perception across sensory systems, where it can mask relevant signals and disrupt behaviour. In mechanosensory modalities like hearing, masking is typically attributed to either energetic interference at the receptor level or informational interference during neural processing—both generally linked to spectral overlap between noise and signal. We explored how substrate-borne vibrational noise of different spectral compositions affects communication during courtship in the stink bug *Nezara viridula* (Hemiptera, Pentatomidae), integrating behavioural assays, neurophysiology, and computational modelling. Surprisingly, we found that not only spectrally overlapping but also non-overlapping noise impaired both signal recognition and source localisation. Overlapping noise reduced sensitivity of receptor neurons and disrupted phase-locked signal frequency encoding, while non-overlapping noise altered frequency encoding without affecting sensitivity. Modelling showed that these effects arise from noise-induced distortion of the signal waveform, which also compromised the temporal cues necessary for spatial orientation. Our results reveal a previously undescribed mechanism of masking by spectrally non-overlapping noise, likely rooted in the specific biophysical constraints of insect vibrational sensing. The results highlight a broader vulnerability of vibration-mediated behaviour to noise interference, with implications for insect interactions in increasingly noisy environments.

Connectome of the Early Visual Pathway of a Butterfly Elucidates the Neural Toolkit for the Green-Red Colour Opponent Axis

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Colour vision in nymphalid butterflies is either limited to the ancestral insect UV-blue-green trichromatism or expanded to the red spectral range. The trichromatic set is based upon UV and blue photoreceptors with long axons (named R1&2), which receive opponent inhibitory input from green photoreceptors (R3-8), while the tetrachromatic set entails additional green photoreceptors R1&2, which receive opponent inhibitory input from the basal red receptors R9. The synaptic connectivity of the photoreceptors and the neural set which underlies both versions of colour vision are unknown. To elucidate this neural substrate, we have produced the connectome of a butterfly, *Argynnis paphia*, which has a sexually dimorphic retina: females have a trichromatic set, while males have a tetrachromatic set of photoreceptors. The proximal retina and the first optical ganglion, the lamina, of both sexes, were sectioned using a serial block-face scanning electron microscope. Serial sections with 8x8x50 nm resolution were segmented by the Connectome Annotation Versioning Engine CAVE using a convolutional neural network trained on EM data and proofread manually. We found that the cells R1&2 have a sex-specific geometry and membraneous structures. In the male we identified the putative intra-retinal inhibitory synapses, located in male-specific cellular processes that connect R9 and R1&2. The photoreceptor axons contain numerous processes with synapses that form the inter-photoreceptor opponent network and project to the downstream interneurons. The lamina contains extensive horizontal and centrifugal networks, which actively modulate the photoreceptors and the first-order visual interneurons. The early visual pathway of the butterfly is a remarkable neural network with numerous attributes of efficient neural wiring and computation.

The Role of Vision and Visual Cues in Host Plant Location by the Meadow Spittlebug Philaenus Spumarius

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Since the first detection of *Xylella fastidiosa* in Europe in 2013 on olive plants as the causal agent of the Olive Quick Decline Syndrome (OQDS), the meadow spittlebug *Philaenus spumarius* has drawn significant attention due to its role in spreading the bacterium across agroecosystems. While research has largely focused on its biology, ecology, and pest management, the host plant location and acceptance mechanisms have remained largely overlooked. However, this knowledge is paramount for devising control strategies to interfere with vector-plant contact and subsequent pathogen transmission. Research conducted so far on the cues underlying host plant location by meadow spittlebugs has primarily focused on olfactory cues, while the visual system of *P. spumarius* remains unexplored.
This study provides the first comprehensive investigation into the visual capabilities of *P. spumarius* and the visual cues driving host plant location, as well as the potential discrimination of different olive varieties by the spittlebug. We examined spittlebug visual capabilities, olive plants' optical properties, and behavioral responses to visual stimuli. X-ray micro-CT analysis revealed that *P. spumarius* possesses aerodynamically shaped eyes, deviating from the optically optimal spherical form. Some ommatidia appeared skewed, suggesting specialized visual zones adapted for distinct tasks, such as high spatial resolution, motion detection, or sensitivity to specific wavelengths and polarized light. Visual acuity was assessed by measuring the interommatidial angles ($\Delta\Phi$) using tracking of the pseudopupil movement. Spittlebugs exhibited $\Delta\Phi$ ranging from 4 to 8° with slightly increased resolution in forward-looking ommatidia forming a frontal acute zone. This is comparable to other herbivorous insects, allowing them to detect larger, high-contrast features rather than fine details at long distances. Single-cell electrophysiological measurements revealed three distinct photoreceptor types with peak sensitivities in UV, blue, and green spectral ranges, providing a strong basis for trichromatic vision, though this requires further confirmation through behavioural tests. Transmission electron microscopy of ommatidial cross-sections showed perpendicularly aligned microvilli, a key structural feature for polarization vision. Electrophysiological recordings further demonstrated strong polarization sensitivity in UV- and blue-sensitive photoreceptors. Behavioural assays in a dual-choice arena revealed a significant preference for linearly polarized over diffuse light (61% vs. 28%, respectively), indicating that polarization cues might be crucial in host-seeking behaviour. To examine the visual properties of olive plants, we measured spectral reflectance from 14 olive varieties. Combined with spittlebug spectral sensitivity, these data were integrated into a perceptual model quantifying colour differences as perceived by the insect. Additionally, polarization imaging revealed substantial variation in the degree of polarized light reflection, particularly on the adaxial leaf surfaces, with distinct inter-varietal differences.
This study highlights the crucial role of vision in host plant location by spittlebugs. Furthermore, our data lay the groundwork for developing sustainable strategies to curb vector activity and the spittlebug-mediated spread of *X. fastidiosa* through the use of repellent and attractive visual cues.

Age-Related Dysregulation of Octopaminergic Ca^{2+} Signalling and Metabolism in *Drosophila* Brain

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Aging leads to a gradual decline in nervous system function, which includes cognitive deterioration and reduced motor coordination. These changes may be associated with altered brain metabolism and signalling and may result from dysfunction in the noradrenergic system, which regulates brain metabolism and behaviour. Noradrenaline, released from noradrenergic neurons, activates adrenoceptors, triggering cytosolic Ca^{2+} and cAMP signalling in brain cells. In astrocytes, this signalling facilitates glucose uptake, glycogen degradation, and aerobic glycolysis, leading to production of lactate. The latter can be transported to neurons, serving as neuronal fuel during heightened brain activity and is essential for learning and memory formation. Whether aging impairs the noradrenergic regulation of brain cell metabolism, contributing to behavioural dysfunction, remains unclear.
To investigate this, we expressed fluorescent sensors for Ca^{2+}, cAMP, glucose and lactate in neurons or glial cells and monitored changes in cytosolic second messengers and metabolites in response to octopamine (an invertebrate analogue of noradrenaline) in the optic lobes of brains isolated from young and aged *Drosophila*. Aging in *Drosophila* was associated with neurodegenerative brain lesions, reduced locomotor activity and altered whole-brain energy metabolism. Octopamine elicited Ca^{2+} increases in neurons and glial cells only in young but not in aged brains, suggesting age-related impairment of cytosolic Ca^{2+} signalling, possibly due to alterations in octopamine/tyramine receptor expression, as suggested by observed downregulation of the Tyr_1 receptors. Octopamine also triggered increases in intracellular cAMP and lactate that were more pronounced in neurons and not affected by aging, suggesting that in *Drosophila* aerobic glycolysis occurs predominantly in neurons. Astrocytes, but not neurons, exhibited an octopamine-mediated increase in cytosolic glucose, that was absent in aged brains. Both neurons and glia were able to take up extracellular glucose and lactate, however, neuronal glucose uptake was reduced in aged brains.
Our results suggest that neurons, rather than glial cells, are the primary site of regulated aerobic glycolysis in *Drosophila* brains. In the brains of aged *Drosophila*, impaired octopaminergic Ca^{2+} signalling, glial glucose uptake and its delivery to neurons were observed, which may contribute to age-related behavioral dysfunction.

Funding: This work was supported by Slovenian Research and Innovation Agency (P3-0310, J3-50104, I0-0034, I0-0022, I0-0048 (CIPKeBiP)), COST Action CA18133 (ERNEST), and Deutsche Forschungsgemeinschaft (FOR 2149:265903901/P01, CRC 1423:421152132/B06).

S08: New Frontiers in Cardiovascular Research

Mitochondrial ROS Formation in Cardiac Disease

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Mitochondrial reactive oxygen species (ROS) play a major role in tissue homeostasis and contribute to the development of several cardiovascular pathologies. Within mitochondria, ROS can be produced at several sites. One of the most prominent sites for ROS formation in the mitochondria are monoamine oxidases (MAOs), flavoenzymes located in the outer mitochondrial membrane. MAOs are responsible for the degradation of neurotransmitters and biogenic amines and during this process they generate hydrogen peroxide, aldehydes and ammonia, species that can target mitochondria and induce mitochondrial dysfunction and cardiomyocyte death. Indeed, accumulating evidence highlighted the role of MAOs in cardiovascular diseases, such as ischemia/reperfusion, heart failure, diabetes and doxorubicin-induced cardiotoxicity. Here, I will present findings linking MAO activation to cardiac alterations in pathological conditions, such as cardiomyopathy associated with Duchenne muscular dystrophy.

Immune Checkpoint Signalling and Heart Failure

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Immune checkpoint molecules are physiological regulators of the adaptive immune response. Immune checkpoint inhibitors (ICIs), such as anti-PD-1 or anti-CTLA-4 monoclonal antibodies, have revolutionized the treatment of cancer and their clinical application is rapidly increasing, as reflected by thousands of ongoing clinical trials. However, ICIs cause various immune-related side effects, including acute and chronic cardiovascular toxicities. Among these, ICI-induced acute fulminant myocarditis is the most studied direct cardiotoxicity, although emerging clinical and preclinical data suggest that other ICI-related chronic cardiac toxicities are important, such as accelerated atherosclerosis, heart failure, arrhythmias and additional forms of cardiac dysfunction. These chronic cardiovascular toxicities are often hidden as they may only occur in the presence of preexisting injuries (ischemic necrosis, hypertrophy) and comorbidities (metabolic disease). It is therefore of high clinical importance to identify the risk factors and comorbidities that may precipitate ICI induced cardiotoxicity. The mechanisms of ICI-induced myocarditis and cardiac dysfunction appear to be distinct: myocarditis develops as a specific autoimmune response against cardiac myocytes involving expansion of autoreactive CD8+ T cells to cardiac self antigens (e.g. against alpha myosin heavy chain), whereas cardiac dysfunction/heart failure following ICI therapy is associated with disturbances in cardiac transcriptomic and metabolic effects, likely due to increased expression of pro-inflammatory cytokines.
The occurrence of these profound effects on the heart suggests a possible role for immune checkpoint molecules in the maintenance of cardiovascular homeostasis, and therefore disruption of physiological immune checkpoint signalling may lead to cardiac diseases, including heart failure.

Human Microtissue Models for Studying Cardiolaminopathies

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Lamin A/C is a nuclear envelope protein essential for maintaining nuclear structure and chromatin organization. Mutations in the Lamin A/C coding gene (*LMNA)* contribute to dilated familial cardiomyopathies (LMNA-DCM), also known as cardiolaminopathies. Mechanistically, this is driven by an interplay of impaired nuclear mechanoresistance, altered Lamin A/C-controlled signalling pathways and dysregulated chromatin organization. Development of a targeted approach to threat LMNA-DCM remains challenging due to the multiphenotipic nature of *LMNA* mutations. Here, we will leverage an innovative multicellular human cardiac microtissue model (cMT) to better understand LMNA-DCM and identify novel therapeutic options.
We generated human induced pluripotent stem cells (hiPSCs) from a patient with a family history of DCM, driven by a heterozygous mutation in the *LMNA* gene. Using the STRAIGHT-IN platform, we also introduced the same LMNA mutation in a wild-type hiPSC control line. Next, we differentiated the mutated and isogenic control hiPSCs into cardiomyocytes, cardiac fibroblasts and cardiac endothelial cells, which will be used to generate cMTs. The cMT system promotes hiPSC-CM maturation to postnatal levels. Our preliminary data show that monocultures of hiPSC-derived cardiomyocytes manifest decreased protein levels of both Lamin A and Lamin C. To study the cardiac phenotype in the context of increased hiPSC-CM maturity, we built cMTs and allowed them to mature in the presence of fibroblasts. Immunofluorescent imaging of cMTs showed decreased Lamin A/C in the lamina and nucleoplasm, and chromatin decompaction of mature hiPSC-derived cardiomyocytes. Upcoming experiments aim to address the cell-type specific contribution to LMNA-DCM of all generated cardiac cells by analyzing molecular and functional properties of the cMTs. We will combine the different cardiac cell types to build cMTs for further molecular and functional characterization of LMNA-DCM.
In conclusion, our preliminary data show that our models reproduce key features of *LMNA* mutations such as altered Lamin A/C content that impacts chromatin structure. We will exploit our model to define cell type-specific contribution to LMNA-DCM and identify potential novel targets and approaches for future therapeutic intervention.

Calcium Signalling in Early and Late Hypertensive Cardiac Remodelling

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Chronic arterial hypertension initiates a clinically silent sequence of structural and functional changes in the left ventricle that progresses from compensatory hypertrophy to overt failure. A pivotal mediator of this transition is Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), yet the spatiotemporal context in which the kinase becomes pathogenic remains insufficiently defined. Recent work indicates that disease progression is governed less by total CaMKII abundance than by its subcellular redistribution. During sustained pressure overload, CaMKII activity relocates from membrane-associated compartments to the nucleoplasm, coincident with perturbed nuclear Ca^{2+} transients and enhanced mechanical stress. This nuclear accumulation acts as a molecular switch, triggering gene-expression programs that drive maladaptive growth and impaired cardiomyocyte relaxation. Normalization of nuclear Ca^{2+} signalling may, therefore, offer new therapeutic avenues to halt or reverse adverse cardiac remodelling.

S09: Endocrine Physiology: From Mechanisms to Phenotypes

Endocrine-Exocrine Interactions in the Pancreas: Impact of Diabetes on Ductal Function

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While type 1 diabetes mellitus is classically defined by its effects on the endocrine pancreas, accumulating evidence underscores its significant impact on the exocrine compartment as well. Beyond the well-documented acinar cell alterations, recent insights reveal that pancreatic ductal cells—key regulators of luminal pH and enzyme transport—are also markedly affected. This evolving perspective on endocrine-exocrine interplay reshapes our understanding of pancreatic physiology in diabetes.
Emerging data suggest that chronic hyperglycemia enhances ductal secretory function, characterized by elevated bicarbonate and fluid output, accompanied by upregulation of key ion and water transporters, notably CFTR (cystic fibrosis transmembrane conductance regulator), Na^+/H^+ exchangers, anoctamine-1, and aquaporins. Intriguingly, these changes occur independently of classical secretagogues like secretin or cholecystokinin, although an upregulation of secretin receptor expression hints at altered paracrine responsiveness in the diabetic state.
These findings illuminate a previously underappreciated plasticity of ductal cells in response to metabolic stress, with implications for both pancreatic homeostasis and the progression of diabetes-related complications. Understanding these mechanisms offers novel angles for therapeutic exploration, particularly targeting ductal ion transport to mitigate exocrine dysfunction in diabetes.

This research received funding from the CFRD-SRC Grant (SRC007) and the Albert Szent-Györgyi Research Grant of the Albert Szent-Györgyi Medical School, University of Szeged.

Dynamical time-to-event analysis provides insight into the role of syntaxin in docking of insulin granules

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Live cell imaging can simultaneously visualize protein kinetics and the behaviour of secretory granules as they approach, attach to ("dock") or detach ("undock") from the cell membrane. These processes underlie hormone secretion and neurotransmitter release and are disturbed in several diseases. Current data analysis approaches do not exploit the rich temporal and spatial information in the data, and we show here how advanced statistical methods can provide insight into the dynamic effects of proteins on granule docking. We analysed live cell data of insulin secretory granules and the protein syntaxin. Automatic image analysis was used to extract individual docking and undocking events, as well as dynamic, single-granule syntaxin levels, from the movies. We adapted dynamical time-to-event analysis to account for the time-varying effect of syntaxin on the undocking rate and found that syntaxin has a significant stabilizing effect of the rate of undocking, in particular in the first few minutes after granules appear at the cell membrane. Our methodology extracts the dynamics of this time-varying effect, providing insight that remains hidden when simpler approaches are applied. Importantly, the conclusions are robust to the choice of parameters in the image analysis algorithm, suggesting that automatic image analysis combined with advanced statistics are highly useful for analysing live cell imaging movies.

