Nutraceuticals in balancing redox status in ageing and age-related diseases

WGs Meeting of the NutRedOx COST Action CA16112

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Book of Abstracts

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The word of welcome

Dear colleagues,

We would like to welcome you to the 3rd Group meeting within the NutRedOx CA16112 COST Action, which is entitled: “Nutraceuticals in balancing redox status in ageing and age-related diseases”. We hope that this gathering will enable us to shed more light on the healing nature of proper nutrition. Since ancient times, food was regarded as something more than a fuel for survival. The Greek doctor Hippocrates once said: “Let food be thy medicine and medicine be thy food.” Nutraceuticals or “nutritional medicines” could be the answer to difficulties encountered during aging, without neglect of official medications. In a society living longer than ever, health has become one of the most valuable assets. It would be comforting to know that in the near future old age is not associated with deteriorating quality of life.

This COST action was initiated in 2017, as a consortium of countries and scientists whose primary goal was to “focus on the impact of redox active compounds in food on healthy ageing, chemoprevention and redox control in the context of major age-related diseases”. By now, 34 COST participating countries and 6 Near Neighborhood Countries took part in this project, showing that there is great interest in this problem.

We are pleased that you have decided to take part in this mutual conversation, where many will present their recent work, through poster sessions, oral communications or simply by asking questions. One of the goals of this action is cooperation between laboratories by short term scientific missions, so we look forward hearing the results of these encounters. Although we are approaching the end of this joint venture, it is satisfying to know that participants are not yet tired, which is supported by the number of registrations and abstracts that will be presented. On this meeting 67 participants from 24 countries will take part.

Belgrade, an old city which is always young, embraced by two rivers, will be your host. We hope that you will enjoy its rugged charm and warm hospitality, its streets, restaurants and cultural heritage.

At the confluence of new ideas and experiences we again wish you a warm welcome.

Your Local Organizing Committee

INVITED LECTURES

IL1. EFFECTS OF NUTRITION ON EPIGENETIC REGULATION OF AGING

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Aging and the development of age-related complex diseases is controlled by a hallmark of molecular mechanisms where epigenetic regulation is considered to be involved in the regulation of telomere attrition, gene expression, autophagy, senescence and DNA repair. Fasting, but also plant ingredients such as anthocyans, EGCG, spermidine have been shown to modulate epigenetic regulation of these mechanisms by modification of AMPK, SIRT and mTOR pathways. Furthermore, microbiota-derived butyrate and BHB have strong effects on epigenetic methylation, histone deacetylation and miRNAs. We analyzed markers for aging such as telomere length, mitochondrial DNA, expression of IL6, TNFa, epigenetic DNA methylation and miRNAs, DNA breaks and DNA repair, GI microbiota composition as well as autophagy and senolysis in human, cell models, and high fat diet mouse model. Supplements included shots with high EGCG or sirtuin-activating plant ingredients. Hight fat diet induced high amounts of DNA breaks in various tissues which could be significantly reduced by fasting, EGCG, gallic acid in mice. A polyphenol rich diet increased expression of DNA repair enyzyme MLH1 and shifted GI microbiota towards a beneficial *Prevotella* dominated phenotype. In vitro and in a human study including a drink with high EGCG content we found increased length of telomers correlating with altered changes of cMYC and hTERT, and improved epigenetic markers of aging. Expression of microRNAs related to inflammation was improved. EGCG and butyrate shows significant effects of BRDU induced senescence in fibroblasts and on expression of genes with relevance for autophagy and senescence. In conclusion, fasting diets which induce ketogenesis and production of short chain fatty acids as well as food additives which modify SIRT and mTOR pathways hold the promise to improve healthy aging and health prevention especially for metabolic related diseases. To fully implement an individualized, preventive health care, analysis of aging and epigenetic parameters could help to guide an individualized health prevention.

IL2. POLYPHENOLS AND COGNITIVE HEALTH: THE "GUT-BRAIN AXIS" BEHIND THE (HAPPY ENDING) STORY?

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Globally, the population is ageing, and it is estimated that by 2050 there will be more than 2 billion people aged over 60 years. This demographic age-shift has been accompanied by an increase in cognitive dysfunction ranging from mild cognitive impairment (MCI) to dementia. Several epidemiological studies pointed out that lifestyle, and particularly dietary habits, may have a role in the prevention of such chronic diseases. Specifically, (poly)phenol-rich foods including nuts, berries, grapes, cocoa, red wine and tea beverages have been suggested to be associated with reduced cognitive decline. These foods are particularly rich in flavan-3-ols, among which (epi)catechin and procyanidins are the most represented. However, these compounds are poorly absorbed in the upper gastro-intestinal tract and are metabolized by colonic bacteria to phenyl-γ-valerolactones (PVLs), which show biological activities and might represent better dietary exposure biomarkers compared to (epi)catechin conjugates, as they reach higher plasma Cmax and tmax. These compounds might be the protagonists of the so called "gut-brain axis": this theory hypothesizes that gut microbial (poly)phenols metabolites reach the brain from the gut to activate signaling cascades and to generate molecular signals that return to the gut and other target organs. In the recent years our group, in collaboration with several national and international groups, was deeply involved in unraveling the putative role of PVLs in the maintenance of brain health. In vitro and in vivo studies have been performed to demonstrate the ability of PVLs to cross the blood brain barrier and reach the brain cells. Then, in vitro models of dementia have been set-up to investigate whether PVLs might have a role in the prevention and progression of the disease.
Finally, the VALID "Valerolactones and healthy Ageing: LInking Dietary factors, nutrient biomarkers, metabolic status and inflammation with cognition in older adults" project aimed to validate PVLs as stable biomarkers of catechin and procyanidin-rich foods and demonstrate their association with markers of inflammation and metabolic health status, and ultimately cognitive function in the Trinity Ulster Department of Agriculture Ageing Cohort (TUDA) cohort, comprising 5,186 adults aged 60-102 years with mild cognitive decline from the two countries within the island of Ireland. In conclusion, an increasing amount of literature is focusing on the neuroprotective effect of polyphenol gut microbial metabolites, with supporting evidence. However, further studies are needed to clarify the molecular mechanisms underneath PVLs blood brain barrier crossing, the inter-individual variability in the production of such metabolites and, more importantly, the biological activity at brain level.

IL3. FLAVANOIDS MODULATE ENDOTHELIAL FUNCTION THROUGH COMPLEX MOLECULAR MECHANISMS OF ACTION

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Accumulating studies suggest the cardioprotective properties of flavonoids, phytochemicals widely present in plant-derived foods. Flavonoids seem to reduce cardiovascular diseases risk through the improvements in endothelial function and reduction of inflammation and atherosclerosis development. Data are particularly convincing for berry flavonoids, namely anthocyanins. However, their molecular mechanisms of action are not fully understood. Therefore, this study aimed to investigate the impact of blueberry anthocyanins and their gut metabolites (shared metabolites with other flavonoids) on endothelial cell function using omics approaches. Endothelial cells were exposed to delphinidin-3-glucoside, peonidin-3-glucoside, cyanidin-3-arabinoside, cyanidin-3-galactoside, cyanidin-3-glucoside, or gut metabolites: vanillic, ferulic, protocatechuic, and hippuric acid, at physiological levels. Inflammation was induced with TNFα and monocytes added to assess cell adhesion and migration. The effects on endothelial cell gene and miRNA expressions and cell signalling were evaluated. Examined compounds induced significant 1.3-fold and 1.9-fold reductions in the adhesion and transendothelial migration of monocytes, respectively. Gene expression analysis showed that anthocyanins and gut metabolites modulated the expression of various genes implicated in the regulation of cell motility, focal adhesion, cytoskeleton reorganisation, and chemokine signalling. This was accompanied by reduced phosphorylation of transcription factor Nf-κB-p65 and ERK signalling protein, the effects that were in line with the molecular docking results. Tested compounds also significantly modulated the expression of various miRNAs, particularly those regulating endothelial permeability to immune cells and atherosclerosis development, contributing to the observed changes in endothelial function. Integration of these results showed that anthocyanins and their metabolites improve endothelial function trough multi-target and multi-layered mode of action.

ORAL COMMUNICATIONS

O1. BENEFICIAL EFFECTS OF *Centaurium erythraea* EXTRACT ON GLYCEMIC CONTROL AND INSULIN LEVEL IN DIABETIC RATS

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Centaurium erythraea (CE) is traditionally used for diabetes treatment due to its anti-diabetic properties. Previously we have reported that the major constituents of CE methanol extract (CEE) are secoiridoids and polyphenols. Here we analyzed the protective effect of CEE on pancreatic β-cells in streptozotocin (STZ)-induced diabetic rats. CEE (100 mg/kg) was administered daily and orally to control or diabetic rats for two weeks before diabetes induction, during five days of STZ treatment (40 mg/kg/day), and for four weeks after last STZ injection (pre-treated group), or for four weeks after diabetes induction (post-treated group). Histological and immunohistochemical examination of the pancreas revealed disturbed morphology of pancreatic islets, a decrease in their number and size which was accompanied by the reduction of insulin-positive β-cells in diabetic rats when compared to control or control/CEE-treated rats. Islet morphology and mass, as well as the number of insulin-positive β-cells, were improved in CEE-treated diabetic rats, particularly in a pre-treated group. In pre- and post-treated groups we observed the increase of GLUT-2 transporter and p-Akt kinase, that were absent in diabetic pancreas pointing to impaired glucose-stimulated insulin secretion in remnant β-cells. CEE-mediated increase of β-cell mass, GLUT-2 and p-Akt levels in diabetic rats contributed to the elevation of serum insulin level and reduction of glucose level which was more prominent in pre- than in a post-treated group. The results of this study suggest that improved insulin production and glycemic control in CEE-treated diabetic rats may result from the structural/functional preservation of pancreatic islets.

