4th Regional Congress of Physiological Societies
and
6th Congress of Croatian Physiological Society

PROGRAMME and BOOK OF ABSTRACTS

October 14 – 16, 2026, Osijek, Croatia

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Hungarian Physiological Society

Slovenian Physiological Society

Faculty of Medicine Osijek

School of Medicine, University of Zagreb

Faculty of Agrobiotechnical Sciences Osijek

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Venue:

Faculty of Agrobiotechnical Sciences Osijek,
University J. J. Strossmayer Osijek,
Vladimira Preloga 1, 31000, Osijek

Contact:

Congress organization board (prof. dr. sc. Ines Drenjančević)
info@hdf.hr

Please note: ***On-site registration will be possible.***

All oral scientific sessions will be in AULA MAGNA, second floor;
All coffee breaks, poster sessions, presenting sponsors and social program will be in Educational-presentation Atrium, ground floor

PRE-CONGRESS WORKSHOP

Funkcionalna dijagnostika kardiorespiratornog sustava I funkcionalnih sposobnosti (spiroergometrija, CPET)

– radionica –

8:30 – 9:15 Teoretska izlaganja

(Seminarska učionica S1 - prizemlje, MEFOS)

1. **Energetski kapaciteti na razini stanice i mišićnog vlakna (Ines Drenjančević)**

2. **Spiroergometrija, CPET (eng. cardiopulmonary exercise testing) (Vladimir Ivančev)**
   - princip rada
   - parametri koje mjerimo
   - interpretacija rezultata

3. **Zdravstvene indikacije i kontraindikacije kod izvođenja testova maksimalnog opterećenja (Ana Stupin)**

4. **Priprema ispitanika i postupaka, mjere opreza i postupci pri izvođenju testova opterećenja (Ivana Jukić)**

9:30 – 11:00 Praktični dio (Vladimir Ivančev i Petar Šušnjara)

(Laboratorij za fiziologiju cirkulacije i kliničku fiziologiju MEFOS, podrum)

1. Izvođenje testa maksimalnog opterećenja na pokretnom sagu
2. Praćenje parametara od interesa
3. Interpretacija rezultata
4. Pisanje završnog nalaza i preporuka

Napomena: Radionica se održava na hrvatskom jeziku. Radionica je besplatna za sudionike kongresa i pokrivena je kotizacijom. Za ostale sudionike, kotizacija je 100 eura.

Prijave: info@hdf.hr **do 13.10.2026.**

Broj polaznika ograničen je na 12 i na principu najbržeg prsta.

CONGRESS PROGRAMME

Wednesday, 14 October 2026

11:00 - 13:00 Registration and Welcome Coffee, Atrium, ground floor

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13:00 - 13:05 Welcome (Aula Magna)

SESSION 1 — KEYNOTE & IMMUNOPHYSIOLOGY

Chairpersons: **Ines Mrakovčić Šutić** (Faculty of Medicine University of Rijeka, Croatia), **Ivan Ćavar** (University of Mostar School of Medicine, Mostar, Bosnia and Herzegovina))

13:10 - 13:45 Bojan Polić (Faculty of Medicine University of Rijeka, Rijeka, Croatia): *The role of IFNg in systemic body adaptations to acute viral infections. KEYNOTE LECTURE* (35 min)

13:45 - 14:00 Dora Višnjić (University of Zagreb School of Medicine, Zagreb, Croatia): *From Metabolic Stress to Cell Cycle Arrest and Differentiation. INVITED LECTURE* (15)

14:00 - 14:15 Ivan Ćavar (University of Mostar School of Medicine, Mostar, Bosnia and Herzegovina):
Serum concentrations of cytokines and PSA in patients with prostate adenocarcinoma. INVITED LECTURE (15*)*

14:15 - 14:25 Vilma Dembitz (University of Zagreb School of Medicine, Zagreb, Croatia):
Plasma Triglycerides Associate with Sensitivity to Stearoyl-CoA Desaturase Inhibition in Acute Myeloid Leukemia. ORAL COMMUNICATION (10)

14:25 - 15:00 (35 min)

Coffee Break and Discussion, Atrium, ground floor

SESSION 2 — NEUROSCIENCE AND CEREBRAL PHYSIOLOGY

Chairpersons: **Robert Zorec** (Faculty of Medicine University of Ljubljana, Slovenia), **Marija Renić** (University of Zagreb School of Medicine, Zagreb, Croatia)

15:00 - 15:15 Marija Renić (University of Zagreb School of Medicine, Zagreb, Croatia): *Physiology of Cerebral Blood Flow INVITED LECTURE (15)*

15:15 - 15:30 Robert Zorec (**Slovenian Physiological Society,** University of Ljubljana, Ljubljana, Slovenia):
Adrenergic Regulation of Astrocytes in Health and Disease. INVITED LECTURE (15)

15:30 - 15:45 Nina Vardjan (**Slovenian Physiological Society,** University of Ljubljana, Ljubljana, Slovenia):
Lipid Droplets and Signalling in Astrocyte Stress Response. INVITED LECTURE (15)

15:45 - 15:55 Vedrana Karan Rakić (Faculty of Medicine University of Novi Sad, Novi Sad, Serbia):
Evaluation and Reliability of SSEP and MEP in the Neurophysiological Assessment of Patients with Myelopathy. ORAL COMMUNICATION (10)

15:55 - 16:05 Anemari Horvat (Slovenian Physiological Society, University of Ljubljana, Slovenia):
Elevated K^+ Triggers Glycolytic Lactate Mobilisation Contributing to Astrocyte Swelling. ORAL COMMUNICATION (10)

16:05 - 16:15 Antonio Markotić (University of Mostar School of Medicine, Mostar, Bosnia and Herzegovina):
Effects of "Prime Energy" drink on cognitive and psychomotor functions. ORAL COMMUNICATION (10)

16:15 - 16:30 Pause and Discussion

SESSION 3: ION CHANNELS AND DISEASE

Chairpersons: **Balázs István Tóth** (Institute of Physiology, Medical School, University of Pécs, Pécs, Hungary), **Hrvoje Lalić** (University of Zagreb School of Medicine, Zagreb, Croatia)

16:30 - 16:45 Balázs István Tóth (Institute of Physiology, University of Pécs Medical School, Pécs; and Department of Physiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary):
TRP channels in human dental pulp-derived cells and their role in pulpitis. INVITED LECTURE (15)

16:45 - 17:00 Aleksandar Kibel (Faculty of Medicine Osijek, Priora International Čepin, Croatia)
The role of SGLT2 inhibitors in the treatment of heart failure. INVITED LECTURE (15)

17:00 - 17:15 Erika Lisztes (Department of Physiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary):
Role of TRPV4 in dermal skin regeneration. INVITED LECTURE (15)

17:15 - 17:30 Viktória Kormos (Department of Pharmacology and Pharmacotherapy, Medical School University of Pécs, Pécs, Hungary):
Examination of Edinger-Westphal nucleus-specific TRPA1 cKO mice in migraine. INVITED LECTURE (15)

17:30 - 17:45 Miklós Kecskés (Institute of Physiology, Medical School University of Pécs, Pécs, Hungary):
Role of TRPM3 in epilepsy. INVITED LECTURE (15)

17:45 - 18:00 Zrinka Mihaljević (Faculty of Medicine Osijek, Osijek, Croatia):
Hyperbaric Oxygenation as a Modulator of Endothelial Function: Insights into Redox and Vasodilatory Pathways. INVITED LECTURE (15)

18:00 - 18:30 Discussion

19:00 - 21:00 Welcome Reception and music program – Atrium, ground floor

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Thursday, 15 October 2026

SESSION 4 — VASCULAR PHYSIOLOGY, MICROCIRCULATION & ENDOTHELIAL FUNCTION (PART I)

Chairpersons: **Helena Lenasi** (Slovenian Physiological Society, Faculty of Medicine University of Ljubljana, Slovenia), **Ana Stupin** (Faculty of Medicine Osijek, Osijek, Croatia)

08:30 - 08:45 Aleksandra Dugandžić (University of Zagreb School of Medicine, Zagreb, Croatia):
Different roles of brown adipose tissue and brain uroguanylin in males and females. INVITED LECTURE (15)

08:45 - 09:00 Nataša Popović (University of Montenegro, Podgorica, Montenegro):
The Retina as a Window to Systemic Physiology: Translating Physiological Signals into Digital Biomarkers. INVITED LECTURE (15)

09:00 - 09:10 Nikolina Kolobarić (Faculty of Medicine Osijek, Croatia):
α-Linolenic Acid and Vascular Endothelial Senescence: From Previous Findings to Current Research. ORAL COMMUNICATION (10)

09:10 - 09:25 Akos Koller (Semmelweis University, Budapest, Hungary, New York Medical College, Valhalla, NY, USA):
The molecular mechanisms reflect the different flow-dependent responses of small extra- and intracerebral arteries. INVITED LECTURE (15)

09:25 - 09:40 Vladimir Ivančev (Faculty of Kinesiology, School of Medicine University of Split, Croatia):
Physiological health predictors – health as prediction of functionality or functionality as a health predictor. INVITED LECTURE (15)

09:40 - 9:55 Dragan M. Mirkov (Faculty of Sport and Physical Education, University of Belgrade, Serbia):
Neuromechanics of the Countermovement Jump: From Neural Drive to Take-Off Velocity. INVITED LECTURE (15)

9:55 - 10:25 Discussion

10:30 - 11:20 (50 min)

Coffee Break & Poster Session I (P1-P21, Totem A and Totem B, parallel sessions) Atrium, ground floor

Chairs Totem A: **Nikolina Kolobarić** (Faculty of Medicine Osijek, Croatia), **Vilma Dembitz** (University of Zagreb School of Medicine, Zagreb, Croatia);

Chairs Totem B: **Ana Shek Vugrovečki** (Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia), **Filip Sedlić** (University of Zagreb School of Medicine, Zagreb, Croatia, Virtual Research Centre All4Life)

SESSION 5 — VASCULAR PHYSIOLOGY, MICROCIRCULATION & ENDOTHELIAL FUNCTION (PART II)

Chairpersons: **Akos Koller** (Semmelweis University, Budapest, Hungary, New York Medical College, Valhalla, NY, USA), **Danilo Radunović** (University of Montenegro, Podgorica, Montenegro)

11:30 - 11:45 Bari Ferenc (University of Szeged, Szeged, Hungary):
Nimodipine reduces microglial activation as shown by morphological phenotype, phagocytic activity and next generation RNA sequencing. INVITED LECTURE (15)

11:45 - 12:00 Helena Lenasi (University of Ljubljana, Ljubljana, Slovenia):
Impact of thyroid dysfunction on (micro)vascular and autonomic nervous system (re)activity. INVITED LECTURE (15)

12:00 - 12:10 Danilo Radunović (University of Montenegro, Podgorica, Montenegro):
Molecular basis of pulse wave velocity (PWV) and its clinical significance. ORAL COMMUNICATION (10)

12:10 - 12:20 Vladimir Prelević (University of Montenegro, Podgorica, Montenegro):
Chronic kidney disease as a model of early vascular ageing. ORAL COMMUNICATION (10)

12:20 - 12:30 Danijel Pravdić (University of Mostar School of Medicine, Mostar, Bosnia and Herzegovina):
The role of mitochondria in cardioprotection. ORAL COMMUNICATION (10)

12:30 - 12:45 Filip Sedlić ((University of Zagreb School of Medicine, Zagreb, Croatia), Virtual Research Centre All4Life):
Human myocardial miRNA expression in heart failure. INVITED LECTURE (15)

12:45 - 13:00 Discussion

SESSION 6 — AI, BIOMEDICAL SIGNALS & TRANSLATIONAL TECHNOLOGIES

Chairpersons: **Igor V. Pantić** (University of Belgrade, Faculty of Medicine Belgrade, Serbia)), **Mirza Žižak** (University of Zagreb School of Medicine, Zagreb, Croatia)

13:00 - 13:30 Mirza Žižak (University of Zagreb School of Medicine, Zagreb, Croatia):
AI -Supported Flipped Classroom approach: Transforming the role of teachers in medical education. INVITED LECTURE (30)

13:30 - 13:45 Igor V. Pantić (University of Belgrade, Faculty of Medicine Belgrade, Serbia): *A Fractal-Feature Neural Network for Quantitative Analysis of Two-Dimensional Physiological Signals. INVITED LECTURE (15)*

13:45 - 13:55 Béla Z. Debreczeni (Doctoral School, Semmelweis University and DrD Plastic Surgery Studio Budapest, Hungary):
Microcirculatory and immunohistopathological assessment of the outcomes of different nipple–areola complex reconstructive surgeries. ORAL COMMUNICATION (10)

13:55 - 14:10 Pero Lučin (Faculty of Medicine Rijeka, Croatia):
AI-Assisted Longitudinal Single-Cell Profiling of Murine Cytomegalovirus Infection by Correlative Digital Holotomographic and Fluorescence Microscopy. INVITED LECTURE (15)

14:10 - 14:20 Carmen Rožmanić (Faculty of Medicine, University of Rijeka, Croatia):
Extracellular Vesicle Profiling in Human Cerebrospinal Fluid Following Traumatic Brain Injury: Automated Size-Exclusion Chromatography Coupled with Single-EV Microflow Cytometry. *ORAL COMMUNICATION (10)*

14:20 - 14:30 Ines Mrakovčić-Šutić (Faculty of Medicine University of Rijeka, Croatia):
Cross-talk between matrix-metalloproteinases and granulysin and perforin-mediated cytotoxicity in malignancies and environmental diseases. *ORAL COMMUNICATION (10)*

14:30 - 14:40 Discussion

14:40 - 15:15 (35 min) Lunch Break, Atrium, ground floor

SESSION 7 – POSTTRAUMATIC STRESS DISORDER: FROM BENCH TO BEDSIDE (session organized by Hungarian Physiological Society)

Chairpersons: **Dora Timea Zelena** (University of Pecs, Institute of Physiology, Pecs, Hungary) and **Nikola Habek** (Faculty of Medicine Zagreb, Croatia)

15:15 - 15:30 Dubravka Švob-Štrac (Institute Rudjer Boskovic, Zagreb, Croatia):
Title: The Biology of Trauma: Searching for PTSD Biomarkers. INVITED LECTURE (15)

15:30 - 15:45 Dóra Timena Zelena (University of Pécs, Institute of Physiology, Pécs, Hungary):
Title: Metabolic consideration of PTSD. INVITED LECTURE (15)

15:45 - 16:00 Ana Havelka Meštrović (Algebra Bernays University, Zagreb, Croatia):
Title: Psychophysiological studies in combat PTSD. INVITED LECTURE (15)

16:00 - 16:15 Viktória Kormos (University of Pécs, Department of Pharmacology and Pharmacotherapy, Pécs, Hungary):
Title: Transient receptor potential ankyrin 1 ion channels may control the stress adaptation and fear conditioning in a mouse model of posttraumatic stress disorder. INVITED LECTURE (15)

16:15 - 16:30 Steering board meeting Croatian Physiological Society

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16:30 - 17:15 Annual meeting of General Assembly of Croatian Physiological Society (members of CPS only)

17:15 Free afternoon - a guided tour of the historic Tvrđa district and Osijek city centre - meeting at the reception desk with a guide

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19:00 - 23:00 Conference Dinner
(Restaurant Projekt 9, Gornjodravska obala bb, 31000, Osijek)

Friday, 16 October 2026

SESSION 8 — EXPERIMENTAL MODELS, EDUCATION & ETHICS

Chairpersons: **Vladiana Crljen** (University of Zagreb School of Medicine, Zagreb, Croatia), **Ivana Jukić** (Faculty of Medicine Osijek, Croatia)

08:30 - 08:45 Mario Ostović (Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia):
Using animals in biomedical research: Welfare issues and alternatives. INVITED LECTURE (15)

08:45 - 09:00 Ivan Alić (Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia):
In vitro models in biomedical research. INVITED LECTURE (15)

09:00 - 09:10 Ana Shek Vugrovečki (Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia):
Are Non-Animal Models Effective in Veterinary Education? Insights from Our Teaching Practice. ORAL COMMUNICATION (10)

09:10 - 09:20 Vladiana Crljen (Faculty of Medicine Zagreb, Croatia):
Ethical approach to handling laboratory rodent. ORAL COMMUNICATION (10)

09:20 - 09:30 Stefani Fruk Šimunec ((Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia):
Changes in Beta-Hydroxybutyrate, Non-Esterified Fatty Acids and Glutamate Dehydrogenase During the Periparturient Period in Saanen Goats. ORAL COMMUNICATION (10)

09:30 - 10:30 (60 min)

Coffee Break & Poster Session I (P22-P42, Totem A and Totem B, parallel sessions) Atrium, ground floor

Chairs Totem A: **Nina Vardjan** (Slovenian Physiological Society, University of Ljubljana, Ljubljana, Slovenia), **Jovana Paunović Pantić** (University of Belgrade, Faculty of Medicine Belgrade, Serbia)

Chairs Totem B: **Zrinka Mihaljević** (Faculty of Medicine Osijek, Croatia), **Danijel Pravdić** (University of Mostar School of Medicine, Mostar, Bosnia and Herzegovina)

SESSION 9 – MICROBIOTA

Chairs: **Ines Drenjančević** (Faculty of Medicine Osijek, Croatia), **Vladimir Prelević** (University of Montenegro, Podgorica, Montenegro)

10:35 - 11:05 Janoš Terzić (Faculty of Medicine Split, Croatia):
The role of microbiota and inflammation in urinary bladder cancer. *PLENARY LECTURE (30 + 10 discussion)*

SESSION 10 - FROM IMMUNOBIOLOGY OF PSORIASIS TO CLINICAL DISEASE CONTROL: IL-23 BLOCKADE WITH RISANOKIZUMAB AND THE POTENTIAL OF DIETARY INTERVENTIONS

Chairpersons: **Melita Vukšić Polić** (Faculty of Medicine Osijek, Clinical Hospital Centre Osijek, Croatia), **Martina Mihalj** (Faculty of Medicine Osijek, Clinical Hospital Centre Osijek, Croatia)

11:20 - 12:30

Martina Mihalj (Clinical Hospital Centre Osijek and Faculty of Medicine Osijek, Croatia):
Sodium Chloride as an Environmental Modifier of Th17 Immunity in Psoriasis Vulgaris.

Ivana Krajina (Clinical Hospital Centre Osijek and Faculty of Medicine Osijek, Croatia):
***IL**-23 as the Master Regulator in Psoriasis: The Role of Dietary Intervention*.

Vera Plužarić (Clinical Hospital Centre Osijek and Faculty of Medicine Osijek, Croatia):
Targeting IL-23 in Psoriasis: Immunologic Rationale for IL-23 Blockade and Dietary Modulation of the Inflammatory Circuit.

Stana Tokić: (Faculty of Medicine Osijek, Croatia):
γδ T and MAIT T Cell Repertoire and Functional Remodeling in Psoriasis.

12:30 - 13:00 (30 min) ***Lunch Break***

SESSION 11 — CELL PHYSIOLOGY, IMMUNOLOGY & TRANSLATIONAL RESEARCH (part I)

Chairpersons: **Maja Miškulin** (Faculty of Medicine Osijek, Croatia), **Dora Višnjić** (Faculty of Medicine Zagreb, Croatia)

13:00 - 13:15 Markus Ritter (Paracelsus Medical University, Salzburg, Austria):
On the manifold functions of the non-gastric H^+/K^+-ATPase ATP12A. INVITED LECTURE (15)

13:15 - 13:30 Aleksandra Popović (University of Novi Sad, Novi Sad, Serbia):
Functional Analysis of Mitochondria in Permeabilized Muscle Fibers, Tissues, and Cultured Cells. INVITED LECTURE (15)

13:30 - 13:40 Ivo Krešić (University of Mostar School of Medicine, Mostar, Bosnia and Herzegovina):
Monocyte functional heterogeneity in psoriatic arthritis and ankylosing spondylitis. ORAL COMMUNICATION (10)

13:40 - 13:50 Pavao Planinić (University of Mostar School of Medicine, Mostar, Bosnia and Herzegovina):
Bone biology in murine models of cholestatic liver injury. ORAL COMMUNICATION (10)

13:50 - 14:05 Jelena Nesovic Ostojic (Faculty of Medicine, University of Belgrade, Serbia):
Hyperbaric Oxygen and Acute Physical Activity: Organ-Specific Effects on Redox Status and Elemental Homeostasis. INVITED LECTURE (15)

14:05 - 14:15 Maja Miškulin (Faculty of Medicine Osijek, Croatia):
Vitamin B_{12} and Depressive Symptoms: A Cross-Sectional Study Among Croatian Older Adults. ORAL COMMUNICATION (10)

SESSION 12 — CELL PHYSIOLOGY, IMMUNOLOGY & TRANSLATIONAL RESEARCH (part II)

Chairpersons: **Ines Mrakovčić Šutić** (Faculty of Medicine Rijeka, Croatia), **Dora Višnjić** (Faculty of Medicine Zagreb, Croatia)

14:20 - 14:35 Jovana Paunović Pantić (University of Belgrade, Faculty of Medicine Belgrade, Serbia):
Recent Advances in Laser-Based Technologies for Tissue Regeneration. INVITED LECTURE (15)

14:35 - 14:50 Vlatka Sotošek (University of Rijeka, Rijeka, Croatia):
Endothelial glycocalyx in health and disease. INVITED LECTURE (15)

14:50 - 15:05 Sanjin Kovacevic (Faculty of Medicine University of Belgrade, Serbia):
Hyperbaric Oxygen Preconditioning in Experimental Acute Kidney Injury: Current Findings and Future Perspectives. INVITED LECTURE (15)

15:05 - 15:15 Ida Ivek (Faculty of Medicine University of Zagreb, Croatia):
Inhibitory Receptor Expression and T-Lymphocyte Recovery After Chimeric Antigen Receptor T-Cell or Bispecific Antibody Therapy: A Within-Subject Longitudinal Study. ORAL COMMUNICATION (10)

15:15 - 15:30 Helena Haque Chowdhury (Faculty of Medicine University of Ljubljana, Slovenia):
Advanced Therapy Medicinal Products Are Transforming the Future of Medicine: Clinical Outcomes and Future Directions of the Cell-Based ATMP aHyc for Prostate Cancer. INVITED LECTURE (15)

15:30 - 15:45 Andraž Stožer (Faculty of Medicine University of Maribor, Slovenia):
From spikes to strikes: Calcium oscillations in beta cells and their changes during development of type 2 diabetes. INVITED LECTURE (15)

15:45 - 16.00 ***Closing Remarks and Awards***

LIST OF POSTERS BY DATES AND POSITIONS

Thursday 15.10.2026 P1. - P11.
Totem A

1. Anemari Horvat*, Ena Begić, Anemari Horvat, Bojana Lazović, Urška Černe, Robert Zorec, Nina Vardjan**.** *Elevated K^+ Triggers Glycolytic Lactate Mobilisation Contributing to Astrocyte Swelling.*

2. Ivan Bagić*, Marko Bagić. *Postoperative C-Reactive Protein Levels After Total Hip Arthroplasty: A Comparison of the Direct Anterior and Anterolateral Approaches.*

3. Marko Bagić*, Ivan Bagić. *Red Cell Distribution Width Differs Between Intracapsular and Extracapsular Proximal Femoral Fractures.*

4. Marta Balog*, Vedran Nemet, Senka Blažetić, Viktoria Čurila, Suzana Matić, Barbara Viljetić, Matija Fenrich, Kristijan Nenadić, Marija Heffer. *Effects of Chronic Aripiprazole and Trazodone Treatment on Lens Opacity and Oxidative Stress in Female Rats.*

5. Klara Bardač*, Alojzija Brčić, Hrvoje Lalić, Vilma Dembitz, Romana Penker, Giovanny Rodriguez Blanco, Dora Višnjić. *Dissecting the Role of Nucleotide Availability in Hydroxyurea-Induced Differentiation of Acute Myeloid Leukemia Cells.*

6. Anja Barić*, Helena Justić, Marina Dobrivojević Radmilović. *Longitudinal Optical Imaging of Fluorescent Glucose Analog Uptake in the Mouse Brain.*

7. Sara Bilić*, Sara Pavičić, Igor Štimac, Marina Marcelić, Gordana Blagojević Zagorac, Pero Lučin, Hana Mahmutefendić Lučin. *Rab11 FIP Proteins are Recruited into the Murine Cytomegalovirus Assembly Compartment.*

8. Alojzija Brčić*, Klara Bardač, Hrvoje Lalić, Vilma Dembitz, Tomislav Smoljo, Romana Penker, Giovanny Rodriguez Blanco, Dora Visnjic. *Nucleotide Homeostasis Determines Differentiation Responses to Replication Stress in Acute Myeloid Leukemia.*

9. Jakov Brozović*, Bruna Baljak, Nikola Habek. *Astrocytic GABA transporter-3 is dynamically regulated during disrupted glutamate–GABA homeostasis.*

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BOOK OF ABSTRACTS

KEYNOTE

Title: The Role of IFNy in Systemic Body Adaptations to Acute Viral Infections

Author(s): Bojan Polić[1]*

Affiliation(s):
[1] Faculty of Medicine, University of Rijeka, Rijeka, Croatia
*Presenting author

Background:
During the acute phase of a viral infection, symptoms such as fever, fatigue, and loss of appetite commonly appear. Although we tend to view these changes as signs of illness, they are actually components of a deeply conserved physiological program orchestrated by the immune system. Our work shows that several of these systemic adjustments enhance the immune system's ability to fight infection more effectively.

Methods:
We employ a broad suite of immunological and metabolic techniques to characterize and functionally assess immune cells both in vivo and in vitro. To uncover the key molecular mechanisms that govern feedback loops between the immune and metabolic systems, we use genetically modified mice and viruses, along with targeted interventions such as monoclonal antibodies.

Results:
Our findings reveal that viral infections such as MCMV, LCMV, and IAV transiently lower serum glucose levels. Splenic γδ T cells produce IFN-γ, which directly drives hyperinsulinemia and consequently reduces circulating glucose. This decrease in glucose availability limits cellular lactate production, enhancing type I interferon induction, a pathway with potent antiviral activity.

During viral infection, NK-cell–derived IFN-γ in visceral adipose tissue promotes lipolysis and elevates serum free-fatty-acid levels. These fatty acids fuel B cells, enhancing their capacity to prime T cells and thereby improving viral control. In addition, our preliminary data in mice indicate that, during the resolution phase of an immune response to viral infection, the body actively lowers its temperature to restrain T-cell activation and limit immune-mediated tissue damage. This process, known as anapyrexia, appears to be triggered by immune sensing of pathogen-free inflammation in peripheral tissues and requires modulation of central thermoregulatory circuits.

Conclusion:
IFN-γ plays a central role in systemic adaptations to viral infection by reallocating energetic resources and by limiting tissue damage through the induction of anapyrexia.

Keywords: *IFNy; viral infection; glucose; lipids; temperature*

KEYNOTE

Title: The Role of Microbiota and Inflammation in Urinary Bladder Cancer

Author(s): Janoš Terzić[1]*

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*Presenting author

Background:

Bladder cancer (BC) is the fourth most common malignancy in men, with an incidence three to four times higher than in women. Beyond sex and age, cigarette smoking is the principal environmental risk factor. Nitrosamines are abundant in cigarette smoke, raising the possibility that the gut microbiome influences BC risk by metabolizing environmental carcinogens.

Methods:

We used the N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN) mouse model of bladder carcinogenesis to examine the role of the gut microbiota in tumor initiation and progression. We assessed the effect of microbiota depletion on tumor development and analyzed the microbial conversion of BBN into its carcinogenic metabolite. We also analyzed human stool samples to measure nitrosamine-converting capacity across individuals, and examined microbiota-dependent signaling through MyD88 and the IL-6/Stat3 axis.

Results:

Depletion of the gut microbiota prevented tumor development. Gut bacteria converted the procarcinogen BBN into the carcinogenic metabolite N-butyl-N-(3-carboxypropyl)nitrosamine (BCPN), identifying microbial procarcinogen activation as a previously unrecognized mechanism of BC initiation. Human stool samples showed substantial interindividual variability in nitrosamine-converting capacity. Beyond initiation, microbiota-dependent signaling via MyD88 and the proinflammatory IL-6/Stat3 axis promoted tumor growth and invasiveness.