Preclinical Models for a Rare Cav1.3 Channelopathy: Insights into the Pathophysiology and Therapeutic Options

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Activity-enhancing genetic variants of *CACNA1D*, the gene encoding the pore-forming α1 subunit of Cav1.3 L-type Ca^{2+} channels, are associated with a rare neurodevelopmental syndrome often accompanied by endocrine symptoms, i.e. hyperaldosteronism and hyperinsulinemic hypoglycemia. These mutations result in specific changes in channel gating, suggesting that the broad clinical presentation – including endocrine features – may reflect distinct biophysical changes. Current treatments predominantly rely on off-label use of unselective L-type Ca^{2+} channel blockers (e.g. dihydropyridines, DHPs), but their therapeutic efficacy remains ambiguous. Thus, to better understand the underlying disease-causing mechanisms and develop tailored therapeutic strategies, model systems reflecting the broad clinical spectrum are urgently required.
We characterized the biophysical and pharmacological properties of human wildtype (WT) and mutant Cav1.3 channels (co-expressed with β3 and α2δ1 subunits) using whole-cell patch-clamp recordings in tsA201 cells. To investigate pathophysiology and therapeutic approaches in vivo, we developed mouse models carrying the pathogenic *CACNA1D* p.A749G variant either alone or in combination with DHP-insensitive Cav1.2 channels (to isolate Cav1.3-selective drug effects). In adrenal chromaffin cells, we performed perforated patch-clamp recordings to assess alterations in Ca^{2+} currents and excitability.
Mutant channels exhibited variant-specific gating changes, with stronger negative shifts in the voltage-dependence of activation correlating with more severe phenotypes, while slowed inactivation kinetics were associated with endocrine symptoms. All tested variants retained or exhibited increased sensitivity in vitro to both tested Ca^{2+} channel inhibitors (isradipine and verapamil). Notably, the enhanced isradipine sensitivity at negative voltages reflected increased voltage-dependent inactivation, i.e. more inactivated channels available, due to gating shifts. A749G mutant mice displayed behavioural abnormalities such as induced hyperlocomotion and social deficits, as well as sex-dependent mild endocrine phenotypes: lower blood glucose levels in male homozygous mutants and increased aldosterone levels in female mutants. Despite achieving (supra)therapeutic plasma levels, oral administration of isradipine did not normalize hyperlocomotion in vivo. Using the double mutant model, we confirmed that 3 µM isradipine preferentially inhibited Cav1.3 currents in adrenal chromaffin cells.
Our findings highlight a critical link between mutant Cav1.3 gating changes and clinical phenotypes, and thereby underscore the need for personalized medicine approaches. We confirmed the pathogenicity of the A749G variant in mice and the feasibility of this mouse model to study both neurodevelopmental and endocrine features. Although mutant channels remain sensitive to DHPs, the lack of meaningful therapeutic benefits supports the exploration of alternative pharmacological approaches. Verapamil, with its use-dependent profile, may offer improved benefit in high-frequency action potential firing conditions relevant to both neurological and endocrine manifestations. Furthermore, the establishment of our novel double mutant mouse model provides a unique tool to selectively inhibit Cav1.3 channels, serving as an important proof-of-principle given the current lack of Cav1.3-selective inhibitors. Together,

these insights pave the way toward personalized strategies addressing both neurodevelopmental and endocrine symptoms in this rare channelopathy.

FWF DOI 10.55776/P35722, 10.55776/DOC178, 10.55776/P35087, Marie Skłodowska-Curie Actions COFUND (ARDRE, H2020 No. 847681), Land Tirol (F.49714/7-2023)

S10: Workshop on Structural Biology and Physiology

All Roads Lead to Cathepsins

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The recent global health crises underscored the urgency in developing effective treatments against SARS-CoV-2 and other emergent viruses. While vaccines have played a pivotal role in controlling the spread of diseases, therapeutic drugs offer a critical advantage by targeting the infected fraction of the population directly. In the emergency of situation back in 2020 we set a comprehensive drug repurposing campaign, using X-ray crystallography to screen over 5000 compounds—spanning approved drugs and those in clinical trials—for their ability to bind to the main protease (Mpro) of SARS-CoV-2. Our approach identified 37 compounds with binding affinity to Mpro, highlighting potential allosteric sites beyond the active site, which could be targeted for therapeutic intervention. Subsequent cell-based assays and treatments of SARS-CoV-2-infected Golden Syrian hamsters with selected compounds provided insights into their antiviral efficacy and safety profiles.
Among the screened compounds, Calpeptin emerged as a leading candidate, demonstrating potent antiviral activity. However, the kinetics studies revealed that instead of Mpro, cysteine cathepsins seemed to be its major target. This strongly suggested that the host cysteine cathepsins are involved in the cell entry of SARS-CoV-2. This finding is significant given the mutational stability of host proteins compared to the highly mutable viral targets, suggesting a reduced likelihood of resistance development. A continuation study identified further compounds with improved cathepsin L potency.
As cathepsins are involved in cell entry mechanisms of viruses such as Ebola, Human papilloma virus type 16, Reoviruses, Dengue, SARS-CoV-2, the identification of Calpeptin and other cysteine cathepsin inhibitors as potent antivirals offers a promising avenue for the treatment of viral infections using endosomal cell entry pathway This strategy not only accelerates the drug development process by possible repurposing existing compounds but also highlights the importance of targeting host cell processes as a means to combat viral diseases in general. Our findings advocate for a broader application of drug promiscuity, targeting multiple proteins and diseases, thereby expanding the potential arsenal against current and future viral threats.

Engineering Endogenous Cathepsin Inhibitor for Subcellular Targeting and Antiviral Intervention

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A detailed understanding of how host proteases mediate viral entry is essential for the development of next-generation antiviral strategies. Among host proteases, cathepsin L plays a central role. By activating the SARS-CoV-2 spike protein within endosomes, cathepsin L presumably initiates membrane fusion and viral genome release. Although small-molecule inhibitors of cathepsin L have shown antiviral activity, their systemic and off-target activity limit their clinical use. To develop a more selective approach and achieve compartment-restricted inhibition, we employed the p41 fragment. The p41 fragment is a part MHC class associated p41 form of invariant chain. It is a potent endogenous cathepsin inhibitor. A series of GFP-tagged constructs were engineered based on the p41 sequence. Optimized linkers and compartment-specific localization signals were incorporated to direct the constructs to the endosomes, the endoplasmic reticulum, or the cytoplasm. Confocal microscopy revealed that not only the localization signals but also linker architecture critically influenced proper subcellular targeting and retention. In pseudovirus entry assays, only endosomally localized constructs achieved significant suppression of SARS-CoV-2 infection, highlighting the necessity of spatial precision in protease inhibition. These results emphasize the functional importance of intracellular compartmentalization and offer a platform for the development of localized antiviral strategies based on native protein inhibitors.

Searching for a New Lactate Receptor Mediating Metabolic Excitability in Astrocytes

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We aim to understand how subcellular vesicle traffic, membrane fusion, second messenger signalling, cytoskeletal dynamics, and cell metabolism contribute to ageing and neurological disorders, and to translate the findings. In the domain of CANCER, which shares aerobic glycolysis with neuroglial astrocytes, and based on electrophysiological studies of lysosomal fusion, we have developed autologous cell-based immunotherapy (an ATMP, i.e. hybrids of immune and cancer cells) to treat prostate cancer, available for patients in Slovenia, with technology applicable to treating solid tumours and rare diseases. In the field of NEUROLOGICAL INDICATIONS, we have developed novel animal models (mice, Drosophila) and small molecules targeting neuroglial aerobic glycolysis, antigen presentation in neurodegeneration by engaging a novel orphan G-protein-coupled receptor. In the lecture, based on searching for a brain L-lactate receptor, we will present preliminary results of bioinformatic analyses identifying new candidate genes. Together with in-silico (molecular dynamics and structural biology), in-vitro and in-vivo data, we will emphasise the need to address the biology and pathophysiology of neurodegeneration based on the demise of locus coeruleus, the primary noradrenaline-releasing structure in the central nervous system, and one of the earliest structures to degenerate in Alzheimer's disease and several other neuro-degenerative disorders.

S11: Pathways in Physiology: From Wellness to Illness

The Role of Uroguanylin in Genetic Predisposition and the Development of Severe Forms of Diabetes Type 2

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Brian uroguanylin (UGN) plays a significant role in the regulation of glucose metabolism and body weight. In our preliminary research, we found a single point mutation that changes the amino acid sequence of UGN. In people who suffer from type 2 diabetes mellitus (T2DM) and do not have a mutation, there is an increase in the activity of brown adipose tissue (BAT), consequently lower blood glucose concentration after a meal and lower triglyceride and higher HDL levels in plasma when compared to patients with a mutation. In this study, we determined proUGN and GC-C (guanylate cyclase C, receptor for UGN) expressions in the prefrontal and cerebellar cortex, arcuate nucleus of hypothalamus and substantia nigra by ELISA, immunohistochemistry and *in situ* hybridisation. The study was performed on 21 male (10 subjects with obesity) and 13 female brains (6 subjects with obesity) with postmortem delay less than 24 h. In the human prefrontal cortex, proUGN was expressed in several interneuron subpopulations and in male BA9 and 10 was downregulated if subjects died shortly after a

meal. In male subjects with obesity, proUGN expression in BA10 was not regulated. Furthermore, in the hypothalamus of male subjects with obesity, expression of proUGN was lower when compared to subjects with normal body weight. Changes in UGN effects by feeding and obesity may occur also by changes in GC-C. In BA10, BA11 and hypothalamus, GC-C expressions were in negative correlation to the volume of stomach content but only in male brain. This study suggests different roles of brain UGN and GC-C in male and female individuals. At least, in male brain UGN and GC-C are possibly involved in feeding regulation, glucose homeostasis, obesity and development of severe forms of T2DM.

This study was funded by the Croatian Science Foundation research grant (IP-2018-01- 7416).

The Physiology of Differentiation Under Stress: Insights from Nucleotide Metabolism

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Differentiation therapy has emerged as a promising strategy for treating acute myeloid leukemia (AML), a malignancy characterized by a block in myeloid maturation. Our early research on the role of AMP-activated protein kinase (AMPK) in the differentiation of monocytic cell lines demonstrated that the purine analog 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR) inhibited proliferation and promoted differentiation of monocytic leukemia cell lines. Although AICAR is widely used as an AMPK activator, our findings revealed that its effects were independent of AMPK activation, and that metformin and other AMPK agonists failed to induce differentiation.

To uncover the underlying mechanism, we investigated alternative pathways and found that AICAR increased autophagic flux. However, genetic inactivation of core autophagy components such as Beclin 1 and hVPS34 did not impair differentiation, indicating that although differentiation and autophagy occurred concomitantly, differentiation did not depend on the classical autophagy pathway.

Metabolomic profiling revealed that AICAR disrupted pyrimidine metabolism, raised orotate levels, and depleted uridine monophosphate (UMP), consistent with inhibition of UMP synthase downstream of dihydroorotate dehydrogenase (DHODH). AICAR acted synergistically with the DHODH inhibitor brequinar, recently described as a novel differentiation agent. This pyrimidine stress activated checkpoint kinase 1 (Chk1), triggering a DNA damage-like response and S-phase arrest. Pharmacologic or genetic inactivation of Chk1 abrogated differentiation induced by both AICAR and brequinar.

Extending this to primary AML blasts from 35 patients, we found that AICAR induced ex vivo differentiation in a subset of primary samples, and sensitivity to AICAR correlated with both proliferative status and responsiveness to brequinar. RNA sequencing of AICAR-treated primary blasts revealed coordinated downregulation of pyrimidine metabolism and upregulation of genes involved in hematopoietic lineage commitment, consistent with metabolically driven differentiation.

Interestingly, low-dose cytarabine, an established chemotherapeutic, elicited a similar differentiation response via Chk1 activation, both in cell lines and in a subset of primary AML samples that also responded to AICAR or brequinar. These findings suggest that diverse agents can converge on a common replication stress to drive leukemic differentiation.

We also investigated the impact of the bone marrow microenvironment. Human stromal cell lines (HS-5, HS-27) and patient-derived mesenchymal stromal cells enhanced AML blast differentiation in response to AICAR and brequinar without compromising stromal viability. This suggests that stromal support may facilitate, rather than inhibit, differentiation under nucleotide stress.

Another interesting aspect of AICAR was its biphasic effect, which differed from the response observed with brequinar. We are currently investigating the possibility that differentiation is driven not merely by pyrimidine depletion but by an imbalance between purine and pyrimidine pools. In this context, we are examining the role of ribonucleotide reductase (RNR), which is activated in response to nucleotide imbalance.

Together, our findings demonstrate how nucleotide metabolism and replication stress intersect with differentiation pathways. These insights not only broaden our understanding of

physiological differentiation control under stress but also point to new possibilities for developing targeted treatments for AML based on metabolism.

Monocyte-Driven Pathways in Chronic Graft-versus-Host Disease

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Chronic graft-versus-host disease (cGVHD) remains a major complication following hematopoietic stem cell transplantation. Previous studies on monocytes in cGVHD have shown inconsistencies in the proportions of monocyte subsets, but have identified alterations in activation markers and a potential role for CX3CR1/fractalkine signalling in monocyte recruitment. Our study aimed to assess the association between cGVHD development and clinical features with the frequency, absolute numbers of monocyte subpopulations, and the concentration of monocyte-related cytokines in peripheral blood. In this study, we characterized monocyte subsets and their associated cytokines in 47 cGVHD patients and 30 control patients, all of whom underwent allogeneic hematopoietic stem cell transplantation at the University Hospital Centre Zagreb, Croatia, between 2017 and 2023. Flow cytometry was used to assess monocyte populations, including classical ($CD14^{++}CD16^{-}$), intermediate ($CD14^{++}CD16^{+}$), non-classical monocytes ($CD14^{+}CD16^{++}$), and their expression of CCR2 and CX3CR1, as well as $CD14^{++}$HLA-DR^{-} monocytes, and 12 cytokines (IL-4, IL-2, CXCL10, IL-1β, TNF-α, MCP-1, IL-17A, IL-6, IL-10, IFN-γ, IL-12p70, IL-8) in plasma samples using a microsphere bead array. Our analysis revealed no significant differences in monocyte percentages between cGVHD patients and controls. However, cGVHD patients exhibited a significantly higher absolute number of classical monocytes, possibly due to increased leukocyte counts, which may be a result of corticosteroid treatment. Patients with cGVHD had a higher percentage of $CD14^{++}$HLA-DR^{-} monocytes, with the loss of HLA-DR being a well-established marker of monocytic functional deactivation. Additionally, cGVHD patients had lower mean fluorescence intensity (MFI) of HLA-DR on both classical and intermediate monocytes, while the expression on non-classical monocytes remained comparable. No differences were observed in the expression of CCR2 or CX3CR1 between groups. Regarding cytokine profiles, CXCL10 and MCP-1 were significantly elevated in cGVHD patients compared to controls. IL-6 and IL-8 were strongly associated with worse clinical features, including higher global cGVHD scores, poorer performance status, and specific organ involvement. Unexpectedly, IL-10, IL-6, IL-8, CXCL10, and MCP-1 showed positive correlations with overall survival in cGVHD patients, despite some of them being markers of more severe disease. These findings underscore the crucial role of monocyte activation and associated cytokine profiles in the pathogenesis of cGVHD. Elevated CXCL10 and MCP-1, along with specific cytokine-monocyte subset interactions, may serve as potential biomarkers for monitoring disease progression and guiding therapeutic strategies in cGVHD.

Are Widespread Hypoxic Chambers Undermining the Use of Hypobaric Altitude Training and Its Associated Adaptations?

