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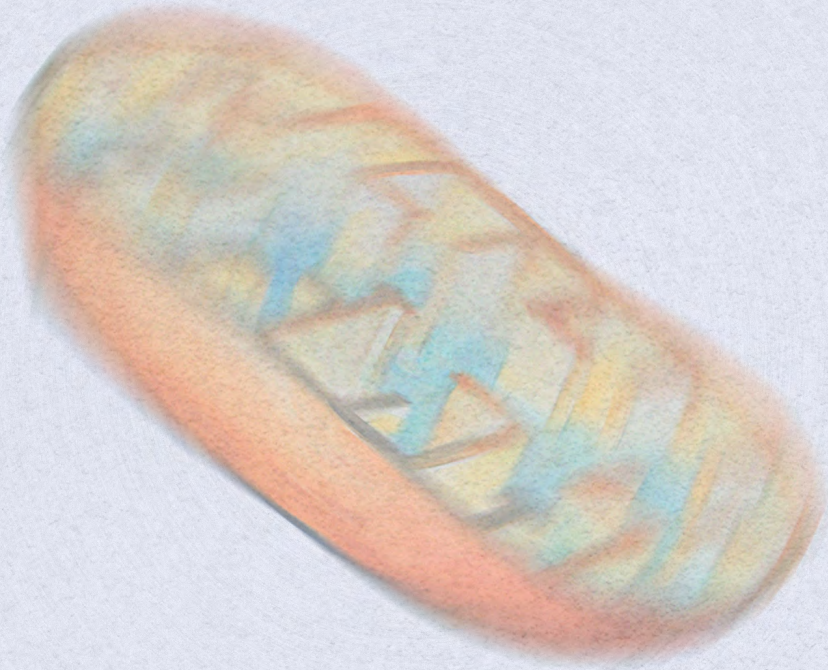
RedoXplore



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Editors' word

RedoXplore is the official Journal of Serbian Society for Mitochondrial and Free Radical Physiology.

RedoXplore is a peer-reviewed Open Access Journal that covers a broad range of topics in the field of Redox Biology, aiming to be of interest and use to both researchers and practitioners.

The aim of **RedoXplore** is to publish research that has the potential to significantly impact the field of Redox Biology, Medicine, and Chemistry, to benefit society, human and animal health, and the conservation of microorganisms and flora.

RedoXplore is particularly interested in innovative research and future trends within Redox Biology. This includes *in vivo* and *in vitro* studies of redox homeostasis in plants and microorganisms, animal models, and human studies, from inflammatory, cardiovascular, neurological, mitochondrial, and metabolic diseases to aging and cancer. The research focus of the journal is to unravel redox-dependent molecular mechanisms in these and numerous other conditions, as well as the influence of environmental factors, lifestyle, nutrition, drugs, and the development of new pharmacological approaches.

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REDOX BIOLOGY:
A PARADIGM OF THE FOUNDATION OF LIFE

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ANTIOXIDANTS AND FREE RADICALS IN HUMAN HEALTH AND DISEASE

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Increased damage by ROS plays a role in the development of neurodegenerative diseases, especially Alzheimer's Disease and other dementias, and diets rich in antioxidants (high intake of fruits and vegetables) seem neuroprotective (as well as being protective against many other age-related diseases). However, attempts to treat/prevent such diseases by giving high doses of antioxidants such as vitamins E and C and carotenoids have, overall, been unsuccessful. Reasons for this will be discussed. A major focus of our work is a unique diet-derived thiol/thione with antioxidant properties, namely ergothioneine (ET). Low blood levels of ET are a risk factor for the development of neurodegenerative and cardiovascular diseases, frailty, eye disease, pre-eclampsia and age-related diseases generally. We have identified "adequate levels" of plasma ET in humans, levels below which are associated with increased disease occurrence, and the reasons leading to these low levels are under investigation. In animal studies, ET has exhibited the ability to modulate inflammation, scavenge certain ROS, protect against acute respiratory distress syndrome, decrease brain damage in models of Parkinson and Alzheimer diseases and stroke, prevent endothelial dysfunction, protect against ischemia-reperfusion injury, counteract iron dysregulation, hinder lung and liver fibrosis, and mitigate damage to the lungs, kidneys, liver, gastrointestinal tract, and testis. ET may also influence the gut microbiome. There is evidence that ET is specifically accumulated at sites of tissue injury, so we have called it an "adaptive antioxidant" that may not interfere with the normal physiological roles of ROS. But does low ET predispose to age-related diseases or is it a spurious correlation? Extensive cell and animal studies strongly suggest the former. Caveats in the use of ergothioneine supplements to prevent/ameliorate aged-related diseases include its potential to generate trimethylamine-N-oxide by the action of ergothionase enzymes in gut bacteria and its ability to be taken up by many bacteria, a few of which are pathogenic (e.g. *H. pylori, M. tuberculosis*). These caveats will be discussed.

