

3rd Regional Congress of Physiological Societies

and

5th Congress of Croatian Physiological Society

22nd - 24th September 2022, Plitvička Jezera, Croatia

HRVATSKO DRUŠTVO FIZIOLOGA

HDF CPS

CROATIAN PHYSIOLOGICAL SOCIETY

Venue

Hotel Jezero

Josipa Jovića 19
Plitvička jezera

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Co-organizing society

Slovenian Physiological Society

Participating societies

IUPS (International Union of Physiological Societies)
FEPS (Federation of European Physiological Societies)
Hungarian Physiological Society
Austrian Physiological Society
French Society of Physiology
Slovak Physiological Society
American Physiological Society
Bulgarian Physiological Society
The Physiological Society of Italy
Macedonian Society of Physiologists and Anthropologists
The Serbian Physiological Society
Turkish Society of Physiological Sciences

Scientific program

Thursday, September 22nd 2022

10:00 - 16:00 Registration

16:00 - 16:30 Opening ceremony

16:30 - 17:30 Keynote lecture:

Walter F. Boron, MD, PhD (IUPS, CWRU, Cleveland, OH, USA):

Gas diffusion through aquaporins and other membrane proteins

17:30 - 19:05 **Session 1**–Sport physiology–Chairwomen: Lana Ružić, Ines Mrakovčić–Šutić

17:30 - 18:00 Guest speaker: Akos Koller (Hungarian University of Sports Science, Semmelweis University, Budapest, Hungary, and New York Medical College, USA): Increased hemodynamic forces drive the adaptions of the coronary circulation to exercise

18:00 - 18:15 Ines Mrakovčić-Šutić: Perforin-mediated cytotoxicity in professional athletes

18:15 - 18:30 Erna Davidović Cvetko: Sex differences in effects of eight-week increment aerobic exercise program on bone metabolism in young non-athletes

18:30 - 18:45 Marul Ivandić: Effects of aerobic versus resistance exercise on glycemic control stability in patients with diabetes type 1-a cross-over study

18:45 - 18:55 Lana Ružić: Is recovery in hyperoxia after high intensity training in hypoxia an added value?

18:55 - 19:05 Lana Kralj: Skin microvascular response to graded dynamic exercise assessed by wavelet analysis

19:05–19:30 Coffee break

19:30 - 21:35 **Session 2**–Cardiovascular physiology–Chairwomen: Ines Drenjančević, Ana Stupin

19:30 - 20:00 Guest speaker: Ines Drenjančević (University of Osijek, Croatia): Permissive role of angiotensin II in maintaining microvascular reactivity to physiological stimuli

20:00 - 20:15 Ana Stupin: Short-term high-salt loading effects on microvascular endothelial function in salt-resistant humans–specific role of oxidative stress and sympathovagal imbalance

20:15 - 20:45 Guest speaker: Nandu Goswami (Medical University of Graz, Graz, Austria): Circardian rhythms, cardiovascular regulation and falls: current updates and perspectives – online

20:45 - 21:00 Julia Nikolova: Investigation of arterial stiffness and clinical and chemical indicators in Bulgarian young people with high–normal arterial pressure

21:00 - 21:15 Kamil Javorka: Mechanisms of cardiovascular changes of phototherapy in newborns with hyperbilirubinemia

21:15 - 21:25 Nataša Kozina: Assessment of the effect of carnosine supplementation on flow induced dilation mechanisms of a middle cerebral arteries (MCAs) in low salt and high salt fed Sprague-Dawley rats

21:25 - 21:35 Ivana Jukić: Oxidative stress level in children with essential arterial hypertension

Friday, September 23rd 2022

08:30 - 10:50 **Session 3**–Neurophysiology–Chairwomen: Aleksandra Dugandžić, Nina Vardjan

08:30 - 09:00 Guest speaker: Zoltan Ungvari (University of Oklahoma, OK, USA): Age-related cognitive decline: role of endothelial senescence

09:00 - 09:30 Guest speaker: Dmitri Rusakov (University College London, London, UK): Activity--dependent astroglial control of glutamatergic inter–synaptic crosstalk in the brain

09:30 - 10:00 Guest speaker: Nina Vardjan (University of Ljubljana, Ljubljana, Slovenia): Noradrenergic regulation of astroglial glucose and lipid droplet metabolism

10:00 - 10:15 Marina Radmilović: Immunomodulatory and metabolic changes after brain and retinal ischemia in bradykinin receptor type 2–deficient diabetic mice

10:15 - 10:30 Erika Pintér: TRPA1 receptor may play modulatory role in the CNS diseases

10:30 - 10:40 Urška Černe: Intracellular signaling and metabolism in neuronal and astroglial cells in Drosophila aging brain

10:40 - 10:50 Andrea Tamas: Examination of pituitary adenylate cyclase-activating polypeptide in Parkinson's disease focusing on correlations with motor symptoms

10:50 - 11:15 Coffee break

11:15 - 13:20 **Session 4**–Cell physiology– Chairwomen: Aleksandra Dugandžić, Ines Drenjančević

11:15 - 11:45 Guest speaker: Michael F. Romero (Mayo Clinic College of Medicine and Science, Rochester, USA): Bicarbonate transporters and how to measure them

11:45 - 12:15 Guest speaker: Markus Ritter (Institute for Physiology and Pathophysiology, Salzburg, Austria): Volume-and-acid activated chloride channels in microglia and chondrocytes

12:15 - 12:30 Jurij Dolenšek: Three different time scales of $[Ca^{2+}]_i$ oscillations from in mouse beta cells in tissue slices

12:30 - 12:45 Beti Dejanova: Oxidative stress state in health and disease

12:45 - 13:00 Dino Šisl: Protective effect of tamoxifen on cholestatic liver fibrosis in mice

13:00 - 13:10 Éva Szőke: Evidence for the role of lipid raft disruption in membrane fluidity in vitro and Transient Receptor Potential Melastatin 8 ion channel activation in in vivo mouse model

13:10 - 13:20 Barbara Tomić: WEE1 kinase is involved in differentiation of acute myeloid leukemia cells in response to pyrimidine synthesis inhibitors

13:20 - 14:00 CPS Assembly

13:20 - 15:00 Lunch

15:00 - 16:00 Poster session #1

16:00 - 20:00 Plitvice lakes Excursion

20:00 - 23:00 Dinner

Saturday, September 24th 2022

08:30 - 11:00 **Session 5**–Endocrinology–Chairwomen: Helena Lenasi, Ana Shek–Vugrovečki

08:30 - 09:00 Guest speaker: Bayram Yilmaz (Yeditepe University, Istanbul, Turkey): Endocrine disruptors and their effects on human health: update on obesogens

09:00 - 09:30 Guest speaker: Helena Lenasi (University of Ljubljana, Ljubljana, Slovenia): Does an oral glucose load affect endothelial function and how to evaluate it?

09:30 - 10:00 Guest speaker: Naim A. Khan (representative of French physiological society): Physiology of 6th sense of taste: fat taste & obesity

10:00 - 10:15 Andraž Stožer: Calcium oscillations in human islets of Langerhans and their changes in Diabetes

10:15 - 10:30 Eglantina Idrizaj: Resistin affects the mechanical responses in strips from the mouse gastric fundus – online

10:30 - 10:40 Nikola Hadzi Petrushev: Serum redox markers in uncomplicated type 2 diabetes mellitus accompanied with abnormal iron levels

10:40 - 10:50 Jan Kopecky: Caloric restriction improves diet–induced beta cell dysfunction in mouse model of type 2 diabetes

10:50 - 11:00 Miova Biljana: Nicotinamide pre-treatment of streptozotocin-induced diabetic rats prevent the changes in liver carbohydrate metabolism

11:00 - 12:00 Poster session #2

12:00 - 13:00 Lunch

13:00 - 15:05 **Session 6**–Immunology and other topics–Chair(wo)man: Hrvoje Lalić, Ines Mrakovčić-Šutić

13:00 - 13:15 Michela Terlizzi: Sphingosine - 1-Phosphate in Lung Physiopathology: exacerbator of circulating cells–mediated inflammation?

13:15 - 13:30 Sara Aničić: RNA-sequencing of osteoclast progenitor subsets in a murine model of arthritis

13:30 - 13:45 Igor Štimac: Rab10 GTPase is involved in membrane remodeling to form assembly compartment in the early phase of murine cytomegalovirus infection

13:45 - 14:00 Katalin Böröcz: Humoral and cellular immune response induced by novel SARS-COV-2 vaccines

14:00 - 14:15 Silvana Petretić Majnarić: T lymphocyte subpopulation in peripheral blood and follicular fluid of patients treated with medically assisted fertilization with and without thyroid autoimmunity

14:15 - 14:25 Ana-Marija Laškarin: Diagnostic accuracy of salivary matrix-metalloproteinase-3 to diagnose temporomandibular disorders in patients with axial spondyloarthritis

14:25 - 14:35 Hrvoje Jakovac: Detection of Sars-Cov-2 antigens in thyroid gland showing histopathological features of subacute thyroiditis

14:35 - 15:05 Guest speaker: Oksana Zayachkivska (representative of Ukraine physiological society): The physiology-based risks and perspectives – online

15:05 - 15:30 Taking photo

15:30 - 16:00 Awards and closing ceremony

Poster session #1: Friday, September 23rd 2022, 15:00 - 16:00

Sport physiology–poster committee: Lana Ružić, Ines Mrakovčić-Šutić

1. Jakus Tadeja: The effect of acute exercise and CHO intake on antiinflammatory adiponectin and inflammatory markers in elite cyclists
2. Zekić Josip: Team sports and ACTN 3/R577X, ADRB 3 and ACE polymorphisms
3. Šeper Vesna: Influence of creatine supplementation on level of menstrual pain in females
4. Nestorova Brazhanska Mihaela: Comparison of the values of resting metabolic rate by indirect calorimetry and by predictive equations in sport athletes
5. Potočnik Nejka: Endurance exercise affects the cardiac parasympathetic response to subsequent moderate exercise
6. Jozinović Mirna: Physical activity of children and young people in health promotion
7. Klašnja Aleksandar: The effects of regular physical activity and playing video games on reaction time in adolescents
8. Čebulj-Kadunc Nina: Measurement of heart rates and skin temperatures in exercise testing of lipizzan horses
9. Babić Nermina: Gender differences in the effects of breathing exercises on chest excursions in healthy students
10. Spirkoska Vangelovska Biljana: Different sport disciplines related to respiratory function
11. Mancevska Sanja: Levels of physical activity in medical students in North Macedonia
12. Pluncevic Gligoroska Jasmina: Cardio-physiological adaptation in handball players during Bruce treadmill test
13. Tomaj Mark: Effect of the general mountaineering school on the body weight and mass of recreational mountaineers
14. Perić Nina: The impact of neurorehabilitation with robotics on the rehabilitation of hand function in people after a stroke

Cardiovascular physiology–poster committee: Ines Drenjančević, Ana Shek-Vugrovečki

15. Đambić Vedran: Adenosine A1 and A2a receptor protein expression in cerebral blood vessels of Sprague-Dawley rats exposed to hyperbaric oxygen
16. Švec Dávid: Arterial compliance in obese adolescents and its dynamics
17. Jakovac Hrvoje: Wearing protective masks does not affect hemoglobin oxygen saturation in young healthy subjects

18. Čerňanová Krohová Jana: The impact of obesity on the cardiac chronotropic and vascular resistance baroreflex arms
19. Mihaljević Zrinka: Effect of carnosine on vascular function of Sprague-Dawley rats on a high-salt diet–a pilot study
20. Czippelová Barbora: Cardio–ankle vascular index (CAVI) and its modified versions (kCAVI, CAVI0, kCAVI0) in obese adolescents with and without metabolic syndrome
21. Withdrawn
22. Švorc Pavol: Rat ECG parameters under general anesthesia
23. Božović Bjanka: Circadian patterns of occurrence of acute coronary events in coronary unit of the clinical center of Montenegro

Neurophysiology–poster committee: Aleksandra Dugandžić, Nina Vardjan

24. Začiragić Asija: The difference in novel lipid indices between patients with Alzheimer's disease and vascular dementia stratified by the degree of cognitive impairment
25. Ratko Martina: Effects of guanylate cyclase–C activation on ischemic stroke
26. Javorka Michal: Early stage of Parkinson disease–an impact on the cardiovascular autonomic control
27. Pirnat Samo: Astrocyte arborization enhances Ca^{2+} but not cAMP signaling plasticity
28. Habek Nikola: Guanylate cyclase C independent signaling pathway for uroguanylin in astrocytes
29. Jakovac Hrvoje: A single dose injection of carbon tetrachloride causes inflammation, neuronal loss, massive demyelination and microglial response in the mouse brain
30. Križančić Bombek Lidija: Inhibition of NMDA receptors increases, synchronizes, and stabilizes the collective activity of pancreatic beta cells
31. Popović Nataša: Retinal microvascular complexity reflects accelerated aging associated with severe chronic diseases including Alzheimer's dementia
32. Helyes Zsuzsanna: Preclinical evelopment of a novel multi-target drug candidate for neuropathic pain
33. Smole Zala: Monitoring noradrenaline–induced changes in cytosolic L–lactate in rat locus coeruleus neurons
34. Konkoly János: The transient receptor potential ankyrin 1 (TRPA1) ion channel expressed by the Edinger–Westphal nucleus plays role in posttraumatic stress disorder (PTSD) in mice
35. Kormos Viktória: TRPA1 ion channel is downregulated in peptidergic neurons of the Edinger–Westphal nucleus upon acute alcohol exposure
36. Žugec Maja: Impaired migration of plectin–deficient astrocytes

37. Göntér Kitti: Investigation of the effect of dimethyl trisulfide exerted via TRPA1 ion channels on depression-like behavior
38. Justić Helena: The Koizumi and Longa methods of middle cerebral artery occlusion led to different outcomes after cerebral and retinal ischemic injury in mice
39. Ranđelović Pavle: The effect of reducing blue light from smartphone screen on subjective quality of sleep among students
40. Barić Anja: The effects of bradykinin type 2 receptor loss on cerebral and retinal ischemic injury in mice with type I diabetes
41. Stojanović Nikola: The role of GABAergic neurotransmission in the anxiolytic action of Melissa officinalis essential oil–in silico, in vitro, and in vivo studies
42. László Szabolcs: Development of analgesic silicone-based transdermal patches
43. Krušić Alić Vedrana: Identification of microRNA cargo of extracellular vesicles from cerebrospinal fluid of severe traumatic brain injury patients
44. Al Omari Ammar: Transient Receptor Potential Ankyrin 1 cation channel–expressing cells of the Edinger-Westphal nucleus are activated in a mouse migraine model
45. Petrovska Suncica: Neuroprotective and neurotrophic effects of estrogen
46. Panayotova Gabriela: Love addiction: How does it affect our metanoia?
55. Gosak Marko: Unveiling the functional characteristics and interrelations of hub and pacemaker cells in the pancreatic islets of Langerhans: Insights from network analysis

Poster session #2: Saturday, September 24th 2022, 11:00 - 12:00

Cell physiology–poster committee: Aleksandra Dugandžić, Ines Drenjančević

47. Suzana Branković: Smooth muscle relaxant activity of *Ribes nigrum* L: juice variety Ben Sarek on spontaneous and spasmogen-induced contraction of the isolated rat ileum
48. Žura Žaja Ivona: Effect of in vitro exposure to 3500 MHz radiofrequency electromagnetic field on spermatozoa DNA integrity and vitality indicators in ejaculates of breeding boars
49. Jakovac Domagoj: The uroguanylin effect on stimulated saliva volume flow, the pH and concentration of Na^+, K^+ and Cl^- in mice
50. Kolomazník Maroš: Modified pulmonary surfactant and N-acetylcysteine in the experimental double--hit model of lung injury in rats
51. Skelin Klemen Maša: cAMP-mediated increase in calcium activity in pancreatic beta cell networks: role of Epac2A

52. Paradiž Leitgeb Eva: Incretins modulate beta cell calcium dynamics in mouse islets from acute pancreas tissue slices
53. Grigoryan Armine: Full-thickness skin wound healing after application of regenerating cream combined with silver nanoparticles on purulent wounds
54. Popović Hadžija Marijana: Caco-2 monolayer on permeable membrane develops a stronger connection between cells than the cells within spheroids
55. Presented yesterday
56. Gjorgoski Icko: The effect of CoenzymeQ10 on the cells of the bone marrow in the presence of cisplatin
57. Pohorec Viljem: Glucose stimulated calcium dynamics in acute pancreas tissue slices of inbred and outbred mouse strains
58. Veljković Milica: Green tea effect on the basal membrane of the rat kidney

Endocrinology–poster committee: Helena Lenasi, Ana Shek-Vugrovečki

59. Petkova-Marinova Tsvetelina: Algorithm for diagnosis and treatment of iron deficiency anemia in adolescent children based on hepcidin and selenium status
60. Suzana Dinevska Kjofkarova: Nicotinamide post–treatment of diabetic rats and its effects on liver carbohydrate metabolism
61. Khan Amira: Obesity is associated with changes in taste bud papillae microbiota, junctional permeability and oro-sensory perception of dietary lipids in mice
62. Kordić Milan: Infrared thermography is a new method for detection of brown adipose tissue activity
63. Beadini Albulena: Menopausal status, BMI and vitamin D in women in Tetovo and Pollog region in N. Macedonia
64. Turianiková Zuzana: Breathing pattern characteristics in young obese patients
65. Polšak Nika: Sex–dependent behavioural responses caused by dietary manipulations in mice
66. Jurdana Mihaela: Identification of sacropenic obesity in patients undergoing orthopaedic surgery: The predictive power of a body shape index (ABSI)
67. Withdrawn
68. Zhaku Vegim: Double combination of antioxidant formula can improve the oxidative stress status and motility in men with asthenozoospermia: double blind placebo controlled trial
69. Hachmeriyan Antoniya: Non–nutritive sweeteners and sweet cravings– is there a link?
70. Zadravec Mateja: Seasonal variations of glycemia in patients with type 1 diabetes mellitus
71. Zarezovski Vesna: Biological mechanisms of the effect of body mass reduction on insulin resistance in type 2 diabetes

Immunology–poster committee: Hrvoje Lalić, Ines Mrakovčić-Šutić

72. Pavišić Valentino: Arf1 and Arf6 GTPases are required for intracellular transport of cytomegalovirus virions
73. Drvar Vedrana: Correlation of fecal calprotectin with salivary inflammatory markers
74. Lukanović Jurić Silvija: Predicting biomolecular condensate formation of cytomegalovirus-associated proteins using bioinformatics tools
75. Grubić Kezele Tanja: Sex-related differences in cartilaginous degradation and BMP-4 and Noggin expressions during pristane induced arthritis
76. Gulić Tamara: The tumour associated macrophages affect NK cell dysfunction in more malignant breast cancer subtypes
77. Marcelić Marina: Role of the endosomal phosphatidylinositol-3-phosphate in the Cytomegalovirus life cycle
78. Uravić Bursać Iva: Interrelationship of monocyte chemoattractant protein-1 and parameters of diastolic dysfunction in patients with psoriatic arthritis
79. Ćurko-Cofek Božena: Characterization of lipids in osteoarthritis patients
80. Smoljo Tomislav: Stromal cells affect response of leukemia cells to differentiation therapy
81. Withdrawn
82. Laškarin Gordana: Frequency of temporomandibular disorders in patients with axial spondyloarthritis and neck pain
83. Karleuša Ljerka: The read-out challenge: how to speed up the research and bypass time-consuming protocols?
84. Berki Timea: Imbalance of the natural autoantibody network in pregnant women with Hashimoto's thyroiditis
85. Erdő–Bonyár Szabina: Ligation of TLR homologue CD180 activates the PI3K/Akt/mTOR pathway and alters the expression of BAFF–binding receptors of B cells to levels observed in patients with systemic sclerosis
86. Jakovac Hrvoje: SARS-CoV-2 can infect cardiac conduction system–A Case Report
87. Akyol Sibel: Comparison of immune defense mechanisms that are first triggered against different implant materials
88. Aladrović Jasna: Effect of supplementation with yeast fermentation product on hematological parameters in growing to finishing pigs
89. Jakovac Hrvoje: Gp96 Expression in mice exposed to psychosocial stress: spotlight on immunological changes

90. Nemeth Peter: Dynamic features of herd immunity: similarities in age-specific anti-measles sero--prevalence data between two countries of different epidemiological history
91. Viduka Ivona: Early endosomal sorting Nexins are required for murine Cytomegalovirus envelopment and egress
92. Radić Barbara: Deubiquitinilation function of murine cytomegalovirus M48 protein influence remodeling of WASH1 endosomal network in the early phase of infection
93. Adamčáková Jana: Comparison of different animal models of silicosis through histological and biochemical analyses
94. Čalkovská Andrea: Pulmonary surfactant in COVID-19 era
95. Miljković Josip: Serum concentrations of selected minerals in Lika pramenka lambs fed by feed suplemeted with white button mushroom (Agaricus bisporus)
96. Pađen Lana: Insight into free-ranging gray wolves' physiological status from fatty acid composition perspective of different tissues

Keynote lecture:

Gas diffusion through aquaporins and other membrane proteins

Thomas Kowatz*, Fraser J. Moss*, Rossana Occhipinti*, Xue Qin*, Eric Shinn†, Archit Vasan†, Dengke Wang*, Pan Zhao*, Heath Gasier‡, Ardi Vahedi-Faridi*, Emad Tajkhorshid†, & Walter F. Boron*

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The dogma had been that gases cross biological membranes simply by dissolving in and then diffusing through the lipid phase of membranes. However, the last two decades have seen a major paradigm shift, with the discoveries of (1) gas-impermeable biological membranes and (2) membrane proteins ("gas channels") that conduct CO_2, NH_3, NO, and/or O_2. Physiological studies on a wide range of such channels, together with mathematical simulations, X-ray crystallography, and molecular-dynamics studies have begun to clarify the molecular mechanism of two gas-channel families, aquaporins (AQPs) and Rhesus (Rh) proteins. These approaches have also begun to develop evidence that classical ion-transport proteins can sometimes conduct dissolved gases. Gas channels can have biological impact only if underlying membrane permeability is relatively low. Indeed, measurements on red blood cells (RBCs) and Xenopus oocytes indicate that—in the absence of gas-channel activity—membrane permeabilities to CO_2 or O_2 are far less than expected. In this lecture, I will present 4 vignettes describing recent work of our group and our collaborators on: (1) molecular mechanism of CO_2 movement through AQPs 2 and 5; (2) a novel neutral-buoyancy assay (NBA) to detect N_2, CO_2, or O_2 influxes through Xenopus oocyte plasma membranes and into an injected gas bubble; (3) surprising evidence of a pathway for CO_2 diffusion through Na-coupled "HCO^{3-}" transporters like NBCe1-A; and (4) O_2 channels in RBCs and alveolar type I cells (ATICs). We will see, for example, that different AQPs exhibit characteristic selectivities for various gases, that point mutations can confer or eliminate gas permeability, and that blocking agents can target specific pathways through an AQP. One practical application in mammalian RBCs, nearly all CO_2 and O_2 move through membrane channels, and that these appear to have a surprising impact on exercise tolerance. In the lung, the knockout of AQP5 may be a strategy for protecting ATICs from pulmonary oxygen toxicity.

Session 1 - Sport physiology

Increased hemodynamic forces drive the adaptions of the coronary circulation to exercise

Akos Koller

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Research Center for Sports Physiology, Hungarian University of Sports Science, Budapest, Hungary;
Department of Physiology, New York Medical College, Valhalla, NY, USA

It has been well established that aerobic exercise elicits several positive adaptations in all organs and tissues of the body, which improve health. During exercise the increased hemodynamic forces (intraluminal pressure flow/shear stress) activate a wide range of mechanisms eliciting functional and structural adaptations in the coronary circulation. These adaptations optimize myocardial oxygen and nutritional supply during increased demand.
Adaptations of large and microvessels of coronary circulation include increased diameter of epicardial coronary arteries, and arterioles, and the density of microvessels, such as capillaries, increasing thereby their exchange capacities.
On the cellular level there are several adaptations, including enhanced smooth muscle-dependent pressure-induced myogenic mechanism and upregulated endothelium-dependent/shear-stress-induced dilation; together increasing the range of diameter change.
At the molecular level, ion channels are key players in the local coronary vascular adaptations to exercise, with enhanced activation of influx of Ca^{2+} contributing to the increased myogenic tone (via voltage-gated Ca-channels) as well as the enhanced endothelium-dependent dilation [via nitric oxide (NO) and TRPV4 channels]. Increased flow delivers NO via the red blood cells to dilates distal microvascular networks. Also, NO released diffuses to cardiac mitochondria and - by reacting with cytochrome c oxidase - can alter oxidative metabolism.
Thus, increased hemodynamic forces during aerobic exercise elicits a number of beneficial effects on network-, cellular- and molecular levels, which ensure a greater coronary blood flow, delivery/exchange of oxygen and nutrients and optimal mitochondrial use of oxygen during exercise. Activation of these mechanisms can also be used to prevent and/or delay the development of coronary and cardiac diseases.

Supports: NKFIH-1281-2/2020 TKP2020-NKA-17 and TKP2021-EGA-37, National Research, Development and Innovation Fund, OTKA-132596, MTA/HAS-POST-COVID 34.

Perforin-mediated cytotoxicity in professional athletes

Ines Mrakovčić-Šutić[1,2], Aleksandar Bulog[3] , Karlo Tudor[4], Sandro Gržančić[5], Miljenko Kovačević[6]
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[4] University Hospital for Orthopedic Surgery Lovran, Lovran, Croatia
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[6] Department of Surgery, University of Rijeka, Medical Faculty , Rijeka, Croatia

Introduction: Exercise-induced factors such as increased metabolic rate, oxidative stress, heat shock proteins, catecholamines, etc. may influence pathogen recognition by altering expression of recognition molecules such as Toll-like or scavenger receptors, cell trafficking by altering hematopoiesis and adhesion molecule expression. Some evidence has shown that athletes included in heavy training programs were susceptible to infection. Immune system's capacity to distinguish between innocuous and harmful foreign antigens is controlled by mechanisms of central and peripheral tolerance. Lymphatic cells may be stimulated to release perforin and initiate the apoptosis. Regulatory T cells (Tregs) play a crucial role in peripheral T-cell tolerance.

The aim and methods: The aim of this study was to investigate perforin-mediated cytotoxicity and the percentage of innate immune cells and perforin positive cells in lymphocyte subpopulations of peripheral blood of professional athletes. The phenotypic profiles were done by flow cytometry. NK-mediated cytotoxicity of peripheral blood mononuclear cells was examines against human NK-sensitive K-562 tumor cell line.

Results: preliminary data showed that the percentage of innate immune cells were significantly elevated in professional athletes in comparison with healthy controls and noncompetitive athletes. Total perforin positive cells and double positive (perforin+ NK+cells) in professional athletes were increased in comparison with blood donors. Perforin-mediated cytotoxicity in professional athletes is not statistically changed in comparison with healthy controls and noncompetitive athletes.

Conclusion: Intensive physical activity in professionally trained athletes had a positive impact on immune response, particularly intensive in innate immunity, which is the first line of defense in the body against the effects of adverse factors. Tracking changes in the percentage of T regulatory cells may contribute to better understanding the interdependence of hormonal and immunological network during physical activity.

Acknowledgement: This work is supported by grants from the University of Rijeka (18-220, 18-146 and 18-301).

Sex differences in effects of eight-week increment aerobic exercise program on bone metabolism in young non-athletes

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ABSTRACT

Purpose: Regular exercise and physical activity play an important role in preserving bone health, mass, and strength. However, it remains unclear how much load is necessary and for how long should it be applied in order to induce bone remodeling. Evidence on the influence of interval aerobic training with variations in intensity to the bone remodeling process is scarce. Thus, the main goal of this study was to explore if there was an effect of the short-term aerobic exercise program on the bone remodeling process and if there were sex differences in the effect of the training program on bone metabolism.

Methods: Twenty-one participants (11 men and 10 women) aged 20-23 performed an eight-week aerobic exercise program three times per week in one-hour sessions with increases in the exercise load every two weeks. Bone density, bone mineral content and concentration of markers of bone metabolism: osteocalcin, C-terminal procollagen type I peptide, pyridinoline, parathyroid hormone, osteoprotegerin, and the receptor activator of nuclear kappa B ligand by ELISA were measured at the start and at the end of the study.

Results: The aerobic exercise program increased the concentration of osteocalcin (11.34 vs 14.24 ng/ml), pyridinoline (67.51 vs 73.99 nmol/l), and the receptor activator of nuclear kappa B ligand (95.122 vs 158.15 pg/ml). A statistically significant increase in bone density at neck mean (1.122 vs 1.176 g/cm^3) and in bone mineral content at dual femur (33.485 vs 33.700 g) was found in women, while there was no statistically significant change at any site in men.

Conclusion: The interval aerobic exercise protocol implemented for 8 weeks affected the bone remodeling process, which was evident from the increase in concentration of some bone remodeling biomarkers. The results also revealed different effects of the exercise program on the bone metabolism in men vs women.

Keywords: Aerobic exercise, bone turnover, biomarkers of bone metabolism, sex differences

Effects of aerobic versus resistance exercise on glycemic control stability in patients with diabetes type 1 – a cross-over study

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Abstract

The increased risk of hypoglycemia and worsening of glycemic control during exercise is a concern for type 1 diabetic (T1DM) patients. Aim of this randomized cross-over prospective study was to assess glycemic control and hypoglycemic events during and after aerobic versus resistance exercises using flash glucose monitoring system.

We included 14 adult T1DM patients. They were randomized according to the type of exercise (aerobic vs resistance) with a washout period of three days in between. Glucose control, hypoglycemic episodes were evaluated during and 24-hours after the exercise. Growth hormone (GH), cortisol and lactate levels were determined at rest, 0, 30 and 60 min post-exercise. Median age of patients was 53 years, HbA1c 7.1% and duration of diabetes 30 years.

During both training sessions there was a drop in glucose immediately after the exercise (0') followed by an increase at 30' and 60' although the difference was only numerical, not statistically significant. However, glucose levels significantly decreased from 60' to 24-h time point (P=0,001) for both types of exercise. Glycemic control was comparable prior and after exercise for both training sessions. No differences in number of hypoglycemic events, duration of hypoglycemia and average glucose level in 24-h post exercise period was observed. Time to hypoglycemia onset was prolonged after the resistance as opposed to aerobic training (13 vs 8 hours, P=NS). Nocturnal hypoglycemic episodes were not recorded (between 00-06h) after the resistance compared to aerobic exercise (4 vs 0, P=NS). GH and cortisol responses were similar, while lactate levels increased significantly more after resistance training.

Both exercise regimes induced similar glucose response during and immediately after acute bout. However, resistance training seems to be more favorable for patients exercising in the afternoon or evening considering prolonged time to hypoglycemia onset and lack of nocturnal hypoglycemia.

Keywords: Type 1 diabetes mellitus, aerobic training, resistance training, flash glucose monitoring, hypoglycemia

Is recovery in hyperoxia after high intensity training in hypoxia an added value?

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Methods to improve athletic performance include the hypoxic and recently also the hyperoxic training. This study hypothesized that a combination of high intensity interval hypoxic training (HIIT) followed by a recovery in hyperoxia improves the aerobic endurance and oxygen transport parameters better than recovery in normoxia.

Forty-nine fit men, 18-20y, were randomly divided into: Hypo+Hyper group (N=16) performing HIIT in hypoxia with 15min recovery in hyperoxia at FiO2=0.40; Hypo group (N=16) HIIT in hypoxia/recovery in normoxia and Control group (N=17) performing everyday activities. The intervention lasted 4 weeks;3x/week. Each week, the intensity was increased by 5%. HIIT in hypoxia exercise protocol consisted of six bouts of 1min at 80-95% of HRmax, each week at higher rate, respectively, followed by 2min at 60% of HRmax. Pre- and postintervention the blood was analyzed related to oxygen transport parameters and an all-out-treadmill test carried out.

At maximal exertion Hypo-Hyper group had a larger increase in maximal oxygen uptake-VO_{2max}, (pre- 49.34 and post- 50,79ml/kgmin-1) as opposed to controls (pre- 48.04 and post- 46,71ml/kgmin-1; $p<0.05$) as expected, and somewhat larger than only Hypo group (pre- 46.89 post- 47,56 ml/kg min-1, $p=0.176$). There was also larger difference in maximal speed reached in Hypo-Hyper group vs both groups ($p<0.01$). Significant changes at anaerobic threshold involved only increase in oxygen uptake values at threshold in Hypo-Hyper group. Changes in blood oxygen transport parameters occurred as hemoglobin mass increase the most in Hypo+Hyper group (+11.8 in Hyper+Hypo vs +0.8 in Hypo and -0.7 g/L in Controls; $p<0.05$ and $p<0.01$ respectively). Slight hematocrit changes were also noted.

The study innovation lays in design, as both methods, hyperoxia and hypoxia training were usually studied separately. It seems that the optimal training for short periods might be HIIT with hyperoxia recovery but further studies, involving better understanding of underlying mechanisms, should follow.