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The decrease in prices of normobaric hypoxia chambers, commonly known as "altitude rooms," has allowed many athletes to experience the benefits of training in an environment that simulates real altitude exposure. These altitude tents, rooms, chambers, beds, and masks serve as complementary tools for athletes, patients, and even recreational users seeking improvements in their physical performance without the expense and inconvenience of traveling to high-altitude locations. The growing popularity of these tools poses a challenge to the traditional approach of "staying at altitude." This lecture will be based on research conducted at the Laboratory of Applied Physiology at the Faculty of Kinesiology in Zagreb, focusing on both short- and long-term exposures to normobaric and/or hypobaric hypoxia.
Our study on hormonal dynamics during short-term normobaric hypoxia found significant increases in cortisol and prolactin levels following acute exposure ($FiO_2 \approx 14.5\%$). This indicates that the activation of stress hormones is a crucial endocrine response during the initial stages of adaptation. In contrast, no changes in testosterone levels were observed in normobaric conditions, which differs from our previous findings during a Himalayan expedition, where free testosterone decreased in correlation with reduced hemoglobin oxygen saturation.
In those hypobaric conditions, the stimulation of erythropoiesis exhibited considerable individual variability, similar to our Alps-based study where baseline erythropoietin levels predicted the reticulocyte response in skiers exposed to moderate altitude. This finding is valuable for developing personalized altitude training protocols. In that prior study, spending several hours a day at 2,000 meters over 10 days while sleeping at 1,250 meters resulted in measurable changes in oxygen transport parameters. These effects are unlikely to be achieved under normobaric hypoxia.
From a neuromuscular point of view, incremental progressive normobaric hypoxia surprisingly led to a decrease in handgrip isometric strength, despite the ATP/CP energy pathway being involved. This implies the neuromuscular stress experienced under combined physical and hypoxic conditions. This finding is in concordance to our 2021 study, which showed a decrease in isometric forearm flexors strength after a short-term normobaric hypoxia, while reaction time remained unchanged. This suggests a selective neuromuscular sensitivity to hypoxic stress.
Even based on our limited research, which is supported by extensive global studies, it can be concluded that hypoxia chambers are not a direct substitute for natural altitude. While they can produce many of the beneficial adaptations, like increased red blood cell production, hormonal changes some important differences still remain. Factors like pressure, psychological stress and the adaptations to prolonged stay are different at natural high altitude and cannot be fully simulated under normobaric conditions. This for example helps to explain why Gammow bags are generally only a temporary solution for treating altitude sickness and are seldom effective as a final cure for more complex cases.

S12: Cellular Strategies in Cancer and Neural Pathologies

Autologous Immunohybridoma Therapy: A Platform for Solid Tumour Treatment

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The connection between cancer development and the immune system has been recognized for a long time. Because the immune system plays a fundamental role in the onset and progression of cancer, it is no surprise that recent years have seen a surge in therapies aimed at modulating immune responses. The rise of cancer immunotherapy reflects the urgent need for innovative and more effective methods to eliminate malignant cells through immune mechanisms. Traditional treatments like surgery, chemotherapy, and radiation often fall short—failing to eradicate all cancer cells, overlooking the disease's immunosuppressive characteristics, and potentially weakening the body's overall immune capacity. Immunotherapy (IT) has become a rapidly expanding field within cancer treatment, encompassing various approaches. The overarching aim of cancer immunotherapy is to restore immune system control by initiating targeted immune responses akin to those observed in cases of spontaneous tumour regression. We developed a personalized immunotherapeutic strategy utilizing dendritic cells (DCs), which are professional antigen-presenting cells capable of initiating robust adaptive immune responses upon encountering foreign or mutated antigens. Upon activation, DCs process and present these antigens to lymphocytes—predominantly T cells—leading to their proliferation and activation against antigen-expressing targets. DCs were generated ex vivo and specifically primed with tumour antigens by fusion with autologous tumour cells. This fusion enabled the incorporation of the complete repertoire of tumour-associated antigens, including both characterized and patient-specific neoantigens, resulting in the formation of autologous immunohybridoma cells (aHyCs). These aHyCs were administered to patients enrolled in a clinical study evaluating treatment efficacy in castration-resistant prostate cancer (CRPC). Here we report on the safety profile, immune system modulation, and overall survival outcome of this therapy.

Given that aHyC is a fully autologous cell-based immunotherapy, this platform has the potential to be adapted for the treatment of other solid tumour types beyond prostate cancer. Preparations are currently underway to evaluate the safety and efficacy of aHyC in patients with triple-negative breast cancer (TNBC) in an upcoming clinical trial.

In summary, treatment with autologous immunohybridoma cells (aHyC) has demonstrated a favourable safety profile and promising immunomodulatory activity in patients with castration-resistant prostate cancer. Our findings support the potential of aHyC as a novel therapeutic approach for solid tumours, warranting further investigation in other malignancies such as TNBC.

Insights into Plectin's Potential as a Glioblastoma Biomarker

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Glioblastoma (GBM) is a highly invasive brain tumour with a predicted average survival time of 1–1.5 years. The spread of GBM cells to the surrounding brain tissue occurs along blood vessels and axons, but the mechanisms underlying tumour progression remain incompletely understood. Our research highlights the crucial role of the cytolinker protein plectin in promoting the migration of GBM cells, as well as the migration of astrocytes, one of their cells of origin. During malignant transformation, astrocytes are reprogrammed into an oncogenic phenotype through extensive changes in the expression and distribution of numerous proteins, including those that form the intermediate filaments (IFs) of the cytoskeleton. A key regulator of IF cytoskeletal plasticity is plectin, which links IFs among each other and to actin filaments, microtubules, the plasma membrane, and intracellular organelles. In addition, plectin also functions as a scaffolding platform for signalling, and has the potential to regulate several astrocyte processes, including cell migration, and the regulation of ion and water homeostasis. Although alterations in plectin expression have been found in certain cancers, expression and the specific role of plectin in GBM has not been investigated yet.
In this talk, I will present the results showing the importance of plectin in the migration of immortalized astrocytes and GBM cells. The contribution of plectin to cell migration will be highlighted by measurements of cell migration in plectin-expressing and plectin-null astrocytes. In addition, the expression of plectin at the cell periphery of astrocytes influences the dynamic changes in cell volume by modulating the expression of the water channel aquaporin-4 in the plasma membrane. Aquaporin-4 has also been proposed to contribute to cell mobility of GBM cells. We here show that in human GBM samples gene expression and protein distribution of aquaporin-4 and plectin show a positive correlation in comparison with healthy tissue. Moreover, the amount of aquaporin-4 aggregates at the plasma membrane is increased in malignant GBM cell lines compared to primary human astrocytes.
The abundant presence of plectin at the cell periphery of GBM cells suggests that plectin could serve as a novel biomarker for the detection and monitoring of GBM. Targeting plectin may represent a novel therapeutic approach to impair the invasive capabilities of GBM cells. Taken together, our findings emphasize the importance of cytoskeletal remodelling in GBM progression and position plectin as a central player in these processes, with potential applications in both diagnostics and therapy.

Connexin 43-based Intercellular Communication and Its Role in Neuropathic Pain

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Neuropathic pain, a persistent condition affecting millions of people worldwide, represents a major challenge due to neuroinflammation, chronicization, and difficulties in developing effective treatments. Neuroinflammatory and neurodegenerative diseases, although distinct in their clinical and pathophysiological features, share remarkable convergence in the mechanisms leading to chronicization and neurotoxicity.
In the context of neuropathic pain, intercellular communication has emerged as a critical player during the chronicization process, and recent evidence support the hypothesis of an interconnected functional role of hyperactivation of poly(ADP-ribose) polymerase 1 (PARP1) and concomitant dysregulated intercellular communication. PARP1 in pathological conditions is involved in NAD^+ depletion, accumulation of PAR polymers, and pro-inflammatory signals activation.
We found that PARP1 inhibition mediated by olaparib induced a marked reduction in mechanical allodynia and an improvement of motor coordination. Moreover, using metabolomic and proteomic analyses, we observed a significant reduction of reactive astrocytes and microglia at the spinal level, coupled with a distinct signature in intercellular communication, oxidative and cellular stress response. Given that our findings suggest a mutual influence between PARP1 and connexin 43 (Cx43), a critical mediator of intercellular communication in fueling neuropathic pain chronicization, we performed a proof-of-concept experiment on astroglial Cx43 conditional knockout (cKO) model. We found that Cx43-based channels are mediating heterocellular coupling between astrocytes and microglial cells and that Cx43-cKO mice showed increased resilience to neuropathic pain development.
Taken together our findings highlight the therapeutic potential of targeting PARP1 and Cx43, suggesting new perspectives for neuropathic pain management.

Poster Abstracts

* Posters Selected for Short Oral Presentations (in Sections with Open Time Slots)

Cardiovascular Physiology

P01*: Dynamic Changes of Interleukin-18 Concentration in Peripheral and Coronary Circulation Following Coronary Artery Bypass Surgery

Lara Batičić[1], Božena Ćurko-Cofek[2], Danijel Knežević[3], Tanja Batinac[4], Aleksandra Ljubačev[5], Lara Valenčić Seršić[3], Gordana Laškarin[1,6], Marko Zdravković[7], Maja Šoštarič[8,9], Vlatka Sotošek[3,4]

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Cardiovascular diseases, including ischemic heart disease, are the most common diseases and the leading cause of death worldwide. When medication is not sufficient, invasive treatments range from percutaneous coronary intervention to open heart surgery. There are two surgical techniques: one in which extracorporeal circulation is used, on-pump, and the other in which the operation is performed on the beating heart, off-pump. Regardless of the technique used, ischemia–reperfusion injury to the myocardium occurs, leading to a strong inflammatory response and the release of inflammatory mediators, including interleukin (IL)–18. IL-18 is an important cytokine regulator involved in innate and adoptive immunity and is produced by various cell types, including cardiomyocytes.
The aim of this study was to analyse the effect of on-pump and off-pump coronary artery bypass surgery on the concentration of IL-18 in peripheral blood, sinus coronarius and aortic bulb in patients during open heart surgery and in the early postoperative period.
In this prospective study, the patients were divided into two groups according to the surgical technique used. The on-pump group consisted of patients who had undergone coronary bypass surgery using the on-pump technique, while the off-pump group consisted of patients who had undergone surgery using the off-pump technique. Five millilitres of peripheral venous blood were collected before surgery (T1), 24 (T2) and 72 hours (T3) after surgery, while blood was collected from the coronary sinus and aortic bulb during surgery before bypass was performed. Plasma was collected by centrifugation and the concentrations of IL-18 were quantified using highly sensitive enzyme-linked immunosorbent assays (ELISAs).

The plasma concentration of IL-18 was statistically significantly higher at time points T2 and T3 compared to time point T1 in both groups. In addition, a statistically higher plasma concentration of IL-18 was observed in the on-pump group than in the off-pump group at time points T2 and T3. The concentration of IL-18 in the coronary sinus and in the aortic root did not differ between the two groups.
In conclusion, both on-pump and off-pump coronary artery bypass grafting lead to a significant increase in plasma IL-18 concentration in the early postoperative period, indicating a systemic inflammatory response after myocardial ischemia–reperfusion. However, the on-pump technique is associated with a significantly higher increase in IL-18, suggesting that the on-pump technique triggers a stronger inflammatory response.

This work was supported by the Croatian Science Foundation under the project number HRZZ-IPS-2023-02-9650, by Slovenian Research and Innovation Agency under the project number ARIS-J3-50120 and by the scientific project approved by University of Rijeka under the project number uniri-iskusni-biomed-23-88-3035.

P02: Age-Confounding Differences in Hemodynamic and Vascular Parameters and Body Composition Profiles in Chronic Kidney Disease Patients Compared to Healthy Controls

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Chronic kidney disease (CKD) is associated with progressive cardiovascular and metabolic alterations that are initially asymptomatic. Impedance cardiography (ICG) and bioimpedance-based body composition analysis are non-invasive methods that can provide insight into hemodynamic function and body composition changes in this population. This cross-sectional study aimed to compare these parameters between patients with CKD stages 3–5 and healthy individuals, with a particular focus on the confounding effect of age.

In this pilot cross-sectional study, a total of 9 patients with CKD stages 3–5 and 8 healthy controls were included in the impedance cardiography analysis. Body composition analysis was conducted using a Maltron Bioscan 920-II bio-electrical impedance analyser in a slightly larger sample of 9 CKD patients and 11 healthy controls. Hemodynamic parameters measured included systolic and mean arterial pressure, systemic vascular resistance index (SVRI), and total arterial compliance index (TACI). Body composition parameters included body mass index (BMI), resting metabolic rate (RMR), fat mass percentage, fat-free mass percentage (FFM), total body water (TBW) and plasma fluid volume. Due to a significant age difference between groups, ANCOVA (analysis of covariance) was used to adjust for age as a covariate in all comparisons.

When groups were not adjusted for age, few significant differences were observed between CKD patients and healthy controls. However, after age matching and statistical adjustment using ANCOVA, clear distinctions emerged. CKD patients demonstrated significantly higher systolic blood pressure and mean arterial pressure compared to healthy controls. Furthermore, they exhibited elevated systemic vascular resistance index and a significantly lower total arterial compliance index, suggesting increased vascular stiffness and impaired hemodynamic regulation.

In terms of body composition, age-adjusted analysis revealed that CKD patients had significantly higher BMI and resting metabolic rate (RMR), as well as a higher percentage of body fat. Conversely, fat-free mass percentage, total body water (TBW), and plasma fluid volume were significantly lower in the CKD group compared to controls. These findings point to a characteristic shift toward increased adiposity and reduced lean tissue and hydration status in CKD.

This study demonstrates that significant differences in hemodynamic, vascular, and body composition parameters between CKD patients and healthy individuals become apparent only after controlling for age. Age was identified as a key confounder that initially masked these differences, highlighting the importance of proper statistical adjustment in small-sample

clinical studies. The findings suggest that even in non-dialysis CKD stages, patients exhibit early vascular dysfunction and unfavourable body composition profiles, which may have implications for cardiovascular risk stratification and nutritional management. Impedance cardiography and body composition analysis could serve as useful, accessible tools in the monitoring of CKD-related systemic changes. Further studies with larger, longitudinal cohorts are needed to validate these preliminary results.

P03: Limitations of the Standardized Myocardial Work Analysis: Role of the Peak Pressure Timing

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Myocardial work (MW), an emerging index of cardiac performance, is derived from the left ventricular (LV) strain curves and a pressure template. However, current technique assumes a fixed pressure shape – specifically a constant timing of peak systolic pressure – across all cardiovascular states. This assumption does not reflect physiological variability, as the shape and timing of the LV pressure curve can shift significantly with hemodynamic alterations. Furthermore, the extent to which this assumption influences the MW index, and its derived metrics of the myocardial performance is not well understood.
The following case study has utilized a standard EchoPac pressure template and an alternative pressure template which were generated by featuring temporally shifted systolic peak while maintaining an identical surface area and peak amplitude. The strains were measured with the speckle tracking echocardiography in 4-chamber, 3-chamber, and 2-chamber views (in total 18 sectors) obtained from a patient with myocarditis. Then, by using both the pressure templates, the calculations of MW index and instantaneous MW as a pressure – strain loop integral and time series from mitral valve closure to mitral valve opening were performed. The MW index values variability was analysed, while in instantaneous MW the mean of absolute difference was calculated and compared.
The MW values showed excellent agreement ($r = 0.99$, $p < 0.05$) between the EchoPac suite and alternative pressure template i.e., 1589 vs 1650 mmHg% respectively across the entire views in all the sectors, with an average discrepancy of 3.8 ± 4.2 %. In contrast, a substantial discrepancy was noted in the instantaneous MW with a mean absolute discrepancy of 11 ± 1.3 %.
These findings reveal a limitation in the current myocardial work (MW) estimation approach: while the cumulative MW is relatively stable to temporal changes in pressure shape, the derived instantaneous MW metric is highly sensitive. This research highlighted the importance of an accurate temporal alignment in the pressure-strain analysis for the precise assessment of instantaneous cardiac performance. This precision becomes important in the diffuse myocardial pathologies, where the subtle regional changes may go undetected with the standard approaches.