Conclusion:

The gut microbiota influences bladder carcinogenesis, driving tumor initiation through metabolic activation of procarcinogens and promoting progression through inflammatory signaling. Because nitrosamines are also present in cigarette smoke, and because microbial conversion capacity varies between individuals, differences in gut microbiota composition may contribute to individual susceptibility to BC.

Keywords: *microbiota; bladder cancer; nitrosamine*

INVITED

Title: Applications of In Vitro Models in Biomedical Research

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*Presenting author

Background:

In vitro models play an important role in modern biomedical research, particularly in neuroscience. One of the major breakthroughs in science was the discovery of induced pluripotent stem cells (iPSCs), cerebral organoids (COs), followed by the generation of region-specific organoids and assembloids. The hypothesis of our research is that organoids can reliably recapitulate key events occurring in the human brain in the context of Down syndrome. To test this hypothesis, we established the following objectives: (1) to generate neurons in a 2D culture system, (2) to generate COs and (3) to generate cortico-striatal assembloids (CSAs).

Methods:

We routinely use several isogenic iPSC lines in which specific genes have been genetically corrected, including DYRK1A, APP, BACE2 and SOD1. COs and CSAs were generated from these cell lines. In addition to organoid models, we differentiated neurons and astrocytes in 2D.

Results:

Our results demonstrated abnormal neuronal morphology in trisomic neurons compared with their isogenic controls, both following differentiation in 2D cultures and in organoid models. Trisomic cells also exhibited a glial shift, characterised by an increased tendency toward glial differentiation. Furthermore, organoids expressed markers corresponding to all six layers of the human cerebral cortex. They also contained different neuronal populations, including GABAergic neurons and interneurons, dopaminergic neurons, and cholinergic neurons. Importantly, trisomic cells exhibited significantly reduced synaptic activity, as well as abnormalities in mitochondrial morphology.

Conclusion:

Our results demonstrate that organoids represent a powerful tool for biomedical research. Furthermore, organoids have great potential to contribute to the reduction of animals. One of the major limitations is the lack of a functional vascular system. Therefore, organoids cannot fully replace a living organ or an entire organism. Ultimately, modern biomedical research requires the complementary use of both in vitro and in vivo models to achieve a comprehensive understanding of the development, function, and pathology of individual organ systems.

Keywords: *iPSCs; organoids; assembloids; neurons*

INVITED

Title: Nimodipine reduces microglial activation as shown by morphological phenotype, phagocytic activity and next generation RNA sequencing

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*Presenting author

Background:

Nimodipine, an L-type voltage-gated calcium channel blocker, is an approved cerebral vasorelaxant. We hypothesized that nimodipine attenuates the pro-inflammatory shift in microglial phenotypes. Here, we investigated in primary microglial cultures and acute live brain slice preparations the effects of nimodipine on microglial morphology and function, as well as on the microglial transcriptomic profile.

Methods:

Live brain slice preparations from C57BL/6 mice and primary microglia cultures from neonatal Sprague Dawley rats were used. Microglia were activated in brain slice preparations by ischemia or in cell cultures with lipopolysaccharide (LPS). The preparations were treated with nimodipine (5–10–20 uM). Microglial morphological phenotypes, phagocytic activity, Iba1 expression and TNF-α levels were evaluated. Total RNA was isolated from microglial monocultures and processed for next generation RNA sequencing.

Results:

Nimodipine reversed microglial activation in ischemia-challenged brain slices, as evidenced by the restoration of a resting morphological phenotype. LPS induced a pro-inflammatory microglial phenotype, affecting the expression of genes associated with cytokine signaling, the complement system, and phagocytosis. LPS increased the transcription of ionotropic purinergic receptors and TRP channels but decreased the expression of voltage- and ligand-gated calcium channels, downregulated RyR and IP3 receptor expression, and increased transcription of the SERCA calcium pump. Nimodipine suppressed amoeboid morphological transformation and phagocytic activity and altered the expression of 110 genes in the direction opposite to that induced by LPS. Of these genes, at least 20 were associated with the microglial immune response, seven with cell adhesion, and two with autophagy regulation.

Conclusion:

The effects of nimodipine extend beyond cerebral vasorelaxation. Nimodipine attenuates microglial activation and modulates the expression of genes involved in Ca^{2+}-dependent intracellular signaling and microglial immune responses. These findings suggest that the therapeutic actions of nimodipine may extend beyond its established vasorelaxant effects.

Keywords: *calcium channel; ischemia; microglia; neuroinflammation; nimodipine*

INVITED

Title: Advanced Therapy Medicinal Products Are Transforming the Future of Medicine: Clinical Outcomes and Future Directions of the Cell-Based ATMP aHyC for Prostate Cancer

Author(s): Helena H. Chowdhury[1,2]*, Simon Hawlina[3,4], Mateja Gabrijel[1,2], Marko Kreft[1,2,5], Matjaž Jeras[2,6], Robert Zorec[1,2]

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Background:

Immunotherapy (IT) has become an integral component of cancer management, with ongoing efforts to optimize its integration into existing treatment protocols, enhance established approaches, and develop novel strategies. The primary goal of cancer IT is to restore immune control by triggering targeted immune responses analogous to those observed during spontaneous tumour rejection. Dendritic cells (DCs) — professional antigen-presenting cells that activate lymphocytes upon encountering foreign or mutated antigens — represent a promising platform for personalized IT.

Methods:

We developed an autologous DC-based immunotherapy in which patient-derived DCs were fused with autologous tumour cells, thereby exposing them to the full repertoire of patient-specific tumour antigens, both known and unknown. The resulting autologous immunohybridoma cells (aHyC) were administered to patients with castration-resistant prostate cancer (CRPC) in a clinical trial.

Results:

aHyC treatment was well tolerated with no serious adverse effects and preserved quality of life. In the treatment group, the NK cell subpopulation $CD56^{bright}CD16^{-}$ remained stable, whereas it increased in the placebo group ($P = 0.004$). The change in $CD56^{bright}CD16^{-}$ NK cells was negatively correlated with survival time in deceased patients ($r = -0.80$, 95% CI 0.95–0.34, $P = 0.005$), suggesting NK cell involvement in the mechanism of action. Median overall survival from first aHyC application was 58.5 months (95% CI 38.8–78.2), with greater efficacy observed in earlier disease stages.

Conclusion:

aHyC immunotherapy is safe, immunologically active, and associated with prolonged survival in CRPC. As a fully autologous approach, it is adaptable to other solid tumours. A clinical trial in triple-negative breast cancer (TNBC) — an aggressive subtype lacking ER, PR, and HER-2 expression, accounting for 15–20% of all breast cancers — is currently in preparation.

Keywords: *dendritic cells; cancer immunotherapy; immunohybridoma; autologous cells; solid tumors; clinical trial*

INVITED

Title: Serum concentrations of cytokines and PSA in patients with prostate adenocarcinoma

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*Presenting author

Background:

Prostate cancer development and progression involve complex interactions among numerous growth factors, hormones, and cytokines. The aim of this study was to assess serum concentrations of selected cytokines and examine their associations with prostate-specific antigen (PSA) levels and disease grade in patients diagnosed with prostate adenocarcinoma.

Methods:

A cross-sectional study was carried out between March 2023 and March 2024 at the Clinic of Oncology, University Hospital Center Mostar, Bosnia and Herzegovina. The study included 50 men with prostate adenocarcinoma. Of these, 28 patients had no confirmed metastatic disease (PC group), whereas 22 patients had metastatic prostate cancer (MPC group). Serum levels of total PSA (tPSA), free PSA (fPSA), and complexed PSA (cPSA) were analyzed using a chemiluminescent microparticle immunoassay. Cytokine concentrations were determined by a bead-based flow cytometric assay.

Results:

Serum concentrations of tPSA, fPSA, and cPSA were higher in patients with metastatic disease compared with those in the PC group. In contrast, serum monocyte chemotactic protein (MCP)-1 concentrations were significantly higher in the PC group than in the MPC group (P=0.008). Among patients without confirmed metastases, interleukin (IL)-10 levels showed a significant correlation with cPSA concentrations. In patients with metastatic prostate cancer, serum levels of IL-1β, tumor necrosis factor (TNF)-α, and IL-23 were significantly associated with disease grade.

Conclusion:

These findings indicate a potential role of MCP-1 in prostate cancer pathophysiology. Among the analyzed cytokines, IL-10 was the only one significantly associated with serum cPSA levels. Furthermore, IL-1β, TNF-α, and IL-23 may represent potentially useful biomarkers associated with disease grade in patients with metastatic prostate cancer.

Keywords: c*ytokines; PSA; prostatic neoplasms; serum; humans*

INVITED

Title: Different roles of brown adipose tissue and brain uroguanylin in males and females

Author(s): Nikola Habek[1,2,3], Martina Ratko[1,2]*, Milan Kordić[4], Marina Dobrivojević Radmilović[1], Siniša Škokić[1], Vladiana Crljen[1,2,3], Martina Tkalčić[5], Anton Mažuranić[5], Pero Bubalo[5], Petar Škavić[5], Aleksandra Dugandžić[1,2,3]*

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*Presenting author

Background:

Obesity and type 2 diabetes mellitus (T2DM) are major burdens on global health today. A novel therapeutic approach could involve increasing postprandial glucose utilization through the activation of brown adipose tissue (BAT). The aim of this study was to determine role of brain uroguanylin (UGN) in sex differences in BAT function.

Methods:

BAT activity was assessed by infrared thermography in wild-type C57Bl/6NCrl mice and UGN KO littermates. The volume of BAT was determined by MRI, while markers of browning were analysed by qPCR. The expression of proUGN in mouse and human brain tissue was determined by ELISA.

Results:

In the human brain, decreased proUGN expression in subjects with obesity was observed only in the male hypothalamus, BA10, and BA11. The effects of centrally applied UGN depend not only on sex, but also on the phase of the estrous cycle. Sex differences in the postprandial regulation of BAT activity in mice may be caused by differential postprandial regulation of hypothalamic proUGN expression, which was observed only in male mice. In young male mice, intranasal administration of a GLP-1 analogue, as possible regulator of UGN expression in the brain, increased BAT activity and decreased hypothalamic proUGN expression only in males. Although chronic UGN administration also increased postprandial BAT activation in both sexes, increased BAT volume was observed only in male mice.

Conclusion:

Due to the different physiological and pathophysiological functions of BAT and its activators, the introduction of brain BAT activators as a therapy for obesity and T2DM must take sex/gender into account.

Keywords: *Estrus vs. diestrus; GLP-1; i.n. vs. i.p. application*

INVITED

Title: Psychophysiology of Posttraumatic Stress Disorder

Author(s): Ana Havelka Meštrović[1]*

Affiliation(s):

[1] University Algebra Bernays

*Presenting author

Background:

Posttraumatic Stress Disorder (PTSD) represents a complex interplay of neurobiological, autonomic, and endocrine dysregulation following exposure to extreme traumatic stress. This study examines core physiological markers of PTSD in a clinical sample of Croatian combat veterans, a population characterized by prolonged and repeated exposure to high-intensity wartime stressors.

Methods:

A clinical cohort of Croatian combat veterans diagnosed with chronic PTSD underwent standardized psychophysiological assessment. Autonomic nervous system activity was evaluated using heart rate variability (HRV) and startle reflex modulation. Endocrine function was assessed through basal cortisol sampling to characterize hypothalamic–pituitary–adrenal (HPA) axis responsiveness. Neurocognitive correlates of hyperarousal were examined through trauma-cue reactivity paradigms.

Results:

Participants demonstrated markedly reduced parasympathetic tone and elevated sympathetic activation, reflected in diminished HRV and exaggerated startle responses. Basal cortisol levels were consistently blunted, indicating chronic HPA axis dysregulation and sustained allostatic load. Trauma-related cues elicited pronounced autonomic reactivity, supporting the presence of conditioned fear circuitry involving amygdala hyperresponsivity and impaired prefrontal regulatory control.

Conclusion:

Croatian combat veterans with chronic PTSD exhibit a distinct psychophysiological profile characterized by autonomic imbalance, endocrine dysregulation, and heightened cue-induced hyperarousal. These findings reinforce contemporary neurobiological models of persistent threat sensitivity and impaired top-down regulation. Integrating physiological assessment into clinical practice may enhance diagnostic precision, support individualized treatment planning, and improve long-term outcomes for combat-related PTSD.

Keywords: *psychophysiology; PTSD; fear response*

INVITED

Title: Physiological health predictors – health as prediction of functionality or functionality as a health predictor

Author(s): Vladimir Ivančev[1]*

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*Presenting author

Background:

Following the definition of health and functionality and their impact on longevity, a question arises as of which tools, methods and findings can be more specific, sensitive and finally useful to make conclusions and decisions about our health, functionality and longevity. There are many methods (physiological, biochemical etc.) but constant existing point is availability and costs. Some methods such as cardiopulmonary exercise testing (CPET) are available, but the question is are their results really presentable and do they precisely show the real cell health and functional status. Other tests of some motoric abilities (agility, balance, coordination, flexibility, accuracy, speed, strength, power) provide extra data about our functionality, but all of it are external presentation of our body function, and question is how we can measure internal body, organ, tissue, and cell response as a presentation of the real picture of our functionality.

Methods:

CPET is an excellent method to present aerobic capacity through cardiovascular and respiratory parameters. Via these parameters we can calculate energy expenditure, the kind and the amount of aerobic and anaerobic energy production, working zones related to thresholds. CPET inaccuracy comes from a fact that we measure gas exchange at the body surface, not directly into working muscles and tissues, but it could be appropriate estimation method as a measure of cardiorespiratory fitness. Measuring mitochondrial health via oxidative phosphorylation enzymes activity can be useful.

Results:

Level of NAD^+ and nicotinamide phosphoribosyltransferase (NAMPT) could be useful as markers of mitochondrial aerobic activity. VO_2max, respiratory metabolic thresholds are used as very valuable parameters presenting functional cardiorespiratory and aerobic capacity.

Conclusion:

A positive relationship between health, functionality and possible longevity could be a valuable screening marker for population regarding ''healthy life style'' and useful recommendation or a prognostic parameter.

Keywords: *cardiopulmonary fitness; aerobic capacity; oxidative phosphorylation; functionality*

INVITED

Title: Neuromechanics of the Countermovement Jump: From Neural Drive to Take-Off Velocity

Author(s): Dragan M. Mirkov[1]*

Affiliation(s):

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*Presenting author

Background:

Contemporary countermovement jump (CMJ) testing yields dozens of kinetic, kinematic, and performance variables from force platforms and, increasingly, markerless video. The central problem is no longer how much can be measured, but how to interpret those measurements. CMJ performance reflects neuromuscular force-generating function expressed through a particular movement strategy; therefore, measured variables occupy different levels of the same neuromechanical chain and should not be treated as independent physiological qualities.

Methods:

This lecture develops an instrument-agnostic framework linking neural activation, muscle-tendon force production, and intermuscular coordination to movement strategy, mechanical output, and jump performance, using Newtonian mechanics and the impulse-momentum and work-energy theorems to separate mechanically determined quantities from physiological inference. The framework is illustrated by two laboratory studies: reliability and principal component analysis of 45 CMJ-derived metrics in 79 participants, and concurrent validity of net propulsive force across force plates, optical motion capture, and iPhone-based markerless pose estimation.

Results:

Of 45 candidate metrics, 24 were sufficiently reliable, and four principal components explained 91.8% of their variance, indicating substantial statistical redundancy. Mechanically, movement strategy shapes how force-generating capacity is expressed through the ground-reaction-force profile, whose net impulse determines take-off velocity and jump height. Net propulsive force agreed closely across force plates and optical motion capture when derived via the impulse-momentum equation (ICC up to 0.97), but not when the same optical system used a flight-time-based equation instead: agreement tracked the governing equation, not the recording instrument. Neither system, however, can uniquely decompose mechanical output into neural, muscular, tendon, and coordination contributions.

Conclusion:

CMJ assessment should move from a flat catalog of metrics toward a causal interpretive hierarchy in which force-generating capacity and movement strategy jointly determine mechanical output, and hence performance outcome. Its practical value lies in reducing redundancy, assigning each variable a mechanistic meaning, and defining the limits of legitimate diagnosis.

Keywords: *countermovement jump; neuromechanics; movement strategy; concurrent validity; diagnostic framework*

INVITED

Title: Role of TRPM3 in epilepsy

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Background:

TRPM3 is best known for its role as a peripheral sensor of pain and heat. Recent studies reporting gain-of-function TRPM3 mutations in patients with neurodevelopmental and epilepsy-associated disorders suggest a previously unrecognized role of this channel in the brain. However, the function of TRPM3 in neurons of the brain remain largely unexplored.

Methods:

Using a battery of *in vivo* and *in vitro* electrophysiological and histological experiments we studied the effect of TRPM3 channel on amygdala related functions.

Results:

Here, we identify TRPM3 expression in excitatory neurons of the lateral nucleus of the amygdala (LA), a brain region critical for fear learning and implicated in neuropsychiatric and epileptic disorders. We show that TRPM3 is functionally active in LA neurons and regulates their intrinsic electrophysiological properties. Consistent with this role, genetic ablation of TRPM3 in mice impairs fear memory formation. Moreover, in a kainic acid–induced epilepsy model, loss of TRPM3 reduces seizure severity, whereas pharmacological activation of the channel exacerbates seizure phenotypes. Finally, we demonstrate the presence of TRPM3 transcripts in neurons of the human LA.

Conclusion:

Together, these findings uncover a central role for TRPM3 in amygdala function and identify this ion channel as a modulator of fear memory and seizure susceptibility.

Keywords: *TRPM3; epilepsy; amygdala*

INVITED

Title: The role of SGLT2 inhibitors in the treatment of heart failure

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This invited lecture aims to present the role of SGLT2 (sodium/glucose cotransporter 2) inhibitors as one of the most important treatment modalities in the management of heart failure.

SGLT2 is a protein which facilitates glucose transport and is regulated by sodium ions. It is the major cotransporter involved in glucose reabsorption in the kidneys and thus has a major physiological role. Inhibitors, such as empagliflozin and dapagliflozin, were initially developed as medications for diabetes mellitus, but have since been recognized as one of the most important options for the medical treatment of heart failure (including heart failure with reduced ejection fraction, mildly reduced ejection fraction and preserved ejection fraction). These inhibitors improve cardiovascular outcomes in patients with and without diabetes and some studies implied they show an antiarrhythmic activity. Our understanding of the mechanisms through which SGLT2 inhibitors contribute to the improvement of cardiovascular outcomes is still incomplete and there is a number of potential mechanisms that are in play. Interestingly, there are also studies that suggest that SGLT2 inhibitors may change ion channel activity and electrophysiological characteristics in the myocardium.

Keywords: *SGLT2; empagliflozin; dapagliflozin; heart failure*

INVITED

Title: Flow-Induced Responses and Underlying Molecular Mechanisms Differ Between Pre- and Post-Circle of Willis Arteries

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Background:

The Circle of Willis, described by Thomas Willis in 1664, plays an important role in maintaining a constant blood supply to the brain. In this ring-like network of interconnected arteries, blood flow and pressure are distributed and equalized before the distal intracranial arteries branch to supply the brain within the confined intracranial space. Indeed, recognition of the impact of the closed cranium on cerebral blood flow (CBF), as described by the Monro–Kellie doctrine (~1800), was important for our understanding of cerebral circulation: the total volume of the brain, blood, and cerebrospinal fluid must remain constant, and an increase in one component can occur only at the expense of the others. Thus, we hypothesized that the function and underlying molecular mechanisms of pre- and post-Circle of Willis arteries (pre-CWa and post-CWa, respectively) differ.

Methods:

Changes in the internal diameter of isolated arteries in response to pressure and flow were measured in a pressure-flow chamber by a video-microscopy. The protein expression of key components of the arachidonic acid (AA) pathway—including cyclooxygenases (COX-1 and COX-2), cytochrome P450 hydroxylases (CYP450), thromboxane synthase (TXAS), the thromboxane A_2 (TP) receptor, prostacyclin synthase (PGIS), and the prostacyclin (IP) receptor—as well as neuronal nitric oxide synthase (nNOS) and endothelial nitric oxide synthase (eNOS), was determined by Western blotting in the basilar artery (BA) and middle cerebral artery (MCA) obtained from rats (n = 20). Transcriptome analysis of pre-CWa and post-CWa from rat brains (n = 25) was performed using RNA sequencing.

Results:

Increasing intraluminal pressure elicited constriction in both types of arteries, whereas increases in flow elicited dilation in the basilar artery (BA; pre-CWa). In contrast, increased flow primarily elicited constriction in post-Circle of Willis arteries (post-CWa), represented by the intracranial middle cerebral artery (MCA). In both types of arteries, mediators of flow-dependent responses included arachidonic acid (AA) metabolites and nitric oxide (NO), although their relative contributions differed. Compared with post-CWa, pre-CWa exhibited lower protein expression of COX-1/COX-2 and CYP450, higher expression of PGIS, similar expression of TXAS and nNOS/eNOS, lower expression of TP receptors, and higher expression of IP receptors. Gene expressions associated with canonical vasodilator pathways was higher in pre-CWa, whereas canonical vasoconstrictor pathways were more highly expressed in post-CWa.

Conclusion:

Pressure-induced constriction in both types of arteries may contribute to the autoregulation of cerebral blood flow. Flow-induced dilation of pre-CWa may ensure efficient blood supply to the Circle of Willis, whereas flow-induced constriction of post-CWa may further contribute to CBF autoregulation. Consistent with these physiological responses, differences in gene and enzyme expression between pre- and post-Circle of Willis arteries (extra- and intracranial arteries, respectively) correspond to their distinct flow-induced vasomotor functions.

Keywords: *cerebral blood flow regulation; small cerebral arteries; mechanotransduction; arachidonic acid metabolites; nitric oxide*

INVITED

Title: Examination of Edinger-Westphal Nucleus-specific TRPA1 cKO Mice in Migraine

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Background:

The urocortin 1 (UCN1)-expressing neurons of the centrally projecting Edinger-Westphal nucleus (EWcp) regulate the function of migraine-related brain areas *via* direct urocortinergic connections. In the central nervous system, EWcp/UCN1 neurons co-expresses transient receptor potential ankyrin 1 (TRPA1) cation channel, which has also been linked to migraine. Here we aimed to investigate the site-specific deletion of TRPA1 in the urocortinergic EWcp neurons in a mouse model of migraine.

Methods:

We have constructed an adeno-associated virus vector (AAV) that carries an UCN1 promoter-driven *Cre* recombinase (*Ucn-Cre*-AAV), which was validated with stopflox-td-Tomato mice. The intraperitoneal calcitonin gene related peptide (CGRP) injection model of migraine was implemented and validated using light-dark box and von Frey assays in sham operated floxed *Trpa1* and Trpa1 conditional knock out (cKO) male mice. RNAscope *in situ* hybridization and immunofluorescence were used to examine the *Ucn1* mRNA expression and UCN1 peptide content in the EWcp.

Results:

We proved that *Ucn1-Cre*-AAV was specific to the urocortinergic neurons of the EWcp, moreover it decreased the expression of *Trpa1*. Following CGRP treatment, the pain threshold decreased significantly in both the floxed and cKO genotypes; however, this decrease was significantly greater in the cKO animals than in the floxed mice. In the EWcp, we found higher *Ucn1* mRNA levels in response to CGRP treatment in the control sham-operated group treated with the vehicle; in contrast, this increase was absent in cKO mice. We observed a similar pattern when examining UCN1 peptide levels, where we also found lower peptide levels in CGRP-treated cKO mice compared to sham-operated animals.

Conclusion:

These findings suggest a possible role of central TRPA1 in migraine by regulating UCN1 dynamics in the EWcp. Targeting TRPA1 ion channels through pharmacological interventions may offer a new strategy for migraine treatment.

Keywords: *TRPA1; migraine; CGRPM; EWcp*

INVITED

Title: Hyperbaric Oxygen Preconditioning in Experimental Acute Kidney Injury: Current Findings and Future Perspectives

Author(s): Sanjin Kovacevic[1]*, Aleksandra Nenadovic[1], Milan Ivanov[2], Rada Jeremic[3], Predrag Brkic[3], Maja Zivotic[4], Una Jovana Vujacic[2], Danijela Karanovic[2], Nevena Mihailovic Stanojevic[2], Zoran Miloradovic[2], Jelena Nesovic Ostojic[1]

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Background:

Ischemia–reperfusion injury is a major cause of acute kidney injury (AKI), particularly under hypertensive conditions, where oxidative stress, renal hypoperfusion, inflammation, tubular injury, DNA damage, and maladaptive molecular signaling contribute to functional deterioration. This integrative experimental overview aimed to summarize the renoprotective effects of hyperbaric oxygen (HBO) preconditioning in postischemic AKI induced in spontaneously hypertensive rats.

Methods:

Across a series of experimental studies, spontaneously hypertensive rats were allocated to sham-operated controls (SHAM), untreated postischemic AKI, or AKI preceded by HBO preconditioning. HBO consisted of exposure to 100% oxygen at 2.0 atmospheres absolute for 60 min, twice daily at 12-h intervals over two consecutive days. AKI was induced by right nephrectomy and 45-min atraumatic occlusion of the left renal artery. Renal function, renal hemodynamics, histopathology, oxidative stress, antioxidant defense, tubular injury biomarkers, DNA damage, inflammation, apoptosis-related markers, and hypoxia-adaptive signaling were evaluated.

Results:

Compared with SHAM, AKI markedly impaired renal function, reduced renal blood flow, increased renal vascular resistance, intensified tubular necrosis and cast formation, and increased oxidative, inflammatory, and injury-related markers. HBO preconditioning improved renal function, increased estimated glomerular filtration rate, reduced renal vascular resistance, and attenuated histopathological injury. These functional and structural effects were accompanied by lower plasma lipid peroxidation, reduced renal expression of 4-hydroxynonenal, 3-nitrotyrosine, NGAL, KIM-1, γH2AX(S139), and NF-κB, as well as partial restoration of HO-1 expression. HBO preconditioning also modulated Bax/Bcl-2 signaling, consistent with an effect on apoptosis-related pathways, while markedly increasing renal HIF-1α expression.

Conclusion:

Taken together, these findings indicate that HBO preconditioning exerts a multifaceted renoprotective effect in experimental postischemic AKI, integrating hemodynamic improvement, antioxidant and anti-inflammatory actions, reduced tubular and DNA damage, and activation of adaptive cytoprotective pathways.

Keywords: *hyperbaric oxygen preconditioning; acute kidney injury; ischemia–reperfusion injury; oxidative stress; renal protection*

INVITED

Title: Impact of thyroid dysfunction on (micro)vascular and autonomic nervous system (re)activity

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*Presenting author

Background:

Thyroid hormones profoundly affect the cardiovascular system (CVS) in many intertwined, direct, and indirect ways. Moreover, thyroid diseases are among the most frequent endocrine and metabolic diseases exerting an important impact on the CVS, whose incidence is increasing. The talk will present the interactions between thyroid hormones and microcirculation, potential influences on its regulation mediated by the endothelium and autonomic nervous system, and some recent experimental findings from our studies in patients with hyper- and hypothyroidism.

Methods:

In two independent studies, 25 hyperthyroid patients with Graves disease (GD), 15 hypothyroid patients, and 30 healthy volunteers were enrolled. Using laser Doppler flowmetry, we assessed the reactivity of skin microcirculation at two representative measurement sites, finger pulp and volar forearm, to physiological challenges: postocclusive reactive hyperemia (PORH) for endothelium assessment, and local cooling to provoke the autonomic nervous system. We simultaneously recorded digital artery blood pressure and electrocardiogram, and determined heart rate variability (HRV) by spectral analysis. In GD patients, peak systolic velocity (PSV) of intrathyroid arteries was assessed by ultrasound and correlated with LDF.

Results:

GD patients showed significantly higher resting heart rate, systolic blood pressure, and the LF/HF ratio of HRV, along with an increased LDF response to cooling, suggesting greater sympathetic nervous system activity and altered PORH parameters indicating increased vasodilator capacity. Moreover, PSV was higher and correlated with LDF in GD patients. In hypothyroid patients, the PORH parameters indicated an increased vasodilator capacity compared to healthy controls, contrasting the hypothesis of endothelial dysfunction associated with hypothyroidism. We also observed increased sympathetic reactivity in hypothyroid patients after local cooling.

Conclusions:

Thyroid dysfunction may be a useful model for studying the effects of thyroid hormones on the cardiovascular system; however, further studies are warranted to elucidate the delicate interplay between mechanisms affecting blood (micro)vessels.

