HRVATSKO DRUŠTVO FIZIOLOGA

HDF CPS

CROATIAN PHYSIOLOGICAL SOCIETY

14th Annual Meeting of Croatian Physiological Society with International Participation

Program and Abstract book

online (MS Teams) September 25th – 26th, 2020

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Friday, September 25th 2020

10,15-10,30: Opening and welcome talk

Ines Mrakovčić-Šutić, president of CPS

INVITED LECTURES:

10,30-10,50: Hana Mahmutefendić Lučin: **Dynamin-2 plays a role in assimilation of endocytic recycling compartment into viral assembly compartment in murine cytomegalovirus (MCMV) infection**

10,55-11,15: Andrej Ovca: **A comprehensive historical overview of public and environmental health as a means of promotion**

11,20-11,40: Helena Lenasi**: Microvascular reactivity after oral glucose load: an interplay between high glucose and high insulin concentration**

SELECTED ORAL PRESENTATIONS:

11,45-11,55: Jasna Aladrović**: Ceruloplasmin and haptoglobin in bovine follicular fluid**

12,00-12,10: Anemari Horvat**: Cytoplasmic ALS/FTD-linked TDP-43 inclusions dysregulate astroglial noradrenergic signalling and metabolism**

12,15-12,25: Ana Shek Vugrovečki: **Effect of *Agaricus bisporus* supplementation in a feed of Lika pramenka lambs on vitamin A and D serum concentrations**

12,30-12,40: Jerneja Filipič: **Caffeine for apnoea treatment does not affect the heart rate variability in newborns**

12,45-12,55: Martina Ratko: **The role of uroguanylin's GC-C independent signalling pathway in the development of ischemic stroke**

13,00-15,00: Refreshments

INVITED LECTURES:

15,00-15,20: Ines Drenjančević: **The effects of n-3 PUFAs enriched chicken eggs' consumption on biochemical markers of microvascular function in human subjects**

SELECTED ORAL PRESENTATIONS:

15,25-15,35: Ana Stupin: **Vitamins C and E supplementation during 7-day high-salt intake prevents impairment of microvascular endothelium-dependent vasodilation in young healthy individuals**

15,40-15,50: Hrvoje Lalić: **5-aminoimidazole-4-carboxamide ribonucleoside induces differentiation in blasts isolated from patients suffering from non-APL acute myeloid leukemia**

15,55-16,05: Marina Marcelić: **Endosomal PI3P domains are not required for the development of the cytomegalovirus pre-assembly compartment but are essential for efficient virion assembly**

16,10-16,20: Željka Minić: **TRPV1-Dependent Mechanisms are Involved in Modulating Viscerosensory Sympathetic Reflexes in the Spinalized Rat**

16,25-16,35: Barbara Tomić: **Nucleotide metabolism and activation of Chk1 in monocytic differentiation**

16,40-16,50: Nikolina Kolobarić: **Positive effect of *n*-3 pufas consumation on serum levels of anti-inflammatory cytokines in young sedentary participants**

16,55-17,05: Ingrid Šutić Udović: **Quantification of the mouse cytomegalovirus by novel fluorescence signal-based approach**

POSTER PRESENTATIONS (SHORT TALKS):

17,15-17,20: Petar Šušnjara, Zrinka Mihaljević, Anita Matić, Nikolina Kolobarić, Iva Šafer, Ines Drenjančević: **The effect of n-3 polyunsaturated fatty acids (n-3 PUFAs) chicken eggs consumption on antioxidative enzymes gene exrpression in mononuclear blood cells in young healthy individuals**

17,25-17,30: Erna Davidović Cvetko, Matea Trogrlić, Vanesa Sesar: **Bioelectrical impendance phase angle change in soccer players during spring preparatory period**

17,30-18,00: CROATIAN PHYSIOLOGICAL SOCIETY: General assembly

Saturday, September 26th 2020

INVITED LECTURES:

10,00-10,20: Ines Mrakovčić-Šutić: **Inflammatory cytokines and immune system response to weight reduction in patients with obesity after anti-inflammatory diet**

SELECTED ORAL PRESENTATIONS:

10,25-10,35: Zrinka Mihaljević: **Ion channels modulation after acute and intermittent hyperbaric oxygen exposure – changes in vascular relaxation mechanism**

10,40-10,50: Mirela Šunda: **Physical activity of teachers**

10,55-11,05: Martina Mihalj: **Leukocytes' Surface Expression of Integrins is affected by Short-Term High-NaCl Dietary Intake in Both Healthy Humans and Sprague-Dawley Rats: A Comparative Study**

11,10-11,20: Tamara Gulić: **Peripheral blood NK Cells from Breast Cancer Patients are tumour-primed**

11,25-11,35: Hrvoje Omrčen: **Hippocampal Neurogenesis and Expression of BMP-4 and Noggin in Pristane Induced Rheumatoid Arthritis**

11,40-11,50: Gordana Laškarin: **The expression of Tumor Associated Glycoprotein 72 in endometrioid endometrial cancer with mucinous component**

POSTER PRESENTATIONS (SHORT TALKS):

12,00-12,05: L. Pađen, I. Pipal, J. Aladrović, B. Beer Ljubić, N. Prvanović Babić, Z. Stojević**: Energy metabolism during the early postpartum period in cows and calves kept in a beef suckler system**

12,10-12,15: Tina Zavidić, Marija Rogoznica, Tatjana Kehler, Božena Ćurko Cofek, Ines Diminić Lisica, Viktor Peršić, Daniel Rukavina, Gordana Laškarin: **Granulysin expression in peripheral blood lymphocyte in women with knee osteoarthritis correlated with pain and restricted daily physical activities**

12,20-12,25: M. Vučenović, N. Salać, V. Mićović, I. Mrakovčić-Šutić, I. Brčić- Karačonji, I. Šutić, A. Bulog: **Analysis of s-phenylmercapturic acid and s-benzylmercapturic acid in human urine by liquid chromatography coupled with tandem mass spectrometry**

12,30-12,35: Sara Bilić Knežević, Damir Grebić, Manuela Alvirović, Tamara Gulić: **The role of heat shock 70 (HSP70) on cancer cell invasion-related activities**

12,40-12,45: Igor Štimac, Natalia Jug Vučko, Gordana Blagojević Zagorac, Zsolt Ruzsics, Pero Lučin, Hana Mahmutefendić Lučin: **Remodelling of the endosomal recycling compartment interfaces with early endosomes and *trans* golgi network during murine cytomegalovirus (mcmv) infection is dynamin-2 dependent**

12,50-13,00: Clossing remarks

POSTERS which will be included in the (digital) abstract book (due to the limited setup of the online meeting this year, only selected authors will present their work)

1. Natalia Jug Vučko, Ljerka Karleuša, Hana Mahmutefendić Lučin, Gordana Blagojević Zagorac, Marina Marcelić, Igor Štimac, Pero Lučin: **Golgi apparatus redistribution and cVAC formation in the early phase of MCMV infection**
2. Ivana Šutić, Aleksandar Bulog, Ingrid Šutić Udović: **Adverse effect of gaseous and particulate emissions on the concentration of BTEX in people with environmental diseases**
3. Valentino Pavišić, Hana Mahmutefendić Lučin, Tamara Gulić, Ljerka Karleuša, Natalia Jug Vučko,, Pero Lučin, Gordana Blagojević Zagorac: **Role of Arf GTPases in MCMV transport to the cell nucleus**
4. Ingrid Šutić Udović, Ludvig Letica, Zdrinko Brekalo, Ivona Letica, Aleksandar Bulog, Iva Mikulić: **Expression profile of MMP 2 and 9 in patients with colorectal cancer**

5. Vedrana Krušić, Maša Biberić, Siniša Zrna, Lara Valenčić, Mladenka Malenica, Lada Kalagac Fabris, Aleksandar Šuput, Kristina Grabušić: **Size-exclusion chromatography based separation of extracellular vesicles from cerebrospinal fluid of severe traumatic brain injury patients**
6. Ivana Kotri Mihajić, Karlo Tudor, Aleksandar Bulog, Ivana Šutić: **Comparation of enzyme expression of matrix metalloproteinase 2 and 9 (MMP 2 and 9) after total hip joint endoprosthesis by minimally invasive and classical approach**
7. Miroslav Župčić, Sandra Graf Župčić, Tatjana Šimurina, Viktor Đuzel, Igor Grubješić, Dinko Tonković, Livija Šakić, Ivana Šutić, Višnja Ivančan, Stjepan Barišin, Ingrid Šutić Udović: **The influence of different anaesthetic approaches on the immune response**
8. Mirela Vučković, Lana Ružić, Anton Tudor, Ivana Šutić: **Quality of life in patients after 8 years of total hip arthroplasty with minimally invasive or classic approach**
9. Karleuša Lj, Lukanović Jurić S, Jug Vučko N, Pavišić V, Mahmutefendić Lučin H, Blagojević Zagorac G, Lisnić B, Lučin P: **Cytokinesis failure in MCMV infection**
10. Ingrid Šutić Udović, Miljenko Kovačević, Zdrinko Brekalo: **Assessment of the role of heat protein 70 in patients with atherosclerosis of carotid arteries**
11. Zrinka Mihaljević, Anita Matić, Ana Stupin, Martina Mihalj, Kristina Selthofer-Relatić, Aleksandar Kibel, Željka Breškić Ćurić, Anamarija Lukinac, Nikolina Kolobarić, Petar Šušnjara, Ines Drenjančević: **Effect of n-3 polyunsaturated fatty acid-enriched chicken eggs consumption in patients with known atherosclerotic coronary artery disease on pro-inflammatory cytokines**
12. Silvana Petreić Majnarić, Neda Smiljan Severinski, Ingrid Šutić Udović, Sandro Gržančić: **Immunological changes in follicular fluid of women with thyroid autoimmuniy**

ABSTRACTS

INVITED LECTURE -1

Dynamin-2 plays a role in assimilation of endocytic recycling compartment into viral assembly compartment in murine cytomegalovirus (mcmv) infection

Hana Mahmutefendić Lučin[1,2], Igor Štimac [1], Natalia Jug Vučko[1], Gordana Blagojević Zagorac[1,2] Pero Lučin[1,2]