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OXYSTEROLS: FROM MOLECULAR BIOLOGY TO MEDICINE AND INDUSTRY

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Oxysterols are oxidized derivatives of cholesterol initially considered as simple metabolic byproducts, nowadays recognized to play significant roles in various biological and pathological processes. In physiology, they are involved in the regulation of cellular processes beyond cholesterol metabolism, influencing cell proliferation, differentiation, apoptosis, and inflammation through various signaling pathways. In medicine, the study of oxysterols holds promise for understanding and treating various diseases, particularly those associated with dysregulated cholesterol metabolism and inflammation. Indeed, some oxysterols have been associated with adverse health effects, including cytotoxicity, pro-inflammatory effects, and potential contributions to the development of chronic diseases. Dysfunctions in oxysterol metabolism have been implicated in the pathogenesis of cardiovascular diseases, neurodegenerative disorders, and certain cancers. Targeting oxysterol pathways could therefore offer novel therapeutic strategies for these conditions. Oxysterols have potential applications in the pharmaceutical and biotechnology industries. Those generated by cholesterol autoxidation can be used as biomarkers for assessing oxidative stress conditions. Additionally, defined oxysterols of enzymatic origin and/or synthetic oxysterol analogs might be developed as antiviral agents. Oxysterols generated through autoxidation processes can serve as markers of lipid oxidation in cholesterol-containing foods and their quantification can help assess the quality and shelf life of food products, and also for ensuring food safety and consumer health. Finally, with regard to skin health and cosmetics industry, prolonged or excessive exposure to and/or formation of certain toxic oxysterols could potentially damage skin cells and disrupt skin barrier function. Therefore, careful formulation and dosage control are essential to ensure the safety of skincare products. Overall, the study of oxysterols spans molecular biology, medicine, and industry, with implications for understanding fundamental biological processes, developing new medical, industrial, and advancing biotechnological applications.

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NITRIC OXIDE, SUPEROXIDE AND PEROXYNITRITE – REDOX REGULATION OF THE CARDIOVASCULAR SYSTEM BY NITRO-OXIDATIVE STRESS AND S-NITROS(YL)ATION

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Oxidative stress is characterized by an excessive and prolonged formation of oxidants, causing an accumulating load of irreversible oxidative modifications of proteins, lipids, and nucleic acids that compromise cell integrity. This competes with the concept of redox regulation, combining the regulatory influence of nitric oxide (•NO), superoxide ($O_2^{\bullet-}$), and their derivatives on redox-sensitive signaling pathways in the cell. The transition from redox regulation to oxidative stress is not only determined by the absolute amount of oxidants formed, but also by the respective intracellular site of formation, by the capacity of the defense machinery of the respective cell type, and by the ratio between •NO and $O_2^{\bullet-}$ that determines the nature of secondary radical species formed. Equimolar and concomitant fluxes of •NO and $O_2^{\bullet-}$, for instance, favor the formation of the oxidant peroxynitrite making $O_2^{\bullet-}$ an antagonist of •NO as well as an inhibitor of prostacyclin synthesis, while an excess of •NO over $O_2^{\bullet-}$ supports the formation of nitrosating species. Secondary •NO-derived species hence not only define cellular targets affected but also the nature of posttranslational modifications. A profound knowledge of redox regulation and the conditions supporting its fluent transition into oxidative stress is hence of outermost importance in molecular cardiovascular medicine. The present overview therefore aims to determine the spectrum of •NO-derived reactive species and the cellular conditions characteristic for reversible modifications and their modulation of cellular targets in redox regulation. The second objective is to define preconditions in cardiovascular cells culminating in an expenditure of the cellular antioxidant system and an accumulation of irreversible modifications that compromise cellular functions to a point of no return.