Keywords: thyroid; microcirculation; endothelium; laser-Doppler fluxmetry

INVITED

Title: Role of TRVP4 in Dermal Regeneration

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Background:

Transient Receptor Potential Vanilloid 4 (TRPV4) is a thermo- and osmosensitive, chiefly Ca^{2+}-permeable ion channel influencing epidermal barrier formation, hair growth, and sebaceous lipogenesis. However, its physiological role in the dermis remains largely unknown. Therefore, in the current study, we aimed to investigate the regenerative and immunological roles of TRPV4 in primary human dermal fibroblasts (HDFs).

Methods:

Channel functionality was assessed via Fura-2 AM-based intracellular Ca^{2+}-measurements using hyposmotic challenges and the TRPV4 agonist GSK1016790A, alongside the antagonist HC-067047. Cell viability was monitored via MTT assay. To determine TRPV4's immunomodulatory effects, pro-inflammatory cytokine mRNA and protein levels were quantified via RT-qPCR, cytokine array, and ELISA. Extracellular matrix (ECM) remodeling and regenerative potential were evaluated using RT-qPCR, Sirius Red staining, and invasion assays.

Results:

TRPV4 is robustly expressed and functionally active in primary HDFs, as both hyposmotic stimuli and GSK1016790A significantly elevated the intracellular Ca^{2+}-level. Importantly, the effect was completely abolished by the co-administration of HC-067047. Regarding regeneration, TRPV4 activation promoted cell migration, increased matrix metallopeptidase expression, and significantly reduced transforming growth factor (TGF)-β1-induced collagen synthesis, indicating a strong regulatory role in ECM remodeling. Furthermore, GSK1016790A differentially modulated the immune phenotype by down-regulating the mRNA expression of tumor necrosis factor (TNF)-α and up-regulating the "itch-relevant" interleukin (IL)-8 and IL-33. Protein-level analyses confirmed marked elevation of IL-8, IL-33, and CXCL1 secretion upon GSK1016790A-treatment. All observed regenerative and immunomodulatory effects were fully prevented by the co-administration of the TRPV4-specific antagonist, confirming receptor specificity.

Conclusion:

Our findings demonstrate that TRPV4 is functionally active in HDFs and actively shapes their immune phenotype and ECM remodeling. Consequently, TRPV4 emerges as a promising pharmacological target in wound healing disorders as well as in certain pruritic and inflammatory skin diseases.

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Keywords: *TRPV4; human dermal fibroblasts; skin inflammation; wound healing*

INVITED

Title: Hyperbaric Oxygenation as a Modulator of Endothelial Function: Insights into Redox and Vasodilatory Pathways

Author(s): Zrinka Mihaljević[1]*, Andrija Labaj[1], Ivana Jukić[1], Ana Stupin[1], Nikolina Kolobarić[1], Ines Drenjančević[1], Aleksandar Kibel[1,2,3]

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Background:

Hyperbaric oxygenation (HBO_2) profoundly alters vascular oxygen availability and redox homeostasis, but its effects on endothelial function are highly dependent on the exposure protocol. We investigated how acute and intermittent HBO_2 influence endothelial vasodilatory responses and the underlying redox-sensitive mechanisms, with particular emphasis on nitric oxide (NO), cytochrome P450 (CYP450)-derived metabolites, adenosine signaling, and the antioxidant transcription factor NRF2.

Methods:

Vascular reactivity was studied ex vivo in isolated aortic and cerebral arteries from healthy and diabetic Sprague–Dawley rats exposed to acute or intermittent HBO_2. Endothelium-dependent relaxation was assessed in response to acetylcholine and other vasoactive stimuli. Pharmacological approaches were used to evaluate the contribution of NO synthase, CYP450 enzymes, adenosine A1 and A2A receptors, and NRF2. Vascular oxidative stress, antioxidant responses, and vasoactive arachidonic acid metabolites were also investigated.

Results:

Acute HBO_2 increased vascular oxidative stress and impaired endothelium-dependent vasorelaxation, whereas intermittent HBO_2 induced an adaptive response associated with preserved or enhanced vascular reactivity. NO-dependent mechanisms represented an important component of acetylcholine-induced relaxation, while CYP450-derived epoxyeicosatrienoic acids contributed to the regulation of vascular tone. HBO_2 additionally altered vasodilatory responses mediated by angiotensin-(1–7) and adenosine receptors, demonstrating that hyperoxia affects multiple interacting pathways regulating vascular function. Importantly, recent findings indicate that inhibition of NRF2 markedly attenuates vasorelaxation following intermittent HBO_2, providing evidence that NRF2-dependent antioxidant signaling contributes to vascular adaptation to repeated hyperoxic exposure.

Conclusion:

Our findings demonstrate that HBO_2 acts as a potent modulator of endothelial function through coordinated changes in redox homeostasis and vasodilatory signaling. While acute exposure may promote oxidative stress and endothelial dysfunction, repeated exposure appears to activate adaptive antioxidant mechanisms. The identification of NRF2 as an important mediator of this adaptation provides new insight into the mechanisms underlying vascular responses to HBO_2 and highlights the redox–vasodilation interplay as a key determinant of endothelial function.

Keywords: *hyperbaric oxygenation; endothelial function; oxidative stress; NRF2; vasorelaxation*

INVITED

Title: Hyperbaric Oxygen and Acute Physical Activity: Organ-Specific Effects on Redox Status and Elemental Homeostasis

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Background:

Oxidative stress and elemental homeostasis are both influenced by physical activity and hyperbaric oxygen exposure, but their combined effects remain insufficiently characterized. This study investigated whether hyperbaric oxygen (HBO) preconditioning modifies systemic and tissue-specific redox responses and elemental distribution following acute physical activity (PA) in previously untrained rats.

Methods:

Thirty-two male Wistar rats were randomized into four groups (n=8 each): control, PA, HBO, and HBO + PA. HBO comprised four 60-min exposures to 100% oxygen at 2.0 atmospheres absolute, administered twice daily at 12-h intervals over two consecutive days. The final HBO exposure occurred 12 h before sacrifice. PA consisted of a single 60-min swimming session, followed by immediate sacrifice. Hematological, biochemical, redox, and kidney and liver elemental parameters were assessed. Main and interaction effects were analyzed by two-way analysis of variance with Tukey *post hoc* testing.

Results:

Most outcomes showed no significant HBO × PA interaction. HBO increased erythrocyte count, hemoglobin, hematocrit, total protein, albumin, and urea and was associated with lower leukocyte counts, while both HBO and PA reduced lymphocyte counts. Systemic oxidative stress markers were largely unchanged, although catalase activity showed a significant interaction. HBO increased hepatic lipid peroxidation but reduced renal lipid peroxidation, while PA reduced renal total and protein sulfhydryl groups. Elemental changes were tissue-specific. In the kidney, HBO predominantly affected potassium, magnesium, phosphorus, zinc, and lead, whereas PA affected iron and arsenic; sodium and mercury were influenced by both factors. In the liver, both factors affected sodium, HBO affected potassium, and PA affected magnesium. Significant interactions were also detected for renal iron and mercury.

Conclusion:

Short-term HBO and acute PA induced distinct organ-specific changes in redox balance and elemental homeostasis, with most effects occurring independently. These findings highlight tissue-specific adaptive responses to intermittent HBO exposure and acute physical activity

Keywords: *hyperbaric oxygen; physical activity; oxidative stress; elemental homeostasis; redox balance*

INVITED

Title: AI-Assisted Longitudinal Single-Cell Profiling of Murine Cytomegalovirus Infection by Correlative Digital Holotomographic and Fluorescence Microscopy

Author(s): Pero Lučin[1]*, Alen Omerović[1], Ivona Viduka[1], Marina Marcelić[1], Ljerka Karleuša[1], Silvija Lukanović Jurić[1], Hana Mahmutefendić Lučin[1]

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Background:

Murine cytomegalovirus (MCMV) infection induces extensive remodeling of host-cell architecture and formation of a cytoplasmic viral assembly compartment (vAC), where virion maturation and secondary envelopment occur. We hypothesized that vAC development generates characteristic spatial and temporal changes in cellular refractive index (RI) that can be detected and quantified without fluorescent labeling.

Methods:

NIH 3T3 cells were infected at MOI 10 with recombinant mCherry-SCP-MCMV and monitored by correlative digital holotomographic microscopy (DHTM) and fluorescence imaging for up to 72 h. The mCherry-tagged small capsid protein enabled visualization of capsid redistribution from the nucleus to the cytoplasmic vAC. DHTM provided label-free 3D RI maps, from which approximately 40 morphological and biophysical parameters were quantified. More than 5,000 cells were analyzed using CellPose-based segmentation and machine-learning approaches for longitudinal single-cell profiling. RI-defined structures were correlated with EdU-labeled replicated viral DNA, viral tegument protein M25, and glycoprotein M55. Viral genome copy numbers in nuclear, cytoplasmic, and extracellular fractions were quantified by qPCR. Membrane compartments involved in secondary envelopment were investigated using inducible GFP-tagged probes for PI3P, PI4P, and SNX27 and additional organelle markers.

Results:

MCMV-induced cytoplasmic remodeling could be continuously followed from 0 to 72 h post-infection. Development of the vAC was associated with distinct spatial and temporal RI changes detectable independently of fluorescent viral reporters. The temporal evolution of RI-defined remodeling was analyzed in relation to changes in viral genome distribution between nuclear, cytoplasmic, and extracellular compartments measured by qPCR. Single-cell analysis revealed substantial heterogeneity in the timing, magnitude, and progression of these biophysical changes.

Conclusion:

MCMV infection generates a distinct four-dimensional biophysical phenotype that can be resolved by label-free DHTM. Combined with fluorescence imaging, quantitative virological measurements, and AI-assisted single-cell analysis, this approach enables longitudinal characterization of viral assembly and infection-associated cellular heterogeneity.

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Keywords: m*urine cytomegalovirus (MCMV); digital holotomographic microscopy (DHTM); single-cell analysis; viral assembly compartment*

INVITED

Title: Using Animals in Biomedical Research: Welfare Issues and Alternatives

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Animal experimentation has been a cornerstone of biomedical science, contributing to major advances that benefit both human and veterinary medicine. However, the use of animals in research raises significant ethical and welfare considerations, making the protection of animal well-being a fundamental scientific responsibility. This review focuses on welfare issues associated with research animals, including pain, distress and suffering, housing and environmental limitations, handling and restraint concerns, and the impact of experimental procedures. Maintaining high standards of animal welfare is vital not only from an ethical perspective but also for improving the reproducibility, reliability and validity of scientific outcomes by minimizing stress-related physiological and behavioral variability. Therefore, addressing welfare issues requires continued improvement of husbandry practices, enhanced recognition and management of pain, and broader implementation of the principles of Replacement, Reduction and Refinement (3Rs) as essential components of responsible and ethical research. The development of alternative approaches, such as *in vitro* testing, three-dimensional cell cultures, organ-on-a-chip technologies, *in silico* methods, microdosing strategies and advanced imaging techniques, represents an important pathway toward reducing reliance on animal models in biomedical research. Nevertheless, complete replacement of animal experimentation remains challenging in many research areas because complex biological interactions within living organisms cannot yet be fully replicated using current alternatives. Furthermore, ongoing concerns regarding the extent to which findings obtained from animal models can accurately predict human responses highlight the need for careful evaluation of their scientific relevance. Consequently, achieving an optimal balance between ethical responsibility and scientific necessity is a key consideration. Future progress will depend on sustained investment in innovative technologies, strengthened regulatory frameworks, greater transparency in research practices and interdisciplinary collaboration among researchers, veterinarians, physicians, ethicists and policymakers. These efforts will be critical for advancing animal welfare and promoting the development and adoption of reliable non-animal approaches wherever scientifically appropriate.

Keywords: *biomedical science; animal experimentation; animal welfare; alternative methods; research ethics*

INVITED

Title: Recent Advances in Laser-Based Technologies for Tissue Regeneration

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Laser-based technologies are increasingly investigated for modulating cellular behavior and promoting tissue regeneration. Current approaches include photobiomodulation, low-level laser therapy, laser-assisted scaffold modification, and controlled stimulation of proliferation, migration, differentiation, and extracellular matrix remodeling. Cell-based models are essential for defining therapeutic windows and distinguishing regenerative effects from laser-induced toxicity, which may involve oxidative stress, mitochondrial dysfunction, photothermal injury, and, at shorter wavelengths, mechanisms partly overlapping with UV-induced cellular damage. Artificial intelligence can support automated image analysis, treatment-response prediction, and optimization of irradiation parameters. Textural approaches incorporating gray-level co-occurrence matrices, run-length matrices, fractal descriptors, and wavelet features may detect subtle laser-induced cellular and subcellular alterations. Yeast cells could hypothetically provide a simplified model for AI-assisted characterization of laser-induced structural and toxic effects, although substantial biological differences limit direct translation to mammalian tissue regeneration. Integration of laser technologies with quantitative imaging and AI may facilitate safer and more standardized regenerative strategies.

Keywords: *laser therapy; tissue regeneration; artificial intelligence; textural analysis; photobiomodulation*

INVITED

Title: A Fractal-Feature Neural Network for Quantitative Analysis of Two-Dimensional Physiological Signals

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Fractal analysis offers a quantitative framework for characterizing structural complexity in two-dimensional physiological signals, while artificial intelligence enables nonlinear integration of complementary descriptors. We propose a fractal-feature neural network combining fractal dimension and lacunarity with gray-level co-occurrence matrix (GLCM), run-length matrix (RLM), and discrete wavelet transform (DWT) features. These descriptors can be integrated within convolutional neural networks incorporating novel attention-based mechanisms to identify multiscale patterns associated with physiological and pathological alterations. Potential applications include detection and quantification of apoptosis, sublethal cellular damage, oxidative injury, and other subtle structural changes that may precede overt morphological abnormalities. The framework can support both classification tasks, including discrimination between control and damaged states, and regression tasks for predicting continuous measures of injury severity or physiological response. Combination of interpretable handcrafted features with attention-guided deep learning, enables approach to offer a flexible strategy for quantitative analysis of complex two-dimensional biomedical signals.

Keywords: *fractal analysis; attention mechanisms; convolutional neural networks; physiological signals; cellular damage*

INVITED

Title: Functional Analysis of Mitochondria in Permeabilized Muscle Fibers, Tissues, and Cultured Cells

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Mitochondrial functional analysis is a valuable tool for elucidating cellular energy metabolism, the molecular mechanisms of cell death, and the mitochondrial contribution to the pathophysiology of various diseases. Although researchers traditionally assess mitochondrial function after organelle isolation, this procedure may alter mitochondrial properties and disrupt important interactions with other cellular structures. Therefore, assessing mitochondria in their native intracellular environment may provide more physiologically relevant insight into their functional states.

In contrast, our investigations directly assess mitochondrial function in selectively permeabilized cultured cells or muscle fibers, without prior organelle isolation, using digitonin or saponin. A specifically designed substrate and inhibitor titration protocol enables the sequential assessment of components of the mitochondrial respiratory chain and detailed characterization of mitochondrial respiratory capacity “in situ”.

By assessing parameters such as mitochondrial respiration, membrane potential, ATP production, reactive oxygen species generation, and mitochondrial integrity, this approach can provide insights into alterations in mitochondrial function in human diseases under both physiological and pathological conditions.

The low tissue requirement and relatively small number of cultured cells make this method promising for clinical and translational applications, including the investigation of mitochondrial dysfunction as a potential diagnostic parameter in both research and clinical settings.

Keywords: m*itochondrial function; mitochondrial respiration; cellular bioenergetics*

INVITED

Title: The Retina as a Window to Systemic Physiology: Translating Physiological Signals into Digital Biomarkers

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Background:
The retina is a highly vascularized neural tissue whose microvascular and neuroretinal characteristics reflect systemic physiological state and disease-related processes. Its unique accessibility to non-invasive imaging provides an opportunity to capture vascular, metabolic, inflammatory, and neurodegenerative signals in vivo. Advances in artificial intelligence (AI) are extending this concept beyond conventional retinal assessment by enabling extraction of complex image-derived patterns as potential digital biomarkers of systemic health. To illustrate this translational approach, we investigated whether deep learning could identify systemic disease-associated signatures in retinal fundus photographs, using colorectal cancer (CRC) as a proof-of-concept.
Methods:
A retrospective case-control study included 1,600 UK Biobank fundus photographs (800 CRC and 800 non-CRC). An ImageNet-pretrained EfficientNet-B3 convolutional neural network was fine-tuned for binary classification using participant-level training, validation, and test splits.
Results:
The retinal image-based model achieved an area under the receiver operating characteristic curve (AUROC) of 0.945 on the independent test set, with 87.08% accuracy, 90.00% sensitivity, and 84.17% specificity. A covariates-only model incorporating age and vascular/heart disease achieved substantially lower discrimination (AUROC 0.629). At an estimated CRC prevalence of 0.5%, the negative predictive value remained 99.94%, while the positive predictive value decreased to 2.8%. Attribution analyses indicated predominant reliance on central retinal regions rather than peripheral illumination artifacts.
Conclusion:
These findings illustrate how physiological information encoded within the retinal neurovascular network can be translated into non-invasive digital biomarkers. Rather than serving solely as an ophthalmic endpoint, the retina may function as a multisystem biomarker platform linking systemic physiology with AI-enabled phenotyping. Although prospective validation is required before clinical application, retinal imaging combined with AI offers a promising framework for future risk stratification and triage across systemic diseases.
Keywords: *retina; systemic physiology; digital biomarkers; artificial intelligence; retinal imaging*

INVITED

Title: 20-HETE in the Cerebral Circulation: From Cerebrovascular Regulation to the Human Neurovascular Unit

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20-Hydroxyeicosatetraenoic acid (20-HETE) is a metabolite of arachidonic acid formed by cytochrome P450 ω-hydroxylases. It is an important regulator of cerebral vascular tone and contributes to the myogenic response and autoregulation of cerebral blood flow. Dysregulation of the 20-HETE pathway has been implicated in cerebrovascular disorders, including cerebral ischemia and subarachnoid hemorrhage. Experimental studies have demonstrated that inhibition of 20-HETE formation attenuates cerebral vasoconstriction and reduces ischemic brain injury. Emerging evidence further indicates that the actions of 20-HETE are not restricted to vascular smooth muscle cells but involve multiple cellular components of the neurovascular unit. Recent studies have therefore focused on the cellular organization of the 20-HETE pathway in the brain. CYP4A enzymes and GPR75, a receptor implicated in mediating the actions of 20-HETE, have been identified in several cell types within the neurovascular unit, suggesting that 20-HETE may regulate the cerebral microcirculation through multiple cellular mechanisms. Current studies in the human cerebral cortex are extending these observations by examining the cellular distribution of CYP4A within the neurovascular unit. Defining the cellular organization of the 20-HETE pathway in the human brain may provide an important link between its established roles in cerebrovascular regulation and its contribution to cerebrovascular disease.

Keywords: *20-HETE; CYP4A; cerebral circulation; neurovascular unit; cerebrovascular disease*

INVITED

Title: On the manifold functions of the non-gastric H^+/K^+-ATPase ATP12A

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ATP12A (ATP1AL1) encodes the catalytic α-subunit of the non-gastric H^+/K^+-ATPase (HKα2, HKA2, colonic or renal H^+/K^+-ATPase). It is structurally related to the gastric H^+/K^+-ATPase and Na^+/K^+-ATPases. Although no ATP12A-specific β-subunit has been identified, ATP12A can associate with β-subunits of other P-type ATPases, which is required for membrane targeting and function. The ATPase mediates H^+/K^+ exchange but can also transport NH_4^+ and Na^+. It is sensitive to proton-pump-inhibitors and upregulated by Th2 cytokines. ATP12A is widely expressed across organs, tissues, and cell types and across species. Its functions have been studied mainly in the colon and kidney, where it contributes to K^+ and acid-base homeostasis, serves for blood pressure regulation and gestational volume expansion, and contributes to K^+-restriction-induced salt-sensitive hypertension. In the airways, ATP12A regulates airway surface liquid pH and mucus properties, and its dysregulation has been implicated in diseases including cystic fibrosis and idiopathic pulmonary fibrosis. In the exocrine pancreas, ATP12A contributes to HCO_3^- secretion and appears to be involved in the pathogenesis of pancreatic ductal adenocarcinoma. ATP12A is also expressed in female and male reproductive tissues, blastocysts, and mammalian spermatozoa, where it is involved in the acrosome reaction. In the rat prostate, ATP12A contributes to seminal fluid acidification. In human prostate tissue, altered ATP12A protein expression has been shown in benign prostatic hyperplasia and cancer.

In insulin-secreting rat insulinoma INS-1E cells, promyelocytic HL-60 cells, and neutrophil granulocytes ATP12A has been implicated in the regulation of cell volume, intracellular pH, and cell migration. Furthermore, ATP12A-mediated K^+ uptake may contribute to an anti-apoptotic mechanism by counteracting the K^+ loss, intracellular acidification, and apoptotic volume decrease. Despite this broad expression pattern, the (dys)functions of ATP12A in several tissues and cell types, including the skin, heart, brain, and plasma cells, remain largely elusive, but might be higly relevant under physiological and pathophysiological conditions.

Keywords: *ATP12A; non-gastric H^+/K^+-ATPase; ion transport; cell volume regulation; acid-base homeostasis*

INVITED

Title: Human myocardial miRNA expression in heart failure

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Background:

MicroRNAs (miRNAs) have important role in pathogenesis of different types of cardiomyopathy. Mitochondrial unfolded protein response (UPRmt) is a mechanism of mitochondrial quality control responsible for maintaining normal mitochondrial function.

Methods:

We investigated myocardial expression of miRNAs and genes involved in the UPRmt in six control hearts and 65 failing human hearts. The expression levels of miR-1-3p, miR-21-3p, miR-30a-5p, miR-34a-5p, miR-133a-3p, miR-208a-3p, and miR-423-5p, together with the UPRmt-related genes *HSPA9, HSPD1, LONP1, OMA1,* and *YME1L*, were quantified using quantitative reverse-transcription PCR. Age at heart transplantation (HTx) or left ventricular assist device (LVAD) implantation was considered a surrogate measure of the rate of HF progression.

Results:

Compared with control hearts, failing myocardium exhibited increased expression of miR-208a-3p and the UPRmt genes *HSPA9, HSPD1, OMA1,* and *YME1L*, whereas miR-1-3p expression was reduced. Higher myocardial levels of miR-1-3p, miR-30a-5p, and miR-34a-5p, as well as lower miR-208a-3p expression, were associated with a younger age at HTx or LVAD implantation. When cardiomyopathy subtypes were compared, hearts from patients with dilated cardiomyopathy (DCM) demonstrated higher expression of miR-1-3p, miR-30a-5p, *HSPD1,* and *LONP1* than those from patients with ischemic cardiomyopathy (ICM).

Conclusion:

Overall, these results suggest that myocardial levels of miR-1-3p, miR-30a-5p, miR-34a-5p, and miR-208a-3p may be linked to the progression of HF, while miR-1-3p, miR-30a-5p, *HSPD1*, and *LONP1* may be differentially expressed between DCM and ICM.

Keywords: *miRNA; mitochondrial unfolded protein response; cardiomyopathy*

INVITED

Title: Endothelial glycocalyx in health and disease

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The endothelial glycocalyx (EG) is a gel-like coating layer that covers the luminal surface of endothelial cells, creating a protective barrier between the cells and the blood. It is composed of membrane-bound proteoglycans, glycosaminoglycans (heparan sulfate, chondroitin sulfate, hyaluronan), glycoproteins, and adsorbed plasma proteins that coat the surface of the vascular endothelium. The EG plays a central role in regulating vascular permeability, mechanotransduction of shear stress, leukocyte and platelet adhesion, coagulation, and the maintenance of a non-thrombogenic endothelial surface. Under physiological conditions, an intact EG contributes to the Starling forces governing transcapillary fluid exchange, modulates nitric oxide production, and shields the endothelium from direct contact with circulating blood cells, thereby limiting inappropriate inflammatory and thrombotic responses.

A wide range of pathological stimuli and numerous conditions, including sepsis, diabetes mellitus, atherosclerosis, chronic kidney disease, acute respiratory distress syndrome, trauma, and surgery, can lead to EG shedding, resulting in exposure of underlying adhesion molecules that, in turn, promote capillary leak and interstitial edema, impair microvascular perfusion, and shift the endothelium toward a pro-inflammatory, pro-thrombotic phenotype. Detection of EG shedding by sublingual microscopy and measurement of circulating EG degradation products, such as syndecans, heparan sulfate, chondroitin sulfate, and hyaluronan, have recently attracted attention as tools for diagnosing disease severity.

The endothelial glycocalyx is a critical, dynamic structure that safeguards vascular homeostasis, and its degradation is a common pathway linking diverse acute and chronic diseases to endothelial dysfunction. Growing recognition of EG shedding as both a marker and a mediator of disease severity underscores its potential as a diagnostic tool and therapeutic target. Further research into strategies to preserve or restore EG integrity may improve the diagnosis and treatment of patients with various pathological conditions.

Keywords: *disease; endothelial glycocalyx; health*

INVITED

Title: The Biology of Trauma: Searching for PTSD Biomarkers

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Background:

Posttraumatic stress disorder (PTSD) is a severe trauma- and stressor-related disorder that develops in only a subset of individuals exposed to traumatic events, highlighting substantial interindividual differences in vulnerability and resilience to trauma. The biological mechanisms underlying these differences remain incompletely understood and likely reflect complex interactions between genetic predisposition, environmental exposures, and trauma-related alterations across multiple biological systems. Despite considerable progress in understanding PTSD neurobiology, specific and clinically validated biomarkers for PTSD diagnosis, prognosis, or symptom stratification are still lacking.

Methods:

Our research in war veterans with PTSD and healthy control participants has focused on identifying biological mechanisms associated with vulnerability and resilience to trauma and exploring potential, readily accessible peripheral markers of PTSD. Using (epi)genetic, biochemical, glycomic, and metabolomic approaches, we investigated the associations between genetic variations, platelet- and plasma-derived molecular measures, and PTSD diagnosis, symptom severity, and specific clinical dimensions.

Results:

Our findings revealed associations of specific genetic variants and alterations in platelet and plasma measures with PTSD and with clinical features, including cognitive, positive, psychotic, and depressive symptoms. These findings point to disturbances across multiple biological systems and pathways, including serotonergic and dopaminergic neurotransmission, brain-derived neurotrophic factor (BDNF) signaling, and glycomic and lipidomic profiles.

Conclusion:

The complex trauma-related psychopathology of PTSD is associated with alterations in biological processes involving neurotransmission, neuroplasticity, inflammation, mitochondrial function, membrane integrity, and oxidative stress. Our findings also highlight the potential of peripheral molecular signatures to advance our understanding of individual differences in vulnerability to trauma and the biological heterogeneity of PTSD. Further validation in independent and prospective cohorts is needed to determine whether such markers across multiple biological levels can provide diagnostic and prognostic value and contribute to biologically informed stratification and personalized approaches to PTSD.

Keywords: *PTSD; genetics; platelets; plasma; biomarkers*

INVITED

Title: From spikes to strikes: Calcium oscillations in beta cells and their changes during development of type 2 diabetes

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Background:

Within the beta cell stimulus-secretion coupling cascade, changes in intracellular concentration of the secondary messenger Ca^{2+} are well synchronized with proximal membrane potential oscillations and serve as the triggering signal for the distal exocytosis of insulin. Under certain conditions, they are also synchronized between different beta cells by means of intercellular waves. These important aspects of Ca^{2+} messages were described more than 30 years ago in pioneering Ca^{2+} imaging studies, but some important details about their exact content remain to be described, especially with regard to the translational relevance of these findings for the human beta cells and the development of diabetes.

Methods:

In the last decade, we have employed high-frequency confocal Ca^{2+} imaging of beta cells to investigate these aspects of Ca^{2+} messages in human islets of Langerhans.

Results:

The shape and active time of so-called fast Ca^{2+} oscillations or bursts of Ca^{2+} spikes encode the concentration of the stimulus. These fast oscillations usually originate in groups of cells at the periphery of islets and are transmitted to some or many other cells by means of intercellular waves that typically traverse more centrally located hub cells. Finally, their main characteristics seem to be conserved in human beta cells, and they seem to become attenuated in type 2 diabetes.

Conclusion:

The stimulus-secretion coupling cascade in human beta cells shows the same main characteristics as in mouse beta cells. Intracellular Ca^{2+} dynamics become deranged in diabetes in a manner that is consistent with the increased basal and decreased stimulated insulin secretion observed in patients in vivo.