P04: The Influence of Different Coronary Artery Bypass Surgeries on the Vascular Endothelial Glycocalyx Degradation

Božena Ćurko-Cofek[1], Lara Batičić[2], Danijel Knežević[3], Tanja Batinac[4], Aleksandra Ljubačev[5], Lara Valenčić Seršić[3], Gordana Laškarin[1,6], Marko Zdravković[7], Maja Šoštarič[8,9], Vlatka Sotošek[3,4]

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The endothelial glycocalyx is a dynamic gel-like layer that lines the luminal surfaces of endothelial cells. It plays a crucial role in the regulation of permeability, mechanotransduction and the maintenance of vascular homeostasis. The endothelial glycocalyx contains proteoglycans, glycosaminoglycans, glycoproteins and associated plasma proteins that are in direct contact with the bloodstream and are therefore exposed to shear stress-induced damage. In addition, surgical procedures, especially high-risk operations such as cardiac surgery, can affect the integrity of the endothelial glycocalyx and the release of endothelial glycocalyx degradation products, such as syndecans and hyaluronic acid, into the blood. Syndecans are the most important proteoglycan core proteins of the endothelial glycocalyx, which are involved in the promotion of inflammation, infectious diseases and tumours. Hyaluronic acid is a part of the endothelial glycocalyx that is most susceptible to structural and chemical changes caused by reactive oxygen species, which are produced in large quantities during cardiac surgery and thus increase the intensity of inflammation.
The aim of the study was to analyse the effects of different techniques of coronary bypass surgery on the degradation of endothelial glycocalyx products (syndecan-1, hyaluronic acid) in the peripheral and cardiac circulation during surgery and in the early postoperative period.
The study included 60 patients who had undergone surgical myocardial revascularisation. The patients were divided into two groups according to the surgical technique used. The on-pump group consisted of patients who had undergone coronary bypass surgery using the on-pump technique (with use of extracorporeal circulation), while the off-pump group consisted of patients who had undergone surgery using the off-pump technique. Five millilitres of peripheral venous blood were collected before surgery (T1), 24 (T2) and 72 hours (T3) after surgery, while blood was collected from the coronary sinus and aortic root during surgery, before the bypass surgery. Plasma was collected by centrifugation and the concentrations of syndecan-1 and hyaluronic acid were quantified using highly sensitive enzyme-linked immunosorbent assays (ELISAs). In the on-pump group, the plasma concentration of syndecan-1 increased

significantly at time T2 compared to time T1 and decreased at time T3, while the plasma concentration of syndecan-1 in the off-pump group did not change significantly at any time point. At time point T2, a statistically higher plasma concentration of syndecan-1 was observed in the on-pump group than in the off-pump group. The plasma concentration of hyaluronic acid increased significantly at time T3 in the on-pump group, but not in the off-pump group. The concentration of syndecan-1 and hyaluronic acid in the coronary sinus and aortic root did not differ between the groups.
In conclusion, the on-pump surgical technique is associated with a significant increase in plasma concentrations of the markers of endothelial glycocalyx degradation, syndecan-1 and hyaluronic acid, suggesting greater damage to the endothelium compared to the off-pump technique.

This work was supported by the Croatian Science Foundation under the project number HRZZ-IPS-2023-02-9650, by Slovenian Research and Innovation Agency under the project number ARIS-J3-50120 and by the scientific project approved by University of Rijeka under the project number uniri-iskusni-biomed-23-88-3035.

P05: n-3 PUFAs-Enriched Functional Food Enhances Macrovascular Endothelium-Dependent Vasodilation in Young Healthy Individuals – a Randomized Study

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Our previous research demonstrated that consumption of hen eggs enriched with n-3 polyunsaturated fatty acids (n-3 PUFAs) had a positive influence on endothelium-dependent vasodilation. This study aimed to evaluate the impact of consuming n-3 PUFAs-enriched chicken meat on brachial artery vasodilation in healthy young individuals. Thirty-nine participants (age 20-26 years) were included in this randomized, placebo-controlled interventional study, and divided into two groups: the Control group (n=20) or n-3 PUFAs group (n=19) who consumed regular (~118 mg of n-3 PUFAs/day) or n-3 PUFAs enriched chicken meat (~1500 mg of n-3 PUFAs/day) for 3 weeks. Brachial artery flow-mediated dilation (FMD) and nitroglycerine-mediated dilation (NTG-MD) were measured to assess macrovascular reactivity. Biochemical parameters and blood pressure measurements were obtained before and after the respective study protocols. All measurements were performed before and after each dietary protocol. Endothelium-dependent vasodilation of the brachial artery was significantly increased following consumption of n-3 PUFAs enriched chicken meat for three weeks, but not regular chicken meat, compared to baseline measurements. FMD was significantly higher in the n-3 PUFAs group compared to the Control group after the study protocol. In contrast, the endothelium-independent vasodilatory response of the brachial artery to nitroglycerin (NTG) remained unchanged following the consumption of either regular or n-3 PUFAs-enriched chicken meat, with no significant differences observed between groups or compared to baseline measurements. Consumption of either regular or n-3 PUFAs enriched chicken did not cause significant changes in blood pressure or biochemical markers among participants. In conclusion, in healthy young adults, a three-week consumption of chicken meat enriched with n-3 PUFAs significantly enhances endothelium-dependent vasodilation in the macrovasculature, without altering endothelium-independent vascular responses. These results support the notion that n-3 PUFAs exert a favourable and potentially protective effect on vascular function under resting, disease-free conditions. Clinical Trial Registration: NCT04564690.

This research was supported by the European Structural and Investment Funds grant for the Croatian National Scientific Center of Excellence for Personalized Health Care, Josip Juraj Strossmayer University of Osijek (Grant Number: KK.01.1.1.01.0010), and by the Faculty of Medicine Osijek institutional research projects IP-05-MEFOS-2023 and IP-08_MEFOS-2025 (PI Ivana Jukić).

P06: Integrating Inverse Problem Reasoning into Complex System Solving: A Computational Approach to Hemodynamic Parameter Estimation

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Physiology traditionally addresses the forward problem: predicting system behaviour under known parameters. However, research and clinical practice rely on solving the inverse problem — inferring system states from limited, uncertain measurements. Despite its centrality, the implications of inverse problem-solving in complex systems remain underexplored. In hemodynamics, the inverse problem involves estimating cardiovascular system (CVS) states, defined by cardiac contractility (CC), systemic vascular resistance (SVR), and venous compliance (VC, proxy for blood volume), from arterial pressure measurements. Using the CircAdapt computational model, we mapped arterial blood pressure (ABP) feature uncertainties into a CC–SVR–VC parametric space. We visualized the distributions of mean arterial pressure (MAP) and pulse pressure (PP) within this space. We demonstrate that feature uncertainties, measurement errors, and transfer function distortions enlarge solution volumes and shift centroids under clinical interventions. Notably, the MAP iso-plane was smooth and convex, the PP iso-plane was twisted, and their intersections formed three distinct geometric patterns. These findings highlight degeneracy, insufficient independent measurements, multi-parameter coupling, and model simplification as key drivers of non-uniqueness in physiological inverse solutions. We propose an Open Forward-Inverse (OFI) framework, combining forward simulations with inverse exploration, to systematically analyse and visualize degenerate solution spaces. Integrating this paradigm into physiology would advance conceptual and analytical capacities for interpreting complex system behaviours.

P38: Immunoregulatory Role of Enzymes Matrix Metalloproteinases 2 and 9, their Tissue Inhibitors TIMP-1 and TIMP-2 and Heat Schock Protein 70 in Atherogenesis

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Matrix metalloproteinases (MMPs) play a key role in the physiology of connective tissue development, morphogenesis and wound healing. Their unregulated activity has been implicated in numerous disease processes including arthritis, tumour cell metastasis and atherosclerosis. Morphology and plaque material play an important role in initiating the atherosclerotic carotid artery symptoms. Ulceration and rupture of the covers change the histological image of the plaque which could be a suitable sign for assessing possible risk. The risk factors for the development of atherosclerosis are: hypercholesterolemia, hypertension, infection and oxidative stress that increase the expression of HSPs in atherosclerotic lesions, i.e., endothelial cells, smooth muscle cells, and macrophages.
Immunohistochemically expression of the heat shock 70 (hsp 70) protein on paraffinic atherosclerotic alterations of carotid arteries were done. We used the method of enzyme immunoassay (ELISA) for the investigation of the concentration of enzyme MMPs and their tissue inhibitors in urine.
Our data showed a rise in the expression of heat shock protein 70 in atherosclerotic-modified carotid arteries. Patients with developed atherosclerosis had statistically significant increases in MMP 2 and 9.
High concentration of MMP 2 and 9 may represent a new method for monitoring atherosclerotic changes. The role of heat shock protein (HSPs) in the atherosclerotic process is insufficiently clarified and controversial. They act as autoantigens and stimulate cellular and humoral immune responses. Since they possess immunoregulatory properties, they may be useful in atherosclerosis as immune modulators of the acquired or inherited immune response.

Acknowledgement: This work was supported by grants from University of Rijeka No. ((uniri-biomed-18-220, 23-288 and 23-303).

Cerebral Circulation

P07: Adenosine A2a Receptor-Mediated Vasodilation is Maintained by Intermittent but not Acute Hyperbaric Oxygenation

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Adenosine and its receptors play a crucial role in the regulation of vascular tone during hypoxia. Hypoxia-induced dilation (HID) is largely mediated through adenosine A2a receptors (A2aRs), which induce vasodilation via the synthesis of vasodilatory metabolites such as epoxyeicosatrienoic acids (EETs). In contrast, adenosine A1 receptors (A1Rs) typically promote vasoconstriction. The present study investigated how acute (Ac-HBO_2), and intermittent (In-HBO_2) hyperbaric oxygenation affect the adenosinergic pathway and vascular reactivity to hypoxia in the middle cerebral arteries (MCAs) of Sprague-Dawley rats. Male and female Sprague-Dawley rats (8–10 weeks old) were randomly assigned to three experimental groups: control (CTRL), Ac-HBO_2 (single 2-hour exposure to 100% O_2 at 2 bars), and In-HBO_2 (daily 2-hour exposures for four consecutive days). MCAs were isolated immediately after sacrifice and mounted in a pressure myograph system for continuous diameter recording under physiological conditions. HID was assessed by exposing arteries to hypoxic gas (0% O_2, 5% CO_2, balance N_2) at 80 mmHg intraluminal pressure. Vessels (N = 7 per group) were tested in baseline conditions and after 30 minutes' incubation of A1R and A2aR agonists (CCPA, Abcam, 10^{-6} M and CGS-21680, Abcam, 10^{-6} M, respectively) or antagonists (8 – Cyclopentyl - 1, 3 - dipropylxanthine (DPCPX), Abcam, 10^{-6} M and 5 – Amino – 7 - (2 - phenylethyl) - 2 - (2 - furyl) - pyrazolo (4, 3 - e) - 1, 2, 4 - triazolo (1, 5 - c) pyrimidine (SCH-58261), Abcam, 10^{-6} M, respectively). Our findings demonstrate that Ac-HBO_2 significantly impairs HID, while In-HBO_2 preserves or even enhances this response. The administration of A1R agonists or antagonists did not significantly alter HID in any group, supporting the notion that A1Rs have a limited functional role in hypoxic conditions. In contrast, A2aR stimulation with the selective agonist CGS-21680 led to a significantly greater HID in the In-HBO_2 group compared to Ac-HBO_2, highlighting the beneficial role of intermittent exposure in preserving A2aR-mediated vasodilation. Notably, no significant differences were observed in response to A2aR antagonism (SCH-58261), suggesting that the vasodilatory effect during hypoxia is strongly dependent on active A2aR signalling. In conclusion, HID is significantly impaired after acute HBO_2, whereas intermittent HBO_2 preserves or enhances HID likely due through upregulated A2aR signalling and favourable metabolic adaptations. These findings suggest that modulation of the adenosinergic pathway by varying HBO_2 protocols has translational relevance for optimizing therapeutic strategies aimed at preserving cerebrovascular function under hypoxic conditions.

This work was supported by institutional grants of Faculty of Medicine Osijek IP8-MEFOS-2020 (to Aleksandar Kibel) and IP5-MEFOS-2021, IP25-MEFOS-2024 and IP7-MEFOS-2025 (to Zrinka Mihaljević).

P08*: Integrating Imaging and Network Science to Decode Capillary Architecture in Health and Disease

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Understanding the structural organization of vascular networks is essential for deciphering tissue physiology and pathology. With recent advances in high-resolution imaging modalities, including confocal and light-sheet fluorescence microscopy, it is now possible to visualize the vascular system in unprecedented detail, including its intricate three-dimensional microarchitecture. However, this progress introduces a growing need for robust computational tools capable of translating complex image data into meaningful structural descriptors. In this context, network-based representations have emerged as highly effective: by abstracting the vasculature as a graph composed of nodes (bifurcations or endpoints) and edges (vessel segments), one can quantitatively assess topological and morphological features across spatial scales—from large arteries down to microcapillaries.
In our study, we developed a computational framework for extracting vascular networks from imperfect imaging datasets. We first demonstrate this methodology on two-dimensional confocal images of retinal capillaries, showing how skeletonized vessel structures can serve as a foundation for graph construction. The key processing challenges—such as ambiguous bifurcations, spurious endpoints, and connectivity gaps—are systematically addressed through geometric criteria and topological refinement algorithms. We then extend our approach to analyse three-dimensional microvascular networks obtained from optically cleared mouse organs—specifically, the heart and brain—using light-sheet fluorescence microscopy. The tissues were collected from 8-week-old C57BL/6J mice and fluorescently labelled to reveal the capillary architecture. Network analysis revealed a highly modular organization of the capillary bed, with strong local integration within modules and limited intermodular connectivity, which acts as a bottleneck for flow. Notably, modules in the cardiac microvasculature were markedly elongated and aligned in a preferential direction. In contrast, brain vasculature exhibited only moderate elongation and lacked directional alignment. To investigate disease-related structural alterations, we applied the same methodology to light-sheet images of the heart tissue from 12-week-old db/db mice, a well-established model of type 2 diabetes. Compared to control animals, diabetic networks were significantly sparser and exhibited longer extravascular distances—indicative of impaired perfusion capacity. However, the modular organization and directional features of the network remained largely unchanged. Importantly, these novel insights into capillary network architecture could only be achieved through the interdisciplinary integration of cutting-edge imaging techniques with computational network-based analysis, underscoring the value of such interdisciplinary approaches in cardiovascular research.

Exercise/Space Physiology

P09: Estimates of Persistent Inward Currents in Young and Older Adults Following Bed Rest and Recovery

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Understanding the role of neuromodulation as a contributor of muscle force reduction with disuse may have important implications to the preservation/recovery of muscle functionality in several conditions such as prolonged hospitalization due to injuries or diseases. Persistent inward current (PIC) is an intrinsic motor neuron property that amplifies the synaptic input to motor neurons proportionally to monoaminergic neuromodulation. Here we assessed PIC contribution to muscle force reduction after prolonged bed rest (BR). Nine young (22±4 years) and 10 older (69±3 years) males underwent 21 and 10 days of BR, respectively, followed by 21 days of endurance training (ET). High-density surface electromyography (HDsEMG) was recorded from the vastus lateralis muscle during isometric contractions. After decomposing individual motor unit (MU) firing rates from HDsEMG signals, PICs were estimated using the paired motor unit technique as the delta frequency between lower and higher threshold MUs. Results showed PIC values decreased after BR in both age groups compared to baseline ($p<0.001$ for young, $p<0.002$ for older) and were restored after ET ($p<0.001$ for young, $p<0.03$ for older). Our findings suggest that PIC significantly contributes to muscle force generation loss after disuse and its recovery following active training. Future research could explore alternative treatments targeting the neuromodulatory system to improve recovery after muscle disuse. This study was cofinanced by the Slovenian Research and Innovation Agency (project J5-4593 and programme P2-0041), the Italian Space Agency (n.2024-5-E.0 - CUP n.I53D24000060005), and by NextGenerationEU/Italian Ministry of University and Research (MUR) PRIN 2022 PNRR, (ReActiveAge, Project n. P2022FNCPR).