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Murine cytomegalovirus (MCMV) generates assembly compartment (AC) in the early phase of infection at the Early endosome/Endosomal recycling compartment/*Trans*-Golgi interface (EE/ERC/*trans*GA). The formation of AC is inhibited after treatment with dynasore (dynamin-2 inhibitor). In healthy cells, dynamin-2 plays important roles in endocytosis, vesicular budding from Golgi, and actin bundling. However, its role in endosomal recycling is still unresolved.
Therefore, we were interested to better investigate the role of dynamin-2 on formation of AC in MCMV infection.
In order to determine the site where MCMV uses dynamin-2 for reorganization of the intracellular system, dynasore has been added 4hpi, and selected markers have been visualized by confocal microscopy during early phase of infection (6 and 16hpi). The markers has been divided in several groups: (1) EE (Rab5 and EEA1); (2) EE-ERC intermediate compartment (Rab10, Rab8A); (3) ERC (Rab11, Arf6); (4) ERC-TGN intermediate compartment (evectin-2); (5) *cis*Golgi (GM130); (6) *trans*Golgi network – TGN (TGN38, Golgin97, Rab6). The influence of the inhibitor has been dominantly visible on markers of EE-ERC interface, ERC and EE-TGN interface. Importantly, dynasore has also prevented Golgi disintegration - the first hallmark that precedes preAC formation.
Considering that MCMV inhibits endosomal recycling of surface glycoproteins, we tested the influence of dynasore treatment on recycling of transferrin receptor (TfR) in noninfected and MCMV infected cells. Interestingly, dynasore have diminished the inhibitory effect of MCMV on TfR recycling, suggesting that dynamin-2 is important for TfR reaching the AC, where recycling kinetics slows down.
Importantly, the presence of dynasore only during early (4-14hpi), but not in the late phase of MCMV infection has prevented formation of AC, but also of late phase proteins
Therefore, we can conclude that dynamin-2 is important transport of recyling molecules from EE to ERC. In cytomegalovirus infection, ERC membranes form the part of AC from where recycling processes are inhibited.

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

INVITED LECTURE -2

A comprehensive historical overview of public and environmental health as a means of promotion

Andrej OVCA[1,2], Sara TAJNIKAR[2], Andreja KUKEC[3], Mateja DOVJAK[4] , Aleš KRULEC[2]

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Introduction/Aim: With the aim of providing younger generations of graduates and the wider professional and general public a comprehensive review of public and environmental health development, the Chamber of Public and Environmental Health Professionals of Slovenia / Institute of Public and Environmental Health have developed an online platform entitled "Historia Sanitaria". **Methods:** Two approaches for data collection were applied. First, we collected available online data. During the selection of online sources, we (in addition to the accessibility of the data) considered the relevance for public and environmental health area in general. Additionally, relevance for the professionals in Slovenia, who integrate public health and environmental health area by their professional work, was also considered. Second, we contacted members of EFEH/IFEH and EUPHA to provide us with contributions relevant for their countries. **Results:** After data collection and preparation of contributions, an online platform available at https://www.wiki.sanitarc.si/ was released. Important historical / current events and achievements of individuals are chronologically arranged across different time periods. Currently, there are 217 contributions related to nine different countries in a timeline from the year 18 AD to 2017. Visitors can browse through the published contributions, either considering timeline or country of origin. The developed platform is an ongoing project, which is expanding with new contributions whereas also already published are continuously updated and upgraded. **Conclusion:** We would like to invite all to participate and to send us part of their history or current important achievements in the field of public and/or environmental health.

Key words: *Public health, Environmental health, Historia sanitaria*

INVITED LECTURE -3

Microvascular reactivity after oral glucose load: an interplay between high glucose and high insulin concentration

Helena Lenasi, Jernej Šorli

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Background: Acute hyperglycemia has been shown to impair endothelial function; on the other hand, insulin interferes with the release of endothelial autacoids, thus modulating vascular reactivity. Moreover, glucose and insulin are both suggested to affect the sympathetic nervous system. How these effects integrate at the level of microcirculation *in situ* has not been elucidated. To this end, we aimed at establishing the kinetics of plasma glucose and insulin concentration after a standard oral glucose tolerance test (OGTT) and evaluating the effect of acute hyperglycemia on cutaneous microvascular reactivity.
Methods: We performed a 3-min occlusion of the brachial artery and traced the parameters of postocclusive reactive hyperaemia (PORH) using laser Doppler fluxmetry in 20 healthy volunteers, before, and 40 minutes after OGTT, or water load as a control, respectively. Simultaneously, ECG, and arterial blood pressure were recorded and heart rate variability (HRV) assessed by spectral analysis. In another set of experiments, blood samples were drawn from the cubital vein every 10-min after OGTT to determine glucose and insulin kinetics.
Results: Glucose and insulin displayed similar kinetics after OGTT, with a 10-min time delay between the peak of glucose and insulin. We found a lesser increase in diastolic pressure ($p = 0.045$, Wilcoxon test), low frequency power ($p = 0.022$) and low-to-high frequency power ratio ($p = 0.022$) of HRV, and a borderline significance of PORH ($p = 0.067$) following OGTT compared to control. A negative correlation between the blood glucose concentration after OGTT and hyperaemic response was established ($p = 0.004$; linear regression).
Conclusions: In healthy subjects, OGTT might affect microvascular reactivity through modulation of the sympathetic activity, rather than through endothelium-dependent vasodilation. We suggest that insulin sensitivity, indirectly assessed by glucose concentration after OGTT should be taken into consideration when interpreting the effects of acute hyperglycemia on vascular function.

INVITED LECTURE -4

The effects of n-3 PUFAs enriched chicken eggs' consumption on biochemical markers of microvascular function in human subjects

Ines Drenjančević[1*], Ana Stupin[1,2], Ivana Jukić[1], Marko Stupin[1,3], Aleksandar Kibel[1,3], Kristina Selthofer-Relatić[1,3], Martina Mihalj[1,3]

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Introduction: n-3 polyunsaturated fatty acids (n-3 PUFAs) have the potential to prevent development and progression of various (CV) diseases, including atherosclerosis and atherosclerosis related diseases, in part, by improving vascular (endothelial) function as demonstrated in numerous epidemiological and clinical trials.
Aim: Present study aimed to examine the effects of n-3 PUFAs enriched hen eggs consumption on microvascular function of healthy young individuals (age 24±2 yrs) of both sexes, before and 3 weeks after standard eggs' consumption (control group, n=21) and n-3 PUFAs enriched eggs (experimental group, n=19).
Materials and Methods: The subjects consumed three boiled eggs daily for three weeks, the total amount of n-3 PUFA per day was 249 mg for the control group, and for the experimental group 1053 mg n-3PUFA / day. Lipid profile, hsCRP were measured from blood samples. Microvascular reactivity was determined by Laser Doppler Flowmetry, before and after dietary protocol. Statistical analysis was conducted by t-test (between groups) and paired t-test (within the group comparisons); statistical significance was set as $p<0.05$. Study was approved by Ethical Committee of Scientific Centre of Excellence, and all participants gave written informed consent.
Results: Participants on n-3 PUFAs eggs had significanlty lower cholesterol and higher HDL-cholesterol after dietary protocol compared to control group and slightly higher LDL-cholesterol, compared to their starting values. Control participants had significanlty higher body fat %, without changes in body mass indeks (BMI). Systolic and mean arterial blood pressure decreased in control subjects after dietary protocol. Subject in experimental group did not have changes in arterial blood pressure, BMI or body tissue composition. Microvascular flow was significantly increased after n-3 PUFAs consumption (acetylcholine-induced dilation).
Conclusions: n-3 PUFAs enriched eggs' consumption can affect biochemical values and improve endothelium-dependent vascular function even in young, healthy subjects.

Support: The 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.

Key words: *microvascular reactivity, n-3 PUFAs, acetylcholine, endothelium*

INVITED LECTURE -5

Inflammatory cytokines and immune system response to weight reduction in patients with obesity after anti-inflammatory diet

Ines Mrakovčić-Šutić[1,2], Gordana Kenđel Jovanović[3], Sanja Klobučar Majanović[4], Ingrid Šutić Udović[1], Ivana Šutić[5,6]

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Introduction: Obesity is accompanied with an inflammation and may cause different metabolic disease (type 2 diabetes, non-alcoholic fatty liver disease, metabolic syndrome) and can increase the risk for developing cardiovascular disease (CVD). The Dietary Inflammatory Index (DII) was developed and validated as a scoring algorithm of 45 food parameters to investigate the inflammatory potential of an individual's diet. It has been shown that DII is valuable tool for assessing a diet's inflammatory potential.
Objective of this study: To analyses the association between diet inflammatory potential, inflammation and immunology state of obese people.
Patients and methods: During six months 81 people with a body mass index BMI>25 kg/m^2 with or without obesity complication were monitored. Participants are divided into group with anti-inflammatory diet nutritional intervention (intervention subjects IS) and into control group (CG) with the KBC Rijeka standard education protocol and energy and nutritional restriction of the diet. For the first time in Croatia, the Dietary Inflammatory Index was used for assessing diet quality and its inflammation potential by which can determine its applicability in the clinical setting on Croatian population.
Inflammatory status was assessed by concentration of hs-CRP, IL-6 and TNF-α, and immune status by immunophenotypic profile of subgroups of regulatory T lymphocytes. The inflammatory potential of the diet was assessed by the Dietary Inflammatory Index (DII).
Results: Both studied groups statistically significantly reduced markers of inflammation, hs-CRP, IL-6 and TNF-α. The innate immunity (the percentage of NK and NKT cells) were significantly decreased in intervention group.
Conclusion: Intervention with anti-inflammatory diet showed the greatest reduction of these parameters in IS subjects with the highest obesity degree.

Acknowledgement: This work is supported by grant from the University of Rijeka (uniri-biomed-18-220)

ORAL -1

Ceruloplasmin and haptoglobin in bovine follicular fluid

J. Aladrović[1], M. Lojkić[1], R. Laškaj[2], L. Pađen[1], B. Beer Ljubić[1]

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Follicular fluid (FF) proteins originate from blood plasma and theca, granulosa cells and oocyte. The FF proteins regulate follicular development and oocyte maturation. Among numerous functions, they are acute phase proteins (APP) and proteins with antioxidant properties. Ceruloplasmin and haptoglobin are APP and protect follicular cells from oxidative damage binding copper and haemoglobin. The aim of the study was to determine follicle size and estrous cycle phase related changes in concentration of ceruloplasmin and haptoglobin. For the better insight in non-protein antioxidative molecules, biological antioxidant potential (BAP) was determined. Oxidative processes intensity was examined by reactive oxygen metabolites (ROMs).