Keywords: *human beta cell; calcium dynamics; type 2 diabetes*

INVITED

Title: TRP channels in human dental pulp-derived cells and their role in pulpitis

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Background:
Pulpitis, the most common inflammatory disease of the teeth, is often associated with hyperalgesia toward mechanical and thermal stimuli. In the transduction of the algogenic physical stimuli, transient receptor potential (TRP) ion channels of the somatosensory fibers play a crucial role. Changes in their expression and sensitivity contribute to the development of hyperalgesia or allodynia. However, TRP channels are also expressed in non-neuronal cells of the dental pulp, which can also shape sensory and inflammatory processes. In this study, we investigated sensory TRP channels in human dental pulp-derived cells isolated from healthy molar teeth.
Methods:
We investigated the expression of warm- and cold-sensitive TRP channels in primary human dental pulp cells (hDPCs) and the expression of mechanosensitive ion channels in odontoblast-like cells (OBLCs) in vitro. The functionality of TRP channels was investigated by measuring changes in intracellular Ca^{2+} concentration upon applying pharmacological agonists and antagonists. The mechanosensitivity of OBLCs was also investigated. We established inflammatory conditions in hDPC cultures by applying various pathogen-associated molecular patterns (PAMPs) activating pattern recognition Toll-like receptors.
Results:
Poly(I:C), a ligand of Toll-like receptor 3 (TLR3), induced robust inflammatory responses, whereas other PAMPs were less effective. Poly(I:C) stimulated the production of pro-inflammatory cytokines, induced oxidative stress, and highly upregulated TRPA1, a multimodal nociceptor of the TRP family. In poly(I:C) induced inflammatory conditions, intracellular Ca^{2+} signals evoked by TRPA1 ligands were highly potentiated. Poly(I:C)-treated cells displayed increased Ca^{2+} responses to H2O2, which was abolished by TRPA1 antagonism. Moreover, poly(I:C) resulted in diminished cellular viability and mitochondrial functions. Mitochondrial dysfunctions were alleviated by the antioxidant glutathione and partly by TRPA1 antagonism or silencing. We also investigated novel pharmacological tools to alleviate poly(I:C) induced pulpal inflammation.
Conclusion:
TRP channels in the dental pulp are promising pharmacological targets to combat pulpitis and alleviate inflammation-related pulpal tissue damage.
Keywords: *dental pulp cells; pulpitis; oxidative stress; TRPA1*

INVITED

Title: Lipid Droplets and Signalling in Astrocyte Stress Response

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Background:

In pathological conditions, the brain exhibits increased accumulation of lipid droplets (LDs), lipid-storage organelles found predominantly in glial cells but rarely in neurons. The molecular mechanisms underlying LD accumulation in astroglia, key homeostatic cells of the brain, remain poorly understood. LDs are important regulators of free fatty acid (FFA) levels, which may increase during metabolic and hypoxic stress. In the extracellular space, FFAs can act as signaling molecules by activating G-protein-coupled FFA receptors (FFARs). Whether FFA–FFAR signaling affects astrocytic intracellular signaling and modulates LD turnover and glucose metabolism remains unclear.

Methods:

We used fluorescence microscopy to image labeled LDs in astrocytes in rat brain tissue and glial cells in the *Drosophila* brain. LD subcellular localization, mobility, and content were assessed under stress conditions associated with brain pathologies. We also examined the effects of excess extracellular FFAs on astrocytic intracellular signaling and metabolism using genetically encoded fluorescent sensors for second messengers and metabolites combined with real-time confocal microscopy.

Results:

LDs displayed confined mobility near mitochondria and the endoplasmic reticulum, which was further restricted by metabolic stress and ATP-induced increases in intracellular Ca^{2+}, consistent with enhanced LD–organelle interactions observed by electron microscopy. Inhibition of de novo LD biogenesis through DGAT1 and DGAT2 inhibition reduced astrocyte cell number by ~40%, suggesting an important role of LD turnover in astrocyte survival and/or proliferation. Noradrenaline, a neuromodulator involved in the brain stress response, as well as metabolic and hypoxic stress and excess FFAs, promoted LD accumulation. FFAs also increased cAMP signaling and L-lactate levels and decreased cytosolic and mitochondrial citrate, whereas intracellular free D-glucose levels remained unchanged.

Conclusion:

Stress-induced LD accumulation may provide an energy reservoir while protecting astrocytes from FFA-induced lipotoxicity. FFA-induced cAMP and metabolic responses are consistent with FFAR-mediated signaling, enhanced aerobic glycolysis, and redistribution of citrate toward lipid metabolism, suggesting functional coupling between lipid and glucose metabolism in astrocytes under stress.

Keywords: *astrocytes; lipid droplets; free fatty acid signalling; glucose metabolism; stress response*

INVITED

Title: From Metabolic Stress to Cell Cycle Arrest and Differentiation

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*Presenting author

Metabolic stress profoundly affects cell proliferation, yet how metabolic signals are translated into cell-cycle arrest and differentiation remains incompletely understood. Our interest in this question began with AMP-activated protein kinase (AMPK), a central sensor of cellular energy status, and the unexpected observation that the widely used AMPK activator 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR) induced differentiation of myeloid leukemia cells independently of AMPK.

Following this unexpected phenotype led us from classical metabolic signaling to nucleotide metabolism. AICAR perturbed pyrimidine synthesis, induced replication stress and S-phase arrest, and activated checkpoint kinase 1 (Chk1), which was required for differentiation. Similar responses to inhibition of dihydroorotate dehydrogenase and to low-dose cytarabine suggested that distinct metabolic and pharmacological perturbations can converge on replication stress to promote differentiation.

More recent metabolomic and functional studies indicate that disruption of nucleotide pool balance, rather than simple nucleotide depletion, is a critical determinant of replication stress and differentiation. Ribonucleotide reductase, a key regulator of deoxyribonucleotide homeostasis, modulates this response in a cell context-dependent manner. An important unresolved question is how replication stress and checkpoint activation are translated into a differentiation response, and how cell-cycle arrest contributes to this cell-fate decision.

Keywords: *replication stress; nucleotide metabolism; cell differentiation*

INVITED

Title: Metabolic consideration of PTSD

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*Presenting author

Background:

Posttraumatic stress disorder (PTSD) develops only in a subset of trauma-exposed individuals, highlighting the need to identify biological mechanisms underlying vulnerability. As increasing evidence implicates the gut–brain axis in stress-related psychopathology, we investigated both naturally occurring PTSD-like vulnerability in rats and the effects of experimental microbiome manipulation in mice.

Methods:

Male Long-Evans rats were exposed to electric footshock trauma and classified as control, resilient, or vulnerable according to behavioral outcomes. Post-mortem analyses included ileal histology, short-chain fatty acid (SCFA) measurements, microbiome characterization, and expression analyses of barrier-related molecules in the intestine and prefrontal cortex (PFC). In a parallel study, male CD1 mice received an antibiotic (AB) cocktail or control treatment before and after footshock trauma. PTSD-like behaviors were assessed two weeks later.

Results:

Vulnerable rats exhibited significantly thinner intestinal villi, elevated intestinal Muc2 expression, and approximately tenfold higher abundance of *Akkermansia muciniphila* compared with resilient and control animals. SCFA concentrations remained unchanged. In the PFC, expression of zonula occludens-1 (ZO-1) and claudin-11 positively correlated with behavioral vulnerability and *A. muciniphila* abundance. In mice, AB treatment altered freezing behavior 2 weeks later, but not at 24h.

Conclusion:

PTSD-like vulnerability is associated with coordinated gut–brain alterations involving intestinal remodeling, microbial shifts, and changes in brain barrier-related pathways. Experimental microbiome manipulation further supports a role for gut-derived signals in trauma-related behavioral regulation. Together, these findings highlight the gut–brain axis as a promising target for understanding vulnerability and developing preventive interventions for PTSD.

Keywords: *posttraumatic stress disorder; gut–brain axis; microbiome; Akkermansia muciniphila; stress vulnerability*

INVITED

Title: Adrenergic Regulation of Astrocytes in Health and Disease

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Background:

Ageing is a key factor in the development of cognitive decline and Alzheimer's disease (AD), the most commonly diagnosed form of dementia. The pathophysiology of AD is poorly understood but likely involves many processes, including those linked to the noradrenergic system. The primary source of noradrenaline (NA) in the central nervous system (CNS) is the locus coeruleus (LC) axons, which project widely from the LC nucleus in the medulla near the fourth ventricle and innervate most parts of the CNS. There is ample evidence that LC neurons degenerate in AD and other neurodegenerative diseases. The demise of the LC is linked to a hypometabolic CNS state, observed clinically in AD. This is likely due to impaired NA release in the AD brain during states of arousal, attention, and awareness, which are needed for learning and memory formation and for activating energy metabolism. Although dementia is associated with neurodegeneration and loss of neurons, this likely develops due to a primary failure of homeostatic cells, astrocytes, and abundant and heterogeneous neuroglial cells in the CNS.

Methods:

We use single-cell approaches, including electrophysiological methods to monitor membrane capacitance (a readout of membrane area changes) and quantitative microscopy to monitor second messengers and metabolites. Astrocytes support neural network viability by controlling ionic balance, neurotransmitter turnover, synaptic connectivity, and energy balance.

Results:

Experiments revealed that metabolism is regulated by NA as is the morphologic plasticity modulating synaptic transmission and inflammation-associated antigen presentation by reactive astrocytes in pathological states.

Conclusions:

Studying adrenergic control of astrocyte function led us to discover an orphan G-protein coupled receptor, which augments aerobic glycolysis in astrocytes, a process robustly activated by NA.

Keywords: *astrocyte; aerobic glycolysis; antigen presentation*

INVITED

Title: AI-Supported Flipped Classroom approach: Transforming the role of teachers in medical education

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*Presenting author

The flipped classroom (FC) is an innovative model in higher education, especially medical education, where students must master complex theory and apply it in practice. Unlike traditional lectures, FC moves initial knowledge acquisition outside the classroom through videos, podcasts, quizzes, and problem-based tasks. Class time then focuses on active learning, collaboration, problem-solving, and applying concepts to clinical cases. Research and our surveys of medical students indicate that FC can prepare students for final examinations more effectively than traditional teaching by promoting deeper understanding, self-directed learning, and long-term retention.

However, many teachers hesitate to adopt FC. It requires a change in mindset: teachers shift from lecturers to facilitators and mentors. Some are uncomfortable with this role or lack training in instructional design and flipped-learning strategies. Implementation also demands initial time to define learning outcomes and create quizzes, podcasts, videos, and problem-based activities. For overburdened faculty, this may increase stress. Producing high-quality materials also requires technical skills and equipment that may be unavailable.

Success also depends on students taking greater responsibility. If they do not study assigned materials before class, classroom activities become less effective. Some may resist collaborative learning because they find it more demanding. Major barriers therefore include workload, inadequate institutional support and training, technical difficulties, and uncertainty about student preparedness.

Artificial intelligence (AI) can address many barriers by acting as a teaching assistant. It can help formulate learning outcomes, generate quizzes, draft podcast and video scripts, propose problem-based assignments, and suggest classroom activities. This reduces administrative and preparation time, may improve teacher well-being, supports the facilitator role, and strengthens interaction and engagement.

In conclusion, integrating AI with the flipped classroom can reduce implementation barriers while promoting active learning, student engagement, and the practical application of knowledge. This synergy offers a more efficient, sustainable, and student-centered approach to contemporary medical education.

Keywords: *flipped classroom; artificial intelligence; active learning; self-directed learning; innovative medical education*

ORAL

Title: Plasma Triglycerides Associate with Sensitivity to Stearoyl-CoA Desaturase Inhibition in Acute Myeloid Leukemia

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Background:

Stearoyl-CoA desaturase (SCD) catalyzes monounsaturated fatty acid synthesis and plays a central role in cellular lipid homeostasis. SCD inhibition is a promising therapeutic strategy in acute myeloid leukemia (AML). We previously demonstrated potent anti-leukemic activity of the clinical-stage SCD inhibitor SSI-4 (MTI-301) in vitro and in vivo, but observed substantial heterogeneity in response. We therefore investigated physiological and molecular determinants of AML sensitivity to SCD inhibition.

Methods:

We initiated a prospective multiomic study of approximately 100 primary AML samples from patients with de novo, relapsed, secondary or therapy-related disease. Ex vivo sensitivity to SSI-4 was assessed after 7 days of treatment at 1 and 10 µM using Annexin V/propidium iodide staining. Viability was normalized to matched untreated controls. An integrated killing score was calculated as 100 minus the mean normalized viability across the two concentrations, with higher scores indicating greater cytotoxic activity. Samples were classified as sensitive or resistant using a predefined cohort-based cutoff. Clinical characteristics, immunophenotyping, whole-exome sequencing, proteomics and metabolomics were integrated with drug-response data. Here, we report findings from the first 50 samples.

Results:

SSI-4 markedly reduced AML cell viability, while 11/50 samples met the criteria for resistance. Responses were highly reproducible and recapitulated by the structurally unrelated SCD inhibitor A939572, supporting SCD-dependent activity. SCD inhibition induced blast cell death without substantial differentiation. Sensitivity was not associated with disease origin, FAB subtype or myeloid neoplasm-associated mutations, although more differentiated samples tended to be more sensitive. Notably, SSI-4 sensitivity positively correlated with plasma triglyceride concentrations, whereas plasma cholesterol levels and fatty acid transporter CD36 expression were not associated with response.

Conclusions:

Systemic lipid metabolism may influence AML dependence on SCD activity. The association between plasma triglycerides and SSI-4 sensitivity highlights interactions between the extracellular lipid environment and leukemic cellular metabolism as potential determinants of vulnerability to SCD inhibition.
Keywords: s*tearoyl-CoA desaturase; acute myeloid leukemia; lipotoxicity*

ORAL

Title: Ethical approach to handling laboratory rodents

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Background:

Restraining laboratory rodents is an everyday procedure. The choice of method is important for making animals cooperative, minimizing effects on homeostasis, and ensuring reliable results. Mice and rats require specific restraining methods and may be awake or anesthetized. To support an ethical approach, recommended methods for both species are presented here.

Methods:

To restrain awaken mouse, a skin fold is made on the back of the neck exposing the abdomen while grasping the tail. The "cup" or tube method is appropriate to move the mouse from the cage without causing adverse effects. For non-injectable substances, gavage can be avoided by dissolving them in a palatable drinking solution. To restrain the rat with one hand, each finger of the hand is placed in such a way as to surround the head and upper limbs, preventing the animal from moving. The abdominal surface becomes accessible for injection after turning the hand. The Camille position is another option, in which the upper body is wrapped in cloth, keeping the rat still while allowing access to the tail for blood sampling or injection. For I.P. injection, the rat is grasped with the hands, holding it close to the body. This relaxes the rat's abdominal wall, making the injection less painful. The specific method uses a restraining device with two tubes of different diameters, making the tail accessible for injection or blood sampling. This device follows rats' behavior to hide, as in a litter. Tubes can be in the cage for enrichment purposes, so the rats are familiar with them.

Results:

Compared with using Ketamine/Xylazine anaesthesia, the restraining device reduces the stress effect on the animal's blood glucose level.

Conclusion:

Based on the planned experimental procedure and expected outcomes, it is highly important to choose the least aversive and stressful restraint method.

Keywords: *laboratory rodents; restrain; methods; restraining device*

ORAL

Title: Microcirculatory assessment of the outcomes of different nipple–areola complex reconstructive surgeries

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Background:

The microvascular plexus is critical for supplying the nipple–areola complex (NAC) of the breast. Thus, preservation of its viability is a key challenge during reconstructive breast surgery, aiming to limit surgery-induced ischemic injury. Few data are available on the recovery of microcirculation and the early tissue response of the NAC following different surgical techniques. Thus, we evaluated the impact of several NAC surgical techniques in an experimental porcine model on local microcirculation changes and wound-healing dynamics.

Methods:

Five NAC surgical techniques were performed on six female porcine breast models (n= 72 nipples). (1) NAC circumareolar incision, (2) Benelli mastopexy, (3) circumvertical mastopexy, (4) NAC transplantation, (5) NAC bisection. The non-operated NACs served as controls (C). Tissue perfusion (arbitrary units, AU) was assessed on postoperative days (PODs) 7 and 14 using laser speckle contrast imaging (LSCI). Blood flux values were compared among the five surgical techniques, between POD 7 and 14, and with the non-operated control group. Flux distribution was analyzed across the following blood flux ranges: 0–100, 100–200, 200–300, 300–400, and >400 AU, and descriptive statistics and ANOVA analysis were calculated.

Results:

On POD 7, the circumareolar NAC incision technique showed the highest blood flux (266.7 ± 161.0 AU), whereas NAC transplantation exhibited the lowest blood flux (111.5 ± 101.4 AU). By POD 14, circumvertical mastopexy demonstrated the highest blood flux (460.8 ± 211.9 AU), whereas NAC transplantation showed the lowest blood flux (116.0 ± 141.4 AU).

Conclusion:

Among the NAC surgical techniques investigated, it seems circumareolar NAC incision (1), Benelli mastopexy (2), and circumvertical mastopexy (3) demonstrated the best preservation of microcirculation and wound healing characteristics compared with NAC transplantation (5). The highest blood flux at POD 14 was observed after circumvertical mastopexy (3) and the next-best flow rate with the Benelli mastopexy (2), in contrast, NAC transplantation (5) resulted in persistently impaired perfusion. The results suggest that preservation of microcirculation during nipple–areola complex surgery is a key determinant of the outcomes for tissue viability and healing.

Keywords: *NAC surgical techniques; laser speckle contrast imaging (LSCI); preservation of microcirculation*

ORAL

Title: Changes in Beta-Hydroxybutyrate, Non-Esterified Fatty Acids and Glutamate Dehydrogenase During the Periparturient Period in Saanen Goats

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Background:

The periparturient period in dairy goats is characterized by metabolic adaptations associated with parturition and the onset of lactation. Following parturition, the high energy requirements for milk production may exceed dietary energy supply, resulting in mobilization of body fat reserves and increased circulating concentrations of non-esterified fatty acids (NEFA). The liver plays a central role in NEFA metabolism and ketone body formation, including beta-hydroxybutyrate (BHB), while glutamate dehydrogenase (GLDH) provides information on hepatocellular status. The aim of this study was to evaluate changes in BHB, NEFA and GLDH during the periparturient period in Saanen goats.

Methods:

Blood samples were collected from the jugular vein of 20 healthy fourth-parity Saanen goats two weeks before parturition (S1), and at two (S2) and six weeks (S3) postpartum. Serum BHB and NEFA concentrations and GLDH activity were determined spectrophotometrically using an automated biochemical analyzer. Repeated measurements were analyzed using the Friedman test, with P-values adjusted across variables according to the Benjamini–Hochberg false discovery rate (FDR) procedure. Pairwise comparisons among sampling times were performed using the Wilcoxon signed-rank test with Holm adjustment.

Results:

NEFA concentrations differed significantly across sampling time points (Friedman P = 0.0013; FDR-adjusted P = 0.0044), with higher values at S2 compared with S1 (Holm-adjusted P = 0.0006). GLDH activity also differed significantly (Friedman P = 0.0004; FDR-adjusted P = 0.0017), being higher at S2 and S3 compared with S1 (Holm-adjusted P = 0.0006 and 0.0005, respectively). BHB concentrations did not differ significantly among sampling time points (Friedman P = 0.1504; FDR-adjusted P = 0.1704).

Conclusion:

The early postpartum increase in NEFA indicates enhanced lipid mobilization, while higher postpartum GLDH activity suggests concurrent changes in hepatic status. In contrast, BHB remained stable throughout the examined period. These findings demonstrate different temporal responses of metabolic and hepatic parameters during the periparturient period in Saanen goats.

Keywords: *Saanen goat; periparturient period; beta-hydroxybutyrate; non-esterified fatty acids; glutamate dehydrogenase*

ORAL

Title: Elevated K^+ Triggers Glycolytic Lactate Mobilisation Contributing to Astrocyte Swelling

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Background:

The brain has exceptionally high energy demands, yet how neuronal activity is coupled with the rapid delivery of energy remains incompletely understood. During intense activity and pathological states, extracellular concentration of K^+ ($[K^+]_o$) rises substantially. Elevated $[K^+]_o$ has been linked to astrocytic K^+ clearance, lactate mobilization, and astrocyte swelling; however, these processes have largely been studied separately. Here, we investigated whether elevated $[K^+]_o$ acts as an integrated metabolic–osmotic signal coordinating astrocytic glycolysis, neuronal lactate supply, and astrocyte volume remodelling.

Methods:

We used genetically encoded fluorescent sensors to perform live-cell imaging in isolated rat astrocytes and in astrocytes and neurons in the living *Drosophila* brain, complemented by automated quantitative analysis of astrocytes in suspension based on the Coulter principle. We examined how elevated $[K^+]_o$ affects intracellular second messenger signalling, metabolic coupling, and cell volume dynamics.

Results:

Elevated $[K^+]_o$ induced robust, sustained increases in neuronal Ca^{2+} and cAMP, whereas astrocytic responses were smaller and delayed. Elevated $[K^+]_o$ also caused a persistent decrease in neuronal cytosolic glucose, consistent with enhanced glucose consumption, while increasing cytosolic L-lactate in both neurons and astrocytes. Inhibition of monocarboxylate transporters (MCTs) abolished the K^+-induced lactate rise in neurons, but not in astrocytes, indicating enhanced astrocytic lactate production and neuronal lactate import. Importantly, MCT inhibition attenuated K^+-induced astrocyte swelling, showing that lactate transport contributes to both metabolic exchange and astrocytic osmotic responses. In isolated astrocytes, lactate accumulation coincided with swelling and process remodelling, what likely alters the architecture of extracellular space and influence neurotransmission.

Conclusion:

Our findings identify a K^+-driven metabolic–osmotic coupling mechanism, in which lactate metabolism is functionally linked to volume regulation and extracellular space remodelling, extending the classical astrocyte–neuron lactate shuttle concept. Under conditions of energy deprivation and/or neuronal hyperactivity, dysfunction of this mechanism may contribute to cytotoxic oedema and neurotoxicity.

Keywords: *potassium; astrocytes; glycolysis; lactate transport; cell shape*

ORAL

Title: Inhibitory Receptor Expression and T-Lymphocyte Recovery After Chimeric Antigen Receptor T-Cell or Bispecific Antibody Therapy: A Within-Subject Longitudinal Study

Author(s): Ida Ivek[1]*, Josip Knežević[2], Antonija Babić[2], Marijana Nehutni[2], Jozefina Palić[2], Ida Hude Dragičević[1], Barbara Dreta[1], Josip Batinić[1], Dino Dujmović[1], Sandra Bašić-Kinda[1], Igor Aurer[1,3], Hrvoje Lalić[2,4]

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Background:

T lymphocytes maintain homeostasis through balanced differentiation and inhibitory receptor signaling. Therapies engaging T cells directly provide a human model of contrasting stimulation: chimeric antigen receptor (CAR) T cells are given once, whereas bispecific antibodies are dosed repeatedly. We compared T-cell subset redistribution, inhibitory receptor expression and immune recovery between these modalities, hypothesising that sustained engagement causes more persistent changes and stronger inhibitory receptor expression.

Methods:

Twenty-four adults with relapsed or refractory lymphoma or multiple myeloma were studied prospectively before treatment and every three months for one year (CAR T cells, n=12; bispecific antibodies, n=10; both, n=2). Flow cytometry quantified T, B and natural killer cells, CD4+ and CD8+ maturation subsets and the inhibitory receptors programmed cell death protein 1 (PD-1), TIGIT, LAG-3 and TIM-3. Each participant served as their own control, values being anchored to their pre-treatment sample. Non-parametric tests were applied.

Results:

After CAR T cells, PD-1, TIGIT and their co-expression on CD4+ cells decreased progressively (PD-1 on effector memory CD4+ cells, p=0.02 versus baseline at month three), whereas bispecific antibodies caused an early rise. TIGIT on CD8+ central memory cells was higher after CAR T cells at month three (p=0.004). CD8+ counts and memory pools expanded above each patient's own baseline after CAR T cells, and recovery thresholds were reached more often (CD4+ ≥200 cells/µL: 83% versus 50%). Higher pre-treatment TIGIT on CD8+ cells predicted more infections (rho=0.56, p=0.001) and greater severity (p=0.03); a lower pre-treatment CD4+ count accompanied more infections (rho=−0.39, p=0.05).

Conclusion:

Sustained T-cell engagement was accompanied by maintained or rising inhibitory receptor expression and slower restoration of T-cell homeostasis, whereas a single CAR T-cell infusion permitted progressive loss of inhibitory receptors and broader recovery. Pre-treatment inhibitory receptor expression marked impaired host defense, linking T-cell regulatory physiology to infection susceptibility.

Keywords: *T-lymphocyte subsets; immune reconstitution; inhibitory receptors; immunotherapy; infection*

ORAL

Title: Evaluation and Potential Reliability of SSEP and MEP in the Neurophysiological Assessment of Patients with Cervical Myelopathy

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Background:
Degenerative changes of the cervical spine can manifest as cervical myelopathy, potentially resulting in progressive spinal cord dysfunction. However, there is currently no consensus regarding the optimal timing of surgical intervention to prevent or delay neurological deterioration. The aim of this study was to evaluate preoperative somatosensory evoked potentials (SSEPs) and motor evoked potentials (MEPs) and to assess their correlation with clinical findings in patients with cervical spondylotic myelopathy.

Methods:
This study included 27 patients diagnosed with cervical spondylotic myelopathy who underwent surgical treatment for this condition. All patients underwent a detailed clinical assessment prior to surgery. Somatosensory evoked potentials (SSEPs) and motor evoked potentials (MEPs) were recorded intraoperatively.

Results:
Somatosensory impairment was present in 85% of patients, while manual muscle testing revealed mild motor deficits in 44% of patients prior to surgery. The latency of SSEPs from the right and left median nerves was 21.2 and 21.5 ms, respectively, with amplitudes of 1.6 and 1.2 µV. For the right and left tibial nerves, SSEP latencies were 46.3 and 41.9 ms, respectively, with amplitudes of 0.5 and 0.6 µV. Tibial nerve SSEPs could not be recorded in 8 patients, while 7 patients demonstrated significantly prolonged latencies. The threshold for eliciting MEPs was also increased. In 13 patients, some standard MEP responses could not be elicited. A correlation was found between the latencies of right and left tibial nerve SSEPs and somatosensory impairment; however, no correlation was observed between manual muscle testing findings and MEP parameters.

Conclusion:
These results suggest that clinical signs alone may not be reliable indicators of the severity of spinal cord dysfunction. Neurophysiological testing may provide additional and more objective insight into the functional status of affected neural structures and may therefore assist in clinical decision-making.

Keywords: *cervical spondylotic myelopathy; somatosensory evoked potentials; motor evoked potentials*

ORAL

Title: α-Linolenic Acid and Vascular Endothelial Senescence: From Previous Findings to Current Research

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Background:

Vascular endothelial dysfunction, inflammation, oxidative stress, and cellular senescence are closely interconnected processes involved in vascular aging and cardiovascular disease. This future research focuses on the potential of dietary omega-3 fatty acids to modulate endothelial responses and preserve vascular homeostasis. In our previous work, we investigated the effects of α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) on human aortic endothelial cells exposed to inflammatory stress. The findings demonstrated molecule-specific effects, with ALA and DHA showing consistent anti-inflammatory and antioxidative responses, providing a basis for further investigation of ALA.

Methods:

Building on these findings, our current research comprises two complementary projects focused specifically on the potential senomorphic effects of ALA. The projects investigate its influence on endothelial senescence, the senescence-associated secretory phenotype, oxidative stress, nitric oxide bioavailability, and antioxidant defence, with particular interest in the Nrf2 and NF-κB signalling pathways.

Conclusion:

Together, this research represents a continuation of our work on omega-3 fatty acids, extending the focus from endothelial inflammation and oxidative stress toward the mechanisms underlying vascular aging and endothelial senescence.

Acknowledgements: This research was funded by the Bohdan Malaniak RECOOP – CSMC Young Scientists Research Grant #043 Reprogramming the Endothelium: Vasoprotective and Anti-aenescent Effects of Alpha-Linolenic Acid (PI N.K.); and the University of Osijek research grant through internal program UNIOS – Projects for Capacity Building of Young Scientists and Artists, under the projct VASO-ALA, grant number: MZU-2026-24 (PI N.K.).