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Grant No. 173020
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O2. INTERACTION OF FIBRINOGEN WITH BIOLOGICALLY ACTIVE SMALL LIGANDS

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Fibrinogen is a plasma protein whose main biological role is in hemostasis, both primary and secondary. This protein is the most susceptible plasma protein to oxidation. Oxidatively modified fibrinogen has thrombogenic characteristics such as the formation of fibrin clots with thinner fibers, reduced permeability and reduced fibrinolysis rate. For this reason, it is relevant to investigate possible interactions of fibrinogen with small molecules that are potent antioxidants and to reveal their capacity to protect this protein from oxidation and their effect on the formation of the fibrin clot. Our tested ligands, resveratrol, dihydro-alpha-lipoic acid and bilirubin bind purified fibrinogen with moderate affinity as determined by spectrofluorimetry. Detected ligands had no significant effect on the structure and stability of fibrinogen as determined by several spectroscopic techniques. Dihydro-alpha-lipoic acid and bilirubin influenced the formation of fibrin clot, as formed fibrin fibers are thicker. Thicker fibers are usually associated with more permeable clots which are lysed more quickly by plasmin. All of the tested ligands were capable to protect fibrinogen from free radical-induced oxidation. In the presence of fibrinogen, bioavailability/solubility of resveratrol in aqueous environment was increased. Mutual protection from the oxidation of fibrinogen and resveratrol was detected in the case of their complex formation. Thus, interactions between fibrinogen and analyzed small molecules have mutual benefits, protecting partners in complex formation from harmful oxidation and increasing the bioavailability of ligands which, in turn, increases their antioxidant potential.

This research work was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia, and is part of the activity within COST action CA16112 - Personalized nutrition in aging society: redox control of major age-related diseases.

O3. APPLYING A PEPTIDOME BASED KINASE ACTIVITY PROFILING PLATFORM IN ALZHEIMER'S DISEASE MODELS

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Alzheimer's dementia (AD) is an important medical and social problem in elderly population worldwide. Today, kinase inhibitors acting upstream or downstream of amyloid-β signaling hold promise as effective strategy to prevent or attenuate AD progression. However, little is known about the regulation of protein kinase activity in the disease's pathogenesis. We sttudied the Serine/ Threonine/ Tyrosine kinase activity and the related signal transduction pathways in different regions of the brain linked to AD; and explore the possibility of using kinase activity changes in this context as possible disease biomarker or as target for therapeutic interventions. Protein lysates were prepared from cortex and hypothalamus tissue of different AD mice models: APP23, Tau 58/4 and Tg 2576, at both pre-plaque and post-plaque stages). The activity of Serine/ Threonine and Tyrosine kinases was assayed using the PamChip-peptidome platform. Data analysis was performed with the Bionavigator software and Enrichment pathway analysis was performed using WEB-GesTAlt. Activity of FYN ad FER Tyrosine and RSK1, MSK1, AMPK and p70S6K Serine/Threonine Kinase families was increased in AD compare to wild type mice. which are related to synapse, Axon and neuronal processes. Kinase activity in the cortex and hippocampus regions of post-plaque APP23 mice was higher than the one observed in animals without plaques. The pathways linked to theses kinases were related to regulation of oligodendrocyte precursor and survival, and synapse, Axon and neuronal processes and others. Peptide phosphorylation changes may be considered as a future biomarker tool for the disease.

O4. FROM DIETARY RESVERATROL TO DERIVATIVES. ATTEMPTS FOR NEW TARGETS AND FOR IMPROVEMENT OF BIOAVAILABILITY

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Resveratrol is considered as one of the most interesting dietary and natural preventive agent against physiological disorders. To overcome the problem of its limited efficiency due to its poor bioavailability and its pleiotropic effects, it is interesting to investigate new strategies involving chemical derivatives of the parental molecule. Due to this, structural modifications of resveratrol analogues combined with structure-activity relationships and computing docking studies should enable to limit the clearance and to explore new molecular targets (1-5). For instance, we previously reported synthesis of a polymethoxy stilbenes analogues: *cis* (*Z*)-3,5,4'-trimethoxystilbene, exhibiting strong antiproliferative activity by acting as inhibitor of polymerization of tubulin (1). The relative weak effect of the natural *trans* (E)-RSV is not exclusively due to its high metabolic rate and efflux since the masking of hydroxyl groups by methylation does not improve significantly resveratrol analogue efficacy (2). Moreover, most of the synthetic methylated derivatives stop mitosis and lead to polyploid cells, while *trans* (*E*)-resveratrol inhibits cells in S phase. Conversely, methylation of *trans* (*E*)-resveratrol significantly decreases the efficiency. Resveratrol analogues open new perspectives for new targets and for improvement of bioavailability, and in the inhibition of cell proliferation (3-5).

References

1-Mazué F, Colin D, Gobbo J, Wegner M, Rescifina A, Spatafora C, Fasseur D, Delmas D, Meunier P, Tringali C, Latruffe N. Structural determinants of resveratrol for cell proliferation inhibition potency: experimental and docking studies of new analogs. *Eur J Med Chem*. 2010 Jul;45(7):2972-80. 2-Chalal M, Delmas D, Meunier P, Latruffe N, Vervandier-Fasseur D. Inhibition of cancer derived cell lines proliferation by synthesized hydroxylated stilbenes and new ferrocenyl-stilbene analogs. Comparison with resveratrol. *Molecules* 2014 Jun 11;19(6):7850-68. 3-Latruffe N, Vervandier-Fasseur D. Strategic Syntheses of Vine and Wine Resveratrol Derivatives to Explore their Effects on Cell Functions and Dysfunctions. *Diseases*. 2018 Dec 11;6 (4). 4-Vervandier-Fasseur D, Latruffe N. The Potential Use of Resveratrol for Cancer Prevention. *Molecules*. 2019 Dec 9;24 (24). 5-Vervandier-Fasseur D, Lizard G, Latruffe N. Aza- and Azo-stilbenes: bio-isosteric analogs of resveratrol. *Molecules*. 2020 (accepted).

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O5. THE ROLE OF P66SHC SIGNALING AXIS IN DIALLYL TRISULFIDE INDUCED CELL DEATH AND FORMATION OF REACTIVE OXYGEN SPECIES

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Diallyl trisulfide (DATS) which is highly promising anticancer constituent of garlic has been shown to induce cancer cell death and cell cycle arrest and these are accompanied by increase formation of reactive oxygen species (ROS). The purpose of this study is to evaluate the role of adaptor protein P66Shc and iron in DATS induced signaling in prostate cancer cell lines. PC-3, DU145 prostate cancer cells and PNT1A noncancerous epithelial cell line were used in this study. Stable cell lines expressing p66ShcS36A or an empty vector have been obtained. Cell viability, level of ROS, changing in P-p66Shc, ferritin H, ferritin L, P-Akt and DNA damage were determined. We observed that DATS treatment increased p66Shc phosphorylation at serine 36 in cancer cells but the changes were minute in PNT1A cells. Cancer cells expressing plasmid-encoded variant of p66ShcS36A showed much higher resistance to DATS-induced cell death and ROS formation. In addition, DATS-induced Akt inactivation and ferritin degradation was blunted in cancer cells expressing p66ShcS36A and in normal PNT1A cells. In conclusions, our results uncover a novel signaling pathway with p66Shc being indispensable for DATS-induced inactivation of Akt, ferritin L and H degradation, increase in labile iron pool and ROS formation in prostate cancer cells.

O6. ANTHOCYANINS: FROM BIOLOGICAL PROPERTIES TO FLUOROPHORES FOR CELL IMAGING

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Anthocyanins form a class of glycosylated/hydroxylated derivatives of 2-phenylbenzopyrylium salts. These pigments are responsible for most of the wide variety of yellow to blue colours displayed by flowers, fruits and leaves of many plants, where they are naturally produced[1]. They provide the plant with protective functions and are of increasing interest because of their implications for the maintenance of human health. Their main property is antioxidant activity, which plays a key role in the prevention of a myriad of human non-infectious diseases and infections (stress conditions, inflammation, neuronal and cardiovascular complications, cognitive decline, liver damage, tumor proliferation or diabetes)[2]. In the first part of investigations, we evaluated the effects of anthocyanins on erythrocytes isolated from normal and β-thalassemic subjects. The erythrocytes were treated *in vitro* with TBHP, thus promoting oxidative events that mimic the pathophysiological pathway leading to cell hemolysis. Cell viability, reduction of glutathione and malondialdehyde levels were measured. Radical scavenging capacities of these pigments were also estimated. In the second part of investigations, we demonstrated how subtle structural modifications can lead to bright ratiometric pH and/or redox fluorescent probes. Reversible pH detection is provided by phenolic substituents, while response to redox alterations is obtained by fusing an electroactive scaffold to a fluorescent skeleton[3]. Selection of the best candidate fluorophores was performed using a virtual dye library and optimization was guided by imaging of living cells (e.g., plant, RBC and cancer cells). These bioinspired functional dyes were designed to answer critical biological questions, e.g., study of *P. falciparum* and understanding of MoA of antimalarial drugs[4].

[1] a) R. Brouillard, P. Figueiredo, M. Elhabiri, O. Dangles, "*Proceedings of The Phytochemical Society of Europe-41: Phytochemistry of Fruit and Vegetables*", Eds Tomas-Barberan et Robins, Oxford University Press, **1997**, Chapter 3, 29-49. b) Iwashina, T., *Natural Product Communications*, **2015**, *10*, 1934578X1501000335.
[2] Dini, C.; Zaro, M.J.; Vina, S. Z., *Current Bioactive Compounds*, **2019**, *15*, 507-523.
[3] Sutter, A.; Elhabiri, M.; G. Ulrich, *Chemistry-A European Journal*, **2018**, *24*, 11119-11130.
[4] Jida, M.; Sanchez, C.P.; Mounien, S.; Urgin, K.; Ehrhardt, K.; Elhabiri, M.; Lanzer, M.; Davioud-Charvet, E., *ACS Infectious Disease*, **2017**, *3*, 119-131.