The bovine ovaries were collected from naturally cycling animals at a local abattoir. The stage of estrous cycle (follicular or luteal) was identified according to presence or absence of the corpus luteum on the ovary. Follicular diameter was measured and follicles were classified according to diameter: small (≤5 mm; N=18 luteal phase; N=14 follicular phase), medium (6-10 mm; N=14 luteal phase; N=12 follicular phase) or large (>10 mm; N=12 luteal phase; N=14 follicular phase) follicles from ovaries in both follicular and luteal stage of estrous cycle. Ceruloplasmin and haptoglobin concentrations and biological antioxidant potential (BAP) and concentration of ROMs were analysed in FF punctured from ovaries in follicular and luteal phase of estrous cycle.

Concentrations of BAP and haptoglobin significantly decrease from small to large follicles collected in both luteal and follicular phase. The highest concentration of ceruloplasmin in FF was determined in medium size follicles but changes was not statistically significant compare to small and large follicles. During folliculogenesis, in luteal phase follicles ROM concentration significantly decreased from small to large follicles. During folliculogenesis ceruloplasmin negatively correlate with haptoglobin but significant correlation was noted only in small follicles from luteal phase ($r = -0.70$; $p<0.013$). Ceruloplasmin as protein with antioxidant capacity and BAP as a sum of uric acid, bilirubin, vitamin C and E antioxidant potential significantly corelated in FF of small follicles in follicular phase of estrous cycle ($r=0.66$; $p<0.05$).

Results indicate similar antioxidative properties of bovine follicles during both luteal/follicular phases of estrous cycle. Ceruloplasmin and haptoglobin concentrations showed different trends during folliculogenesis and negative correlation indicates their specific functions in FF.

Keywords: *bovine follicular fluid, luteal phase, follicular phase, ceruloplasmin, haptoglobin*

ORAL -2

Cytoplasmic ALS/FTD-linked TDP-43 inclusions dysregulate astroglial noradrenergic signalling and metabolism

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In the majority of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) cases, cytoplasmic TDP-43 (TAR DNA-binding protein 43) inclusions are the main histopathological feature present not only in neurons, but also in non-neuronal cells, including astrocytes. Astrocytes metabolically support neurons with nutrients, such as glucose and its derivatives, as well as with lipids. During intense brain activity neuronal metabolism largely depends on the activation of *locus coeruleus* noradrenergic system and astroglial adrenergic receptors (ARs), the primary target of noradrenaline (NA) released from the noradrenergic neurons. Activation of astroglial ARs with NA triggers Ca^{2+} and cAMP signaling and augments aerobic glycolysis with production of lactate, an important neuronal energy fuel and signal. Cytoplasmic TDP-43 inclusions in astrocytes alone can cause motor neuron death, however, whether astroglial metabolism and metabolic support of neurons is altered in astrocytes with TDP-43 inclusions, is unclear. We measured lipid droplet and glucose metabolisms in astrocytes expressing the inclusion-forming C-terminal fragment of TDP-43 or the WT TDP-43 using fluorescent dyes or genetically encoded nanosensors. Astrocytes with TDP-43 inclusions exhibited a 3-fold increase in the accumulation of lipid droplets vs. astrocytes expressing WT TDP-43, indicating altered lipid droplet metabolism. In these cells the NA-mediated increases in intracellular Ca^{2+} and cAMP were reduced by ~30%, likely due to the downregulation of β_2-ARs. NA triggered a similar intracellular lactate increase in astrocytes with and without TDP-43 inclusions, but the probability of activating aerobic glycolysis was facilitated by 1.6-fold in astrocytes with TDP-43 inclusions and lactate MCT1 transporters were downregulated. Thus, although noradrenergic signaling is reduced in astrocytes with TDP-43 inclusions, aerobic glycolysis and lipid droplet accumulation are facilitated, suggesting dysregulation of metabolism that likely impairs astroglial capacity to metabolically support neurons in ALS and FTD.

ORAL -3

Effect of *Agaricus bisporus* supplementation in a feed of Lika pramenka lambs on vitamin A and D serum concentrations

Shek Vugrovečki A.[1], I. Žura Žaja[1], S. Milinković Tur[1], M. Šimpraga[1],B. Špoljarić[1], S. Vince[1], M. M. Kardum Paro[2], D. Špoljarić[1], L. Pajurin[1], K. Vlahović[1], M. Popović[1]

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The supplementation of sheep diets with vitamins, minerals and trace elements (in the form of a premix) is key as they have a positive effects on maintenance, growth, health and milk production. Mushrooms are an excellent source of vitamins, e.g. B vitamins and vitamin D and A, and also minerals, e.g. phosphorus, magnesium, selenium, copper, and potassium and are also rich in dietary fiber, chitin and β-glucans. *Agaricus biporus* (white button) mushroom is the common produced mushroom in Croatia and Europe. This study was performed on 45 clinically healthy, three-month-old lambs (24 females and 21 males), randomly divided into three 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* and the third group was fed for six weeks with commercial lamb food supplemented with 1.5% dried *Agaricus bisporus.* The aim of this study was to determine whether intake of the dry and raw fruiting bodies of white button mushrooms has effect on Vitamin A and vitamin D concentrations in sera of Lika pramenka sheep lambs. The results showed that supplementation within both treatment groups significantly elevated blood vitamins A and D concentrations in both sexes compared to the control group and baseline. We can conclude that *A. bisporus* supplementation in lambs could be used as a vitamins source and have beneficial effect on overall animal health and growth.

Key words: *dietary supplementation, Agaricus bisporus, lambs, vitamin A, vitamin D*

This research was funded by the Croatian Science Foundation with the project "Innovative functional lamb products" (IP-2016-06-3685).

ORAL -4

Caffeine for apnoea treatment does not affect the heart rate variability in newborns

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BACKGROUND. Caffeine is the drug of choice for treating neonatal apnoea, affecting the respiratory and cardiovascular systems. Heart rate variability (HRV) reflects the change of duration of consecutive RR intervals. Spectral analysis of HRV is a significant clinical tool for evaluating the maturation and influence of the autonomic nervous system (ANS) on the newborns' heart. A higher HRV reflects a more matured ANS.
AIM. The aim of our research was to establish whether caffeine affects cardiorespiratory parameters in newborns. By analyzing HRV we indirectly assessed the activity of ANS, also regarding the postmenstrual age (PMA).
METHODS. A prospective clinical study was performed in 25 newborns treated with caffeine citrate in a maintenance dose of 2,5 mg/kg body mass. While the newborns were sleeping supine after feeding, initially in a horizontal bed position (without tilt) for 20 minutes and afterwards in a tilted bed position for 30 degress for 20 minutes, we measured breathing frequency (BF), heart rate (HR), arterial oxygen saturation (SpO2), body temperature (T) and HRV. At least 72 hours after caffeine withdrawal, the same protocol of measurements was applied again.
RESULTS. We determined a correlation between caffeine and a higher BF (p=0,023) without tilt. No significant differences in HR, SpO2 or T between the caffeine or without it were found. HRV was not significantly different either regarding caffeine treatment or bed tilt. HRV increased with PMA; TP (total power spectral density) and LF (low frequency power) both increased with caffeine while tilted (TP: p=0,044) and LF: p=0,039) and increased after caffeine withdrawal without tilt (TP: p=0,041 and LF: p=0,017).
CONCLUSIONS. We confirmed that caffeine increases BF, probably by stimulating the respiratory centre. The effect of caffeine on HRV was not established. We assume that the caffeine maintenance dose of 2,5 mg/kg body mass is too low to affect HR and HRV. HRV parameters increased with increasing PMA, probably due to maturation of ANS.

ORAL -5

The role of uroguanylin's GC-C independent signalling pathway in the development of ischemic stroke

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INTRODUCTION: Recent research has shown that the activation of guanylate cyclase (GC)-A, but not guanylate cyclase B (GC-B), leads to a decrease of brain lesion size following middle cerebral artery occlusion (MCAO).
AIM: Our aim was to investigate the potential role of uroguanylin (UGN)-dependent signalling pathways (GC-C or Ca^{2+}-dependent) in the development of ischemic stroke.
METHODS: Lesion volumes were measured in WT, GC-C and UGN KO mice by MR imaging 1 day after MCAO. Intracellular Ca^{2+} concentrations were measured in slices of ipsilateral and contralateral hemispheres.
RESULTS: GC-C KO animals exhibited significantly smaller lesion volumes 1 day after MCAO than WT mice. This difference was not present between UGN KO and WT littermates. The hemisphere with stroke shows a greater increase in intracellular Ca^{2+} concentrations following UGN stimulation compared to the healthy hemisphere of the same animal. This difference is absent in GC-C KO animals, while UGN KO animals exhibit a prolonged Ca^{2+} response compared to their WT littermates.
CONCLUSION: The reduction in stroke volume present in GC-C KO but not UGN KO mice indicates that the activation of the Ca^{2+}-dependent signalling pathway may have potentially harming effects. The possible mechanism could be increased UGN activity on pH regulation in astrocytes via Ca^{2+} signalling pathway and worsening of existing interstitial post ischemic acidosis.

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ORAL -6

Vitamins C and E supplementation during 7-day high-salt intake prevents impairment of microvascular endothelium-dependent vasodilation in young healthy individuals

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Objective: We have earlier reported that 7-day high-salt (HS) intake increases oxidative stress and impairs microvascular endothelium-dependent vasodilation in young healthy individuals, independently of blood pressure (BP) changes. The aim of the present study was to test whether such disturbed oxidative balance has direct adverse effect on microvascular endothelial function by functionally testing if administration of vitamins C and E during 7-day HS diet will prevent observed alterations in endothelium-dependent vasodilation of skin microcirculation in healthy individuals.
Materials and Methods: 51 young healthy individuals of both sexes (26 women and 25 men, age ranged 18-24) were assigned to a 7-day low-salt diet (<3.5 g salt/day) followed by a 7-day HS diet (~14 g salt/day) without (HS group) or with the daily supplementation of vitamins C (1000 mg) and E (800 IU) (HS+C+E group). Laser Doppler flowmetry measurement of skin microvascular blood flow following iontophoresis of acetylcholine (ACh) or sodium nitroprusside (SNP) was used to examine skin microvascular endothelium-dependent and –independent vasodilation, respectively. 24h urinary sodium excretion and BP were measured before and after diet protocols.
Results: There was no change in BP after HS or HS+C+E protocol compared to LS. Changes in 24h urinary sodium excretion confirmed that subjects conformed to the diet protocols. ACh-induced dilation (AChID) of forearm skin microcirculation was significantly impaired in HS group, but not in HS+C+E group compared to LS. SNP-induced dilation (SNPID) remain unchanged after the protocol in both HS and HS+C+E group compared to LS.
Conclusion: The finding of this study that enhanced antioxidative defense by vitamin C and E administration during 7-day HS loading prevented impairment of forearm skin microvascular endothelium-dependent vasodilation, provides functional evidence that increased oxidative stress level has the pivotal role in mediating HS diet-induced impairment of endothelial function in healthy individuals. (HRZZ #IP-2016-06-8744).