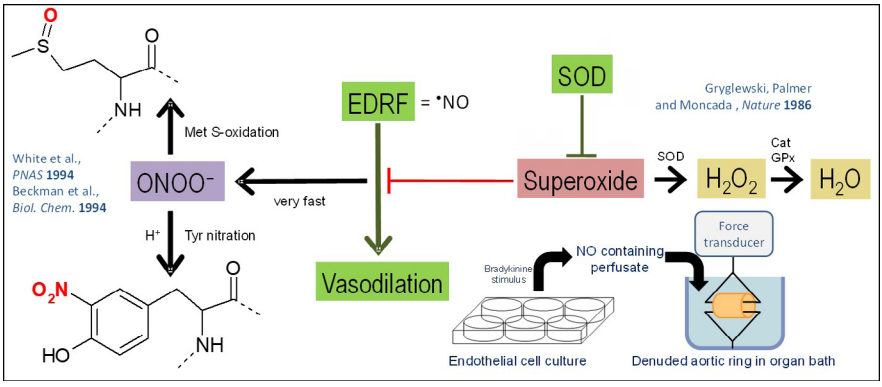

Figure 1. *Superoxide anion-induced loss of "endothelium-derived relaxing factor (EDRF)" (Daiber et al. Redox Biol. 12:35-49, 2017).*

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OXYGEN, SULFUR, SELENIUM AND LIPID PEROXIDATION: HOW GPx4 CONTROLS LIFE AND DEATH

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The selenoperoxidase GPx4, discovered in 1982, plays a pivotal role in preventing ferroptosis. In a moonlighting function, GPx4, in its mitochondrial and nuclear forms, also contributes to spermatogenesis. The critical advantage of Selenium *vs.* Sulfur catalysis is the stability of the oxidized form of the chalcogen in the catalytic cycle. While the mechanisms of catalytic cycle are understood, its regulation remains largely unknown. Existing evidence supports the notion that ferroptosis is activated when GPx4 is inhibited, glutathione (GSH) concentration is lowered, or the labile iron pool is expanded. The outcome is framed in the context of oxygen toxicity playing the physiological function of controlling cell death. GPx4 stands out as the sole peroxidase indispensable to aerobic life. Moreover, a recent study exploring the role of the residue Arg152 in GPx4, linked to a fatal although not embryonically lethal disease, revealed that the wild-type enzyme exhibits surface-sensing and positive cooperativity in the presence of cardiolipin. This adds complexity to the mechanism of physiological function encompassing the interaction with acidic phospholipids in mitochondrial membranes. Ferroptosis is implicated in both physio-pathological conditions, including embryogenesis, cancer suppression, neurodegenerations, inflammatory disorders, metabolic syndrome, heart and kidney diseases. No antioxidant enzymatic system can substitute for GPx4 in inhibiting ferroptosis, emphasizing the vital role of selenium. Phenolic antioxidants, which reduce lipid hydroperoxyl radicals, can only inhibit lipid peroxidation under physiological conditions, and thus ferroptosis, when the lipid hydroperoxides formed are immediately reduced by GPx4. In contrast, the ferroptosis inhibitor Ferrostatin-1 (Fer-1) proves to be significantly more efficient than phenolic antioxidants. Analytical and computational evidence supports the notion of a pseudo-catalytic cycle where the ferrostatin-iron complex, both produces and reduces lipid alkoxyl radicals from lipid hydroperoxides. This discloses the roadmap for the identification of innovative antioxidants competent for preventing ferroptosis.

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$CO_3^{\bullet-}$, THE RADICAL THAT CONNECTS PEROXYNITRITE AND FENTON CHEMISTRY

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Oxidative biochemistry centered about 35 years ago on the one-electron reduction of H_2O_2 by Fe^{2+}, the Fenton reaction, to yield $HO^{\bullet}$ and a Fe(III)-complex. The discovery that $NO^{\bullet}$ is formed *in vivo* and that it reacts with $O_2^{\bullet-}$ at a diffusion-controlled rate led to $ONOO^-$ as an additional oxidant. The rate constant of the Fenton reaction is 53 $M^{-1}s^{-1}$ up to about pH 4, but above it the rate constant increases linearly with pH. This acceleration of the Fenton reaction led to the hypothesis that above pH 5 formation of FeO^{2+} predominates. Thermodynamically, this species is comparable to $HO^{\bullet}$ as an oxidant. HCO_3^- accelerates the reaction even more, and convincing evidence has been presented that the complex of Fe^{2+} with CO_3^{2-} reacts with H_2O_2 to form $CO_3^{\bullet-}$ and a Fe(III)-complex, conceivably *via* FeO^{2+} as an intermediate. The rapid reaction of $ONOO^-$ with CO_2 ($k > 10^7$ $M^{-1}s^{-1}$) leads to $ONOOCO_2^-$ that, depending on the CO_2 concentration, yields varying amounts of $NO_2^{\bullet}$ and $CO_3^{\bullet-}$. These two oxidizing radicals together nitrate aromatic residues. Compared to 35 years ago, oxidative biochemistry is no longer concerned with the indiscriminate oxidations and additions of $HO^{\bullet}$, but with the more selective reactions of $CO_3^{\bullet-}$ and $NO_2^{\bullet}$.