Skin microvascular response to graded dynamic exercise assessed by wavelet analysis: a pilot study

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Abstract

Brief introduction: Wavelet analysis (WA) of laser Doppler flowmetry (LDF)-measured microcirculatory signals reveals six characteristic frequency intervals, ranging from 0.005-2 Hz, each of them related to a particular physiological influence modulating skin microcirculation, making WA a valuable tool for a more thorough analysis of the LDF signals.

Aim: We aimed to analyze LDF signals, acquired during resting and in the early recovery phase to submaximal dynamic exercise by applying WA, hypothesizing that this approach might reveal potential physiological mechanisms reflecting the thermoregulatory response of skin microcirculation.

Methods: LDF signals were acquired at the finger pulp and at the volar forearm in six healthy volunteers undergoing graded dynamic exercise on a cycloergometer (starting at 40W, increased by 50W-increments, till submaximal heart rate was reached).
WA of the signals obtained during resting and in the recovery to exercise was performed. To compare the contribution of different physiological mechanisms regulating microcirculatory responses in these phases, relative power (RP = mean power of each frequency interval/ mean power of the total spectrum) was determined for each frequency interval in the corresponding phase.

Results: Periodic oscillations with frequencies corresponding to cardiac, respiratory, myogenic, neurogenic, and endothelial NO-dependent influences were detected in the LDF signals at both measuring sites.
During the recovery phase, the largest RP was obtained in the interval, reflecting NO-dependent endothelial component (0.0095-0.021Hz), at the finger pulp (recovery: 3.9±0.3 vs. resting: 1.0±0.3; p=0.0007; paired t-test), but not at the forearm.

Conclusions: Our findings indicate that during early recovery phase to exercise, the endothelial, NO-dependent component most importantly contributes to microvascular response in the finger pulp.
The contribution of different physiological mechanisms controlling skin microcirculation in early recovery significantly differs between glabrous and nonglabrous skin.
Further research including larger sample size and a longer measurement time is required to confirm our findings.

Keywords:
skin microcirculation, laser Doppler flowmetry, wavelet analysis, spectral analysis, physical exercise

Session 2 - Cardiovascular physiology

Permissive role of angiotensin II in maintaining microvascular reactivity to physiological stimuli

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OBJECTIVE: A number of data suggest, rather controversial, permissive role of angiotensin II (ANGII) in maintaining vasodilator responses in microcirculation. In CSF grant: V-ELI Athero, #IP-2014-09-6380, we aimed to elucidate the effect of RAS restoration on the mechanisms of flow-induced dilation and incidence of oxidative stress in the cerebral vasculature of Sprague-Dawley rats on HS diet.

DESIGN AND METHOD: Eleven-week old, male Sprague-Dawley rats were divided to control group (0.4% NaCl in rat chow); HS group (7 days 4% NaCl in rat chow); Losartan group (rats on a standard diet given AT1R blocker losartan (1 mg/mL)); or HS+ANGII group (7 days HS with 3 days ANGII via osmotic minipumps (100 ng/kg/min on days 4–7)). FID of MCA was determined in absence/presence of indomethacin, L-NAME, and TEMPOL. Gene and protein expression of enzymes involved in FID mechanisms and antioxidative enzymes were determined by RT-qPCR and Western blot. Direct fluorescence was used for vascular NO and superoxide/ROS levels measurements. All experimental procedures conformed to the EU Directive 2010/63/EU.

RESULTS: All rats remained normotensive. HS or AT1R blockade impaired FID and increased oxidative stress. Supplementation of ANGII restored the mechanisms of FID in MCA of rats fed HS diet to the ones present in control rats by decreasing systemic oxidative stress, increasing NO bioavailability and decreasing superoxide/ROS levels in the vasculature. The AT1R blockade resulted in attenuated and altered mechanisms of FID, increased systemic and vascular oxidative stress and decreased expression/activity of antioxidative enzymes.

CONCLUSION: Suppression of ANGII is a key link between HS intake and development of endothelial dysfunction. The results provide evidence for the new paradigm of crucial role of normally modulated RAS in the preservation of vascular oxidative stress-antioxidant system balance and, and in maintenance of physiological vasodilation mechanisms of MCA via AT1R.
Acknowledgements: This study was supportedby Croatian Science Fundation grant: V-ELI Athero, #IP-2014-09-6380 and by the Faculty of Medicine Osijek Institutional grant IP-1- MEFOS (2020; PI Ines Drenjančević), IP3-MEFOS (2021; PI Ines Drenjančević)

Short-term high-salt loading effects on microvascular endothelial function in salt-resistant humans – specific role of oxidative stress and sympathovagal imbalance

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Introduction and Aim: This project tested the hypothesis that suppression of renin-angiotensin system (RAS) and sympathetic activity by 7 day high-salt (HS) diet lead to endothelial and leukocyte activation and further, impaired microvascular reactivity in humans, by increasing oxidative stress.

Materials and Methods: Project lasted for 54 months (April 2017-October 2021). Overall 132 young healthy individuals were included in project studies. All subjects adhered to a 7-day low-salt diet (3.5g salt/day), followed by a 7-day HS diet (~14.7g salt/day) protocol. Functional assessment of microvascular endothelial function, adrenergic system activity, body composition and fluid status, RAS activity, catecholamines concentration, biomarkers of oxidative status, pro- and anti-inflammatory cytokines, frequencies of peripheral blood lymphocytes, and biomarkers of endothelial activation were measured before and after HS diet. To specifically test the role of oxidative stress and sympathetic system activity in changes of microvascular reactivity due to the HS diet one group of subjects was supplemented with oral antioxidants during the HS diet, while the other group was subjected to a mental stress test.

Results and Conclusions: 7-day HS diet suppresses RAS and impairs microvascular endothelium-dependent vasodilation in healthy individuals; these changes are independent of blood pressure, body composition or fluid retention, but are the consequences of the unique effect of HS on endothelial function. HS diet induces oxidative disbalance, with antioxidant supplementation during HS diet preserving both oxidative status and endothelial microvascular reactivity. HS diet also suppresses sympathetic activity which represents a physiological response, rather than direct pathophysiological mechanism by which HS diet affects microvascular endothelial function in healthy individuals. HS diet induces lymphocyte activation, resulting in enhanced production of pro-inflammatory, but also anti-inflammatory cytokines, while increased oxidative stress, due to HS loading, alters immune regulatory mechanisms. Taken together, oxidative disbalance has the pivotal role in HS diet-induced impairment of endothelial and microvascular function in healthy individuals.

Investigation of arterial stiffness and clinical and chemical indicators in Bulgarian young people with high-normal arterial pressure

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Abstract:
High normal arterial pressure (HNAP) according to European Society of Cardiology (ESC) is a category between the norm and pathology with systolic blood pressure (SBP) of 130-139 mmHg and diastolic blood pressure (DBP) of 85-89 mmHg. According to literature data HNAP in combination with endothelial activation and metabolic changes suggests increased cardiovascular risk (CVR). There is still insufficient data for young individuals with HNAP and their CVR. According to the TROPHY (Trial of prevention Hypertension) study, vascular changes are preceded by clinical manifestations of arterial hypertension (AH).

Purpose: to answer are there any early structural or functional changes in the vascular wall that precede be changes in arterial pressure values.

Material and metods: 56 young health individuals were studied, 32 with HNAP and 24 controls with normal arterial pressure. Anthropometric analysis, arterial stiffness (Tensioclinic, "Tensiomed", Hungary), intracellular adhesion molecule-1 (iCAM-1), vascular adhesion molecule-1 (vCAM-1) and asymmetric dimethylarginine (ADMA) were examinated. Arterial pressure (AP) monitoring with CNAP (CNSystems, Austria) (averaged 214 ± 37 values of SBP and DBP) was performed.

Results and conclusion: The anthropometric characteristics of HNAP individuals determine increased CVR. Arterial stiffness (central systolic AP) at HNAP gives signs of more significant "vascular aging" (110.04±10.33 vs 102.45±12.45). In HNAP, was found endothelial activity- elevated iCAM-1 (366.07±93.49 in comparison with controls: 323.18±72.82).

Key words: high normal arterial pressure (HNAP), CNAP, arterial stiffness, iCAM-1.

Mechanisms of Cardiovascular Changes of Phototherapy in Newborns with Hyperbilirubinemia

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During phototherapy of jaundiced newborns, vasodilation occurs in the skin circulation compensated by vasoconstriction in the renal and mesenteric circulation. Furthermore, there is a slight decrease in cardiac systolic volume, and blood pressure, as well as an increase in heart rate and discrete changes in the heart rate variability (HRV).
The primary change during phototherapy is the skin vasodilatation mediated by multiple mechanisms: 1) Passive vasodilatation induced by direct skin heating of the body surface and subcutaneous blood vessels, modified by myogenic autoregulation. 2) Active vasodilatation mediated via mechanism provided by axon reflexes through nerve C - fibres and humoral mechanism via nitric oxide (NO) and endothelin 1 (ET-1). During and after phototherapy is a rise in the NO:ET-1 ratio. 3) Regulation of the skin circulation by the sympathetic nerves is unique, but their role in skin vasodilation during phototherapy was not studied. 4) Special mechanism is a photovasorelaxation independent of the skin heating. Melanopsin (opsin 4) - is thought to play a major role in systemic vascular photorelaxation. Signalling cascade of the photorelaxation is specific, independent of endothelium and NO.
The increased skin blood flow during phototherapy is promoted by the restriction of blood flow in the renal and mesenteric circulation. An increase in heart rate besides a fall of systemic blood pressure indicates activation of the sympathetic system as it is also seen in the measures of the HRV. High-pressure, as well as low-pressure baroreflexes, may play important role in these adaptation responses.
During neonatal phototherapy, cardiovascular changes occur which are important for maintaining vital functions as well as for improving the effect of phototherapy by increasing skin blood flow. The integrated complex and specific mechanisms responsible for the hemodynamic changes during phototherapy confirm adequate and functioning regulation of the neonatal cardiovascular system, including baroreflexes.

Keywords: Newborns, phototherapy, photovasorelaxation, cardiovascular changes, skin vasodilation, baroreflexes

Assessment of the effect of carnosine supplementation on flow induced dilation mechanisms of a middle cerebral arteries (MCAs) in low salt and high salt fed Sprague-Dawley rats

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Introduction: This pilot study aimed to determine the effects of carnosine supplementation on the mechanisms of flow-induced dilation (FID) in isolated middle cerebral arteries (MCAs) of Sprague-Dawley rats on LS and HS diet.

Methods: Ten-weeks-old Sprague-Dawley rats (N=3-4 per group) were divided to low salt group (LS; 0.4% NaCl in rodent chow), HS group (4% NaCl in rodent chow), LS+carnosine group (carnosine by oral gavage 150 mg/kg per day/7 days) and HS+carnosine group (oral gavage 150 mg/kg per day/7 days), fed for 1 week, water ad libidum. Rats were anesthetized with ketamine-chloride (75 mg/kg) and midazolam (0.5 mg/kg), decapitated and MCA was isolated, cannulated on glass pipettes and pressurized (DMT pressure myograph, Denmark). Reactivity of MCAs in response to stepwise increases in pressure gradients (Δ10-Δ100 mmHg) was determined in absence/presence of Dimethyl fumarate (DMF), the nuclear factor erythroid 2–related factor 2 (Nrf2) activator. The endothelium-independent response was tested using the NO donor sodium nitroprusside (SNP; 10-6 mol/L) and endothelium-dependent vasodilation was tested using acetylcholine (ACh; 10-6 mol/L). Statistical analyses were performed with Two-way ANOVA tests; $p<0.05$ was considered significant. All experiments conformed to EU directive 86/609 and were approved by the local (No. 2158-61-46-22-14, 2158-61-46-22-13) and national (355/2022, 356/2022) Ethical Committee.

Results: FID was significantly reduced in HS group compared to LS group. FID was similar between the LS and LS +carnosine groups while carnosine administration in HS group restored FID at ∆40 -100 mm Hg. ACh-induced dilation was significantly decreased in HS group compared to the other study groups. SNP-induced vasodilation was preserved and similar in all groups. In HS group, DMF significantly increased FID at ∆40 mm Hg, while had no effect in other groups.

Conclusion: Administration of carnosine restores vasodilation impaired by HS diet. HS diet might possibly suppress /inhibit Nrf2 pathway(s).

Acknowledgements: This study was supported by the Faculty of Medicine Osijek Institutional grant IP-1 (2020; PI Ines Drenjančević), IP3 (2021; PI Ines Drenjančević), IP1 (2022; PI Ines Drenjančević), IP16 (2022; PI Zrinka Mihaljević) and by European Structural and Investment Funds through a grant to the Croatian National Science Center of Excellence for Personalized Health Care, Josip Juraj Strossmayer University of Osijek # KK.01.1.1.01.0010.

Oxidative stress level in children with essential arterial hypertension

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Objective: Oxidative stress may play an important role in the pathophysiology of hypertension. Isoprostanes, a group of prostaglandin-like compounds, active products of arachidonic acid, have proved to be representative biomarkers of lipid peroxidation. This study aimed to determine the influence of essential arterial hypertension on serum concentration of 8-iso-prostaglandin F2alpha, (8-iso-PGF2α), as an in vivo oxidative stress marker in children.

Design and Method: Total of 52 children participated in this study: 26 children with essential arterial hypertension (HT) and 26 normotensive children (NT) (both sexes, age ranged 8-17). In addition to arterial blood pressure and heart rate, body mass index (BMI) was measured for all subjects. In the venous blood sample, the concentration of serum 8-iso-PGF2α was determined using the ELISA method. Statistical analysis was conducted by nonparametric Mann-Whitney U test (between groups), and Pearson's correlation test was used to determine the correlations between 8-iso-PGF2α and blood pressure. $p< 0.05$ was considered statistically significant.

Results: Body mass index (BMI) was significantly higher in hypertensive children compared to normotensive children, but waist to hip ratio (WHR) was similar between groups. Children with essential arterial hypertension had significantly higher systolic (SBP), diastolic (DBP) and mean (MAP) arterial blood pressure compared to normotensive children, while there was no difference in heart rate.
Serum concentration of 8-iso-PGF2α was significantly higher in HT group compared to the NT group (adjusted for BMI). Futhermore, 8-iso-PGF2α was positively associated with SBP (r=0,468) and MAP (r=0,366).

Conclusions: Results support the hypothesis that already in childhood, arterial hypertension is accompanied by an elevated level of oxidative stress, independently of BMI.

Acknowledgement: This study was supported by institutional grants from the Faculty of Medicine Osijek IP9-2021-MEFOS grant (PI Ivana Jukić) and IP17-2022-MEFOS grant (PI Ivana Jukić).

Session 3 - Neurophysiology

Age-related cognitive decline: role of endothelial senescence

Zoltan Ungvari (University of Oklahoma, OK, USA)

Age-related phenotypic changes of cerebromicrovascular endothelial cells lead to dysregulation of cerebral blood flow and blood brain barrier disruption, promoting the pathogenesis of vascular cognitive impairment (VCI). In recent years endothelial cell senescence has emerged as a potential mechanism contributing to microvascular pathologies opening the avenue to the therapeutic exploitation of senolytic drugs in preclinical studies. To detect senescent endothelial cells in the aging mouse brain, we analyzed cell fractions enriched for cerebromicrovascular endothelial cells and other cells associated with the neurovascular unit obtained from young (3-month-old) and aged (28-month-old) C57BL/6 mice. 13 transcriptomic cell types were identified by deep, single-cell RNA sequencing.
Transcriptomic signatures of cellular senescence were matched to endothelial cells identified on the basis of their gene expression profile. Our study demonstrates that with advanced aging there is an increased ratio of senescent endothelial cells (~10%) in the mouse cerebral microcirculation. To test the hypothesis that a validated senolytic treatment can improve endothelium-dependent neurovascular coupling (NVC) responses and cognitive performance in aged mice, aged mice were treated with ABT263/Navitoclax, a potent senolytic agent known to eliminate senescent cells. Mice were behaviorally evaluated and NVC was assessed by measuring CBF responses in the somatosensory whisker barrel cortex evoked by contralateral whisker stimulation. We found that NVC responses were significantly impaired in aged mice. ABT263/Navitoclax treatment improved NVC response, which was associated with significantly improved hippocampal-encoded functions of learning and memory. Thus, increased presence of senescent cells in the aged brain likely contributes to age-related neurovascular dysfunction, exacerbating cognitive decline.

Activity-dependent astroglial control of glutamatergic inter-synaptic crosstalk in the brain

Dmitri Rusakov (University College London, London, UK)

The actions of the excitatory neurotransmitter glutamate outside the synaptic cleft are constrained by high-affinity transporters expressed by perisynaptic astroglia. Learning and memory formation in the brain involve synaptic remodelling, but how this affects perisynaptic astroglial processes (PAPs) and therefore extrasynaptic glutamate actions is poorly understood. We used advanced methods of multiplexed imaging, in situ and in vivo, to find that long-term potentiation (LTP) of excitatory transmission, triggers withdrawal of PAPs from the potentiated synapses. LTP induction appears to trigger spatial rearrangement of astroglial glutamate transporters, boosting extrasynaptic glutamate actions and NMDA receptor-mediated inter-synaptic cross-talk. The LTP-triggered PAP withdrawal involves astroglial NKCC1 transporters and the actin-controlling protein cofilin but does not depend on major Ca^{2+}-dependent cascades in astrocytes. Combining targeted viral transduction with multiplexed imaging in vivo reveals a similar phenomenon under the sensory LTP paradigm involving rhythmic whisker stimulation, in the intact brain.

Noradrenergic Regulation of Astroglial Glucose and Lipid Droplet Metabolism

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Astrocytes, neuroglial cells with key homeostatic brain functions, are positioned in the brain between blood vessels and neurons to deliver nutrients from the circulation to the energy-consuming neurons. Neurons rely heavily on the availability of astroglial-derived metabolite L-lactate, which is produced in astrocytes from D-glucose in the process of aerobic glycolysis. Aerobic glycolysis in astrocytes is regulated by activation of plasma membrane receptors, including adrenergic receptors (ARs). Activation of α_1- and β-ARs with noradrenaline increases intracellular Ca^{2+} and cAMP signals, respectively, however the extent to which the Ca^{2+} and cAMP signals regulate astroglial glucose and lipid metabolism is not well understood.

We performed real-time fluorescence microscopy using glucose and lactate nanosensors and measured the cytosolic concentration of free D-glucose ($[glc]_i$) and L-lactate ($[lac]_i$) in rat cortical astrocytes upon selective AR activation. We also determined the effect of selective AR activation on lipid droplet formation by labeling lipid droplets with Nile Red dye. We have showed that intracellular Ca^{2+}, but not cAMP signals, trigger an increase in intracellular $[glc]_i$ and $[lac]_i$ in astrocytes, suggesting Ca^{2+}-mediated glucose uptake and aerobic glycolysis in astrocytes. Moreover, an increase in lipid droplet accumulation, typically seen in brain pathologies, was observed in astrocytes upon chronic exposure to noradrenaline, the effects of which were mediated by activation of β-AR, but not α1-ARs, implying a role of cAMP, but not Ca^{2+} signals.

Taken together, an increase in the intracellular Ca^{2+} upon noradrenergic activation is the prime mechanism of augmented aerobic glycolysis in astrocytes, while cAMP has only a moderate role, but importantly contributes to regulation of lipid metabolism in astrocytes. The results provide a novel information on the signals regulating brain metabolism and open new avenues to explore whether astroglial Ca^{2+} or cAMP signals are dysregulated and contribute to neuropathologies with impaired brain metabolism.

Immunomodulatory and metabolic changes after brain and retinal ischemia in bradykinin receptor type 2 – deficient diabetic mice

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INTRODUCTION AND AIM
Diabetes mellitus, characterized by hyperglycemia is associated with an increased risk of ischemic injury. Bradykinin and bradykinin receptor type 2 (B2R), known for the role in vasodilation and edema formation after ischemia, have been recently related to glucose metabolism modulation. This study aimed to determine the long-term effects of B2R-deficiency on brain and retinal ischemic injury in a mouse model of type 2 diabetes.

METHODS
Simultaneous brain and retinal ischemia were induced in male B2R-deficient (B2R-KO) and control (WT) mice by 30-minute middle cerebral artery occlusion (MCAO) after 16 weeks on high-fat diet (HFD) or standard diet (SD). Blood glucose and HbA1C levels were measured before surgery and before the last imaging point. Seven days before and 2, 9, and 35 days after MCAO the animals were scored for neurological deficit followed by fundus photography, fluorescein angiography and magnetic resonance imaging of the brain and the ipsilateral eye.

RESULTS
Diabetes was confirmed in both HFD groups. WT on HFD showed significantly larger brain ischemic lesions in the acute phase and larger tissue atrophy in the chronic phase compared to B2R-KO on HFD. Oppositely, WT on SD showed significantly smaller tissue loss than B2R-KO on SD. There were no significant differences in the neurological status nor retinal thickness between groups, however delayed resolution of retinal edema was observed in B2R-KO mice.

CONCLUSION
Activation of B2R in non-diabetic animals promotes cerebral tissue recovery in the chronic phase of ischemic injury, in contrast to diabetic animals where B2R activation promotes tissue atrophy.

ACKNOWLEDGEMENTS
The study is supported by the Croatian Science Foundation project BRADISCHEMIA (UIP-2017-05-8082). The work of doctoral students Anja Barić and Rok Ister has been fully supported by the “Young researchers' career development project – training of doctoral students” of the Croatian Science Foundation funded by the European Union from the European Social Fund.

TRPA1 receptor may play modulatory role in the CNS diseases

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The Transient Receptor Potential Ankyrin 1 (TRPA1) non-selective cation channel expressed by the primary sensory neurons participates in mediation of pain and inflammation. However, there are only few data about its localization and importance in the central nervous system. RNAscope in situ hybridization technique showed the expression of Trpa1 mRNA in the mouse EWcp-UCN1 neurons and in the piriform cortex. TRPA1 might participate in stress adaptation, mood regulation, as well as in the smell loss which is an early sign of several neurodegenerative disorders. In the present study, we focused on its role in olfaction, social behaviour and chronic variable mild stress (CVMS) model of depression using Trpa1 knockout (KO) mice.

Trpa1 KO mice showed significantly greater immobility in the forced swim test compared to WTs, but CVMS increased the depression-like behavioural parameter only in the WT group. CVMS down-regulated Trpa1 expression in the EWcp of WT mice. Fox, but not cat odour activated directly the TRPA1 channels in TRPA1-overexpressing Chinese Hamster Ovary cells. Accordingly, KO animals showed less aversion against fox, but not cat odour. The social interest of KO mice was reduced during social habituation-dishabituation and social interaction, but not in the resident-intruder tests.

We conclude that TRPA1 receptors expressed in EWcp-UCN1 neurons possess physiological role in behavioural and stress adaptation responses in mice. TRPA1 may also contribute to odour processing in the olfactory tract and may influence the social behaviour of mice. Thus, TRPA1 could be a potential drug target in the pharmacotherapy of neurodenerative and mood disorders.

Funding: Thematic Excellence Program 2021 Health Sub-programme of the Ministry for Innovation and Technology in Hungary, within the framework of the EGA-16 project.

Intracellular signaling and metabolism in neuronal and astroglial cells in *Drosophila* aging brain

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During aging the brain energy utilization is reduced, likely contributing to the progression of cognitive decline. This may occur due to a malfunction of the noradrenergic system which controls the brain metabolism. Memory formation and learning are considered to depend on the release of noradrenaline from the neurons, triggering Ca^{2+} and cAMP signals in astrocytes. This then leads in astrocytes to an increased glucose uptake, glycogen degradation and stimulation of aerobic glycolysis with the end-product lactate, which is shuttled to neurons as fuel. However, how the noradrenergic system controls neurons and astrocytes in the aged brain is poorly understood.

We have designed *Drosophila* flies that express fluorescent sensors for Ca^{2+}, cAMP, free glucose and lactate in the brain, selectively in neurons or astrocytes. By confocal microscopy the changes in intracellular second messengers and metabolites were monitored upon the exposure of young and aged brains from *Drosophila* to octopamine (an invertebrate analogue of noradrenaline) or increased extracellular glucose and lactate levels. Interestingly, the results revealed that octopamine triggers Ca^{2+} and cAMP signals in neurons, but in astrocytes only Ca^{2+} signals, suggesting a difference in octopaminergic signaling between astrocytes and neurons. Neurons respond to octopamine application with an increase in cytosolic lactate. However, in astrocytes octopamine application induced an increase in cytosolic glucose, indicating that aerobic glycolysis occurs primarily in neurons. In aged brains in astrocytes the Ca^{2+} and glucose signals were absent, while in neurons the octopamine-induced signals were unaltered. Both neurons and astrocytes were able to uptake extracellular glucose and lactate, but in neurons the glucose uptake was reduced in aged brains.

Taken together our results suggest that the octopaminergic regulation of glucose metabolism in astrocytes and the glucose delivery to neurons are impaired in aged brain, which may contribute to the age-related cognitive deficits, representing a novel therapeutic target.

Examination of pituitary adenylate cyclase-activating polypeptide in Parkinson's disease focusing on correlations with motor symptoms

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The neuroprotective effects of pituitary adenylate cyclase-activating polypeptide (PACAP) have been shown in numerous in vitro and in vivo models of Parkinson's disease (PD) supporting the theory that PACAP could have an important role in the pathomechanism of the disorder affecting mostly older patients. Earlier studies found changes in PACAP levels in neurological disorders, therefore, the aim of our study was to examine PACAP in plasma samples of PD patients. Peptide levels were measured with ELISA and correlated with clinical parameters e.g. age, stage of the disorder based on the Hoehn and Yahr (HY) scale, subtype of the disease, treatment and specific scores measuring motor and non-motor symptoms, such as Movement Disorder Society-Unified Parkinson's Disease Rating Scale (MDS-UPDRS), Epworth sleepiness scale (ESS), Parkinson's disease sleep scale (PDSS-2) and Beck-Depression Inventory (BDI). Our results showed significantly decreased PACAP levels in PD patients without deep brain stimulation (DBS) therapy and in akinetic-rigid subtype, additionally we also described further decrease in the HY stage 3 and 4. Elevated PACAP levels were found in patients with DBS. There were no significant correlations in PACAP level with MDS-UPDRS, type of pharmacological treatment, PDSS-2 sleepiness and depression (BDI) scales, but we found increased PACAP level in patients with more severe sleepiness problems based on the ESS scale. Based on these results we suggest that following the alterations of PACAP with other frequently used clinical biomarkers in PD patients might improve strategic planning of further therapeutic interventions and help to provide a clearer prognosis regarding the future perspective of the disease.

Session 4 - Cell physiology

Bicarbonate transporters and how to measure them

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Mammalian life exists between blood pH broadly of 7.0 to 7.8. CO_2 and HCO_3^- are the major plasma buffers making this possible, and this equilibrium between pH, CO_2 and HCO_3^- are dynamically controlled by the predominantly the kidneys and the lungs. However, genetic, dietary, pharmacological and systemic physiological controls integrate the final outcome.
About 500 g of $NaHCO_3$ is absorbed in the kidney proximal tubule daily via SLC4A4 (the electrogenic Na^+ bicarbonate cotransporter, NBCe1). Human mutations cause severe metabolic acidosis and ocular problems. We will discuss NBCe1 transporter biophysics, human mutations, mouse phenotypes and emerging pH-sensing methodologies. The biophysics of human NBCe1 mutations tells us if there are functional or trafficking problems. Our recent data reveal that inappropriate NBCe1 expression occurs in pancreatic β-cells in type 2 diabetes. Current approaches use genetically encoded-pH sensors to track buffering and metabolic disturbances in β-cells as well as kidney epithelia.

Volume- and Acid-activated Chloride Channels in Microglia and Chondrocytes

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Cell volume (CV) needs to be precisely regulated. Cell swelling elicits regulatory volume decrease (RVD) and cell shrinkage by regulatory volume increase (RVI), respectively. Canonically, RVD is accomplished by activation of K^+- and anion channels which leads to cellular efflux of K^+, Cl^- and HCO_3^- ions as well as other osmolytes and osmotically obligated water, thus restoring CV. The anion currents/channels activated during RVD have been termed volume-regulated anion current (VRAC) or volume-sensitive outwardly rectifying (VSOR) anion current. Besides Cl^-, they are also permeable to other molecules including taurine, glutamate, glutathione, ATP or cGAMP, or drugs like cisplatin. Hence, VRACs serve for diverse cellular functions including, e.g., inflammation, phagocytosis, migration and programmed cell death. Different subunit combinations of LRRC8A-E proteins (with LRRC8A being indispensable) have been identified to underlie these functions on the molecular level. Similarly, strong extracellular acidification (pHe ≤ 5.0) activates anion currents which are referred to as acid-sensitive outwardly rectifying (ASOR) anion currents or proton-activated Cl^- (PAC) currents. ASOR channels are made up by TMEM206 proteins. The function of ASOR/TMEM206 channels are less clear, but recent research has revealed a crucial role in shrinkage of macropinocytotic vesicles, which is a prerequisite for proper content processing. Both VSOR and ASOR channels are widely expressed and share many electrophysiological properties. We and others have shown that ASOR currents are carried by anions (Cl^-) and not H^+ ions, are independent of extracellular Na^+, temperature-sensitive, activated by extra- but not intracellular acidification and that they depolarize the cell membrane potential. Simultaneous hypotonic and acidic stimulation, leads to VSOR and ASOR coactivation with a mixed current phenotype and a deactivation of VSOR over time, leaving a pure ASOR current. Strong acidification associated with ASOR current activation and VSOR current deactivation causes an isotonic CV gain and suppresses of RVD. Furthermore, in C28/I2 chondrocytes extracellular acidification blunts caspase 3/7 activation and enhances cell viability following apoptosis induction with staurosporine, eventually by inhibiting apoptotic volume decrease (AVD).

Kittl, M. et al. (2019) Int J Mol Sci *20*.; Kittl, M. et al. (2020) Front Cell Dev Biol 8.; Kittl, M., et al., (2021) Front Cell Dev Biol 9, 804105.

Three Different Time Scales of $[Ca^{2+}]_i$ oscillations from in Mouse Beta Cells in Tissue Slices

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Stimulus-secretion coupling (SSC) in beta cells regulates exocytosis of insulin in response to metabolic demands. The crucial steps in SSC upon stimulation with glucose are oscillatory changes in membrane potential (MP), intracellular calcium concentration, metabolism, and finally exocytosis of insulin granules. The changes in MP consist of bursts of action potentials (APs, also spikes), each burst being followed by a silent phase dominated by membrane repolarization and absence of APs. In mice, the shape of the bursts that last a few seconds and spikes that last a few tens of milliseconds, is brought about by an interplay between ATP-sensitive potassium channels, voltage-dependent calcium channels, and voltage- and calcium-dependent potassium channels. Underlying these fast bursts and ultrafast spikes are slow oscillations with a typical period of 5-15 minutes that most probably reflect oscillations in cellular metabolism. Multicellular calcium imaging of spikes would resolve coupling between changes in MP, calcium, and exocytosis within each beta cell, as well as between different cells within an islet. To this aim we combined the acute tissue slice method with a new fast calcium sensitive dye, highly sensitive hybrid detectors, and resonant laser-scanning confocal microscopy. We were able to resolve and quantify individual spikes superimposed on bursts at a temporal resolution of >100 Hz over prolonged periods with minimum photodamage. Using simultaneous patch-clamp recordings we correlated these fastest calcium changes with MP changes. By analyzing spikes from multiple subsequent bursts in many different beta cells, we quantified the relationship between bursting and spiking dynamics. To the best of our knowledge, this is the first detailed report of spiking behavior at the multicellular level. Once extended to tissue from diabetic mice and humans, it could help us better understand the mechanistic basis of diabetes and find new molecular targets.

Oxidative stress state in health and disease

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Oxidative stress (OS) is a state of free radicals (FR) overproduction that may damage cells, cause disease and accelerate aging process. Antioxidative defense may prevent FR damage by cell and plasma antioxidants. Since many factors have been already identified to induce OS, the aim of the study was to present the OS markers in healthy subjects and in chronic disease patients. A number of 80 healthy subjects were submitted regarding gender, age, body mass index, physical activity habits and living environment. Out of 115 chronic disease patients, 85 were related to chronic renal failure and 30 to diabetes mellitus. Patients were divided regarding gender, age, and chronic disease duration. Following parameters were investigated: free radicals – FR (D-ROM), Oxi-adsorbent test, SH-groups (by DIACRON, Italy) and lipid peroxidation by its end product malonyldialdehyde was determined by tiobarbituric acid method. For the statistical analysis Student t-test was performed. The results showed that women had lower OS compared to men, probably due to their protective estrogen effect ($p<0.05$). Aging process was related to an increased OS as the antioxidant defense obviously declines with the age ($p<0.05$) and also the obesity showed higher OS, due to the possible impaired metabolism ($p<0.01$). Modest physical activity contributed for better health condition according to lower OS markers, compared to the sedentary subjects. There was no difference in OS markers between city and village citizens probably because of their environment adaptation. According to different age and gender groups of the patients, no significantly difference was found, but the longer chronic disease, the higher OS was noticed ($p<0.01$). Due to the obtained results, it can be concluded that OS depends on many issues, so some may be modified in order to prevent further OS damage, but some have to be in charge when choosing and estimating appropriate therapy.