ORAL -7

5-aminoimidazole-4-carboxamide ribonucleoside induces differentiation in blasts isolated from patients suffering from non-APL acute myeloid leukemia

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INTRODUCTION: The most successful treatment of acute myeloid leukemia (AML) to this day is a differentiation therapy of acute promyelocytic leukemia (APL) carrying typical t(15;17) translocation with pharmacological doses of all-trans retinoic acid (ATRA). Since the recent discoveries of beneficial effects of inhibitors of mutated isocitrate dehydrogenase and dihydroorotate dehydrogenase (DHODH), differentiation therapy of non-APL AML has come into limelight. Our previous studies demonstrated that 5-aminoimidazole-4carboxamide ribonucleoside (AICAr), a precursor in purine biosynthesis and an activator of AMP-kinase, induced differentiation of monocytic cell lines by activating the ATR/Chk1 via pyrimidine depletion.
AIM: The aim of this study was to test anti-leukemic and differentiative properties of AICAr in blasts isolated from bone marrow of patients suffering from non-APL AML in comparison to ATRA and DHODH inhibitor brequinar.
MATERIALS AND METHODS: Mononuclear cells from bone marrow of 35 non-APL AML patients were obtained by density gradient separation and seeded in liquid medium containing 50 ng/mL interleukin-3, interleukin-6, stem cell factor and FLT3-ligand. Cell viability was assessed by MTT assay and AML cell differentiation was determined by flow cytometry and morphological analyses. RNA sequencing and partial data analysis were conducted using ClusterProfiler package. Statistical analysis was performed using GraphPad Prism 6.0.
RESULTS: AICAr increses the expression of differentiation markers and induces the accumulation of cells that morphologicaly resemble macrophages in subset of samples of bone marrow blasts that were resistant to ATRA. AICAr-induced differentiation correlates with proliferation and sensitivity to DHODH inhibition. RNA-sequencing data obtained in primary AML blasts confirmed that AICAr treatment induced downregulation of pyrimidine metabolism pathways together with an upregulation of gene set involved in hematopoietic cell lineage.
CONCLUSION: AICAr induces differentiation in a subset of primary AML blasts that carry no t(15;17) translocation and these effects correlate with sensitivity to a well-known potent inhibitor of DHODH.

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ORAL -8

Endosomal PI3P domains are not required for the development of the cytomegalovirus pre-assembly compartment but are essential for efficient virion assembly

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Introduction: Cytomegaloviruses (CMV) rearrange cellular endosomal system to develop a site for assembly and release of progeny virion, known as cytoplasmic assembly compartment (cVAC). Generally, remodeling of the endosomal system depends on the adaptation of the content of membrane phospholipids, especially phosphoinositides. Remodeling of early endosomal domains (EE) begins already from the earliest stages of infection, where dynamics of phosphoinositide phosphatidylinositol 3-monophosphate (PI(3)P) has a principal role in the endosomal flow. Therefore, our aim was to determine expression and localization of PI(3)P and its main effectors during endosomal rearrangements in the course of CMV infection as well as to determine the effect of PI(3)P impaired function on cVAC formation.
Materials and methods: We analyzed localization of PI(3)P by confocal microscopy using retroviral vectors with subcloned PI(3)P-binding domains (GFP-tagged 2xFYVE and YFP-tagged PX) in Balb3T3 fibroblasts infected with murine cytomegalovirus (Δm138-MCMV). Impaired function of PI(3)P was achieved with retroviral vectors and pharmacological inhibitors, and its effect on MCMV replication cycle was monitored by Western blot and flow cytometry. Release of progeny virions was followed by plaque assay.
Results: Overexpression of the PI(3)P binding domains did not affect establishment of the infection. PI(3)P localized in the rearranged endosomes in the juxtanuclear agglomerate of MCMV infected cells, partially colocalized with EE markers Rab5 and EEA1. Overexpression of the PI(3)P binding domains, but not the expression of YFP/GFP and mutated domains with depleted PI(3)P binding altered membrane flow into endosomal recycling compartment, as demonstrated by transferrin retention in the enlarged $PI(3)P^{+}$ $Rab5^{+}$ $Rab11^{-}$ endosomes. Impaired PI(3)P function did not prevent development of the pre-AC, but affected MCMV replication cycle in the late phase of the infection, including the release of progeny virions. These results were further confirmed by inhibition of PI(3)P production (PI3KCIII inhibitor).
Conclusion: These results suggest that PI(3)P membrane domains contribute to biogenesis of the cVAC and final release of newly formed virions by a mechanism that affect entry into the late phase of infection.

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ORAL -9

TRPV1-Dependent Mechanisms are Involved in Modulating Viscerosensory-Sympathetic Reflexes in the Spinalized Rat

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Background: The viscerosensory-sympathetic network is a functional neural circuit connecting afferent sensory fibers of the gut and efferent vasoconstrictor neurons at the level of the spinal cord. This neural circuitry is critical in development of hypertensive crises in patients living with spinal cord injury (SCI) which is known as autonomic dysreflexia. While both mylinated Aδ and unmylinated C-fibers can elicit sympathetic vasoconstrictor responses, the functional significance of afferent mechanisms involved in the viscerosensory-sympathetic network remains poorly understood. Sensory afferent neurons of the gastrointestinal tract exhibit chemo-, thermo-, and mechano-sensitivity, and are generally polymodal in character. The transient receptor potential cation channel V1 (TRPV1) is a member of a larger family of TRP channels located on C-fibers that serve many sensory functions. In addition to noxious heat, TRPV1 is activated by capsaicin and plays an important role in mechano transduction within the gastrointestinal tract.

Objective: Assess the involvement of C-fibers expressing TRPV1 channels in modulating viscerosensory-sympathetic reflex reactivity in a rodent model of SCI.

Methods: The effect of exogenously supplied TRPV1 ligands on viscerosensory-sympathetic reflex reactivity was assessed in spinalized Sprague Dawley rats. Quantification of sympathetic reflex responses to colorectal distension following SCI was achieved using a decerebrate preparation, which enables all anesthesia to be discontinued prior to electrophysiological assessment.

Results: Acute administration of the TRPV1 agonist, capsaicin, potentiates pressor responses to visceral stimulation. Pretreatment with the selective TRPV1 antagonist, A-784168, significantly attenuates viscerosensory-sympathetic reflex reactivity to colorectal distension in spinalized animals.

Conclusion: TRPV1 channels, modulate viscerosensory-sympathetic reflex reactivity in animals following SCI and may provide a novel target for the management of autonomic dysreflexia.

ORAL -10

Nucleotide metabolism and activation of Chk1 in monocytic differentiation

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INTRODUCTION:
Inhibitors of pyrimidine synthesis are known to induce monocytic differentiation in leukemia cells. Our previous studies show that 5-aminoimidazol-4-carboxamide ribonucleoside (AICAr) inhibits UMP synthesis and induces S-phase arrest as well as cellular differentiation. Brequinar, inhibitor of dihydroorotate dehydrogenase, one of the key enzymes in pyrimidine *de novo* synthesis, exerts similar effects.

AIM:
The aim of this study is to further test for the role of Chk1 DNA-damage pathway in differentiation by comparing response to cytarabine, an antimetabolite analogue of cytidine, to the effects of pyrimidine synthesis inhibitors.

MATERIALS AND METHODS:
U937 cells were incubated in the presence of AICAr, brequinar and cytarabine. The expression of differentiation markers and DNA content for cell cycle analysis were determined by flow cytometry. Total cell lysates were analyzed for the level of specific proteins using western blot. Protein level was downregulated using RNA-interference.

RESULTS:
Results of our study show that the effects of cytarabine on cell viability and the expression of differentiation markers in monocytic leukemia cells are similar to the effects of AICAr and brequinar. Furthermore, western blot analysis confirmed the activation of Chk1 and inhibition of Cdk1 corelated with cell cycle arrest. Use of Torin2 and VE-821, pharmacological inhibitors of ATR/Chk1 DNA-damage signaling pathway, as well as transfection of U937 cells with siRNA targeting Chk1, reduced differentiative effects of all agents tested.

CONCLUSION:
Nucleotide metabolism perturbation induces monocytic differentiation through activation of ATR/Chk1 signaling pathway.

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ORAL -11

Positive effect of *n*-3 pufas consumation on serum levels of anti-inflammatory cytokines in young sedentary participants

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Introduction: Unbalanced, nutrient-poor diet followed by sedentary lifestyle and harmful habits leads to an increased risk of developing diabetes, obesity and cardiovascular disease. Previous studies have shown a positive effect of *n*-3 polyunsaturated fatty acids (PUFAs) on microvascular reactivity, lipid metabolism and reduction of oxidative stress. **Aim:** The aim of this study was to determine serum concentrations of inflammatory and anti-inflammatory cytokines in young healthy subjects of both sexes (n = 38; age 23.8 ± 2.4 years, min = 19, max = 28) after three weeks of consumption of *n*-3 PUFAs enriched eggs (experimental group, n = 18) or standard eggs (control group, n = 21). **Materials and Methods:** Cytokine concentrations were determined by the Luminex method at the time of enrollment (baseline levels) and at the end of the three-week dietary protocol (end-point levels). A t-test was used for group comparisons, while paired t-test or Wilcoxon test were used within the group; statistical significance was set as $P < 0.05$. The study was approved by the Ethics Committee of the Faculty of Medicine, University of Osijek, and all respondents signed informed consent. **Results:** At the end of the protocol, compared to the baseline levels, subjects from control group had significantly increased end-point serum concentrations of IL-17A and VEGF-A, while subjects from experimental group had significantly reduced serum IFN-gamma concentration. The end-point concentration of IL-10 increased significantly in both groups. In addition, end-point serum levels of IL-10 and IL-21 were significantly higher in the experimental group compared to the control group. **Conclusion:** Consumption of *n*-3 PUFA enriched eggs resulted in increased levels of anti-inflammatory cytokines and decreased levels of serum inflammatory cytokines, indicating that functional foods may have an immunomodulatory effect.