P10: Diet Quality and Nutrient Adequacy in Endurance-Trained Male Athletes: Insights from an Epic-Norfolk Food Frequency Questionnaire

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This cross-sectional study aimed to assess the dietary quality of competitive male endurance athletes by analyzing their energy, macro- and micronutrient intake using the validated EPIC-Norfolk Food Frequency Questionnaire (FFQ), and to compare these values with current dietary recommendations established by the European Food Safety Authority (EFSA). A secondary objective was to evaluate the sodium-to-potassium (Na/K) ratio, a recognized marker of cardiovascular risk.
Thirty-one healthy male endurance athletes (aged 18–45 years), training a minimum of five times per week, were enrolled in this study. Subjects' body mass index, arterial blood pressure, as well as red blood count (RBC) and serum lipid profile was measured. Translated in Croatian, cross-culturally adapted and validated EPIC-Norfolk food frequency questionnaire (FFQ) was used to determine average daily total energy, food groups, and micro-/macronutrient intake in athletes.
All subjects were lean and normotensive, and had normal RBC and serum lipid levels. The reported energy intake was significantly lower than recommended (mean 2105 ± 666 kcal/day vs. 3350 kcal/day), with 71% of participants identified as low-energy reporters using the Goldberg cut-off method. Protein intake (1.25 ± 0.47 g/kg/day) was within recommended levels, while carbohydrate intake (2.8 ± 1.1 g/kg/day) fell markedly below the target range (5–12 g/kg/day). Total fat intake (38.0% of total energy) exceeded recommendations, particularly saturated fat (14.4%), which surpassed the recommended <10% of energy. Micronutrient analysis revealed adequate intake for most nutrients; however, suboptimal intakes of vitamin D and folate were observed. Conversely, intakes of sodium and iodine exceeded recommended thresholds, contributing to a mean molar Na/K ratio of 1.53 ± 0.36. Regression analysis identified that protein intake was predominantly derived from cereals, eggs, and meat, while carbohydrates were sourced from cereals, fruits, and potatoes. Fat intake primarily originated from meat, nuts, soups, snacks, and fruit, while sodium (and iodine) intake was mainly contributed by cereals, nuts, potatoes, and soups.
Although the athletes demonstrated a generally favourable body composition and adequate intake of several key nutrients, notable imbalances were detected. These include insufficient carbohydrate intake, excessive saturated fat and sodium consumption, and inadequate intake of vitamin D and folate. These findings highlight the need for individualized nutritional strategies tailored to training demands, exercise intensity, and electrolyte losses. Suboptimal intakes of vitamin D and folate indicate the necessity for monitoring (and supplementing) in this population. Furthermore, high sodium intake, which despite adequate potassium intake resulted in a higher Na-to-K ratio, highlighted concerns over excessive salt consumption, but also accentuated the need for monitoring sodium (and potassium) intake in competitive athletes with consideration for sweat-related losses.

Neurophysiology

P11: L-Lactate in Astrocytes and Neurons: More than Just Fuel in Brain

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L-lactate (LL) in the central nervous system has long been considered merely as a metabolic byproduct or a signal of cellular distress, particularly during hypoxia. Recent evidence has highlighted its role in energy metabolism and signalling in the brain. According to the astrocyte-neuron lactate shuttle (ANLS) hypothesis, intracellular LL (LL_i) produced in astrocytes leaves the cell via monocarboxylate transporter 4 (MCT4) and enters neurons via MCT2, where it is used as an energy source. However, studies have shown that downregulation of MCT2 also causes a drop in extracellular LL (LL_e), challenging this unidirectional view and raising questions about LL dynamics and signalling. In addition, LL_e has been shown to activate aerobic glycolysis and thus LL_i production in astrocytes and depolarize locus coeruleus neurons (LCn) via a receptor-mediated mechanism. Whether LL_e can also regulate LL_i production in neurons, as in astrocytes, remains unclear.
Here we aimed to test and compare the effects of LL_e (2 mM) on the dynamics of LL_i changes in LCn and astrocytes. To isolate receptor-mediated effects, we blocked MCTs using α-cyano-4-hydroxycinnamic acid (CHC). Since LL_e levels can be significantly increased in certain pathological conditions (epilepsy, hypoxia), in the aging brain, and during strenuous exercise, we compared the effects of 2 mM with the effects of 10 mM LL on LL_i dynamics in both cell types.
Isolated rat LCn and cortical astrocytes were transduced with a fluorescent L-lactate sensor (*Laconic*) based on Förster resonance energy transfer (FRET). We used 2 mM or 10 mM LL, and for experiments with blocked MCTs, 6 mM CHC was applied before the addition of LL. Changes in the ratio of FRET signals, which reflected real-time changes in LL_i after stimulation, and the rate of LL_i change upon stimulation were measured and analyzed.
The results show that the physiological (2 mM) concentration of LL elicits different dynamics of LL_i changes in both LCn and astrocytes, which can be grouped into three groups of responses, with individual responses being differently represented in each cell type. In both cell types, 10 mM LL elicited significantly larger changes in LL_i compared to physiological concentration, but the response was different – in LCn, LL_i stabilized, while in astrocytes, LL_i continuously increased. In both cell types, blockade of MCT with CHC caused a rapid and significant increase in LL_i. Astrocytes responded markedly to the subsequent addition of 2 mM LL, while in LCn, changes were not significantly higher compared to controls. While LL_i handling under basal conditions appears to be comparable in LCn and astrocytes, LL receptor-mediated LL production is part of astrocyte physiology alone.

P12: Remimazolam Induced Cytotoxicity and Cell Death in Rat Astrocytes

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Remimazolam is a novel, ultra-short-acting anesthetic belonging to the benzodiazepine class. It is commonly used for the induction and maintenance of anesthesia during day surgeries, serving as an effective alternative to traditional anesthetics such as ketamine and propofol. Although data on remimazolam's toxicity are currently limited, the use of general anesthetics has often been associated with toxic effects, particularly in the developing brain, where they may contribute to long-term neurocognitive decline. One proposed mechanism involves the cytotoxic effects of anesthetics on various brain cells. Astrocytes, among the most abundant cell types in the brain, play a key role in maintaining neural homeostasis, and reduced astrocyte viability could significantly impair central nervous system function. This study aims to investigate the potential cytotoxic effects and cell death induced by remimazolam in primary cortical rat astrocytes using flow cytometry.

Primary cultures of cortical astrocytes obtained from newborn rats were used as an experimental model. Cells were exposed for 24 hours to increasing concentrations of remimazolam, including clinically relevant plasma concentrations observed during anesthesia. Apoptosis, necrosis, and necroptosis were assessed as forms of cell death using flow cytometric analysis.

The viability of cultured astrocytes was not affected by remimazolam at concentrations up to 350 μM. Cytotoxic effects, indicated by an increased fraction of dead cells, were dose-dependent and observed only at concentrations approximately 300 times higher than therapeutic levels (350 μM –5,3-fold increase vs. control, $p < 0.0001$). The majority of cell death at 350 μM occurred via apoptosis (58%), whereas at higher concentrations, necrosis became the predominant form. Necroptosis was not detected.

Remimazolam, at plasma concentrations achievable during general anesthesia, does not seem to exert cytotoxic effects on cultured astrocytes. Toxicity was dose-dependent and observed only at concentrations several hundred times higher than therapeutic levels. Although astrocytes are relatively resistant to apoptosis, remimazolam at high concentrations appears to primarily induce apoptotic, rather than necroptotic, cell death. At even higher concentrations, apoptosis appears to progress to secondary necrosis.

P13*: Neuropsin, TRPV4 and Intracellular Calcium Mediate Intrinsic Photosensitivity in Corneal Epithelial Cells

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Our aim was to investigate intrinsic phototransduction in the corneal epithelium and its role in intracellular and inflammatory signalling.
Optical imaging in isolated corneal epithelial cells (CECs) and debrided epithelia was combined with molecular, biochemical, pharmacological assays and gene deletion studies to track UVB-induced calcium signalling and release of cytokines, chemokines and matrix remodelling enzymes. Results from wild type mouse CECs were compared to data obtained from *Opn5*$^{-/-}$ and *Trpv4*$^{-/-}$ cells.
UVB stimuli and TRPV4 activity induced epithelial release of IL-1β, IL-17, matrix metalloproteinases MMP-3/MMP-9, and thymic stromal lymphopoietin (TSLP). UVB stimuli evoked $[Ca^{2+}]_i$ elevations in dissociated mouse CECs that were partially reduced by inhibition of TRPV4 channels, *Trpv4* knockdown and replacement of control saline with Ca^{2+}-free saline. UVB-induced Ca^{2+} responses were significantly suppressed by OPN5 deletion and by inhibition of phospholipase C signalling, and responses were abrogated in cells with depleted intracellular Ca^{2+} stores.
Mammalian CECs are intrinsically and constitutively photosensitive. UVB photons are transduced by neuropsin, phospholipase C and CICR signalling, with mouse but not human CE transduction exhibiting a UVB-sensitive TRPV4 component. TRPV4 activity and UVB transduction are linked to cell-autonomous release of proinflammatory, matrix remodelling and nociceptive interleukins and MMPS. TRPV4-induced cytokine release may contribute to the pain induced by mechanical injury of the cornea and CEC photosensing may alert and protect the visual system from ultraviolet B (UVB) radiation -induced snow blindness, injury, vision loss and cancer.

P14: L-lactate and GPR27 Agonists Modulate Citrate Production in 3T3 Cells and Astrocytes

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Astrocytes are neuroglial cells with many homeostatic functions, including the regulation of brain energy metabolism. Astrocytes can convert D-glucose to L-lactate in a process known as aerobic glycolysis. In culture, and likely *in vivo*, astrocytes represent the main source of mitochondrial citrate production and its secretion into the intercellular space. Citrate, which is produced in the Krebs cycle, is involved in the regulation of glycolysis and gluconeogenesis. Although the concentration of citrate in the cerebrospinal fluid is relatively high, ranging from several tens to several hundred μmol/L, its precise role remains unclear. It has been shown that aerobic glycolysis in astrocytes can potentially be activated with certain G-protein coupled receptor (GPCR) agonists, including those activated by noradrenaline and L-lactate. GPR27 is an orphan GPCR and a member of the super-conserved receptors expressed in the brain (SREB). It has been shown that stimulation of GPR27 enhances aerobic glycolysis and L-lactate production in 3T3 murine embryonic fibroblasts (MEF) and astrocytes. Here, we investigate the impact of the stimulation with extracellular L-lactate and GPCR surrogate agonists on the production of citrate in astrocytes and 3T3 cells. We used a genetically encoded fluorescent biosensor to monitor cytosolic citrate with high temporal resolution in single cells. Cells were stimulated with L-lactate (2 mM) and GPR27 surrogate agonists (1 μM). Our preliminary results show that extracellular L-lactate significantly increases $[citrate]_i$ in 3T3 cells and rat astrocytes. Similarly, stimulation of GPR27 with surrogate agonists also increases $[citrate]_i$ in 3T3 cells and rat astrocytes. These results indicate that L-lactate and GPR27 receptor activation modulate mitochondrial citrate production, which may affect energy metabolism in the cell itself and, under *in vivo* conditions, more broadly in the brain.

P15: Regulation of Second Messenger Dynamics and Mechanosensitivity in 3T3 Cells and Astrocytes by the Orphan Receptor GPR27

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GPR27 is an orphan receptor and belongs to a family of super-conserved receptors expressed in the brain (SREB). GPR27 appears to play a role in insulin production, lipid metabolism and is also involved in L-lactate homeostasis (LL). It has been shown that GPR27 stimulation enhances aerobic glycolysis (AG) and LL production in NIH-3T3 MEF cells and primary rat cortical astrocytes. Here, we investigated whether activation of the GPR27 receptor by GPR27 surrogate agonist (1 μM) involves signalling via the second messengers Ca^{2+} and cAMP. We used a Förster resonance energy transfer (FRET)-based cAMP nanosensor to monitor cytosolic cAMP with high temporal resolution in single cells. Intracellular Ca^{2+} was monitored with Ca^{2+} indicator Calbryte 520AM in real time. Our preliminary results show that stimulation of GPR27 with a surrogate agonist increases $[Ca^{2+}]_i$ but not $[cAMP]_i$ in WT 3T3 cells. In astrocytes, the GPR27-surrogate agonist also caused an increase in $[Ca^{2+}]_i$. We have observed that GPR27 modulates $[cAMP]_i$ responses following L-lactate stimulation in 3T3 cells. Interestingly, in control experiments, we also observed an increase in $[cAMP]_i$ in both 3T3 cells and astrocytes in response to the addition of a vehicle (extracellular solution). In 3T3 cells with CRISPR-Cas9 GPR27 knockout, the vehicle-induced increase in $[cAMP]_i$ was greater than WT controls. Transfection of GPR27KO 3T3 cells with a plasmid encoding GPR27 attenuated the vehicle-induced increase in $[cAMP]_i$. Additionally, we observed that the addition of a vehicle also induced an increase in $[Ca^{2+}]_i$ in GPR27KO 3T3 cells. Previous studies of mechanosensitive signalling in mouse and rat astrocytes have suggested the possible involvement of mechanically activated channels, including PIEZO channels and most notably TRPV4 channels. We observed that both TRPV4 and Piezo1 channels are more abundantly expressed in GPR27KO 3T3 cells, which could explain the observed responses after vehicle stimulation. It appears that GPR27 not only plays a role in AG and LL production but is also likely involved in the mechanosensitivity of cells.

P16: Morphological Remodelling of Astrocytes Potentiates Calcium but not cAMP Plasticity

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Astrocyte plasticity is essential for lifelong homeostasis in the central nervous system and for adaptive responses to environmental and exposomal challenges. Here, we compared astrocytes cultured in serum-containing DMEM ($DMEM^+$; flattened, pathology-like) and astrocytes cultured in serum-free, hbEGF-supplemented neurobasal medium (NB^+; process-bearing/arborized) to examine how structural state influences subcellular Ca^{2+} and cAMP signalling, and vesicle dynamics. Using ratiometric (Fura-2) and non-ratiometric confocal Ca^{2+} imaging (Fluo-4), FRET-based cAMP nanoreporting (Epac1-camps), single-vesicle membrane capacitance measurements, and quantitative tracking of endocytotic vesicles, we assessed resting states and agonist-evoked cellular responses. Arborized astrocytes displayed higher resting $[Ca^{2+}]_i$ and greater subcellular heterogeneity, accompanied by reduced spontaneous vesicle mobility and altered parameters of reversible exocytosis (larger fusion-pore conductance and longer dwell time). Purinergic stimulation elicited a larger integrated Ca^{2+} signal with a shift toward sustained responses in NB^+ astrocytes, whereas $DMEM^+$ cells predominantly exhibited oscillatory Ca^{2+} responses. In contrast, global cAMP dynamics were largely comparable: resting $[cAMP]_i$ was modestly elevated in NB+ astrocytes, but the amplitude and kinetics of noradrenaline-evoked cAMP increases, microdomain-level heterogeneity, and overall production capacity were similar in both culture conditions. Our findings indicate that increased morphological complexity selectively augments Ca^{2+} signalling plasticity and reconfigures vesicle trafficking, whereas cAMP signalling remains comparatively resilient to structural changes. We propose that the Ca^{2+} toolkit is the primary locus of morphology-dependent functional remodelling in astrocytes shaping their homeostatic adaptations across physiological and pathological contexts.