Key words: oxidative stress, healthy subjects, chronic patients

Protective effect of tamoxifen on cholestatic liver fibrosis in mice

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INTRODUCTION. Available treatments for cholestatic liver fibrosis are limited, and the disease often progresses to liver cirrhosis. Tamoxifen is a selective modulator of estrogen receptors, commonly used in breast cancer therapy. A recent in vitro study showed that tamoxifen deactivates hepatic stellate cells, suggesting its potential as an antifibrotic therapeutic, but its effects in vivo remain poorly investigated.

AIM. The aim of this study was to investigate the effect of tamoxifen *per se* on the development of liver fibrosis.

METHODS. C57Bl/6 mice were fed either 0.025% 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC)-supplemented diet or standard diet for 4 weeks and treated with either tamoxifen or vehicle (corn oil). Liver tissue and sera were analysed histologically (Sirius red staining, immunohistochemistry), genetically (qPCR) and biochemically (liver hydroxyproline content).

RESULTS. Mice fed with a DDC-supplemented diet and treated with tamoxifen developed a significantly milder degree of liver fibrosis than vehicle-treated mice, as evidenced by a lower percentage of Sirius red-stained area (60.4% decrease in stained area in male and 42% decrease in female mice, $p < 0.001$ and $p < 0.01$, respectively) and by lower hydroxyproline content. qPCR analysis showed a lower expression of genes for *Col1a1, Acta2, Sox9, Pdgf*, and *Krt19*, indicating the inhibitory effect on hepatic stellate cells, collagen production, and biliary duct proliferation. The degree of protection was similar in male and female mice. Tamoxifen per se, injected into standard-diet-fed mice, increased the expression of genes for *Il6* ($p < 0.01$ and $p < 0.001$ in male and female mice, respectively) and *Tgfβ* ($p < 0.01$ for both sexes), and had no adverse effects.

CONCLUSION. We showed that tamoxifen sex-independently protects against cholestatic DDC-induced liver fibrosis. The increased expression of Il6 and Tgfβ seems to be a plausible protective mechanism that should be the primary focus of further research.

Research has recently been accepted for publication, full text of the article is available at: https://www.mdpi.com/2227-9059/10/5/1209/htm

Evidence for the role of lipid raft disruption in membrane fluidity *in vitro* and Transient Receptor Potential Melastatin 8 ion channel activation in *in vivo* mouse model

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Transient Receptor Potential (TRP) cation channels as the Vanilloid 1 and Ankyrin 1 (TRPV1 and TRPA1) are playing important role in pain sensation, they are thermosensors. The „melastatin" TRP receptor TRPM8 is also expressed in subgroups of primary sensory neurons. TRP channels are proven to be embedded in lipid rafts, the specific microdomains of the cell membrane that are rich in cholesterol, sphingolipids, and gangliosides. Their integrity can be broken by methyl-β-cyclodextrin (MCD), sphingomyelinase (SMase) and myriocin (Myr). We previously described that lipid raft disruptors inhibit the activation of TRPV1, TRPA1 and TRPM8 channels in vitro, and we also demonstrated this effect *in vivo* for TRPV1/TRPA1. We aimed to test the effect of lipid raft disruptors on the activation of the TRPM8 ion channel *in vivo*, and their effects on membrane fluidity using fluorescence spectroscopic methods.

In *in vivo* experiments, mice were pretreated intraplantarly with MCD, SMase and Myr before the TRPM8 agonist icilin injection into the hindpaw of animals and we measured the duration of the pain reaction (raising, licking, chewing, shaking) for 20 minutes. For the *in vitro* studies, native CHO cells were treated with lipid raft disruptors, then they were incubated with 40 μM Laurdan and the decay curves of the Laurdan time-lapse emission spectrum between 410-540 nm were recorded. The microviscosity and the parameters characteristic of Laurdan and its environment were determined, from which we can deduce the membrane fluidity. The duration of the icilin-induced acute pain reaction was significantly reduced by MCD and SMase, but Myr was not effective. MCD decreased, while SMase and Myr increased the membrane fluidity. We suggest that the hydrophobic interactions between the TRP channel and lipid raft interfaces modulate the opening properties of these channels and therfore, targeting this interaction might be a promising tool for drug developmental puposes.

Support: TKP2021-EGA-16, TKP2021-EGA-13, NKFIH-138936, PTE-ÁOK-KA-2021-09, KTIA_NAP_20017-1.2.1-NKP-2017-00002, ÚNKP-21-3-II New National Excellence Program of the Ministry for Innovation and Technology from the source of the National Research, Development and Innovation Fund, Á. Horváth was supported by the Gedeon Richter Talentum Foundation.

Session 5 - Endocrinology

Endocrine disruptors and their effects on human health: update on obesogens

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Endocrine Disrupting Chemicals (EDCs) are a global problem for environmental and human health. They are defined as “an exogenous chemical, or mixture of chemicals, that can interfere with any aspect of hormone action”. It is estimated that there are more than 1000 chemicals with endocrine-acting properties. EDCs comprise pesticides, fungicides, industrial chemicals, plasticizers, nonylphenols, metals, pharmaceutical agents and phytoestrogens. Human exposure to EDCs mainly occurs by ingestion and to some extent by inhalation and dermal uptake. Most EDCs are lipophilic and bioaccumulate in the adipose tissue, thus they have a very long half-life in the body. It is difficult to assess the full impact of human exposure to EDCs because adverse effects develop latently and manifest at later ages, and in some people do not present. EDCs may interfere with synthesis, action and metabolism of sex steroid hormones that in turn cause developmental and fertility problems, infertility and hormone-sensitive cancers in women and men.

Recently it has been suggested that some EDCs promote adipogenesis and cause obesity. These EDCs are called “obesogens”. They may cause disturbance in energy homeostasis. Disruption of lipid homeostasis may involve multiple mechanisms: 1) increasing the number of adipocytes, 2) increasing the size of adipocytes, 3) altering endocrine regulation of adipose tissue development, 4) changing hypothalamic regulation of appetite, satiety and food preference, 5) affecting basal metabolic rate and 6) energy balance in favor of calorie storage and 7) altering insulin sensitivity in the liver, skeletal muscle, pancreas, gastrointestinal system and the brain. It has been shown that various EDCs alter adipogenesis through interfering with peroxisome proliferator-activated receptor-γ (PPAR-γ) function. In this talk, potential EDCs, their obesogenic effects, epidemiological studies to analyze their effects on human health, analysis methods and recommendations for prevention will be reviewed.

Does an oral glucose load affect endothelial function and how to evaluate it?

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While the long-term adverse effects of chronic hyperglycemia on microcirculation are well established, the effects of acute hyperglycemia are less well understood, *in vitro* studies reporting increased level of oxidative stress, altered activity of the autonomic nervous system, reduced activity of endothelial nitric oxide synthase. On the other hand, insulin was shown to augment, or diminish the endothelium-dependent vasodilation, additionally interfering with the activity of the sympathetic nervous system. The evidence of a concomitant impact of glucose and insulin on human microcirculation and its regulation *in vivo* remain scarce and contradictory. The most appropriate approach to induce hyperglycemia remains questionable: is the glucose-clamp really the most appropriate one or could it be replaced by an oral glucose load, possibly better mimicking physiological response? Moreover, the exact time-frame of glucose and insulin increase after an oral glucose load and their impact on vascular function have not accurately been established. To this end, we aim to investigate the effect of an oral glucose load (plasma glucose concentration > 6 mmol/L) on (micro)vascular function and elucidate possible mechanisms involved, focusing on skin microcirculation and some other hemodynamic parameters and heart rate variability, as well as the level of oxidative stress. The talk would expose the results of recent studies performed in young healthy volunteers, addressing some methodological issues of inducing hyperglycemia, assessment of microvascular function and oxidative stress, and expose some weaknesses and strengths of our research. Additionally, we will present a rather new tool for evaluating microcirculatory signals that enables a more thorough insight into the mechanisms of microvascular tone regulation, evaluating endothelial, myogenic, neurogenic, cardiac and respiratory influence, namely the wavelet analysis of the laser Doppler signal. Our results might help to better understand the underlying physiological changes following oral glucose load and, possibly, reveal pathologic implications in diabetic vascular disease.

Key words: hyperglycemia, oral glucose load, microcirculation, oxidative stress, endothelial function, laser Doppler flowmetry, wavelet transform

Physiology of 6th sense of taste: fat taste & obesity

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Obesity is a pathology that is arithmetically increasing worldwide. It is responsible for several pathologies, such as cancer, metabolic syndrome and cardiovascular diseases. The excessive consumption of lipid products is considered as a key factor, involved in this pathology.

It has been well propounded that there exists *five basic taste* modalities, *e.g.*, sweet, sour, bitter, salty and umami. Recent compelling evidence from rodent and human studies raise the possibility for an additional *sixth taste* modality devoted to the perception of lipids. A number of studies have recently suggested that lingual CD36, a glycoprotein, mainly expressed by circumvallate papillae of the tongue, might be implicated in the perception of dietary fat taste.
Our recent studies have not only supported the existence of the 6th taste modality, destined for the perception of fat, but also explored the intracellular signaling mechanisms, involved in this phenomenon. We have shown that lingual CD36, after activation by free fatty acids, induces increases in free intracellular calcium concentrations, ($[Ca^{2+}]_i$), phosphorylation of protein-tyrosine kinase (PTK) and release of the neurotransmitters like serotonin and nor-adrenaline into synaptic clefts. This signaling cascade is likely responsible for physiologic responses, induced by the detection of lipids in the oral cavity. The lipid-mediated regulation of feeding behaviour which is very critical in the development of several diseases like obesity and other metabolic disorders. Our studies show that fat taste signaling is altered in obese animals and there is a polymorphism of CD36 in obese subjects.

Calcium oscillations in human islets of langerhans and their changes in diabetes

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Introduction:
Calcium ions couple stimuli with secretion and coordinate intercellular activity in islets of Langerhans, but both intra- and intercellular coupling are altered in type 2 diabetes mellitus [1]. Most of our knowledge on calcium signaling is based on mouse models [2] and it is not entirely clear whether and how glucose controls calcium oscillations in human islets, to what extent they are synchronized in different beta cells, whether global calcium waves support small-world or some other type of functional network features, and which of these parameters change in diabetes.

Aim, Methods, and Results:
To address the above questions, we combined widefield calcium imaging in human islets isolated from control and diabetic donors with classical physiological and advanced network analyses. Glucose evoked regular calcium oscillations that were synchronized in different regions through intercellular calcium waves. Higher glucose facilitated the recruitment of beta cells, increased their active time, elicited a greater proportion of global waves from more stable initiator regions, and brought about denser and less fragmented functional networks. Islets from diabetic donors exhibited a slower recruitment, a shorter active time, a greater proportion of local waves, less stable initiator regions, and more segregated functional networks. Finally, while some diabetic islets displayed striking pathophysiological changes others from the same donor behaved rather normally, supporting the view that different islets are differently susceptible to diabetogenic insults.

Conclusion:
Our study is the first to combine classical and network measures in human islets and is able to account for and reconcile practically all of the features observed in previous studies addressing different aspects of calcium signaling. In conceivable future, our approach could be improved by a better resolution, additional physiological and pharmacological stimuli, and islets from patients achieving remission of diabetes, to importantly deepen our pathophysiological understanding of type 2 diabetes [3].

References
1. Benninger, R.K.P. and D.J. Hodson, *New Understanding of β-Cell Heterogeneity and In Situ Islet Function.* Diabetes, 2018. 67(4): p. 537-547.
2. Skelin Klemen, M., et al., *The triggering pathway to insulin secretion: Functional similarities and differences between the human and the mouse β cells and their translational relevance*. Islets, 2017: p. e1342022.
3. White, M.G., J.A.M. Shaw, and R. Taylor, *Type 2 Diabetes: The Pathologic Basis of Reversible β-Cell Dysfunction*. Diabetes Care, 2016. 39(11): p. 2080-2088.

Resistin affects the mechanical responses in strips from the mouse gastric fundus

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Introduction
Resistin, among its several actions, has been reported to display central anorexigenic effects in rodents. Some adipokines, which centrally influence feeding, have also been reported to affect the motor phenomena of the stomach. The latter are a source of peripheral signals involved in the control of the hunger-satiety cycle through the gut-brain axis. We previously observed that adiponectin influenced the mechanical responses of the mouse gastric fundus, through a nitric oxide (NO)-dependent mechanism.

Aim
To investigate whether resistin too could influence the mechanical responses in preparations from the mouse gastric fundus and the possible involvement of NO through a combined functional and immunohistochemical approach.

Methods
Full-thickness longitudinal strips were dissected from the mouse gastric fundus and mounted in organ baths for continuous recording of isometric tension. Western blotting and immunofluorescence analysis were also performed.

Results
At basal tension, electrical field stimulation (EFS) elicited tetrodotoxin- and atropine-sensitive contractile responses. Resistin reduced the amplitude of the EFS-induced contractile responses. This effect was no longer detected in the presence of the NO synthesis inhibitor, L-NNA. Resistin did not influence the direct muscular response to methacholine. In the presence of carbachol and guanethidine, EFS elicited NO-mediated relaxant responses whose amplitude was increased by resistin which also caused a decay of the strip basal tension. Western blot and immunofluorescence analysis revealed a significant increase in neuronal nitric oxide synthase (nNOS) expression in neurons of the myenteric plexus following resistin exposure.

Conclusions
The present results offer the first evidence that resistin acts on the gastric fundus, likely through a modulatory action on the nitrergic neurotransmission. From a physiological perspective, it could be speculated that the inhibitory action of resistin on the mouse proximal stomach represents a source of satiety peripheral signals, which could fit well with the central anorexigenic effects of the hormone.

Serum redox markers in uncomplicated type 2 diabetes mellitus accompanied with abnormal iron levels

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Abstract

This study aimed at evaluating the serum redox status in type 2 diabetes mellitus (T2DM) accompanied with an imbalance in iron concentrations.

Ninety diabetic patients were grouped according to serum iron levels [normal (DNFe), low (DLFe), and high (DHFe)], and their clinical and redox parameters [total sulfhydryl groups (tSH), uric acid (UA), and total bilirubin (tBILI) as non-enzymatic antioxidants, and malondialdehyde (MDA) and advanced oxidation products of proteins (AOPP) as markers of oxidative stress] were determined and compared to thirty healthy controls. Data were analyzed by two-way ANCOVA, taking the group and gender as factors, and controlling for the age of the subjects.

Glucose and HbA1c levels in the T2DM patients did not differ in function of serum iron. T2DM was associated with reduced tSH levels. In the diabetic patients, tSH, UA, and tBILI negatively correlated with MDA, as well as HbA1c with UA. Accordingly, AOPP and MDA were higher in the diabetic groups compared to the controls. The reduced antioxidant capacity was particularly pronounced in the DLFe group, which was further characterized by lower levels of UA and tBILI compared to the other groups. Subsequently, the level of MDA in the DLFe group was higher compared to the DNFe and DHFe groups. The positive correlation between serum iron levels and the antioxidants UA and tBILI, in conjunction with the negative correlation between serum iron levels and the markers of oxidative stress in the diabetic patients corroborated the indication that comparatively higher level of oxidative stress is present when T2DM coexists with decreased iron levels, irrespectively of the gender of the patients. The relatively non-invasive and simple determination of these parameters may be of considerable interest in monitoring the pathophysiological processes in T2DM patients, and may provide useful insights into the effects of potential therapeutic or nutritional interventions.

Keywords: type 2 diabetes mellitus; serum iron; oxidative stress; total sulfhydryl groups; uric acid; total bilirubin.

Caloric restriction improves diet-induced beta cell dysfunction in mouse model of type 2 diabetes

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

Nicotinamide pre-treatement of streptozotocin-induced diabetic rats prevent the changes in liver carbohydrate metabolism

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It was found that mixed administration of nicotinamide (NA) 15 minutes before streptozotocin (STZ) in adult rats not only had a protective effect but also led to the development of a new model of experimental diabetes, characterized by stable hyperglycemia and reduced insulin stores. In this work, we have investigated the alterations in liver carbohydrate metabolism in diabetic rats pretreated with NA.
In this work, we investigated the changes in rate-limiting enzymes and substrates related to hepatic carbohydrate metabolism in NA-pretreated diabetic rats. Experimental diabetes was induced by a single intraperitoneal injection of STZ (65 mg/kg body weight) and NA was given at two different doses (250 and 500 mg/kg/i.p./single injection, 15 minutes before STZ). Substrate concentration and enzyme activity measurements as well as Western blot and mRNA expression were performed.
STZ-induced diabetes caused a decrease in serum insulin and an increase in blood and hepatic glucose, as well as increased gluconeogenic enzymes (G6P-ase, F16BP-ase and PEPCK); decreased glycogen content and glycogenolytic enzyme (GP*a*), intermediate substrates (G6P, G1P and F6P) and glycolytic (HK, PK, PFK) enzymes.
Pretreatment of diabetic animals with both doses of NA showed a significantly increased insulin concentration compared to both diabetic and healthy (control) animals. It is important to emphasize that NA pretreatment did not allow the development of diabetic changes in enzymes and substrates. In fact, NA pretreatment showed significant changes compared to diabetic animals and non-significant changes compared to healthy animals in all parameters. In general, there were only minor differences between the two NA doses in a dose-dependent manner.
It could be concluded that NA pretreatment, has a protective effect against the development of diabetic alterations on all examined key carbohydrate-related enzymes and substrates. We propose that these protective NA- effects are based on the elevated serum insulin level caused by NA pretreatment.

Session 6 - Immunology and other topics

Sphingosine-1-Phosphate in Lung Physiopathology: exacerbator of circulating cells-mediated inflammation?

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Abstract

Motivation/problem statement: Sphingosine-1-phosphate (S1P) is a bioactive lipid mediator involve in inflammatory signaling/s associated with the development of respiratory disorders, including inflammatory disease and cancer, although many doubts about the precise mechanism are still to be clarified. In this study we aim to investigate the role of S1P, highly released from epithelial lung cancer cells, in circulating cells-mediated inflammatory processes.

Methods/procedure/approach: We used peripheral blood mononuclear cells (PBMCs) isolated from healthy volunteers and lung cancer patients to discriminate between the physiological and pathological role of S1P.

Results: We found that S1P exacerbates the pro-inflammatory milieu by inducing the release of TNF-α and IL-6 from lung cancer-, but not healthy-derived PBMCs in a S1P receptor 3 (S1PR3)-dependent manner. The pharmacological blockade of ceramidase and sphingosine kinases (SPHKs), key enzymes of S1P metabolism, in lung cancer-derived PBMCs completely reduced the release of both TNF-α and IL-6 after exogenous S1P stimulation. Furthermore, S1P-induced IL-6 release from PBMCs of lung cancer patients was mTOR- and K-Ras-, but not NF-κB-dependent.

Conclusion/implications: These data exalt S1P as one of the main orchestrators of lung cancer-associated inflammatory pathways, shedding light on a new pro-inflammatory mechanism mediated by immune circulating cells which sees S1P/IL-6 axis as a keystone in a complex landscape such as lung cancer.

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RNA-sequencing of osteoclast progenitor subsets in a murine model of arthritis

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Osteoclasts are multinuclear bone resorbing cells differentiated from monocyte/macrophage lineage of hematopoietic cells. They form by fusion of mononuclear osteoclast progenitors (OCPs), and mediate increased bone loss in arthritis. Our research group identified murine periarticular bone marrow OCPs as $CD45^{+}Ly6G^{-}CD3^{-}B220^{-}NK1.1^{-}CD11b^{-/lo}CD115^{+}$ cells using flow cytometry. They were further separated by the level of chemokine receptor CCR2 expression, into $CCR2^{hi}$ and $CCR2^{lo}$ subsets. We previously showed that these subsets are significantly expanded in collagen-induced arthrtis (CIA). Our aim was to characterize these OCP subsets by RNA-sequencing. Therefore, we sorted $CCR2^{hi}$ and $CCR2^{lo}$ OCPs from control and CIA mice (n=4 for every group), and performed RNA extraction, library preparation and RNA sequencing. For transcriptome profiling, we first aligned raw sequencing reads to the mouse reference genome, and then performed gene set enrichment analysis to evaluate the differential gene expression profile across samples. Principal component analysis showed that transcriptomes differed mostly based on CCR2 expression, and less depending on intervention (CIA/control). Our stringent analysis criteria ($\log_2$ fold change≥2) revealed total of 862 differentially expressed genes between $CCR2^{hi}$ and $CCR2^{lo}$ OCPs, included within several biological pathways. Top enriched pathways in $CCR2^{hi}$ OCPs in both CIA and control were osteoclast differentiation, chemokine and NOD-like receptor signaling pathways, while ribosomal biosynthetic pathways were downregulated compared to $CCR2^{lo}$ OCPs. In addition, several inflammatory pathways were enriched in $CCR2^{hi}$ subset only in CIA, including Staphylococcus aureus infection, Toll-like receptor and TNF signaling pathways. Genes selected from the enriched pathways were further validated by qPCR. To conclude, $CCR2^{hi}$ was more altered than $CCR2^{lo}$ subset in CIA compared to control. $CCR2^{hi}$ OCPs posses greater osteoclastogenic and migratory potential than $CCR2^{lo}$ OCPs. Furthermore, $CCR2^{hi}$ subset becomes specifically responsive to inflammatory conditions in CIA. These results indicate that $CCR2^{hi}$ OCPs could be mainly responsible for enhanced osteoresorption in arthritis.

Rab10 GTPase is involved in membrane remodeling to form assembly compartment in the early phase of murine cytomegalovirus infection

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Murine cytomegalovirus (MCMV), like other members of the herpesvirus family, forms a viral assembly compartment (AC), where synthesis of new virions takes place. The process of AC formation is initiated in the early phase of infection and requires intense remodeling of host membranes at the interface between early endosomes, endosomal recycling compartment, and trans-Golgi network (EE-ERC-TGN). In uninfected cells, transport between EE-ERC compartments is regulated by a sufficiently resolved function of Rab10 GTPase.

The aim of our study was to clarify the role of Rab10 in AC biogenesis. With that purpose, we analysed the localization and function of Rab10 and its regulators and effectors in MCMV-infected fibroblasts.

By confocal analysis, we found that Rab10 and its regulators and effectors (ACAP1, ACAP2, EHBP1, EHD1, Rabin8, Rab5, Rabex5) showed pericentriolar accumulation in the inner AC (iAC) during the early phase of MCMV infection (6/16 hr p.i.). In addition, western blot analysis showed that total expression, as well as membrane mobilization, of Rab10 and its regulators did not change during the 40 hours period. Interestingly, Rab10 disappeared from the iAC in cells treated with Dynasore (a reversible dynamin inhibitor), indicating the role of dynamin-2 for AC biogenesis. Finally, we tested the importance of the GTPase function of Rab10 in the early phase of AC formation. For this purpose, cells were transfected with the Rab10-EGFP constructs (EGFP-Rab10, EGFP-Rab10Q67L (dominant active), and EGFP-Rab10T23N (dominant inactive)). Interestingly, the both constructs with mutations in the Rab10 GTPase domains inhibited iAC formation, resulting in decreased accumulation of the iAC marker PLEKHB2.

We can conclude that, although the expression of Rab10 and its effectors has not been changed in MCMV infection, they are are accumulated in AC in the early phase of MCMV infection. Importantly, proper dynamin-2 and Rab10 GTPase function are involved in this process.

This work was supported by Croatian Science Foundation grants (IP-2020-02-2916, IP-2020-02-1323, and IP-2019-04-3582), and by the UNIRI (uniri biomed-18-180, uniri-biomed-18-88, uniri-biomed 18-229).

Humoral and cellular immune response induced by novel SARS-COV-2 vaccines

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INTRODUCTION. Three types of SARS-CoV-2 vaccines are available in Hungary; vector-, inactivated virus-and mRNA-based. The relationship between vaccine types and the humoral and cellular response has been investigated.

MATERIALS AND METHODS. Antibody levels of 255 healthy individuals were tested using SARS-CoV-2 anti-S1 protein IgA and IgG ELISAs, and NeutraLISA (ACE2 receptor S1 RBD inhibition assay). T cell response was measured using SARS-CoV-2 Interferon Gamma Release Assay (IGRA) (Kits from: Euro-immun GmBh). Anti-citrate synthase (anti-CS) IgG autoantibodies were determined using in-house ELISAs. Blood samples were taken 2-3 months after vaccination. Vector vaccine (n=61), inactivated pathogen (n=28), mRNA-based (n=71). Controls: unvaccinated healthy (n=10), unvaccinated PCR-confirmed disease-experienced individuals (n=26).

RESULTS. Significant correlations were found between vaccine types and seropositivity rates in terms of antibody (IgA, IgG), and cellular response (anti-human IFN-γ) (Khi^2 test: $p < 0.01$). Positivity rates for vector, mRNA and inactivated virus vaccines: anti-S1 IgA: 41%, 73% and 25%; IgG: 74%, 94% and 50% (respectively). IFN-γ positivity: 84%, 82% and 39% (respectively).

CONCLUSION. In terms of antibody response mRNA vaccines were significantly more effective than both vector-based (IgA, IgG: $p<0.001$) and inactivated virus-based vaccines (IgA, IgG: $p<0.001$). Regarding IgG titres, mRNA vaccine was also significantly more effective than COVID infection ($p< 0.001$). Considering the cellular response wild-type infection, mRNA and adenovirus-based vaccines were equally effective. A weaker IFN-γ response was found in individuals immunized with inactivated virus vaccine. Importantly, the latter group had the highest mean age (64 years).

The research was financed by the Thematic Excellence Program 2021 Health Sub-programme of the Ministry for Innovation and Technology in Hungary, within the framework of the EGA-10 project of the Pécs of University.

T lymphocyte subpopulation in peripheral blood and follicular fluid of patients treated with medically assisted fertilization with and without thyroid autoimmunity

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AIM: Analyze lymphocyte subpopulation in peripheral blood and follicular fluid (T and B lymphocyte, natural killer cells, natural killer T cells) of the patients with positive thyroid autoantibodies compared to patients with no thyroid autoantibodies.

MATERIALS AND METHODS: One hundred and sixty seven female in assisted reproduction were included in the research at the Department of gynecology and human reproduction and the Clinical department of nuclear medicine, Clinical hospital centre Rijeka. Peripheral blood and follicular fluid samples were analyzed at the Department of physiology, immunology and pathophysiology of the Medical faculty, University of Rijeka, using facscalibur flow cytometer for direct flow cytometry. From the samples, after centrifugation on a gradient of density, mononuclear cells were isolated.

RESULTS: The percentage of total T lymphocyte (CD3+) is statistically significantly lower in the follicular fluid of the patient compared to the peripheral blood regardless of the presence of antibodies.
The percentage of T helper cells (CD4+ CD3+) is statistically lower in peripheral blood and follicular fluid of a patient with positive thyroid antibodies compared to patients without ATA.
The percentage of T cytotoxic cells (CD8 + CD3 +) is statistically significantly lower in peripheral blood and follicular fluid of the patient regardless of the presence of antibodies.
The percentage of total B lymphocytes (CD19 +) is statistically lower in the follicular fluid of the patient regardless of the presence of antibodies. In the blood of a patient with elevated thyroid antibodies, the share of these cells is significantly lower.

CONCLUSION: Percentage of NKT cells, other subpopulation of cells of innate immunity, is statistically significantly higher in the blood and follicular fluid of a patient with ATA compared to patients without ATA. In the follicular fluid of patients with autoimmune thyroid diseases there are changes in cells of innate and acquired immunity.

Diagnostic accuracy of salivary matrix-metalloproteinase-3 to diagnose temporomandibular disorders in patients with axial spondyloarthritis

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Background: Th17 immune response underlying osteoarthritis acts proinflammatory inducing osteoclastic process with osteolysis, which is partially mediated by the activity of matrix metalloproteinase-3 (MMP-3). Temporomandibular disorders (TMDs) correlate with neck pain and makes spondyloarthritis (SpA) clinically more severe.

The aim of this study was to analyze salivary MMP-3 diagnostic accuracy for TMDs in patients with SpA with neck pain, as well as MMP-3 correlation with laboratory and functional status of patients.

Methods: Patients with non-radiographic axial SpA (N 90), diagnosed according to ASAS (Assessment of Spondyloarthritis International Society) criteria, who reported neck pain, were examined according to the Diagnostic Criteria for temporomandibular disorders (DC/TMD). SpA was assessed with BASDAI (Bath Ankylosing Spondylitis Disease Activity Index) and BASFI (Bath Ankylosing Spondylitis Functional Index). Unstimulated saliva was sampled for MMP-3 analysis (ELISA) and serum for C-reactive protein (CRP) and antistreptolysin-O titer (automatic analyzer).

Results: Salivary MMP-3 increased in patients with TMDs in comparison with patients without TMDs (P= 0.045) and showed diagnostic accuracy for TMDs with cut-off value at 104 pg/mL, specificity 100%, and sensitivity 29%. Salivary MMP-3 positively correlated with antistreptolysin-O titer, BASFI, limitation of mandibular movement, and with physical symptoms of general health measured with DC/TMP protocol. It did not correlate with BASDAI, CRP, chronic pain, anxiety and depressive disorder.

Conclusion: Salivary MMP-3 could help in diagnosis of TMDs in patients with non-radiografic axial SpA with 100% specificity. It mirrors local and systemic functional status of patients, particularly with enhanced antistreptolysin-O titer. However, due to a low sensitivity of the test, a large proportion of patients might remain undiagnosed this way.

Funding: University grant Uni-ri-biomed-18-110 and Uni-ri-biomed-18-160.

Detection of Sars-Cov-2 antigens in thyroid gland showing histopathological features of subacute thyroiditis

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Clinical and laboratory findings of subacute thyroiditis have been repeatedly reported as being associated with acute Sars-Cov-2 infection and post- COVID-19 syndrome. Exact mechanisms and histopathological correlates underlying thyroid involvement remained unresolved, but current insights suggest either direct viral damage, systemic inflammatory reaction, or an autoimmune response as possible noxious effectors. Here we present findings of immunohistochemical/immunofluorescence detection of Sars-Cov-2 viral proteins in relation to histoarchitectonic changes of autoptic thyroid tissue obtained from patient who deceased from COVID-19. Samples of thyroid gland were found with striking Sars-Cov-2 spike glycoprotein immunohistochemical/immunofluorescent positivity that was present throughout the thyroid tissue and localized diffusely in cytoplasm of follicular cells. This finding was associated with the presence of copious mononuclear infiltrate forming granuloma-like structures with giant multinucleated cells, as well with extensive follicular disruption/collapse along with epithelial desquamation and colloid depletion, being consistent with histopathological characteristics of subacute thyroiditis. Spike protein positivity was also found in epithelioid cells within the granuloma fulfilling destroyed follicles. In contrast, nucleocapsid protein immunopositivity was noticeably sparser, forming punctiform clusters with predominant perinuclear localization in thyrocytes. Such expression discordance between these Sars-Cov-2 antigens suggests possibility of S protein shedding in thyroid gland, leaving behind targets of the immune system even after viral clearance. Interestingly, the vast majority of remnant thyrocytes were immunopositive for cleaved caspase-3, pointing to the apoptosis as an important mechanism in SARS-CoV-2-induced thyroid pathology.

Long COVID-19: The physiology-based risks and perspectives

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Long COVID-19 (**co**rona**vi**rus **d**isease – 20**19**) is novel disorder which present in about 30% of survivors which were diagnosed with a neurological illness within six months of infection. The neurological signs and symptoms of long-COVID-19 may include fatigue, myalgia, headache, cognitive impairment ('brain fog'), while anxiety, depression, sleep disturbances, and post-traumatic stress syndrome (PTSD). Other organ systems involved in 'long-COVID-19' include the respiratory, cardiovascular, hematologic, gastroenterological, endocrine, and renal systems. The long-COVID-19 is complex and multifactorial disorders which predictors are still unknown.

The physiological basis of post-COVID-19 syndrome is still under discussion. Recent investigated physiology-based mechanisms of long-COVID-19 complications which involve microvascular thrombosis, immune dysregulation, and inflammation will discussed. Risk factors which could be predictors of long COVID-19 detected in original study of cohort survivors with SARS-CoV-2 infection. The complexity of the long-COVID-19 syndrome requires a multidisciplinary coordinated research approach, sufficiently powered to provide robust evidence to develop patient care, rehabilitation, support, and the formulation of health policies based on detected physiology-based risk factors.

Keywords: Long COVID-19, SARS-CoV-2, physiology, inflammation

Poster session #1: Sport physiology

The effect of acute exercise and CHO intake on antiinflammatory adiponectin and inflammatory markers in elite cyclists

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Abstract

Introduction
High-intensity exercise can elicit acute changes in the biochemical and physiological processes of the athlete body, including increased oxidative stress and inflammation. Also, the carbohydrate (CHO) intake may influence performance and inflammatory responses in elite athletes.