Keywords: *cytokines, inflammation, n-3 PUFAs, functional foods*

ORAL -12

Quantification of the mouse cytomegalovirus by novel fluorescence signal-based approach

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Introduction: Mouse cytomegalovirus (MCMV) enables a valuable animal model to better understand the biology of the related human CMV. Technical prerequisite for viral study is virion quantification. MCMV quantifications mostly relies on standard plaque forming unit (PFU) assay. In the PFU assay the number of viral particles is indirectly determined by viral infectivity of fibroblasts. Although the PFU assay is widely used, it suffers from poor reproducibility. Other commonly used method for quantification of MCMV is polymerase chain reaction (PCR). PCR can detect the number of viral genome copies in highly precise manner, but it poorly correlates to the PFU assay, i.e. the variable genome-to-PFU ratio indicates that methods are inaccurate in detecting infectious viral particles. Therefore, it is necessary to improve methods for virion quantification.
Objective: To establish a novel, specific, fast and machine readable MCMV quantification. The method will be based on spectrophotometry and introduced in 2 phases: 1) quantification of fluorescent protein expressing recombinant MCMVs; 2) quantification of immuno-captured virions by already available and proven antibody against the capsid protein gB.
Materials and methods: Balb 3T3 cells were infected with 1 PFU of recombinant C3X-GFP MCMV per cell. Serial dilutions of cells infected at 0, 6, 24, 48 and 72 hours after infection were analyzed by flow cytometry and 96-well spectrophotometer to detect and compare fluorescence signal of GFP expressed from the recombinant viral genome.
Results: Serial dilution in range of 200 000 to 1 562 infected cells resulted in 284 177,5 to 106 relative light units detected on 96-well spectrophotometer for all hours of infection, and 630,37 to 4,74 ΔMFU range detected on flow cytometry.
Conclusion: Preliminary data showed that spectrophotometer is sensitive enough to detect fluorescent signal from MCMV infected cells and suitable for the next phase.

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ORAL -13

Ion channels modulation after acute and intermittent hyperbaric oxygen exposure – changes in vascular relaxation mechanism

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INTRODUCTION: Hyperbaric oxygenation (HBO_2) effects on vascular reactivity in aortic rings vary, depending on the applied protocols. Acute changes in pO_2 affect K^+ channels, and HBO_2 seems to modulate transient potential receptor channel (TRP) on the endothelial cell membrane.
AIM: This study assessed the effect of different HBO_2 protocols on the mechanisms of flow induced dilation (FID) and the role of K+ and TRP channels in middle cerebral arteries (MCA) of Sprague-Dawley rats.
MATERIALS AND METHODS: 11 week old male rats (N=6 per group) were divided in: CTRL (control group of rats); A-HBO_2 (acute exposure to 100% oxygen; 2bar/2hrs) and 4D-HBO_2 (rats exposed to HBO_2 for four consecutive days and examined on the 5th day). Rats were anesthetized with ketamine-chloride (75 mg/kg) and midazolam (0.5 mg/kg), decapitated and blood vessels were collected. FID was assessed in isolated cannulated and pressurized MCA, (Myograph Pressure System Model 110P MyoView Version 1.2.0 DMT; Danish Myo Technology). FID was evaluated over pressure gradient at Δ10 mmHg, Δ20 mmHg, Δ40 mmHg, Δ60 mmHg and Δ100 mmHg, with/without agonist and antagonists for large conductance Ca^{2+}-activated K^+-channel ($K_{Ca2+,}$ NS-1619 and iberiotoxin); ATP-sensitive potassium channels (K_{ATP}, cromakalim and glibenclamide) and TRPV4 (GSK1016790A and RN-1734). Protein levels of TRPV4 and KCNMB1 were assessed by 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.
RESULTS: FID in A-HBO_2 group was significantly impaired compared to CTRL and 4DHBO_2 groups. Agonists of ion channels restored FID in A-HBO_2 groups and antagonists (iberiotoxin, glibenclamide and RN-1734) inhibit FID without additional effect in 4DHBO_2 group compared to CTRL. Protein levels of KCNMB1 and TRPV4 were increased in 4DHBO_2 group compared to CTRL.
CONCLUSION: Results suggest ion channels' modulation depending on the HBO_2 protocol.

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Keywords: *hyperbaric oxygen, flow induced dilation, cerebral circulation, endothelium, membrane ion channels*

ORAL -14

Physical activity of teachers

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In the era of modern technologies dominated by a sedentary lifestyle and stress, a silent killer of the contemporary time, physical activity and its importance should have a significant and indispensable role. Numerous studies testify of an increase of physical inactivity caused by the way of life and a lack of free time. Sindik, Andrijašević and Ćurković (2009) believe that the physical activity should be a social priority, since the appearance of modern technology has eliminated the fundamental human biological need to be physically active, consequently resulting in a decrease of physical and physiological potential of humans. The aim of this paper is to determine the level of physical activity of high school teachers, and establish whether there are differences in physical activity of female and male teachers during their professional work, travel to work and free time taking into consideration their age, gender and work experience. The study included teachers of a high school in Slavonija. The sample included 41 high school teachers aged from 25 to 64 with the average age being 45 years and 8 months. This study used the Croatian version of the Global Physical Activity Questionnaire (GPAQ) as the measuring instrument. The descriptive statistical analysis resulted in the following parameters: arithmetic mean and standard deviation of the total physical activity of teachers, physical activity in the workplace, physical activity during the travel to work and free time, sports and leisure activities. In order to determine the difference between the named arithmetic means the two-way analysis of variance, i.e. ANOVA was used. The results presented in the paper show that the overall level of physical activity among the respondents was high, amounting to 4.753,85 MET- min/week for male and 2.137,14 MET- min/week for female teacher. 76 % of teachers engaged in medium and high intensity physical activity which benefits health, while 24 % of teachers engaged in low intensity physical activities.

Key words: *physical activity, teachers, high school, GPAQ*

ORAL -15

Leukocytes' Surface Expression of Integrins is affected by Short-Term High-NaCl Dietary Intake in Both Healthy Humans and Sprague-Dawley Rats: A Comparative Study

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Introduction: Growing body of evidence suggests a strong link between the increased dietary salt load and the atherosclerosis leading to the development of cardiovascular diseases. However, data on the effects of acute high salt intake on the immune system activation in otherwise healthy animals and human subjects are scarce.
Aims: This study is aimed at assessing the effects of a short-term high-salt (HS) diet on the peripheral blood leukocyte (PBL) activation status in healthy rats and young human individuals.
Materials and Methods: Distribution of PBL subpopulations and surface expression of integrins were determined using flow cytometry in 36 men and women on a 7-day low-salt diet (<3.2 g salt/day) immediately followed by a 7-day HS diet (~14 g salt/day) or in Sprague-Dawley (SD) rats (n = 24) on a 0.4% NaCl diet (aLS group) or a 4% NaCl diet (aHS group) for 7 days.
Results: The aHS group presented with an increased frequency of granulocytes, while the frequency of lymphocytes was reduced. Although in humans HS diet reduced the expression of CD11b(act) integrin on lymphocytes, the frequency of CD11b(act)-bearing cells among all PBL subsets was increased. The aHS group of rats exhibited increased expression of total CD11b/c in granulocytes and CD3 lymphocytes. The expression of CD11a was significantly reduced in all PBL subsets from human subjects and increased in the aHS group. CD49d expression on all PBL subsets was significantly decreased in both humans and rats. In human subjects, we found reduced frequencies of intermediate monocytes accompanied by a reciprocal increase in classical monocytes.
Conclusion: Present results suggest that a short-term HS diet can alter leukocytes' activation status and promote vascular low-grade inflammation.

This study has been published as full length paper:
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ORAL -16

Peripheral blood NK Cells from Breast Cancer Patients are tumour-primed

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INTRODUCTION: Breast cancer is one of the leading causes of cancer related deaths. Immune infiltration of breast tumors has been shown to be related to clinical outcome. Natural killer (NK) cells are effector lymphocytes involved in tumor immunosurveillance. In solid malignancies, tumor-associated NK cells in peripheral blood and tumor-infiltrating NK cells show altered phenotypes and are characterized by either anergy or reduced cytotoxicity. The goal of study was to investigate the immune activation profile of NK cells in order to gain a better understanding of pathological behaviour of different breast cancer subtypes.

MATERIAL AND METHODS: Immunohistology was used to detect presence and localization of CD56 and IL-15 in paraffin embedded normal and tumoral breast tissue sections. The distribution and frequency of NKG2A, NKG2C, NKp46, CD94, CD69 and CD107a, was investigated in population of NK cells in mononuclear cell suspensions from peripheral blood by flow cytometry. Cytolitic mediator's mRNA was detected by quantitative RT-qPCR.

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

CONCLUSION: Modulation of NK cells activity in peripheral blood and tumor-infiltrating NK could be involved in pathogenesis of breast cancer. This highlights the importance of NK cells as a suitable target to modulate the immunosuppressive tumour microenvironment and try to trigger a more potent anti-tumor response.

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ORAL -17

Hippocampal Neurogenesis and Expression of BMP-4 and Noggin in Pristane Induced Rheumatoid Arthritis

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Introduction. Rheumatoid arthritis (RA) is an inflammatory joint disease with a neurological component including depression, cognitive deficits, and pain, which substantially affect patients' quality of daily life. Bone morphogenetic protein 4 (BMP4) is one of the factors in RA pathogenesis as well as a known regulator of adult neurogenesis.
Aim. This study examines the effects of animal model of RA (pristane induced arthritis, PIA) on adult neurogenesis and on expression profile of BMP4 and its inhibitor Noggin.
Materials and Methods. 8 Dark Agouti rats were injected with pristane in the base of the tail which lead to arthritis 9 days after immunization. Animals were sacrificed at acute phase of disease. 4 rats were left as untreated control and were injected with saline. Brain paraffin sections were stained with BMP4 and its' inhibitor Noggin. Additionally, sections were also stained with Ki-67 and doublecortin (DCX) to monitor neural progenitor cell development. The number of positive cells (cells/mm^2) and quantification of expression (mean gray value) are presented as mean ± standard deviation. The statistical significance is proposed at $p \leq 0.05$.
Results: Adult neurogenesis is reduced in acute phase of PIA, i.e. number of proliferating cells, i.e., Ki67$^+$ cells (22±8) and number of new generated neurons, i.e., DCX$^+$ cells (77±9) unlike in the control rats (Ki67=45±5; DCX=130±22) with the statistical significance. The number of Ki67+BMP4 and DCX$^+$Noggin$^+$ cells is lower in PIA (2±3; 6±5) than in control rats (40±6; 71±8). The BMP4 expression (17±1) is higher and the Noggin expression (6±1) is lower in PIA than in control rats (BMP4=13±2; Noggin=26±5) with the statistical significance.
Conclusions: The PIA has a negative impact on adult neurogenesis during the acute phase, i.e., reduces number of proliferating cells and young neurons. Furthermore, PIA increases BMP4 and reduces Noggin expression in young neurons in acute phase.