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NUTRIGENOMICS OF VITAMIN E AND FATTY ACID METABOLISM IN LIPOTOXICITY AND OXIDATIVE STRESS-RELATED DISEASES

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Vitamin E (alpha-tocopherol, VE) is a nutrient essential to prevent the severe neurological symptoms of a genetic form of ataxia associated with vitamin deficiency (AVED). Its essentiality is also proven in secondary deficiencies associated with malnutrition and/or malabsorption syndromes that besides to neurological abnormalities may induce metabolic, musculoskeletal, hematological and immune symptoms, especially in vulnerable subjects such as the elderly. Mechanistic aspects of such an essentiality are far from being understood. VE is the most abundant fat-soluble hydrogen atom donor of the plasmalemma and its relative concentrations with respect to phospholipid residues is sufficient to influencing the flux of lipoperoxyl radicals generated during both the enzymatic and non-enzymatic processes of lipid peroxidation. Consequently, VE affects membrane stability and the lipid signaling of inflammatory eicosanoids and ferroptosis mediators. Also, VE directly or indirectly interacts with different proteins with enzymatic, signal transduction and transcriptional function. All these VE biological roles might concur to some extent to the pathophysiology of deficiency symptoms or to explain the therapeutic potential of this vitamin, or may be none of them. New insights in the biological complexity of this vitamin came from a recent series of studies that supported the participation of the long-chain metabolites of this vitamin in at least some of its “non-antioxidant” functions. Omics technologies are now offering a great opportunity to deal with such a complexity, exploring with unprecedent efficacy the molecular properties of this vitamin and its effects in clinical trials on deficiency syndromes and other human diseases. Transcriptomics and lipidomics have been utilized in our laboratories, either separate or in multiomics protocols, to develop personalized and precision nutrition (i.e. nutrigenomics) platforms of investigation dedicated to this vitamin. Examples of their potential for innovation in VE research will be given in this presentation, including studies on the liver metabolism of free fatty acids and hepatocyte lipotoxicity, the key etiologic factor of non-alcoholic fatty liver disease, and studies in kidney disease patients that develop a characteristic form of VE deficiency, along with oxidative stress and lipid peroxidation symptoms.

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HEPATOCYTE SEIPIN SILENCING REDUCES CHOLESTEROL-MEDIATED LIPID DROPLET MATURATION IN FATTY LIVER MODEL

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The incidence of non-alcoholic fatty liver disease (NAFLD) is gradually increasing with the prevalence of obesity, which is the strongest risk factor for steatosis. Lipid droplet (LD) accumulation in hepatocytes is a hallmark of NAFLD. Seipin protein, which is LD related protein, resides in the endoplasmic reticulum membrane and a shortage of this protein leads to accumulation of abnormal LDs in adipose tissue. Although it has been shown that adipose-specific Seipin deficiency causes increased lipid accumulation in liver and muscle tissue following abnormal LD formation and loss of adipose tissue function, Seipin protein deficiency in liver tissue and its effect on lipid accumulation have not been investigated. Our study aimed to investigate the effect of Seipin deficiency on ER stress and lipophagy in cholesterol-accumulated mouse hepatocyte cells (AML12 cell line). In this direction cholesterol accumulation in mouse hepatocyte cells was established by administrating cholesterol-containing liposome and Seipin levels were reduced using siRNA transfection. Following liposome-cholesterol and siRNA administrations, lipophagy was determined by confocal microscopy, and mRNA levels of GRP78, GRP94, and ATF4 were examined by qRT-PCR. Our findings show that cholesterol-containing liposome administration in hepatocytes increases both Seipin protein and number of large LDs. However, Seipin silencing reduced the increase of cholesterol-mediated large LDs and GRP78 mRNA. Additionally, lysosome-LD colocalization increased only in cells treated with cholesterol-containing liposome, while the siRNA against Seipin did not lead to any significant difference. According to our results, we hypothesise that Seipin silencing in hepatocytes reduced cholesterol-mediated LD maturation as well as GRP78 levels, but not lipophagy.