P17*: Chronic 5-HT$_2$A Receptor Activation Differentially Affects Mitochondrial Biogenesis and Stress Tolerance in Neonatal and Adult Rat Cortical Astrocytes

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Astrocytes, the most abundant glial cells in the central nervous system, play a central role in maintaining neuronal function. Mitochondria enable astrocytic neuroprotective functions, particularly during periods of increased demand or injury. Emerging evidence suggests that serotonergic signalling, notably via the 5-HT$_2$A receptor, may modulate astrocyte physiology, including mitochondrial capacity. However, the extent to which 5-HT$_2$A receptor activation influences mitochondrial biogenesis remains unclear. Our objective was to investigate mitochondrial biogenesis following 5-HT$_{2A}$ receptor activation under conditions of chronic stress in primary cortical astrocytes derived from neonatal and adult rats.
Astrocyte cell cultures were derived using the method of Schwartz and Wilson (1992) from the cerebral cortex of neonatal (1–3 days old) and adult (8–10 weeks old) Wistar rats. Cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1 mM sodium pyruvate, 2 mM L-glutamine, and 50 µg/mL gentamicin. Adult rat astrocytes required a longer cultivation period to reach maturity. After micorglia removal, cell cultures were replated onto 12-well plates for gene expression analysis or 96-well plates for functional assays. At approximately 80% confluency, cells were treated for either 3 or 7 days with 0.1-10 µM 2,5-dimethoxy-4-iodoamphetamine (DOI), a selective 5HT2A agonist. For induction of metabolic stress (starvation), cells were maintained in glucose-free and serum-free DMEM during the treatment period. Experimental conditions included untreated control, DOI in standard medium, starvation alone, and DOI under starvation. Mitochondrial biomass was evaluated by MitoTracker Green FM staining, and cell viability was assessed via resazurin reduction using the alamarBlue assay. Gene expression of PGC-1α, GFAP, and 5-HT$_{2A}$ receptor was quantified using TaqMan qPCR and analyzed by the comparative Ct (ΔΔCt) method, with β-actin as the endogenous control. Statistical analysis was performed using one-way ANOVA, with $p < 0.05$ considered statistically significant.
Chronic DOI treatment significantly increased mitochondrial biomass and improved viability under both normal conditions and under metabolic stress in neonatal rat astrocytes, suggesting enhanced mitochondrial biogenesis. In contrast, adult rat astrocytes showed no significant changes in mitochondrial mass or viability following DOI treatment. qPCR confirmed the expression of PGC-1α, GFAP, and 5-HT$_{2A}$ receptor in both tissue and cell cultures, with age-related differences in expression levels.
Chronic activation of the 5-HT$_2$A receptor enhances mitochondrial biogenesis and stress resilience in neonatal cortical astrocytes, but not in those derived from adult rats, indicating an age-dependent effect. These differences may stem from developmental regulation of serotonergic signalling or reflect reduced plasticity in adult astrocytes, possibly influenced by prolonged in vitro cultivation. Understanding such age-specific responses is essential for advancing glia-targeted therapeutic approaches in neurodevelopmental and neurodegenerative disorders.

Cellular and Molecular Physiology

P18: Plectin Plays a Role in the Distribution of Mitochondria and Mitophagy in Mouse Astrocytes

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Mitophagy is a specialized form of macroautophagy that selectively targets damaged or dysfunctional mitochondria (those with impaired membrane potential) for removal and recycling. This tightly regulated process varies depending on cell type and physiological conditions. Impaired mitophagy has been linked to cellular dysfunction, aging, and chronic diseases, particularly in high-energy-demand cells such as neurons, cardiomyocytes, and skeletal muscle cells. Dysregulation of mitophagy is implicated in diverse pathologies, including lung, cardiovascular, liver, and kidney diseases, as well as cancers, immune disorders, metabolic disturbances, and sensory impairments. Notably, mitophagy activity declines with age, by up to 70 % in some cases.
Astrocytes, once overlooked as passive glial cells, are now recognized for their critical roles in neuronal support and neurodegeneration. Investigating mitochondrial function and mitophagy in astrocytes may provide novel therapeutic insights for age- and disease-related brain changes. In our study, we have assessed mitophagy in astrocytes under various physiological and pharmacological conditions using the mKeima fluorescent protein. mKeima, derived from the marine coral *Montipora*, exhibits pH-dependent excitation shifts. At mitochondrial pH (8.0), it is optimally excited at shorter wavelengths, whereas in acidic lysosomes (pH 4.5) following mitophagy, its excitation peak shifts toward longer wavelengths. This property enables long-term tracking of mitophagy flux in live cells, as mKeima resists lysosomal degradation. Using mitochondria-targeted mKeima, we quantified mitophagy in mouse astrocytes by detecting mitophagosome-lysosome fusion.
By expressing the pH-sensitive fluorescent protein mKeima, we established a robust method for monitoring mitophagy in live astrocytes. Confocal microscopy revealed distinct regions of varying mitophagic activity. The mitochondrial network exhibited considerable morphological diversity, ranging from rounded, globular structures to elongated and branched filaments. As shown in previous research, the distribution, fusion and fission of mitochondria depends on the cytoskeleton. We have here addressed how plectin influences mitophagy and distribution of mitochondria in mouse astrocytes. We show that plectin closely envelopes the mitochondria and influences mitochondrial density. Astrocytes without plectin contained fewer mitochondria and exhibited reduced mitophagic activity. These results suggest a role for plectin in shaping mitochondrial dynamics and regulating mitophagy in astrocytes.

P37: Astrocytes Exhibit Higher Ca^{2+} Excitability to Neuromodulator Octopamine than Neurons in *Drosophila* Living Brain

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Octopamine, the invertebrate analogue of noradrenaline, exerts its neuromodulatory effects in *Drosophila* brain via G protein-coupled adrenoceptor-like receptors, octopamine/tyramine receptors (OctR/TyrR). Upon release from octopaminergic neurons, octopamine by binding to its receptors activates intracellular Ca^{2+} signalling pathways that modulate diverse brain functions, including neurotransmission. Despite the expression of these receptors in both neurons and glial cells, the cellular specificity of octopamine-induced Ca^{2+} responses in the brain remains incompletely understood.
In this study, we investigated octopamine-induced Ca^{2+} signalling in neurons and glial cells in the *Drosophila* brain using confocal imaging and genetically encoded Ca^{2+} indicator jGCaMP7b, selectively expressed in each cell type. Application of octopamine evoked Ca^{2+} transients in both astrocytes and neurons. Although the amplitude of neuronal Ca^{2+} signals was 3.4-fold higher than in astrocytes, astrocytes exhibited greater sensitivity to octopamine. The median effective concentration (EC_{50}) required to induce Ca^{2+} responses was nearly six times lower in astrocytes than neurons. Pharmacological blockade of α_1- and β-adrenoceptor homologs with antagonists terazosin and mianserin, respectively, significantly attenuated octopamine-induced Ca^{2+} responses in both cell types, suggesting the involvement of both Gq/Ca^{2+} and Gs/cAMP signalling cascades. The inhibitory effects of the antagonists were more pronounced in neurons. *Drosophila* single-nucleus RNA-sequencing (snRNA-seq) database screening revealed distinct expression patterns of OctR and TyrR across neurons and glia, which likely underlie the observed differences in Ca^{2+} signalling between different brain cell types in *Drosophila* brain.
Taken together, our findings indicate that in the optic lobes of the *Drosophila* brain, astrocytes, but not neurons, respond to low concentrations of octopamine, and are therefore likely to act as drivers of synaptic plasticity and visual processing. Given the connectivity of the optic lobes with other brain regions, astrocyte-mediated responses to octopamine may also influence higher-order brain functions, including learning and memory.

P19: GOReverseLookup: A Novel Tool for Candidate Gene Identification from Gene Ontology

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The Gene Ontology (GO) project provides a critical framework for gene annotation and biological characterization. Traditional annotation methods map genes to GO terms, yet there is an increasing demand for reverse querying tools, which allow the identification of genes enriched in user-defined phenotypes described by sets of GO terms. This study presents GOReverseLookup, a novel bioinformatics tool enabling reverse gene ontology queries. By integrating orthologous gene querying capabilities with databases such as Ensembl and UniProtKB, GOReverseLookup facilitates the identification of genes significantly associated with specific biological states across various species.
GOReverseLookup operates through a three-stage algorithm: initially querying genes associated with selected GO terms representing the phenotypes of interest, subsequently retrieving all GO terms linked to these genes, and finally employing statistical analysis to evaluate the significance of gene associations with the defined phenotypes. This tool is freely available, written in Python, and designed for straightforward user implementation.
Two use cases demonstrate the effectiveness of GOReverseLookup. The first use case identified genes significantly associated with rheumatoid arthritis, including validated genes such as IL10, ACP5, and HLA-DRB1. The second use case explored genes linked to the concurrent states of chronic inflammation and tumorigenesis, uncovering established genes like NF-κB, IL6R, and novel candidates such as TNFRSF21. GOReverseLookup successfully captured known pathways and identified potential new gene targets, illustrating its utility in hypothesis generation and exploration.
GOReverseLookup offers a robust, accessible solution for the identification of candidate genes associated with defined phenotypes. Its ability to integrate cross-species orthologous data significantly enhances its predictive power, making it particularly valuable for early-stage research in functional genomics, systems biology, and drug discovery. We anticipate that GOReverseLookup will substantially facilitate the exploration of genetic mechanisms underlying complex biological phenotypes.

P20: Potential Gene Candidates Involved in Pathophysiology of Osteosarcopenia

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With advancing age, decline of muscle and bone tissues leads to sarcopenia and osteoporosis – conditions that commonly coexist, sharing numerous risk factors and pathophysiological mechanisms. Recognizing their intertwined nature, researchers have introduced a new concept of osteosarcopenia. However, the underlying pathophysiological mechanisms of osteosarcopenia as a clinical entity remain poorly understood. The aim of this study was to use bioinformatics tools to identify novel genetic candidates simultaneously involved in the pathogenesis of both osteoporosis and sarcopenia. We utilized recently developed bioinformatics tool, GOReverseLookup, which performs reverse gene ontology (GO) analyses. This tool identifies candidate genes enriched within user-defined states of interest (SOIs), based on phenotype-associated GO terms. Initially, we manually curated a comprehensive set of GO terms representing our target SOIs – osteoporosis and sarcopenia – using AmiGO2 and QuickGO databases. GOReverseLookup subsequently queried genes linked to these GO terms, incorporating orthologous gene data across multiple species (*Danio rerio, Rattus norvegicus, Mus musculus*, and *Xenopus tropicalis*). Indirect annotations were included up to three hierarchical parent terms. Statistical significance was determined using Fisher's exact test with a stringent genome-wide *p*-value threshold (5×10^{-8}). Finally, candidate genes underwent a robust validation process consisting of manual literature curation, DisGeNET scoring, tissue-specific expression analyses and pathway enrichment analysis (KEGG). We identified 37 genes associated with osteosarcopenia. Among these, five genes – *RBCK1, TERF2IP, LGALS9, ZBTB7A,* and *RPS3* have not previously been documented in the literature regarding osteoporosis or sarcopenia. Though, these genes exhibited at least moderate expression levels in both bone and muscle tissues. Additionally, four genes – *PYCARD, MALT1, BCL2L11,* and *PRKCQ* were identified with lower expression levels in both tissues and had not been previously associated with osteoporosis or sarcopenia. Ten of our identified genes were previously described in the literature as associated with both osteoporosis and sarcopenia, validating the effectiveness and accuracy of our identification methodology. KEGG pathway enrichment analysis indicated significant enrichment predominantly within the NF-kappa B signalling pathway, NOD-like receptor signalling pathway, and necroptosis pathway. Each of the 5 most promising and unknown genes plays a fundamental role in pathways that regulate inflammation and proteostasis. While direct evidence linking these genes to osteosarcopenia is not yet available, their established functions in human and animal studies strongly suggest new potential connections. This research provides a comprehensive list of novel genetic candidates and highlights molecular pathways potentially pivotal in osteosarcopenia. The identified genes are promising targets for future research aimed at explaining disease mechanisms, discovering early biomarkers, and developing effective therapeutic strategies. Enrichment analysis identified three pathways involved in immune system regulation and function, supporting the concept of chronic low-grade inflammation as a common driver in the pathogenesis of osteosarcopenia.

P21: Molecular Biology of Rheumatoid Arthritis – Studying the Role of Galectins

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Galectins belong to the family of glycan binding proteins. Although they are usually located in the extracellular space, where they bind glycoconjugates, they have a profound influence on intracellular processes, such as autophagy, cell activation, cell signalling, phagocytosis, and survival. Galectins demonstrate a plethora of immunoregulatory properties. For instance, they regulate the activity of T cells by engaging glycoproteins that participate in T cell activation and signalling. Therefore, dysregulated expression of galectins can significantly affect immune responses, leading to pathological conditions. For example, they mediate the secretion of IL-17, a crucial cytokine involved in autoimmune processes. Early studies suggested that administration of recombinant galectins can suppress excess pro-inflammatory conditions. The aim of the present study is to shed light on the potential role of galectins in pathophysiology of rheumatoid arthritis (RA), a highly prevalent autoimmune disease.
To perform this study, samples of synovium and blood were collected from patients who provided written consent for the study during routine hospitalizations. The synovial expression and plasma concentrations of Gal-1, Gal-3, Gal-4, and Gal-7 were studied. The results were correlated with clinical parameters. The study was approved by the bioethics committee of the Pomeranian Medical University in Szczecin, Poland.
The synovial expression of Gal-1 and Gal-3 were significantly greater in the control group than in the cohort with RA. However, there were no significant differences in the expression of Gal-4 and Gal-7. Furthermore, differences in plasma concentrations of all four galectins were not significant. Except for age, no significant correlations between clinical parameters and galectins were observed. Interestingly, Gal synovial expression and plasma concentrations demonstrated significant correlations with each other and other immune regulators, such as alarmins.
Current analyses demonstrate the potential involvement of galectins within the local inflammatory responses within the joint. However, it should be noted that samples were collected from hand joints and not from the knees. Perhaps, patients with advanced RA who would require joint replacement would demonstrate different levels of galectins in plasma as well.

P22: The Complex Role of Adiponectin in Joints – The Examples of Osteoarthritis and Rheumatoid Arthritis

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Adipose tissue secretes an enormous number of biologically active peptides, known as adipokines. These molecules are strongly involved in mediating metabolic processes, such as glucose and lipid metabolism. However, they also demonstrate immunoregulatory properties which attracted attention of researchers. Dysregulated levels of adipokines observed in inflammatory conditions are considered to contribute to the pathogenesis of diseases. Adiponectin is an insulin-sensitizing hormone which promotes glucose uptake and suppresses gluconeogenesis. It stimulates AMP-activated protein kinase (AMPK) and proliferator-activated receptor α (PPAR α), important signalling pathways implicated in metabolism regulation. Moreover, studies demonstrated that adiponectin can exert both pro- and anti-inflammatory features. Adiponectin and its receptors are expressed in the synovial tissue within the joints. Nevertheless, its role and involvement in articular diseases is not fully understood. The role of the present study is to analyse the expression of adiponectin in the tissues within the joints and study the association with circulating cytokines in patients with osteoarthritis (OA) and rheumatoid arthritis (RA).
The study includes OA and RA patients that underwent routine joint replacement surgery. During hospitalization and treatment, blood, synovial membranes and fat pad samples were collected. RNA isolation, RT-PCR, qRT-PCR, and analysis of cytokine levels were performed using commercially available research kits. All experiments were performed in accordance with manufacturers' protocols. STATISTICA 13.3 software was used to perform statistical analyses. The study was approved by the Bioethics Committee of the Pomeranian Medical University in Szczecin (KB-0012/39/17), and written consent was obtained from all participants.
Eighteen patients with OA and 18 patients with RA were included. In patients with OA, synovial expression of adiponectin demonstrated significant positive correlations with IL-1β, IL-4, G-CSF and GM-CSF plasma levels. Furthermore, a significant positive correlation between infrapatellar fat pad adiponectin expression and plasma GM-CSF concentrations were observed. Additional analyses of adiponectin receptors revealed significant negative correlations between synovial AdipoR1 and AdipoR2 expression and IL-6 plasma levels. In addition, a negative association between synovial AdipoR2 expression and MCP-1 and TNFα plasma concentrations were found. In RA patients, no significant correlations between adiponectin synovial expression and studied cytokines were found. However, a significant negative relationship between synovial AdipoR1 and TNFα, IL-7, IL-12, and IL-13 were observed.
The presented study is a continuation of our experiments studying the role of adiponectin within OA and RA. The present results demonstrate that plasma cytokines affect the expression of adiponectin within joint tissues. Interestingly, a positive correlation was observed regarding levels of pro-inflammatory cytokine IL-1β and synovial adiponectin expression in OA patients,

while no significant results were observed in the case of RA. Such findings could indicate the involvement of adiponectin in pathological responses observed in joints in OA. Perhaps, chronic inflammatory conditions present in RA are a result why pro-inflammatory plasma cytokines do not show positive correlations with adiponectin. Furthermore, negative correlations were found between plasma cytokines and adiponectin receptors. To conclude, the involvement of adiponectin in articular inflammation is complex and context dependent. Further experiments are needed to understand these associations.

P23: The Role and Signalling of Transient Receptor Potential Vanilloid-4 (TRPV4) Ion Channels in the Mouse Bladder

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Overactive bladder syndrome (OAB) is a common clinical condition affecting around 17 % of the population. Its symptoms include frequent urinary urgency, often accompanied by incontinence and nocturia. These symptoms significantly affect patients' quality of life, making this condition a matter of public health importance. Currently, antimuscarinic drugs are the first-line medical therapy for the management of OAB. However, their application is limited due to their several side effects. According to the literature, Transient Receptor Potential Vanilloid 4 ($TRPV_4$) ion channels play a role in the regulation of smooth muscle contraction. Moreover, studies indicate that the activation of $TRPV_4$ can result in the secretion of prostanoids.