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ORAL -18

The expression of Tumor Associated Glycoprotein 72 in endometrioid endometrial cancer with mucinous component

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Introduction: Tumor Associated Glycoprotein 72 (TAG-72) is expressed in various tumors. The aim of this study was to analyze the expression of TAG-72 in endometrioid endometrial cancer in relationship with cytokeratin (CK) 19 and immunohistological properties of the tumor.
Material and methods: Archival biopsy material of endometrioid endometrial cancer with mucinous component (N 56) was obtained from patients during surgery and embedded in paraffin, in the period from 2002 to 2006. Histological analysis (histological type, proportion of mucinous component) was performed in hemalaun-eosin labeled sections and tissue microarrays were selected for further labeling. CK19, TAG-72, CD68, arginase-1 and iNOS (from inducible nitric oxide synthase) were labeled by immunohistology to quantify cell number by counting positive cells per field at magnification 400x. Double immunofluorescence CD68/arginase-1, CD68/CC ligand (CCL) 2, CD68/CCL22] was used to analyze the polarization of macrophages and spatial arrangement of TAG-72/CK19 and TAG-72/CD68 positive cells.
Results: TAG-72 was expressed in 96.4 8.3 % of cancer cells and their distribution slightly differed from CK19+ cells. The number of TAG-72+ cells and the number of CK19+ cells or the total number of CD68+ cells positively correlated, as well as the percentage of tumor mucinous component did. Arginase-1 and CCL22 were intensely labeled; CCL2 was moderately expressed, while iNOS was not detected in CD68+ cells in endometrioid cancer with mucinous component. TAG-72+ cells positively correlated with tumor size.
Conclusion: Abundant expression of TAG-72 in endometrioid endometrial cancer with mucinous component drive the M2 macrophage maturation that could support uncontrolled local tumor growth.

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POSTER -1

The effect of n-3 polyunsaturated fatty acids (n-3 PUFAs) chicken eggs consumption on antioxidative enzymes gene exrpression in mononuclear blood cells in young healthy individuals

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Introduction: Oxidative stress underlies the mechanisms of impaired vascular reactivity associated with the development of cardiovascular disease. In previous studies (Appl Physiol Nutr Metab. 2018) we showed that there is a change in oxidative stress levels when consuming n-3 PUFA enriched eggs in healthy young subjects.
Aim: The aim of the study was to determine the expression of the gene for SOD1-superoxy dismutase GPX1-glutathione peroxidase CAT-catalase MPO-myeloperoxidase
Materials and methods: Gene expression of SOD1-superoxy dismutase (control group n = 7, experimental group n = 11), GPX1-glutathione peroxidase (control group n = 8, experimental group n = 9), CAT-catalase (control group n = 5, experimental group n = 6) and MPO-myeloperoxidase (control group n = 3, experimental group n = 5) in healthy young subjects who consumed n-3 PUFA-enriched eggs or plain, control eggs before and after the dietary protocol. Subjects consumed three boiled eggs daily for three weeks, the total amount of n-3 PUFA per day was 249 mg for the control group and 1053 mg n-3PUFA / day for the experimental group. Gene expression was determined by real-time PCR on a Bio Rad CFX96 device.
Results: These preliminary results of gene expression of antioxidant enzymes SOD1, CAT, Gpx1 and MPO before and after consumption of n-3 PUFA results do not show significant statistical differences.
Conclusion: As our previous studies have shown the impact of n-3 PUFA-enriched eggs on oxidative stress levels, it is possible that changes at the gene level come later and more slowly. In order to determine which antioxidant enzymes mediate such a reduction, in addition to genetic expression, the protein expression of antioxidant enzymes should be determined and / or the activity of antioxidant enzymes should be determined. To draw concrete conclusions, the study should be repeated on a larger number of respondents.

Keywords: *antioxidant enzymes, gene expression, n-3 PUFA, vascular reactivity*

POSTER -2

Bioelectrical impendance phase angle change in soccer players during spring preparatory period

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AIM: Phase angle is derived measure obtained from direct measures of BIA method: resistance and reactance. Its biological meaning is not completely understood, but it has been interpreted as an indicator of membrane integrity and water distribution between intra and extracellular spaces, as well as predictor of body cell mass. Some researchers connected it with sport performance. The aim of this paper is to show values of the phase angle and how they change in a sample of football players during the spring preparatory period.
MATERIALS AND METHODS: A total of 22 male football players from two football clubs from the Vukovar area who played in the 3rd Croatian Football League East participated in the study. For the purpose of the study, body composition was estimated using the bioelectrical impedance method (BIA) at the beggining of the preparatory period, and after 8 weeks of regular trainings.
RESULTS: Although body mass and body composition, measured by BMI, % of body fat and fat-free mass did not change significantly, we found statistically significant change in BIA phase angle, which was higher at the end of the study compared to the beggining ($p=0.039$).
CONCLUSION: Phase angle is very sensitive measure of changes in body of soccer players generated by trainings during the preparatory period.

Key words: *bioelectric impedance, soccer, phase angle*

POSTER -3

Energy metabolism during the early postpartum period in cows and calves kept in a beef suckler system

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Background. The early lactation and early neonatal period are characterized by intense changes in energy metabolism and the aim of this study was to evaluate blood biochemical parameters of energy metabolism.
Methods. The study was carried out on 13 Simmental cows and 13 beef suckler calves in cow-calf operations. Blood samples were obtained at 6, 12 and 48 h (hours) and 7, 14 and 30 d (days) postpartum (pp). Concentrations of glucose, total protein, albumin, urea, triacylglycerols, total, HDL (high density lipoproteins) and LDL (low density lipoproteins) cholesterol, BHB (beta-hydroxybutyric acid) and NEFA (non-esterified fatty acids), insulin and IGF-I were determined.
Results. In cows, glucose concentration was lower at 30 d pp than at 6 h pp ($P<0.001$). Triacylglycerol concentration was lower at 48 h pp than at 30 d pp ($P=0.05$). Total and LDL cholesterol concentrations were the highest at 30 d pp ($P<0.02$). Concentrations of HDL cholesterol and BHB were higher at 30 d pp than at 48 h pp ($P<0.03$, $P<0.01$). Concentration of NEFA was lower at 30 d pp than at 48 h pp ($P=0.02$). Albumin and urea concentrations were lower, while globulin concentration was higher at 30 d pp than at 6 h pp ($P<0.03$, $P=0.03$, $P=0.02$). Insulin concentration was higher at 7 d pp than at 6 h pp ($P=0.008$). In calves, glucose concentration was the lowest at 6 h pp ($P<0.001$). Concentrations of total, HDL and LDL cholesterol as well as BHB were higher at 30 d pp than at 6 h pp ($P<0.03$, $P<0.02$, $P<0.03$, $P<0.01$), while concentration of NEFA was lower ($P<0.01$). Concentration of albumin was higher, while concentration of globulin was lower at 30 d pp than at 6h pp ($P<0.01$, $P=0.008$). Urea concentration was higher at 7 d pp that the other points of measurements ($P=0.003$). Concentration of IGF-I was lower at 12 h pp than at 14 d pp ($P=0.04$).
Conclusions. In cows investigated period is characterized by rapid and significant changes in energy metabolism, and in calves by more intensive energy metabolism and greater substrate requirements at a later stage. The measured parameters, especially in the first hours after birth, are valuable indicators for future research of energy metabolism, as they are, to our knowledge, not currently available in the literature.

Key words: *energy metabolism, blood biochemical parameters, cow, calf*

POSTER -4

Granulysin expression in peripheral blood lymphocyte in women with knee osteoarthritis correlated with pain and restricted daily physical activities

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Introduction: Patients with osteoarthritis (OA) of the knee have limited daily activities due to local pain which correlates well with the serum pro-inflammatory cytokine concentrations. Pro-inflammatory cytokines support granulysin (GNLY) expression.
The aim of this study was to evaluate whether GNLY correlates with the pain and with the daily activities in patients with knee OA.
Patients and methods: In women with knee OA (no. 16), who agreed to participate in the study, age, pain intensity by visual analogue scale and daily activities tested by a 6-minute walk were evaluated during clinical examination. The women donated 10 ml of peripheral venous blood for intracellular labelling of GNLY protein in T and NK cells by triple immunofluorescence and flow cytometry. C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) were also analysed.
Results: Frequency of total $GNLY^+$ cells in patients with OA did not correlate with CRP and ESR. Total $GNLY^+$ cells, $GNLY^+$ NK cells and $GNLY^+$ T cells positively correlated with the pain intensity. The intensity of pain negatively correlated with the 6-minute walk test. Neither the pain, nor the 6-minute walk test were affected by age in our investigation group. Total $GNLY^+$ cells, $GNLY^+$ NK cells and $GNLY^+$ T cells negatively correlated with the 6-minute walk test.
Conclusion: The intracellular GNLY protein expression in the peripheral blood lymphocytes, as markers of inflammation independent of the CRP and ESR, contributes to the development of local pain and shortens the 6-minute walk distance in patients with knee OA.

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POSTER -5

Analysis of s-phenylmercapturic acid and s-benzylmercapturic acid in human urine by liquid chromatography coupled with tandem mass spectrometry

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This study reported a method to simultaneously determine *S*-phenyl mercapturic acid (*S*-PMA) and *S*-benzyl mercapturic acid (*S*-BMA) in human urine.

Since constant exposure to benzene isomers it is necessary to quantify this issue. S-PMA is believed to be a sensitive and specific biomarker for evaluating benzene exposure at low levels but also a metabolite of sorbic acid. Specific metabolite of toluene is excreted in urine in the form of S-BMA. Urine samples were analyzed by ultra-high performance liquid chromatography/tandem mass spectrometer in negative electrospray ionization mode. To perform matrix match calibration standards were prepared in synthetic urine. Target analytes eluted within 15 min in multiple reaction monitoring mode. In order to achieve maximum sensitivity instrument conditions were automatically optimized. Urine samples were refrigerated until analysis and stability was observed within 15 days.

This study was made as a part of the scientific research projects of the university in Rijeka uniri-biomed-18-146 1292 i uniri-biomed-18-220-6107.

POSTER -6

The role of heat shock 70 (HSP70) on cancer cell invasion-related activities

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INTRODUCTION: Breast cancer is one of the leading cause of cancer-related deaths in women worldwide and increasing rapidly in developing countries. Heat shock proteins (HSP) and heat shock factor 1 (HSF1), key factors in the heat shock response (HSR) have been implicated in the etiology of breast cancer. Available data indicate that extracellular HSP70 has a potent motogenic activity for fibroblasts, vascular and epithelial cells, and can induce angiogenesis and matrix remodelling, suggesting a possible role in cancer development and dissemination. In the present study, we investigated the potential role of HSP70 on migration and invasion capability of breast cancer cell line in vitro.