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DIETARY NITRATE AS PIVOT ON THE GUT MICROBIOTA-HOST REDOX COMMUNICATION

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Humans are complex holobionts in which many physiological functions are ensured by the gut microbiota. The communication between the microbiota and its human host relies on immune, neural, metabolic and endocrine pathways and the derailment of this interaction can lead to gastrointestinal and systemic diseases. Here, we propose a novel form of communication between the microbiota and the host, based on the production of redox species by gut bacteria and the activation of signaling cascades in host mucosa. The biological significance of such a pathway is further highlighted by the observation that these inter-kingdom interactions are modulated by dietary nitrate, the major precursor of nitrite and NO *in vivo*. We demonstrate that nitrate has a positive metabolic effect in a murine model of antibiotic-induced dysbiosis by regulating cecum morphology and body weight ($p<0.05$). In agreement with these observations, shallow shotgun sequencing analysis showed that nitrate modulates the metabolic function of bacteria involved in the metabolism of carbohydrates, likely aiding in food digestion and substrate delivery to the host. Furthermore, we observed that the exposure to antibiotics decreases the expression of tight junction proteins in the colon and that nitrate recovers the expression of both occludin ($p<0.05$) and claudin-5 ($p<0.01$). The activation of the Nrf2/ARE pathway was also investigated by the downstream expression of detoxifying enzymes including NQO1 and GCLM/GCLC. Here, dietary nitrate emerges as a pivot regulating microbiota-host interactions through redox pathways. Nitrate modulates the function of gut microbiota during dysbiosis by enhancing bacterial metabolic performance with positive effects on host body weight and prevents the loss of tight junction proteins likely reinforcing gut barrier integrity. Given that increased epithelial permeability may lead to *leaky gut syndrome*, triggering local and systemic disorders, this study has the potential to transform the way Redox Biology expands from the bench to patient's bedside.

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AFFECTING CUTANEOUS NRF2-KEAP1 PATHWAY BY UNIQUE EXOGENOUS AND ENDOGENOUS ACTIVATORS

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The human skin, being our outermost protective barrier, sustains continuous contact with the environment. As such, its cells must be kept in a state of constant alert against external increased oxidative stress and massive environmental insults (e.g. sunlight and UV radiation, air pollution, and mechanical stress). All these insults ultimately result in an impaired redox balance and increased cellular oxidation. One of the pivotal oxidation regulation mechanisms in the skin is the Nrf2–Keap1 pathway, and its activity leads to cutaneous redox maintenance which evidently sustains the principle of hormesis. We suggest that moderate environmental stressors and skin microbiome can provide the necessary continuous stimuli for the activation of the Nrf2 pathway. We also suggest that endogenous neurotransmitters play a major role in this activation.

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TRIAL READINESS IN MITOCHONDRIAL MEDICINE

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Mitochondrial diseases, characterized by dysfunction in the cellular powerhouse, the mitochondria, present a complex and heterogeneous group of disorders. As research in mitochondrial medicine advances, the need for effective therapies becomes increasingly apparent. Collaborative efforts among researchers, clinicians, regulatory bodies, patient advocacy groups and other stakeholders are crucial to overcome the challenges linked to the complexity of mitochondrial medicine, and to ensure the successful implementation of clinical trials in this field. This lecture explores the key aspects of trial readiness in the context of mitochondrial medicine, emphasizing the challenges and opportunities in designing and executing successful clinical trials. An overview of the ongoing clinical trials will be also provided.