O7. MAY THIRTEEN WEEKS OF OCTACOSANOL SUPPLEMENTATION AFFECT PROOXIDANT-ANTIOXIDANT BALANCE IN PATIENTS ON ATORVASTATIN THERAPY

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Statins reduce the risk of cardiovascular disease mainly due to cholesterol reduction, but also pleiotropic effects are present. Co-administration of statins with bioactive molecules may obtain a synergistic benefit. The aim of this randomized, placebo-controlled, double-blind study was to investigate whether 13-week long supplementation of policosanol affected antioxidant status and markers of oxidative stress in patients on atorvastatin therapy. Eighty-seven patients aged 40–80 years on chronic (>4 months) atorvastatin therapy (20 mg) were randomly allocated to policosanol (n=42) or placebo (n=45). At baseline, after 8 and 13 weeks, markers of oxidative stress and antioxidant defence include total antioxidant status (TAS), total oxidant status (TOS), advanced oxidation protein products (AOPP) and activity of superoxide dismutase (SOD) were measured. Creatinine phosphokinase and hepatic enzymes were the main safety endpoints. The mean age of the patients was 62.6 ± 0.8 years. Patients in supplemented group had higher TAS and TOS levels 1640 (963–1752) vs 927 (481–1305), [μmol/L], $p < 0.001$ and 18 (13—20) vs 6 (5–11), [μmol/L], $p < 0.001$], respectively, compared to baseline. In placebo group, subjects had also higher TAS and TOS levels after 13-week compared to baseline. SOD and AOPP were not influenced by the intervention. The investigated supplement possessed a good safety profile. Combination of policosanol with atorvastatin may be useful in an attempt to avoid potential adverse effects associated with statins. 13-week supplementation was not sufficient to induce remarkably changes in SOD and AOPP. TAS and TOS were increased in both groups.

O8. STUDIES ON S-NITROSATION IN HEK293 CELLS AFTER TREATMENT WITH NO DONORS

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Oxidative stress and metabolic inflexibility are well-known hallmarks of the ageing process. The human forkhead box, class O (FOXO) proteins are involved in the regulation of both redox homeostasis and fuel metabolism. As targets of the insulin signaling cascade, FOXOs regulate the expression of genes encoding key proteins in carbohydrate and trace element metabolism. On the other hand, FOXO activity is affected by oxidative stress through posttranslational modifications, including oxidation of redox-sensitive cysteines. As a premise to elucidate such modifications of FOXO1 in the future, we here tested for the S-nitrosation of proteins in the human embryonic kidney cell line HEK293. HEK293 cells grown in 6-well plates were exposed to varying concentrations of S-nitroso-glutathione (GSNO) and S-nitroso-N-acetyl-L-cysteine (NACNO). After 1 h, cell lysates were analyzed for nitrite and for nitrosated proteins using 2,3-diaminonaphthalene in the absence or presence of mercuric (Hg^{2+}) chloride, respectively. Both GSNO and NACNO caused a concentration-dependent increase in nitrite levels in exposed HEK293 cells. GSNO treatment resulted in a slight increase in nitrosation, albeit with high variation among experiments. Nevertheless, these findings were somewhat surprising, as, according to literature, the expression of γ-glutamyl-transferase, an enzyme enhancing the release of NO from GSNO, is very low in HEK293 cells. Considerably less variation was observed after exposure to NACNO, and general protein nitrosation was well detectable using 100 µM of NO donor. Taken together, we demonstrate the suitability of cultured HEK293 cells for the in-depth analysis of nitrosation of regulatory proteins such as FOXO transcription factors.

O9. DETERMINATION OF TOTAL LIPIDS AND FATTY ACIDS PROFILE IN COCOA-BASED PRODUCTS AND CONFECTIONERY PRODUCTS

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Confectionery and cocoa-based products represents food often consumed among young people, so is very important that they are health-safe and prepared from nutritionally valuable nutrients. This research analyzed the fatty acids (FA) composition of 25 different confectionery and cocoa-based products available in market. Confectionery products included: biscuits, waffles, tea cakes, chocolates, chocolate bars, sweet pastries, sweet crackers. The total fat (TF) content was determined using Soxhlet method and then FA composition was analyzed by gas chromatography. Obtained results showed very variable FA profile, linked to the difference in the products composition and to the conditions of production process. The TF content ranged from 12.6% (tea biscuits) to as high as 47.1% in waffles and chocolate. Discrepancies between the analyzed TF and declared values were observed. The average content of saturated fatty acids (SFA) was over 50% of total fatty acids content ranging from 3.9 g/100g to 23.6 g/100g (chocolate). The most prevalent SFAs were palmitic and stearic acids, and the maximum value for oleic acid was observed in chocolate-bar (9.9 g/100g). Linoleic fatty acid was the only polyunsaturated FAs identified, ranging from 0.7 g/100g to 5.2 g/100g (*elvita* cake). To evaluate the potential effects of fatty acids on the occurrence of atheroma and/or thrombus formation, atherogenic (AI) and thrombogenic index (TI) were calculated. Atherogenic index ranged from 0.2-2.6, while TI ranged from 0.8-8.1. Due to potentially harmful effects of SFA, it is necessary to limit the intake of these products, especially among children and adolescent population.

Key words: confectionery and cocoa-based products, fatty acid profile, atherogenic and thrombogenic index

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SHORT TERM SCIENTIFIC MISSIONS

S1. MEDITERRANEAN DIET AND DESIRED WEIGHT LOSS IN OVERWEIGHT ADULTS

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Background: Weight perception and ideal weight are likely to boost weight management.
Objectives: To assess the associations between adherence to the Mediterranean Diet and body image in an overweight adult Mediterranean population.
Methods: Cross-sectional analysis in 6561 participants aged 55-75, with metabolic syndrome from the PREDIMED-PLUS trial. Ideal weight loss was defined as the difference between reported ideal weight and actual measured weight at baseline. Body image was expressed as the percentage that ideal body weight loss represents over the actual weight and was categorized into four cut-offs of this percentage. Diet was assessed using a validated food frequency questionnaire and a 17-item Mediterranean dietary questionnaire.
Results: Ideal weight losses were higher among young participants and participants with high real or perceived BMI and abdominal obesity. Physical activity and adherence to the Mediterranean Diet were higher among participants seeking to lose up to 10% of their current weight compared to those seeking to lose over 15%. Items related to the consumption of vegetables, fruits, nuts, sugar sweetened beverages and red meat played a key role in adhering to the Mediterranean Diet.
Conclusions: The adherence to the Mediterranean Diet was lower among those whose desired weight loss represented a higher percentage of their current body weight, also showing lower dietary quality and physical activity.

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S2. BRAIN UPTAKE OF LOW MOLECULAR WEIGHT (POLY)PHENOL METABOLITES

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(Poly)phenols have been extensively studied considering their beneficial effects on brain's health, particularly in the context of neurodegenerative disorders. Importantly, the low molecular weight (LMW) (poly)phenol metabolites, that result from the metabolism of dietary (poly)phenols, are those most abundantly found in circulation but underexplored concerning their bioactivity and bioavailability in the brain. These LMW metabolites might be the main players of neuroprotective effects associated with (poly)phenols intake. However, the major limiting factor could be reaching the brain**.** The ability of LMW (poly)phenol metabolites to cross the BBB, of which much is still unknown, is a key step towards the neuronal tissue. From 72 small metabolites available in our lab, we selected the three most effective in attenuating neuroinflammation in microglial cells submitted to an inflammatory insult, and tested their ability to cross the BBB following both *in silico* and *in vivo* approaches. The predictive results from the model of permeation indicated that metabolites are able to passively cross the BBB endothelium. In living rats, after the intravenous injection of the metabolites through the dorsal penis vein, we have shown at different extents their ability to reach the brain, liver and kidney, and the concentration levels present in blood and urine, 15 seconds and 5 minutes after the administration. Despite the potential of metabolites to produce neuroprotective effects, their capacity to reach the brain has been the major limitation to assign them a biological function. Here we demonstrated the capacity of three relevant LMW metabolites to reach the brain in living organisms.

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S3. LIMBO PROJECT: LOW MOLECULAR WEIGHT (POLY)PHENOL METABOLITES AS MODULATORS OF MICROGLIA INFLAMMATORY RESPONSE

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Neurodegeneration resides on multifactorial changes with complex mechanisms and no existing cure. Nevertheless, evidences suggest that neuroinflammation is a key mechanism in the development of neurodegenerative diseases[1]. Prevention and treatment will require multi-targeted therapeutics with a focus on their anti-inflammatory properties. (Poly)phenols have proven such properties. However, for a huge number of (poly)phenols originating from our diet, much is still unknown about their low molecular weight polyphenol metabolites[2]. Blood concentrations for some of these low molecular weight (poly)phenol metabolites reach high concentrations and studies have shown their ability to reach the brain[3]. Yet, our understanding of their effects is still low. In this work we focus on the ability of several low molecular weight (poly)phenol metabolites, present in human circulation upon dietary interventions, to modulate neuroinflammation in microglia.[2] We have tested their ability to impair TNFα release by microglia cells upon an inflammatory stimulus. Dose response studies have been conducted and a kinetic study is undergoing. Meanwhile the mechanisms by which these molecules can alter TNFα release are being elucidated. In this respect we conducted a kinase microarray study and demonstrated the impact of these molecules on several key points of inflammatory pathways, such as NFκB, MAPK and Jak-Stat. In conclusion, we are deciphering the role of low molecular weight polyphenol metabolites at physiological relevant conditions, tracking their anti-inflammatory effects, and exploring the mechanism of action in microglia cells[4]. Altogether we hope to understand how these molecules could potentially be a useful tool to modulate neuroinflammation and limit neurodegeneration.