MATERIAL AND METHODS: Breast cancer cell line MDA-MB-231 was used for in vitro model study to reflect in vivo immunophenotypic features of triple negative breast cancer. Stimulation with HSP70 was tested on cancer cells lines (PANC-1, MDA-MB-231, JAR) by migration and invasion assays. The gelatinase zymography method will be used to investigate the functional activity of matrix metalloproteases 2 and 9 (MMP-2 and-9) after the invasion assay. Double immunofluorescence of actin/tubulin was investigated on HSP70 stimulated breast cancer cell lines MDA-MB-231 .

RESULTS: Extracellular HSP70 induce migration and invasion in a different human cancer cell line (PANC-1, MDA-MB-231, JAR) as well as remodel of extracellular matrix by MMP-2 and-9 upregulation.

CONCLUSION: These results suggest that HSP70 may facilitate cancer cell extravasation and invasion, a crucial event for carcinogenesis. Modulating cancer cell migration and invasion as well as upregulation of MMP-2 and-9, HSP70 might promote and/or sustain a permissive microenvironment for cancer cell invasion and metastasis.

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POSTER -7

Remodelling of the endosomal recycling compartment interfaces with early endosomes and *trans* golgi network during murine cytomegalovirus (mcmv) infection is dynamin-2 dependent

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Murine cytomegalovirus (MCMV) remodels cellular compartments of infected fibroblasts with the purpose of formation of viral assembly compartment (AC) for its own synthesis. With the use of Rab10 (marker of early endosome(s) – endosomal recycling compartment interface (EE-ERC)), we have previously shown that dynasore is important for generation of preAC in early phase of infection. However, for better understanding the mechanism(s) of preAC formation during MCMV infection, we have investigated the influence of dynasore on different markers of cellular endosomal compartments. Furthermore, we have additionally used the advantage of dynasore as a reversible inhibitor to assay the kinetics of (pre)AC formation with Rab10 as a marker.
In order to determine the site where MCMV uses dynamin-2 for reorganization of the intracellular system, dynasore has been added 4hpi, and selected markers have been visualized by confocal microscopy during early phase of infection (6 and 16hpi). The markers has been divided in several groups: (1) EE (Rab5 and EEA1); (2) EE-ERC intermediate compartment (Rab10, Rab8A); (3) ERC (Rab11, Arf6); (4) ERC-TGN intermediate compartment (evectin-2); (5) *cis*Golgi (GM130); (6) *trans*Golgi network – TGN (TGN38, Golgin97, Rab6). The influence of the inhibitor has been dominantly visible on markers of EE-ERC interface, ERC and EE-TGN interface. Importantly, dynasore has also prevented Golgi disintegration - the first hallmark that precedes preAC formation. Finally, the presence of dynasore only during early (4-14hpi), but not in the late phase of MCMV infection has prevented formation of AC (Rab10 visualized 24 and 48hpi by confocal microscopy). Interestingly, we have proved by flow cytometry and western blot analysis that the synthesis of MCMV proteins of the late phase of infection has also be prevented.
Therefore, we can conclude that dynasore acts during early phase of MCMV infection at the level of late EEs or EE-ERC interface. However, it could also affect directly or indirectly ERC-TGN remodeling and GA disintegration.

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POSTER -8

Golgi apparatus redistribution and cvac formation in the early phase of mcmv infection

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Introduction: Cytomegaloviruses (CMVs) as other members of the herpesvirus family, extensively rearrange the cellular membrane system to develop assembly compartment (AC). The AC area is large, approximately size of the nucleus, composed of host-cell-derived membranous elements delimited at its periphery by the Golgi complex. The loss of normal Golgi compartment morphology and its relocalization from a juxtanuclear ribbonlike structure to a series of concentric rings on the periphery of the AC represents one of the earliest landmarks of membranous system reorganization during MCMV infection.The AC is fully formed at late stages of infection after late viral genes encoding tegument, and envelope proteins are expressed. However, the reorganization of the host-cell organelles could be initiated earlier in the infection

Objective: The objective of our study was to characterize Golgi transformations as the first step in the formation of the AC.

Materials and methods: Mouse fibroblasts, Balb3T3 were infected with recombinant murine cytomegalovirus Δm138-MCMV (ΔMC95.15), 1PFU/cell for in 10% DMEM. Using confocal imaging analysis, we analyzed different Gogli markers, Rab6 (trans – Golgi and TNG), CM130 (cis/medial Golgi), Grasp55 (medial trans Golgi), Grasp65 (cis Golgi), GS15 (medial and trans), Golgin 97 (trans-Golgi-TGN interface) and Rab41 through several stages of MCMV replication cycle. Also, changes in Golgi apparatus were followed using specific inhibitor of protein trafficking to Golgi, Brefeldin A.

Results: In MCMV infected cells, the cis-, medial-, and trans-Golgi were vacuolized, fragmented and displaced from the nucleus to form the outer ring of the AC, they colocalized with different late viral proteins. Also, the analysis of the Golgi demonstrates that oAC is mainly build by the C2-C7 Golgi cisternae, whereas the iAC is composed of membrane intermediates derived at the interface the Golgi and post-Golgi linker compartments that are oriented toward the cell center.

Conclusion: We have shown that unlinking od Golgi ribbon and transformation of cis and medial Gogli cistens in Golgi stacks in the early phase of MCMV infection are the earliest events in the AC biogenesis.

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POSTER -9

Adverse effect of gaseous and particulate emissions on the concentration of BTEX in humans

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Introduction: Nowdays in majority of metropolises urban air pollution represents a common and difficult problem with high levels of traffic congestion consequently leading to release great amounts of genotoxic substances. Volatile organic compounds (VOCs) play an important role in ecological damages, disturbing the ecosystem and human health. It is well-known that the blood-air relationships of benzene, toluene, ethylbenzene, and the xylenes (BTEX) were dependent by smoking, exposure-smoking interactions, and by gender and age, while the other VOCs were not.
The objectivities and methods of the study: to determine the correlation between the concentration of BTEX in urine and respiratory illnesses in people from industrial area in comparison with the subjects from rural fields.
Results: people living in urban industrial areas have significantly diminished values of PIF (inspiratory peak flow), FIVC (inspiratory forced vital capacity), FIV1 (volume inspired in the first second of the test) and FVC (forced vital capacity) and significantly increased the concentration of BTEX (o-ksilen, benzene, toluene and etilbenzene).
Conclusion: BTEX-analyze in urine, as an important and easy method represents a good marker in environmental monitoring which contributes better protection of air pollutants and quicker diagnosis of COPD and asthma.

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POSTER -10

Role of Arf GTPases in MCMV transport to the cell nucleus

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INTRODUCTION

Cytomegaloviruses (CMVs) are widely spread DNA viruses from herpesvirus family that cause massive reorganization of the cellular functions in the early phase of infection. Reorganization of the membranes and cellular cytoskeleton finally results in formation of a large, cylindrical, juxtanuclear compartment with phenotype characteristics of several intracellular compartments- cytoplasmic virion assembly compartment (cVAC). Arf proteins are small GTPases that, by the recruitment of their effector proteins organize cellular cytoskeleton and change the membrane dynamics, and thus regulate traffic within secretory and endocytic system, cellular division and migration. Considering that the role of Arf proteins in pathogenesis of CMV infection is still poorly understood, the aim of this study was to determine how impaired function of Arfs affects MCMV infection, especially MCMV entry from the plasma membrane to the cell nucleus.

MATERIAL AND METHODS

Murine embryonic fibroblasts (Balb/3T3) were infected with recombinant murine cytomegalovirus Δm138-MCMV in conditions of impaired Arf function (siRNA or chemical inhibitors) and 0, 6, 16, and 30 hours post infection expression of viral proteins was determined by WB. cVAC formation was determined by confocal microscopy. Viral replication was monitored also by C3X GFP MCMV.

RESULTS

Impaired function of Arf proteins by siRNA resulted in decreased expression of analysed viral proteins and lack of cVAC formation.

CONCLUSIONS

Arf proteins are important for MCMV transport from plasma membrane to the cell nucleus.

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POSTER -11

Expression profile of MMP 2 and 9 in patients with colorectal cancer

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Background: Matrix metalloproteinases 2 and 9 (MMP-2 and 9) belong to the family of enzymes that commonly called matrix metalloproteinases. There are a total of 24 more or less explored matrix metalloproteinases, which are primarily divided by the structure of their domain. Gelatinize B (MMP-9) is synthesized in many cell types, such as: keratinocytes, monocytes, tissue macrophages, polymorphonuclear leukocytes and many types of tumor cells. The intensity of release of active enzyme is dependent on the amount of the enzymes stored in granules of these cells. MMPs comprise a large family of structurally related zinc metalloendopeptidases with different substrate specificities and possibilities to degrade protein constituents of the extracellular matrix. Statistically significant expression of matrix metalloproteinase-9 is demonstrated in various cases of: lung cancer and in inflammatory conditions, where is involved in many processes of proliferation, differentiation and migration of mast cells.
Patients and methods: we hypothesized that circulating levels of MMP-9 were abnormal in patients with colorectal cancer and these levels were compared with those in matched controls. The method of enzyme immunoassay (ELISA) was used to determine enzyme expression of matrix metalloproteinase-9 (MMP-9).
Results: The concentration of MMP 2 and 9 are significantly increased in colorectal cancer patient in comparison with healthy controls.

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POSTER -12

Size-exclusion chromatography based separation of extracellular vesicles from cerebrospinal fluid of severe traumatic brain injury patients

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Introduction: Extracellular vesicles (EVs) are cell-secreted membranous nanoparticles found in nearly every human body fluid, including blood and cerebrospinal fluid (CSF). In the last two decades, EVs have gained immense interest, both as mediators of intercellular communication and as a potential source for biomarkers. However, EV isolation and characterisation proved to be challenging due to EV nanosize and high variability in composition and physical traits. Recent progress shows that method of choice to purify intact EVs in sufficient amounts and purity might be size-exclusion chromatography (SEC). SEC encompasses a vast range of possible designs including different stationary phases, separation lengths and mobile phases. Although several SEC designs have been employed with various success, standardized SEC and its applicability for different body fluids is still lacking.
Objective: To compare commercial and in-house SEC methods for isolation of EVs from CSF samples of patients with severe traumatic brain injury (TBI).
Materials and methods: CSF samples will be collected on days 1-10 after injury from severe TBI patients while they are requiring external drainage for monitoring intracranial pressure. SEC will be performed using gravity-flow columns packed with different stationary phases. To compare the efficiency of separation, collected SEC fractions will be analysed by measuring: 1) concentration and size by qNano; 2) morphological appearance of EVs by electron microscopy; 3) concentration and enzymatic activity of EV protein markers, and 4) total protein concentration by Bradford assay.
Results: The results of this study are expected to provide a novel protocol for efficient EV isolation and identification from clinical CSF samples.
Conclusion: Isolation of intact EVs from CSF and their thorough characterisation will provide a better insight into EV subpopulations distinctive for the central nervous system. Such characterisation of intact EV will also enable future functional assays to better understand cell communication during brain recovery.