Keywords: *α-linolenic acid; endothelial senescence; vascular aging; oxidative stress*

ORAL

Title: IL-23 as the Master Regulator in Psoriasis: The Role of Dietary Intervention

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Background:

Interleukin (IL)-23 is regarded as a master regulator of psoriatic inflammation because it sustains the expansion, survival, and pathogenic activity of Th17 cells and thereby maintains downstream IL-17A-mediated responses. Although selective IL-23 blockade is highly effective in psoriasis, the potential of dietary interventions to modulate this pathway remains insufficiently explored. We assessed whether short-term dietary sodium restriction affects serum IL-23 and IL-17A levels and psoriasis severity.

Methods:

This prospective cohort study included patients with psoriasis and a Psoriasis Area and Severity Index (PASI) score ≥5 who received no systemic or biologic therapy for at least 3 months before or during the study. Participants followed a standardized 2-week low-salt diet providing 1,500 mg sodium (3.75 g salt) daily. Adherence was evaluated using 24-hour urinary sodium excretion. Serum IL-23 and IL-17A concentrations were measured using commercial sandwich ELISA kits.

Results:

Nineteen participants completed the protocol, and 24-hour natriuresis confirmed adherence. Serum IL-23 decreased significantly after the intervention, from 58.79 (52.31–76.78) to 53.77 (50.64–58.58) pg/mL (p=0.02), whereas IL-17A remained unchanged [1.39 (0.81–3.17) vs. 1.39 (0.83–2.42) pg/mL; p=0.68]. Changes in IL-23 and IL-17A were not correlated (r=0.098; p=0.68). PASI improved by 12%, decreasing from 6.8 (5.1–11.2) at baseline to 6.4 (5.0–9.5) after the low-salt diet (Wilcoxon signed-rank test, p=0.002).

Conclusion:

A 2-week low-salt diet was associated with reduced serum IL-23 and modest clinical improvement, without a measurable change in IL-17A. These preliminary findings support dietary sodium restriction as a potential adjunctive strategy targeting an upstream regulator of psoriatic inflammation and warrant confirmation in larger controlled studies.

Keywords: *psoriasis; interleukin-23; diet; sodium-restricted*

ORAL

Title: Monocyte-derived dendritic cells in spondyloarthropathy: a stereotyped differentiation programme

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Background:

Monocyte-derived dendritic cells (moDC) model antigen presentation in inflammatory arthritis, yet uptake and presentation are rarely measured in the same cells, and uptake is usually reported as percentage-positive cells, a readout that saturates.

Methods:

We FACS-sorted classical monocytes ($CD3^{-}CD14^{+}$) and autologous $CD3^{+}$ lymphocytes from healthy donors and patients with active ankylosing spondylitis (AS) or psoriatic arthritis (PsA). Monocytes were cultured five days spontaneously or with M-CSF, IL-4 and TNF-α, then phenotyped by flow cytometry (17 participants). pHrodo uptake was read as percentage-positive cells and median fluorescence intensity (MFI). Antigen presentation used 8-day co-culture with sorted lymphocytes (8 donors) given CEFT pool, MOG pool (irrelevant-antigen control) or PMA/ionomycin, with intracellular cytokines in $CD4^{+}$ and $CD8^{+}$ T cells. Analysis used paired Wilcoxon tests with FDR correction and PERMANOVA.

Results:

Relative to spontaneous culture, the cocktail shifted CD14 90 to 75%, CD206 73 to 52%, CD16 33 to 14%, CD209 39 to 69% and CD1c 6 to 15% (all corrected $p<0.001$), while CD86, HLA-DR and CD141 were unchanged. Particle uptake fell to 0.37 of spontaneous by MFI (95% CI 0.29–0.44; $p<0.001$). Because almost every cell took up particles, percentage-positive moved only 96 to 83% while MFI fell 63%, indicating fewer particles per cell, not fewer cells. Co-culture was antigen-specific, as 6 of 10 cytokine readouts separated CEFT from MOG (corrected $p=0.013$), all eight donors concordant, $CD8^{+}IFN\text{-}\gamma^{+}$ rising 0.05 to 0.69%. PERMANOVA showed no difference between controls, AS and PsA at baseline ($p=0.87$) or in differentiation response ($p=0.31$).

Conclusion:

The monocyte-to-moDC transition is a stereotyped programme, reproduced in nearly every participant and unaltered in AS or PsA, with a large functional cost: a two-thirds fall in particle uptake that percentage-positive readouts conceal. These cells drive antigen-specific T-cell responses. Disease effects, if any, more likely lie in function than differentiation.

Keywords: *monocyte-derived dendritic cells; antigen presentation; particle uptake; flow cytometry; spondyloarthropathy*

ORAL

Title: Vitamin B_{12} and Depressive Symptoms: A Cross-Sectional Study Among Croatian Older Adults

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Background:

Subclinical vitamin B_{12} deficiency is highly prevalent in older populations, occurring in 15–40% of the elderly. The association between serum vitamin B_{12} levels and depressive symptoms in older adults is supported by clear biological mechanisms, although epidemiological studies on this topic have shown mixed results. Understanding the link between vitamin B_{12} and depressive symptoms in later life is important because depression is a risk factor for functional decline, admission to residential care, and early mortality. Therefore, this study aimed to determine the frequency of vitamin B_{12} deficiency among the elderly in Eastern Croatia and to assess whether there is a connection between serum vitamin B_{12} levels and the occurrence of depressive symptoms in this population.

Methods:

A cross-sectional study was conducted on a convenience sample of 140 older adults from Vukovar-Srijem County. An anonymous questionnaire was used to collect demographic data, information on socioeconomic status and personal medical history, and data on the presence of depressive symptoms among the study participants. A competitive immunoassay vitamin B_{12} kit was used to determine serum vitamin B_{12} levels.

Results:

The study sample comprised 47.9% males and 52.1% females, with a mean age of 71.0 ± 6.7 years. Among all participants, 7.1% had measured serum vitamin B_{12} concentrations below the reference range, 87.2% had concentrations within the reference range, and 5.7% had concentrations above the reference range. Depressive symptoms were present in 67.7% of participants whose serum vitamin B_{12} concentrations were within or above the reference range and in 100.0% of participants whose serum vitamin B_{12} concentrations were below the reference range ($p = 0.033$).

Conclusion:

The study showed a positive association between depressive symptoms and vitamin B_{12} deficiency among older adults. This finding highlights the need for regular evaluation of vitamin status in this age group and appropriate treatment, which could improve the overall health of this vulnerable subgroup.

Keywords: *depression; elderly; vitamin B_{12}; deficiency; Croatia*

ORAL

Title: Targeting IL-23 in Psoriasis: Immunologic Rationale for IL-23 Blockade and Dietary Modulation of the Inflammatory Circuit

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Background:

Psoriasis is a chronic, immune-mediated disease driven by the interleukin-23 (IL-23)/T-helper-17 (Th17) axis. IL-23, produced by myeloid dendritic cells, sustains pathogenic Th17 cells and interleukin-17 (IL-17) production, generating a self-amplifying feed-forward circuit and long-lived cutaneous tissue-resident memory (TRM17) cells that underlie disease recurrence. This axis is also modulated by metabolic and environmental factors—obesity, diet composition, salt intake, and the gut microbiota—positioning psoriasis at the interface of immunology and metabolism. The objective was to integrate current evidence on selective IL-23 blockade and dietary interventions that converge on this shared pathogenic pathway.

Methods:

A narrative review of pivotal and mechanistic literature (2013–2026) was performed. Sources included phase 3 randomized trials with long-term extensions, network meta-analyses, single-cell and spatial transcriptomic studies, controlled dietary trials, and translational studies of the gut–skin axis and sodium-driven Th17 pathogenicity. Findings were synthesized qualitatively.

Results:

Selective anti-p19 IL-23 blockers (risankizumab, guselkumab, tildrakizumab) achieved durable, high-level skin clearance in phase 3 trials and long-term extensions, and a recent network meta-analysis supported the class as among the most effective biologics for plaque psoriasis. Longitudinal transcriptomic data demonstrated stable remodeling of the type 17 circuit—reduced IL-17-producing TRM cells, pro-inflammatory fibroblasts, and vascular chemokine expression—persisting after treatment cessation. Dietary evidence showed that weight loss nearly doubled Psoriasis Area and Severity Index (PASI) 75 response, the Mediterranean diet conferred benefit independent of weight loss, glucagon-like peptide-1 (GLP-1) receptor agonists reduced disease severity, and gut-derived indoxyl sulfate drove skin Th17 inflammation via aryl hydrocarbon receptor signaling

Conclusion:

IL-23-targeted biologic therapy and dietary modulation act on a shared IL-23/Th17 axis. Optimal, durable psoriasis control integrates selective IL-23 blockade with weight management and anti-inflammatory dietary strategies.

Keywords: *psoriasis; IL-23/Th17 axis; IL-23 blockade; dietary intervention; gut–skin axis*

ORAL

Title: The role of mitochondria in cardioprotection

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*Presenting author

Background:

Mitochondria are known to play a key role in heart cells metabolism. They produce cell energy, and may be involved in apoptosis. Therefore, mitochondrial function is vital in short term, and, as well as, in long term function of heart cells. Thus, the aim of this study is to show the role of mitochondria in short-term and long-term cardiac cell function under the conditions of cellular hypoxia.

Methods:

Cardiac myocytes and mitochondria were isolated from hearts of adult Wistar rats using standard techniques. Cardiomyocytes survival, and mitochondrial function were analyzed using confocal microscope, and spectrofluorometric techniques under conditions of cellular ischemia in the presence or absence of volatile anesthetic isoflurane.

Results:

The cells that were exposed to isoflurane have a higher survival rate. The mitochondria that were exposed to isoflurane had better preserved ATP production, less reactive oxygen species production, less accumulation of calcium, and had better preserved mitochondrial membrane potential. When analyzed long-term effects, in a separate experiment, animals that have better preserved cardiac function, showed better preserved ATP production.

Conclusion:

Our results show that mitochondria may play a key role in acute cardiac injury as well as in chronic disfunction of the heart muscle. Therefore, preserving mitochondrial function is important mechanism in preserving the vitality of the heart.

Keywords: *mirochondria; cardiac myocites; apoptosis*

ORAL

Title: Chronic Kidney Disease as a Prototype for Early Vascular Ageing: Pathophysiological Mechanisms and Multimodal Management

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Background:

Chronic kidney disease (CKD) affects more than 850 million people worldwide and is projected to impact up to 16.5% of the population by 2032, with stage 3–5 prevalence rising by 25% and dialysis demand by over 75%. By 2040, CKD is forecasted to become the 5th leading cause of life years lost globally. International consensus guidelines (ASN-ERA-ISN) highlight CKD as an urgent global public health priority.

Methods:

Pathophysiology of Early Vascular Ageing (EVA): CKD serves as a primary clinical model for Early Vascular Ageing (EVA), a trajectory marked by accelerated arterial stiffening compared to normal or supranormal (SUPERNOVA) vascular ageing phenotypes. At the cellular and tissue level, uremic toxins, oxidative stress, and chronic low-grade inflammation trigger endothelial dysfunction, vascular smooth muscle cell phenotypic transition, extracellular matrix remodeling, and vascular calcification. Even in early CKD (stages 1–2), cardiovascular event risk increases approximately 10-fold compared to non-CKD populations. Albuminuria acts as a central morphological and pathophysiological biomarker of microvascular damage and directly correlates with pulse wave velocity, central aortic pressure, and augmentation index. Diagnostic Assessment: Non-invasive evaluation of arterial stiffness relies on carotid-femoral pulse wave velocity (cfPWV) as the gold standard, alongside central hemodynamic indicators such as Central Aortic Pressure (CAP) and Augmentation Index (AiX). Clinical evidence indicates that while various office and 24-hour non-invasive measurement methods are compatible, they are not interchangeable for clinical assessment. Therapeutic Strategies: Modern management integrates CKD within the Cardiovascular-Kidney-Metabolic (CKM) syndrome framework. Treatment rests on Lifestyle Medicine pillars combined with four core disease-modifying pharmacological pillars: 1. RAS Inhibitors (ACEi / ARB), 2. SGLT-2 Inhibitors (reducing intraglomerular pressure and endothelial injury), 3. Non-steroidal Mineralocorticoid Receptor Antagonists (*finerenone*, counteracting inflammation and fibrosis), 4. GLP-1 Receptor Agonists (*semaglutide*, which significantly attenuates eGFR loss and reduces albuminuria by up to 32–40%). Emerging targeted senolytics and anti-inflammatory agents hold further promise in dampening vascular ageing cascades.

Conclusion:

CKD exemplifies accelerated vascular ageing driven by systemic metabolic, inflammatory, and hemodynamic stress. Early recognition through vascular biomarkers and prompt initiation of multimodal, four-pillar disease-modifying therapy are critical to improving renal and cardiovascular outcomes.

Keywords: *chronic kidney disease; arterial stiffness; albuminiura*

ORAL

Title: Extracellular Vesicle Profiling in Human Cerebrospinal Fluid Following Traumatic Brain Injury: Automated Size-Exclusion Chromatography Coupled with Single-EV Microflow Cytometry

Author(s): Carmen Rožmanić[1]*, Anton Bilić[2], Emina Fodolović[1], Siniša Zrna[3], Branka Balaban[4], Janja Tarčuković[5,6], Kristina Grabušić[1]

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Background:

Extracellular vesicles (EVs) have gained significant attention as promising biomarkers because their molecular cargo reflects the physiological and pathological state of their cells of origin. However, their clinical application in central nervous system disorders is challenged by their extremely low abundance in cerebrospinal fluid (CSF) and high contamination with co-isolated proteins and other particles. This study aimed to optimize a size-exclusion chromatography (SEC) protocol for EV enrichment from human CSF following traumatic brain injury (TBI), providing suitable sample preparation for downstream analyses, including microflow cytometry.

Methods:

Following ethical approval, longitudinal CSF samples were collected daily from patients with severe TBI undergoing therapeutic external ventricular drainage. Size exclusion chromatography (SEC) was optimized and standardized for EV separation using qEV columns coupled to an Automated Fraction Collector. From each sample, 24 consecutive fractions were collected and systematically screened by slot blot and western blot for EV-associated protein markers (CD9, Flotillin-1) against abundant soluble protein (albumin) and lipoproteins (apolipoprotein A1, ApoA1). Additionally, a lipophilic membrane-dye staining protocol was developed and validated on synthetic liposomes as an EV model system for microflow cytometry.

Results:

EV-associated proteins were markedly enriched in early SEC fractions (7-10), whereas albumin and ApoA1 were predominantly detected in later fractions (13-18), demonstrating effective separation of EVs from major protein and lipoprotein contaminants. Liposome-based assay optimization established the optimal membrane-dye staining parameters and gating strategies required to discriminate true vesicular events from dye aggregates during microflow cytometric single-EV profiling.

Conclusion:

Standardization of automated SEC coupled with immunodetection provides a robust and practical method for enriching high-purity EVs from human CSF. Integrating this workflow with optimized membrane staining could facilitate the discrimination of EVs from non-vesicular artifacts in microflow cytometry, enabling single-EV characterization and the identification of EV-associated molecular markers of neurorecovery following TBI.

Keywords: *extracellular vesicles; size-exclusion chromatography; microflow cytometry*

ORAL

Title: Are Non-Animal Models Effective in Veterinary Education? Insights from Our Teaching Practice

Author(s): Ana Shek Vugrovečki[1]*, Suzana Milinković Tur[1], Jasna Aladrović[1], Lana Pađen[1], Dražen Đuričić[1], Jadranka Pejaković Hlede[1], Stefani Fruk[1], Mario Ostović[2], Nikola Rošić[3], Ivona Žura Žaja[1]

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Non-animal models are increasingly used in veterinary education due to ethical considerations, limited availability of teaching animals, student safety, and the need for standardized and reproducible practical training. In physiology teaching, such models may support the acquisition of theoretical knowledge, practical skills, and analytical reasoning before students proceed to animal-based or clinical work. This communication presents teaching experiences from Physiology of Domestic Animals I and II at the Faculty of Veterinary Medicine, University of Zagreb. Practical classes include the use of PhysioEx virtual physiology simulations and the BIOPAC physiological data acquisition system. PhysioEx is used for practicals in cell membrane transport, endocrinology, muscles and nerves, and renal physiology. BIOPAC is used to introduce students to the acquisition, analysis, and interpretation of physiological recordings, including ECG, EEG, EMG, and spirometry. Non-animal models allow students to explore physiological mechanisms in a safe, repeatable, and time-efficient learning environment. Simulations enable comparison of experimental conditions that would be difficult, time-consuming, or ethically problematic to demonstrate *in vivo* during regular practical sessions. BIOPAC-based practicals promote active learning by requiring students to collect real physiological data, perform basic analysis, consider calibration and electrode or sensor placement, and interpret results using professional terminology. However, simulations cannot fully reproduce animal handling, behavioral responses, biological variability, species-specific differences, or clinical unpredictability. Some students may also approach simulations procedurally, which requires additional teacher guidance. Non-animal models effectively support physiology teaching, particularly conceptual understanding, data interpretation, methodological awareness, and safe practical training. Nevertheless, they should be viewed as complementary rather than replacement tools. A blended educational model combining theory, simulation, physiological data acquisition, animal-based practical work, and clinical application appears most appropriate for veterinary education.

Keywords: *veterinary education; non-animal models; physiology teaching*

ORAL

Title: Cross-talk between matrix-metalloproteinases and granulysin and perforin-mediated cytotoxicity in malignancies and environmental diseases

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Background:

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, tumor cell metastasis and atherosclerosis. Innate and adaptive immunity play a pivotal role in the tumor response, but many of these interactions are still not well understood. Granulysin (GNLY) is an effector, cytolytic molecule, presents in human cytotoxic granules of different lymphocyte subpopulations, mainly in cytotoxic T cells and NK cells. Pore-forming proteins: granulysin, perforin and granzymes play a key role in cell-mediated immune responses against tumors and infections. Interactions between matrix metaloproteinases and perforin and granulysin cytotoxicity is still not investigated.

Methods:

We analysed perforin (P) and granulysin (GNLY)-mediated cytotoxicity in the peripheral blood of patients with colorectal cancer (CRC) by flow cytometry. Phenotype of lymphocyte subpopulation and expression of perforin and granulysin were analyzed using intracellular and surface immunofluorescency. We also hypothesized that circulating levels of MMP-2 and 9 were abnormal in patients wih colorectal cancer and these levels were compared with those in matched controls. The method of enzyme immunoassay (ELISA) was used to determine enzyme expression of MMP-2 and 9.

Results and Conclusion:

High concentration of MMP 2 and 9 enzymes followed by diminished values of G and P cells in CRC patients highlights the importance of this regulatory immunological pathway and indicated the determination of MMPs as easy marker for the monitoring of malignancies. The concentrations of the enzyme MMP-2 and 9 were significantly elevated in the urban population in comparison with the rural one as well as in the occupationally exposed cohort, suggesting that the rise of MMPs might be non-specific early marker for tissue damages.

Acknowledgement: This work was supported by grants from University of Rijeka (No. uniri-iz 25-239 and uniri-iz-25-72).

Keywords: *colorectal cancer; environmental diseases; granulysin; matrix metalloproteinases; perforin*

ORAL

Title: Bone Degeneration in Murine Models of Cholestatic Liver Injury

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Background:

Chronic liver diseases, especially cholestatic liver diseases, are often associated with osteoporosis, which represents a significant clinical concern. Animal models of cholestasis are well established, but bone phenotype in these models still remains largely uninvestigated. Thus, our goal was to determine bone structure changes and bone cells function shifts in 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) and 1-naphthylisothiocyanate (ANIT) mouse models.

Methods:

C57BL/6 female mice were fed with either standard food, DDC-enriched food or ANIT-enriched food for 4-8 weeks. Liver, blood, spine and femora were sampled for further analyses. Liver fibrosis was assessed by Sirius red histology and RNA levels of *Col1a1*, *Acta2* and *Krt19*. Femora and spines were scanned by micro-CT and scans were reconstructed and analysed in order to assess bone mass changes. Frequencies of osteoclast (OCPs) and osteoblast progenitors (OBPs) in bone marrow were determined by flow cytometry, while concentrations of serum markers of active osteoclasts (TRAP) and osteoblasts (osteocalcin) were determined by ELISA. Direct effect on osteoclastogenesis was examined by adding various concentrations of DDC or ANIT in osteoclast cultures.

Results:

Both DDC- and ANIT-fed mice developed liver fibrosis, as evidenced by higher proportions of Sirius red-stained liver sections and elevated fibrosis markers. ANIT-fed mice had significant trabecular and cortical bone loss, as indicated by decreased bone volume/tissue volume (BV/TV), trabecular number and cortical thickness, as well as by increased trabecular separation. DDC-fed mice didn't show such a unidirectional bone phenotype, exhibiting decreased femoral cortical thickness, but somewhat increased spinal trabecular BV/TV. Bone marrow frequencies of both OCPs and OBPs were elevated, but serum TRAP and osteocalcin levels were unchanged in both models. However, DDC showed strong inhibitory effect on osteoclastogenesis *in vitro*, while the effect of ANIT was not so pronounced.

Conclusion:

Both DDC and ANIT models exhibit changed bone phenotype, with ANIT model better resembling clinical findings.

Keywords: *mice; cholestasis; osteoporosis; DDC; ANIT*

ORAL

Title: Molecular Basics of Pulse Wave Velocity and Clinical Implications

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Background:

Arterial stiffness is a hallmark of vascular aging and an important predictor of cardiovascular morbidity and mortality. Carotid–femoral pulse wave velocity (cfPWV), the gold-standard non-invasive measure of arterial stiffness, provides valuable prognostic information beyond conventional cardiovascular risk factors. Increased PWV independently predicts myocardial infarction, stroke, heart failure, chronic kidney disease progression, dementia, and all-cause mortality.

Methods:

The molecular basis of arterial stiffness involves complex interactions between extracellular matrix remodeling, endothelial dysfunction, vascular smooth muscle cell alterations, oxidative stress, chronic inflammation, metabolic disturbances, and genetic susceptibility. Physiological arterial elasticity depends on the balance between elastin and collagen. Aging and disease promote collagen accumulation, elastin degradation by matrix metalloproteinases, and irreversible collagen cross-linking mediated by advanced glycation end products. Activation of AGE-related pathways enhances oxidative stress, inflammation, and vascular remodeling. Endothelial dysfunction, characterized by reduced nitric oxide bioavailability, activation of the renin–angiotensin–aldosterone system, and increased endothelin-1 activity, further promotes vasoconstriction, fibrosis, and arterial remodeling. Diabetes and chronic kidney disease substantially accelerate these processes through metabolic disturbances, inflammation, vascular calcification, and oxidative stress. Genetic factors also contribute significantly to PWV variability.

Results:

Clinically, cfPWV enables early detection of subclinical vascular damage and improved cardiovascular risk stratification. Arterial stiffness has also emerged as a potential therapeutic target. Current treatments, including ACE inhibitors, angiotensin receptor blockers, mineralocorticoid receptor antagonists, calcium channel blockers, and newer antidiabetic agents, may improve arterial compliance by reducing fibrosis, inflammation, and oxidative stress. Emerging strategies include AGE cross-link breakers, Klotho-based therapies, senolytics, microRNA-based approaches, and interventions targeting vascular calcification and endothelial dysfunction.

Conclusion:

In conclusion, PWV represents both an important biomarker of vascular aging and a promising surrogate endpoint for evaluating novel precision therapies targeting the molecular mechanisms of arterial stiffness.

Keywords: *arterial stiffness; pulse wave velocity; endothelial dysfunction*

ORAL

Title: γδ T and MAIT T Cell Repertoire and Functional Remodeling in Psoriasis

Author(s): Stana Tokić[1]*, Maja Jirouš Drulak[2], Martina Mihalj[3,4], Vera Plužarić[1,4], Marija Šola[4], Maja Tolušić Levak[1,3], Mario Štefanić[5]

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Background:

Psoriasis vulgaris (PV) involves immune activation with contributions from innate-like and adaptive T-cell subsets. γδ T cells and mucosa-associated invariant T (MAIT) cells can rapidly mediate inflammation, but their phenotypic, transcriptional and T-cell receptor (TCR) repertoire alterations in human psoriasis remain incompletely defined. We aimed to characterize γδ T and MAIT cells in blood and psoriatic skin and relate their profiles to disease severity.

Methods:

Patients with PV (n=60) and healthy controls (HC=30) were studied cross-sectionally. Flow cytometry quantified γδ T-cell and MAIT-cell subsets in blood and tissue. γδ and MAIT TCR repertoires were profiled by targeted next-generation sequencing (NGS) of complementary determining region 3 (CDR3) regions and analyzed using MiXCR, VDJTools and immunarch. Immune-transcriptomic profiling used the Illumina Immune Response Panel with differential expression and pathway analyses. Spectral cytometry characterized detailed phenotypes.

Results:

Male patients with PV had reduced circulating γδ T-cell and Vδ2+ proportions, while lesional skin showed expansion of Vδ1−Vδ2− γδ T cells. Circulating γδ TCR repertoire diversity decreased and clonal dominance increased with higher PASI. Thirty-six genes were differentially expressed in γδ T cells (30 upregulated, 6 downregulated), involving activation, cytotoxicity, interferon-γ responses, migration and survival. MAIT-cell frequencies were largely preserved, but CD69 expression was increased; CLA+ and PD-1+ MAIT subsets positively correlated with PASI. MAIT TCR repertoires remained diverse but showed age- and severity-related contraction, particularly in men, with skewing of selected TRBV/TRBJ combinations. CD4+ and double-positive MAIT subsets showed the strongest tissue-homing phenotype.

Conclusion:

Psoriasis is associated with coordinated phenotypic, transcriptional and TCR repertoire remodeling of γδ T cells, consistent with adaptive-like clonal selection and redistribution between blood and skin. MAIT cells remain numerically stable but acquire activation and tissue-homing features linked to disease severity. Together, these findings support complementary roles of γδ T and MAIT cells in psoriasis.

Keywords: *psoriasis vulgaris; γδ T cells; MAIT; TCR repertoire; NGS*

ORAL

Title: Sodium Chloride as an Environmental Modifier of Th17 Immunity in Psoriasis Vulgaris

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Background:

Psoriasis vulgaris (PV) is a chronic, immune-mediated skin disease driven by the interleukin (IL)-23/T helper 17 (Th17) axis. Genetic susceptibility is well established, yet environmental modifiers determine disease expression, severity and comorbidity. Sodium chloride has emerged as one such modifier, since the skin stores sodium at concentrations exceeding those in serum. This lecture reviews the evidence linking dietary salt and cutaneous sodium storage to the pathogenesis of PV and to its cardiovascular burden.

Scope of the lecture:

Three converging lines of evidence are presented. The first concerns the skin as a sodium reservoir: sodium is bound to dermal glycosaminoglycans in an osmotically inactive form, and sodium-23 magnetic resonance imaging has shown increased cutaneous sodium and water content in patients with higher disease activity, confined to the skin rather than to other tissues. The second concerns immunology: a high extracellular sodium concentration amplifies Th17 differentiation and IL-17A production through the p38/nuclear factor of activated T cells 5–serum/glucocorticoid-regulated kinase 1 pathway, impairs regulatory T cell function and promotes pro-inflammatory macrophage polarisation, while IL-17A itself drives dermal sodium accumulation, suggesting a feed-forward loop. The third concerns vascular biology: high salt intake increases systemic oxidative stress, reduces antioxidant capacity and induces endothelial dysfunction, mechanisms shared with the elevated cardiovascular risk of PV. Our own data on the effects of a short-term low-salt diet on disease activity and cutaneous microvascular function in patients with PV support this concept. Current limitations, including scarce interventional data and the possibility of a salt-sensitive subgroup, are addressed.

Conclusion:

Cutaneous sodium accumulation is a measurable pathophysiological feature of PV that links Th17-mediated inflammation with oxidative stress and vascular dysfunction, and provides a rationale for testing salt reduction in randomised controlled trials.

Keywords: *psoriasis; sodium chloride; Th17 cells; oxidative stress; endothelial dysfunction*

POSTER

Title: Role of TRVP4 in Dermal Regeneration

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Background:

Transient Receptor Potential Vanilloid 4 (TRPV4) is a thermo- and osmosensitive, chiefly Ca^{2+}-permeable ion channel influencing epidermal barrier formation, hair growth, and sebaceous lipogenesis. However, its physiological role in the dermis remains largely unknown. Therefore, in the current study, we aimed to investigate the regenerative and immunological roles of TRPV4 in primary human dermal fibroblasts (HDFs).