Acknowledgments

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(1) Figueira, I.; Fernandes, A.; Djordjevic, A. M.; Lopez-Contreras, A.; Henriques, C. M.; Selman, C.; Ferreiro, E.; Gonos, E. S.; Trejo, J. L.; Misra, J.; et al. Interventions for Age-Related Diseases: Shifting the Paradigm. *Mech. Ageing Dev*. 2016, 160, 69–92. https://doi.org/10.1016/j.mad.2016.09.009.
(2) Carregosa, D.; Carecho, R.; Figueira, I.; N Santos, C. Low-Molecular Weight Metabolites from Polyphenols as Effectors for Attenuating Neuroinflammation. *J. Agric. Food Chem*. 2019. https://doi.org/10.1021/acs.jafc.9b02155.
(3) Carecho, R.; Carregosa, D.; dos Santos, C. N. Low Molecular Weight Polyphenol Metabolites across the Blood-Brain Barrier: The Underexplored Journey. 2020.*[Submitte*d]
(4) Figueira, I.; Garcia, G.; Pimpão, R. C.; Terrasso, A. P.; Costa, I.; Almeida, A. F.; Tavares, L.; Pais, T. F.; Pinto, P.; Ventura, M. R.; et al. Polyphenols Journey through Blood-Brain Barrier towards Neuronal Protection. *Sci. Rep.* 2017, 7 (1), 11456. https://doi.org/10.1038/s41598-017-11512-6.

S4. ASSESSING NUTRITIONAL AND LIFESTYLE DETERMINANTS IN HUMAN POPULATIONS

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When conducting a research on human population using biomarkers of effect, there are multiple parameters affecting results that should be taken into account such as nutrition and physical exercise. However, the use of self-reporting to describe those parameters led to potential bias, imprecisions, and statistical difficulties. During the STSM, as the part of the team the grantee evaluated nutrition and physical ability of 18 patients. Eleven of them were male, at mean age of 49.7±7.0 years, BMI of 33.5±3.1 kg/m^2, and fat mass of 34.0±7.7 kg. The mean Mediterranean diet score was 7.8±3.3 and the weakest point of adherence were determined for the too low red wine, vegetables, and legumes consumption. As for the fitness, according to the Chester Step Test, they were in average physical shape. Average one-leg stand time was 39.5±22.0 s, handgrip mass was 42.2±8.6 kg, jump reach was 29.6±2.4 cm, and could have completed 9.0±2.4 push-ups. Seven of female participants were evaluated at mean age of 55.1±5.8 years, BMI of 35.4±1.6 kg/m^2 and fat mass of 37.0±6.4 kg. The mean Mediterranean diet score was 8.3±3.2 and the weakest point of adherence were determined for the too low legumes and fish consumption. According to the Chester Step Test, they were also in average physical shape. Average one-leg stand time was 35.8±22.2 s, handgrip mass was 26.1±6.7 kg, jump reach was 18.2±4.8 cm, and could have completed 5.8±2.6 push-ups.

The STSM resulted in building of a new network and knowledge transfer.

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S5. EVALUATION OF SULFUR CONTAINING SYNTHETIC ISOSTERIC ANALOGUES OF CHALCONES AND AURONES AS POSSIBLE REDOX MODULATORS

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Synthetic isosteric analogues of natural compounds such as chalcones and bioflavonoids, especially rare types such as aurones, are of considerable interest from the point of view of designing novel redox-modulatory compounds with additional complementary features that may have pharmacological potential. Evaluation of the antioxidant, redox-modulatory, antimicrobial and herbicidal activities/properties of a library (*i.e.*, 27 derivatives) of isosteric synthetic sulfur-containing analogues of natural compounds namely 5-ene-thiazolo[3,2-b][1,2,4]triazole-6-ones with a previously demonstrated favourable anticancer activity/toxicity profile. Investigation was performed using 5-Ene-thiazolo[3,2-b][1,2,4]triazole-6-ones; DPPH assay; cyclic voltammetry; antimicrobial and herbicidal activities screening. Low molecular weight radical scavengers with IC50 = 5.624 mM (**1**) and 1.409 mM (**2**) *(i.e,* for ascorbic acid IC50=0.045 mM) were identified in DPPH tests (stock solutions in methanol C = 4mM +Tris-HCl buffer pH=7.40, measurements after 60 min). No redox peaks were observed for most of the compounds by cyclic voltammetry experiments (BAS 100W potentiostat; GC working electrode, Pt wire counter and SCE reference electrodes, potential range from -1.5 V to 1.5 V, scan rates of 10 and 100 mV/s; stock solutions in methanol C = 5 mM, PBS pH = 6.40; measurements at 0 and after 60 min). Almost all compounds demonstrate satisfactory activity level against *E. coli* and *Candida albicans*; however, they were inactive against *Lactobacillus* and did not demonstrate herbicidal effect to grass species *Agrostis stolonifera*. The correlation between structure and studied properties was established which will provide deeper insights into the detailed mode and mechanism of action of these compounds.

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S6. THE IMPACT OF CATECHINS ON DNA METHYLATION LEVEL WITHIN PROMOTER AREA OF SULFIREDOXIN-1 GENE IN HT29 CELL LINE

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In our previous study, treatment of HT29 cell line with catechins induced dose-dependent changes in the expression of redox-related genes. Uniquely, only one gene (SRXN1, sulfiredoxin-1) was down-regulated upon treatment with 10 μM (-)-epigallocatechin (EGC). The aim of the current study was to investigate whether the observed down-regulation of SRXN1 expression was affected by epigenetic changes. HT29 cells were treated with catechins at different concentrations for 24 h and subsequently genomic DNA was isolated and bisulfite converted. DNA methylation profiles of selected regions within SRXN1 promoter were examined using Methylation-Specific PCR (MSP) and Methylation-Sensitive High Resolution Melting (MS-HRM). MSP analysis showed no differences in DNA methylation level between any of the treatments compared to control. However, the difference was observed when the bigger area of CpG island was analyzed by MS-HRM. Significant increase in DNA methylation level was observed after cell treatment with higher doses of EGC and (-)-epicatechin gallate (ECG). DNA demethylation requires oxidative modifications of methylated cytosine. Catechins, which are strong antioxidants, may lead to inhibition of DNA demethylation by changing cellular environment to more reduced state, especially in the case of higher doses. Thus, we report that catechins can act as methylation inducers and probably this function derives from their ability to influence cellular redox homeostasis.

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S7. EXOSOME-MEDIATED COMMUNICATION BETWEEN HEPATOCELLULAR CANCER CELLS AND NEIGHBORING CELLS

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Hepatocellular carcinoma is a primary malignancy of hepatocytes that is rapidly fatal. There is growing evidence indicating that exosomes modulate intracellular communication and tumor progression. Cancer cell-derived exosomes may present alterations of the proteasome amount and activity. Besides the proteasome, heat shock proteins are also involved in the regulation of signaling pathways that may be related to cancer development. These factors may be linked to many related signaling pathways, including cell death, cellular stress response, cell cycle, and oxidative stress. We studied the exosomal content of hepatocellular cancer cells and primary hepatocytes following stress conditions induced by hydrogen peroxide and quercetin. HepG2 cells and primary hepatocytes were cultured and divided into four groups (control, H_2O_2-treated, quercetin-treated, and H_2O_2 + quercetin treated). Cell viability, apoptosis, cell cycle phase analysis, and proteasome activities were determined. The amount of stress was assessed by ROS, glutathione, and mitochondrial membrane potential measurements. In the second part of the study, exosomes were isolated from HepG2 cells and were applied to primary human hepatocyte cells in co-culture. These cells were used for RNA isolation, RT^2 profiler arrays, and proteasome activity analyses. Gene expression experiments showed that expression of twenty genes was changed significantly under stress conditions in HepG2 cells. Primary human hepatocyte results showed that the expression of eleven genes significantly changed. Five of these genes were related to molecular chaperones, four to xenobiotic metabolism, and one to anti/pro-oxidant enzyme mechanisms.

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S8. THE GENOMIC RESPONSE OF MURINE THYROID TO EXCESS IODINE: ROLE OF THE NRF2 ANTIOXIDANT RESPONSE

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Nrf2 (Nfe2l2) is a transcription factor that regulates a series of cytoprotective and antioxidant enzymes. Upon exposure to oxidative or electrophilic stress, Nrf2 enters the nucleus and induces transcription of several genes. Follicular thyroid cells have physiologically high levels of reactive oxygen species, as oxidation of iodine is essential for iodination of thyroglobulin and thyroid hormones synthesis. We have shown previously that Nrf2 pathway is active in thyroid and regulates the transcription of thyroglobulin. We thus hypothesized that the response of thyroid to iodine excess should comprise Nrf2-dependent and -independent pathways. To this end, 3 months-old male C57Bl6J wild-type (WT) or Nrf2 knockout (Nrf2KO) mice were exposed to 0.05% sodium iodide in their water for 7 days. RNA-Seq was performed by Exiqon for thyroidal mRNAs and miRNAs. Ingenuity Pathway analysis was performed on genes that showed at least a 2-fold expression change. Iodine exposure led to differential expression of 728 mRNAs and 71 miRNAs that regulate pathways related to inflammatory-immune response, antioxidant response, xenobiotic metabolism and fibrosis. Deletion of Nrf2 affected 274 mRNAs and 38 miRNAs regulating mainly glutathione and xenobiotic metabolism. 133 mRNAs and 44 miRNAs were found to change by exposure to iodine in an Nrf2-dependent manner and regulate mainly inflammatory and cytoprotective pathways. Iodine induces inflammatory and cytoprotective pathways and Nrf2 can partially mediate this response. Natural compounds, such as broccoli-derived sulforaphane, that activate the Nrf2 pathway, should be studied for their protective effects against oxidative-stress related thyroid diseases.

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S9. DETERMINATION OF ADVANCED GLYCATION END-PRODUCTS IN INFANT FORMULA PRODUCED BY THERMAL OR CASCADE MEMBRANE FILTRATION

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Advanced Glycation End-products (AGEs) are non-enzymatic by-products formed when sugars and proteins are heated together. These protein modifications occur during dairy processing at high temperatures and are considered undesirable. The Thermal Or Membrane processing for Infant formula project (TOMI) proposes an alternative method utilising cascade membrane filtration (CMF) to produce infant formula (IF) at pilot plant scale. The objective of this study was to quantify chemical modifications to protein structure in IF produced by either the standard high thermal processes or by the alternative CMF. The extent of chemical modification was also quantified over a 3 month storage period, and during (in vitro) gastrointestinal digestion. Standard thermal and CMF-IF were produced at pilot scale. Microbial load was quantified by plate counts on standard media. AGE products were quantified by LC-MS/MS, using a Bruker Impact-II Q-TOF mass spectrometer as per (Chen et al. 2019). Static simulated gastric and intestinal digestion was performed using INFOGEST protocol (Brodkorb et al. 2019). From a microbial prospective, CMF-IF was comparable to IF produced by standard thermal processing. Significant differences were observed in protein content ($p<0.05$). The availability of several important amino acids, including arginine (-0.02µM, $p<0.05$) and histidine (-0.035µM, $p<0.05$) were reduced in thermal IF. The established AGE marker, N^{ε}-carboxymethyllysine (CML), showed a trend (although not significantly), towards higher levels in IF produced by thermal processing. Interestingly post gastro-intestinal digestion, levels of AGEs were significantly higher in IF produced by thermal processing compared to CMF (+1.4 nM, $p<0.05$).