We aimed to analyse the intracellular signalling pathways of $TRPV_4$ receptors in the urinary bladder (UB) contraction with the goal of better understanding the intracellular regulation of micturition and identifying potential novel therapeutic targets of OAB.

Urinary bladder (UB) tissues were obtained from adult (90-120 days) male and female C57Bl6/N wild-type (WT) and COX_1 knockout (KO) mice. Urinary bladder slices were prepared under a microscope. The contractile force was measured with a wire-myograph under isometric conditions and normalized to contractions induced by 124 mM K^+. The release of prostaglandin E_2 (PGE_2) or thromboxane A_2 (TXA_2) from the tissue was quantified using ELISA.

The $TRPV_4$ agonist GSK101 (GSK) evoked contractions in the mouse UB. The contractions were more pronounced in slices isolated from male than female mice. These findings confirm the critical role of $TRPV_4$ ion channels in bladder contractions. Interestingly, the GSK-induced contractions were completely abolished in the presence of the non-specific COX enzyme inhibitor indomethacin, demonstrating the role of COX enzymes in bladder contractions induced by GSK. Furthermore, the contractile responses were significantly reduced in bladders from mice deficient in the COX_1 enzyme compared to those of the wild-type control. The COX_2 inhibitor NS-398 significantly reduced the GSK-induced bladder contractions. Moreover, the treatment of NS-398 was able to diminish the contractile responses in UB isolated from COX_1-KO animals, suggesting the critical role of the COX_1 and COX_2 enzymes in the $TRPV_4$-mediated bladder contractions. In addition, elevated levels of TXA_2 and PGE_2 release were measured in GSK-treated bladder samples. Finally, to identify the receptors mediating GSK-induced bladder contractions, we tested the effects of EP_1 (SC19220), EP_3 (L798106), and TP receptor (SQ29548) antagonists. Neither EP_1 nor EP_3 receptor inhibition had any effect on the contractions induced by GSK. However, GSK-induced bladder contractions were almost completely abolished by SQ29548. These findings suggest that the $TRPV_4$-mediated bladder contractions are transmitted mostly by the TP receptors.

In summary, the TRPV4 ion channel plays an important role in the regulation of bladder contractions. Moreover, GSK induces PGE_2 and TXA_2 release in the mouse bladder involving the COX_1 and COX_2 enzymes. Furthermore, GSK-induced contractions are mediated mostly by the TP receptors. The deeper understanding of the $TRPV_4$ signalling in the mouse UB may aid the identification of more specific therapeutic targets for bladder dysfunctions.

Grant support: K-135683, K-139230, PD-143327 and TKP2021-EGA-25 from the Hungarian NRDIO.

Metabolic/Endocrine Physiology

P24: Functional Food Intervention Alters Correlation Patterns Between Vascular, Inflammatory, and Antioxidant Markers in Healthy Individuals

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The investigation of the potential of nutritionally enriched foods, particularly in healthy individuals, has in recent years become a growing focus of scientific research aimed at reducing systemic inflammation and the risk of future chronic diseases development. This study was conducted on 60 young healthy participants of both sexes, aged 19–27 years, with normal body mass index (BMI, ~24 kg/m²) and serum biochemical parameters within reference range for the general population. Participants were divided into: 1) CONTROL GROUP – consuming regular food; 2) EXPERIMENTAL GROUP – consuming food enriched with omega-3 fatty acids, vitamin E, selenium, and lutein. Samples were collected on the first and last day of the 21-day dietary protocol. Markers of inflammation and oxidative stress, serum activity and gene expression of antioxidant enzymes, and the serum concentration of vascular markers were analyzed. Statistical analysis included the Student's t-test and correlation analysis, performed using GraphPad Prism 8 and Microsoft Excel 2016 softwares. A p-value < 0.05 was considered statistically significant. No significant differences were observed between control and experimental group in all measured parameters. However, in the control group, a significant positive correlation was observed between serum iron and the pro-inflammatory cytokine IL-17A ($r = 0.54$; $p = 0.044$), and a negative correlation between transferrin and endoglin ($r = –0.53$; $p = 0.024$). In contrast, in the experimental group, iron negatively correlated with VCAM-1 ($r = –0.48$; $p = 0.05$), while transferrin positively correlated with ICAM-1 ($r = 0.53$; $p = 0.03$). Additionally, in the control group, antioxidant capacity (FRAP) showed a significant positive correlation with E-selectin ($r = 0.48$; $p = 0.05$) and a negative correlation with hsCRP ($r = –0.54$; $p = 0.008$). In the experimental group, FRAP positively correlated with IL-17A ($r = 0.44$; $p = 0.04$), while TBARS (a measure of lipid oxidation) negatively correlated with endoglin ($r = –0.46$; $p = 0.05$). Furthermore, in the experimental group, serum catalase activity further showed a positive correlation with serum GPx activity ($r = 0.52$; $p < 0.0001$) and gene expression of GPx4 ($r = 0.47$; $p = 0.004$), eNOS ($r = 0.49$; $p = 0.002$), and iNOS ($r = 0.54$; $p = 0.0006$), while it negatively correlated with serum SOD activity ($r = –0.42$; $p = 0.002$). These results indicate distinct patterns of association between oxidative stress, inflammation, and vascular function depending on the type of diet. In the experimental group, specific correlations suggest a potential modulation of inflammatory and antioxidant responses influenced by functional foods. Despite the absence of significant differences in biomarker values between the groups, the presence of different correlation patterns suggests possible subtle effects of functional foods on antioxidant defense and inflammatory pathways.

P25*: Effects of Arginine Vasopressin on Pancreatic α and β Cells: Glucose-Dependent Modulation and Receptor-Specific Responses

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The role of arginine vasopressin (AVP) in the regulation of pancreatic α and β cell function has been controversial. We investigated the effect of AVP and its designed receptor agonists and antagonists on α and β cells. Acute pancreatic tissue slices from C57BL/6J mice were imaged with confocal microscopy. Slices were stimulated with 8 mM glucose with AVP or AVP receptor agonist/antagonist and cAMP modulating agents, cytosolic Ca^{2+} events were recorded and analysed as described before (1). AVP exerts glucose-dependent effects on both cell types. At low glucose concentrations AVP selectively activated α cells, without significantly affecting β cells. At stimulatory glucose concentrations AVP enhanced β and α cell activity, leading to increased intracellular Ca^{2+} activity. Epinephrine-dependent depletion of cAMP levels in β cells suppressed the AVP effects. AVP displayed a bell-shaped concentration dependence, with lower concentrations stimulating and higher concentrations diminishing responses, consistent with IP_3 receptor activation and inactivation properties. Furthermore, our results indicate that AVP acts primarily through V_{1b} receptors. The effects of AVP are contingent on the intracellular cAMP environment enabling it to significantly modulate the response of α and β cells to other stimuli. These findings provide new insights into the glucose-dependent modulation of pancreatic hormones by AVP, highlighting its potential role in metabolic regulation.

P26: Sex-Based Differences in Iron Metabolism, Oxidative Stress, and Antioxidant Enzyme Activity in Healthy Young Adults

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Oxidative stress occurs when there is an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defenses, potentially leading to cellular damage. Thus, an excess of iron can lead to the production of ROS through the Fenton reaction. At the same time, sex hormones such as estrogen can influence its distribution and availability by regulating hepcidin. Iron metabolism is essential for cellular and tissue homeostasis. Therefore, it is important to understand whether there are sex-based differences in the regulation of iron between men and women. In this study, we explored the correlation between biological parameters (iron, ferritin, BMI), markers of inflammation (cytokines), oxidative stress (FRAP, TBARS, dcf-da), activity and gene expression of antioxidant enzymes (GPx, SOD) to better understand their interactions. The study included sixty young, healthy participants - 30 women and 30 men, aged between 20 and 25 years. The study population had normal body mass index (women 22.8 ± 3.3 kg/m^2; men 24.7 ± 3.0 kg/m^2). Biochemical parameters were within normal reference. All participants had normal levels of lipid profile (total cholesterol: 4.4 ± 0.9 mmol/L; triglycerides: 0.95 ± 0.6 mmol/L; HDL: 1.45 ± 0.3 mmol/L; LDL: 2.7 ± 0.6 mmol/L), iron, transferrin, inflammation markers and oxidative stress markers. In the group of women participants, the level of the anti-inflammatory cytokine IL-10 positively corelated with serum iron levels ($r = 0.47$; $p = 0.024$) and negatively correlated with transferrin ($r = -0.49$; $p = 0.017$). While transferrin levels were significantly higher in women compared to men ($p = 0.0002$). Higher BMI in the group with women participants positively correlated with lower expression of mitochondrial SOD2 ($r = -0.64$; $p = 0.020$). Furthermore, in women a significant positive correlation was observed between pro-inflammatory cytokine TNF-α and serum SOD activity ($r = 0.76$; $p = 0.030$), and a negative correlation of lipid peroxidation (measured as TBARS) with expression of GPx1 ($r = -0.89$; $p = 0.003$) and GPx4 ($r = -0.62$; $p = 0.035$). In contrast, in the group of men participants, FRAP positively correlated with SOD activity ($r = 0.623$; $p < 0.0001$) and iNOS expression ($r = 0.526$; $p = 0.006$), while SOD activity showed a positive correlation with ROS production inside cells ($r = 0.47$; $p = 0.050$) and a negative correlation with IL-17A ($r = -0.62$; $p = 0.050$). Still, SOD activity was significantly higher in women compared to men ($p=0.001$). Women demonstrated a stronger antioxidant-inflammatory profile, where iron, IL-10 and the antioxidant enzymes gene expression are connected in a pattern that could be linked to estrogen regulation. In men, a reactive-redox response is more dominant, while the activity of SOD is associated with intracellular ROS and IL-17, which suggests a different hormonal effect on the immune-redox balance. These findings suggest sex-specific patterns in redox regulation: in women, immune and metabolic components are more evident, likely influenced by hormonal modulation, while in men, functional adaptation to oxidative stress dominates through the activation of antioxidant mechanisms.

P27: Beta cell recovery drives remission of type 2 diabetes following caloric restriction in mice

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Diet-induced obesity (DIO) mouse models are a common and valuable research tool in studying pathophysiology of type 2 diabetes mellitus (T2D). Currently used animal models (e.g., high fat diet, high fat high sucrose) have some inherent methodological drawbacks, mostly due to beta cell plasticity when used from an early age, and due to the composition of the diet used to induce T2DM. On the other hand, clinical studies by Taylor et al. strongly suggest that caloric restriction results in effective remission of T2D in humans, however limited mechanistical explanation is available at the level of beta cell function. We therefore constructed a novel mouse model of DIO that more closely reflects T2D in humans in an attempt to decipher functional and/or morphological changes following caloric restriction-induced remission of T2D. Male and female C57BL/6J mice were fed a western diet (WD) for 12 weeks starting from the age of 12 weeks, after which they exhibited a T2D phenotype in the form of fasting hyperglycemia, impaired glucose clearance during ipGTT and increased insulin resistance during ipITT. 7 days of caloric restriction (i.e., intake of 35 % of the caloric intake of the control group) completely reversed the diabetic phenotype, with normalization of body mass, normalization of glucose handling and insulin sensitivity. In an attempt to provide a mechanistical explanation for both the DIO and remission following caloric restriction at the level of beta cell function, we performed functional multicellular confocal calcium imaging on acutely prepared pancreatic tissue slices to assess the effects of both DIO and caloric restriction on the glucose sensitivity of beta cells. A left shift in the glucose dependence was detected in the DIO group, which together with hyperglycemia could account for hyperinsulinemia observed *in vivo*. Short term caloric restriction completely reversed the above compensatory left shift in beta cells and their oscillatory activity at a given glucose concentration decreased to that of the control group. Our findings further elucidate the impact of caloric restriction on T2D and our animal model provides a novel platform for studying T2D.

P28: Spatially Organized Functional Heterogeneity and Modular Structure in Beta Cell Networks

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Multicellular structures interpret external stimuli through the collective behaviour of heterogeneous cell populations. This is also true for the islets of Langerhans, where networks of beta cells respond in a coordinated manner to changes in extracellular nutrient levels to regulate insulin secretion. However, due to their functional heterogeneity, the resulting spatiotemporal activity patterns are highly complex and remain incompletely understood. In this study, we investigated the spatial distribution of beta cell heterogeneity and its relationship to the modular organization of functional networks. These networks were constructed based on multicellular calcium activity measured by confocal microscopy in pancreatic tissue slices from mice and isolated human donor islets. Our analyses revealed that beta cells with similar Ca^{2+} signalling properties are not randomly distributed but tend to form spatial clusters. These clusters also showed strong correspondence with network-defined communities, suggesting a close link between spatial proximity and functional connectivity. Moreover, during the transition from non-stimulatory to stimulatory glucose levels, beta cell activation occurred in spatially localized groups, further supporting the presence of underlying functional submodules. Functional networks were highly modular in both mouse and human islets, with spatial position emerging as a key determinant of community structure. These network modules showed partial overlap with clusters identified by Ca^{2+} signal similarity and even stronger overlap with groups of cells that activated simultaneously during glucose elevation. We also assessed the distribution of specialized beta cell subpopulations across these network communities. Hub cells, which facilitate intercellular communication due to their central role in the multicellular network; wave-initiator cells, which trigger intercellular calcium waves; and first-responder cells, which initiate the first-phase response to glucose stimulation, were generally found in different communities rather than co-localizing within a single module. Interestingly, in mouse islets, the spatial distribution of these subpopulations was primarily driven by their location within the islet, whereas in human islets, their presence was more strongly associated with regional activity levels.

P29: Temporal Stability in Pancreatic Beta Cell Networks: Are Specialized Subpopulations Functionally Persistent?

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Healthy pancreatic islets contain hundreds of β cells that act in synchrony to generate pulsatile insulin secretion, thereby ensuring metabolic homeostasis. Their collective behaviour is shaped by the interplay of at least three specialized subpopulations of cells which have recently gained attention: (i) first-responder cells, which initiate the first-phase response to glucose stimulation; (ii) wave-initiator cells, which trigger intercellular Ca^{2+} waves—the primary synchronizing mechanism for fast Ca^{2+} oscillations during the plateau phase of response; and (iii) hub cells, which facilitate intercellular communication due to their central role in the multicellular network. Yet, important questions remain regarding the stability and interplay of these subpopulations. In this study, we investigated the temporal stability of these subpopulations using functional multicellular Ca^{2+} imaging in acute mouse (NMRI, 8-12 weeks old) pancreatic tissue slices, combined with methods from complex network theory. We employed a protocol consisting of three consecutive 20-minute stimulations with 10 mM glucose, interspersed with recovery intervals of 20 and 180 minutes in substimulatory 6 mM glucose. Our findings suggest that, over this time frame, the roles of the three subpopulations are largely maintained across successive stimulations, albeit with some exceptions. However, there were important distinctions in their temporal dynamics. Hub cells consistently retained their network-central positions both during each individual stimulation and across all three stimulations. In contrast, the identities of first responder and wave-initiator cells showed more temporal variability, with roles occasionally shifting between cells and regions within and across stimulations. These findings suggest that the role of hub cells is more strongly determined by features that stay stable on the time scale of several hours, such as gap junction coupling, whereas the emergence of wave-initiators and first-responders is more flexible, shaped by their spatial location and the dynamic socio-cellular context.

P30: Porcine Pancreatic Slices as a Model for Functional and Physiological Research

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Pancreatic tissue slices offer a valuable *ex vivo* model for investigating both the physiology and pathology of the pancreas while maintaining the native microenvironment. Due to their anatomical and physiological similarities to humans, pigs are considered a suitable model for pancreatic research. This technique allows for the simultaneous assessment of various pancreatic cell types, such as beta and acinar cells, under near-physiological conditions. Despite its potential, the application of this method in the porcine pancreas remains largely unexplored.

The aim of this study was to develop a robust protocol for preparing viable porcine pancreatic slices and to evaluate their suitability for calcium imaging and immunohistochemical analyses. Fresh porcine pancreas was sectioned into thin slices using a vibratome. Tissue viability was assessed using live/dead staining. Functional imaging of beta and acinar cells was performed using calcium-sensitive fluorescent dyes and confocal microscopy, while immunohistochemistry was employed to identify specific cell markers.