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POSTER -13

Comparation of enzyme expression of matrix metalloproteinase 2 and 9 (MMP 2 and 9) after total hip joint endoprosthesis by minimally invasive and classical approach

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Introduction: The anterolateral minimally invasive approach in total hip endoprosthesis, compared to the classic operative approach causes less tissue damage and stress for the organism. An increase in perioperative cytokine level has been demonstrated to correlate with surgical trauma. Nonetheless, these findings may however be dependent on the surgical approach adopted and the degree of soft tissue dissection. For instance, in a mini-posterior if the femoral head can be excised without dislocating the hip, there will be less trauma to muscles and capsule unlike the anterolateral approach
The aim of the study: In this investigation, for the first time, we want to examine differences in early tissue recovery following the changes in expression of the matrix metalloproteinase 2 and 9 (MMP-2 and 9) enzymes and their tissue inhibitors in urine by ELISA method.
Results: Our preliminary data has shown that the patients underwent anterolateral minimally invasive approach in total hip endoprosthesis have diminished values of matrix metalloproteinases in urine in comparisom with those patients who had classicall approach, suggesting that minimally invasive technique is accompanied with less damages of soft tisue and shorter recovery period.

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POSTER -14

The influence of different anaesthetic approaches on the immune response

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Introduction: The immune response during the perioperative period can be modulated by trauma, pain, hypothermia, blood transfusion fear/anxiety, medication, hypo/hyperglycaemia, as well as infection. Anaesthesia can influence the stress response by central modulation (general anaesthesia), afferent blockade (regional anaesthesia), or by interaction with the endocrine system. The anaesthetic technique have a big influence on cancer cells and their possible dissemination.

The aims of this study: was to review the effects of regional anaesthesia in comparison to general anaesthesia (intravenous and inhalational) on the immune response to surgical stress and malignant disease progression.

Nowdays, it is wellknown that general anaesthesia can suppress cellular and humoral immune response by acting on immune-competent cells, gene expression and secretion of inflammatory mediators. Opioids as well as their mode of administration have shown a different effect on the immune system: immune suppression and immune stimulation or both.Contrary, local anaesthetics are very good in treating acute and chronic inflammation because they deactivate inflammatory processes on different levels, accompanied with the activation of monocyte-macrophage system, decrease of oxygen metabolites and free radical formation.

Conclusion: Thanks to these significant advantages, the use of regional anaesthesia is widespread in oncological surgery.

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POSTER -15

Quality of life in patients after 8 years of total hip arthroplasty with minimally invasive or classic approach

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Introduction: The modern, specially adapted hip endoprothesis which is implanted by a minimally invasive approach enables the faster functional recovery of the patients. Replacing the hip joint with an artificial joint is a routine procedure considered to be the highest quality endoprosthetic orthopedic surgery in general. It is also the most commonly implanted endoprosthesis today.
The goal of this reserch is to investigate the differences in quality of life and incidence of limping 8 years after total hip arthroplasty with a minimally invasive or a classical approach.
Patients and methods: A cross-sectional retrospective study included 68 patients underwent an elective surgery for a hip replacement. 36 patients were operated with the minimally invasive while 36 patients with the classical approach. All patients completed the SF-36 questionnaire (Short Form Survey Instrument).
Results: The higher number of limping patients in classical approach group may contribute to the differences in quality of life. Minimally invasive approach enable a long-term quality of life and a better functional recovery.

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POSTER -16

Cytokinesis failure in mcmv infection

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INTRODUCTION: Mouse cytomegalovirus (MCMV) is a member of *Herepesviridiae* family. Once it infects the host cell, it alters its life cycle and functions to promote viral replication and virion production. The infection interferes with normal cellular processes including cell division. Here we investigated the changes that may lead to cytokinesis failure in cells infected with MCMV.
OBJECTIVES: The aim of this study was to elucidate the steps that lead to cytokinesis failer during MCMV infection.
MATERIALS AND METHODS: Balb3T3 fibroblasts were infected with recombinant MCMV, Δm138-MCMV, and the samples were taken at different time points during the infection. Intracellular distribution and expression of protein markers was analysed by immunofluorescent confocal microscopy. Percentage of binucelar cells was calculated in comparison to total cell number. Transcriptome analysis of the genes connected to cytokinesis was performed at 3 and 18 hours post infection. Cells infected with MCMV were recorded on Nanolive 3D Cell Explorer-fluo.
RESULTS: MCMV infection leads to formation of binucelar cells in up to 8% of the infected cells during the first hours of infection. Transcriptome analysis of MCMV infected cells shows downregulation of several genes responsible for different phases of cytokinesis. Dysregulation of Golgi apparatus can be seen as transformation of Golgi ribbon into Golgi stacks. Probable dysregulation of cleavage plane formation is demonstrated by Arf6 and Epi64 accumulation. The formation of the γ tubulin rings occurs in more copies than in cells that undergo regular cytokinesis with no detectable mitotic spindles.
CONCLUSION: MCMV infection leads to cytokinesis failure probably by several mechanisms: downregulation of transcription of genes with role in different phases of cytokinesis, dysregulation of Golgi apparatus and dysregulation of cleavage plane formation.

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POSTER -17

Assessment of the role of heat protein 70 in patients with atherosclerosis of carotid arteries

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Background: Atherosclerosis represents an inflammatory, autoimmune, and chronic metabolic disorder of the vessel wall, with genetic and environmental factors in its etiopathogenesis. Three proteins play a key role as autoantigens: heat shock proteins (HSPs), oxidized low density lipoprotein (oxLDL) and beta 2 glycoprotein 1 (b2GP1).
The aim of this study: was to examine the interactions between the changes in clinical status and the expression of hsp 70 in patients with mild atherosclerosis (A patients) and with carotid arteries stenosis (CAS) patients who were undergoing the surgical procedure.
Patients and methods: Patients were selected from a stratified sample of the population of adult patients of both sexes with diagnosed atherosclerotic changes in medium and large-sized arteries. Immunohistochemically expression of the heat shock 70 (hsp 70) protein on paraffinic atherosclerotic alterations of carotid arteries were done.
Results: our data have shown an increased expression of heat shock protein 70 in atherosclerotic-modified carotid arteries.
Conclusion: Heat shock proteins (HSPs) may act as autoantigens and consequently stimulate cellular and humoral immune responses. The expression of hsp 70 correlates with the clinical stages of the disease.

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POSTER -18

Effect of n-3 polyunsaturated fatty acid-enriched chicken eggs consumption in patients with known atherosclerotic coronary artery disease on pro-inflammatory cytokines

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INTRODUCTION: Chronic inflammation plays a key role in the pathogenesis of atherosclerosis and other cardiovascular diseases. N-6 and n-3 polyunsaturated fatty acids (PUFAs) are precursors of eicosanoids, lipid mediators of inflammation. Eicosanoids from n-6 PUFA (e.g. arachidonic acid) have pro-inflammatory and immunoactive activity, whereas eicosanoids from n-3 PUFA (e.g. eicosapentanoic and dochexanoic acid) have anti-inflammatory properties.
AIM: The aim of this study was to determine serum concentrations of inflammatory cytokines and hsCRP in chronic cardiovascular patients of both sexes (n_{total} = 19) before and three weeks after consumption of standard eggs (control group, n = 9) or n-3 PUFAs enriched chicken eggs (experimental group, n = 10), and examine their interrelation.
MATERIALS AND METHODS: The subjects consumed three boiled eggs daily for three weeks, the total amount of n-3 PUFA per day was 249 mg for the control group, and for the experimental group 1053 mg n-3PUFA / day. The concentrations of inflammatory cytokines IL-1α, IL-10, IL-6, MCP-1 and TNF-α were determined by the ProCarta Multiplex kit on the Luminex device. For group comparisons a t-test was used, while within-group comparisons were performed using paired t-test or Wilcoxon test, statistical significance was set as $P < 0.05$. The study was approved by the Ethics Committee of the Clinical Hospital Center Osijek, and all subjects signed an informed consent.
RESULTS: hsCRP was significantly decreased after consumption of n-3 PUFAs enriched eggs, whereas it increased significantly when consuming ordinary eggs. The results show no significant difference in the concentrations of proinflammatory cytokines before and after the consumption of n-3 PUFA enriched eggs or in comparison with the consumption of ordinary eggs. However, there was a

difference in the relative change (delta) of IL-1α and hsCRP, which decreased significantly more in the experimental group compared to the control group. Furthermore, IL-1α concentration positively correlated with hsCRP in both groups.
CONCLUSION: These results suggest that the consumption of n-3 PUFAs enriched eggs reduces the tendency for inflammation.

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Keywords: *cytokines, functional food, atherosclerosis; coronary artery disease, n-3 PUFAs, inflammation*

POSTER -19

Immunological changes in follicular fluid of women with thyroid autoimmuniy

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Background: Thyroid autoimmunity (hyper-and hypothyroidism) in women in reproductive age are very often accompanied with increased risk for infertility, as well as, adverse pregnancy outcomes.
Aim of the study: to estimate the influence of the serum concentration of thyroid hormones, antithyroid antibodies, ultrasonographic examination of thyroid gland, the presence of thyroid antibodies and changes in immunological status in follicular fluid of women with thyroid autoimmunity.
Patients and methods: The mononuclear lymphatic cells from follicular fluid as well as from peripheral blood were analysed by flow cytometry and compared between the infertile patients and the patients with normal hormone levels, negative thyroid antibody tests and normal ultrasound findings.
Results: There was a significantly lower proportion of natural killer and natural killer T cells in the blood and in the follicular fluid of the patients with positive thyroid autoantibodies compared to patients with no thyroid autoantibodies.
Conclusion: The presence of anti-thyroid antibodies and diminished percentage of innate immune cells in follicular fluid may play a critical role in female infertility related to thyroid autoimmunity.

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Notes