Methods:

Channel functionality was assessed via Fura-2 AM-based intracellular Ca^{2+}-measurements using hyposmotic challenges and the TRPV4 agonist GSK1016790A, alongside the antagonist HC-067047. Cell viability was monitored via MTT assay. To determine TRPV4's immunomodulatory effects, pro-inflammatory cytokine mRNA and protein levels were quantified via RT-qPCR, cytokine array, and ELISA. Extracellular matrix (ECM) remodeling and regenerative potential were evaluated using RT-qPCR, Sirius Red staining, and invasion assays.

Results:

TRPV4 is robustly expressed and functionally active in primary HDFs, as both hyposmotic stimuli and GSK1016790A significantly elevated the intracellular Ca^{2+}-level. Importantly, the effect was completely abolished by the co-administration of HC-067047. Regarding regeneration, TRPV4 activation promoted cell migration, increased matrix metallopeptidase expression, and significantly reduced transforming growth factor (TGF)-β1-induced collagen synthesis, indicating a strong regulatory role in ECM remodeling. Furthermore, GSK1016790A differentially modulated the immune phenotype by down-regulating the mRNA expression of tumor necrosis factor (TNF)-α and up-regulating the "itch-relevant" interleukin (IL)-8 and IL-33. Protein-level analyses confirmed marked elevation of IL-8, IL-33, and CXCL1 secretion upon GSK1016790A-treatment. All observed regenerative and immunomodulatory effects were fully prevented by the co-administration of the TRPV4-specific antagonist, confirming receptor specificity.

Conclusion:

Our findings demonstrate that TRPV4 is functionally active in HDFs and actively shapes their immune phenotype and ECM remodeling. Consequently, TRPV4 emerges as a promising pharmacological target in wound healing disorders as well as in certain pruritic and inflammatory skin diseases.

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Keywords: *TRPV4, human dermal fibroblasts, skin inflammation, wound healing*

POSTER

Title: Postoperative C-Reactive Protein Levels After Total Hip Arthroplasty: A Comparison of the Direct Anterior and Anterolateral Approaches

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Background:

The surgical approach used for total hip arthroplasty (THA) may influence the extent of soft-tissue trauma and the postoperative inflammatory response. C-reactive protein (CRP) is a commonly used marker of systemic inflammation after surgery. This study aimed to compare serum CRP levels on postoperative day 4 in patients undergoing primary THA through the direct anterior approach (DAA) or anterolateral approach (ALA).

Methods:

This retrospective comparative study included 163 patients who underwent primary THA from January 1 through December 31, 2025. Patients were categorized according to surgical approach into the ALA group (n=80) and DAA group (n=83). The primary outcome was serum CRP concentration on postoperative day 4. CRP distributions were assessed for normality. Because the assumption of normality was not met, between-group differences were evaluated using the Mann–Whitney U test. Welch's independent-samples t-test was additionally performed as a sensitivity analysis to assess the consistency of the findings when comparing mean CRP values.

Results:

Mean CRP concentration on postoperative day 4 was 90.94 ± 41.71 mg/L in the ALA group and 75.63 ± 35.76 mg/L in the DAA group. Median CRP was 84.75 mg/L (interquartile range [IQR], 59.18–114.70) following ALA and 73.90 mg/L (IQR, 49.25–101.80) following DAA. CRP levels were significantly higher in the ALA group (Mann–Whitney U = 4063.5, p = 0.014). The sensitivity analysis using Welch's t-test yielded consistent results (p = 0.013), with a mean between-group difference of 15.31 mg/L (95% confidence interval, 3.27–27.35).

Conclusion:

Patients undergoing primary THA through the anterolateral approach had significantly higher CRP levels on postoperative day 4 compared with patients undergoing the direct anterior approach. These findings suggest that surgical approach may be associated with differences in the early postoperative inflammatory response. Further studies incorporating additional perioperative and patient-related factors are needed to determine the clinical significance of this difference.

Keywords: *total hip arthroplasty; direct anterior approach; anterolateral approach; C-reactive protein; postoperative inflammatory response*

POSTER

Title: Red Cell Distribution Width Differs Between Intracapsular and Extracapsular Proximal Femoral Fractures

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Background:

Proximal femoral fractures are common in older adults and differ in anatomical pattern and clinical course. Extracapsular fractures have been associated with greater blood loss than intracapsular femoral neck fractures. Red cell distribution width (RDW) has also been associated with adverse outcomes after hip fracture. This study aimed to compare hematological parameters between intracapsular and extracapsular proximal femoral fractures and explore their relevance.

Methods:

This retrospective comparative study included 74 patients with proximal femoral fractures: 37 with femoral neck fractures and 37 with pertrochanteric fractures. Age, sex, RDW, hemoglobin (Hb), and mean corpuscular volume (MCV) from blood tests at hospital admission were analyzed. Groups were compared using Welch's t-test. Multivariable linear regression assessed associations between fracture type and hematological parameters after adjustment for age and sex.

Results:

Patients with pertrochanteric fractures had higher RDW values than those with femoral neck fractures (13.98 ± 1.11 vs. 13.31 ± 0.70; $p = 0.003$). Hb was lower in the pertrochanteric group (127.1 ± 14.3 vs. 134.2 ± 12.7 g/L; $p = 0.023$), as was MCV (88.95 ± 5.50 vs. 91.26 ± 4.62 fL; $p = 0.045$). After adjustment for age and sex, fracture type remained significantly associated with RDW ($\beta = 0.67$; $p = 0.003$), Hb ($\beta = -7.04$ g/L; $p = 0.020$), and MCV ($\beta = -2.33$ fL; $p = 0.044$).

Conclusion:

Pertrochanteric fractures were associated with higher RDW and lower Hb and MCV levels than femoral neck fractures, independent of age and sex. These findings suggest that proximal femoral fracture patterns may be associated with distinct hematological profiles, potentially reflecting differences in the systemic impact of the injury. As these parameters are readily available from blood testing, they may have relevance for early perioperative assessment. Prospective studies incorporating serial measurements and clinical outcomes are warranted to determine their prognostic value.

Keywords: *red cell distribution width; hemoglobin; proximal femoral fracture; femoral neck fracture; pertrochanteric fracture*

POSTER

Title: Effects of Chronic Aripiprazole and Trazodone Treatment on Lens Opacity and Oxidative Stress in Female Rats

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Background:

Oxidative stress is a key mechanism contributing to lens protein and lipid damage and subsequent loss of lens transparency. Although psychotropic medications are widely used, their potential effects on lens redox homeostasis remain insufficiently investigated. This study aimed to determine whether chronic administration of aripiprazole and trazodone, alone or in combination, affected lens opacity and oxidative stress in middle-aged female rats.

Methods:

In this experimental study, 41 female Sprague–Dawley rats aged 11–12 months were randomly assigned to four groups: control (n=10), aripiprazole (ARI; 2.5 mg/kg; n=10), trazodone (TRZ; 5 mg/kg; n=10), and combined aripiprazole and trazodone (ARI+TRZ; n=11). Treatments were administered intraperitoneally for seven weeks. Following euthanasia, the right lenses were dissected, photographed under standardized conditions, weighed, snap-frozen, and stored at −80 °C. Lens opacity was quantified using mean grayscale intensity (0–255) in ImageJ, with higher values indicating greater opacity. Oxidative stress was assessed by measuring malondialdehyde (MDA) and the activities of superoxide dismutase (SOD) and catalase (CAT). Between-group differences and associations between oxidative stress markers and lens opacity were analyzed using appropriate statistical methods.

Results:

Biochemical analyses are ongoing. Final results will report group specific values for lens opacity, oxidative damage and antioxidant enzyme activities, together with statistical comparisons among the four groups and associations between oxidative stress parameters and lens opacity.

Conclusion:

The findings will clarify whether chronic exposure to aripiprazole and trazodone, individually or combined, is associated with altered lens redox homeostasis and transparency, providing insight into potential ocular effects of prolonged psychotropic treatment.

Keywords: *aripiprazole; trazodone; oxidative stress; lens opacity; cataract*

POSTER

Title: Dissecting the Role of Nucleotide Availability in Hydroxyurea-Induced Differentiation of Acute Myeloid Leukemia Cells

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Background:

Hydroxyurea (HU) is used for cytoreduction in hematological malignancies and canonically inhibits ribonucleotide reductase (RNR), thereby disrupting deoxyribonucleotide homeostasis, impairing DNA synthesis, and causing S-phase arrest. HU also induces differentiation in acute myeloid leukemia (AML) cell lines, but whether this response is simply a consequence of RNR inhibition and replication-associated cell-cycle arrest or reflects broader alterations in nucleotide homeostasis remains unclear.

Methods:

U937, THP-1, and MOLM-13 AML cell lines were treated with HU for up to 72 hours. Differentiation markers, viability, and cell-cycle distribution were assessed by flow cytometry. In U937 cells, morphological and functional maturation were evaluated by May-Grünwald-Giemsa and α-naphthyl acetate esterase staining plus a phagocytosis assay. Targeted LC-MS metabolomics was performed after 24 hours. Ribo- and deoxyribonucleosides, alone or combined, were supplemented to probe nucleotide precursor availability. CHK1 phosphorylation at Ser345 [p-CHK1(S345)], γH2AX, and RNR subunit M2 (RRM2) were assessed by Western blotting. RRM2 was silenced using siRNA.

Results:

HU induced myeloid differentiation across AML cell lines, characterized by increased CD11b and CD64 expression and supported by morphological and functional maturation. Cell-line-specific cell-cycle arrest was also observed. In U937 cells, S-phase arrest was accompanied by increased γH2AX and p-CHK1(S345), consistent with replication stress, as well as increased RRM2. However, RRM2 silencing failed to mimic or modify HU-induced differentiation. Metabolomic profiling of U937 cells revealed selective nucleotide-pool remodeling, including decreased dATP, rather than generalized deoxyribonucleotide depletion. Ribonucleoside supplementation, alone or combined with deoxyribonucleosides, attenuated differentiation without reversing HU-induced cell-cycle arrest. Despite strongly increasing CD11b in untreated cells, deoxyribonucleosides did not significantly affect HU-induced differentiation or cell-cycle arrest.

Conclusion:

HU induces myeloid differentiation across AML cell lines together with replication stress and S-phase arrest. However, the differentiation response cannot be explained solely by RRM2 suppression, deoxyribonucleotide insufficiency, or replication-associated cell-cycle arrest, suggesting a broader role for nucleotide homeostasis in regulating differentiation.

Keywords: *acute myeloid leukemia; hydroxyurea; nucleotide homeostasis; replication stress; ribonucleotide reductase*

POSTER

Title: Longitudinal Optical Imaging of Fluorescent Glucose Analog Uptake in the Mouse Brain

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Background:

Cerebral glucose uptake is critical for maintaining normal brain function, yet its assessment in preclinical models often relies on radioactive tracers or invasive procedures. We aimed to establish an accessible optical imaging protocol for longitudinal monitoring of fluorescent glucose analog uptake kinetics in the mouse brain.

Methods:

The protocol was established using 6-month-old male C57BL/6J mice (n = 12). Animals were anesthetized, and the head was shaved to reduce optical attenuation. GlucoseCy5 was administered subcutaneously (1.2 µg/kg) in the lower back, away from the cranial imaging field, while tail vein intravenous administration was evaluated for comparison. Animals were imaged at baseline and 1, 5, 15, 30, 45, and 60 minutes post-injection using the IVIS Spectrum imaging system (ex: 640nm; em: 680nm). The body below the neck was masked during image acquisition, and fluorescence was quantified within a predefined cranial region of interest using Living Image software. Following transcardial PBS perfusion, brains were isolated and imaged *ex vivo.*

Results:

In vivo imaging revealed a progressive increase in cranial fluorescence during the first 30 minutes after subcutaneous administration, followed by a gradual decline consistent with tracer redistribution and systemic clearance. Intravenous administration produced rapid probe distribution that limited temporal characterization within the predefined imaging schedule, whereas subcutaneous administration enabled monitoring over time. Repeated imaging significantly reduced fluorescence, consistent with photobleaching and limiting acquisition frequency. Fluorescence remained detectable in isolated brains after transcardial perfusion, supporting the passage of the GlucoseCy5 probe across the blood–brain barrier and retention within cerebral tissue.

Conclusion:

This optical imaging protocol provides a standardized framework for longitudinal assessment of fluorescent glucose analog uptake and temporal fluorescence distribution in the mouse brain. It avoids radioactive tracers and invasive cranial surgeries, making it an accessible complementary approach for studying alterations in cerebral glucose handling in experimental mouse models.

Acknowledgement: This work was supported by the Croatian Science Foundation project HRZZ IP 2025-02-7524 “The role of bradykinin and glucose transporters in diabetes induced cognitive and neurovascular dysfunction” (CANDy).

Keywords: *fluorescence imaging; glucose uptake; brain metabolism*

POSTER

Title: Rab11 FIP Proteins are Recruited into the Murine Cytomegalovirus Assembly Compartment

Author(s): Sara Bilić[1]*, Sara Pavičić[1], Igor Štimac[1], Marina Marcelić[1], Gordana Blagojević Zagorac[1], Pero Lučin[1], Hana Mahmutefendić Lučin[1]

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Background:

Murine cytomegalovirus (MCMV) infection induces extensive remodeling of the recycling endosomal and Golgi membranes, leading to the formation of the viral assembly compartment (vAC/AC). Our previous studies identified expansion and accumulation of Rab10-positive membranes as an early hallmark of AC biogenesis. Rab10 is a marker of intermediate recycling vesicles, and acts upstream of Rab11, which regulates endosomal recycling through Rab11 family-interacting proteins (Rab11-FIPs). Here, we investigated the localization and recruitment of Rab11-FIPs during AC biogenesis in MCMV-infected fibroblasts, as well as their interactions with Rab10.

Methods:

NIH3T3 and NIH3T3 Rab10-GFP cells were infected with MCMV and analyzed by immunofluorescence and confocal microscopy to determine the localization, expression, and colocalization of Rab11 and Rab11-FIP proteins with Rab10 during early AC formation. Protein expression dynamics were assessed by Western blot at early and late phase of infection. Rab10-associated proteins were identified using proximity-dependent biotin identification (BioID).

Results:

Immunofluorescence and confocal microscopy demonstrated the accumulation of Rab11 and Rab11-FIP proteins within the perinuclear region during the early phase of MCMV infection. Rab10-GFP was found highly colocalized with Rab11, Rab11-FIP3, and Rab11-FIP5, and less colocalized with Rab11-FIP1 and Rab11-FIP2, indicating their recruitment to Rab10-expanded compartments during AC formation. Western blot analysis showed no significant changes in the expression levels of Rab11 or Rab11-FIP proteins during infection. Furthermore, BioID analysis on Rab10 BioID2-HA cells identified Rab11-FIP3 and Rab11-FIP5, among Rab10-associated proteins, suggesting their association with the Rab10 interactome during AC biogenesis.

Conclusion:

These findings suggest that Rab11-FIP proteins contribute to the expansion and reorganization of the endosomal recycling compartment during MCMV infection. The significant colocalization rate of Rab11-FIP3 and Rab11-FIP5 with Rab10, as well as their close interactions with Rab10 demonstrated by the BioID method, indicate their role in Rab10-dependent events required for the expansion of recycling compartments during AC formation.

Acknowledgement: This work was funded by the European Union – NextGenerationEU – RRRAC - uniri-iz-25-179, and the Croatian Science Foundation - IP-2024-05-4995.

Keywords: *murine cytomegalovirus; assembly compartment; Rab11FIPs; Rab10*

POSTER

Title: Nucleotide Homeostasis Determines Differentiation Responses to Replication Stress in Acute Myeloid Leukemia

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Background:

Replication stress can promote differentiation of acute myeloid leukemia (AML) cells, but the mechanisms determining whether it induces differentiation or loss of viability remain incompletely understood. We investigated how perturbations of nucleotide homeostasis and adaptive regulation of ribonucleotide reductase (RNR) influence AML cell fate.

Methods:

U937 and MOLM-13 AML cells were treated with 5-aminoimidazole-4-carboxamide ribonucleoside (AICAr), the dihydroorotate dehydrogenase inhibitor brequinar, or low-dose cytarabine, with or without nucleoside supplementation or N-acetylcysteine (NAC). Differentiation, viability, and cell-cycle distribution were assessed using flow cytometry and cell-based assays. Nucleotide metabolism was analyzed by LC-MS. Expression and functional roles of the RNR subunits RRM2 and RRM2B were examined by immunoblotting and siRNA-mediated depletion.

Results:

Despite acting through distinct upstream mechanisms, all three treatments induced myeloid differentiation associated with disrupted nucleotide homeostasis and replication stress. Ribo- and deoxyribonucleoside supplementation abolished cell-cycle arrest and differentiation, demonstrating a requirement for nucleotide imbalance. Adaptive responses differed between AML models. Treatments increased RRM2 in p53-mutant U937 cells, whereas the p53-responsive RRM2B isoform predominated in p53-wild-type MOLM-13 cells. Accordingly, RRM2 depletion enhanced differentiation in U937 cells without substantially affecting viability, but reduced survival and impaired differentiation in MOLM-13 cells. NAC markedly suppressed treatment-induced myeloid differentiation-marker expression in U937 cells, whereas only modest effects were observed in MOLM-13 cells, indicating that the redox-sensitive regulation of differentiation is cell-context dependent.

Conclusion:

AML cell fate during replication stress depends on the balance between nucleotide perturbation and cellular adaptation. Differential use of RRM2 and RRM2B provides distinct mechanisms for maintaining nucleotide homeostasis, and redox-sensitive signaling may further modulate the differentiation response to replication stress.

Keywords: *AICAr; replication stress; nucleotide homeostasis*

POSTER

Title: Astrocytic GABA transporter-3 is dynamically regulated during disrupted glutamate–GABA homeostasis

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Background:
The balance between glutamatergic excitation and GABAergic inhibition is essential for neuronal network stability. Astrocytes contribute to this balance by regulating extracellular neurotransmitter availability, yet how astrocytic GABA handling responds to altered network activity remains poorly understood. GABA transporter 3 (GAT-3), predominantly expressed in astrocytic processes, regulates extracellular GABA and is distributed beyond the immediate vicinity of GABAergic terminals, suggesting a broader role in neuronal network homeostasis. We investigated whether two distinct forms of network dysfunction, and NMDA receptor hypofunction, converge on altered astrocytic GAT-3.

Methods:
Male C57BL/6 mice underwent pilocarpine-induced SE or repeated administration of the NMDA receptor antagonist MK-801, which exhibits anticonvulsant activity, with corresponding control groups. GAT-3 and IP3R2 expression was quantified by Western blotting. Immunofluorescence and confocal microscopy were used to assess hippocampal GAT-3 distribution relative to GABAergic synaptic markers using distance-based intensity analysis and a GAT-3 proximity index. MK-801-induced behavioral changes were assessed using open-field and Y-maze tests.

Results:
Following SE, hippocampal GAT-3 showed time-dependent regulation, with a significant reduction at day 14. GAT-3 expression strongly correlated with IP3R2 expression. Confocal analysis following SE suggested differences in the spatial organization of GAT-3 relative to GABAergic synaptic structures. Repeated MK-801 treatment reduced GAT-3 expression in the prefrontal cortex (PFC) and the hippocampus, while IP3R2 remained unchanged. MK-801 induced acute hyperlocomotion and reduced spontaneous alternation in the Y-maze, with Y-maze performance positively correlating with PFC GAT-3 expression.

Conclusion:
The association between GAT-3 and IP3R2 suggests potential regulation by astrocytic Ca^{2+} signaling, while its relationship with behavioral performance points to functional consequences extending beyond local GABA homeostasis. Together, these results support GAT-3 as a shared and dynamic astrocytic response to disrupted glutamate–GABA homeostasis.

Acknowledgment: This research was funded by the National Recovery and Resilience Plan 2021–2026, research grant ASTROVERSE (10106-25-3012).

Keywords: *astrocytes; GAT-3; GABA; status epilepticus; NMDA receptor hypofunction*

POSTER

Title: Cardiorespiratory Fitness in High School Students: A One-Year Follow-Up

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Background:

Cardiorespiratory fitness is an important indicator of functional capacity and overall health in adolescents. Longitudinal monitoring of estimated maximal oxygen uptake (VO2max) may help track changes in aerobic capacity over time and identify unfavorable trends in cardiorespiratory fitness that may be associated with future cardiovascular risk. This study aimed to assess changes in cardiorespiratory fitness, evaluated by estimated VO_2max, in high school students over a one-year period.

Methods:

The study included 33 high school students aged 16–17 years who were followed longitudinally over two consecutive school years. The first assessment was conducted in 2025 and the second in 2026. At both assessment points, the same students performed the 20-m shuttle run (Beep) test as part of their regular Physical Education classes. VO2max was estimated based on the test results, and differences between the two measurements were analyzed using a paired-samples t-test, with statistical significance set at $p < 0.05$.

Results:

Analysis revealed changes in estimated VO2max between the two assessment points. Overall, mean VO2max decreased from 41.02 mL/kg/min in 2025 to 39.90 mL/kg/min in 2026; however, this difference did not reach statistical significance ($p = 0.057$). Sex-specific analysis showed a slight decrease in boys, from 42.65 mL/kg/min in 2025 to 42.00 mL/kg/min in 2026, with no statistically significant difference ($p = 0.34$). In contrast, girls showed a more pronounced decrease in mean VO2max, from 35.91 mL/kg/min in 2025 to 33.40 mL/kg/min in 2026, with a statistically significant difference between the two measurements ($p = 0.02$).

Conclusion:

The findings indicate that cardiorespiratory fitness remained relatively stable in boys, whereas girls showed a significant decline in VO2max over the one-year period. The observed decrease in girls may reflect a less favorable cardiorespiratory fitness profile and may indicate a potentially increased cardiovascular health risk.

Keywords: *VO2max; adolescents; 20-m shuttle run test*

POSTER

Title: Expression of uroguanylin in the brain is regulated by feeding

Author(s): Nikola Habek[1,2,3], Vladiana Crljen[2,3]*, Dora Sedmak[4], Martina Ratko[1,2], Martina Tkalčić[5], Anton Mažuranić[5], Pero Bubalo[5], Petar Škavić[5], Ivan Banovac[4], Aleksandra Dugandžić[1-3]

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Background:

The expression of guanylate cyclase C (GC-C) in the brain is known for several decades. However, the presence of GC-C agonists, like uroguanylin (UGN), has been disputed. Insulin resistance and glucose homeostasis are essential for metabolic disorders according to preclinical and clinical research. The aim of the study was to determine presence of UGN and its importance in metabolic disorders.

Methods:

UGN expression was confirmed at mRNA (RT PCR, qPCR, RNAScope) and protein (Western blot, ELISA, immunohistochemistry) level, both in mouse and human brain.

Results:

Two hours after a meal UGN mRNA in mouse cerebral cortex was in positive correlation to glucose concentration in cerebrospinal fluid (CSF). In mouse hypothalamus, UGN mRNA was in negative correlation to glucose concertation in CSF. We also found UGN in human prefrontal and cerebellar cortex, arcuate nucleus of hypothalamus and substantia nigra in brain samples of 21 human male brains. UGN is expressed in all cortical layers in several interneuron subpopulations.

Conclusion:

These results confirm presence of UGN in the mouse and human brain. UGN expression is regulated by feeding suggesting possible role of UGN signaling pathway during feeding process regulation in mouse and cognitive regulation of feeding in humans.

Keywords: *guanylate cyclase C; uroguanylin; brain; feeding*

POSTER

Title: The impact of virtual reality on sleep physiology in patients with epilepsy

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Background:

During a certain phase, the brain changes electrical activity, which can facilitate the occurrence of seizures in some people. Lack of sleep is one of the most common triggers of epileptic seizures. Seizures in some people occur mainly: during sleep, during deep sleep, immediately after waking up. Poor sleep quality after a seizure can further increase the risk of new seizures the next day. The most common triggers associated with screens include: rapidly flashing lights (usually 3–30 Hz), high-contrast lights, rapidly changing colors or patterns, video games with intense visual effects, viewing the screen in a dark room, fatigue and lack of sleep while using the device.

Methods:

The research included 20 men and 25 women with an average age of 25.15±5.1 as a pilot study. The previously mentioned sociodemographic data of all subjects were taken. The Pittsburgh Sleep Quality Index (PSQI) was also applied - an instrument that measures 7 components of sleep quality using 19 items: 1. sleep duration, 2. sleep disturbances, 3. sleep latency, 4. difficulties in daytime functioning due to sleep deprivation, 5. usual sleep efficiency, 6. subjective sleep quality, 7. use of pharmacological agents. The participant provides a self-assessment on all 7 components.

Results:

Later bedtime, less sleep, lower level of physical activity, later bedtime, polytherapy (in our population, especially lamotrigine, topiramate, and carbamazepine), generalized seizures, lower level of education, and unemployment were correlated with poorer PSQI scores.

Conclusion:

Ensure assistance from a multidisciplinary team as well as the broader community.

Tailor AET to the individual's specific characteristics—the best medical treatment. Perform polysomnography if necessary and adjust therapy or address comorbidities. Advise on lifestyle and dietary measures; provide education on the use of electronic media and necessary precautions; emphasize the importance of physical activity and better organization of leisure time through alternative activities. Do not underestimate the impact of VR in the treatment of sleep disorders.

Keywords: *virtual reality; sleep; epilepsy*

POSTER

Title: Perioperative Microcirculatory Assessment in Total Hip Arthroplasty: A Comparative Pictorial Case Report Using Sublingual Glycocalyx Imaging and Laser Doppler Flowmetry

Author(s): Slavko Čičak[1,2]*, Irena Kmoniček-Krajina[2,3], Nikolina Kolobarić [4], Ana Marinčić Žagar[4], Silvija Midenjak[4], Ines Drenjančević[4], Ivana Haršanji Drenjančević [2,3]

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*Presenting author

Background:

Total hip arthroplasty may be accompanied by changes in microcirculation and endothelial function. This case report aimed to provide a comparative pictorial presentation of sublingual GlycoCheck imaging and cutaneous laser Doppler flowmetry (LDF), illustrating their complementary application in perioperative assessment.

Methods:

A patient undergoing total hip arthroplasty was assessed preoperatively (T1), on postoperative day 1 (T2), and on postoperative day 5 (T3). Sublingual GlycoCheck imaging assessed the perfused boundary region (PBR), an indirect marker of glycocalyx barrier properties. Cutaneous LDF was combined with post-occlusive reactive hyperemia (PORH) testing to characterize microvascular reactivity. Representative sublingual images, PBR values, and LDF–PORH curves were compared descriptively across the three time points.

Results:

PBR progressively decreased from 2.70 µm at T1 to 2.52 µm at T2 and 2.39 µm at T3. This direction of change was compatible with reduced red blood cell penetration into the glycocalyx boundary region, but did not establish glycocalyx recovery. LDF recordings demonstrated a post-occlusive perfusion increase at all time points, with a less pronounced recorded response at T3 than at earlier assessments. The progressive reduction in PBR was therefore accompanied by a different temporal pattern in the LDF–PORH curves.

Conclusion:

This comparative pictorial case report illustrates the use of two complementary methods to characterize perioperative microcirculatory changes and aspects of endothelial function during total hip arthroplasty. The differing patterns highlight the value of examining glycocalyx-related properties alongside cutaneous microvascular reactivity.

Keywords: *microcirculation; endothelial glycocalyx; total hip arthroplasty*

POSTER

Title: Effects of Sex, Age, and Gonadectomy on Systemic Oxidative Stress in Sprague–Dawley Rats

Author(s): Viktoria Čurila[1]*, Senka Blažetić[2], Marta Balog[1], Barbara Viljetić[3], Lovro Mihajlović[2], Robert Blažeković[4], Ozana Katarina Tot[5], Marija Heffer[1]

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Background:

Aging and sex hormones influence metabolic homeostasis and may contribute to changes in systemic redox balance. Alterations in oxidative stress have been implicated in age-related metabolic dysfunction. The individual and combined effects of biological sex, aging, and loss of gonadal function remain insufficiently understood. This study aimed to analyse sex- and age-related differences in oxidative stress and to determine whether gonadectomy modifies redox homeostasis in middle-aged rats.