Methods
Ten elite men cyclists (age 15–26 years, body mass index (BMI) 19.4–24.7 kg/m^2) participated in this study. Each subject performed the maximal oxygen uptake test (V_{O_2max}) and completed a ten-minute cycling exercise at a workload of 50% of the peak of V_{O_2max}. Blood samples were collected on three different occasions: after an overnight fasting and at the exercise workloads of 50 % and 100 % V_{O_2max}. We measured APN, inflammatory markers (CRP, IL-6, TNF- α) as well as assessed nutrient and energy intake for each participant by 3-day dietary protocols.

Results
IL-6 increased 2.3-fold at 50 % V_{O_2max}, and 4.1-fold at 100 % V_{O_2max} from the baseline level of 4.3 pg/mL. TNF-α increased 1.5-fold at 50 % $V_{O_2max}$and 1.3-fold at 100 % V_{O_2max} from the baseline value of 1.1 pg/mL. Baseline concentration of serum APN before the load was 10.9 µg/mL and increased 1.4-fold at 50 % V_{O_2max} and 1.5-fold at 100 % V_{O_2max}. The concentration of APN at 50 % V_{O_2max} positively correlated with the CRP and negatively correlated with TNF- α ($p < 0.05$). The average daily CHO intake was 6.1 g/kg body mass. Basal IL-6 levels were lower when the daily CHO (> 7 g/kg body mass) and post-exercise (> 1.3 g/kg BM) intakes were within recommended levels.

Conclusion
Exercise increases muscle production of IL-6, which is responsible to reduce the pro-inflammatory TNF-α production, and to increase the anti-inflammatory APN. Finally, adequate CHO intake reduces pro-inflammatory IL -6. These observations are of importance in planning recovery training after strenuous exercise, or during disease rehabilitation.

KEY WORDS: Inflammation, adiponectin, nutrition, cycling

Team sports and ACTN 3/R577X, ADRB 3 and ACE polymorphisms

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In the last thirty years, in the context of sports, the association of more than 200 gene polymorphisms with motor and functional abilities has been confirmed. The purpose of this study was to determine the occurrence of different genotypic polymorphisms of ACTN3/ R577X, ADRB 3 and ACE genes in players of team sports of the highest competitive rank in Croatia. For these genetic polymorphisms, research to date has suggested a possible correlation with success in energy-extreme spots such as power lifting and sprinting, and on the other hand, marathon and high-endurance sports.
The sample consisted of 127 athletes: 56 football players, 40 basketball players, 31 volleyball players of top competition levels who underwent the genetic testing. For the purpose of this study we calculated so-called “sport index” from polymorphisms previously in literature related to sport success.
“Superior” gene polymorphism RR-ACTN3 R577X's for explosive ability and strength was represented in 37% of the selected sample of top athletes. The study showed statistically significant differences in the distribution of the RR polymorphism of the ACTN3 gene. There was no difference in the incidence of Ins/Ins ACE gene polymorphisms and Trp/Trp and Trp/Arg ADRB3 gene polymorphisms, which were assumed to be more common in sports with a pronounced aerobic component. The results showed statistically higher “sport index” in midfield footballers in comparison to other soccer positions, but no statistically significant difference in the incidence of certain polymorphisms between three team sports athletes and the general population of this region.
This research raises the question of determining the coefficient of influence of a particular gene polymorphism on the possibility of developing a certain ability and their interaction, as well as their influence on the expression of other abilities and skills that determine the sport result and relate to all elements of the complex multifactorial nature of sports success.

Influence of creatine supplementation on level of menstrual pain in females

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ABSTRACT

Introduction: creatine is a dietary supplement that has a number of confirmed positive effects. The aim of this study was to determine the effects of supplementation with the new formulation of creatine on the level of menstrual pain in the female population.

Materials and Methods: the study included 20 participants (age 23.7±2.8 years, BMI 21.5±2 kg/m², body weight 61.8±8.1kg). The supplementation lasted 8 weeks in a regimen of a double-blind, placebo-controlled randomized study. The experimental group (n=10) consumed a combination of creatine monohydrate (Cr) and guanidino-acetic acid (GAA) in equal proportion (2.5gr Cr + 2.5gr GAA), and the placebo group (n=10) an equivalent amount of maltodextrin. Following measurements were done: body mass composition using bioimpednace, heart rate and blood pressure, the upper arm, waist, hips and thighs circumference, skin folds on the upper arm and abdomen, height and body weight, and questionnaires for estimating the amount of physical activity in the last week (IPAQ short form), pain assessment (VAS pain scale ranging from 1 to 10), and a questionnaire on the monthly period and the monthly cycle drawn up only for the purposes of this paper.

Results: pain in the Cr+GAA group was lower post supplementation (before 7.1±1.9; VAS1 3.8±2.4; VAS2 4.1±2.5; p>0.05). Total amount of body water was significantly lower in the Cr+GAA group (32.5±2.8kg vs. placebo 35.3±3.5kg; p=0.04) and intracellular fluid (19±1.7kg vs. placebo 20.7±2.2kg; p=0.04). The body mass index was significantly lower in the Cr+GAA group post supplementation (20.8±1.8kg/m² vs. placebo 22.8±1.9kg/m²; p=0.02) as well as waist circumference (73.6±5.9cm vs. placebo 76.4±6.6cm; p≤0,001), while the skin fold on the abdomen was significantly higher (14.8.8±3.5mm vs. placebo 10.8±3.3mm; p=0.04).

Conclusion: Supplementation with a combination of creatine and guanidino-acetic acid led to changes in the body mass composition of women. Level of pain was not significantly affected.

Key words: creatine, guanidino-acetic acid, dysmenorrhea, menstrual pain

Comparison of the values of resting metabolic rate by indirect calorimetry and by predictive equations in sport athletes

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Resting metabolic rate (RMR) is very important in sport medicine, in order to support adequate nutritional prescription of energy and macronutrient requirements. As RMR is often measured by indirect calorimetry (IC), thus theoretical calculation of prediction equations for RMR may be a faster and easier tool to be used in routine examination of sport athletes. The aim of this study was to compare the accuracy of the predictive equations for RMR with the values obtained by the IC in sport athletes.
A number of 51 male (28±5 years old) and 39 female (27±3 years old) subjects of different sport disciplines were included in the study. The body composition measurements (body weight, body mass index–BMI, fat mass, lean muscle mass) were determined by the Body Composition Analyser with bioelectric impedance In Body 720, Great Britain, and the RMR was obtained by Fit Mate device, COSMED, Italy. A complete anamnesis of the daily habits of the subjects was taken prior calculation of RMR with following prediction equations: Harris and Benedict, Mifflin-St.Jeor, Katch-McArdle, Henry-Oxford, Schofield and Cunningham. To compare the measured and predictive values of RMR, the Bland-Altman statistical analysis was used.
Male subjects showed higher values of body height and weight, BMI and lean muscle mass ($p<0.05$) and significantly lower value of fat mass ($p<0.05$), compared to female subjects. All predictive equations for RMR showed significantly lower values, compared to those values measured by IC, with exception for the equation of Cunningham, probably due to the fact that it includes the parameter of muscle mass, which is more present in athletes, compared to the general population. Thus, we can conclude, the Cunningham’s equation may be used as a method of choice for more appropriate and more precise value of RMR in sport athletes’ examinations.

Key words: resting metabolic rate; indirect calorimetry; predictive equations; sport athletes.

Endurance exercise affects the cardiac parasympathetic response to subsequent moderate exercise

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INTRODUCTION:
Endurance exercise (EE) alters the activity of autonomic nervous system (ANS) for days to completely recover. Thus the ANS response to subsequent moderate aerobic exercise performed repeatedly during recovery to EE as a frequently used training regime in recreational athletes may be affected in time dependant manner. The aim of our study was to examine the impact of EE on the cardiac ANS response to subsequent moderate cycling with respect to time elapsed since EE cessation.

METHODS: Young healthy subjects (N = 19) performed a 21-km run as a model of EE, a moderate cycling bout (MCB) before EE (baseline) and four MCBs after EE subsequently at the same day, one, two and seven days after EE. Heart rate, heart rate variability, heart rate recovery and the rate of perceived exertion were monitored before and after each MCB.

RESULTS: On the same day after EE there was a reduced activation of the parasympathetic nervous system to the heart (slower hear rate recovery, smaller heart rate variability at rest and during recovery to MCB) compared to baseline indicating a cardiac vulnerable period. One day after EE, an increased activation of cardiac parasympathetic activity was found in the recovery after MCB but not at rest (faster hear rate recovery, lower heart rate in the recovery to MCB) compared to baseline. Two days after EE, lower heart rate in the recovery to MCB compared to baseline was detected. The cardiac ANS response at day 7 did not differ from the baseline response. MCB was considered more difficult immediately after EE compared to baseline.

DISCUSSION: Performing moderate exercise at the same day after EE may potentially lead to adverse cardiac events while moderate exercising one day after EE could be optimal to achieve the best cardiac adjustments to exercise and potentially improve cardiac performance.
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Physical activity of children and young people in health promotion

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ABSTRACT
Insufficient physical activity is a global problem due to its adverse effects on health and the increased costs of treatment. In addition to physical inactivity, health is affected by other factors such as stress, poor quality of life, and modern lifestyle. Scientific evidence indicates a reduction in the loss of functionality and an increase in chronic diseases and injuries associated with a sedentary lifestyle. Given the importance of physical activity in the prevention of many chronic non-communicable diseases and its role in reducing overall health care costs, the level of physical activity is a frequent topic of study on a global level.
The justification for the implementation of national strategies and action plans aimed at increasing physical activity is manifested in the reduction of the frequency of disease occurrence, and cost-effectiveness compared to other preventive measures. Therefore, the goal of this review is a general insight into the level of physical activity of children and young people as well as the influence of sedentary behavior on the incidence of painful symptoms.
Databases and complete texts from the fields of biomedicine and health and social sciences were searched using questionnaires to assess the level of physical activity and musculoskeletal health. The link between physical activity and the state of human health is indisputable, it is the basic factor that determines the level of changes in the body and the state of its skeletal, muscular, cardiovascular, and nervous systems. Therefore, physical activity is an integral part of everyday life and a key factor in maintaining a healthy organism, as well as preventing the appearance of acute pain symptoms.

Keywords: physical activity, pain symptoms, musculoskeletal system, students

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The effects of regular physical activity and playing video games on reaction time in adolescents

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Introduction: Reaction time is of the great importance in life. In both sports and video games, movements of participants are conditioned by different visual, acoustic and somatosensory signals.

Aim: The aim of this research was to determine whether the reaction time is influenced by regular physical activity and playing video games in adolescents.

Methods: The study included 41 female and 26 male students, aged 10-14 years. Questionnaires about habits related to chronic physical activity and playing video games were given to the examinees. Afterwards, the reaction time was determined for visual stimuli, via a computer program.

Results: The obtained results show that there is a statistically significant difference in the value of the reaction time of children who are regularly engaged in physical activity relative to those who play video games (0.327 ± 0.081 sec vs. 0.403 ± 0.137 sec, p=0.013), while there is no statistically significant difference in reaction time between children who equally participate in physical activity and video games (0.386 ± 0.134 sec) compared to those who regularly practice physical activity (p=0.156), and those who only play video games (p=0.610).

Conclusion: Physical activity can decrease reaction time in children, but further studies are needed to elucidate the impact of regular physical activity and gaming on the developing adolescent brain.

Keywords: physical activity; video games; reaction time; adolescent

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Measurement of heart rates and skin temperatures in exercise testing of Lipizzan horses

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Introduction: As with human athletes, the fitness level of sport horses can be assessed by exercise testing (ExT), which monitors the responses of organism to a workload. The performance of the horse during the ExT is reflected by the changes in heart rate (HR) and can be influenced by disturbances in thermoregulation. Therefore, determination of HR and body surface temperature (BST) is a useful indicator for evaluation of fitness level and health status of the sports horses. Since the physiological responses of Lipizzan horses to workload are poorly known, the aim of this study was to investigate the changes in HR and BST during graded ExT.

Material and methods: Ten Lipizzan fillies were lunged for 15 min in four ExTs, repeated every 2-weeks. BSTs were measured before (BEx) and after exercise (AEx) by infrared thermography (IRT) with a thermal imaging camera FLIER E40bx (Wilsonville, OR, USA) and HR throughout the ExT with a commercial detector for continuous monitoring and real-time recording of HR (Polar electro, Kempele, Finland).

Results: HRs and BSTs were within the physiological ranges for warm-blooded horses BEx and increased AEx significantly in all 4 tests due to the increased metabolic demands, although HRmax (200 bpm) was not reached, which indicates good fitness of the fillies. Bilateral symmetry of BSTs was confirmed BEx and AEx. The highest BSTs measured AEx were at the cranial, followed by the caudal and distal body regions, indicating differences in their muscle load.

Conclusions: The results of our study indicate an improvement in the physical condition of the fillies, their adaptation to the environment and habituation to the work routine. The study contributes to the knowledge of the physiological processes in young horses during exercise and provides a basis for further research in the equine ExT, sports medicine and animal welfare.

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Gender differences in the effects of breathing exercises on chest excursions in healthy students

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Introduction: From clinical practice and research it is known that breathing exercises have a positive effect on breathing patterns and thoracoabdominal movement.

Aim: This study aims to examine the effects of breathing exercise on chest exursions in healthy students and to assess gender differences in chest circumferences.

Methods: Prospective study was conducted at the Department of Human Physiology at the University of Sarajevo - Faculty of Medicine. The 60 healthy students were divided into male (n=30) and female (n=30) groups. Set of four breathing exercises was practically demonstrated to all of the subjects and written instructions were given to them. The chest circumferences (axilla-acromion, axilla-axilla, circumference at the height of processus xiphoideus and circumference at the costal margin) were measured during the maximum inspiration and maximum expiration before and after three months of practicing breathing exercises.

Results: In the male group following circumferences measured during the maximum inspiration had statistically significantly higher values after three months of practicing breathing exercises: axilla-acromion (p=0.001), axilla-axilla (p=0,004), circumference at the height of processus xiphoideus (p=0,004) and circumference at the height of costal margin (p=0,001), while during the maximum expiration it was measured statistically significantly lower values of: axilla-axilla circumference (p<0,0005) and circumference at the height of processus xiphoideus (p=0,002). In the female group, statistically significantly higher values were found in following circumferences measured during the maximum inspiration: axilla-acromion (p=0,011), axilla-axilla (p<0,0005), circumference at the height of processus xiphoideus (p<0,0005) and circumference at the height of costal margin (p<0,0005), while statistically significantly lower values were found in circumferences measured during the maximum expiration: axilla-acromion (p=0,002), axilla-axilla (p=0,001) and circumference at the height of costal margin (p=0,015) after tree months of practicing breathing exercises.

Conclusion: The range of respiratory mobility of subjects' ribcage significantly increased after three months of practicing breathing exercises.

Key words: breathing, breathing exercises, anthropometric measurements, chest excursions

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Diferrent sport disciplines related to respratory function

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It is well known that sport activity may have beneficial effect on respiratory function. Thus, spirometric reference equations for general population may not be applicable to elite athletes. The aim of the study was to investigate the respiratory function parameters of the elite athletes, and also among different sport disciplines.
A number of 59 male athletes (25±4 years old), aged matched to a control group of 30 male healthy subjects, were included in the study. The athletes were divided in 4 groups, concerning the sport disciplines: I group – skills (tennis); II group – power (bodybuilding); III group – mixed (basketball/ football); IV group – endurance (swimming). In all groups, the following parameters were examined: body weight (kg), body height (cm), body mass index - BMI (kg/m^2), skeletal muscle mass - SMM (kg) and the amount of proteins (kg) and minerals (kg). The anthropometric measurements were performed by the the Body composition analyzer InBody 720, Great Britain. The spirometry method was performed with the Spirobank II spirometer (Rome, Italy), for the following parameters: forced vital capacity (FVC) in L, forced expiratory volume in the first second (FEV1) in L, and the FEV1/FVC ratio.
The anthropometric measurement showed better body composition in all groups of sport disciplines compared to the control group. Among all, the IV group showed the best spirometric results for FCV, FEV1 and FEV1/FVC ratio ($p<0.05$). It is probably due to the elasticity of the respiratory tissue, intermittent hypoxia between respiratory cycles, due to the increased respiratory excursions. Additionally, it may be due to obtaining the increased pressure of diaphragm and all respiratory muscles in water immersion.
From the obtained results, it may be concluded that swimming is a sport of choice to strengthen respiratory function and to give better response during the sport activity need.

Key words: sport disciplines; spirometry; sport athletes

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Levels of physical activity in medical students in North Macedonia

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Abstract
The aim of the study was to assess the levels of physical activity in a cohort of medical students enrolled on Medical Faculty, University "Ss. Cyril and Methodius", in Skopje during 2018/2019 study year. A total of 637 (217 first year, 195 second year, 124 third year, 61 fourth , 69 fifth and 20 sixth year) medical students aged 18-33 years received and answered the IPAQ questionnaire for evaluation of the intensity of physical activity. Gender structure of the cohort was 242 (38%) male and 395 (62%) females.
Metabolic equivalent minutes per week (MET minutes) for physical activity and “hours per week” for sedentary behavior were analyzed parameters. Mean values of calculated MET minutes per week in students from all study years were categorized as high level of physical activity. Highest mean values were evident in students from the sixth and fifth study year compared to first year students (F (ANOVA= 9.09531; $p<0.0001$)). Highest mean values of the parameter “hours a week” for sedentary behavior were achieved in the third and fourth year students, while students form first and second year showed the lowest sitting hours. Almost 20% of female and 14.4% of male students showed insufficient physical activity (< 600 MET minutes a week), accompanied with intense sedentary behavior.
It is very important that ways of proper, adequate, sustainable and successful involvement of all medical students in regular physical activity should be designed and implemented in medical schools curricula especially at the start of the medical education.

Key words: medical students, IPAQ, physical activity

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Cardio-physiological adaptation in handball players during Bruce treadmill test

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Abstract

Background: Ergometrical testing in athletes is method for monitoring the cardiophysiological parameters as indicator of the cardiovascular health and the physical fitness. The ultimate product of Bruce protocol is maxymal oxygen consumption (VO_{2max}). A grafical presentation of the Bruce test's result is the heart rates curve which is made of mean values of heart frequency for each minute, starting with heart rate during resting period, through the ten consequent minutes of the treadmil testing, until the end of 3 minutes recovery period. Material and methods: 28 male HB players from two top ranking teams from RNM were tested ergometrically with Bruce protocol for determination of maximal oxygen consumption. Body analysis was made with bioelectrical impedance analyzer, InBody 720. Results: Anthropometric parameters were as follows: mean height was 190.4 ±7.8 cm and weight 96.3 ±15.5 kg, skeletal muscle mass (SMM) = 47.11±6.69 kg; BMI = 26.38 ±3.1; BF%=15.04 ±6.01 and WHR = 0.9 ±1.8. The result of ergometrical test produce mean VO_{2max}= 43.92 ml/kg/min which is 100.46% of reference value. Conclusion: Bruce protocol heart rates curve showed succesful cardiovascular adaptation to submaximal effort in handball players from elite macedonian handball teams. The general endurance expressed with maxymal oxygen consumpiton was at level of healthy active people.

Key words: ergometry, cardiac adaptations, handball players, heart rates

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Effect of the general mountaineering school on the body weight and mass of recreational mountaineers

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Introduction: Hiking burns an average of 100 kilocalories for every kilometer traveled. Anthropological measurements are non-invasive quantitative measurements of the body. According to the CDC, anthropometry provides a valuable nutritional and health assessment, body composition determination and assesment of future disease risk.

Aim of the work: To determine the differences between the initial and final measurement results and to prove a favorable connection between recreational hiking and the optimization of anthropological measures - body mass, mass of adipose tissue and subcutaneous adipose tissue of the subjects.

Methods: The research was conducted on a sample of 20 people from General mountaineering school, who were recreationally engaged in hiking in a three-month period. The t-test for dependent samples was used to test the hypothesis set forth in the research. The Shapiro-Wilk test was used to determine the normality of the distribution, and the Levene test was used for the homogeneity of variances.

Results: Determining the difference between the initial and final measurement and analyzing them in the observed variables, a statistically significant difference between the initial and final measurement of fat tissue mass was obtained ($p=0.04$). No statistically significant difference was obtained between the initial and final measurements of the subjects subcutaneous fat tissue ($p=0.06$), but a trend towards the existence of statistically significant differences is shown.

Conclusion: Recreational hiking did not have a favorable effect on the optimization of the anthropological measures monitored in the research. The percentage of the total mass of adipose tissue and subcutaneous adipose tissue of the subjects increased during the period of conducting the research, which did not confirm the initial hypothesis. The possible causes of the obtained results are the research carried out in the autumn/winter period and the adaptation of the subjects to other activities, which can consequently lead to NEAT (Non Exercise Activity Thermogenesis).

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The impact of neurorehabilitation with robotics on the rehabilitation of hand function in people after a stroke

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Introduction: A stroke is a disease of the brain structures, which results in a reduced ability to move, disturbances in the coordination of movements and gait. People who have suffered a stroke are known to have severe damage and limited movement and perception problems. Robotics in the neurorehabilitation of people after a stroke has its place in the rehabilitation protocol.
The aim of the work: To examine the effect of robotic therapeutic devices on the increase of gross and fine motor skills in a person after a stroke.

Methods: The research was conducted on a sample of 19 people who recovered from a stroke. To check the effectiveness, measurements were taken at the beginning and at the end of the therapy. By analyzing the differences in the variables of interest, statistically significant differences were obtained between the initial and final measurements in the Box and blocks test (p=0.00) and the Nine hole peg test (p=0.03).

Results: The results of hand strength measurements showed a statistically significant difference between the measurement results at the beginning and end of the therapy. Based on these results, it can be concluded that the application of robotics in the neurorehabilitation of the upper extremities in people after a stroke had a positive effect on the performance in the observed tests.

Conclusions: Considering the presented results, it can be concluded that the application of robotics should find a place in the neurorehabilitation of the upper extremities in people after a stroke. Future research should be conducted on larger samples. The best approach for neurorehabilitation requires the cooperation of several professions applying modern knowledge about the functioning of the nervous system (neurophysiology).

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Poster session #1: Cardiovascular physiology

Adenosine A1 and A2a receptor protein expression in cerebral blood vessels of Sprague-Dawley rats exposed to hyperbaric oxygen

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Introduction: Hyperbaric oxygenation (HBO_2) affects gene and protein expression, modulate signaling pathways and influence vascular structure and function. Adenosine receptors (AR) may play an important role in the mechanism of action of oxygen as a signaling molecule and vasoactive substance, and their expression may be affected by HBO_2.

Objective: Present study aimed to determine the protein expression of adenosine A1 and A2a receptors in cerebral blood vessels of Sprague-Dawley rats after exposure to acute and intermittent HBO_2.

Methods: Male and femal healthy Sprague-Dawley rats aged 8-10 weeks were divided into 3 groups: CTRL (control non HBO_2), A-HBO_2 (single animals exposed to HBO_2 for 2 hours) and 4D-HBO_2 (animals exposed to HBO_2 at a pressure od 2 bars for 2 hours daily for 4 days, with saple collection on the fifth day). Superficial brain blood vessels were collected to determine the protein expression of adenosine A1 and A2a receptors using the Western blot method. All experimental procedures conformed to the European Guidelines for the Care and Use of Laboratory Animals (directive 86/609) and were approved by the local and national Ethical Committee (#2158-61-07-21-88; EP-348/2021).

Results: A1 receptor protein expression was significantly reduced in A-HBO_2 rats compared to 4D-HBO_2 (relative expression in relation to ß-actin 0.5865 +/- 0.1259 vs 0.9316 +/- 0.1152, p=0.0191). A-HBO_2 group had significantly reduced protein expression of A2a receptors compared to CTRL (0.2380 +/- 0.09028 vs 0.6833 +/- 0.1054, p<0.001) and 4D-HBO_2 (0.2380 +/- 0.09028 vs 0.7204 +/- 0.08244, p<0.001). 4D-HBO_2group had a similar protein expression of both adenosine receptors as the CTRL group.

Conclusion: Acute HBO_2 significantly reduced protein expression of adenosine A1 and A2a receptors, whereas exposure to intermittent HBO_2 render similar protein expression as in control. These results suggest that changes in adenosine receptor protein expression might be involved in the mechanisms through which HBO modifies vascular reactivity.

Acknowledgement: Supported by institutional grants of Faculty of Medicine Osijek IP8-MEFOS-2020 (PI Aleksandar Kibel) and IP5-MEFOS-2021 (PI Zrinka Mihaljević)

Key words: hyperbaric oxygenation, adenosine receptors, cerebral blood vessels, protein expression, Sprague-Dawley rats

Arterial compliance in obese adolescents and its dynamics

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Arterial compliance (AC) decrease with aging is accelerated by factors associated with progression of atherosclerotic process, including obesity. Incidence of obesity increases not only in adult population but also in children and adolescents. The results of studies on the effect of obesity on AC (often indirectly estimated by pulse wave velocity (PWV)) are contradictory. Considering the potential limitation of previously applied methods and the need to interpret AC values in the context of potential confounders or during various physiological states, the aim of this study was to compare AC of control and obese adolescents during four different physiological states – supine rest, head-up tilt (HUT), supine recovery and mental arithmetic (MA).

AC was assessed by the method based on two-element Windkessel model as the ratio of a time constant τ characterizing diastolic blood pressure decay and total peripheral resistance (TPR). In total, fifty healthy and normotensive subjects (40f, 10m, age 17.5 years (SD=1.1 years)) were examined – 25 obese and 25 age and sex matched control subjects.

We observed significantly increased AC values during all phases in obese group. An increase in AC was also preserved after controlling for blood pressure differences. These results were confirmed using another method of AC estimation based on PWV measurement. Interestingly, AC decreased similarly during stress phases (HUT, MA) in both groups. Lastly, TPR was decreased throughout the study protocol in obese subjects.

In conclusion, AC is increased in young obese subjects consistently during various physiological states. Furthermore, changes of physiological states evoke similar response of AC in both groups indicating preserved autonomic control of arteries. Lastly, decreased TPR in obese subjects points towards the influence of different maturation state of the arterial tree and/or changes in vasomotion possibly counterbalancing acceleration of atherosclerosis process.

Supported by grants VEGA 1/0199/19, 1/0200/19, 1/0283/21 and Grant UK/262/2022.

Wearing protective masks does not affect hemoglobin oxygen saturation in young healthy subjects

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During the recent COVID-19 pandemic, wearing protective masks has been common at crowded places, such as on-site meetings and teaching classes. However, some speculations have raised concerns on the possible negative effects of protective masks regarding pulmonary gas exchange. In the present study, we measured hemoglobin oxygen saturation levels in students who were wearing either surgical or FFP2 protective masks during on-site classes. Sample comprised 150 subjects aged 20-30 years, of which 45% were males and 55% were females, while 30% of total subjects were smokers (had not been smoking for minimally 1 h prior the measurement). Exclusion criteria were presence of any pulmonary or hematologic disease, as well as other disabilitating acute or chronic conditions. Participants underwent hemoglobin oxygen saturation measurement by standard pulse oxymeter firstly with no mask at open place (control values), and then 10 minutes and 60 minutes subsequently after the masks have been placed, as well as immediately after mild physical exertion while wearing mask. We found no significant differences of hemoglobin oxygen saturation level between any measurement point compared to control values and each other, neither regarding surgical nor FFP2 mask wearing. Additionally, no differences were found between male and female participants, neither between smokers and non-smokers. In conclusion, data obtained indicates that wearing masks does not affect hemoglobin oxygen saturation in young and healthy people.

Effect of carnosine on vascular function of Sprague-Dawley rats on a high-salt diet – a pilot study

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ABSTRACT

INTRODUCTION: Previously, we showed that increased NaCl dietary intake impairs vascular function via increased oxidative stress. Carnosine (CAR; β-alanyl-L-histidine) is a dipeptide that has anti-inflammatory, antioxidant activity and acts as a "scavenger" of ROS, reduces the levels of lipid peroxidation and low-density lipoprotein in the serum of aged rats. However, there are no studies that have examined the effect of carnosine supplementation on vascular function and oxidative stress levels following high salt intake.

AIM: Present pilot study aimed to investigate the effect of carnosine supplementation on vascular function by examining acetylcholine-induced dilation of isolated aortic rings.

MATERIALS AND METHODS: Healthy Sprague-Dawley rats (8-10 weeks old) were divided in four groups (3 rats per group) – CTRL (control group, 0.4% NaCl), HS (high salt diet, 4% NaCl in diet for 7 days), CTRL+CAR (oral carnosine supplementation, 150 mg/kg/day for 7 days) and HS+CAR group (HS and carnosine supplementation). Response to ACh and SNP (sodium nitro-prusside) was studied in isolated norepinephrine precontracted aortic rings (Isolated Organ Bath (Experimetria LTD)). All experimental procedures conformed to the European Guidelines for the Care and Use of Laboratory Animals (directive 86/609) and were approved by the local and national Ethical Committees (#2158-61-46-22-14, EP355/2022).

RESULTS: These preliminary results showed impaired ACh-induced relaxation of aortic rings in HS group compared to CTRL, and restored relaxation after carnosine supplementation. There are no differences in response to SNP among tested groups.

CONCLUSION: Results suggest positive effect of carnosine on vascular function of rats on high salt diet.

ACKNOWLEDGMENT: This study was supported by European Structural and Investment Funds through a grant to the Croatian National Science Center of Excellence for Personalized Health Care, Josip Juraj Strossmayer University of Osijek # KK.01.1.1.01.0010. and by institutional grants from the Faculty of Medicine Osijek IP1-2022-MEFOS (PI Ines Drenjančević) and IP16-2022-MEFOS grant (PI Zrinka Mihaljević).

Cardio-ankle vascular index (CAVI) and its modified versions (kCAVI, $CAVI_0$, $kCAVI_0$) in obese adolescents with and without metabolic syndrome

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Introduction. Index CAVI (Cardio-ankle vascular index: heart – *a.tibialis*), estimating arterial stiffness, is increasingly used for the early atherosclerotic changes assessment. Several studies surprisingly found lower CAVI values in obese patients [1] (which is inconsistent with other methods e.g., USG). Similar results have also been found when measuring CAVI in patients with metabolic syndrome (MS). Studies show increasing CAVI as a number of MS components increases, but a negative association of CAVI with waist circumference or BMI [2,3]. Alternative indices: kCAVI – measured between the heart and *a.poplitea* (includes smaller part of the vasoactive peripheral circulation), $CAVI_0$ – blood pressure corrected CAVI [4] and $kCAVI_0$ (blood pressure corrected kCAVI) describe arterial stiffness from different points of view. The aim of our study was to compare CAVI and alternative indices kCAVI, $CAVI_0$, and $kCAVI_0$ in the group of obese adolescents, obese adolescents with MS and normal-weight controls.
Methods. We examined 34 obese (17.08 ± 1.02 y., BMI: 35.47 ± 5.44 kg.m-2), 12 obese adolescents with MS (17.37 ± 1.01 y., BMI: 36.38 ± 4.68 kg.m-2) and 46 age-matched controls (17.27 ± 1.01 y., BMI: 20.87 ± 1.70 kg.m-2). MS was defined according to [5]. Arterial stiffness indices CAVI and kCAVI were measured using VaSera VS-1500 (Fukuda Denshi, Japan). $CAVI_0$ and $kCAVI_0$ were calculated according to [4].

Results. The results of our study are shown in Fig.1. Indices CAVI, $CAVI_0$ and kCAVI were significantly lower in obese group compared to both control group and obese+MS group, and in all these indices there was no significant difference between obese+MS group and control group.
Only index $kCAVI_0$ was significantly higher in obese+MS group compared to both obese and control groups.

Conclusion. Only the stiffness parameter $kCAVI_0$ (in contrast to CAVI, kCAVI and $CAVI_0$) indicates increased arterial stiffness in obese+MS adolescents compared to obese and control groups.

Grants: VEGA 1/0200/19, VEGA 1/0283/21

References

[1] Czippelova B, Turianikova Z, Korhová J. et al.: Arterial stiffness and endothelial function in young obese patients – vascular resistance matters, J Atheroscler Thromb. 2019; 26:1015-1025
[2] Gomez-Sanchez L, Garcia-Ortiz L, Patino-Alonso MC et al, Association of metabolic syndrome and its components with arterial stiffness in Caucasian subjects of the MARK study: a cross-sectional trial. Cardiovasc Diabetol. 2016 Oct 24;15(1):148. doi: 10.1186/s12933-016-0465-7. PMID: 27776526; PMCID: PMC5078926.
[3] Topouchian J, Labat C, Gautier S, et al. Effects of metabolic syndrome on arterial function in different age groups: the Advanced Approach to Arterial Stiffness study. J Hypertens. 2018 Apr;36(4):824-833. doi: 10.1097/HJH.0000000000001631. PMID: 29324585; PMCID: PMC5862002.
[4] Spronck B, Avolio AP, Tan I, Butlin M, Reesink KD and Delhaas T: Arterial stiffness index beta and cardio-ankle vascular index inherently depend on blood pressure but can be readily corrected. J Hypertens. 2017;35(1):98-104
[5] Beilby J. Definition of Metabolic Syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association Conference on Scientific Issues Related to Definition. Clin Biochem Rev. 2004 Aug;25(3):195–8. PMCID: PMC1880831.