We successfully established a method for obtaining structurally and functionally intact porcine pancreatic slices. Calcium imaging revealed dynamic activity in both beta and acinar cells. Beta cells exhibited glucose-induced calcium transients, while acinar cells responded to acetylcholine with characteristic calcium signalling. Immunohistochemical staining confirmed the presence of distinct pancreatic cell populations, and viability assays demonstrated high tissue viability.

Porcine pancreatic slices thus represent a promising *ex vivo* model for studying pancreatic function and disease. This approach enables real-time functional imaging and immunohistochemical analysis while preserving the native tissue architecture. Our findings support the applicability of this method in future research focused on diabetes and pancreatic disease modelling.

P31: Short- and Long-Term Ethanol Exposure in Mouse Pancreatic Acinar Slices: Analysis of Calcium Dynamics and Tissue Integrity

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Pancreatic acinar cells play a central role in the initiation of acute pancreatitis, and their response to external stimuli, such as ethanol, is crucial for understanding early pathophysiological changes. Ethanol is a known risk factor for pancreatic damage, yet its direct cellular effects on calcium signalling and tissue integrity remain unclear. In this study, we investigated how different durations of ethanol exposure affect calcium dynamics and tissue morphology in pancreatic acinar tissue slices from adult NMRI mice.
Pancreatic tissue slices were prepared from adult NMRI mice. Some tissue was reserved for histological analysis using hematoxylin and eosin (H&E) staining. Remaining slices were loaded with a calcium-sensitive fluorescent dye and imaged using confocal microscopy to assess calcium dynamics in acinar cells. Slices were divided into three experimental groups: (1) control, exposed only to acetylcholine (ACh) to trigger physiological calcium oscillations; (2) short ethanol exposure group, where 30 mM ethanol was applied for 10 minutes during ACh stimulation; and (3) long ethanol exposure group, where slices were incubated for 5 hours in 30 mM ethanol prior to imaging. Following imaging, live/dead staining was performed to assess tissue viability.
Confocal calcium imaging revealed no significant differences in calcium oscillation patterns between the three groups. All groups responded similarly to ACh stimulation, indicating that short- and long-term exposure to 30 mM ethanol did not impair calcium signalling in acinar cells under the conditions used. However, live/dead staining showed that slices incubated for 5 hours in ethanol displayed marked structural disruption, characterized by the presence of large intracellular spaces. These spaces were not uniformly necrotic in appearance; rather, they suggested a mechanical disintegration or loss of tissue cohesion. In contrast, control and short exposure slices maintained more intact tissue architecture. Histological evaluation with H&E staining corroborated these findings: incubated slices exhibited more extensive necrotic areas and disrupted morphology compared to the other groups.
Our results demonstrate that prolonged exposure to ethanol leads to significant structural alterations in pancreatic acinar tissue without immediately affecting intracellular calcium dynamics. These findings suggest that ethanol-induced damage may involve mechanisms beyond calcium signalling dysregulation, potentially affecting cell adhesion or extracellular matrix integrity.

P32: Glucose-Dependent Calcium Dynamics and Insulin Secretion in Human Diabetic and Non-diabetic Pancreas Tissue Slices

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Animal models have been instrumental in advancing our understanding of pancreatic islet physiology, yet they often fail to replicate species-specific characteristics essential to human type 2 diabetes (T2D). Direct investigation of human pancreatic tissue offers a more relevant platform for elucidating disease mechanisms specific to humans. This study aimed to characterize glucose-stimulated calcium signalling dynamics and insulin secretion in pancreatic tissue slices and isolated islets derived from deceased non-diabetic and diabetic human donors, as well as surgical donors.

Pancreatic tissue samples were obtained from three deceased donors—two non-diabetic and one with T2D. Acute pancreas tissue slices (130–140 μm) were prepared from the tail-end of the pancreas, stained with a calcium indicator and confocal microscopy was used to record beta-cell calcium dynamics during perifusion with stepwise glucose concentrations (3, 6, 9, and 12 mM). In parallel, islets were enzymatically isolated from the same donors for static insulin secretion assays. Subsequently, calcium imaging experiments were conducted using pancreatic slices from surgical donors.

In slices from non-diabetic donors, beta cells exhibited heterogeneous calcium activity, with detectable baseline oscillations at 3 mM glucose and progressively increased oscillatory activity at higher glucose concentrations. These calcium dynamics correlated with robust, glucose-dependent insulin secretion from isolated islets. In contrast, slices and islets from the diabetic donor revealed a marked attenuation of glucose responsiveness. Although a modest increase in calcium activity was observed between 3- and 12 mM glucose, diabetic beta cells failed to demonstrate the clear glucose-dependent responsiveness seen in non-diabetic samples and insulin secretion remained significantly blunted.

This study reveals distinct alterations in glucose-induced calcium signalling and insulin secretion between non-diabetic and diabetic human islets. In diabetic tissue, the diminished calcium oscillatory response to glucose and severely reduced insulin output point to an impairment in stimulus-secretion coupling within beta cells. These deficits likely reflect disruptions in calcium channel function, intracellular signalling pathways, or beta-cell excitability that are central to the pathogenesis of T2D. Our findings underscore the importance of using human pancreatic tissue to uncover disease-specific mechanisms that are not fully captured by animal models.

P39: Illuminating GABA-Mediated Coordination in Pancreatic Beta Cells via Multicellular Imaging and Network Analyses

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Gamma-aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the central nervous system, but also plays important roles in peripheral tissues, including the endocrine pancreas. In pancreatic islets, beta cells release GABA, which acts via ionotropic $GABA_A$ and metabotropic $GABA_B$ receptors to regulate beta cell excitability, calcium dynamics, and insulin secretion, while influencing alpha and delta cells by inhibiting glucagon release and modulating somatostatin secretion. Beyond signalling, GABA metabolism through the GABA shunt links it to mitochondrial function and redox homeostasis.

GABA secretion is pulsatile, with a period of 4–10 minutes, closely resembling insulin oscillations. While metabolically regulated, secretion is not directly triggered by glucose but depends on biosynthesis and catabolism. Perturbing GABA metabolism alters insulin release periodicity, suggesting a role in islet synchronization. However, how GABA contributes to collective islet dynamics remains unclear.

We combined multicellular Ca^{2+} imaging with functional network analysis to explore how GABA shapes beta cell activity and coordination. Acute pancreatic slices from 8–25-week-old male NMRI mice were loaded with the calcium-sensitive dye Calbryte™. Tissues were perifused with oxygenated extracellular solution containing stimulatory glucose (9 mM) ± GABA. Ca^{2+} time series were filtered into fast (electrically driven) and slow (metabolically driven) oscillatory components. We extracted oscillation parameters (frequency, duration, active time) and analysed cellular activation. Functional connectivity networks were constructed from pairwise correlations, separately for fast and slow oscillations.

GABA markedly modulated both individual and collective beta cell activity. Its application significantly shortened activation time upon glucose stimulation, demonstrating a priming effect. GABA also enhanced spontaneous activity at substimulatory glucose, indicating it can promote basal excitability independently of glucose. At the oscillatory level, GABA slightly reduced the frequency of slow oscillations but increased their amplitude, making them more pronounced. In contrast, it strongly enhanced fast oscillatory activity by increasing frequency and active time. Oscillation duration was shortened in a protocol-dependent manner.

Network analyses revealed that GABA promotes stronger synchrony and integration among beta cells. Connectivity maps under GABA exposure were denser and more homogeneous, with higher node degree, increased clustering, and larger connected components in both fast and slow regimes. Modularity was not significantly affected, but overall network architecture shifted toward greater cohesion. These findings demonstrate that GABA not only regulates single-cell excitability but also reorganizes multicellular dynamics, enhancing coordinated responses.

Our study demonstrates that GABA accelerates activation, increases spontaneous and fast oscillatory activity, and strengthens functional integration in beta cell networks. By reinforcing synchrony and connectivity, GABA emerges as a key physiological modulator of coordinated

islet activity. Given the strong link between disrupted islet synchronization and diabetes, these findings highlight GABA's potential role in maintaining metabolic homeostasis and suggest new avenues for understanding islet dysfunction.

Cancer Physiology

P33: Microglia-to-Astrocyte Connexin 43 Transfer Reveals a New Potential Mechanism for Glial Network Organization

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Neuroinflammation involves dynamic crosstalk between glial cells, ranging from classical paracrine mechanisms to direct cell-to-cell communication mediated by gap junctions (GJs). Accumulating evidence suggests a critical role for connexin 43 (Cx43), the main component of GJs in astrocytes, in maintaining homeostatic functions and in modulating glial responses under pathological conditions. In contrast, its role in microglia is less well-characterized and the molecular mechanism of Cx43-mediated microglia-astrocytes interactions remains poorly understood. Bioinformatic analyses of publicly available RNA-seq dataset revealed that Gja1 expression, the gene encoding for Cx43, emerged among the top differentially expressed genes in LPS-stimulated microglia, suggesting a microglia-specific functional role for Cx43 in neuroinflammatory conditions. To explore this hypothesis, we used human microglia (i.e. HMC3) and astrocyte-like (i.e. CCF-STTG1) cells, genetically modified to express GFP or Cx43-Flag. By using a same-well co-culture system, we observed a redistribution of HMC3-derived Cx43-Flag onto CCF-STTG1, indicating a unidirectional transfer that was not observed by co-culturing CCF-STTG1 Cx43-Flag with HMC3 cells. A contact-free trans-well co-culture system was used to determine whether Cx43 transfer was contact dependent or potentially vesicle-mediated. To evaluate the functional relevance of this phenomenon, we conducted an energetic profiling via Seahorse analyses. We observed that co-cultured cells overexpressing Cx43-Flag exhibited distinct metabolic activity, characterized by increased oxidative phosphorylation, overall showing an improved energetic profile. Collectively, this study uncovers a novel aspect of microglia-to-astrocytes signalling and supports a role for Cx43 in shaping the glia response in the central nervous system.

P34: Guanosine and Inosine Restore Chemosensitivity via MFN2-Driven Mitochondrial Fusion in Glioblastoma

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Glioblastoma (GBM) is the most lethal brain tumour, with limited survival due to resistance to current standard therapies. High heterogeneity makes GBM particularly difficult to treat, resulting in chemotherapy resistance and in a poor prognosis for patients. In this context, metabolic reprogramming has emerged as a key mechanism that GBM exploits to promote cell proliferation and overcome treatment-induced toxicity.
In this study, we investigated metabolic alterations in both chemotherapy-sensitive and -resistant GBM cell lines, showing a significant dysregulation in purine metabolism, with different guanosine and inosine levels between sensitive and resistant cells. Through a mesenchymal-like GBM zebrafish model, we further identified alterations in purine metabolism, exhibiting a marked downregulation in nucleotide catabolic processes. Notably, the combined treatment with guanosine and inosine significantly increased chemotherapy-induced cytotoxicity in resistant cells, pointing to a promising therapeutic strategy. This effect was strongly associated with changes in mitochondrial dynamics; indeed, the combinatorial treatment induced the overexpression of Mitofusin-2 (MFN2), a key regulator of mitochondrial fusion. This shift supported a fused mitochondrial network, contrasting with the fragmented mitochondria typically observed in resistant GBM. Thus, promoting mitochondrial fusion through purine metabolites treatment improved chemosensitivity in GBM cells and may contribute to a more favourable prognosis.
Our findings reveal that purine metabolism plays a critical role in GBM therapy resistance and that guanosine and inosine treatment increase chemotherapy efficacy by modulating mitochondrial dynamics and promoting mitochondrial fusion. Targeting these interconnected pathways may offer a potential therapeutic strategy to overcome resistance and improve therapeutic efficacy in GBM.

P35: Impact of Cancer Therapies on IL-6 Signalling during Myogenic Differentiation *In Vitro*

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Electrochemotherapy (ECT) is a clinically established cancer treatment that combines the administration of cytotoxic drugs with the application of electric pulses to enhance drug uptake, primarily used for cutaneous, subcutaneous, and select internal tumours. Radiation therapy (RT), another cornerstone of cancer treatment, utilizes high-energy ionizing radiation to induce DNA damage in tumour cells. Skeletal muscle, often located adjacent to tumour sites, is frequently exposed to both ECT and RT. However, the side effects of ECT and RT on skeletal muscle myokines have not yet been thoroughly investigated. Therefore, the aim of this study was to examine the effects of ECT in a murine muscle cell line (C2C12) and RT in a human muscle cell line on the expression of interleukin-6 (IL-6) during *in vitro* myogenesis. Electrochemotherapy was performed by exposing cells in suspension to cytotoxic drugs (bleomycin and cisplatin), followed by the application of short, high-voltage electric pulses to cells, which were placed between two electrodes. These pulses transiently increase cell membrane permeability, significantly enhancing the intracellular uptake of the drugs and thereby improving their cytotoxic efficacy. Human skeletal muscle myoblasts, obtained from muscle biopsies, were cultured and irradiated using a Darpac 2000 X-ray unit at doses of 4, 6, and 8 Gy.

ECT with bleomycin and cisplatin reduced the viability of C2C12 myoblasts and myotubes in a dose-dependent manner, with myoblasts exhibiting greater sensitivity than myotubes. A significant increase in IL-6 secretion observed three days after ECT confirms its impact on early myogenesis. In contrast, ECT had only minor effects on differentiated myotubes, supporting its safety profile in the context of ECT treatment.

Similarly, the long-term effects of ionizing radiation in human skeletal muscle myoblasts *in vitro* showed a dose-dependent reduction in proliferation, with significant decreases observed three days post-irradiation compared to non-irradiated controls. Lower doses (4 and 6 Gy) suppressed constitutive IL-6 secretion, indicating impaired early myogenic signalling.

To our knowledge, these are the first and only studies to investigate the effects of cancer therapy on the concentration of muscle derived (IL-6). Using both the C2C12 cell line and primary human skeletal muscle cells, the studies provide novel insights into how different therapeutic modalities, ECT and RT affect IL-6 signalling. Given the pivotal role of IL-6 in muscle regeneration and inflammatory responses, these findings are particularly significant for understanding the potential side effects of cancer treatments on skeletal muscle homeostasis. Further research is required to address the discrepancies observed between animal and human cell line responses.

P36: The Regulation of Metallothionein Expression in Breast Cancer

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Breast carcinoma is the most common malignant disease in the female population and one of the leading causes of death among women worldwide. Hormone receptor-positive carcinomas are the less malignant and have a better clinical prognosis, whereas HER-2 positive and triple negative carcinomas are more aggressive with greater malignancy and a poorer prognosis. Metallothionein is the main protein that delivers the tissue metal, especially zinc and is supported to be a new predictive factor for breast cancer.
We conduct an observational retrospective study, processing and analysing patient tissue samples from the archives of the Department of Pathology and Pathological Anatomy (University Hospital Rijeka). The onset of analysis began, with previously immunophenotyped tissue biopsies of the various groups of carcinomas (Luminal A, Luminal B HER2-, Luminal B HER2+, HER2+, triple negative), using "tissue microarray" (TMA) and later, immunohistochemistry staining of these samples was be performed (using metallotionein monoclonal antibodies). After that the number of metallothionein positive cells was quantified microscopically and the statistical analysis was done using Student-t test. These results were compared with clinical data associated with the course and outcome of the disease.
We have found the lowest metallothionein expression in the Luminal A and Luminal B HER2- breast cancers group, the highest metallothionein expression was in HER2+ and triple negative breast cancers group among the molecular subtypes. The statistical analysis of the results has shown the statistical significance difference among the metallothionein expression in the HER-2 positive and triple negative breast cancers compared to hormonal positive breast cancers (luminal A group) – $p<0,05$. The women in the group of HER-2 positive and triple negative breast cancers had poor prognosis (short time to metastasis and recurrence of the disease) when compared to the group of luminal A breast cancers that had better clinical outcome.
The goal of this study was to show the difference in the expression of metallothionein among the different groups of breast carcinomas and to incorporate the obtained results with the clinical course and disease outcome in order to gain a better understanding of the pathological behaviour of various breast cancer immunophenotypes.

This work has been fully supported by the University of Rijeka under the project number (uniri-iskusni-prirod-23-93).

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