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S10. EFFECT OF BIOAVAILABLE WHEY PEPTIDES ON OXIDATIVE BIOMARKERS IN MICROGLIAL CELLS

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Objective: Sarcopenia is defined as the age related loss of muscle mass and strength. Dietary intervention studies for elderly adults have demonstrated that whey protein may benefit ageing muscle and may reverse or attenuate frailty. The objective of this study was to determine whether or not 4 bioavailable whey peptides could decrease oxidative stress in microglial cells. **Methods:** Four peptides, produced during gastrointestinal digestion of whey protein isolate, were chosen based on their ability to cross the intestinal barrier in vitro. The murine microglial cell lines, BV-2 wild type and its redox disrupted BV-2-Acox1 deficient, were exposed for 1 hour to varying concentrations of synthesized whey peptides (VGIN, VAGT, KVPQ, and NLPPL). Nitric oxide (NO) concentration was measured in the supernatant using the Griess assay. Enzymatic catalase activity was also quantified as described by Saih et al. (2017). Expression levels of catalase were measured using western blot and quantification of the catalase mRNA transcript evaluated using qRT-PCR. **Results:** Surprisingly VGIN (2.5mM) significantly induced production of NO compared to untreated controls ($p<0.05$) in both BV-2 wildtype and *Acox1*-deficient cells. Catalase enzyme activity in BV-2 cells was not influenced by whey peptide treatments. VAGT significantly increased catalase activity in *Acox1*-deficient cells compared to the control ($P<0.05$). **Conclusion:** In conclusion, whey peptides can modulate oxidative biomarkers in microglial cells in vitro.

Saih FE et al. Molecules. 2017;22(1). doi: 10.3390/molecules22010102.

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S11. IS ERUCIN A PROMISING BIOACTIVE AGAINST RENAL CELL CARCINOMA?

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A large number of epidemiological studies have linked cruciferous vegetable consumption to a reduced risk of various types of cancer, including renal cell carcinoma. Organosulfur compounds from cruciferous vegetables, glucosinolates and their metabolic breakdown derivatives, isothiocyanates and indole-3-carbinol, rise scientific interest by exerting unique anticancer properties. Erucin (ER) is an isothiocyanate that is generated by enzymatic hydrolyzes of glucoerucin, a glucosinolate predominant found in rocket species, or by *in vivo* reduction of sulforaphane, its structural oxidized analog. In the present study, the inhibitory effects of ER on renal cancer cell viability, migration and invasion were investigated. The 786-O human renal cancer cell line and the Vero normal-like cells were treated with different concentrations of ER (10-100 μM). Cell viability was determined using the MTT and PI assays. The intracellular level of reactive oxygen species was evaluated using dichlorodihydrofluorescein diacetate. The collective cell migration and chemotaxis/chemoinvasion were studied by a wound healing and transwell assay, respectively. ER induced a concentration-dependent decrease in cell viability, with more cytotoxicity for 786-O cells than against Vero cells. Non-cytotoxic concentration of ER significantly reduced cell migration rate, chemotaxis and invasiveness potential of 786-O cells. The observed favorable anticancer potential of ER against human renal carcinoma *in vitro* requires further investigation.

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POSTER PRESENTATIONS

P1. PRIMROSE OIL ON THE CARDIODYNAMICS AND OXIDATIVE STRESS IN MALE AND FEMALE RATS

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The main ingredients of evening primrose oil (EPO) are omega-6 polyunsaturated fatty acids to which its therapeutic effects are mainly attributed. The aim of the present study was to investigate the effects of chronic treatment with EPO on the cardiodynamics and oxidative stress markers in the rat heart. A total of forty *Wistar albino* rats (24 weeks old) were divided into four groups (10 per group): male rats treated with EPO; female rats treated with EPO; control group of female rats and control group of male rats. Animals in the experimental groups were treated with EPO in a dose of 10 mg/kg body weight once a day for 6 weeks via gavage. After sacrificing the animals, hearts were isolated and perfused according to the *Langendorff* technique at gradually increased coronary perfusion pressures (40-120 cmH_2O). The following parameters of cardiac function were continuously recorded: dp/dt max, dp/dt min, SLVP, DLVP, HR. Coronary flow (CF) was measured flowmetrically. Oxidative stress biomarkers (H_2O_2, O_2^-, NO_2^-, TBARS) were measured in the coronary venous effluent spectrophotometrically. Our results demonstrate that treatment with EPO significantly improved diastolic function in male rats. Moreover, slightly better impact of EPO administration on cardiac function was achieved in male rats compared to female rats. Based on our findings, we may conclude that EPO treatment did not change the function of the isolated rat heart, independently of the sex. On the other hand, treatment with EPO reduced production of some molecules responsible for oxidative stress (H_2O_2, NO_2^-).

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P2. CHERRY-THE SOURCE OF POTENTIAL FUNCTIONAL FOOD

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Serbia is among the top world producers of high-quality cherry fruit. Beside it refreshing taste cherries are rich source of many natural active principles. The most interesting among them are those one which exhibit antioxidant properties. It is possible to produce many derived products from cherries and one certainly is fruit wine. Such wine, is rich source of those natural active principles present in cherry fruit and the aim of this study is to investigate antioxidant properties of cherry wine. Fruit wines were produced from Serbian autochthonous sort of cherry. Microvinification was conducted with pure selected yeast culture and enzymatic preparation glycosidase. In the half of microvinifications pits were left, without cracking. Determinations of total phenolic content (TPC) were conducted using Folin-Ciocalteu method. Antiradical activity estimated by DPPH method, while FRAP method was also applied. Selected phenolic compounds were quantified by UPLC TQ-MS/MS. The TPC for the cherry wines were in interval 1552-1787 mg GAE/L while FRAP was 45.3 – 73.5 mmol/L Fe^{2+}. The IC_{50} anti DPPH radical activity was from 3.8 to 7.2%. Also, were quantified compounds which exhibit antioxidant properties which are hydroxybenzoic acid derivatives, such as vanillic, gallic, protocatehuic and parahydroxybenzoic acid. The obtain results indicate that cherry wine is good source of antioxidant compounds. Antioxidant properties and quantity of phenolic compounds depends from the technological process applied in the production of fruit wines. Cherries and their derived products have beneficial health effect for overall health.

P3. IMPACT OF DEFATTING PROCESS ON ANTIOXIDANT POTENTIAL OF GRAPE SEEDS

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Grape seeds contain a wide range of phenolic compounds responsible for its health promoting effects. Interest for grape seed utilization has increased significantly along with a growing recognition that present biologically active compounds were not completely isolated during the extraction process. Numerous studies have been conducted in order to achieve the best extraction recovery. Nevertheless, there is still a question concerning the pretreatment of grape seeds. The aim of the present study was to investigate the impact of defatting process on total polyphenol content and antioxidant activity of grape seed extracts. For that purpose seeds were obtained from eight different grape varieties. Extraction with 70 % ethanol was performed on both, crude and defatted seeds (lipid fraction was removed by *Soxhlet* method of extraction). Determination of total phenolic content (*TPC*) was done using *Folin-Ciocalteu* assay. Antioxidant activity was investigated through four different tests (*FRAP*, *CUPRAC*, *DPPH* and *ABTS*) and obtained results were combined in unique Antioxidant Composite Index (*ACI*). *TPC* values ranged from 50.48 to 121.06 and from 38.04 to 108.17 mg GAE/g dry weight for crude and defatted grape seed extracts, respectively. Higher phenolic content determined in extracts obtained from crude seeds could be explained by the presence of lipophilic biologically active compounds in non-removed lipid fraction. Discrepancies between varieties are presumably present due to different morphological properties of seeds, environmental and agricultural conditions. Concerning antioxidant activity, all tests were significantly correlated with *TPC*, suggesting the strong contribution of phenolic compounds to this biological activity.

Keywords: grape seeds, polyphenols, antioxidants, extraction, defatting process

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P4. CHRONIC IMPACT OF FLAXSEED OIL ON MYOCARDIAL FUNCTION AND OXIDATIVE STRESS: GENDER SPECIFIC DIFFERENCES

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Since flaxseed represents a unique combination of bioactive molecules proven to exert beneficial cardiovascular effects, the present investigation aimed to establish the effects of 6-week administration of flaxseed oil (FSO) on cardiac function and oxidative stress, with an emphasis on gender specific differences. The present study was carried out on 40 adult *Wistar albino* rats (24 weeks old) randomly divided into 4 equal groups: males and females treated with either FSO (300 mg/kg/day) or saline via gavage. After the completion of treatment, animals were sacrificed, hearts were isolated and retrogradely perfused according to *Langendorff*, at gradually increased coronary perfusion pressures (40-120 cmH_2O). Several cardiodynamic parameters were continuously recorded: dp/dt max, dp/dt min, SLVP, DLVP, HR, while CF was measured flowmetrically. Cardiac pro-oxidant markers (O_2^-, H_2O_2, NO_2^-, TBARS) were determined spectrophotometrically from the coronary venous effluent. FSO treatment significantly decreased dp/dt max values compared to control group of female rats at higher pressures (100-120 cmH_2O), while most of measured parameters were similar in males and females. Significant increase in H_2O_2 ($p < 0.05$) was observed in both genders, while increases in TBARS and NO_2^-were observed only in FSO males. In contrast, O_2^- levels significantly dropped in FSO females at higher pressure (120 cmH_2O). Our study results suggest that chronic FSO administration slightly influence cardiac contractility and systolic and diastolic function, independently from the gender, while certain detrimental pro-oxidant effects were noticed in males. Further research is needed to clarify the possible mechanisms of FSO effects on the myocardium.