Rat ECG parameters under general anesthesia

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Abstract

General anesthesia is an established and well known factor with significant impact on cardiac parameters, what can be a problem in the final evaluation of changes of the individual electrophysiological myocardial parameters after various interventions. In the presented poster is compilation the data on electrocardiographic parameters (heart rate, PR interval, P wave duration, P wave amplitude, QRS complex, QT and QTc interval duration, and R wave and T wave amplitude) for in vivo rat experiments under general anesthesia from 130 articles, which were retrieved from a search of the Web of Science database, for articles published mainly between 2000 and 2021. ECG parameters reported as baseline or control values were summarized and averages with ranges were calculated. It is important to be cautious in interpreting the results of such studies and, in discussions addressing the mechanisms underlying a given type of arrhythmia, it is important to acknowledge that initial ECG parameters may already be affected to some extent by general anesthesia as well as by sex and the time of day the experiments are performed. Although, it is not an original research work, researchers, working with rats in laboratory who routinely perform anesthesia can use this as a reference to look into while analyzing their data.

Keywords: ECG parameters, general anesthesia, sex, chronobiology, rat

Circadian patterns of occurrence of acute coronary events in coronary unit of the clinical center of Montenegro

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Cardiovascular diseases (CVD) are the most common cause of death in the world. Montenegro follows this trend, and for the past decade the incidence of CVD has been increasing. Therefore, better prevention, timely diagnosis and treatment of CVD is one of the top healthcare priorities. Scientific data shows that acute coronary events (ACE) peak in the early morning hours, and this is associated with circadial fluctuation of gene expression, metabolic processes and hormone secretion.

The aims of this study were to determine: 1) the number of patients with CVD, the most common presenting diagnosis caused by CVD and the most frequently used treatment at the Coronary Unit of the Clinical Center of Montenegro (CU-CCM); 2) at what time during the circadian cycle the frequency of the acute coronary events is the highest at the CU-CCM.

The data were collected retrospectively at the CU-CCM from January 1st 2018 to June 30th 2018. It is important to note that the data collected in this institution reflect the situation in the whole country, since this is the only tertiary level health care center with capability of coronarography.
During the six-month period 628 patients were admitted due to ACE. The study showed: acute myocardial infarction (AIM) was the most common presentation of CVD in the CU-CCM (44.3% of patients). Non-ST elevation AIM was more frequent compared to ST elevation AIM, but this difference was marginal (52% vs. 48% of all AIM). Primary percutanous coronary intervention is the most common form of treatment for patients with ST elevation AIM. The study showed that 375 patients (59%) were admitted in ante meridiem hours, confirming that ACE most frequently occur during the morning hours. This study sets the foundation for our future research aimed to define circadian patterns of expression of miRNA species as predictors of CVD.

Poster session #1: Neurophysiology

The difference in novel lipid indices between patients with Alzheimer's disease and vascular dementia stratified by the degree of cognitive impairment

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Introduction: Lipids are significantly involved in maintaining structural and functional components of the human brain and neurons, but their role in the development of Alzheimer's disease (AD) and vascular dementia (VD) remains inconclusive.

Aim: The aim of the present study was to explore the differences of novel lipid indices in patients with AD and VD, stratified by the degree of cognitive impairment (CI).

Methods: Study included 66 patients with AD, 50 patients with VD, and 60 controls. For an evaluation of the global cognitive function the Montreal Cognitive Assessment (MoCA) test was used. In order to distinguish patients with VD from those with AD the Hachinski ischemic score was used. Based on the MoCA score patients were further stratified in those with severe and moderate CI. Lipids were measured by standard methods. TG/HDL-C, TC/HDL-C, and LDL-C/HDL-C ratio were separately calculated. The differences between the groups were analyzed with the Kruskal Wallis test followed by the Mann-Whitney test or with ANOVA followed by the Tuckey posthoc test.

Results: Our results have shown that patients in AD group with moderate CI had significantly lower level of TG/HDL-C and TC/HDL-C compared to VD patients with moderate CI. In addition, patients in AD group with severe CI had significantly lower level of TC/HDL-C compared to VD patients with severe CI.

Conclusion: Based on the results of the present study it can be deduced that there is a difference in novel lipid indices between AD and VD patients with different degree of CI. Obtained results suggest that lipids are decreased in AD and in VD to a certain extent. However, since there is an inconsistence in literature whether there is an association between cholesterol and cognition, large prospective studies are required to elucidate this controversy.

Key words: Alzheimer's disease, vascular dementia, cognitive impairment, TG/HDL-C ratio, TC/HDL-C ratio, LDL-C/HDL-C ratio

Effects of guanylate cyclase-C activation on ischemic stroke

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INTRODUCTION: Stroke is one of the leading causes of mortality and disability the world. Guanylate cyclase (GC) A activation after ischemic stroke is neuroprotective. The aim of this study is to investigate what effect GC-C activation by uroguanylin (UGN) has on ischemic stroke.

MATERIALS AND METHODS: Middle cerebral artery occlusion (MCAO) was performed on GC-C knock out (GC-C KO), UGN KO mice and their WT littermates. MR images were acquired before and 24h after stroke induction. Ca^{2+} response was recorded in astrocytes of peri-ischemic and contralateral cortices 48h after MCAO. Systolic (SP), diastolic (DP) and mean arterial blood pressure (MAP) were recorded with a non-invasive tail-cuff method. Immunohistochemical staining and in situ hybridization were used to confirm GC-C expression in neurons and astrocytes before any after stroke induction.

RESULTS: GC-C KO mice develop 35% smaller ischemic lesions compared with their WT littermates. Although higher SP, DP and MAP were recorded in GC-C KO animals when compared with WT mice, blood flow or the reduction of blood flow during MCAO did not differ between those test groups. A stronger Ca^{2+} response to UGN stimulation was recorded in in cortical peri-ischemic astrocytes of UGN KO and WT mice but this stronger activation is absent in GC-C KO mice. The observed difference in response may result in smaller ischemic lesions observed in GC-C KO mice. This difference in Ca^{2+} response could be explained by the shift in GC-C expression that occurs after stroke – under normoxic conditions GC-C is expressed only in neurons, but after ischemic injury GC-C becomes strongly expressed in peri-ischemic astrocytes and is rarely found in neurons.

CONCLUSION: Contrary to GC-A, GC-C activation is not neuroprotective but instead leads to the development of larger ischemic lesions. Its expression in peri-ischemic astrocytes causes a stronger activation of the Ca^{2+}-dependent signalling pathway which likely stimulates the Na^{+}/H^{+} exchanger, leading to tissue acidification and neuronal death.

ACKNOWLEDGEMENTS: Research was funded by the Scientific Centre of Excellence for Basic, Clinical and Translational Neuroscience (project “Experimental and clinical research of hypoxic-ischemic damage in perinatal and adult brain”; GA KK01.1.1.01.0007 funded by the European Union through the European Regional Development Fund). The work of doctoral student Anja Barić has been fully supported by the “Young researchers' career development project – training of doctoral students” and project BRADISCHEMIA (UIP-2017-05-8082) of the Croatian Science Foundation funded by the European Union from the European Social Fund.

Early stage of Parkinson disease – an impact on the cardiovascular autonomic control

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Background and aim: Non-motor symptoms including those reflecting autonomic cardiovascular dysregulation are often present in Parkinson disease. It is unclear whether it is possible to detect cardiovascular autonomic dysregulation in the very early stage of Parkinson disease potentially supporting the concept of the upstream propagation of nervous system damage through autonomic nerves. We hypothesized that cardiovascular dysregulation should precede the motor symptoms and at the time of their occurrence autonomic dysregulation should be clearly demonstrable. Therefore, the aim of this study was to assess the various aspects of autonomic cardiovascular control in the very early stage of Parkinson disease.

Methods: We examined 19 patients with Parkinson disease (< 6 months after motor signs occurrence) and 19 healthy control subjects. For each phase of study protocol (supine, head-up tilt, supine recovery), we calculated a wide array of cardiovascular control related parameters reflecting cardiac chronotropic, cardiac inotropic and vasomotor control and baroreflex mediated cardiovascular response.

Results: We observed the well preserved heart rate and blood pressure control in patients with early stage of Parkinson disease. However, causal analysis of interactions between heart rate and blood pressure oscillations revealed subtle differences in baroreflex function and baroreflex mediated vasoconstriction response to orthostasis. Furthermore, a tendency towards a decreased contraction strength in Parkinson disease was observed.

Conclusion: Considering only subtle cardiovascular control impairment in our study employing a wide array of sensitive methods at the time when motor signs were clearly expressed, we suggest that motor signs dominated in this stage of Parkinson disease.
Supported by grant VEGA no.1/0283/21.

Astrocyte arborization enhances Ca^{2+} but not cAMP signaling plasticity

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Introduction
Astrocytes, a class of morphologically and functionally heterogeneous neuroglial cells, principally provide for homeostasis and defense of the central nervous system. Throughout life they may undergo plastic remodeling that is associated with diverse exposomal challenges.

Aim
Here we employed cultured astrocytes to investigate fundamental physiological processes at a subcellular level under controlled environmental conditions. We examined cell viability, the expression of astrocyte-specific markers, exo−/endocytotic activity, intracellular vesicle mobility as well as spontaneous and evoked cytoplasmic 3′,5′-cyclic adenosine monophosphate ($[cAMP]_i$) and Ca^{2+} ($[Ca^{2+}]_i$) signaling in astrocytes cultured in a serum-free medium, which favors the acquisition of arborized morphology and in a serum-containing medium, which mimics pathologic conditions.

Methods
Using opto-/electrophysiological techniques and (immuno)fluorescent labelling of cultured astrocytes, we studied cell viability, vesicle dynamics, expression of astrocyte-specific markers, cytosolic cyclic adenosine monophosphate ($[cAMP]_i$) and calcium ($[Ca^{2+}]_i$) signaling.

Results
Arborized astrocytes displayed increased resting $[Ca^{2+}]_i$ and increased subcellular heterogeneity in $[Ca^{2+}]_i$ that was causally linked with the attenuated vesicle mobility. In contrast, subcellular dynamic $[cAMP]_i$ was comparable in both cultured astrocytes.

Conclusion
In arborized astrocytes, resting levels of $[Ca^{2+}]_i$ and $[cAMP]_i$ are increased, whereas vesicles exhibit altered mobility and interaction with the plasmalemma. In astrocytes, Ca^{2+} signaling toolkit is more prone to plastic remodeling than cAMP signaling.

Guanylate cyclase C independent signaling pathway for uroguanylin in astrocytes

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Introduction: Guanylate cyclase C (GC-C), today's only known receptor for natriuretic peptide uroguanylin (UGN), upon activation leads to an increase in intracellular cGMP. In the brain GC-C is located only in Substantia nigra compacta (SNc) and Ventral Tegmental Area (VTA), Cerebral cortex (Cx), POMC neurons of Arcuate nucleus (Arc) of Hypothalamus, Amygdala, and Purkinje cells of the cerebellum. UGN effects via GC-C dependent signaling are neuromodulating like increase of firing frequency in SNc and VTA, hyperpolarization and decrease of firing frequency of Purkinje cells, and depolarization of POMC neurons. Here we report the effects of UGN on astrocytes that are independent of GC-C.

Methods: We performed GC-C expression analysis by PCR, electrophysiological recordings, Ca^{2+} imaging, and pH measurements on wild-type (WT) mice primary astrocyte culture isolated by magnetic activated cell separation. Additionally, Ca^{2+} imaging was performed on cortical and cerebellar brain slices of WT and GC-C KO mice. Accelerating-speed rota-rod and hanging wire behavioral testing were performed on WT, GC-C KO, and UGN KO mice.

Results: Expression analysis showed that astrocytes do not express GC-C. UGN hyperpolarizes cell membrane in contrast to cGMP that depolarize cell membrane. Furthermore, UGN increases intracellular Ca^{2+} concentration both on primary astrocyte culture and slices of different brain regions that are also present in slices obtained from GC-C KO mice. pH measurements showed that UGN increases Na^{+}/H^{+} exchanger activity tested by amonic pulse and HCO_3^- transport. Behavioral testing showed that UGN-KO mice have increased performance on both tests than WT and GC-C KO mice (1).

Conclusion: In this research, we showed the existence of GC-C independent, but Ca^{2+} dependent, signaling pathway for UGN in astrocytes that are a suitable model for investigation of this signaling pathway due to lack of GC-C. UGN effects via the Ca^{2+} signaling pathway could modulate neuronal activity and behavior.

Acknowledgment: This study is financed by the Croatian science foundation research grant (IP-2018-01-7416) and co-financed by the European Union through the European Regional Development Fund, Operational Programme Competitiveness and Cohesion, Grant Agreement No. KK.01.1.1.01.0007, CoRE-Neuro.
References: Habek N, Ratko M, Dugandžić A. Uroguanylin increases Ca^{2+} concentration in astrocytes via guanylate cyclase C-independent signaling pathway. Croat Med J. 62(3):250-263. 2021 doi: 10.3325/cmj.2021.62.250.

A single dose injection of carbon tetrachloride causes inflammation, neuronal loss, massive demyelination and microglial response in the mouse brain

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Carbon tetrachloride (CCl4) is an organic solvent known for its high toxicity. Liver and kidney damage are the best characterized consequences of carbon tetrachloride poisoning. Although the neurological aspects of CCl4 toxicity have been known for a long time, underlying pathological changes still remain unclarified and are often considered as being consequences of liver failure. In the present study we aimed to assess pathological changes in the brain tissue of mice undergone single dose CCl4 intoxication. For that purpose, male C57BL6 mice, aged 2-3 months, were injected intraperitoneally with 5 µL/g of 10 % (v/v) dissolved in corn oil. Control animals were injected with corresponding volume of vehicle only. One day and three days after injection, mice (N=5 per group) were assessed for neurological deficits and tissue samples were collected. Both groups of treated animals showed reduction of hindlimb strength and loss of coordination, as observed by hindlimb clasping test and ledge test, respectively. In the brain of treated animals, we found mononuclear cell infiltrates, being most copious in the motor cortex. Some cerebral and cerebellar cortical areas showed complete loss of cellularity, while the vast majority of remnant neurons were found either with chromatolysis, pyknosis or karyorrhexis, appearing to be so called "red neurons" on HE slides. Besides, areas showing complete lack of immunohistochemical PLP staining were being consistently found trough the brain parenchyma of intoxicated mice, while the rest of their brain tissue showed lesser PLP staining intensity compared to control animals. Furthermore, a higher number of Iba1 positive cells was found in the brains of treated mice, implying microglial activation upon CCl4 injection. In conclusion, data obtained indicate CCl4 poisoning can cause brain inflammation, neuronal loss, and massive demyelination.

Inhibition of NMDA receptors increases, synchronizes, and stabilizes the collective activity of pancreatic β-cells

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Abstract

NMDA receptors promote repolarization in pancreatic beta cells and thereby reduce glucose-stimulated insulin secretion. Therefore, NMDA receptors are a potential therapeutic target for diabetes. While the mechanism of NMDA receptor inhibition in beta cells is rather well understood at the molecular level, its possible effects on the collective cellular activity have not been addressed to date, even though proper insulin secretion patterns result from well-synchronized beta cell behavior. The latter is enabled by strong intercellular connectivity, which governs propagating calcium waves across the islets and makes the heterogeneous beta cell population work in synchrony. Since a disrupted collective activity is an important and possibly early contributor to impaired insulin secretion and glucose intolerance, it is of utmost importance to understand possible effects of NMDA receptor inhibition on beta cell functional connectivity.

To address this issue, we combined confocal functional multicellular calcium imaging in mouse tissue slices with network science approaches.

Our results revealed that NMDA receptor inhibition increases, synchronizes, and stabilizes beta cell activity without affecting the velocity or size of calcium waves. To explore intercellular interactions more precisely, we made use of the multilayer network formalism by regarding each calcium wave as an individual network layer, with weighted directed connections portraying the intercellular propagation. NMDA receptor inhibition stabilized both the role of wave initiators and the course of waves. The findings obtained with the experimental antagonist of NMDA receptors, MK-801, were additionally validated with dextrorphan, the active metabolite of the approved drug dextromethorphan, as well as with experiments on NMDA receptor KO mice.

In sum, our results provide additional and new evidence for a possible role of NMDA receptor inhibition in treatment of type 2 diabetes and introduce the multilayer network paradigm as a general strategy to examine effects of drugs on connectivity in multicellular systems.

Keywords: pancreatic islets; beta cells; NMDA receptors; multicellular dynamics, calcium waves; multilayer networks; antidiabetic drugs.

Retinal microvascular complexity reflects accelerated aging associated with severe chronic diseases including Alzheimer's dementia

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Recent research has demonstrated that decreased cognitive performance is associated with decreased retinal vascular complexity measured by retinal microvascular fractal dimension (RMV-Fd). Therefore, RMV-Fd has been proposed as a potential biomarker of Alzheimer's dementia (AD). However, decreased RMV-Fd is found in other systemic and eye-specific diseases such as hypertension, diabetes mellitus and glaucoma. More importantly, RMV-Fd decreases during physiologic aging.

Growing scientific evidence pointed that in people with cancer, the incidence of AD is very low, while in people with AD the incidence of cancer is very low. If RMV-Fd could serve as a biomarker of cognitive performance, this pattern in comorbidities should be reflected through changes in RMV-Fd.

The aim of the presented research was to determine whether RMV-Fd is decreased in patients with AD (group AD), and increased in patients with colon cancer and/or tubular adenoma (group T), compared to the healthy participants who do not have history of cancer or cognitive problems (group H). To test this, non-mydriatic color retinal fundus photographs were captured by using portable fundus camera MiiS HORUS Scope DEC200 at 45°FOV. RMV-Fd of binarized, manually segmented and skeletonized microvascular images was calculated by using ImageJ 1.53a.

There was no difference in the age among the groups (T:AD:H=67.4:69.7:68.5). As expected, the Montreal Cognitive Assessment (MoCA) test showed that patients with tumor had normal, while AD group had decreased MoCA score (T:AD:H=26.7:16:24.6). There was no significant difference in RMV-Fd among the groups, but RMV-Fd was decreased in sick (T+AD) compared to the healthy participants (sick vs. healthy=1.32:1.34, p= 0.04). Decrease in RMV-Fd as a function of age was more pronounced in the participants who are sick, implying that RMV-Fd can detect accelerated aging associated with severe chronic diseases.

RMV-Fd could be used as a biomarker of aging, rather than a specific biomarker of AD.

Preclinical development of a novel multi-target drug candidate for neuropathic pain

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SzV-1287 (3-(4,5-diphenyl-1,3-oxazol-2-yl)propanal oxime) is a novel, metabolism-activated multi-target ("MAMUT") drug, which inhibits semicarbazide-sensitive amine oxidase (SSAO) metabolizing primary amines to irritants like methyglyoxal and formaldehyde activating the transient receptor potential ankyrin 1 (TRPA1) and vanilloid 1 (TRPV1) receptors. It also directly antagonizes these non-selective cation channels located on primary sensory neurons, immune cells and several central nervous system regions, and play roles in pain and inflammation. Furthermore, SzV-1287 is predominantly metabolized to the cyclooxygenase inhibitor oxaprozin, which can act synergistically.

We provided evidence that single SzV-1287 injection significantly reduces acute and chronic pain behaviors including sciatic nerve injury-induced neuropathic hyperalgesia in mice via inhibiting TRPA1 and TRPV1 activation. Repeated SZV-1287 administrations attenuated chronic arthritic changes (oedema, plasma leakage, myeloperoxidase activity) and related hyperalgesia, L4-L6 spinal dorsal horn neuroinflammation (microgliosis) even in the late phase of the model when neuropathic mechanisms are important. Since mainly under acidic conditions, SzV-1287 is transformed to the cyclooxygenase inhibitor oxaprozin, which is ineffective for neuropathy, enterosolvent capsule was formulated and tested during the preclinical development. SzV-1287 is quickly absorbed, its plasma concentration is stable for 2 h, and enters the brain. Although SzV-1287 significantly decreased the proton-induced TRPV1-mediated calcium-influx potentially leading to hyperthermia, it did not alter deep body temperature. It did not show any considerable side effects either in rodents or in dogs.

After the approval of the preclinical dossier by the Hungarian authority, the phase I.A clinical trial (singe ascending dose) was successfully completed in May, phase I.B multiple dosing starts in October 2022. If the candidate proves to be safe in humans with favorable kinetic profile, the compound can progress to phase II. trial in neuropathic pain patients.

Acknowledgements: GINOP-2.2.1-15-2016-00020, EFOP-3.6.1-16-2016-00004, National Brain Research Program 20017-1.2.1-NKP-2017-00002, TKP2021-EGA-16.

Monitoring noradrenaline-induced changes in cytosolic L-lactate in rat *locus coeruleus* neurons

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Introduction:
Cognitive decline (e.g. in Alzheimer's disease) is thought to be associated with the demise of the locus coeruleus (LC) neurons in the brain stem1, the primary source of noradrenaline (NA) in the central nervous system. Release of NA during the state of alertness can elicit an additional energy supply for activated neurons via excitation of astrocytes, activating aerobic glycolysis and production of cytosolic L-lactate (LLi), which can be transported to neurons for oxidation2. The expression of adrenoreceptors on LC neurons (LCn), especially α2, but also α13 may indicate that NA may also affect changes in LLi in the LCn.

Aim:
To test whether NA induces changes in LLi in LCn.

Methods:
Isolated rat LCn and cortical astrocytes were transfected with the L-lactate sensor (laconic). Changes in LLi, reported as changes in fluorescence resonance energy transfer signal ratio were measured upon stimulation of cells with NA [100μM]. P values <0.05 were considered significant.

Results:
The responsiveness to NA was recorded in 86.1% of astrocytes (37/43) and in 71.4% of LCn (10/14). The average NA-induced peak increase in LLi in astrocytes was 5.4 ± 1.2% (mean ± sem; n=43), significantly higher than in controls (application of the vehicle; 0.9 ± 0.4%; n=27; P<0.001). In LCn the NA-induced increase in LLi was 4.2 ± 1.5% (n=14), not different to controls (2.2 ±1.15%; n=10; P =0.661) However, in the responsive neurons the average increase of LLi was significantly higher (7.0 ±1.3%; n=10) than in controls (P=0.038), which may indicate that NA released from LCn may regulate LLi production in the activated LCn.

Conclusion:
Although aerobic glycolysis is considered to be exclusively present in astroglia4, present data indicates, that NA-mediated increase in LLi, a hallmark of aerobic glycolysis, may also be recorded in LCn.

Acknowledgements: This work was supported by the Slovenian Research Agency.

[1]Kelly et al., 2017; [2]Magistretti and Allaman, 2018; [3]Schwartz and Luo, 2015; [4]Barros and Weber, 2018

The transient receptor potential ankyrin 1 (TRPA1) ion channel expressed by the Edinger-Westphal nucleus plays role in posttraumatic stress disorder (PTSD) in mice

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Introduction:
The centrally projecting Edinger-Westphal nucleus (EWcp) is involved in stress adaptation. TRPA1 mRNA was previously shown to be expressed significantly in mouse and human EWcp urocortin1 (UCN1) positive neurons and reacted to chronic stress. As UCN1 neurons are deeply implicated in the stress-related disorders here we hypothesized that TRPA1/UCN1 neurons are also affected in post-traumatic stress disorder (PTSD).

Methods:
We used male TRPA1 wild-type (WT) and gene-deficient (KO) mice in a PTSD model (single prolonged stress (SPS)). Two weeks later the behavioral changes were monitored by forced swim test (FST) and restraint. The Trpa1 and Ucn1 mRNA expression and the UCN1 peptide content were assessed by RNAscope in situ hybridization technique combined with immunofluorescence labeling in EWcp.

Results:
TRPA1 KO animals showed significantly lower immobility after SPS compared to WT mice, both in the FST and restraint stress, which can be considered as depression-like behavior. The copy number of Trpa1 mRNA was significantly increased in EWcp of KO animals in response to SPS. Higher Ucn1 mRNA expression was observed in the EWcp of KO animals, that was not affected by SPS exposure. EWcp neurons of WT animals responded to SPS with significantly increased amount of UCN1 peptide content compared to the controls, whereas such changes were not observable in KO mice.

Conclusion:
Decreased Trpa1 mRNA expression in the SPS model of PTSD associated with increased neuronal UCN1 peptide content suggesting that this cation channel might be involved in the regulation of stress adaptation and may contribute to the pathomechanism of PTSD.

TRPA1 ion channel is downregulated in peptidergic neurons of the Edinger-Westphal nucleus upon acute alcohol exposure

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Introduction: The centrally projecting Edinger-Westphal nucleus (EWcp) contributes to the control of alcohol consumption by its urocortin 1 (UCN1) and cocaine- and amphetamine-regulated transcript (CART) co-expressing peptidergic neurons. Our group most recently published that the urocortinergic EWcp is the primary seat of central transient receptor potential ankyrin 1 (TRPA1) cation channel expression. We aimed to examine the role of TRPA1 in EWcp in a mouse model of acute alcohol exposure.

Methods: Our studies were performed in 12-week-old male *Trpa1* knockout (KO) and wild-type (WT) mice. In both genotypes, half of the animals were treated intraperitoneally with 1g/kg 6% ethanol vs. physiological saline-injected controls. Transcardial perfusion was performed 2 hours after the treatment. In the EWcp area, FOS immunohistochemistry was performed to assess neuronal activation. *Trpa1, Cart* and *Ucn1* mRNA expression as well as UCN1 and CART peptide content was assessed by RNAscope *in situ* hybridization combined with immunofluorescence.

Results: Alcohol treatment resulted in significant FOS activation in both genotypes and *Trpa1* mRNA expression was significantly decreased in WT mice. The assessment of UCN1 peptide immunoreactivity revealed lower basal UCN1 in KO mice compared to WTs. The UCN1 peptide content was affected genotype-dependently by alcohol: the peptide content decreased in WTs while it increased in KO mice. Alcohol exposure influenced neither *Cart* and *Ucn1* mRNA expression nor the EWcp/CART peptide content.

Conclusion: Decreased *Trpa1* expression upon alcohol treatment associated with reduced neuronal UCN1 peptide content suggests that this cation channel is involved in the regulation of the UCN1 release. Further research is in progress to examine the recruitment of TRPA1/UCN1 in chronic alcohol consumption and addiction.

Impaired migration of plectin-deficient astrocytes

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Cell migration is an essential process in the development of the central nervous system (CNS). Astrocytes, the most heterogeneous glial cells in the adult CNS, develop during the embryonal stage when they intensively migrate and proliferate. In early neonatal life their migratory properties appear to change, however the nature of migratory mechanisms is poorly understood. In adults, astrocytes are migratory dormant cells in physiological conditions, however, they may reinstate migrations during various pathologies, including glioblastoma. The prerequisite for migrations of cells are their cytoskeleton elements, the remodeling of which is regulated by adaptor proteins and cytolinkers. Cytolinker plectin is the only plakin cytolinker in astrocytes, interconnecting actin filaments, microtubules and intermediate filaments, and linking cytoskeleton elements to the plasma membrane and to various cellular organelles. As the role of plectin in astrocyte migrations has not been addressed, yet, our aim was to use immortalized astrocytes, a model of migratory glioblastoma cells, to address the involvement of plectin in the migration of astrocytes. We used primary mouse astrocytes isolated from cortices of C57BL/6 neonatal mice, and immortalized mouse astrocytes; those lacking proteins p53 and plectin ($p53^{-/-}/plec^{-/-}$) and those lacking p53 protein, while still expressing plectin ($p53^{-/-}/plec^{+/+}$). Cell migration was assessed with the wound healing assay monitored under a confocal microscope. Plectin was labeled by immunocytochemistry.
The results revealed that: 1) in primary astrocytes and $p53^{-/-}/plec^{+/+}$ astrocytes plectin distributes in the cell cytoplasm, predominantly in the form of filaments, 2) neonatal astrocytes migration is limited in comparison to immortalized astrocytes, which can be considered as a model for migrating glioblastoma cells, and 3) the velocity of astrocyte migration is impaired in the absence of plectin, implying that plectin affects functionally of the cytoskeleton elements involved in the cell motility.

Investigation of the effect of dimethyl trisulfide exerted via TRPA1 ion channels on depression-like behaviour

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Background
Several drugs are available for the treatment of anxiety and depression. However, many patients do not respond to drug treatment. Others develop side effects and discontinue therapy. It has been found recently that sulfide administration relieves depression-like behavior in experimental animals. Dimethyl trisulfide (DMTS) is a polysulfide found in garlic and used as food additive. According to our earlier results, DMTS inhibits movements and breathing of mice. This suggests possible anxiolytic and antidepressant effects of the compound.

Aims
The purpose of the present study was to investigate the effect of DMTS in mouse acute stress model of anxiety and depression, as well as underlying mechanisms.

Methods
TRPA1 gene-knockout (KO) and wild-type (WT) mice were used. We determined the appropriate dose of DMTS in the Open Field test. Then experimental animals participated in forced swim test (FST) to investigate the depression-like behavior and anxiety.

Results
Dose of 50 mg/kg DMTS was the largest which did not diminish the natural activity of the mice. DMTS treatment reduced inactive duration, increased highly active duration and activity frequency in TRPA1 WT animals compared to either naïve mice or the vehicle treated group in the FST. DMTS did not alter parameters in TRPA1 KO animals relative to naïve mice or vehicle treatment.

Conclusion
Since the beneficial effects of DMTS were not presented in TRPA1 KO mice, but we experienced them in TRPA1 WT mice, we conclude that DMTS reduces depression-like behavior in the forced swim test via TRPA1 ion channels. DMTS might offer an alternative for the complementary treatment of depression.

The Koizumi and Longa methods of middle cerebral artery occlusion lead to different outcomes after cerebral and retinal ischemic injury in mice

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INTRODUCTION AND AIM
Ischemic stroke in humans is often accompanied by visual impairments. Preclinical studies reported co-occurrence of retinal and cerebral ischemia in the middle cerebral artery occlusion (MCAO) model, however the outcomes and data about the origin of the ophthalmic artery (OA) are inconsistent. With this study we aimed to clarify whether and how the Koizumi and Longa modifications of the MCAO procedure induce retinal ischemia in mice using longitudinal in vivo magnetic resonance assessment.

METHODS
Four months old male C57Bl/6J mice underwent a transient 30-minute MCAO by filament insertion through the common (Koizumi, CCA group) or external carotid artery (Longa, ECA group). Seven days before and 2, 9, and 35 days after MCAO the animals were scored for neurological deficit followed by magnetic resonance imaging of the brain and the ipsilateral eye, and 3D-TOF whole-brain angiography. The obtained images were analyzed in ImageJ.

RESULTS
Brain angiograms revealed that the OA originates from the pterygopalatine artery and a chronic state of hypoperfusion of the ipsilateral hemisphere in the CCA group. Oppositely, complete reperfusion was reached acutely in the ECA group, which showed a significantly larger ischemic lesionand greater tissue loss in the chronic phase compared to the CCA group. Significant acute thickening of the retina was observed only in the CCA group.

CONCLUSION
Most of the retinal responses to ischemia overlap with those in the brain in the Koizumi method. The Longa method induced only brain ischemia which was more extensive and resulted in greater tissue loss compared to the Koizumi method.

ACKNOWLEDGEMENTS
The study is supported by the Croatian Science Foundation project BRADISCHEMIA (UIP-2017-05-8082). The work of doctoral students Anja Barić and Rok Ister has been fully supported by the “Young researchers' career development project – training of doctoral students” of the Croatian Science Foundation funded by the European Union from the European Social Fund.

The effects of bradykinin type 2 receptor loss on cerebral and retinal ischemic injury in mice with type I diabetes

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Key words: diabetes mellitus, bradykinin type 2 receptor, brain and retinal ischemia

Introduction: The activation of the pro-inflammatory bradykinin signaling pathway which aggravates the secondary injury by promoting edema formation during the acute phase of brain and retinal ischemia is modified by the presence of diabetes mellitus.

Aim: The aim of this study was to determine the effects of bradykinin type 2 receptor (B2R) deficiency on the acute phase of brain and retinal ischemia in diabetic mice.
Materials and methods: Brain and retinal ischemia were induced in male diabetic C57BL/6-Ins2Akita/J (Akita, n=14) and diabetic B2R knockout B6.Cg-Ins2Akita/Bdkrb2tm1Jfh/SmiJ (Akita/B2R-KO, n=15) mice by 30-minute intraluminal middle cerebral artery occlusion (MCAO). Diabetes was confirmed by measuring blood glucose concentration and HbA1C levels. Before surgery, blood and intraocular pressure were measured. Seven days prior, and on the 1st and 3rd day after MCAO the animals were scored for neurological deficit followed by magnetic resonance imaging (MRI) of the brain and the ipsilateral eye using the 7T BioSpec 70/20 US system.