Methods:

Male and female Sprague-Dawley rats were studied at 3 months (young) and 9 months of age (middle-aged). An additional group of middle-aged animals underwent gonadectomy approximately one month before final sampling. All animals were maintained on a standard laboratory diet throughout the experiment. Blood samples were collected under standardized fasting conditions, and serum was isolated and stored for subsequent analyses. Oxidative stress was assessed by evaluating biomarkers reflecting oxidative damage and antioxidant defense. Differences in redox status were analyzed according to sex, age, and gonadal status.

Results:

Analyses are ongoing. The results will characterize differences in systemic oxidative stress between male and female rats across age groups and determine the effects of gonadectomy in middle-aged animals.

Conclusion:

This study will provide insight into the relative contributions of biological sex, aging, and gonadal function to systemic redox homeostasis and may help clarify the role of oxidative stress in sex- and age-dependent metabolic alterations

Keywords: *oxidative stress; aging; sex differences; gonadectomy; metabolic homeostasis*

POSTER

Title: Relationship Between Motor Abilities, Physical Activity Levels, and Screen Time in Early Primary School Children

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*Presenting author

Background:

The aim of the present study was to examine relationship between selected characteristics of the motor development and physical activity level and screen time among children in the first grade of primary school residing in rural areas of Eastern Croatia.

Methods:

The cross-sectional study involved 42 children (25 girls+17 boys, age 7±0.5) recruited from three primary schools across Osijek-Baranja County. Data regarding physical activity levels and screen time were obtained through parent-administered questionnaires. Anthropometric characteristics, as well as motor and functional abilities, were evaluated using a standardized test battery (AST 6–11), which encompasses measurements of body height and body mass (for body mass index [BMI] calculation), alongside tests assessing speed, coordination, precision, flexibility, explosive power, and aerobic endurance.

Results:

The findings revealed that although the majority of children do not participate in organized sports, they routinely engage in daily informal physical activity. Notably, a high prevalence of overweight and obesity was observed, with 53.8% of participants exhibiting elevated body mass. Boys demonstrated statistically significantly superior performance compared to girls in the 20 m sprint (4.97 ± 0.3 s vs. 5.86 ± 0.34 s) and the coordination test (23.4 ± 4.7 vs. 16.4 ± 2.8), whereas no sex-based differences were found in the remaining variables. Although digital technology use was widespread among participants, no clear association with motor or functional test performance was established.

Conclusion:

The results indicate the importance of regular, high-quality physical activity in early childhood, as well as the need to balance active and sedentary behaviors to foster optimal pediatric growth and development.

Keywords: *screen time; physical activity; motor abilities*

POSTER

Title: Continuous Glucose Monitoring Metrics and Circulatory Function in Adults with Type 1 Diabetes: A Cross-Sectional Analysis

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Background:

Continuous glucose monitoring (CGM) provides measures of glycaemic exposure and variability that may capture vascular risk beyond glycated haemoglobin. We examined associations between CGM metrics and arterial stiffness, blood pressure, and haemodynamic parameters in adults with type 1 diabetes.

Methods:

This cross-sectional analysis included 51 participants with CGM and circulatory measurements. Time in range (TIR), time above range (TAR) >13.9 mmol/L, total time below range (TBR) <3.9 mmol/L, glucose management indicator (GMI), coefficient of variation (CV), and mean glucose were analysed continuously and separately over 14, 30, and 90 days. Outcomes comprised pulse wave velocity (PWV), blood pressure, and cardiac and vascular haemodynamic indices. We used Spearman correlations and multivariable linear regression models adjusted for age, sex, diabetes duration, and body mass index. Multiple testing was controlled using the Benjamini–Hochberg false discovery rate (FDR).

Results:

Mean age was 27.7±6.8 years, diabetes duration 10.5±7.5 years, and 54.9% were women. Mean TIR was 70.2%, 69.7%, and 70.1% over 14, 30, and 90 days, respectively. Mean GMI values were 6.94%, 6.98%, and 6.95%, mean CV values were 33.28%, 33.26%, and 34.06%, and mean total TBR values were 2.97%, 2.86%, and 3.08%. No significant unadjusted associations were identified. The strongest unadjusted trend was observed between 30-day TAR >13.9 mmol/L and left cardiac work index (LCWI; ρ=0.254; p=0.072). In adjusted analyses, higher total TBR was nominally associated with higher systolic blood pressure over 14 days (standardized β=0.229, 95% CI 0.012–0.445; p=0.039) and 90 days (β=0.234, 95% CI 0.019–0.448; p=0.033). Neither association remained significant after FDR correction (q=0.963 and q=0.999). No nominally significant adjusted association was found over 30 days.

Conclusion:

CGM metrics were not consistently associated with arterial stiffness or haemodynamic parameters in this young cohort. Recurrent nominal associations between greater hypoglycaemic exposure and systolic blood pressure warrant prospective evaluation but remain hypothesis-generating.

Keywords: *continuous glucose monitoring; time below range; arterial stiffness; haemodynamics; type 1 diabetes*

POSTER

Title: Responsible Sun Behavior During Outdoor Sports Activities: Knowledge and Application of UV Radiation Protection Measures Among Young Athletes

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Background:

Outdoor sports provide important physical and social benefits for children and adolescents but may also result in repeated exposure to ultraviolet (UV) radiation. Responsible sun behavior and appropriate use of protective measures are therefore important components of preventive sports medicine. However, high awareness of UV-related risks does not necessarily translate into consistent protective practices. Aim: To assess knowledge and practices related to responsible sun behavior and UV radiation protection among young athletes, and to explore the perspectives of their parents and sports professionals involved in youth sports.

Materials and Methods:

A cross-sectional survey was conducted in 2025 among 228 participants: 64 young athletes, 132 parents, and 32 teachers, coaches and healthcare professionals. The questionnaires assessed knowledge of UV radiation, outdoor sports participation during summer, exposure between 10:00 and 16:00, use of sunscreen and protective clothing, history of sunburn, and attitudes toward sun-protection education. Data were analyzed descriptively and presented as percentages.

Results:

Knowledge of peak UV exposure was high across all groups, with 95.3% of young athletes, 99.2% of parents and 100% of sports professionals correctly identifying 10:00–16:00 as the period of highest UV radiation. Despite this, 76.5% of parents reported that their child participated at least occasionally in outdoor sports or other activities during this period in summer. Among sports professionals, 75.0% reported participating in outdoor activities during the same period at least occasionally, while 21.9% reported organizing activities for children between 10:00 and 16:00. Consistent sunscreen use, both during leisure time and sports activities, was reported for 50.8% of children, whereas 49.2% did not use sunscreen consistently across both settings. Although 99.2% of parents reported advising their children about sun protection, 84.8% considered sun protection insufficiently represented in children's education. Among sports professionals, 96.9% reported advising children about sun protection, while only 18.8% reported consistent sunscreen use during both leisure time and training. A history of childhood sunburn was reported by 47.0% of parents for their children and by 87.5% of sports professionals for themselves.

Conclusion:

The findings demonstrate a high level of knowledge and awareness regarding UV radiation, accompanied by inconsistent application of protective measures during outdoor activities. Responsible sun behavior should therefore be addressed as a shared responsibility of young athletes, parents and sports professionals. Preventive strategies should focus not on restricting participation in outdoor sports, but on creating conditions that enable children and adolescents to engage in outdoor physical activity safely and responsibly, with appropriate protection from UV radiation.

Keywords: *UV radiation; sun protection; responsible sun behavior; young athletes; outdoor sports; preventive sports medicine; health education*

POSTER

Title: Correlation of tensiomyographic parameters of the quadriceps femoris muscle and maximum isometric force in athletes after anterior cruciate ligament injury

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Background:

Tensiomyography (TMG) non-invasively assesses the contractile properties of the quadriceps femoris, which stabilizes the knee joint and frequently exhibits deficits following ACL injury. Understanding the relationship between contractile parameters and maximum isometric force is crucial for optimizing athletic rehabilitation. This study investigated relationships between TMG parameters of quadriceps heads (VM, VL, RF) and maximum isometric force in athletes with previous ACL injury and healthy controls.

Methods:

The study included 10 athletes aged 18 to 28 years, divided into a group with a previous ACL injury (N = 5) and a healthy control group (N = 5). Muscle contractile properties were measured using a TMG device, while maximum isometric force (PF) was measured using a K-pull dynamometer. The relationship between variables was analyzed using Pearson's correlation test on the total sample and separately by groups

Results:

For the vastus lateralis (VL) muscle, statistically significant negative correlations were found in the total sample between contraction time (TcVL) and maximum force ($r = -0.71$; $p = 0.02$) and between delay time (TdVL) and maximum force ($r = -0.66$; $p = 0.04$). In the ACL injury group, an extremely high negative correlation was recorded between TdVL and maximum force ($r = -0.92$; $p = 0.03$). In the control group and for the vastus medialis (VM) and rectus femoris (RF) muscles, no statistically significant correlations with maximum force were found.

Conclusion:

The negative correlations indicate that shorter contraction and delay times of the vastus lateralis muscle are associated with greater maximum isometric force. The high correlation between the delay time of the vastus lateralis muscle and force in injured athletes highlights the sensitivity of TMG in detecting specific neuromuscular changes and the importance of monitoring this muscle in rehabilitation following an ACL injury.

Keywords: *delay time; contraction time; neuromuscular deficit; rehabilitation*

POSTER

Title: The regulation of glycoprotein 96 expression in breast cancer

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Background:

Breast carcinoma is the most common malignant disease in the female population. Hormone receptors-positive carcinomas are the less malignant and have a better clinical prognosis, whereas HER-2 positive and triple negative carcinomas are more aggressive with a poorer prognosis. GP96 is an endoplasmic reticulum chaperone involved in tumor progression and immune modulation.

Methods:

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 immunohistochemistry staining of these samples was be performed. After that the number of gp96 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.

Results:

We have found the lowest gp96 expression in the luminal A and luminal B HER2- breast cancers group, the highest expression was in HER2+ and tripple negative breast cancer. The statistical analysis of the results have shown the statistical significance difference among the gp96 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 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 group of luminal A breast cancers that had better clinical outcome.

Conclusion:

The goal of this study was to show the difference in the expression of gp96 among the different groups of breast carcinomas and to incorporate the obtained results with the clinical course and disease outcome.

Acknowledgement: This work has been fully supported by the University of Rijeka under the project number (uniri-iz-25-45).

Keywords: *breast cancer; glycoprotein 96; immunohistochemistry*

POSTER

Title: Evaluation of oxidative balance score in athletes and sedentary individuals

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Background:

Oxidative balance reflects the interplay between antioxidant and pro-oxidant factors and may be influenced by physical activity and dietary patterns. The Oxidative Balance Score (OBS) provides an integrated measure of these factors. This study aimed to compare OBS between athletes and sedentary individuals and to investigate its associations with oxidative stress biomarkers and anthropometric parameters.

Methods:

This cross-sectional study included 70 participants, 31 athletes and 39 sedentary individuals. Athletes reported at least five predominantly endurance-based training sessions per week for ≥12 months. Anthropometric and hemodynamic parameters were assessed, while dietary intake was evaluated using the EPIC-Norfolk food frequency questionnaire. OBS was calculated from standardized dietary antioxidant and pro-oxidant components. Serum activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were analyzed as oxidative stress biomarkers. Between-group differences were assessed using independent samples t-tests, and associations were examined using Pearson correlation analysis. Analysis of covariance (ANCOVA) was additionally performed to account for sex.

Results:

Athletes had significantly lower OBS values than sedentary individuals (−1.19 ± 5.46 vs. 1.23 ± 5.98, p=0.0198). GPx activity was also significantly lower in athletes (0.07 ± 0.02 vs. 0.09 ± 0.03 U/mg protein, p=0.0026), whereas SOD and CAT did not differ significantly between groups. OBS was not significantly associated with SOD, CAT, or GPx in either group. Among sedentary individuals, OBS showed weak negative correlations with body mass (r=−0.370, p<0.01) and hip circumference (r=−0.390, p<0.01), while no significant anthropometric associations were observed among athletes.

Conclusion:

Athletes demonstrated lower dietary-derived oxidative balance scores and GPx activity compared with sedentary individuals. The absence of associations between OBS and enzymatic oxidative stress biomarkers suggests that dietary oxidative balance alone may not adequately reflect redox status in physically active individuals.

Keywords: *oxidative balance score; oxidative stress; athletes*

POSTER

Title: Acute Neuromuscular Responses Following 5-km and Half-Marathon Running in Recreational Runners

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Background:

Running distance and intensity place different demands on the neuromuscular system, yet it remains unclear whether shorter, faster running and longer endurance running produce different acute changes in muscle contractile properties and force-generating capacity. This study aimed to compare acute neuromuscular responses following 5-km and half-marathon running in recreational runners.

Methods:

Twenty-eight male recreational runners (36.96 ± 11.35 years) completed both a 5-km and a half-marathon running trial 10 days apart in a within-subject repeated-measures design. Vastus medialis maximal radial displacement (Dm), contraction time (Tc), and contraction velocity (Vc) were assessed using tensiomyography before and after each trial. Quadriceps maximal voluntary isometric contraction (MVIC) was assessed using isometric dynamometry. Pre-to-post changes were examined using repeated-measures analyses of variance, with an additional baseline-adjusted linear mixed-effects model applied to Dm because baseline values differed between trial days.

Results:

The 5-km trial was completed at a faster pace than the half-marathon (245.37 ± 35.25 vs. 275.65 ± 32.06 s/km, $p < 0.001$) and elicited higher mean and peak heart rates. Dm showed a significant running trial × time interaction in the unadjusted analysis ($p = 0.024$), with a greater increase following the half-marathon. However, after adjustment for baseline Dm, the effect of running trial was no longer significant ($p = 0.238$). Across both trials, Tc decreased ($p = 0.012$), Vc increased ($p < 0.001$), and MVIC decreased ($p < 0.001$), with no significant running trial × time interactions for these outcomes.

Conclusion:

Both 5-km and half-marathon running produced immediate changes in vastus medialis contractile properties and quadriceps maximal isometric strength. Although the two trials differed in running pace and cardiovascular response, the magnitude of pre-to-post changes in Tc, Vc, and MVIC did not differ significantly between distances. These findings indicate that acute post-running responses vary according to the neuromuscular parameter assessed rather than consistently according to running distance.

Keywords: *tensiomyography; maximal voluntary isometric contraction; neuromuscular function; endurance running; recreational runners.*

POSTER

Title: From Injury to Recovery: Temporal Dynamics of the Endothelial Glycocalyx After Cardiac Surgery

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Background:

The endothelial glycocalyx (eGC) is a dynamic protective layer involved in vascular permeability, microcirculation, and inflammatory regulation. Cardiac surgery, particularly with cardiopulmonary bypass (CPB), exposes the endothelium to mechanical, inflammatory, oxidative, and ischemia–reperfusion stress, which may lead to eGC degradation. This review aimed to characterize the temporal dynamics of eGC injury and recovery after cardiac surgery using circulating syndecan-1, heparan sulfate, and hyaluronan.

Methods:

A structured literature review was performed in PubMed, Scopus, and Web of Science. The search strategy combined terms related to the eGC and selected biomarkers with cardiac surgery, CPB, coronary artery bypass grafting, and valve surgery. Identified studies were classified according to a predefined MASTER matrix.

Results:

The available evidence was heterogeneous. In some studies, syndecan-1 peaked during or immediately after CPB or upon ICU admission, followed by a partial decline at 24 hours. In contrast, another study reported persistently elevated syndecan-1 levels up to 72 hours after surgery. Findings for heparan sulfate and hyaluronan were similarly heterogeneous, with some studies reporting a decrease in heparan sulfate at 24 hours, while others found persistently elevated levels at 48 hours. Prolonged CPB duration was associated with greater syndecan-1 release. Declining biomarker levels do not necessarily indicate complete structural glycocalyx recovery.

Conclusion:

eGC injury after cardiac surgery is a rapid, phase-dependent process followed by variable temporal changes in circulating degradation products. Multimodal, temporally standardized assessment combining circulating biomarkers with functional and structural measures is needed to better characterize eGC injury and recovery.

Keywords: *glycocalyx; cardiopulmonary bypass; coronary artery bypass; syndecan-1*

POSTER

Title: Cystic Fibrosis Transmembrane Conductance Regulator as a Potential Mediator of Hormone-dependent Cervical Mucus Properties

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Background:

Cystic fibrosis transmembrane conductance regulator (CFTR) is a transmembrane ion channel involved in mucus hydration and function in the lungs, intestine and other tissues. Emerging evidence suggests that CFTR is expressed in the animal cervical tissue and human endocervical cells *in vitro* and its expression and activity may be modulated by sex hormones, particularly estrogens. A study on *Rhesus macaque* model showed that in vitro inhibition of the CFTR ion channel leads to dehydrated and thicker cervical mucus secretions. The aim of this study was to assess the CFTR expression in the human endocervical epithelium and its correlation with circulating sex hormone levels in order to examine if CFTR may be a potential molecule involved in the hormonal regulation of human cervical mucus properties relevant for human fertility.

Methods:

This observational cross-sectional study was performed on the endocervical tissue of 18 women undergoing total hysterectomy for benign indications. CFTR mRNA and protein expression were assessed by RTqPCR and immunofluorescence, respectively, and correlated with serum estradiol concentrations and VAMP8 (vesicle-associated membrane protein 8) mRNA expression in the same samples.

Results:

CFTR mRNA and protein were detected in all endocervical samples, with CFTR immunofluorescence predominantly localized to the apical region of glandular epithelial cells. Estradiol concentration showed positive but non-significant correlations with CFTR mRNA expression and CFTR fluorescence intensity, with stronger associations observed in follicular-phase samples. Additionally, significant positive correlation was found between mRNA expression levels of CFTR and VAMP8, a molecule involved in the vesicular trafficking of CFTR.

Conclusion:

CFTR mRNA and protein expression in human endocervical epithelium shows a positive trend in relation to estradiol levels. These findings suggest that CFTR may contribute to the regulation of cervical mucus properties relevant for fertility, potentially through mechanisms involving intracellular trafficking.

Keywords: *CFTR; estradiol; human endocervix; fertility*

POSTER

Title: Serum Copper and Zinc as Potential Monitoring Biomarkers in Multiple Sclerosis

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Background:

Despite decades of research, serum copper (Cu) and zinc (Zn) have not been established as clinical biomarkers in multiple sclerosis (MS), largely because published studies disagree on the direction of change — some report elevated, others reduced, and others unchanged levels in MS patients. This study aimed to summarize the existing evidence on Cu and Zn in MS and to evaluate these parameters in a previously unstudied population from Eastern Croatia.

Methods:

We conducted a narrative review of previously published case-control and cohort studies reporting serum or plasma Cu and Zn concentrations in MS patients. In parallel, we performed our own case-control study, including 67 MS patients and 67 matched healthy controls from the Clinical Hospital Center Osijek. Serum Cu and Zn were measured by atomic absorption spectroscopy, and individual values were additionally classified as within or outside standard laboratory reference ranges.

Results:

The reviewed literature showed varying, and at times conflicting findings regarding Cu and Zn levels in MS patients. In our population, both Cu and the Cu-to-Zn ratio were significantly reduced in MS patients, while Zn was significantly elevated, compared to healthy controls. Despite these group-level differences, individual classification relative to reference ranges showed no significant difference between MS patients and controls, indicating that the observed shift did not translate into a higher rate of out-of-range values at the individual level.

Conclusion:

The persistent inconsistency across studies, together with the discordance between group-level and individual-level findings in our data, highlights that additional research is needed to determine whether Cu and Zn could serve as reliable monitoring parameters at the individual patient level. Further studies with consistent methodology are needed to clarify their potential clinical value.

Keywords: *multiple sclerosis; copper; zinc; biomarkers; trace elements*

POSTER

Title: Anthropometric Indicators and Physical Fitness in Adolescents: Relevance for Health and Functional Assessment

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Background:

Adolescence is characterized by substantial biological and physiological changes that may influence body composition and physical fitness. Monitoring anthropometric and fitness indicators may provide valuable information on the health and functional status of young people. This study aimed to examine associations between anthropometric characteristics and physical fitness in high school students and to determine sex-related differences.

Methods:

This cross-sectional study included 62 high school students, 31 males and 31 females. Anthropometric measurements included body height, body mass, body mass index (BMI), and waist circumference. Physical fitness was assessed using handgrip strength, standing long jump, 4 × 10 m shuttle run, 20 m sprint, and the 20 m progressive shuttle run test. Sex differences were examined using the Mann–Whitney U test. Associations between variables were assessed using Spearman's rank correlation, while multiple linear regression was used to examine whether anthropometric characteristics predicted physical fitness outcomes.

Results:

Male students had greater body height, body mass, waist circumference, handgrip strength, and standing long jump performance and achieved better results in speed and agility tests ($p < 0.05$). BMI did not significantly differ between sexes ($p = 0.06$). Body mass was positively correlated with handgrip strength in females ($\rho = 0.46$, $p = 0.02$) and males ($\rho = 0.57$, $p = 0.01$). Standing long jump was negatively correlated with 20 m sprint time in females ($\rho = -0.55$, $p = 0.01$) and males ($\rho = -0.65$, $p = 0.01$). No anthropometric variable was a significant predictor of physical fitness outcomes.

Conclusion:

Anthropometric characteristics were associated with several components of physical fitness in adolescents, with clear sex-related differences. Combined assessment of anthropometric and physical fitness indicators may provide useful information for monitoring functional status and supporting health-oriented physical activity strategies in adolescents.

Keywords: *adolescents; anthropometry; physical fitness; functional capacity; health assessment*

POSTER

Title: Effects of Mouthguard Use on Aerobic Capabilities

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* Presenting author

Background:

The aim of this research is to investigate, within the population of athletes engaged in martial arts who regularly use mouthguards during their training, the impact of mouthguards on ventilation, metabolic and functional parameters during a progressive maximal load test. Additionally, we aim to examine the influence on ventilatory thresholds and maximum aerobic capacity (VO2max).

Methods:

This randomized crossover study included 10 healthy male combat sport athletes (boxing, kickboxing, and Brazilian jiu-jitsu), aged 18–28 years, who regularly used mouthguards. Each participant completed two maximal treadmill exercise tests, one with and one without a mouthguard, with the order of conditions randomized and several days between tests. Ventilatory, metabolic, and cardiovascular parameters were measured continuously by cardiopulmonary exercise testing. Maximal voluntary ventilation (MVV), breathing reserve (BR), VO2max, minute ventilation, respiratory frequency, heart rate, exercise duration, achieved speed, and parameters at the first and second ventilatory thresholds were assessed. Perceived exertion was recorded using the Borg scale. Paired t-tests were used for comparisons, with $p<0.05$ considered significant.

Results:

Mouthguard use did not significantly affect VO2max, achieved speed, exercise duration, or heart rate, nor did it significantly affect the measured parameters at the first or second ventilatory threshold. MVV was significantly lower with a mouthguard (189.06±28.17 vs. 217.41±33.58 L/min; P=0.035), as was BR (63.26±29.69 vs. 85.66±29.73 L/min; P=0.020). Perceived exertion was slightly higher with a mouthguard (7.8±1.32 vs. 7.2±0.92), but the difference was not statistically significant (P=0.051).

Conclusion:

Mouthguard use was not associated with clinically relevant reductions in maximal aerobic capacity or exercise performance. Although MVV and BR were significantly reduced, the main cardiopulmonary responses during progressive maximal exercise and at ventilatory thresholds were not significantly altered. These findings support the use of mouthguards in combat sports without a substantial adverse effect on maximal aerobic performance.

Keywords: *mouthguard; VO2max; ventilatory thresholds*

POSTER

Title: Systemic Vascular Dysfunction in Chronic Kidney Disease: Insights from Hemodynamic and Microvascular Assessment

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Background:

Chronic kidney disease (CKD) is associated with an increased risk of cardiovascular complications, but the interaction between systemic hemodynamic, arterial function and microvascular endothelial dysfunction remains incompletely understood. This study aimed to compare macrovascular, microvascular and body composition parameters in patients with CKD, hypertension (HTN), and healthy controls.

Methods:

This cross-sectional study included 75 participants: 22 with CKD, 27 with HTN, and 26 healthy controls. Participants with diabetes or current smoking were excluded. Hemodynamic parameters were assessed using impedance cardiography (ICG). Microvascular function was evaluated by laser Doppler flowmetry (LDF), including sodium nitroprusside-induced dilation (SNPID), acetylcholine-induced dilation (AChID) and post-occlusive reactive hyperemia (PORH) following one-minute vascular occlusion. Body composition was determined using bioelectrical impedance analysis. Differences between groups were assessed using one-way analysis of variance with post hoc testing.

Results:

Compared with controls, CKD patients had higher systolic blood pressure (143±14 vs. 125±8 mmHg, p=0.0001), diastolic blood pressure (85±13 vs. 77±8 mmHg, p=0.0073), mean arterial pressure (100±13 vs. 88±8 mmHg, p=0.0002) and systemic vascular resistance index (2452±501 vs. 1935±549 $dyn \cdot s \cdot cm^{-5} \cdot m^{-2}$, p=0.0057). Stroke volume (95±19 vs. 118±18 mL, p=0.0209) and total arterial compliance index (0.9±0.2 vs. 1.15±0.2 $mL/m^2/mmHg$, p=0.0053) were lower in CKD. HTN patients also demonstrated higher systolic and diastolic blood pressure and mean arterial pressure, together with reduced arterial compliance. CKD patients had impaired endothelium-dependent microvascular dilation, reflected by lower AChID than controls (9.4±5.6 vs. 14.15±5.9, p=0.0116). BMI was higher in the HTN group than in controls (28.4±4.3 vs. 25.8±2.9 kg/m^2, p=0.0435).

Conclusion:

CKD was characterized by concomitant macrovascular and microvascular dysfunction, including increased vascular resistance, reduced arterial compliance, diminished stroke volume and impaired endothelium-dependent vasodilation. These findings support comprehensive vascular assessment as an important component of cardiovascular risk evaluation in patients with CKD.

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Keywords: *chronic kidney disease; vascular dysfunction; microcirculation; endothelial dysfunction; arterial compliance*

POSTER

Title: Combined Renoprotective Effects of Hyperbaric Oxygen Preconditioning and 4,4' Dimethoxychalcone in Ischemia-Reperfusion-Induced Acute Kidney Injury: A Pilot Study

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Background:

Renal ischemia-reperfusion (I/R) injury is an important cause of acute kidney injury (AKI), leading to impaired renal function, altered renal hemodynamics, and tubular damage. Hyperbaric oxygen (HBO) preconditioning may increase renal resistance to I/R injury, whereas 4,4'-dimethoxychalcone (DMC) has cytoprotective effects. This pilot study aimed to evaluate the renoprotective effects of HBO preconditioning combined with DMC in experimental I/R-induced AKI.

Methods:

Thirty adult male Wistar rats were randomized into five groups (n=6 each): SHAM, AKI, DMC, HBO, and HBO+DMC. AKI was induced by right nephrectomy followed by 45-min left renal artery occlusion. HBO preconditioning consisted of four 60-min exposures to 100% O_2 at 2.0 ATA, 12 h apart, with the final session ending 12 h before AKI induction. DMC (100 mg/kg) was administered 5 min before reperfusion and 30-45 min before euthanasia. Renal function (diuresis and creatinine, urea, and phosphate clearances), renal blood flow (RBF), renal vascular resistance (RVR), urinary neutrophil gelatinase-associated lipocalin (NGAL), and renal morphology were assessed after 24 h. SHAM and AKI were compared using an unpaired t-test; treatment groups were analyzed by one-way ANOVA with Dunnett's post hoc test versus AKI.

Results:

Compared with SHAM, AKI significantly reduced diuresis ($p=0.0117$), creatinine clearance ($p<0.0001$), urea clearance ($p<0.0001$), phosphate clearance ($p=0.0002$), and RBF ($p=0.0404$), while increasing RVR ($p=0.0234$) and urinary NGAL ($p=0.0077$). Compared with untreated AKI, DMC increased RBF ($p=0.0026$) and reduced RVR ($p<0.0001$), while HBO increased diuresis ($p=0.0148$), reduced RVR ($p=0.0003$), and decreased NGAL ($p=0.0426$). Combined HBO+DMC improved diuresis ($p=0.0001$), creatinine clearance ($p=0.0236$), urea clearance ($p=0.0283$), phosphate clearance ($p=0.0028$), and RBF ($p=0.0016$), while reducing RVR ($p<0.0001$) and NGAL ($p=0.0238$). Histopathological findings were consistent with the functional results.