This research was supported by JP 01/15 from the Faculty of Medical Sciences University of Kragujevac.

P5. INFLAMMATORY AND ANTIOXIDATIVE RESPONSE OF DIFFERENT CELL LINES AFTER *IN VITRO* FRUCTOSE TREATMENT

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Modern lifestyle, characterized by increased consumption of fructose-enriched beverages, can lead to obesity, type 2 diabetes and cardiovascular diseases. Since increased fructose intake is often associated with chronic low-grade inflammation and increased oxidative stress in various tissues, the aim was to investigate the level of inflammation and antioxidant protection in endothelial, neuroblastoma and preadipocyte cell lines treated with fructose. We examined the effects of 4-hour treatment with different concentrations of fructose (0.5 mM, 2.5 mM and 10 mM) on gene expression of pro-inflammatory cytokines (tumor necrosis factor α (TNFα), interleukin (IL) 1β and 6) in endothelial (EA.hy926), neuroblastoma (SH-SY5Y) and differentiated preadipocyte (3T3-F442A) cells. The protein levels of nuclear factor-κB (NF-κB), IκB, superoxide dismutase (SOD) 1 and 2, catalase, glutathione reductase and glutathione S-transferase, were also analyzed. In endothelial cells, 0.5 mM and 2.5 mM fructose treatment caused significant increase of TNFα mRNA level, while in SH-SY5Y and differentiated 3T3-F442A cells, IL-6 mRNA level was elevated after 0.5 mM fructose treatment. Although fructose increased pro-inflammatory cytokines in cell-type and dose-specific manner, decreased IκB protein level was detected only in adipocytes regardless of the dose. Finally, among all examined antioxidant enzymes, only SOD2 was significantly reduced in all cell types upon 2.5 mM fructose treatment. These preliminary results show that the effects of fructose on inflammation and antioxidant enzymes are both cell-type and dose specific. A marked decrease in the levels of SOD2, observed in all examined cells, can be associated with lower antioxidative defense under moderate fructose concentration.

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P6. BENEFICIAL EFFECTS OF POMEGRANATE PEEL EXTRACT ON PLASMA LIPID PROFILE, FATTY ACIDS LEVELS AND BLOOD PRESSURE IN PATIENTS WITH DIABETES MELLITUS TYPE-2: A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY

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Pomegranates contain high levels of various phytochemicals that have anti-inflammatory, antioxidative, hypoglycemic and lipid-lowering effects that can be used for treatment of cardiovascular diseases and diabetes mellitus. The aim of this study was to evaluate the effects of pomegranate peel extract (PoPEx) consumption on plasma lipid profile, fatty acids level and blood pressure in patients with diabetes mellitus type 2 (DMT2). Thirty-seven subjects were recruited in this double blind, placebo controlled randomized clinical trial. The study group (n=19) received capsules containing PoPEx (250 mg) twice daily (8 weeks), while the placebo group (n=18) received placebo, as visually identical capsules. The treatment with PoPEx induced significant lowering of both systolic and diastolic blood pressure (136.32 ± 19.57 vs 130.26 ± 14.58 mmHg; $p<0.01$ and 82.89 ± 8.22 vs 80.79 ± 7.32 mmHg; $p<0.05$),respectively. The plasma levels of triglycerides ($p<0.01$) and the ratio of low-density lipoprotein cholesterol to high-density lipoprotein cholesterol ($p<0.001$), and HbA1C ($p<0.05$) were significantly increased in the treatment group compared to placebo group. The level of HDL-C was significantly decreased ($p<0.001$) in the treatment group compared to placebo. Moreover, the PoPEX treatment significantly improved the plasma fatty acids content within the total lipids; for instance, it significantly decreased levels of palmitic acid, stearic acid and total saturated fatty acids, as well as the level of arachidonic acid ($p<0.05$). Fasting blood glucose, insulin concentration, total cholesterol, LDL-C, and insulin sensitivity were not significantly different at the end of the study in neither group. Consumption of PoPEx in DMT2 subjects had favourable healthy effects on some metabolic parameters, blood pressure, lipid profile and plasma lipid fatty acids composition.

P7. ASSOCIATION OF REDOX STATUS AND LIFESTYLE FACTORS WITH TELOMERE LENGTH IN HEALTHY SUBJECTS

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Telomeres are DNA-protein structures located at the ends of eukaryotic chromosomes. Since their shortening is a natural process, which repeats with each cell division, telomere lengths have been proposed as a biomarker of aging. Also, there is evidence that telomere shortening and dysfunction can be accelerated by oxidative-stress and unhealthy lifestyle factors, including physical inactivity and inadequate nutrition, Thus, we aimed to evaluate the association of redox status and selected lifestyle factors with telomere length. The study included 94 apparently healthy adults, both genders with average age 46±12 years. Before anthropometric measurements and venous blood sampling, participants were asked to complete a lifestyle questionnaire. Serum antioxidant defense markers (total sulfhydryl groups, paraoxonase activity and total antioxidant status) and prooxidants and products of its activity (malondialdehyde, superoxide anion and total oxidant status) were determined. The Prooxidative score, Antioxidative score and Oxy score were calculated from measured redox status markers by using z-score statistics. Telomere length was determined by qPCR method using genomic DNA from peripheral blood leukocytes. A positive relation was found among telomere length and a moderate level of physical activity, intake of fruits and vegetables, especially for females. Increasing abdominal fat, alcohol and fried food intake, as well the Oxy score (difference between Proxidative and Antioxidative scores) were inversely associated with telomere length. Overall, these results support the impact of healthy lifestyle on healthy aging and redox control in the term of prevention of major age-related diseases.

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P8. VITAMIN D DOWNREGULATES CARDIAC FOXO1 AND INCREASES PGC-1 ALPHA EXPRESSION

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It is well known that vitamin D levels are accompanied with favorable lipid profile and it could explain its beneficial effects on cardiovascular system. Regarding that vitamin D effects on cardiac lipid metabolism are not fully elucidated, we treated male rats with cholecalciferol during 6 weeks (1000IU/kg b.w./day, every second day). Proteins involved in fatty acid metabolism in the rat heart were analyzed by Western blot as well as plasma lipid profile. Cholecalciferol did not alter body and visceral adipose tissue mass as well as triglyceride and cholesterol (total, LDL, HDL) level. However, non-esterified fatty acid (NEFA) level was significantly reduced. Regarding the heart, translocation of vitamin D receptor from cytosol into the nucleus was observed upon cholecalciferol administration. Levels of fatty acid transporters, CD36 and FATP1, were not modified. Accordingly, protein level of lipin 1 (microsomal and nuclear) was not affected by the treatment. However, Forkhead box protein O1 (FOXO1) in cytosolic fraction was significantly down regulated. On the other hand, peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) level was elevated following cholecalciferol treatment. In conclusion, decreased availability of NEFA as a cardiac energy substrate upon vitamin D treatment is accompanied with an alteration of FOXO1/PGC1 pathway regulating fatty acid metabolism in the heart.

This study was supported by the Ministry of Education, Science and Technological Development, Republic of Serbia.

P9. EFFECTS OF POMEGRANATE JUICE CONSUMPTION ON THE MEMBRANE PHOSPHOLIPID COMPOSITION IN ERYTHROCYTES IN SUBJECTS WITH METABOLIC SYNDROME

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Metabolic syndrome (MS) represents a cluster of cardiovascular risk factors related to insulin resistance, disturbed glucose metabolism, obesity, arterial hypertension and atherogenic dyslipidemia. Regular consumption of pomegranate (*Punica granatum* L.) may be associated with lower incidences of cardiovascular diseases. In this study twenty one subjects (12 women, 9 men) with MS, aged from 40 to 60 years, were studied. They were randomly assigned into two groups. The interventional group received 200 mL of pomegranate juice daily for 2 weeks, while control group did not consume the juice. Phospholipid (FA) composition in erythrocytes was not significantly different between intervention and control group, at baseline. There was a significant increase ($p<0.05$) in the relative amounts of dihomo-α-linoleic acid and an increase of relative amounts of docosahexaenoic fatty acid in erythrocytes phospholipids in subjects with MS. Also, activity of Δ6 and Δ5 desaturase in erythrocytes were changed in interventional group. Δ6 desaturase were significantly increased and Δ5 desaturase were significantly decreased. Fatty acid profiles in erythrocytes in control group were not significantly changed during the study. These results suggested that short term consumption of pomegranate juice may change phospholipid fatty acid profile and activities of desaturases in erythrocytes in subjects with MS. Considering the limited data on pomegranate impact on FA status, further research is need in order to clarify the potential mechanism of action, and to confirm the findings of this study in a larger cohort of subjects to allow elimination of other confounding factors.

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P10. THE EXTRACTION SELECTIVITY OF METHANOL, N-HEXANE, DICHLOROMETHANE AND NEAR CRITICAL LIQUID CARBON DIOXIDE TOWARDS CASTICIN AND ROTUNDIFURAN FROM *VITEX AGNUS-CASTUS* FRUIT

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The activity of *Vitex agnus-castus* L. (*VAC*) fruits extract in the treatment of premenstrual syndrome, menopause or disrupted lactation in women, is linked to their content in diterpenes and other constituents [1]. We compared the extractability of the diterpene rotundifurane and flavonoid casticin from *VAC* dried fruits using Soxhlet extraction technique and four different solvents namely near critical liquid carbon dioxide, n-hexane, dichloromethane and methanol. The carbon dioxide extraction is carried out in a Soxhlet type glass vessel inserted in a high-pressure stainless-steel container in temperatures 25-26°C and pressures 62-64 bar. The classical Soxhlet glass apparatus was used to extract the *VAC* fruits by n-hexane, dichloromethane and methanol. The near-critical liquid carbon dioxide extractor, allowed to stop extraction at certain time points (15, 45, 90 min and 12 hours) yielding 0,01 g, 0,14 g, 0,25 and 0,28 g extract respectively, compared to 1,8 g n-hexane, 1,3 g dichloromethane and 0,96 g methanol extracts. HPLC and external standards were used for the quantification of casticin and rotundifurane [4]. Rotundifurane is pharmacologically relevant because of its affinity to dopamine D2 receptor [5]. It was observed that highest casticin concentration was found in the n-hexane extract. Concentrations of casticin varied between 0,002 and 1,067 g/kg in the dried fruits and between 0,13 and 1,18% in the extracts. Rotundifurane content was below limit of quantification for the n-hexane, dichloromethane and methanol extracts but was relatively high (11,75 - 301 mg/100 g drug) in the CO_2 extracts in comparison with the literature [2].