Results: B2R deficiency resulted in significantly higher body weight ($p<0,05$) and HbA1C levels ($p<0,05$) in diabetic Akita/B2R-KO compared to Akita mice but had no effect on blood glucose concertation. Additionally, there were no differences in blood pressure but Akita/B2R-KO mice showed lower intraocular pressure ($p<0,05$) compared to controls. In the first 3 days after MCAO, there were no differences in survival, neurological deficit, or ischemic lesion size between the groups. The MRI volumetric analysis of the 1st and the 3rd day after ischemia induction showed prominent swelling of ipsilateral hemisphere and the measured retinal layer of the ipsilateral eye caused by vasogenic edema formation in both groups, without significant differences between the two genotypes.

Conclusion: The loss of B2R and the resulting change in the glycemic status and IOP of the animal did not affect the acute phase of cerebral and retinal ischemic injury in the presence of type 1 diabetes.
Acknowledgements: The study was supported by the Croatian Science Foundation project BRADISCHEMIA (UIP-2017-05-8082). The work of doctoral student Anja has been fully supported by the “Young researchers’ career development project – training of doctoral students” of the Croatian Science Foundation funded by the European Union from the European Social Fund. Multimodal imaging was done at Laboratory for Regenerative Neuroscience - GlowLab, University of Zagreb School of Medicine.

The role of GABAergic neurotransmission in the anxiolytic action of *Melissa officinalis* essential oil – *in silico*, *in vitro*, and in *vivo studies*

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Anxiety disorders are among the most prevalent mental health disturbances, which are believed to affect around ¼ of the world's population during some period of their lives. Current medicamentous treatment options involve numerous drugs, however, these are sometimes ineffective or their application is associated with various side effects. Lemon balm (*Melissa officinalis* L.) represents one of the most frequently utilized herbal remedies for the treatment of different mental health disturbances worldwide, including anxiety disorders. The aim of the present study was to evaluate the involvement of GABAergic neurotransmission in the anxiolytic action of *M. officinalis* essential oil. This was done using docking studies of the major oil constituents with $GABA_A$ receptor (α1β2γ2), while *in vitro* studies were conducted in order to investigate the inhibitory potential of the oil on GABA transaminase in the mouse brain homogenate. Using a light/dark test in male BALB/c mice, the anxiolytic action of the oil (12.5 mg/kg, i.p.) was investigated after pretreatment with either flumazenil (3 mg/kg) or picrotoxin (1 mg/kg). According to the docking studies, all modeled compounds, although positive and indicating binding could occur, showed significantly lower binding affinity than that of picrotoxin. The activity of GABA transaminase was diminished when the homogenate was incubated with the essential oil and displayed IC_{50} at 97.5 μg/mL. Interestingly, when applied in either combination with a competitive or non-competitive antagonist of $GABA_A$ receptors, the anxiolytic activity of the essential oil was not statistically significantly modified in the light/dark test. The results of the present study indicate that although the essential-oil constituents possess $GABA_A$ receptor binding affinity and *in vitro* GABA transaminase inhibitory action, the anxiolytic potential of the essential oil in the *in vivo* model was not modified by $GABA_A$ receptor antagonists.
Keywords: Lemon balm, anxiety, $GABA_A$ receptor, GABA transaminase, light/dark test

Development of analgesic silicone-based transdermal patches

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Introduction: Transdermal therapeutic systems (TTS) provide an excellent mode of accurate, safe and painless dosing in drug therapy. Modified silicone-polymer-based systems served a well-controlled and cost-effective matrix diffusion system.

Aim: We investigated substance release properties, skin penetration and analgesic effect of a modified silicone-polymer-based system loaded with capsaicin.

Methods: Release properties of silicone-based model TTS loaded with capsaicin were measured in vitro using Franz-cell and flow-through cells. Capsaicin was detected with HPLC-UV and UV spectrophotometry. Raman spectroscopy was conducted on human skin samples exposed to the TTS. Male Wistar rats were anesthetized with sodium pentobarbital. A surgical incision was performed and sutured on one hindpaw. Capsaicin-releasing TTSs were applied to the epilated dorsal skin either immediately after the incision or 18 hours later. Patches were kept on the animal skin for 6 hours. Thereafter, mechanical pain threshold of the hindpaws was detected with dynamic plantar aesthesiometry.

Results: Patches exhibited regulated capsaicin release. According to Raman experiments, capsaicin penetrated into the epidermis and dermis of human skin. Thermal pain threshold of the operated rat paws was elevated by capsaicin treatment compared to that of animals treated with control patch.

Conclusions: Our silicone-based TTS displayed controlled release of capsaicin. Further experiments involving TTS loaded with various detergents and other excipient possessing unexplored potential offer further optimization of substance release and increased therapeutic value.

Acknowledgment: “Project no. TKP2021-EGA-13 has been implemented with the support provided from the National Research, Development and Innovation Fund of Hungary, financed under the EGA-13 funding scheme.” OTKA_NKFI-K-134214, from the National Research, Development and Innovation Office, Hungary

Keywords : capsaicin, silicone, transdermal therapeutic system, analgesia, release kinetics

Identification of microRNA cargo of extracellular vesicles from cerebrospinal fluid of severe traumatic brain injury patients

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Introduction: Severe traumatic brain injury (sTBI) is a life-threatening intracranial injury with a complex pathophysiology which may include increased intracranial pressure (ICP) triggering further brain damage. ICP monitoring and managing by spontaneous drainage of cerebrospinal fluid (CSF) is thus crucial. Moreover, the resulting CSF might provide much needed information about both sTBI pathophysiology and recovery.
Here we focus on CSF-derived miRNAs, small RNA molecules included in gene expression. They are released into CSF due to direct tissue damage or by secretion from living cell, either in free form or as part of extracellular vesicles (EVs), membrane enveloped nanoparticles used for intercellular communication.

Objective: To identify and quantify miRNAs from CSFs of sTBI patients.
Materials and methods: CSFs from 5 sTBI patients were collected daily and subsequently pooled into day (d) 1-2, d3-4, d5-6 and d7-12 samples. Pooled samples were analysed by immunodetection for EV and blood content markers, and separated by size-exclusion chromatography on Sepharose CL-6B columns. Fractions containing CD81-positive EVs were used for miRNA isolation followed by cDNA synthesis and a commercial real-time PCR-array containing primers for detection of 87 miRNAs.

Results: CD9, CD81, Flotillin-1 and -2, Tsg101, albumin and apolipoproten A were detected in all four consecutive CSF-pools in declining amounts. SEC separation resulted in CD81-enriched fractions containing comparable amounts of miRNA in all 4 CSF-pools, but the majority of 87 analysed miRNAs were detected at low levels except for miR-16-5p, miR-21-5p, miR-142-3p, miR-144-3p, miR-204-5p, miR-223-3p and miR-451a whose levels were higher across the four CSF-pools in comparison to other analysed miRNAs, but also showed declining trend with the highest levels at d1-2 followed by lowering levels at later days.

Conclusion: This is a pilot study indicating that CSF-EVs contain RNAs whose identity and quantity change during the 10 days after sTBI. Potential EV-cargo includes miR-16-5p, miR-21-5p, miR-142-3p, miR-144-3p, miR-204-5p, miR-223-3p, and miR-451a.

This work was supported by the Croatian Science Foundation IP-2019-04-1511 to KG and University of Rijeka grant uniri-biomed-18-5 to KG.

Transient Receptor Potential Ankyrin 1 cation channel-expressing cells of the Edinger-Westphal nucleus are activated in a mouse migraine model

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Background: Transient receptor potential cation channel subfamily A member 1 (TRPA1) is involved in nociception, thermoregulation and inflammatory responses. Our previous study has proved that the *Trpa1* expression is very weak throughout the mouse and human brain, except for the urocortin1 (UCN1)-immunoreactive neurons in the centrally-projecting Edinger-Westphal (EWcp) nucleus. Interestingly, our earlier studies showed that EWcp/UCN1 neurons are recruited by acute pain stress. Here we aimed at investigating the role of the TRPA1-expressing EWcp neurons in the neurobiology of migraine.

Methods: A calcitonin gene-related peptide (CGRP) injection model of migraine was applied in C57BL6 mice. Behavioral assessment was performed using light-dark box test. The neuronal activity was measured by FOS immunohistochemistry. EWcp *Trpa1* mRNA expression and UCN1 peptide contents were assessed by RNAscope *in situ* hybridization combined with immunofluorescence.

Results: CGRP treatment increased the time spent in the dark compartment of the light-dark box device suggesting migraine-like state-associated photophobia in mice. The increased number of EWcp/FOS positive neurons suggest the activation of gene expression. Upon CGRP administration, EWcp neurons showed higher *Trpa1* mRNA expression and contained more UCN1 peptide.

Conclusion: TRPA1 in EWcp/UCN1 neurons may play role in neurobiology of migraine. In our ongoing experiments we study the underlying central mechanisms to identify new potential therapeutical targets for the therapy of migraine.

Neuroprotecitive and neurotrophic effects of estrogen

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Abstract

Estrogen is an important steroid hormone signal that regulates multiple tissues and functions in the body. This review focuses on the neurotrophic and neuroprotective actions of estrogen in the brain. The brain is an important target organ for estrogen. In addition to direct effects, estrogen influences brain function through effects on the vasculature and the immune system. Estrogen influences several neurotransmitter systems, including acetylcholine, serotonin, noradrenalin, and glutamate. There are complex mechanisms that underlie estrogen neuroprotective and neurotrophic actions (genomic, non-genomic kinase signalic pathways and membrane estrogen receptors pathways). Estradiol effects on synaptic plasticity and cognitive processes depend on hormone levels and the interaction of different estrogen receptors within neural circuits. Estradiol induces gene transcription and rapid membrane signaling mediated by estrogen receptor alpha (ERα), estrogen receptor-beta (ERβ), and a recently characterized G-protein coupled estrogen receptor, each with distinct distributions and ability to influence estradiol-dependent signaling.Vector-mediated expression of estrogen receptors in the hippocampus provides an innovative research approach and suggests that memory depends on the relative expression of ERα and ERβ interacting with estradiol levels. Sex differences in the risk, onset and severity of neurodegenerative disease such as Alzheimer’s disease, Parkinson’s disease and stroke are well known. The potential role of estrogen as a neuroprotective factor and a multicellular mode of estrogen action in the regulation of neuronal survival and neurotrophism is discussed, as are potential future directions for the field.

Key words: estrogen; steroid hormone; synaptic plasticity; neuroprotection;

Love addiction: how does it affect our metanoia?

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Introduction: The term "metanoia" has a Greek origin and denotes the process of fundamentally changing one's mind, views, opinions, heart, character, lifestyle, and overall self. Every single person has experienced the emotion of love at least once in their life. That has left a lasting mark on their consciousness. However, sometimes our feelings can easily be described as an addiction. That affects not only our mental health but also some physical parameters. All this puts addiction to love almost on a par with addiction to drugs, cigarettes, alcohol, and even gambling. For this reason, many researchers are looking for the connection between love addiction and our metanoia.

Aim: This review analyzes the established links between love addiction and the changes in our organism when we experience strong romantic feelings. Emphasis will also be placed on managing this condition, looking at different types of treatment and recovery options.

Methods: Several refereed articles published on platforms such as PubMed, Google Scholar, and Science Direct were used. The review was based on already established correlations and proven methods in practice.

Results: The studies indicate that romantic love may be called a "natural addiction," which in some ways parallels "diseases" such as drug and behavioral addictions. When the romantic connection is reciprocal, non-toxic, and suitable, passionate love may be a pleasant addiction. Nevertheless, as Paracelsus once said, " The dosage makes it either a poison or a remedy." Because of this, scientists assume that the strength of the feelings and their duration significantly impact the final effect on the body.

Conclusion: We conclude that love addiction may also negatively affect our overall behavior depending on the strength of the feelings. More research is needed to determine what factors influence love addiction.

Poster session #2: Cell physiology

Smooth muscle relaxant activity of *Ribes nigrum* L. juice variety Ben Sarek on spontaneous and spasmogen-induced contraction of the isolated rat ileum

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Black currant (*Ribes nigrum* L.) is a member of the Grossulariaceae family and has been used in diet as a rich source of bioactive compounds. The main objective of this research was to examine the relaxant activity of the juice obtained from a black currant variety, Ben Sarek, and one of its main flavonols, quercetin, on the spontaneous, KCl and acetylcholine-induced ileum smooth muscle contractions. Segments of the isolated rat ileum were suspended in an isolated tissue bath filled with Tyrode's solution that were connected to a force transducer. The results indicated that increasing concentrations of the black currant juice (0.01-3 mg/ml) and quercetin (0.05-15 mM) produced concentration-dependent relaxant effects on the rat ileum spontaneous contractions (EC_{50} value were 0.96± 0.06 mg/ml and 2.2 ± 0.15 mM, respectively). The KCl solution (80 mM) induced a depolarization and tonic smooth muscle contractions of the isolated rat ileum. The black currant juice and quercetin in a concentration-dependent manner significantly relaxed potassium chloride induced contraction in the ileum ($p < 0.01$). Verapamil, a calcium channel blocker, was used as a positive control. Cumulative concentrations of the black currants juice and quercetin concentration-dependently inhibited acetylcholine induced contractions of the isolated rat ileum ($p < 0.01$). From the presented results, we can conclude that the Ben Sarek variety black currant juice and quercetin exhibited significant and dose-dependent relaxant activity, indicating the possibility for further investigation of the black currant as a functional food.

Key words: black currant, *Ribes nigrum* L., quercetin, rat, ileum, relaxant activity

The authors would like to thank the Ministry of Education, Science and Technological Development of Republic of Serbia (Grant No:451-03-68/2022-14/200113 and 451-03-68/2022-14/200003) for financial support.

Effect of *in vitro* exposure to 3500 MHz radiofrequency electromagnetic field on spermatozoa DNA integrity and vitality indicators in ejaculates of breeding boars

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The potentially negative effects of radiofrequency electromagnetic fields (RF-EMF) of higher frequencies have not been investigated on human and animal ejaculates so far. The aim of this study was to investigate the effect of RF-EMF at 3500 MHz on spermatozoa DNA integrity and vitality indicators *in vitro* exposed ejaculates of breeding boars.

Ejaculate samples of 12 breeding boars aged from 1.5 to 3.5 years were obtained by manual fixation of the penis. Freshly diluted ejaculates of each boar were divided into a control (n = 12) and a test sample (n = 12). The test samples were exposed to RF-EMF at 3500 MHz and field strength of 10 V/m for 2 hours using gigahertz transverse electromagnetic chamber. In the both samples, exposed and non-exposed ejaculates, the proportion of spermatozoa with cell and acrosomal membrane damage, the proportion of spermatozoa with damage and depolarization of mitochondria using PI/SYBR-14 LIVE/DEAD Sperm Viability Kit and the proportion of spermatozoa with DNA damage were determined using Halomax DNA Fragmetation Test.

The significantly increase in the proportion of spermatozoa with DNA damage was recorded in ejaculates exposed to RF-EMF compared to non-exposed ejaculates. A significantly higher proportion of spermatozoa with damaged membrane and good mitochondrial potential was recorded in ejaculates exposed to RF-EMF at 3500 MHz. Although there was an increase in the sum of the proportion of spermatozoa with damaged membrane and/or low mitochondrial potential in all exposed ejaculates, but not significant.

In vitro exposure of breading boars' ejaculates to RF-EMF at 3500 MHz has a negative effect on the DNA integrity. Furthermore, the effect of RF-EMF at 3500 MHz on *in vitro* exposed ejaculates, is manifested by an increase in the proportion of spermatozoa with damaged membrane and/or low mitochondrial potential.

Key words: non-ionizing electromagnetic radiation, boar semen, DNA damage

The Uroguanylin effect on stimulated saliva volume flow, the pH and concentration of Na^+, K^+, Cl^- in mice

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Introduction: The physiological role of signalling pathways for guanylin peptides (*GP*) has been demonstrated in the kidney and brain, but their unexplored role in the physiology of the salivary glands and their possible role in the regulation of ion secretion not only in physiological but also in pathophysiological conditions, such as cystic fibrosis, Sjoegren's syndrome or oral mucositis, remains to be seen.

Aim: This work aimed to determine the effect of uroguanylin (*UGN*) on the flow volume of stimulated saliva and the pH and concentration of Na^+, K^+, and Cl^- in wild type (*WT*) animals and animals lacking the guanylate cyclase C (*GC-C KO*).

Materials and methods: A total of 30 wild-type male mice (C57BC/6NCrl), and wild-type mice lacking the GC-C from the same littermates were used. Mice were anesthetized and administered i.p. UGN or physiological solution. After 15 min, pilocarpine was applied to stimulate salivation. Filter paper cut into strips was used to collect saliva. Secreted saliva was determined by the gravimetric method and samples were centrifuged at 15,000g at 24C for sialochemistry. Concentrations of Na^+, K^+, and Cl^- in saliva were measured by indirect potentiometry, while pH was determined by potentiometry on a blood gas analyser using dedicated pH-sensors.

Results: The difference in concentration of Na^+, K^+ and Cl^- after UGN application in saliva of individual groups of animals was not statistically significant. A lower pH was observed in the WT animals upon UGN application, but comparing it to animals lacking GC-C, it was not statistically significant. Salivary flow was reduced during the application of UGN in WT. This effects was missing in same reduction was observed in GC-C WT, whereas animals from the same littermate lacking GC-C were not.

Conclusion: Based on the obtained results, we concluded that UGN can affects saliva composition and flow via both GC-C dependent (pH) or GC-C independent (flow) signalling pathways.

Key words: guanylate cyclase C, saliva flow, ion, and water transport

Modified pulmonary surfactant and N-acetylcysteine in the experimental double-hit model of lung injury in rats

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Hyperoxia and subsequent accumulation of reactive oxygen species (ROS) during supportive ventilation induce an inflammatory response and together lead to lung tissue damage. These processes make the lung tissue more sensitive to secondary bacterial infection. Lung antioxidant mechanisms can significantly reduce the effect of ROS and may be enhanced by therapeutical antioxidants (such as N-acetylcysteine) and/or surfactant preparations. Pulmonary surfactant is a complex and highly surface active material composed of lipids and proteins which is found in the fluid lining the alveolar surface of the lungs.

Our aim was to compare the efficacy of N-acetylcysteine monotherapy and combined therapy of exogenous surfactant and N-acetylcysteine in the experimental double-hit model of lung injury.

Adult Wistar rats were used and divided into four groups: Bacterial lipopolysaccharide (LPS) was instilled intratracheally to induce infection (LPSgroup; 500μg/kg, 1.25ml/kg). Animals with hyperoxia (FiO_2 1.0) and LPS were treated with NAC intravenously (10mg/kg; NACgroup) or with exogenous surfactant intratracheally and NAC intravenously (PSUR+NACgroup; PSUR 50mgPL/kg+NAC10mg/ml). Controls received saline. After the experiment, markers (IL-1ß, TNF-α, IL-6, TBARS, AOPP, TAC) were analyzed in homogenized lung. The total count of leukocytes was evaluated in arterial blood, lung oedema was expressed as wet/dry weight ratio.

In comparison with control, hyperoxia and LPS increase markers (IL-1ß, TNF-α, IL-6, TBARS, AOPP) in lung (all $p<0.01$), lung oedema formation ($p<0.05$) and decrease TAC ($p<0.01$) and total count of leukocytes in the blood. In LPS-treated animals, NAC administration increases TAC ($p<0.05$) and decreases IL-6 ($p<0.05$) in the lung. This effect is potentiated after administration of combined therapy of exogenous surfactant and NAC. In addition, PSUR+NAC improved TNF-α, IL-1ß and TAC ($p<0.05$) compared to NAC.

The combined therapy of exogenous surfactant with NAC suppresses lung inflammation and oxidative stress in the experimental double-hit model of lung injury.

Supported by grants: APVV-17-0250, VEGA 1/0055/19, VEGA 1/0004/21

cAMP-mediated increase in calcium activity in pancreatic beta cell networks: role of Epac2A

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The pancreatic islets of Langerhans are multicellular micro-organs within which communication among a variety of cells with unique functions must occur to ensure proper control of metabolic homeostasis. The insulin-secreting beta cells are the most abundant cell type within the islets and exhibit strong intercellular connectivity required for coordinated responses of beta cells and the subsequent secretion of insulin. Beta cell signaling and cell-to-cell communication in islets have been reported to be modulated by cAMP involving PKA-dependent and PKA-independent mechanisms through Epac2A.To clarify the effect of cAMP and the role of Epac2A in intracellular calcium signaling as well as intercellular communication, we performed functional multicellular calcium imaging of beta cells in pancreas tissue slices of wild-type and Epac2A knock-out mice after stimulation with glucose and forskolin. Increased cAMP evoked calcium signals in otherwise sub-stimulatory glucose, and beta cells from Epac2A knock-out mice displayed faster activation. Our results reveal that beta cells from Epac2A knock-out mice displayed a slightly higher active time in response to glucose compared with wild-type littermates. In Epac2A knock-out and wild-type mice, the increased cAMP levels increased the active time by significantly increasing oscillation frequency and slightly decreasing oscillation duration. Functional network analyses that we used to quantify the multicellular activity patterns have revealed that the network characteristics during stimulation with glucose were similar in wild-type and Epac2A knock-out mice. However, the presence of Epac2A was crucial for the protective effect of increased cAMP in preventing a decline in beta cell functional connectivity with time. Moreover, increased cAMP levels prolonged beta cell activity during deactivation in an Epac2A-independent manner. Our findings provide a deeper insight into the involvement of cAMP signaling in intra- as well as intercellular activity in beta cells, which is vital for developing novel incretin-based pharmacological management strategies for diabetes.

Incretins modulate beta cell calcium dynamics in mouse islets from acute pancreas tissue slices

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Precise control of insulin secretion from pancreatic beta cells is crucial for maintaining normoglycemia. While glucose is the main stimulus regulating this process, glucagon-like peptide-1 (GLP-1), augments beta cell insulin output through both the rise of cytosolic Ca^{2+} and the increased sensitivity of the secretory apparatus to the elevated $[Ca^{2+}]_{IC}$. By combining functional multicellular Ca^{2+} imaging with acute pancreas tissue slices, we were able to assess the effect of GLP-1 receptor pharmacological manipulation on activation, activity, and deactivation of beta cells. GLP-1 receptor agonists exendine-4 (Ex-4) and GLP-1 as well as a GLP-1 receptor antagonist exendine-9 (Ex-9) were used in the experiments. In the presence of an otherwise sub-stimulatory glucose, on average less than 5 % of beta cells per islet were active. However, the addition of Ex-4 triggered individual Ca^{2+} rises in approximately 30 % of beta cells. Stimulation of beta cells with glucose evoked high-frequency Ca^{2+}+ oscillations, superimposed on slower basal oscillations. Throughout prolonged glucose stimulation, the frequency of fast Ca^{2+} oscillations tended to increase with no major changes in their duration, indicating thereby an increase in active time. Adding Ex-4 after a period of introductory glucose-only stimulation induced an additional modest increase in active time. However, activation or blockade of GLP-1 receptor during the initial stepwise rise in glucose considerably raised or declined beta cell active time, respectively. The inhibition of beta cells was reversible, with changes in both fast and slow Ca^{2+} dynamics contributing to enhanced activity after removing the antagonist. To summarize, pharmacological manipulation of GLP-1 receptors affected beta cell Ca^{2+} dynamics, especially when glucose and Ex-4 or Ex-9 were applied concurrently at the onset of stimulation or even before stimulation. This indicates that GLP-1 receptor signaling may primarily be important for the initial part of beta cell response.

Full-thickness skin wound healing after application of regenerating cream combined with silver nanoparticles on purulent wounds

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Introduction: A wound is a pathological process caused by physical, chemical and biological factors (accident, violence or surgery), which usually involves injury to the skin and underlying tissues. The normal healing process for a full-thickness surgical injury involves the following stages: hemostasis, inflammation, recovery and remodeling. Various factors can prolong or speed up the regeneration and recovery of wounds.

Objective: To evaluate the effect of silver nanoparticles (AgNPs) and regenerating cream (RC) on the intensity of the regenerative process in rats.

Materials and methods: A wound defect was modeled on 48 female rats weighing 120 g, on a template 1.0 × 1.5 cm and infected with bacteria S. aureus, E. coli and P. aeruginosa at a concentration of 5 × 10 9 CFU / mL for 72 hours. The treatment lasted 21 days with use of RC - Cantareus Total Recovery of „Kantareus R“ LTD and AgNPs by the company NanoWave, Poland, in the following groups:
• Group 1 (G1) - 14 animals treated with AgNPs + RC
• Group 2 (G2) - 14 animals treated with AgNPs
• Group 3 (G3)- 14 animals treated with RC
• Group 4 (G4) - 5 animals, without treatment
Histological methods were used to assess the effect of the treatment, with biopsy taken at days 3, 7, 14 and 21.

Results: The group treated with AgNPs + PK showed the best results for wound healing. The average number of myofibroblasts was statistically significantly higher on 14th day than on the 7th and 3rd days in G1 compared to G 4 ($p < 0.05$). The mean number of myofibroblasts in G 2 and G3 was statistically significantly higher on day 21 than on day 7 and 3 compared to G 4 ($p < 0.05$).

Conclusions: Our results show that the application of treatment with AgNPs + PK facilitates the differentiation of myofibroblasts during the early stages of the cicatricial repair process and therefore contributes to the full-thickness skin wound healing.

Key words: Silver Nanoparticles, Regenerating cream, Inflammation, Purulent Wound.

Caco-2 monolayer on permeable membrane develops a stronger connection between cells than the cells within spheroids

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INTRODUCTION

Caco-2 cells, derived from human colon adenocarcinoma, have been used as a cellular intestinal (2D) model due to their ability to differentiate into enterocyte-like cells. These cells even can assemble into the 3D structure called spheroids.

AIM

To differentiate Caco-2 cells as a monolayer on Transwell permeable supports, to obtain Caco-2 spheroids using the 3D Petri Dish culture technology and compare them through tight junction analysis between cells.

METHODS

Before freezing, Caco-2 cells were conditioned by harvesting at 50% of density through 10 passages. After thawing, all experiments were done between 4 – 6 passages.

On permeable polyester membrane with pores of 0.4 um were seeded 85x103 or 125x103 cells/well (12 well plate). The cells were grown for 21 days and analyzed by immunofluorescence.

3D Petri Dish system (12 well plate) was used for generating spheroids from 273x103 cells over 10 days, followed by cryo-preservation of spheroids, sections on cryotome, and immunofluorescence analysis.

RESULTS

ZO-1, as a marker of tight junction between cells, was fully developed on cells growing on permeable membrane support, opposite to cells aggregated in spheroids where ZO-1 is partially expressed on cells' membrane, causing the very fragile structure of spheroids and/or its cryo sections without cells' polarity.

Caco-2 cells growing on membrane expressed SGLT1 and Glut2 enterocytes-like markers that we here used for fluorescent glucose derivate absorption into cells.

CONCLUSION

Although differentiated cells on membrane support present 2D model that has some weaknesses, it is still a valuable system that mimics intestinal membrane.

3D Petri Dish system is helpful for forming Caco-2 geometrically controlled aggregates, which should further move in Matrigel for forming intestinal polarized spheroids.

Unveiling the functional characteristics and interrelations of hub and pacemaker cells in the pancreatic islets of Langerhans: Insights from network analysis

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The pancreas is a complex organ that is home to micro-organs called islets of Langerhans which contain different cell types, among which the insulin-producing and excitable beta cells are the most abundant ones. Despite their inherent heterogeneity, gap junctional coupling facilitates their coordinated behavior and coherent Ca^{2+} concentration oscillations when stimulated with glucose. Their synchronous behavior is vital for ensuring proper secretion of insulin secretion patterns. The importance of beta cell heterogeneity and how they ensure coordinated intercellular rhythmicity is still incompletely understood. Recent advances in multicellular imaging, optogenetic and photopharmacological strategies, and network theory have aided the identification of specialized beta cells subpopulations, which drive the collective activity. It has been postulated that hub cells – cells with many functional connections, significantly enhance the communication capacities of the intercellular network and facilitate an efficient spreading of intercellular waves. The latter was triggered within specific beta cell subpopulations – pacemaker regions, defined by their local excitability and metabolic profiles. However, to what extent the roles of these subpopulations overlap, and what are their functional characteristics remains to be elucidated. We addressed these issues by combining multicellular Ca^{2+} imaging in mouse pancreatic tissue slices with complex network-based approaches. We constructed network layers based on individual Ca^{2+} waves to identify wave initiators and functional correlation-based networks to detect hub cells. Our results reveal that both cell types exhibit a higher-than-average activity under physiological and supraphysiological glucose concentrations but differ in other signaling characteristics. Ca^{2+} oscillations in hub cells are more regular, and their role appears to be more stable in time. Most importantly, in contrast to pacemaker cells, hub cells display shorter activation time delays than their neighbors, which implies stronger junctional connectivity. Our findings indicate that both hub and pacemaker cells are natural features of islets, but their roles do not overlap.

The effect of CoenzymeQ10 on the cells of the bone marrow in the presence of cisplatin

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Introduction

- **CoQ10** is a lipid-soluble antioxidant, commonly known as ubiquinone, present in all membranes in the body. It plays a key role in the mitochondria respiratory chain in the synthesis of adenosine triphosphate (ATP).
- **Cisplatin** is an antineoplastic drug used for treating various human malignances. Anyway, because of its cytotoxicity, the therapeutic outcome of Cisplatin is limited. It causes oxidative stress in the body by reducing the amount of ATP in mitochondria.

Mitochondria are the main target for Cisplatin and CoQ10, and therefore CoenzymeQ10 could possibly mitigate the side effects of Cisplatin.

The aim of this study is to evaluate the effect of CoQ10 on the cells of the bone marrow e.g. RDWa, RBC, HGB, in Cisplatin induced cytotoxicity.

Materials and methods

60 normotensive Wistar rats (250-300 g) were included in this study. The animals were divided in **4 groups**, each one 15 rats. Cisplatin group received Cisplatin 5 mg/kg i.p. on day 4, as a single dose. Cisplatin/CoQ10 group received Cisplatin 5 mg/kg i.p. on day 4 as a single dose and CoenzymeQ10 10 mg/kg i.p. for 11 days. CoenzymeQ10 group received only CoenzymeQ10 10 mg/kg i.p. for 11 days. The last one was a control group that received physiological solution (with Tween 80 and Vitamin C), i.p. Blood samples for hematological analyses were obtained on day 2, 6 & 12 of the study.

Results

The CoenzymeQ10 group showed significantly increased levels for **HGB** and **RDWa**, compared to the other three groups. However, there were significantly decreased **RDWa** and **RBC** levels in the Cisplatin group, compared to the Cisplatin/CoenzymeQ10 group.

Conclusion

According to these results, CoenzymeQ10 is a *potential solution* for improving anemia as a side effect of the Cisplatin drug.

Glucose stimulated calcium dynamics in acute pancreas tissue slices of inbred and outbred mouse strains

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Beta cell response to glucose is characterized by a change in intracellular calcium concentration ($[Ca^{2+}]_i$) dynamics that triggers insulin release. Previously, confocal microscopy was used to characterize glucose-stimulated calcium dynamics in acute mouse pancreas tissue slices of the outbred NMRI strain in many beta cells from all layers of islets using physiological and supraphysiological glucose concentrations. Due to the widespread use of the inbred C57BL/6 substrains as models of T2DM, their characterization of glucose stimulated $[Ca^{2+}]_i$ dynamics is necessary. Acute pancreas tissue slices of 12-13-week-old C57BL6/6J, C57BL6N, and NMRI mice were prepared and stained with a calcium sensitive dye. The tissue slices were exposed to single square-pulse-like glucose stimulation (7, 8, 9, 10, 12, or 16 mM, lasting 40, 30, 20, 20, 15, and 15 minutes, respectively), followed by a return to 6 mM glucose. Confocal imaging of beta cell $[Ca^{2+}]_i$ dynamics was preformed to determine the delays in response onset (activation) and response termination (deactivation) as well as frequency and duration of $[Ca^{2+}]_i$ spikes and active time in the plateau phase. Strain- and substrain differences were observed in all three phases. Specifically, A glucose-dependent response was observed both in delays to activation after stimulation and delays to deactivation after cessation of stimulation in C57BL6/6J, C57BL6N, and NMRI mice. A statistically significant difference in responses was also observed between the three groups of mice. In the plateau phase, quantitative and qualitative differences may be observed in calcium dynamics, both between groups of mice and stimulatory glucose concentrations. Differences in glucose-induced $[Ca^{2+}]_i$ dynamics of beta cells are observed both between the inbred (C57BL6/6J and C57BL6N) and the outbred (NMRI) mice, as well as within the two genetically similar inbred substrains, shedding light on the importance of the genetic background in selection of murine models of type 2 diabetes.

Green tea effect on the basal membrane of the rat kidney

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Introduction:
One of the most popular beverages worldwide, green tea, has lately been found in the focus of research. It is documented to have many health promoting effects and could be used as prevention against diseases caused by oxidative stress because of its antioxidant activity.