Conclusion:

Combined HBO preconditioning and DMC produced the broadest renoprotective profile, improving renal filtration and hemodynamics while reducing tubular injury. These preliminary findings support further investigation of this combined approach in I/R-induced AKI.

Keywords: *acute kidney injury; renal ischemia-reperfusion; hyperbaric oxygen preconditioning; 4,4'-dimethoxychalcone; renoprotection*

POSTER

Title: Random Forest Machine Learning Detection of Iron Oxide-Induced Changes in Yeast Nuclear Architecture Using Wavelet Energy Features

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Background:

Sublethal exposure to iron oxide may induce subtle alterations in cellular and nuclear organization before overt morphological damage becomes visible. Conventional visual assessment of microscopy images may therefore lack sufficient sensitivity for early toxicity detection. This study aimed to develop a machine-learning model based on discrete wavelet transform features for identifying subtle changes in the nuclear architecture of *Saccharomyces cerevisiae* cells exposed to sublethal doses of iron oxide.

Methods:

Regions of interest corresponding to cell nuclei were extracted from digital micrographs of control and iron oxide-exposed yeast cell groups. Nuclear image regions were analyzed using the discrete wavelet transform, which decomposed the images through combinations of low-pass and high-pass filtering. Wavelet coefficient energies were calculated for the resulting approximation and detail components. These features were used to train and evaluate a random forest classifier developed in the Google Colab environment using the scikit-learn library. Model performance was assessed using the area under the receiver operating characteristic curve, Matthews correlation coefficient, F1 score, and additional classification metrics.

Results:

All wavelet coefficient energies were significantly increased in iron oxide-exposed cells compared with controls ($p < 0.01$), indicating a marked alteration in nuclear image texture following exposure. The random forest model distinguished iron oxide-exposed cells from controls with an area under the curve exceeding 0.80, despite the absence of clearly detectable nuclear changes by visual inspection. The most informative feature was the wavelet energy derived from the high-high component, obtained through successive high-pass filtering in both image dimensions.

Conclusion:

Wavelet-based image features combined with random forest classification detected subtle iron oxide-associated changes in yeast nuclear architecture that were not readily observable by the human eye. The developed code provides a foundation for more advanced artificial intelligence platforms for sensitive assessment of iron-associated cellular toxicity.

Keywords: *Saccharomyces cerevisiae; iron oxide toxicity; discrete wavelet transform; random forest; nuclear architecture*

POSTER

Title: Stacked Ensemble Detection of Oxidopamine-Induced Nuclear Texture Alterations in Yeast Cells

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Background:

Sublethal cellular injury may produce changes in nuclear architecture that are not sufficiently pronounced for reliable visual detection. Quantitative texture analysis combined with machine learning may therefore provide sensitive markers of early toxic effects. This study investigated whether run-length matrix features could identify alterations in the nuclear organization of *Saccharomyces cerevisiae* cells exposed to sublethal doses of oxidopamine, a proapoptotic agent.

Methods:

Nuclear regions of interest were extracted from digital micrographs of control and oxidopamine-exposed yeast cells. Run-length matrix analysis was used to calculate short-run emphasis, long-run emphasis, run-length non-uniformity, and gray-level non-uniformity, representing fine and coarse texture patterns and the heterogeneity of run lengths and gray-level distributions. A stacked ensemble was constructed using random forest and gradient boosting classifiers as base learners and L2-regularized logistic regression as the meta-learner. Out-of-fold class probabilities generated by the base models were used to train the meta-learner. Model development was performed in Python using scikit-learn, with stratified cross-validation applied to support robust performance estimation and reduce overfitting.

Results:

Highly statistically significant differences in run-length matrix features were detected between the two cell groups ($p < 0.01$), indicating a substantial increase in nuclear textural heterogeneity following oxidopamine treatment. The stacked ensemble distinguished exposed cells from controls with an area under the receiver operating characteristic curve exceeding 0.85. Short-run emphasis was the most informative feature, followed by run-length non-uniformity, suggesting that oxidopamine exposure predominantly affected fine-scale nuclear texture and the variability of homogeneous intensity runs. The F1 score, Matthews correlation coefficient, and other classification metrics also indicated satisfactory predictive performance.

Conclusion:

Run-length matrix features combined with stacked ensemble learning enabled detection of subtle oxidopamine-associated alterations in yeast nuclear architecture. This framework may support the development of advanced artificial intelligence methods for image-derived medical signal analysis and toxicity assessment in physiological sciences.

Keywords: *Saccharomyces cerevisiae; oxidopamine; run-length matrix; stacked ensemble learning; nuclear texture*

POSTER

Title: Within-Session Variability During Indoor Radiofrequency Electromagnetic Field Measurements

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Background:

Radiofrequency electromagnetic fields (RF-EMF) are non-ionizing fields in the 100 kHz–300 GHz range widely used in telecommunications and wireless technologies. Their widespread use sustains interest in exposure characterization and possible biological effects. The NextGenerationEU-funded INDEKS-RF project at the Faculty of Veterinary Medicine, University of Zagreb, investigates RF-EMF exposure across academic environments. Because indoor fields vary spatially and temporally, we assessed whether repeated reference measurements could identify within-session variability relevant to estimated spatial differences.

Methods:

Measurements were conducted in an unoccupied room using a Narda FieldMan with an isotropic EFD-0691 electric-field probe (100 kHz–6 GHz). Broadband electric field strength was measured at a probe height of 1.5 m. Eleven 6-min cycles were recorded at 1-s intervals. Four spatial points were measured sequentially, and a reference position was remeasured throughout the session. In an exploratory sensitivity analysis, consecutive reference measurements were linearly interpolated to estimate time-matched reference values for each spatial measurement. Spatial differences relative to the reference were expressed in decibels.

Results:

Six-minute mean electric-field strength at the repeated reference position ranged from 0.2269 to 0.2836 V/m over the measurement sequence, corresponding to a 1.94 dB difference. Using time-matched reference estimates altered the estimated spatial contrasts relative to the reference by up to 1.33 dB. This degree of within-session variability was sufficient to affect the magnitude of the apparent spatial differences within the same sequential survey.

Conclusion:

Repeated reference measurements provided a practical check of within-session stability and showed that within-session variability can influence estimated spatial differences during sequential indoor RF-EMF measurements. These findings are informing further development of the INDEKS-RF protocol for environmental RF-EMF characterization.

Keywords: *radiofrequency electromagnetic fields; environmental exposure assessment; indoor measurement*

POSTER

Title: Effects of Three-Week Carnosine-Enriched Chicken Consumption on Neuromuscular Function and Physical Performance in Competitive Athletes

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Background:

Carnosine is a naturally occurring dipeptide predominantly found in skeletal muscle and has been suggested to improve muscle function, particularly during high-intensity activity. The growing use of dietary supplements in sports has increased interest in nutritional strategies that may enhance skeletal muscle function, including approaches targeting muscle carnosine availability. This randomized interventional trial investigated whether a three-week intervention with carnosine-enriched chicken meat could influence neuromuscular function, muscle strength and cardiopulmonary exercise capacity in competitive athletes.

Methods:

Thirty-five male athletes aged 18-45 were randomly allocated to a control group receiving conventional chicken (n=16) or an intervention group consuming carnosine-enriched chicken (n=19). Before and after the three-week protocol, participants underwent hand-grip strength assessment, integrated electromyography (iEMG), and cardiopulmonary exercise testing with spiroergometry. Blood samples were additionally analyzed for aldolase A (ALDOA), creatine kinase MM (CK-MM) and citrate synthase (CS).

Results:

Post-intervention, iEMG activity of the non-dominant hand was significantly higher in the carnosine group compared to controls (p=0.041). Hand-grip strength of both arms demonstrated a non-significant tendency toward improvement following carnosine intake. Maximum running velocity was significantly increased in the carnosine group compared to the control group (p=0.003). In contrast, the control group exhibited a significant reduction in time spent above the compensatory anaerobic threshold following the intervention (p=0.024). Post-intervention concentrations of ALDOA and CK-MM were significantly higher in the carnosine group compared with the control group (p=0.045 and p=0.023), whereas post-intervention CS concentrations decreased from the baseline (p=0.012).

Conclusion:

Three weeks of carnosine-enriched chicken meat consumption was associated with enhanced neuromuscular activation and greater maximum running velocity in competitive athletes. These findings support further investigation of dietary carnosine as a potential nutritional strategy for athletes engaged in high-intensity exercise.

Keywords: *athletes; carnosine; functional food; electromyography*

POSTER

Title: Non-neuronal TRPA1 Mediates Communication from Dental Pulp Cells to Sensory Neurons in a Co-Culture Model

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Background:

Dental pulp is a richly innervated tissue that plays an important role in oral sensory physiology by conferring thermo- and mechanosensitivity upon teeth. These sensory functions are of great clinical significance, as pulpal inflammation and injury often manifest in hypersensitivity to external stimuli and may cause severe pain. In a recently published article, our group showed that the cold sensitive ion channel TRPA1 is upregulated in dental pulp cells during inflammation. The aims of the present study were to establish a co-culture model suitable for the study of intercellular signaling, and to show that activation of TRPA1 on dental pulp cells can lead to signal transmission to nearby neurons.

Methods:

Dental pulp cells (DPCs) were isolated from healthy human third molars, and sensory neurons were isolated from the trigeminal ganglia of C57BL/6 mice. To study ion channel activity, intracellular Ca^{2+} imaging was performed using the Ca^{2+}-sensitive fluorescent dye Fura-2 in a microscopy setting. Individual ion channels and receptors were investigated by application of specific pharmacological agonists and antagonists. To differentiate DPCs from murine cells and neurons from non-neuronal cells, a second fluorescent dye and 50 mM KCl were utilized, respectively.

Results:

DPCs and a fraction of WT neurons showed robust $[Ca^{2+}]_{IC}$ transients in response to 100 µM AITC, while TRPA1 KO neurons did not respond when cultured alone. In a co-culture setting however, TPRA1 KO neurons produced smaller, delayed $[Ca^{2+}]_{IC}$ peaks. This response was found to be mediated by ATP release from DPCs, as it was significantly inhibited by application of 1 µM A317491, a specific antagonist of the $P2X_3$ receptor.

Conclusion:

Our model demonstrates that TRPA1 may play an important role in sensory functions of the dental pulp, especially when its expression is greatly increased, such as in inflammatory conditions.

Keywords: *dental pulp cells; TRPA1; co-culture; intercellular communication*

POSTER

Title: Rapid GLP-1 Response after Liquid Meal intake: A single Arm Pre-post Pilot study

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Background:

Glucagon-like peptide-1 (GLP-1) is an incretin hormone released after nutrient ingestion. Preparing and eating mixed-food meals is useful for assessing postprandial enteroendocrine responses but may be less practical in field research where a liquid nutritional supplement may be a simpler solution. Aim: To evaluate whether Fresubin Energy Drink (kcal 300; CHO 37.6g; fat 11.6g; protein 11.2g) induces an early postprandial GLP-1 response.

Methods:

Five adult patients (3F, 2M) received Fresubin Energy Drink after fasting baseline blood sampling. Blood was collected into BD P800 blood collection system for plasma metabolic biomarker preservation. A second blood sample was obtained 30 minutes after intake. Plasma GLP-1 was measured by ELISA on the ThunderBolt platform. Fasting and 30-minute postprandial values were compared descriptively and by exploratory paired analysis.

Results:

GLP-1 increased in all patients 30 minutes after Fresubin Energy Drink intake. Mean fasting GLP-1 was 11.05±3.69 pmol/L, increasing to 22.54±8.98 pmol/L. The mean absolute increase was 11.49±7.37 pmol/L, corresponding to a 2.06±0.53-fold rise from baseline. Individual increases ranged from 5.86 to 22.78. In exploratory paired analysis, the increase was significant by paired t-test, $p=0.025$. The observed paired effect size was large (Cohen’s $dz \approx 1.57$), suggesting possible robust within-subject signal despite the pilot sample size.

Conclusion:

Although the study was limited by the small sample size and absence of a control meal, the uniform GLP-1 increase in all participants and the large paired effect size suggest that Fresubin Energy Drink produces a measurable early GLP-1 response suitable for further evaluation in a larger powered study. These preliminary findings suggest that a standardized liquid nutritional supplement may be a practical alternative stimulus for assessing early GLP-1 secretion, particularly in altitude settings of the project where mixed-meal testing is difficult. Larger studies comparing liquid and mixed-meal protocols are needed.

Acknowledgement: Funded by EU Next generation: HARH project.

Keywords: *GLP-1; energy drink; postprandial response*

POSTER

Title: Do Sprinters and Jumpers Differ in the Acute Countermovement-Jump Response to Isometric Conditioning Protocols? An Exploratory Study

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Background:

Post-activation performance enhancement (PAPE) describes an acute, transient improvement in explosive performance following a high-intensity conditioning activity, and isometric protocols are of particular interest because they require minimal equipment and involve less peripheral fatigue than dynamic alternatives. Sprinters and jumpers present distinct neuromuscular and biomechanical profiles, so the balance between potentiation and fatigue produced by isometric conditioning may differ between disciplines. This exploratory study compared the acute countermovement jump (CMJ) response to isometric conditioning between competitive sprinters and jumpers.

Methods:

Twenty-two competitive track and field athletes (10 sprinters, 12 jumpers; 19.1 ± 3.3 years) were alternately allocated from a randomised list to a 3 × 5 s isometric knee-extension or isometric half-squat protocol (n = 11 each). CMJ height was assessed from flight time using frame-by-frame video analysis, and the percentage change (ΔCMJ) from the best of three pre- and post-conditioning jumps was the primary outcome. Discipline and protocol were analysed jointly in one additive model, with age as a covariate.

Results:

Sprinters increased their CMJ (+2.40 ± 4.46%) whereas jumpers decreased (−2.02 ± 6.44%). The protocol contrast was small and imprecise (−0.25 percentage points, 95% CI −5.50 to 5.00; p = 0.921). The unadjusted discipline difference was 4.42 percentage points (95% CI −0.46 to 9.30; p = 0.074); after age adjustment this attenuated to 2.51 percentage points (95% CI −2.65 to 7.66; p = 0.320), while age itself remained associated with ΔCMJ (−0.86% per year; p = 0.042).

Conclusion:

The primary discipline comparison was inconclusive and materially attenuated by age adjustment, but the direction of the contrast was consistent across protocols and trial summaries. The observed discipline-dependent pattern is hypothesis-generating and warrants confirmation in adequately powered, controlled trials.

Keywords: *countermovement jump; post-activation performance enhancement; track and field*

POSTER

Title: The Role of Systemic Proinflammatory Interleukin-17A in the Somatization–Depression Axis in Patients with Spondyloarthritis during the Coronavirus Disease 19 Pandemic

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Background:

Depressive symptoms are common in patients with spondyloarthritis (SpA). Their occurrence is influenced by complex mechanisms among clinical, psychological and inflammatory factors, of which the cytokine IL-17 is significantly represented. We investigated whether IL-17A moderated the indirect effect of clinical disease activity on depression through somatization, while controlling for anxiety and demographic covariates in the period of the Coronavirus disease 19 (COVID-19) pandemic.

Methods:

Patients with SpA (N=80) aged from 25 to 80 years (median 53), represented with 74% of females were evaluated using psychometric instruments for depression (Patient Health Questionnaire-9), anxiety (Generalized Anxiety Disorder-7), and non-specific physical or functional symptoms – somatisation (Patient Health Questionnaire-15) from 2021 to 2024. SpA disease activity was indexed using Axial Spondyloarthritis Disease Activity Score (ASDAS). Serum IL-17A was quantified using enzyme-linked immunosorbent assay.

Results:

Hierarchical linear regression explained 40.8% of the variance in depression ($p < 0.001$), with somatization severity ($p = 0.002$) and anxiety severity ($p = 0.002$) emerging as the only independent positive predictors. ASDAS, IL-17A, and baseline demographic characteristics were not independently associated with depression. Moderated mediation analysis demonstrated that IL-17A did not significantly moderate either the pathway from ASDAS to somatization or the pathway from somatization to depression.

Conclusion:

Depression severity in patients with SpA was independently driven by somatization and anxiety during the COVID-19 pandemic. Management strategies for affective distress in SpA should target somatic symptom burden and anxiety alongside primary anti-inflammatory therapies.

Keywords: *COVID-19 pandemic; depression; spondyloarthritis*

POSTER

Title: Cardiorespiratory fitness and quality of life in children between the ages of 9 and 11 participating in intensively organized sport and their peers

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Background:

Physical activity and cardiorespiratory fitness are important components of physical and psychosocial development in school-aged children. The aim of this study was to examine differences in fitness index (FI), maximal oxygen uptake (VO_2max), and quality of life (QOL) between children aged 9–11 years participating in intensively organized sport (IOS) and a non-intensive comparison cohort, and to determine the association between FI and QOL.

Methods:

The study included 112 children, categorized into an IOS group (n = 50) (≥360 min/week) and a control group (n = 62) according to weekly organized training duration. The Harvard step test (HST) was used to assess cardiorespiratory fitness, and the self-reported Pediatric Quality of Life Inventory™ (PedsQL™) was used to assess QOL. Student's t-test was used to compare FI and VO_2max, calculated from the HST, as well as QOL scores between groups. Pearson's correlation was used to examine associations between FI and QOL related to physical and psychosocial functioning.

Results:

FI was significantly higher in the IOS group ($p = 0.025$), while no significant difference in VO_2max was found between groups ($p = 0.492$). Higher scores for physical and psychosocial functioning were observed in the IOS group, but these were not statistically significant. No significant association was found between FI and QOL related to physical functioning ($r = –0.12$; $p = 0.207$) or psychosocial functioning ($r = 0.10$; $p = 0.268$).

Conclusion:

Although FI was significantly higher in the intensively PA group, it was not significantly associated with QOL in either domain. These findings suggest a complex relationship between growth and development, cardiorespiratory fitness, and QOL in children, potentially influenced by sociodemographic and cultural factors. Further research with larger samples and different age groups is warranted.

Keywords: *cardiorespiratory fitness; children; physical activity; quality of life*

POSTER

Title: Carnosine-Enriched Chicken Meat Promotes an Anti-Inflammatory Profile in Patients with Chronic Coronary Syndrome

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Background:

Endothelial dysfunction, which plays a central role in the pathogenesis of cardiovascular disease, may result from multiple factors, including dyslipidemia, hypertension, proinflammatory cytokines, and oxidative stress. Together, impaired endothelial function and a proinflammatory profile contribute substantially to the progression of chronic coronary syndrome (CCS). Carnosine supplementation in the form of a functional food could have a favorable effect on inflammation in patients with CCS. This study investigated the effects of the consumption of carnosine-enriched chicken meat on inflammatory mediators in patients with CCS.

Methods:

This was a double-blind, placebo-controlled clinical study that included 38 CCS participants randomized into two groups: the Control group (N = 19), who consumed regular chicken meat and Carnosine group (N = 19), who consumed the carnosine-enriched chicken meat for 3 weeks. Anti- and proinflammatory cytokine levels were assessed before and after the intervention using ProcartaPlex Human TGF-β1 Simplex, ProcartaPlex Human C3a Simplex, and Human ProcartaPlex Mix&Match 7-plex magnetic bead panels on the Luminex 200 platform. Serum concentrations of intercellular adhesion molecule 1, vascular cell adhesion molecule 1, E-selectin, endoglin, monocyte chemoattractant protein 1, transforming growth factor beta 1, tumor necrosis factor alpha (TNF-α), complement component 3a, interferon gamma, interleukin 6 (IL-6), interleukin 10, interleukin 17A, and interleukin 23 were quantified.

Results:

In the Control group, serum cytokine and chemokine levels remained unchanged following the consumption of regular chicken meat, whereas significant decreases in TNF-α and IL-6 levels were observed in the Carnosine group. Following the dietary intervention, endoglin levels were significantly lower in the Carnosine group than in the Control group after adjustment for baseline values.

Conclusion:

The consumption of carnosine-enriched chicken meat reduced serum concentrations of TNF-α, IL-6 and endoglin. Reduced levels of proinflammatory cytokines may suggest a protective effect of carnosine on the progression of atherosclerosis in CCS patients.

Keywords: *carnosine; chronic coronary syndrome; functional food*

POSTER

Title: Characterizing the Vascular Phenotype of Prehypertension: Preliminary Findings

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Background:

Prehypertension (PreHT; SBP 130–139 mmHg and/or DBP 85–89 mmHg) may represent an early stage of vascular impairment preceding the development of overt hypertension. However, vascular alterations may not occur uniformly across different vascular compartments. We aimed to explore whether microvascular reactivity and arterial stiffness show distinct patterns across the blood pressure continuum.

Methods:

Preliminary data from an ongoing study were analyzed in participants (N=38) classified according to 24-hour ambulatory blood pressure monitoring into normotension (NT; SBP ≤129, DBP ≤84 mmHg), prehypertension (PreHT; SBP 130–139, DBP 85–89 mmHg), and untreated stage 1 hypertension (HT; SBP 140–159, DBP 90–99 mmHg). Forearm skin microvascular function was assessed using laser Doppler flowmetry, including post-occlusive reactive hyperemia (PORH), acetylcholine-induced vasodilation (AChID), and sodium nitroprusside-induced vasodilation (SNPID). Arterial stiffness was assessed by pulse wave velocity (PWV).

Results:

PORH differed significantly across groups (P=0.046), decreasing stepwise from NT to PreHT and HT (148.2±22.4, 131.8±35.3, 115.1±30.0): reductions of 11.1% in PreHT and 22.3% in HT versus NT, and a further 12.6% in HT versus PreHT; the NT-HT difference was significant (P=0.042). AChID was lower in PreHT and HT than NT (13.2±4.4, 11.0±4.3, 11.5±3.5; P=0.414), reduced by 16.6% and 13.1%, while SNPID was comparable across groups (16.7±7.5, 16.5±6.9, 16.2±4.4; P=0.984). PWV increased progressively across groups (5.39±0.78, 6.15±0.73, 6.42±0.96 m/s; P=0.020), higher in HT than NT (P=0.019), not significantly different between PreHT and NT (P=0.080) or HT and PreHT (P=0.432).

Conclusion:

These preliminary findings suggest that prehypertension may already be associated with early alterations in multiple components of the vascular system. Further analysis of the ongoing study is needed to confirm these findings.

Keywords: *prehypertension; endothelium; laser Doppler flowmetry; arterial stiffness; pulse wave velocity*

POSTER

Title: The Role of Uroguanylin in Genetic Predisposition of Type 2 Diabetes Mellitus

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Background:

Type 2 diabetes mellitus (T2DM) is one of the most common metabolic diseases with a strong genetic predisposition. We hypothesized that mutations in the *GUCA2B* gene contribute to the genetic predisposition to T2DM.

Methods:

The study included 51 healthy individuals, 50 patients with T2DM, and 13 individuals with prediabetes. DNA was isolated from buccal swab samples, and the GUCA2B gene was amplified and sequenced. The frequency of mutations was analyzed, along with their association with anthropometric, clinical, and laboratory parameters.

Results:

Mutations with higher frequency in affected individuals included rs1646094713 (44% vs. 6%), rs2297566 (35% vs. 29%), rs571840179 (13% vs. 0%), rs1646095639 (10% vs. 0%), rs35728635 (10% vs. 0%), rs1201664458 (6% vs. 0%), rs1329734003 (15% vs. 0%), rs2522569841 (10% vs. 0%) and rs1470615782 (15% vs. 6%). Higher incidence of all mutations was found in participants from northern compared to southern regions of Croatia with pronounced gender differences. All mutations were present in higher percentage in T2DM patients with genetic predisposition except rs35728635. In patients with T2DM with GUCA2B mutations the statistically significant difference was found in the systolic blood pressure, fasting blood glucose concentration and HbA1c (glycated hemoglobin) which were higher in males; triglycerides were higher in females, but LDL (low-density lipoproteins) was higher in both genders.

Conclusion:

GUCA 2B gene participates in development of T2DM with gender dependent consequences in homeostasis of glucose and lipids.

Acknowledge: Financed by Adris Foundation and National Recovery and Resilience Plan 2021-2026 research grant (10106-25-2985.

Keywords: *uroguanylin; GUCA2B; type 2 diabetes mellitus; glucose metabolism regulation*

POSTER

Title: Students' Descriptions of Blood Coagulation in Master's Theses: Anchored in the Textbook?

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Background:

The understanding of *in vivo* hemostasis has evolved substantially since the mid-20th century, leading to the contemporary cell-based model of blood coagulation. However, *Guyton and Hall Textbook of Medical Physiology*, the required textbook for the Physiology course at the Schools of Medicine and Dental Medicine, University of Zagreb, still describes secondary hemostasis primarily through the traditional coagulation cascade model, in which coagulation is initiated via the intrinsic and extrinsic pathways that converge into a common pathway. The aim of this study was to determine whether students' descriptions of coagulation in their master's theses reflect the model presented in the required Physiology textbook.

Methods:

The Digital Academic Archive and Repository of the Schools of Medicine and Dental Medicine was searched in July 2026 for publicly available master's theses in which hemostasis was relevant to the topic and therefore described in the introductory section. The search was performed using the terms *coagulation*, *hemostasis*, *anticoagulants*, *platelets*, *bleeding*, and *physiology*.

Results:

A total of 22 master's theses were included, 14 from the School of Medicine and 8 from the School of Dental Medicine, published between 2014 and 2024. The traditional coagulation model, consistent with that presented in *Guyton and Hall*, was used to describe coagulation in 43% (6/14) of medical and 75% (6/8) of dental students' theses. All students who used this model cited *Guyton and Hall* as either their sole source or one of the references. Even when references describing the contemporary cell-based coagulation model were cited, this information was not incorporated into the students' descriptions.

Conclusion:

The findings suggest that students continue to rely on the required Physiology textbook as a reference source when describing physiological processes, even at the end of their undergraduate education. They also highlight the importance of encouraging and guiding students in the use of primary literature.

Keywords: *blood coagulation; physiology textbooks; physiology education*

POSTER

Title: Establishment of adult mouse brain-derived astrocyte cultures for metabolic studies

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Background:

Astrocytes maintain central nervous system homeostasis through metabolic support, neurotransmitter clearance, extracellular ion buffering, and modulation of neuronal activity. Primary astrocyte cultures are widely used to study glial biology, although culture conditions can substantially alter astrocyte phenotype and metabolism. We aimed to establish an adult mouse brain-derived astrocyte culture with reduced exogenous cAMP and trophic stimulation by omitting forskolin and glial cell line-derived neurotrophic factor, to limit culture-induced metabolic modulation that could confound subsequent metabolic analyses.

Methods:

Brains from three adult C57BL/6J mice (≥6 months) were processed individually following transcardial perfusion with phosphate-buffered saline. Cerebral cortices, hippocampi, and hypothalamic were dissected and pooled within each animal, while white-matter-rich tissue was excluded to reduce myelin debris. Tissue was mechanically minced into ≤ 0.5 mm fragments and enzymatically dissociated using Accutase supplemented with deoxyribonuclease I and hyaluronidase. Following two 10-minute incubations at 37 °C with intermittent mixing and gentle trituration, suspensions were passed through a 70-µm cell strainer and centrifuged at 300×g for 5 minutes. Cells were resuspended in Dulbecco's Modified Eagle Medium/F-12 containing 10% fetal bovine serum and penicillin–streptomycin and plated onto Matrigel-coated culture plates containing glass coverslips. Partial medium replacement began at day *in vitro* 5 (DIV5) and continued every 48 hours through DIV 14. Cultures were characterized by immunofluorescence for GFAP and NeuN.

Results:

Primary mixed glial cultures were successfully established from all three adult mice and maintained through DIV14. Immunofluorescence confirmed the presence of GFAP-positive astrocytes (3.87×10^5) with minimal Neun-positive neuronal contamination (1.6×10^4).

Conclusion:

This protocol supports establishment of adult mouse brain-derives astrocyte cultures without use of glial differentiation or growth factors. The approach may provide a useful platform for studies of adult astrocyte biology and metabolism while reducing exogenous cAMP and trophic stimulation during culture.

Acknowledgement: This work was supported by the Croatian Science Foundation project HRZZ IP 2025-02-7524 "The role of bradykinin and glucose transporters in diabetes induced cognitive and neurovascular dysfunction" (CANDy).

Keywords: *adult astrocytes; primary astrocyte culture; cellular metabolism*

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