Acknowledgements: Reference standards (casticin, rotundifuran) were a kind gift from Bionorica AG.

References:

1. Meier B. et al. (2000) Phytomedicine, 7(5):373-381
2. Cossuta D. et al. (2008) J. of Supercritical Fluids, 47: 188-194
3. Naik S.N. et al. (1989) Fluid Phase Equilibria, 49: 115-126
4. Hoberg E. et al. (2000) Planta Med., 66(4): 352-355
5. Hoberg E. et al. (1999) Phytochemistry, 52: 1555-1558

P11. DETERMINATION OF THE GENOTOXIC EFFECT OF CAPSAICIN ON HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS BY USING A COMET ASSAY

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Capsaicin is a main representative of the group of proto-alkaloids called capsaicinoids, isolated from hot peppers. Despite the positive health effects of capsaicin seen previously, some studies suggest that capsaicin may act as a cytotoxic and genotoxic agent on some human cells. A considerable number of assays exist for the detection of different genotoxic effects of various compounds in experimental systems. The Comet assay is widely used because it is technically straightforward, sensitive, relatively fast, and cost-effective. DNA damage can be detected and quantified in different cell types without the requirement of cell culture. This work aimed to evaluate the genotoxic potential of capsaicin on human peripheral blood mononuclear cells (PBMCs). The cells are embedded in agarose and lysed, generating nucleus-like structures in the gel – nucleoids. Following alkaline electrophoresis, the DNA strands migrate towards the anode, and the extent of migration correlates with the number of strand breaks (SB) in the nucleoid. The migration is visualized and scored with a light microscope after silver staining. Thirty minutes of treatment with capsaicin concentrations in the range of 100 - 200 µmol/L resulted in a high occurrence of single and double SB. The concentration of 50 µmol/L caused moderate DNA damage, and lower concentrations (20 µmol/L) provoked only minor changes in the genome without DNA lesions. Although not all types of genotoxic exposures should be expected to result in DNA damage in PBMCs, the comet assay seems to be a valuable tool for the detection of genotoxic exposure in humans.

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P12. *LAETIPORUS SULPHUREUS* LECTIN INHIBITS ANGIOGENESIS AND TUMOR DEVELOPMENT IN THE ZEBRAFISH XENOGRAFT MODELS OF COLORECTAL CARCINOMA AND MELANOMA

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Laetiporus sulphureus, an edible mushroom, for centuries has been used in traditional European and Asian ethno-medicine. Its therapeutic properties have been attributed to different types of biologically active compounds, such as terpenes, polysaccharides, steroids, proteins and lectins. Recently, mushroom lectins emerged as very potent macromolecules with antiproliferative, cytotoxic, antiviral and immunostimulatory activities. Therefore, we isolated lectin from *L. sulphureus* (LSL) and evaluated its anti-angiogenic and anticancer activity *in vivo*, using the zebrafish model. We found that LSL is not toxic at high doses up to 400-500 μg/mL, while it effectively inhibited angiogenesis and cancer development at much lower doses. Compared to sunitinib-malate, cardiotoxic and myelosupressive anti-angiogenic drug of clinical relevance, therapeutic potential of LSL was 378-fold higher. Wound healing and MTT assays were employed to examine antimigratory effect and endothelial cytotoxicity. Surprisingly, LSL affected human colorectal carcinoma and mouse melanoma cell lines, by almost completely diminishing their growth, neovascularization and metastasis. Moreover, in comparison to the used control (cisplatin), LSL showed markedly greater activity, while its potency turned out to be 8-fold higher towards colorectal carcinoma than melanoma. These encouraging data strongly imply that LSL can be considered for the application as supporting agent in chemotherapy against colorectal carcinoma and melanoma or used in pharmaceutical industry.

P13. EFFECTS OF COMMERCIALLY AVAILABLE POLYPHENOL-RICH ARONIA JUICE ON LIVER FATTY ACID PHOSPHOLIPIDS IN WISTAR RATS

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Aronia melanocarpa (AM) is a medicinal plant commonly named black chokeberry and investigated for wide-array of health-promoting characteristics. The beneficial properties of AM on human health are attributed to high content of non-nutritional phytochemicals i.e. polyphenols. The aim of our study was to compare two doses of Aronia juice on liver phospholipid composition in Wistar rats. The AMJ corresponded to commercial aronia juice product, available on the Serbian market. A commercial product used has been registered as dietary supplement by Serbian Ministry of Health, under the trade name “Aronia Anti-Oxi” (515-04-1339/2011-04).
The animals were divided in 2 groups of 8 animals each, to receive *ad libitum* either full polyphenol aronia dose (AMJ), and polyphenol-lacking placebo beverage (PLB).
The extraction and preparation of FA esters and GC analyses were performed (Folch 1957). Methyl-ester derivatives formed from isolated liver phospholipids fraction were separated by GC (Shimadzu Co, Tokyo, Japan)
Our results showed decreasing in stearic acid ($p<0.01$), increasing in vaccenic acid ($p<0.001$) as well as MUFA($p<0.05$). Dihomo-gama-linolenic (n-6) acid was increased ($p<0.05$) while arachidonic acid (n-6) was decreased ($p<0.05$). n-6/n-3 ratio was decreased but not significantly. Liver enzyme activities were significantly different for elongase ($p = 0.009$) and D5D enzyme ($p = 0.015$), due to the decreasing effect of aronia juice consumption in comparison with control.
Stronger effect of aronia juice was in liver, as a primary site of FAs metabolism and through the decreasing in arachidonic acid, as well as decline in FA metabolic enzymes indicates favorable lipid-metabolism related properties of AMJ consumption.

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P14. ARTEMISIA DRACUNCULUS L. ESSENTIAL OIL: INFLUENCE ON ENZYMATIC ACTIVITY IN MICROGLIAL BV-2 WILD TYPE AND ACYL-COA OXIDASE TYPE 1 DEFICIENT CELL LINES

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The investigation of dietary phytochemicals with antioxidant and anti-inflammatory activities is still of interest due to their ability to reduce the deleterious effects of ROS and associated inflammatory processes. For such investigations the neuroglial cells can serve as a model to reveal some action modes of *Artemisia dracunculus* essential oil (EO).
According to the gas chromatography mass spectrometry (GC MS) analysis, estragol reached 84.9% in essential oil (EO) of *A. dracunculus* cultivated at the altitude of 1700–1800 m above sea level. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay showed that IC_{50} value of investigated EO was 94.2 μgmL^{-1}. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test revealed that the sub-cytotoxic concentration of *A. dracunculus* EO for both BV-2 microglial wild type (WT) cells and acyl-CoA oxidase type 1 (ACOX1) deficient cell ($Acox1^{-/-}$) lines was 5.10^{-1} µg/mL. 72-hour treatment with EO leads to the increased viability (up to 12%) of the cells, in WT cells and up to 14% -in BV-2 $Acox1^{-/-}$cells. The 48-hour treatment increases the ACOX1 activity of BV-2 cells up to 70%. Catalase activity of WT cells increased after the 24-hour period, but in case of $ACOX1^{-/-}$ cells – after the 48-hour treatment. Superoxide dismutase activity in both cell lines increased during the 24 and 48-hour treatments. These results suggest that *A. dracunclus* EO can be considered as potential antioxidant and protective agent.

P15. OLIVE OIL CONSUMPTION AS AN INDICATOR OF MEDITERRANEAN DIET ADHERENCE IS NOT EDUCATION LEVEL-RELATED IN NORTH MACEDONIA

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The consumption of olive oil is one of the hallmarks of the Mediterranean diet (MD). Being rich in polyphenols, olive oil is considered to underlie, at least in part, the much appreciated positive influence of MD on health preservation and longevity. The work to be presented is a part of a larger project, MeDiWeB (Mediterranean Diet and Wellbeing), conducted using an on-line questionnaire. One of the goals of MeDiWeB is to study the adherence to ~~the~~ MD among the citizens of North Macedonia, as a sub-Mediterranean country. The aim of the present work was to study the association between the education level of the participants and the amount of the consumed olive oil per day; therefore, only data obtained from these questions have been analyzed and will be presented. Our results demonstrate that in general, the citizens of North Macedonia do not use ~~the~~ olive oil as main cooking oil, and have a low amount of olive oil intake on a daily basis. This result was expected, since sunflower oil is used traditionally for cooking purposes. The results also demonstrate the lack of association between the level of participants' education and olive oil intake. It is expected that further analyses of the data collected with the MeDiWeB questionnaire will give a deeper understanding of the reasons for the low intake of olive oil in North Macedonia. In order to increase the olive oil intake, the general population should be better informed about the health benefits of regular olive oil consumption.

P16. COMBINATIONS OF COCOA ANTIOXIDANTS: WHAT THEY TELL US ABOUT THE CHEMOPREVENTIVE RELEVANCE OF INTERACTIONS BETWEEN FOOD COMPONENTS?