The aim:
The aim of our study was to examine whether green tea fed rats had the function and structure of their kidneys and its basement membrane impaired by comparing it with a control group of rats given only saline.

Materials and methods:
The study was conducted on two groups of rats: control (C) group and GT group, treated with green tea. Kidney function as well as histopathological studies were assessed.
Results: Green tea didn't cause significant changes in serum urea and creatinine levels when compared to control group. Also, histological analysis found that GT group of rats had normal kidney structure.

Conclusion:
Based on our results we found that green tea diet in dosage of 300 mg/kg daily is safe for rat kidneys and doesn't cause them any structural or functional damage. Basal membrane of rat kidneys stayed intact.

Key words: rat, green tea, oxidative stress

Poster session #2: Endocrinology

Algorithm for diagnosis and treatment of iron deficiency anemia in adolescent children based on hepcidin and selenium status

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Introduction. Adolescent girls are at increased risk of trace element deficiencies due to their higher requirements for micronutrients, such as iron and selenium. Despite its wide spread, iron deficiency anemia (IDA) remains commonly underdiagnosed and undertreated. Treatment with iron is effective in only 40-60% of anemia cases, while the rest are unresponsive to iron supplementation. Hormone hepcidin is the main factor regulating iron homeostasis, but its increase during inflammation and in specific gene mutations limits the intestinal absorption of iron.

Our aim was to study the hepcidin and selenium involvement in the iron homeostasis in adolescent children with IDA and to propose an algorithm for diagnosis and treatment of IDA in adolescent children.

Methods. Twenty-three (23) adolescent children with IDA (n=12) and healthy controls (n=11) were studied. We evaluated the serum concentrations of hepcidin, interleukin-6 (IL-6) and selenium, red blood cell count (RBC), hemoglobin concentration, hematocrit and erythrocyte indices (MCV, MCH and MCHC). Iron metabolism was characterized by the biochemical markers: serum ferritin, serum iron and total iron-binding capacity, with subsequent calculation of transferrin saturation.

Our results demonstrate:

- Statistically significant lower serum concentrations of hepcidin and selenium, whereas higher serum IL-6 levels in the IDA group than in the control group;
- Statistically significant higher proportion of children having below-threshold serum selenium concentrations in the work group compared to the controls;
- Statistically significant correlations of the serum selenium with erythrocyte indices and iron metabolism biochemical markers, as well as between the serum hepcidin and RBC in the IDA children.

Herewith we propose an algorithm for diagnosis and treatment of IDA in adolescent children based on hepcidin and selenium status.

Conclusion. Clarification of factors regulating hepcidin synthesis and secretion, as well as the bounding inter-links between the homeostasis of iron and selenium could contribute to the better management of iron deficiency conditions.

Key words: hepcidin, iron homeostasis, selenium, adolescence

Nicotinamide post-treatment of diabetic rats and its effects on liver carbohydrate metabolism

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As a precursor to NAD synthesis, nicotinamide (NA) increases levels of pyridine nucleotides (which are of great importance in glucose/glycogen metabolism), protects pancreatic islets from inflammation and stimulates endocrine differentiation of rat β-cells *in vivo*. In addition, NA administered as a pretreatment provides protection for animal models of diabetes induced by streptozotocin (STZ). Little is known about the effect of NA when administered as a post-treatment in STZ-diabetic rats.

We examined the changes in hepatic rate-limiting enzymes and substrates associated with carbohydrate metabolism in diabetic rats treated with NA. Experimental diabetes was induced by a single intraperitoneal injection of STZ (65 mg/kg body weight) and NA (500 mg/kg/i.p.) was administered for 14 consecutive days, 7 days after STZ administration. We performed measurements in substrate concentration, enzyme activities, Western blot and mRNA expression.

As expected, STZ-induced diabetes manifests itself with decreased serum insulin levels and increased blood and liver glucose. This further resulted in increased gluconeogenic enzymes (G6P-ase, F16BP-ase and PEPCK) as well as decreased glycogen, intermediate substrates (G6P, G1P and F6P), glycogenolytic (GPa) and glycolytic (HK, PK, PFK) enzymes.

Two-week treatment of diabetic rats with NA caused a slight decrease in blood glucose and non-significant changes in insulin concentration compared to diabetic rats. However, there was significantly higher glycogen content, intermediate substrates (G6P, G1P and F6P) and GS mRNA expression. On the other hand, NA-treated diabetic rats have significantly lower G6P-ase (activity, protein levels and mRNA expression), significantly higher F16BP-ase activity and non-significant changes in PEPCK (activity and mRNA expression). Finally, there was significantly lower PFK activity and non-significant changes in GPa and PK activity.

In summary, NA post-treatment caused slight changes in carbohydrate metabolism and only a tendency to normalize to control values. NA administered as post-treatment in diabetic rats showed no reparative effects.

Obesity is associated with changes in taste bud papillae microbiota, junctional permeability and oro-sensory perception of dietary lipids in mice

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The excessive consumption of lipid products is considered as a key factor involved in obesity. A number of studies have recently suggested that lingual CD36, a glycoprotein, mainly expressed by circumvallate papillae of the tongue, might be implicated in the perception of dietary fat taste. It is well established that chronic high fat intake contributes to obesity, which alters fat taste perception and generates inflammation. We have conducted studied on taste bud inflammation and microbiota in two groups; high fat diet (HFD)-induced obese mice and normal diet, in 2-month old. The obese mice expressed higher levels of mRNA encoding pro-inflammatory cytokines like IL-1α, IL-1β, IL-6, IL-12 and TNFα in taste buds compared to the control group. The diet-induced obesity was also associated with the rupture of junctions, marked by ZO-1, Claudin 4 and Claudin 7 in circumvallate papillae. The obese animals also exhibited low oro-gustatory detection capacity for dietary fatty acids. Furthermore, the taste bud papillae microbiota in obese animals was marked with high number of bacteria of the streptococci family. The metagenomics analysis revealed that obese mice taste buds were prone to the modifications via NOD-like signaling pathway. Our observations suggest that obesity is not only marked by peripheral mild-inflammation, but also an altered taste bud microbiota and its associated local inflammation. This phenomenon along with local papillary inflammation might play a key role in diet-induced obesity.

Infrared thermography is a new method for detection of brown adipose tissue activity

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Introduction: Measuring brown adipose tissue (BAT) activity by PET CT fails as the method of choice. Therefore, to estimate physiological importance of BAT in animals and humans and its sex, age, feeding status and phase of the estrous cycle dependency, new infrared thermography method is applied.

Methods: The BAT activity was measured by infrared thermography before and 30, 60 and 120 min after a meal in human volunteers and male and female C57Bl/6NCrl wild type mice. Phase of the estrous cycle was determined by vaginal smears stained with Cresyl Violet (0.1%) Stain Solution. In healthy volunteers two region of interest at neck area were selected: 1. the supraclavicular area (above BAT) and 2. interclavicular area (without BAT). The BAT activity was determined by subtraction of maximal measured temperature in both regions. To measure BAT activity in mice, the interscapular region of the skin was shaved the day before experiments and animals fasted overnight. Maximal temperature above BAT (interscapular region) was measured. Furthermore, the production of heat by BAT is calculated by Stefan-Boltzmann law. Average temperature of the skin in designated areas could be determined in animals and in human participants.

Results and conclusion: Infrared thermography, a non-invasive more sensitive method for determining the BAT activity is variable tool for determining potential drugs which could re-juvenile BAT activity. The BAT activity after a meal is decreased and almost abolished with age and in different pathological conditions as debates mellitus type 2, especially in women. To find a new pharmacological approach for safe BAT activation is main topic in BAT research today. Furthermore, using infrared thermography will be of great importance for measuring BAT activity in people with increased body weight and diabetes mellitus type 2 with possible differences in glucose plasma concentration as well as in different laboratory animals (knock out mice).

Keywords: Infrared thermography, human and animal subjects, postprandial activation of brown adipose tissue, PET-CT

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

Menopausal status, BMI and vitamin D in women in Tetovo and Pollog region in N.Macedonia

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Abstract

Menopause is known as the end of natural transition in a woman's reproductive life. Otherwise, is the period when progesterone and estrogen production is significantly reduced. The ovaries forbid the cell production and woman loses the ability of getting pregnant. Menopause is defined a period after 12 months without a menstrual cycle or even more. (WHO)

Aims: of this study is to make comparate the level effects of hypovitaminosis D and body mass index, overweight and menopausal and post-menopausal women.

Materials and Methods: This study includes data for 670 female patients, in premenopausal, menopausal and postmenopausal period. The data were obtained from the Medical Scientific Research Laboratory, Medical Faculty and Biochemical Laboratory Albimedika.
Samples of researched patients were analyzed for the determination of parathyroid hormone, vitamin D BioMerieux Vidas - immunoassay fluorescent test and Ca2 + in serum by spectrophotometric method.

Results: vitamin D level compared between winter time and summer time in women is around 10-15 % lower in winter time or 2.88 ± SD. The prevalence of BMI in menopausal patients expresses a significant increase of 2.18% compared to control patients.
Parathyroid hormone is a very important hormone in menopausal women who have a 12-15% (6.70± SD) increase of parathormone in patients with low level of vitamin D and decrease of calcium ions compared with control patients. Women in period of premenopause 28.3% (3.44 ± SD), menopause 49,6% (6.62 ± SD) and postmenopause 23.1% (3.44 ± SD) we conclude that the more problem of osteoporosis is attached a menopausal group and postmenopausal group than premenopausal group.

Conclusions: This study demonstrated that hypovitaminosis D is associated with osteoporosis in menopausal and postmenopausal women and with high level of parathormon, and BMI level in the studied patients.

Key words: menopause, vitamin D, parathormone, calcium, BMI.

Breathing pattern characteristics in young obese patients

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Obesity is a severe and growing medical problem associated with an increased risk of many diseases. It is well known, that excessive adipose tissue adversely affects the function of the respiratory system. Fat deposits in the mediastinum and abdominal cavity alter the mechanical properties of the lungs and chest wall, which reduces the compliance of the respiratory system, leading to restrictive respiratory disorder. The reduction in respiratory system compliance could significantly alter the breathing pattern. The aim of our study was to assess the respiratory pattern changes in young obese individuals, including the variability in respiratory pattern, inspired by the fact that its changes were observed in pathological conditions characterized by restrictive respiratory disorder.

Our study included 51 obese and 51 sex and age matched control subjects, mean age 17.6 years. The respiratory pattern related measures – tidal volume, inspiratory and expiratory times, their ratio (Ti/Te) and respiratory cycle duration – were breath-to-breath recorded using respiratory inductive plethysmography method during 5 consecutive phases – supine rest, head-up tilt to 45°, supine recovery, non-verbal mental arithmetics and supine recovery. We calculated mean values of assessed ventilatory measures and their variability was quantified usig standard deviation (SD) and coefficient of variation (CV).

From mean values Ti/Te ratio was significantly increased in obese patients in all three resting phases and tidal volume was significantly reduced in supine rest and head-up tilt in obese patients compared to control group. Regarding respiratory variability, we found its increase in Ti/Te during recovery and stress phases in obesity.

We conclude that Ti/Te ratio is a parameter mostly differing between obese and control groups. This parameter have the potential to reveal obese patients with imminent respiratory disorder in this high-risk group.

Supported by grants: VEGA 1/0200/19 and VEGA 1/0283/21.

Sex-dependent behavioural responses caused by dietary manipulations in mice

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A typical western society diet is energy-dense and affects physical and mental health. Obesity, depression, and anxiety are the most prevalent co-occurring global health problems. The link between them seems to be bidirectional.

The aim of the study was to examine the occurrence of anxiety and depressive and depressive-like behaviors in mice. In our study, the effect of Western diet (WD) and possible therapeutic effect of restricted-caloric diet (RCD) on behaviour of adult C57BL/6J mice of both sexes were investigated. WD and control group of mice were fed with WD and standard diet, respectively, *ad libitum* for 3 months. RCD group was fed with WD for 2 months, followed by 1 week of RCD and 5 weeks of standard diet. The impact of the diet was assessed using behavioral tests. The Open Field Test (OFT) was used to assess locomotorization, the Elevated Plus Maze (EPM) test to assess anxious-like behavior, and the Forced Swimming Test (FST) to assess depressive-like behavior.

A progression of anxiety-like behaviour was observed in WD mice, which was successfully reversed by RCD in female mice. No significant diet effect in depressive-like behaviour was found. Interestingly, sex-related differences in locomotion activity have been observed in WD mice. WD females were more active than WD males (they were faster, walked farther distances, and spent less time at rest). We observed the impact of habituation on some behavioral tests. In the OFT test, we observed a decrease in research behavior when repeating the test.

WD provoked behavioural changes that were to some extent reversible by reduced caloric intake. Females were less prone to dietary manipulations, suggesting the beneficial role of oestrogens in the control of energy balance.

Identification of sacropenic obesity in patients undergoing orthopaedic surgery. The predictive power of a body shape index (ABSI)

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Abstract

Background:
Sarcopenic obesity is a condition characterised by the coexistence of low muscle mass and function (sarcopenia) and excessive fat mass (obesity). The aim of this study was to determine the prevalence of this condition in patients undergoing orthopaedic surgery and investigated the suitability of a waist circumference-based anthropometric measure, body shape index (ABSI), for predicting sarcopenic obesity and the predictive power of ABSI for fat-free mass index (FFMI), a surrogate marker of lean body mass.

Methods:
A cross-sectional study of overweight and obese orthopaedic patients undergoing knee or hip and spine surgery was conducted between October 2019 and March 2020 in Orthopaedic Hospital Valdoltra, Slovenia. General anthropometric parameters body mass index (BMI) and ABSI = (WC/(BMI2/3x height½) as well as body composition data (fat mass FM, fat-free mass FFM, FFMI, and the ratio FM/FFM as an index of sarcopenic obesity) were determined in 120 women (aged 66.5 ± 9.6 years) and 89 men (aged 65.5 ± 7.8 years) with overweight (25 kg/m2 ≤ BMI < 30 kg/m2) and obesity (BMI ≥ 30 kg/m2) by bioelectrical impedance analysis (BIA).

Results:
Sarcopenic obesity phenotypes based on FM/FFM ratio > 0.80 was present in 15.3% of patients, mainly in female patients undergoing knee surgery. ABSI was significantly associated with age in all women and obese men and with waist circumference (WC) in all patients. ABSI did not correlate with BMI in women and men; however, multiple linear regression analysis showed that BMI independently predicted FFMI in women and men and ABSI in women only. Women with a lower ABSI had a significantly higher FFMI than the group with a higher ABSI.

Conclusions:
Sarcopenic obesity was most prevalent in obese women scheduled for knee surgery. In addition, ABSI, independently predicted FFMI in women and represents a significant predictor of sarcopenic obesity.

Double combination of antioxidant formula can improve the oxidative stress status and motilityin men with asthenozoospermia: double blind placebo controlled trial

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Abstract

The definition of male infertility is clearly described by the World Health Organization, and the number of factors associated with this issue is large, but with particular emphasis most belong to problems of idiopathic etiology alluding to oxidative stress.
Thus, the aim of this study is to verify the effectiveness of combination antioxidant therapy as recommended in some elaborated papers.
A total of 42 infertile men were enrolled in the study after fulfilling the inclusive criteria. Standard semen analysis along with biochemical analysis of malondialdehyde (MDA) and total antioxidant capacity (TAC) levels were done at the beginning and after six months of receiving antioxidant or placebo therapy. The groups were randomly divided into the individuals who received the combination of the antioxidant formula (n=20) and the individuals who received placebo therapy (n=22).
An improvement in sperm motility (mean & SD from 31,4 ± 4,77 to 50,8 ± 9,37, p=0,000014) was detected in the group receiving the double combination of antioxidant formula, while no improvement in terms of motility was seen at the placebo group (31,01 ± 4,41 to 32,92 ± 3,85, p=0,021). In the group treated with antioxidants we had a reduction in MDA levels (5,32 ± 0,73 to 2,53 ± 0,47 ; p <0,001) and increase in TAC levels (44,13 ± 6,20 to 78,87 ± 8,63 ; p <0,001). While in the placebo group the mean MDA and TAC levels had no statistically significant changes (5,94 ± 1,28 to 5,99 ± 1,29 ; p =0,189) and (49,71 ± 8.34 to 49,03 ± 7,56 ; p =0,284), respectively.
Looking at the cost-benefit aspect, administration of such therapy contributes positively in men with asthenozoospermia.

Keywords: Male infertility, asthenozoospermia, antioxidants, malondialdehyde, total antioxidant capacity

Non-nutritive sweeteners and sweet cravings- is there a link?

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Abstract:

Introduction:
Over the last 20 years, the use of non-nutritive sweeteners (NNS) as a "healthy" alternative to sugar is steadily increasing. There is undoubtful relation between high consumption of sugar and sweet beverages and the global epidemic of obesity and metabolic disorders, associated with it. NNS promise the same or even more intense sweet taste with minimum or no calories. However, the number of studies that highlight the potential side effects of NNS and question the health benefits of their use is also growing.

Aim:
This paper aims to summarize the current information about the increasing usage of NNS and the possible link between NNS and the alteration in sweet perception and taste preferences.

Methods:
Articles referenced in PubMed, Google Scholar, and Science Direct have been analyzed, with emphasis on findings from human studies.

Results:
Most of the studies focus on the metabolic effect of the NNS, in terms of weight control. There is a limited number of papers that address the impact of the NNS on the neuroendocrine regulation of eating behavior and the development of addictive-like patterns (sweet cravings).

Conclusion:
More and better-designed studies are warranted to establish the consequences of NNS usage on the alterations of the sweet perception and the development of abnormal appetite response.

Seasonal variations of Glycemia in Patients with Type 1 Diabetes Mellitus

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Abstract

Introduction Blood glucose variability in patients with type 1 diabetes mellitus is reportedly not influenced by the seasons, with level not decreasing or increasing in the summer or warm season and the winter or cold season.

Aims

The aim of the present study is to examine the association between seasonal variation and glucose variability in adult patients with type 1 diabetes mellitus (T1DM).

Methods

We retrospectively reviewed 49 Ambulatory glucose profile for the winter and 24 for the summer, for a period of 28 days. We analyzed by T test for independent samples and Manwitney test.

Results

There was not seasonal variations in glycemic variability during summer and winter. 1) (winter = 61,33 ± 23,38, summer = 55,75 ± 21,51; t = 0,88; p = 0,38) → statistically insignificant difference between seasons in the variable **Time in Range**. 2) (winter = 19,56 ± 11,10, summer = 21,17 ± 12,58; t = 1,39; p = 0,17) → statistically insignificant difference between seasons in the variable **Time above Range**. 3) (U = 318,00; Z = -0,11; p = 0,91) → statistically insignificant difference between seasons in the variable **Glucose Management Indicator**. 4) (winter = 36,83 ± 8,14, summer = 36,02 ± 8,24; t = 0,35; p = 0,73) → statistically insignificant difference between seasons in the variable **Glycemic variability**. 5) (U = 263,00; Z = 1,16; p = 0,25) → statistically insignificant difference between seasons in the variable **Time of active glucose monitoring**. 6) (U = 272,00; Z = 0,99; p = 0,32) → statistically insignificant difference between seasons in the variable **Time below Range**.

Conclusion Patients with type 1 diabetes mellitus should be treated more carefully during the season and control of glycemic variability should be considered in patient education and diabetes management.

Keywords: Diabetes Mellitus Type 1, Glycemic variability, Seasonal variation, Time in Range, Time above Range, Time below Range, Glucose Management Indicator, Time of active glucose monitoring

Biological mechanisms of the effect of body mass reduction on insulin resistance in type 2 diabetes

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Abstract

Obesity is a growing public health problem according to data from the World Health Organization (WHO) obesity worldwide is three times higher than in 1975. Most of the world's population lives in countries where obesity kills more people than malnutrition. The fact that in 2020, 39 million children under the age of 5 are overweight or obese is also alarming. Changes in lifestyle and diet lead to higher intake than energy consumption, which leads to increased energy storage in the form of adipose tissue, which acts as an endocrine organ and secretes a whole series of adipokines that play an important role in the development of insulin resistance, endothelial dysfunction, and accelerated atherosclerosis. The state of insulin resistance is the first step toward type 2 diabetes because it leads to increased production and secretion of insulin to overcome resistance on the target receptors, which leads to an increase in fasting blood glucose and later to permanent hyperglycemia, i.e. the appearance of diabetes. The prevalence of type 2 diabetes has increased alarmingly in recent decades, and it is closely related to the occurrence of microvascular and macrovascular complications that significantly impair the quality of life. The pathophysiological mechanisms that contribute to the development of hyperglycemia and the onset of DM2 are combined into the term ominous octet, which includes the hypothalamus, the islets of Langerhans, the gastrointestinal system, and adipose tissue as the four main culprits for difficult body weight regulation and obesity. Three glands also participate in the octet, namely are thyroid, adrenal and reproductive glands (ovaries and testicles). Skeletal muscles are counted as the eighth member of the octet because their dysfunction is associated with the sarcopenic form of obesity and as such has a poor response to all attempts to rapidly increase physical activity to lose body weight.

Poster session #2: Immunology

Arf1 and Arf6 GTPases are required for intracellular transport of cytomegalovirus virions

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Introduction
Small GTPases of the Arf family are one of the key regulators of endosomal intracellular trafficking and endocytosis. We have previously shown that in cells in which Arf1 or Arf6 protein expression is knocked down by small siRNAs, the establishment of MCMV infection is impaired.

Aims
Because our previous results suggest that the GTPases Arf1 and Arf6 are required at the earliest stages of MCMV infection before the MCMV genome enters the nucleus, this study aimed to determine whether they are required for internalization or intracellular transport of CMV virions.

Materials and methods
Untransfected Balb 3T3 cells or cells transfected with control siRNA or siRNA targeting Arf proteins were infected with recombinant murine cytomegalovirus Δm138-MCMV, and the kinetics of virion internalization was followed by viral m55 envelope protein staining and flow cytometry, whereas intracellular transport was followed by viral m55 envelope protein and viral m25 tegument protein staining and confocal microscopy.

Results
After 30 minutes, approximately 50% of the virions initially bound to the plasma membrane were internalized in untransfected cells, and the kinetics of internalization was not impaired in cells transfected with siRNA targeting Arf proteins. After 60 minutes of internalization and especially after 240 minutes, the viral m25 tegument protein was detected in the nucleus of untransfected or cells transfected with scramble siRNA, whereas in cells transfected with siArf1 or siArf6, m25 was detected predominantly in the cytoplasm.

Conclusion
Our results suggest that Arf1 and Arf6 are not required for internalization of CMV virions but are required for their intracellular transport to the nucleus.

This work was supported in part by the Croatian Science Foundation (grants IP-2020-02-1323 and IP-2019-04-3582) and by the University of Rijeka (grants uniri-biomed-18-180, 18-88, and 18-229).

Correlation of fecal calprotectin with salivary inflammatory markers

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Introduction:
Oral cavity is the initial part of the alimentary canal and saliva is an excellent medium for assessment of local immunity, including inflammatory bowel disease (IBD) due to the abundance of pro-inflammatory markers, especially in dysbiosis. Microbiological and inflammatory changes originating from the gingival niche, might lead to a worsening of IBD via saliva. In turn, it is to be expected that IBD leads to an increase of pro-inflammatory cytokines in saliva. Clinically expressed or asymptomatic intestinal inflammation appears in more than 50% of patients with spondyloarthritis (SpA).

The aim of this study was to analyze correlations between fecal calprotectin and inflammatory markers in saliva depending on C-reactive protein (CRP).

Methods: In this prospective study we enrolled 184 patients with SpA of age from 18 to 80 years, diagnosed by rheumatologist according to the Assessment of Spondyloarthritis International Society non-radiographic axial SpA criteria and signed the Informed Consent Form. Patients completed the Graded Chronic Pain Scale (GCPS) Questionnaire version 2.0 and the Patient Health Questionnaire (PHQ)-15: physical symptoms. Inflammatory markers were analyzed in stool (fecal calprotectin), unstimulated saliva (matrix metalloproteinase-3, IL-12/IL-23 p40, IL-17 and TNF-α), serum (CRP). Fecal calprotectin and CRP were analyzed by routine laboratory methods (automated analyzer) and inflammatory markers in saliva with ELISA.

Results: Fecal calprotectin correlated statistically significantly with matrix metalloproteinase-3 in the saliva of SpA patients (P=0.0005) and physical health symptoms (P=0.028), but not with the degree of chronic pain and the concentration of pro-inflammatory cytokines (IL-12/IL-23 p40, IL-17 and TNF-α). Fecal calprotectin did not correlate with the disease onset and the duration of the disease, but it correlated with the IBD activity measured with CRP.

Conclusion: Specific pro-inflammatory markers in saliva can reflect inflammatory conditions in the intestines.

Acknowledgement: University of Rijeka supported the study by the grants Uni-ri-biomed 18- 110 and Uni-ri-biomed-18-160.

Predicting biomolecular condensate formation of cytomegalovirus-associated proteins using bioinformatics tools

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Introduction: Murine cytomegalovirus (MCMV) extensively rearranges cellular structure and manipulates the regulatory processes of the infected cell in a way that favors viral replication. One of the possible mechanisms of such cellular reorganization and compartmentalization is a formation of biomolecular condensates, a highly variable, dynamic membraneless cellular compartments, which may host hundreds of proteins, organizing molecules that act in a biological spectrum from RNA metabolism to signaling and gene regulation. The organizing principle for these compartments is a process called liquid-liquid phase separation (LLPS), that has recently emerged as a ubiquitous mechanism of cellular organization, underlying many key biological processes.

Aims: The aim of this study was to determine the MCMV virion-associated proteins with a propensity to LLPS, that is, a biomolecular condensate formation, as a possible mechanism by which CMV rearranges the infected cell.

Material and methods: Protein sequences of 58 virion-associated MCMV-encoded proteins were retrieved from the Uniprot database for LLPS bioinformatic prediction analysis by five LLPS- specific prediction methods: PLAAC, PSPer, PScore, catGRANULE and PSPredictor. To identify intrinsically disordered regions (IDRs) in MCMV proteins, we used PONDR platform.

Results: MCMV proteins which assigned a positive LLPS prediction in 3 or more of the 3 tested methods, were considered as LLPS-prone proteins, i.e. those that are likely to form biomolecular condensates. Of the fifteen tegument MCMV proteins analyzed M25, M32 and M69 were found to be LLPS-prone, as well as capsid protein m48.2. Among twenty-seven additional MCMV proteins M80, m18, M57, M44 and M45 had a positive LLPS prediction. None of the viral envelope glycoproteins nor the other viral glycoproteins were shown to be prone to LLPS.

Conclusion: Our results indicate that MCMV proteins M25, M32, M69, m48.2, M80, m18, M57, M44 and M45 have a high propensity to organize biomolecular condensates in the infected cell.

This work was supported by Croatian Science Foundation grants (IP-2020-02-2916 and IP-2019-04-3582) and UNIRI (uniri biomed-18-180, uniri-biomed-18-88, uniri-biomed 18-229).

Sex-related differences in cartilaginous degradation and BMP-4 and Noggin expressions during pristane induced arthritis

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ABSTRACT:

Introduction. Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by the synovial inflammation, cartilage destruction and deformation of joints and adjacent bones.

Aim. This study examines sex differences in cartilaginous degradation and BMP-4 and Noggin expressions during animal model of RA (pristane induced arthritis, PIA).

Materials and Methods. 48 Dark Agouti male and female rats, 7-8 weeks old, were injected with pristane in the base of the tail, which lead to arthritis 9 days after immunization. Animals were sacrificed at onset and peak of disease and in remission phase. Paraffin slides of hind paws were counterstained with Safranin O/Fast green FCF for evaluation of cartilaginous degradation, and separately stained with anti-BMP-4 and anti-Noggin for protein expression evaluation. A semiquantitative histopathological evaluation of cartilaginous degradation was done using Mankin scoring system. Protein expressions (mean gray values) were quantified on 12 regions of interest per field (400x)/3 slides per animal/6 animals per group using Cell F v3.1 software (Olympus). The data were expressed as mean ± SD. Differences between genders and groups were assessed with oneway ANOVA followed by the post hoc Scheffé test. The Pearson correlation (r) was used to determine the association between Mankin scores and BMP-4 and Noggin expressions. The statistical significance is proposed at $p \leq 0.05$.

Results: Females showed slightly higher level of cartilaginous degradation but without the statistical significance. However, expressions of BMP-4 and its inhibitor Noggin were much higher in females (BMP-4 peak: $p<0.0001$ and Noggin onset: $p<0.0001$; peak: $p<0.0001$; remission: $p<0.0001$) with strong positive correlations with Mankin scores (BMP-4 $r=0.70$, $p=0.001$; Noggin $r=0.76$, $p=0.001$).

Conclusions: Our data show that gender differences exist in cartilaginous BMP-4 and Noggin expressions, which could imply differences in mechanisms for the onset and progression of the disease, clinical course severity, and subsequent joint destruction and invalidity between genders.

Key words: rheumatoid arthritis, cartilaginous degradation, bone morphogenetic protein, Noggin, sex differences

This study has been supported by the University of Rijeka (numbers: uniri-biomed-18-41 and uniri-biomed-18-197).

The tumour associated macrophages affect NK cell dysfunction in more malignant breast cancer subtypes

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INTRODUCTION: Breast cancer is the leading cause of death in cancer patients worldwide. Tumour-infiltrating lymphocytes (TILs) and tumour-associated macrophages (TAMs) in breast cancer are associated with a more favourable disease outcome. Natural killer (NK) cells are important cytotoxic cells involved in tumour immune surveillance and directly kill tumour cells. In solid tumours, peripheral NK cells and tumour-infiltrating NK cells show an alteration characterised by decreased cytotoxicity or anergy. The aim of this study was to investigate the phenotype and activation status of TAMs and NK cells to understand the pathological process of different breast cancer subtypes.

MATERIAL AND METHODS: Immunohistology was used to detect the presence and localization of CD68, CD56, and IL -15 in paraffin-embedded normal and tumorous breast tissue sections. The distribution and abundance of NKG2A, NKG2C, NKp46, CD94, CD69, and CD107a in the population of NK cells in peripheral blood mononuclear cell suspensions was investigated by flow cytometry. The mRNA of cytolitic mediators in all subtypes of breast cancer was detected by quantitative RT -qPCR.

RESULTS: The percentage of $CD68^+$, $IL15^+$, and $CD56^+$ cells was significantly higher in triple-negative breast cancer tissue. The frequency of NK cell activating receptors was decreased in breast cancer subtypes, whereas the inhibitory receptor NKG2A was increased. The decreased frequency of $CD69^+/CD107a^+$ NK cell population indicates lower cell function and cytotoxicity. Gene expression of cytolitic mediators at the local level were up regulated in luminal B breast cancer.

CONCLUSION: Modulation of peripheral blood NK cell activity and tumour-infiltrating NK cells by tumour-associated macrophages at the tumour site may be involved in the pathogenesis of more malignant breast cancer. This highlights the importance of the tumour immunosuppressive microenvironment in modulating the activation status of NK cells and providing a suitable target for an effective anti-tumour response.

Acknowledgments: The experiments were funded by grants UNIRI No. 19-171498 and Medical Faculty No. 100.21.0002.

Role of the endosomal phosphatidylinositol-3-phosphate in the cytomegalovirus life cycle

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Shortly after entering the cells, cytomegalovirus begins a massive restructuring of the host membranous compartments to create an intracellular site for virion assembly and egress, called the cytoplasmic virion assembly compartment (cVAC). The formation of the cVAC begins very early in CMV life cycle and it comprises membranes derived from early endosomes (EEs), the endosomal recycling compartment (ERC), and the trans-Golgi network, surrounded by fragmented Golgi cisternae. This membranous organelle reorganization also involves phosphatidylinositol-3-phosphate (PI(3)P)-containing membrane domains, as PI(3)P is the master regulator of the EE pathway. To investigate the importance of the PI(3)P-dependent EE domains in cVAC biogenesis and their role in the consequent progression of the CMV life cycle we used VPS34-IN1, a specific inhibitor of the Vps34 component of class III PI(3)P kinase that causes rapid depletion of PI(3)P at EE membranes, and recombinant PI(3)P-binding domains (2xFYVE, p40PX) that cause saturation of PI(3)P when overexpressed. PI(3)P depletion led to the rapid reorganization of the Rab11a-dependent endosomal pathway, as evidenced by the spread and vacuolization of Rab11a endosomes in the perinuclear region and the disappearance of the ERC. Also, it abolished recycling cargo sorting in this pathway. Despite these alterations and although PI(3)P+ and Vps34+ endosomes build a substantial part of cVAC, the PI(3)P inhibition did not prevent the establishment of infection, progression through the early phase of infection, and membrane reorganization associated with cVAC development, as analyzed by confocal imaging and Western blot analysis. However, the drastically reduced viral growth under PI(3)P depletion and the significant inhibition of late infection events (DNA replication, late protein expression) together with the reduced release of infectious virions suggest that PI(3)P is essential for the entry into the late phase of infection and the final formation of progeny virions.

Supported by the Croatian Science Foundation (IP-2019-4-3582, IP-2020-02-2916, IP-2020-02-1323), and by the University of Rijeka (uniri-biomed-18-88, 18-180, and 18-229).

Interrelationship of monocyte chemoattractant protein-1 and parameters of diastolic dysfunction in patients with psoriatic arthritis

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Introductions: During active disease in psoriatic skin lesions and synovial tissue, activated monocytes represent the major source of proinflammatory mediators, including the chemokine MCP-1 [1]. Patients with PsA show multiple cardiovascular diseases, including heart failure [2].
Aim:. We aimed to evaluate diagnostic accuracy of the proinflammatory monocyte chemotactic protein-1 (MCP-1) in the diagnosis of asymptomatic diastolic dysfunction (DD) in patients with psoriatic arthritis (PsA).
Patients and methods: Sixty-three patients with axial and peripheral PsA without clinical manifestations of heart failure or NYHA (New York Heart Association) class 0 [3], who were treated in rheumatologic office in “Thalassotherapia - Opatija”, Croatia were recruited in study, and signed Informed Consent. Cardiac ultrasound was done according to the current recommendations of the American Society of Echocardiography and the European Association of Cardiovascular Imaging [4,5]. ELISA metod was used for measure MCP-1 concentration.
Results: The serum MCP-1 concentration in patients with PsA did not correlate with the E-wave, whereas it showed a significant positive correlation with the A-wave (P = 0.00001,). The MCP-1 concentration was negatively correlated with the E/A ratio (P = 0.00004). However, it did not show a correlation with the LAVI or TR values. The MCP-1 concentration showed a significant negative correlation with the TDI parameters E' wave (P = 0.0023), whereas the correlation between the MCP-1 concentration and the E/E’ ratio was positive (P = 0.00038). The MCP-1 concentration showed a significant negative correlation with the SA value (P = 0.01) and the SrA waves (P = 0.04), whereas it was not correlated with the SrE wave. MCP-1 concentrations did not correlate with the systolic function parameters EF and GLS.
Conclusion: MCP-1 can serve as a non-invasive, independent prognostic parameter to diagnose isolated asymptomatic DD in patients with PsA and preserved left ventricular EF ≥ 50, as confirmed by echocardiography.

This work was supported by the University of Rijeka, Croatia, Uni-ri-biomed-18-110, and Uni-ri-biomed-18-160

Characterization of lipids in osteoarthritis patients

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Introduction: Osteoarthritis (OA) is a chronic joint disease mainly associated with mechanical damage to the joints. Recent studies point to metabolic factors, especially altered lipid metabolism as an underlying cause, but the relationship between lipids and the pathogenesis of OA is still not fully resolved.

Aim: The aim of this study was to characterize the lipids present in synovial membrane (SM) and to determine the fatty acid (FA) composition of SM, synovial fluid (SF), plasma, and erythrocytes of OA patients.

Methods: The samples were collected from OA patients during knee alloarthroplasty. SM was subjected to enzymatic degradation with collagenase. Lipids were extracted by a modified method of Folch and separated by solid phase extraction into fractions whose FA composition was also determined by the gas chromatography.

Results: In the SM samples, 227 mg of lipids/g SM were determined and 98% of total lipids were neutral lipids, with sterol esters being the predominant fraction (88.8%). Total lipids from SM were predominated by unsaturated FA (UFA) (74.8%, from which oleic and linoleic acid make 46.2% and 19.4%, respectively). Neutral lipids and sterol esters showed a similar FA profile. The phospholipid fraction contained equal proportion of UFAs and saturated FA (SFA). The triglyceride fraction consists of oleic (50%), palmitic (24%), linoleic (13%), and palmitoleic (8%) acid. In SF, the SFA/UFA ratio was 0.66 with almost 60% C18 FA. In the plasma of OA patients, the proportion of UFAs was 56% (26.5% linoleic acid). The erythrocyte membranes had the same proportion of UFAs as the plasma, but a different FA composition (oleic 17.6%, docosahexaenoic and linoleic 10%, arachidonic 8%, erucic acid 6%).

Conclusion: The results show a significant content of pro-inflammatory FA (linoleic, arachidonic, palmitic, and oleic acid) in the samples of OA patients, which may affect immunopathogenesis of OA.

Stromal cells affect response of leukemia cells to differentiation therapy

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Introduction:
Bone marrow stromal (BMS) cells are known to reduce cytarabine (AraC)-mediated toxicity in acute myeloid leukemia (AML) cells in vitro, but the effects on differentiation have not been investigated. Our previous study showed that low-dose AraC (LDAC) induced differentiation of AML cells by activating checkpoint kinase-1 (Chk1) similar to inhibitors of pyrimidine synthesis, 5-aminoimidazole-4-carboxamide ribonucleoside (AICAr) and brequinar.

Aim:
The aim of this study was to investigate the effects of stromal cells on AML differentiation induced by agents that activate Chk1.

Methods:
Human AML cell lines U937 (85011440, ECACC) and THP-1 (ACC16, DSMZ) were seeded 24 hours after MS-5 cells (ACC441, DSMZ), and incubated for 72 hours with AICAr (0.2 mM), brequinar (0.5 μM) or AraC (10, 100, 1000 nM), with or without nucleosides. In some experiments, U937 cells were separated from MS-5 monolayers by a 0.4mM transwell-insert. Viable cells were counted by hemocytometer. Morphology of May-Grünwald-Giemsa-stained cells was analyzed by AxioVert-200 microscope. Cell cycle analysis and the expression of differentiation markers were analyzed by flow cytometry and the protein expression by western blot.

Results:
MS-5 cells reduced cytarabine-mediated toxicity and S-phase arrest caused by pyrimidine synthesis inhibitors, but inhibited the expression of differentiation markers only in U937 cells treated with LDAC, and these effects did not depend on cell-to-cell contact. However, AICAr and high-dose cytarabine induced significant changes in morphology of stromal cells. AICAr induced fibrocyte-like appearance of MS-5 cells that was not prevented by addition of nucleosides. Preliminary data suggest that AICAr activates AMP-kinase (AMPK) in MS-5 cells.

Conclusion:
The presence of stromal cells decreases differentiation of leukemia cells in response to LDAC. AICAr inhibits proliferation and induces fibrocyte-like changes in stromal cells that are independent of pyrimidine synthesis inhibition and probably depends on AMPK. Our results suggest that AICAr effects in stromal cells are exerted via different mechanisms than in AML cells.

Frequency of temporomandibular disorders in patients with axial spondyloarthritis and neck pain

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Background: Neck pain is associated with temporomandibular disorders (TMDs). Patients with spondyloarthritis (SpA) self-report pain or difficulty with neck movements and simultaneous pain and difficulty in opening the mouth at the rheumatological examination. Therefore, patients with SpA with clinically pronounced disturbances of the cervical spine represent a significant group for evaluating TMDs, although we are aware of the fact that patients with SpA and neck pain do not necessarily have TMD. The aim of the study was to investigate the frequency of TMD in patients with SpA and neck pain.

Methods: Eligibility for inclusion in the study was considered in 224 adult patients with axial SpA with regard to neck pain (entry criteria) and the absence of other autoimmune diseases, infections, malignant tumors, pregnancy and decompensated diseases of the heart, lungs, liver, kidneys, central nervous system or endocrine organs (exclusion criteria) during a routine rheumatological examination. The diagnosis of TMD was made at examination by a dental medicine doctor according to the Diagnostic criteria for TMD protocol.

Results: 25% of patients with SpA (n 56) did not have neck pain and did not meet the entry criteria. Additionally, 48 patients were excluded due to exclusion criteria. Six patients were excluded from the research for technical reasons. Therefore 114 patients with SpA and neck pain were allocated to the investigation group. In this group 39.47% (45 patients) did not have TMD, and 60.52% (n 69) have TMD.

Conclusion: Approximately 60% of SpA and neck pain have TMD. Considering their high frequency in patients with SpA, TMD requires the attention of rheumatologists and dentists in daily practice.

Funding: University grant Uni-ri-biomed-18-110 and Uni-ri-biomed-18-160.

The read-out challenge: how to speed up the research and bypass time-consuming protocols?

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INTRODUCTION: When researching the cellular functions that could affect the production of the newly formed virions of murine citomegalovirus, it is essential to determine the amount of the newly released virions and their infectious capacity. Cannonically, the preferred method is plaque assay. It requires primary cell line, with long duration that can hinder the workflow of the overall projects, where the virion production is the read-out of the process itself.

AIM: Our main goal was to test, validate and implement two alternative methods for measuring the production of viral progeny during the MCMV infection to bystep commonly used plaque assay.

METHODS: We infected Balb3T3 fibroblast with either 95.15 MCMV of C3X-GFP MCMV and collected cell culture supernatant in fixed timepoints (0, 24, 48, 72, 96 hpi). For qPCR detection of viral genome we isolated MCMV DNA from the cell culture supernatant and used m86 MCMV gene as readout signal of produced virions. When using C3X-GFP MCMV, we infected freshly cultured Balb3T3 cells with the collected supernatant and used fluorescent signal as readout of the produced infectious units. The readouts from the samples where pure viruses were tested were used to create standard curves.

RESULTS: After extrapolating results from the test samples using standard curves we also tested them in paralell on the standardized plaque assay. Both methods show promising potential and the results derived from them are compatible to the results acquired from the gold-standard method.

CONCLUSION: Both qPCR of m86 MCMV gene isolated from cell culture superantant and detection of fluorescent signal derived from infected cells show promising potential in acquiring precise, quantitative and specific results providing information about number of total viral progeny and infectious progeny, respectively. Implemented together, they will improve and expedite research on the production of infectious viral progeny by bypassing classic and time-consuming plaque assay.

Supported by the Croatian Science Foundation (IP-2019-4-3582, IP-2020-02-2916), and by the University of Rijeka (uniribiomed-18-88, 18-180, and 18-229).

Imbalance of the natural autoantibody network in pregnant women with Hashimoto's thyroiditis

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Objectives: Although Hashimoto's thyroiditis (HT) is classically considered a T cell-mediated disorder, the presence of pathological anti-thyroid autoantibodies indicate that alterations in B cell activation could be present. This can lead to the imbalance of the natural autoantibody network as component of the immunological tolerance. Patients with anti-thyroid antibodies have a 4-times higher risk for miscarriage and 2 times higher risk for preterm birth. The mechanism of infertility of HT is not known. During pregnancy, immunological tolerance to the fetus must be established and the natural autoantibody network may be an important player in the process.

Aims: The purpose of the study was to investigate autoantibodies to heat shock proteins (Hsp) and cytokines in pregnant women with infertility and Hashimoto's thyroiditis (HT) and in healthy pregnant women (HP).

Methods: We collected peripheral blood samples from HT, HP and age-matched healthy controls (HC). Autoantibodies against cytokines were determined from the sera with multiplex bead-based assay (MILLIPLEX Merck), while anti-Hsp60 and anti-Hsp70 autoantibodies were measured with in-house ELISAs.

Results: The elevated anti-Hsp60 IgG and anti-Hsp70 IgG levels detected in HP compared to HC were not present in HT. These differences were observed between HP and HT in the third trimester. Lower levels of anti-cytokine antibodies directed against TNFα, IFNγ and IL-8 were determined in HP compared to HC in the first trimester of pregnancies. However, these alterations were not detectable in HT.

Conclusion: Disturbance of autoantibodies to Hsp and inflammatory cytokines in HT indicates that the changes observed in the natural autoantibody network during physiological pregnancy is missing, which may contribute to an impaired inflammatory processes necessary for a healthy pregnancy.

The research was financed by the Thematic Excellence Program 2021 Health Sub-programme of the Ministry for Innovation and Technology in Hungary, within the framework of the EGA-10 project and the Hungarian National Laboratory on Reproduction of the Pécs of University

Ligation of TLR homologue CD180 activates the PI3K/Akt/mTOR pathway and alters the expression of BAFF-binding receptors of B cells to levels observed in patients with systemic sclerosis

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Objectives: Toll-like receptors (TLRs) can alter the expression of BAFF receptors and also signal via the phosphatidylinositol-3-kinase (PI3K)/Akt /the mammalian target of rapamycin (mTOR) pathways. These pathways are shown to play a key role in B-cell activation in systemic sclerosis (SSc), and B cell activator factor (BAFF) receptors utilize this pathway.

Aims: To investigate the effect of activation via CD180 on the phosphorylation of Akt and S6 (a downstream target of mTOR), and on the expression of BAFF receptors in diffuse cutaneous SSc (dcSSc) patients.

Methods: Peripheral blood mononuclear cells of dcSSc patients and age-matched healthy controls (HC) were isolated using Ficoll gradient centrifugation and the B cells were purified by magnetic bead-based negative selection. To investigate the effect of activation via CD180 the cells were stimulated with anti-human CD180 antibody. The changes in the phosphorylation of Akt and S6 and in the expression of BAFF receptor (BAFF-R) and enhanced transmembrane activator and calcium-modulator and cyclophilin ligand interactor (TACI) were detected by flow cytometry. Autoantibodies to BAFF were measured with Milliplex assay.

Results: Our results show increased phosphorylation of Akt in dcSSc B cells after stimulation via CD180. We found basal downregulated BAFF-R and TACI expression in dcSSc B cells. Additionally, we measured higher serum levels of autoantibodies to BAFF in dcSSc patients. However, anti-CD180 antibody treatment had opposite effects on the expression of BAFF-R and TACI, but only in HC B cells, resulting in similar levels found in SSc without stimulation. Furthermore, the changes were observed in naive B cells.

Conclusion: B-cell activation via CD180 utilizes the PI3K/Akt/mTOR pathway in SSc and may contribute to a pathological shift in BAFF signaling, particularly in naive B cells, which adds to our previous findings proposing that the anti-CD180 antibody therapy suggested in lupus may not be beneficial in SSc.

SARS-CoV-2 can infect cardiac conduction system – A Case Report

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Acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been found as a highly pleiotropic virus causing COVID-19, the systemic disease most strikingly affecting the respiratory system and blood vessels, but the function of almost all body tissues can be impaired in infected persons. Mounting evidence shows that distinct types of new-onset cardiac arrhythmias have emerged as a common finding in COVID-19 patients, posing additional mortality risk in older-aged and critically ill patients. However, underlying mechanisms and cardio pathological substrates of COVID-related arrhythmias have not been clarified yet. Here we report immunohistochemical detection of SARS-CoV-2 spike and nucleocapsid antigens, as well as the finding of viral genes in the cardiac AV-node of the patient who deceased from COVID-19 and had had an abnormal heart rhythm prior to death, pointing to the direct viral infection of the cardiac conduction system as a possible arrhythmogenic mechanism of COVID-19-elicited arrhythmias.

Comparison of immune defense mechanisms that are first triggered against different implant materials

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Aim
In regenerative medicine, biomaterial-focused immunomodulation strategies are being developed for the prevention of inflammation.
In our study, we analyzed the activation of macrophages and sub groups (M1, M2a, M2b, M2c) which are the first triggered cells in the innate immune system mechanism by implanting Stainless Steel (SS), Titanium 500 (Ti500), Coral clean (Cc) into the spinal region in male *Wistar albino rats* in order to control the specific inflammation events that may occur after implantation.
Material and Method
28 male Wistar *albino* rats were divided into four groups (7 rats in each group) as follows: C, SS, Ti 500, Cc. Peripheral blood was collected on the 1st, 3rd and 7th days. CCL3, CCL4, CD163, CD206, CCL1, CD150 parameters were analyzed using ELISA method.
Results
When SS and Ti 500 were compared, a decrease in the CCL1 and CD163 on the 1st day. On the 3rd and 7th days, there was a highly significant increase in the CCL3, and a statistically significant increase in the CCL1.
When C and C_{clean} were compared, we found that there was a significant increase in the CCL3 and CCL4 .
On the day 3, we observed a highly significant increase in the CCL1, CCL3, CD206, and CD163. On day 7, there was a less significant increase in the CD150, CCL1, and CD206 .
When SS and Ti 500 and Cc were compared, there was a highly significant increase in the CCL3 and CCL4 on the 1st day. On the 3rd day, CD163 and CD206 reached the highest level. On the 7th day, the CD150, CCL1, and CD206 increased significantly.
Conlusion
We believe that a controllably low level of immune responses that may develop in Cclean implanted patients will guide the prevention of inflammatory reactions and development of immunological therapeutic methods.

Note; This work was a part of the project School of Life Sciences Translational Biomaterials and Medicine Group at University of Technology, Sydney

Effect of suplementation with yeast fermentation product on hematological parameters in growing to finishing pigs

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Fermented feed products have been investigated to reduce the use of antibiotic growth promoters and decrease feed price in swine production. Fermentation product contains amino acids, nucleotides, minerals, and other bioactive compounds which have an enzymatic effect on plant cell wall non-starch polysaccharides, resulting in increased availability of cytoplasm plant cell protein, starch and fat. The aim of this study was to determine influence of fermentation product of selected Aspergillus spp. strains on hematological parameters of swine. A total of 80 growing-finishing crossbreed pigs were used for the feeding trial. Pigs were randomly assigned to one of two dietary treatments with 40 pigs per treatment in two pens. Control group (initial body weight; BW 32.09 kg) was fed a corn-soybean meal. The diet for experimental group (initial BW 31.13 kg) containing 0.5% dry yeast fermentation product (YFP) and soybean meal was reduced by 2%. Blood was collected at slaughterhouse and total number of blood cells, hemoglobin concentration and hematocrit values were determined by hematology analyzer using veterinary software. Differential leukocyte counts were determined from blood smears stained by the Pappenheim method. All parameters determined in present study were within reference range. Mean corpuscular volume was significantly higher and mean corpuscular haemoglobin concentration was significantly lower in group fed diet supplemented with YFP. Total number of erythrocytes, leucocytes, and platelets as well as differential blood count were not affected with in YFP diet supplementation. Final body weight of control group was 121.05 kg and was similar to body weight of swine fed diet supplemented with YFP and 2% less soybean meal (121.45 kg). Based on results it can be concluded that supplementation with YFP did not alter immune function but it did enable decrease feed price due to reduction in soybean meal ratio.

Keywords: swine, yeast functional product, hematological parameters

Gp96 Expression in Mice Exposed to Psychosocial Stress: Spotlight on Immunological Changes

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Glycoprotein 96 (Gp96) is an endoplasmic reticulum chaperone, which plays essential roles in the proper folding, assembly and glycosylation of membrane and secreted proteins and participates in elimination of unfolded, mutant or defective proteins. Besides, as a carrier of immunogenic peptides it has the ability to modulate the innate and adaptive immune response and stimulate APC cytokine expression and dendritic cell maturation. Here we analyzed some of these aspects in mice subjected to chronic psychosocial stress. The data have shown that closure of group of mice in small metabolic chamber for 1 h/day during 10 days enhances the expression of gp96 in the thymus (cortical thymocytes and epithelial cells), in the liver (hepatocytes and several lymhoid cells) as well as in the spleen (paracortical areas and red pulp). Concurrently, thymic cortex of stressed animals showed the increased number of cells co-expressing Dolichos Biflorus Agglutinin (referred to as a unique marker for NK cell progenitors; pro-NK) and gp96, while lymphoid cells isolated from the livers and spleens of these animals exhibited significantly higher cytotoxic activity against NKT- or NK- sensitive targets.
The data point that during psychosocial stress gp96, in combination with neuroendocrine signaling, may be involved in the maintenance of self-tolerance and in activation of intrahepatic immune cells which, depending on stress severity, might have either protective or harmful effects on the host

Dynamic Features of Herd Immunity: Similarities in Age-Specific Anti-Measles Seroprevalence Data between Two Countries of Different Epidemiological History

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INTRODUCTION. Measles immunization gap(s), aggravated by the current geopolitical conflicts, raise the concern of outbreaks. Croatia and Hungary are proximal to measles-endemic countries. Immunization activities compromised by the COVID-19 surge is an obvious ground for concern.

AIM. We wanted to compare age-stratified seroprevalence results in the cross-border region.

MATERIALS AND METHODS. Anti-MMR specific antibody levels (IgG) of 950 anonymous Croatian samples were compared with previous Hungarian results (n>3500 samples), and former Croatian seroprevalence data (n=1205). Seropositivity ratios were determined using our self-developed anti-MMR indirect ELISA and commercial kits (Euroimmun GmBH).

RESULTS. Seropositivity ratios of the Croatian samples were largely overlapping with earlier published Hungarian data (the lowest seropositivity was found in individuals of age 34–43, with 78% of seropositivity), and are in agreement with earlier published Croatian data. Despite dissimilar epidemiological histories, analogies in age-specific measles susceptibility have been discovered. We assume that besides potential coincidental epidemiological events, as well as inherent biological dynamics of vaccination-based humoral protection might have played a pivotal role.

CONCLUSION. Our findings may also serve as a lesson regarding the current anti-COVID-19 vaccination strategy.

This research was funded by the HUHR-1901-3.1.1-0032, CABCOS3 (Hungary-Croatia Cross-border Co-operation INTERREG-IPA, EU grant and by the Thematic Excellence Program 2021 Health Sub-programme of the Ministry for Innovation and Technology in Hungary, within the framework of the EGA-10 project of the University of Pécs.

Early Endosomal Sorting Nexins Are Required for Murine Cytomegalovirus Envelopment and Egress

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The late stage of murine cytomegalovirus (MCMV) infection is characterized by viral DNA replication and the production of the structural components that assemble into new virions. The final step in virion maturation is secondary envelopment, in which the cell organelle membranes envelop cytoplasmic capsids. The identity and origin of these specialized secondary envelopment compartments are unclear. The enlargement and tubulation of early endosomes (EE) compartments within AC and the retention of cargo proteins within EE-derived compartments suggest a reconfiguration of membrane flux and cargo sorting mechanisms required for efficient envelopment. Therefore, it is important to investigate the proteins that potentially influence tegument and envelope protein sorting, such as EE sorting nexins (SNXs).

We investigated the impact of SNX1, SNX2, SNX3, SNX4, and SNX27 on MCMV infection and virion egress. SNX suppression was performed by siRNA silencing, and the efficiency of suppression was determined by western blot and qPCR. The effect of SNX suppression on virus entry and establishment of infection was monitored by flow cytometry using C3X-GFP MCMV. The progression of the late phase of infection was monitored with semi-quantitative analysis of viral DNA with EdU incorporation and small capsid protein (SCP) expression using S-mCherrry-SCP MCMV. Intracellular virion production and egress of infectious virions were determined by plaque assay. MCMV infection proceeded in all SNX-suppressed cells as in the control with a similar amount of assembled infectious virions in the cytosol. However, the release of extracellular virion release was reduced 5-10-fold after the suppression of SNX3, SNX4, and SNX27, whereas suppression of SNX1 and SNX2 had no effect. These results suggest that early endosomal SNXs play an essential role in secondary envelopment and egress of beta-herpesviruses.

This work was supported in part by Croatian Science Foundation (IP-2019-04-3582 [PL], IP-2020-02-1323 [GBZ], IP-2020-02-2916 [HML]), and by the University of Rijeka (uniri-biomed-18-88 [PL], uniri-biomed-18-229 [GBZ] and uniri biomed-18-180 [HML]).

Deubiquitinilation function of murine cytomegalovirus M48 protein influence remodeling of WASH1 endosomal network in the early phase of infection

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Murine cytomegalovirus (MCMV) generates the viral assembly compartment (AC) for production of new virions after intensive remodeling of intracellular membrane compartments. Although the mechanism of this process is poorly understood, it is known that the function of host cell proteins involved in the regulation of membrane trafficking is affected. Some of these proteins, as WASH1, that is involved in formation of recycling tubules from early endosomes (EE), require proper ubiquitinilation as a regulatory mechanism.
Thea aim of our study was to investigate the role of deubiquitinilation (DUB) function of M48 MCMV on intracellular expression of WASH1 protein and its network, and their role in generation of MCMV AC.
For this purpose, C3X M48^{C23S} MCMV construct with a single mutation in M48 DUB domain was produced, as previously described (Hilterbrand et al, 2017). We showed that viral growth in infected fibroblasts was not affected by inactivation of M48 DUB activity, and viral titar was similar between C3X M48^{C23S} MCMV and C3X wt MCMV, both in cell lysates and cell culture supernatants. Furthermore, the kinetics of total expression of WASH1 and host proteins that are part of EE recycling network, as Vps35, SNX1/2, and MICAL-L1, did not change between C3X M48^{C23S} MCMV and C3X wt MCMV infected fibroblasts. However, the extent of their accumulation in the pericentriolar region of the AC was decreased in fibroblasts infected with C3X M48^{C23S} MCMV. Interestingly, the inhibition of transferrin recycling normally observed in wt MCMV infected cells was relieved after infection with MCMV M48^{C23S} construct, while the accumulation of PLEKHB2 (marker of AC) in the pericentriolar region of infected cell was retarded.
We conclude that DUB M48 function in MCMV induced host cell remodeling cannot be excluded, although further experiments are necessary to clarify the mechanism.

This work was supported by Croatian Science Foundation grants (IP-2020-02-2916, and IP-2019-04-3582), and by the UNIRI (uniri biomed-18-180, uniri-biomed-18-88, uniri-biomed 18-229).

Comparison of different animal models of silicosis through histological and biochemical analyses

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Introduction: Silicosis is a non-infectious lung disease caused by inhaling silica particles where initial pneumonia often progresses into fibrosis. Our study aimed to compare several inflammatory and early fibrotic changes in two animal models of silicosis.
Methods: The study included adult male Wistar rats (n=64) which were divided into 8 subsets. Models of silicosis were prepared by orotracheal instillation of silica suspension with different silica particle sizes at a dose of 50 mg silica/ml/kg of animal weight (model no. 1, n=32), or with uniform silica particle size (1.5 μm) at a dose of 50 mg silica in 1 ml saline/animal (model no. 2, n=32). Control groups were given sterile saline in the same volume as the model groups. To evaluate dynamics of changes, animals were killed 14 or 28 days after administration of silica/saline. At the end of experiment, total and differential numbers of leukocytes were determined in the blood. In the lung tissue, emphysema, atelectasis, and inflammatory cells accumulation in the interstitium, and presence of collagen in the walls of bronchioles and vessels were analysed. Concentrations of inflammation markers in the lung tissue were measured using ELISA method.
Results: In model no. 2, interleukin (IL)-6 significantly increased and chemokine CXCL1 tended to increase 14 days after silica administration while tumour necrosis factor (TNF)α significantly elevated and interferon (IFN)γ tended to increase 28 days after silica administration compared to respective control groups. In model no. 2, blood neutrophil count significantly raised 14 days after silica administration and more marked histopathological lung alterations and collagen reconstruction of bronchioles and vessels were demonstrated.
Conclusion: The comparison of two different animal models of silicosis showed that model no. 2 is more pronounced and stable, and therefore more suitable for continuation of our experiments.
The work was supported by projects VEGA 1/0131/22 and APVV-15-0075.

Pulmonary surfactant in COVID-19 era

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Pulmonary surfactant has an important role in host defense against pathogens, including the novel coronavirus. According to the latest hypothesis, the surfactant may penetrate the virus envelope, split it, and let the content be released into the environment. In the lower respiratory system, the virus uses the protein receptor ACE2 (angiotensin-converting enzyme 2) in conjunction with the serine protease TMPRSS2, expressed primarily by alveolar type II cells (ATII), to enter the cell. ATII cells, as the main source of surfactant, are thus target for SARS-CoV-2. Their infection and subsequent damage can have serious consequences, resulting also from reducing the amount of surfactant, or its inactivation. The damage leads to disruption of the alveolar-capillary barrier, pulmonary edema, inflammation, impaired gas exchange, deterioration of lung mechanics and reduced oxygenation. Symptoms resemble acute respiratory distress syndrome (ARDS) of other etiology. ATII cells infected with the virus lose their ability to repair and participate in fibroproliferation. Secretion of growth factors and pro-inflammatory molecules may result in pulmonary fibrosis as part of interstitial lung disease.
The damage to ATII cells and damage to surfactant function are key events in the pathogenesis of the respiratory form of COVID-19. This knowledge provides a rational basis for the use of exogenous surfactant in patients with SARS-CoV-2-induced ARDS. Administration of surfactant protects the lungs, due to its anti-inflammatory properties. The final effect of surfactant treatment in patients with COVID-19 depends on several variables, including the route and timing of administration, dosage and type of preparation, as well as mechanical ventilation.
Pulmonary surfactant as a target for SARS-CoV-2 infection becomes a mean for suppressing the consequences of this infection.

Supported by VEGA 1/0055/19, VEGA 1/0004/21 and APVV-17-0250.

Serum concentrations of selected minerals in Lika pramenka lambs fed by feed suplemeted with white button mushroom *(Agaricus bisporus)*

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Summary
The adequate amounts of essential minerals are critical to maximizing the productivity and health of livestock. It is known that mineral deficiencies can lead to impaired growth and reproduction of animals and increases in disease, due to impaired immune function, and serious deficiencies may even cause death. Mushrooms are an excellent source of many nutrients including minerals, e.g. phosphorus, potassium and calcium but have a low sodium content. The objective of this study was to examine whether the intake of the dry and raw fruiting bodies of white button mushrooms does influence minerals serum concentration in lambs of the Lika Pramenka sheep breed. For this study, 60 clinically healthy, three-month-old lambs (32 females and 28 males), were randomly divided into four groups comprised of 15 lambs each. The first group was a control group in which lambs were fed for six weeks with a standard diet, the second group of lambs was fed for six weeks with commercial lamb food supplemented with 15% of raw *Agaricus bisporus* the third group was fed for six weeks with commercial lamb food supplemented with 1.5% dried *Agaricus bisporus*, and the forth group was kept extensively on pasture. Blood samples were taken once, and in the obtained sera calcium, potassium, chloride, sodium and phosphorus concentrations were defeminated by using standard commercial kits Beckman Coulter Biomedical Ltd., O'Callaghan's Mills, Co. Clare, Ireland on an automatic Beckman Coulter AU 680 analyzer Beckman Coulter Biomedical Ltd, Ireland. Results showed that feed supplemented with *Agaricus bisporus* mushroom did not have a statistically significant effect on the selected blood minerals concentrations in lambs but also did not have any adverse effect on it. This research was funded by the Croatian Science Foundation with the project "Innovative functional lamb products" (IP-2016-06-3685).
Key words: Agaricus bisporus, lambs, calcium, phosphorus, potassium, sodium, chloride

Insight into free-ranging gray wolves' physiological status from fatty acid composition perspective of different tissues

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Composition of fatty acids (FAs) of different tissues can provide insight into feeding patterns, physiological status, and health assessment, which is especially important for free-ranging top predators. In that regard, this study was conducted on 18 individuals (F, N=12; M, N=6) that mostly died as road or railroad mortalities during 2015-2018. Low sample number can be explained by the fact that gray wolf (*Canis lupus*, GW) in Croatia is strictly protected species and since 2013 there have been no legal interventions in the population. Samples from hind leg muscles (HLM), subcutaneous (SAT) and perirenal (PAT) adipose tissues were collected from different GW habitats throughout Croatia. After homogenization and lipid extraction, the FA composition of tissues was determined by gas chromatography. Saturated FA were the most represented in HLM lipids, followed by polyunsaturated FA and monounsaturated FA (SFA:PUFA:MUFA; 4.1:3.0:2.9 in females vs. 4.1:3.6:2.3 in males). In SAT and PAT occurrences were as follows, MUFA:SFA:PUFA=4.7:4.1:1.3, females vs. 4.5:4.5:1.0, males and 4.8:3.9:1.3, females vs. 4.1:4.8:1.1, males. Younger (<2 year) and heavier females (>25 kg) had significantly higher percentages of C18:2n-6 and C15:0 compared to younger (<2 year) and heavier (>32 kg) males in HLM. Lighter females (<25 kg) had significantly higher percentage of C18:2n-6 compared to lighter males (<32 kg) in HLM. In this research high representation of C18:1n-9 in SAT and presence of *trans* FA, ie. C16:1*t* in tissues, agrees with feeding habits comprising ruminants. Results agree with our previous studies on GW stomach content and FA composition of tissues. Fatty acids such as C15:0, C18:2n-6 have a positive association with an individual's health and are required for normal physiological functions linked to membrane integrity and regulatory cell signals. Larger samples size is needed to provide better health assessments and more precise determination on feed selection supporting the European idea of coexistence between humans and large carnivores.

Keywords: fatty acids, gray wolf, feeding habits, health status





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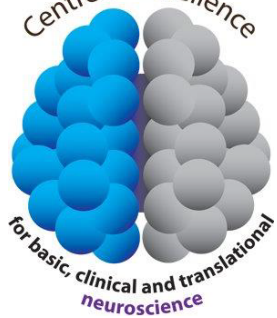

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This publication is co-financed by the Scientific Centre of Excellence for Basic, Clinical and Translational Neuroscience (project “Experimental and clinical research of hypoxic-ischemic damage in perinatal and adult brain”; GA KK01.1.1.01.0007 funded by the European Union through the European Regional Development Fund)