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Antioxidant properties of polyphenols are believed to underlie cocoa chemopreventive potential. However, it has been not recognized if these effects are mainly caused by the most abundant components or result from concerted action of major and minor cocoa bioactives as proposed by food synergy concept. This study was aimed at resolving this question. Initially, the cocoa extract composition was determined by HPLC-DAD-MS. Then, bioactivities of cocoa extract and a series of artificial mixtures of cocoa phytochemicals were tested to compare their redox properties in cell-free system and redox-associated biological effects in human colon cancer HT29 cells serving as a model of human alimentary tract. Under cell-free conditions, DPPH test as well as differential pulse voltammetry showed the highest antioxidant activity for cocoa powder extract (CE), but surprisingly, did not reveal any dose-dependent differences between mixtures despite growing concentration and complexity of antioxidants. Basically, to the same conclusion lead determinations of cellular antioxidant activity; CE was the most efficient in cell protection against ROS whereby concentration of catechins in studied solutions had to be above 10 μM to override cellular redox homeostasis. Cell growth inhibition was dose-dependent only for mixtures that consisted of main catechins at narrow range of low concentrations (0.01 – 1 μM C+EC). Neither clear relationship between composition of cocoa phytochemicals and nutrigenomic activity of CE and matching mixtures was spotted. Therefore, our study indicates that the bioactivity of non-toxic complex natural mixtures such as cocoa is strongly affected by interactions between their components, as predicted by food synergy.

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P17. PROTECTIVE EFFECTS OF ARGAN OIL ON OXIDATIVE STRESS INDUCED BY IRON IN *TETRAHYMENA PYRIFORMIS*

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Recently, the research on the protective powers of medicinal plants has become the focus of several studies. Attention has been directed to the identification of new molecules with antioxidant and chelating properties to counter reactive oxygen species (ROS) involved as key factors in several pathologies. In our study, the protective effect of argan oil (AO) extracted from fruits of *Argania spinosa* was evaluated against oxidative stress induced by ferrous sulfate ($FeSO_4$) in *Tetrahymena pyriformis*. This protozoan was grown in a medium supplemented with 1.85 mM $FeSO_4$ in the presence or absence of 0.1% argan oil. The activities of enzymatic markers of the antioxidant balance - superoxide dismutase (SOD), glutathione peroxidase (GPx) as well as lipid peroxidation (MDA) and glutathione (GSH) level - were evaluated. The results showed an increase in the level of iron-induced SOD and GPx activities. By contrast, treatment of iron-exposed *T. pyriformis* with argan oil caused a significant decrease in the SOD and GPx activities. These results reveal that the iron-induced ROS imbalance can be counteracted by AO, which is probably related to its high content of polyphenols known for their antioxidant activities.

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P18. THERAPEUTIC POTENTIAL OF *S*-NITROSOTHIOLS IN THE PREVENTION OF ATHEROSCLEROSIS: MODULATION OF MONOCYTES METAPLASIA INTO FOAM CELLS

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During atherosclerosis development, oxidative stress and inflammation potentiate the decrease of nitric oxide (NO) bioavailability either in its free or storage forms (*S*-nitrosothiols). As *in vivo*, *S*-nitrosothiols, like *S*-nitrosoglutathione (GSNO), are the physiological form of NO storage and transport, these compounds can be used as therapeutics to restore NO bioavailability and to counteract the different steps of atherosclerosis development. Indeed, GSNO has been shown to modulate protein activity and/or expression by *S*-nitrosation process, which also counteracts oxidative stress (Belcastro *et al*., 2017). So, GSNO may have potential to modulate the differentiation (metaplasia) of human monocytes to macrophages and their ability of OxLDL internalisation (leading to foam cells formation). To that purpose, human peripheral blood mononuclear cells (PBMCs) were isolated from buffy coats from healthy donors. Monocytes were purified by two methods *i*. adhesion and *ii*. negative isolation using magnetic beads. We differentiated monocytes into M0-like macrophages followed by a functional polarization in M1-like pro-inflammatory macrophages (LPS/IFN-γ), or M2-like anti-inflammatory macrophages (IL-4 or IL-10). Polarized M1-like macrophages were $CD80^+$ and M2-like macrophages were $CD206^+$. The activity of GSH/GSNO-related enzymes like glutathione peroxidase and gamma-glutamyl transferase was different upon polarization with a decrease of glutathione peroxidase activity in M2-like macrophages, compared to M0-like macrophages. The induction of CD36 expression, an OxLDL scavenger receptor, by OxLDL was prevented by 40% with a GSNO pre-incubation. The production of foam cells, as well as GSNO potential to limit or reverse the systems described above, is still under investigation.

Reference:
E. Belcastro, W. Wu, I. Fries-Raeth, A. Corti, A. Pompella, P. Leroy, I. Lartaud, C. Gaucher, Oxidative stress enhances and modulates protein *S*-nitrosation in smooth muscle cells exposed to *S*-nitrosoglutathione, Nitric Oxide Biol. Chem. 69 (2017) 10–21.

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P19. HNE-AGE-INDUCED INFLAMMATORY SIGNALS VIA RAGE AND TLR4 AFFECT THE SURVIVAL OF OSTEOARTHRITIC HUMAN CHONDROCYTES AND MATRIX PEPTIDES: COMPARISON BETWEEN OLIVE LEAF EXTRACT ZeyEX AND IBUPROFEN

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Accumulation of advanced glycation-glycoxidation or lipoxidation end products (AGEs, ALEs) in joints is important in the development of cartilage destruction and inflammatory damage in osteoarthritis (OA). ZeyEX, a standardized, patented polyphenolic antioxidant mixture from olive leaves, has been reported to regulate the oxidative injury and inflammation (1). The aim was to investigate the effects of ZeyEX on a number of different biomarkers (AGEs, ALEs, interleukin 1β (IΛ−1β), interleukin 6 (IL-6), toll-like receptor4 (TLR4), and receptor for AGEs (RAGE), cyclooxygenase-2 (COX-2), cartilage oligomeric matrix protein (COMP) and type II collagen (COL2)) in human OA chondrocytes (OACs). OACs were isolated from grade-4 OA patients and cultured. AGEs, ALEs, IL-1β and IL-6 were quantified by ELISA kits and the findings were compared between ZeyEX-treated and ibuprofen-treated groups. The expressions of related proteins were determined by Western blotting (TLR4, RAGE, COMP) or fluorescence immunocytochemical (COX-2, COL2) analysis. Our results showed that 24 h pre-treatment with ZeyEX and ibuprofen decreased AGEs, ALEs, IL-1β, IL-6, RAGE, TLR4 and COMP. ZeyEX enhanced the expression levels of COL2 in OACs at 1-100 nM, but ibuprofen did not. COX-2 was only inhibited by ibuprofen at 1-100 nM. The viability (MTT) of OACs was increased by ibuprofen at 1-100 nM, unchanged by ZeyEX at 1 nM-50 μM, and inhibited by both agents at ≥100 μM. H_2O_2 exposure (200 μM, 2-h) inhibited cell viability in chondrocytes that have lost their pathology (passage10). However, both agents protected the viability and inhibited ROS in chondrocytes (passage10) against H_2O_2-toxicity. These findings suggest that ZeyEX can modify AGE and ALE damage in chondrocytes from osteoarthritic patients.

1. Bali et al., Planta Med. 2014 Aug;80(12):984-92. doi: 10.1055/s-0034-1382881.

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P20. EFFECTS OF SOMATOSTATIN AND ANTIOXIDANTS ON SOMATOSTATIN RECEPTORS IN BREAST CANCER CELLS

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Breast cancer is the second main cause of cancer death in women. No information is available in the literature on the effects of antioxidants and somatostatin on membrane SST receptors in breast cancer cells. The activity of receptors in cell membranes is very important in the development of cancer. The aim of our study was to elucidate the effects of SST and antioxidants on membrane SST receptors in breast cancer cells. We measured Somatostatin Receptor 1 (SSTR-1) and Somatostatin Receptor 5 (SSTR-5) levels using ELISA Kits (SunRed) in MCF-7 and BT-20 breast cancer cell lysates incubated 24 h with Somatostatin(SST), Curcumin(CUR), Quercetin(QUE), Vitamin C(VITC), SST+CUR, SST+QUE or SST+VitC. There was no significant difference in the SSTR-1 levels in both cell lysates among the groups. There were significant differences in the SSTR-5 levels in the MCF-7 cell lysates incubated with SST+CUR, SST+QUE or SST+VitC in comparison to the control cells. Significant differences in the SSTR-5 levels were found in the BT-20 cell lysates incubated with SST, CUR, QUE or VitC in comparison to the control cells. The lowest level was found in the BT-20 cells incubated with CUR in comparison to the controls and SST. Our study has demonstrated the effects of antioxidants and somatostatin on membrane SST receptors in two breast cancer cells. This study contributes to the literature showing the effects of these antioxidants in combination with somatostatin on somatostatin receptors in breast cancer cell signaling.

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P21. EFFECTS OF SOMATOSTATIN AND ANTIOXIDANTS ON LIPID PEROXIDATION IN BREAST CANCER CELLS

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In recent years, studies have focused on the effects of lipids oxidation in cancer development. It is well known that antioxidants reduce lipid oxidation. Somatostatin (SST) inhibits the release of the growth hormone release and has been shown to have antiproliferative and antiangiogenic effects in both *in vivo* and *in vitro* cancer models. However, no information is currently available in the literature on the effect of antioxidants and somatostatin on lipid peroxidation in breast cancer cells. Therefore, we studied the effects of SST and antioxidants on lipid peroxidation in two types of breast cancer cells. Elabscience Malondialdehyde test kit (catalog number E-BC-K028) was used to determine MDA levels in cells incubated 24h with SST, different antioxidants and their combinations with SST. MDA values (nmol/ml) were proportioned to the amounts of protein measured in each group of cell lysates and the results were calculated as nmol MDA/mg protein. The highest MDA level was observed in the MCF-7 cells incubated with SST and SST+ Quercetin (QUE) in comparison to the controls. In BT-20 cells, the highest MDA level was in the VitC group and the MDA level increased further in the SST+VitC group. Our study demonstrated the effects of SST, antioxidants and their combinations on the amount of cell MDA as a marker of lipid peroxidation and oxidative damage in the breast cancer cells. SST enhanced the level of lipid peroxidation demonstrating another beneficial effect of SST in addition to its antiproliferative and antiangiogenic effects in breast cancer.

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