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MITOCHONDRIAL DISEASE: FROM MECHANISMS TO THERAPY

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Mitochondrial diseases are a large family of extremely heterogeneous disorders genetically determined by mutations in either the nuclear genome or the mitochondrial DNA. Most of the mitochondrial disease genes are expressed in all cell types. However, in many conditions, some cell types are more affected than others. However, the reasons for this tissue-specificity remain poorly understood. To investigate the functional basis of the striking tissue-specificity in mitochondrial diseases, we analyzed several bioenergetic parameters, including oxygen consumption rates, Q redox poise, and reactive oxygen species production in mouse brain and liver mitochondria fueled by different substrates. In addition, we determined how these functional parameters are affected by electron transport chain impairment in a tissue-specific manner using pathologically relevant mouse models lacking either *Ndufs4* or *Ttc19*, leading to complex I or III defects, respectively. No cure is currently available for most of the mitochondrial diseases. We previously showed that the coordinated activation of autophagy, lysosomal biogenesis, and mitochondrial biogenesis by rapamycin, ameliorated the myopathic phenotype of a muscle-specific knockout mouse for *Cox15* (*Cox15sm*), encoding an enzyme involved in heme A biosynthesis. However, the role of mitophagy has been poorly investigated. We found that urolithin A, a direct mitophagy inducer, improved motor performance and myopathy in the *Cox15sm* mice, without increasing the activity of the respiratory chain complexes in a 10 week-treatment. These results indicate that activation of mitophagy can be a suitable treatment to ameliorate mitochondrial myopathies.

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INSULIN MODULATES MITOCHONDRIAL STRUCTURAL AND FUNCTIONAL MOSAICISM IN BROWN ADIPOCYTES

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Since the discovery of the thermogenic role of brown adipocytes, there was consensus that the biochemical and metabolic function of their mitochondria is uniform. By switching the ATP production between glycolytic pathway and oxidative phosphorylation, brown adipocytes are able to produce heat in mitochondria through uncoupling protein 1 (UCP1). Thermogenically active brown adipocyte mitochondria are characterized by clear morphological features (long, tightly packed cristae). The process of their biogenesis includes an increased numberof mitochondria (by division), increase of their surface area, and incorporation of UCP1 as well as specific structural organization of the cristae. But, is it true that all brown adipocytes mitochondria within one cell are structurally and functionally the same? Do they harbor the same set of enzymes? Actually, the very first cell mosaicism, e.g. Harlequin appearance was shown in brown adipose tissue. This unique uneven UCP1 expression suggests that brown adipocyte's mitochondria may be heterogeneous regarding production of ATP (bioenergetic) vs. heat (thermogenic) role. This presentation deals with structural and functional mitochondrial mosaicism and changes caused by insulin.

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HIBERNATION AND NEUROPROTECTION: DIFFERENTIAL EXPRESSION OF FERROPTOSIS-RELATED GENES IN ARCTIC GROUND SQUIRRELS

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Ferroptosis, a form of regulated cell death characterized by iron-dependent lipid peroxidation, is linked to neurodegenerative disorders and cold-induced cell death. SLC7A11 (xCT) plays a crucial role in protecting cells against ferroptosis by maintaining intracellular cysteine and glutathione levels. SLC7A11 requires the chaperone protein SLC3A2 for its localization on the plasma membrane to mediate cystine uptake. Arctic ground squirrels (AGS) are known to be protected from cold tissue temperatures and oxidative stress and to resist neuropathology following cerebral ischemia/reperfusion. This study investigated how ferroptosis is influenced by the hibernation season in AGS hippocampus. RNA-Seq, gene expression, and differential gene expression analysis were conducted on hippocampus tissue samples from male and female AGS collected during the summer active season, torpor, and interbout arousal (IBA). Hippocampus was dissected from partially thawed whole brain prior to RNA extraction. Total RNA samples were used for cDNA library construction and sequencing by BGI Americas Corporation (Cambridge, MA) and analyzed using CLC Genomics Workbench (QIAGEN). Genes were mapped to the *Ictidomys tridecemlineatus* reference genome and transcript (HiC_Itri_2, GCF_016881025.1). Results show the highest number of differentially expressed genes (4,042) in torpor compared to summer active animals. Notably, SLC7A11 expression was elevated in torpor compared to summer active animals (fold change: 1.80, FDR-p value: 0.0034). Additionally, SLC3A2 was significantly upregulated in torpor compared to IBA (fold change: 1.24; FDR-p value: 0.030). SLC7A11 transports glutamate(out)/cystine(in). Cystine is rapidly converted into cysteine, a limiting reactant for glutathione synthesis, in the presence of NADPH. These findings suggest that SLC7A11 and SLC3A2 may protect AGS from ferroptosis during the hibernation season. This research provides insights into the molecular mechanisms underlying neuroprotection in hibernating AGS and may have implications for understanding and potentially treating neurodegenerative disorders.

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NRF2/AMPK AXIS IS REQUIRED FOR REDOX-MEDIATED PHASE RESETTING OF MUSCULOSKELETAL CLOCKS UPON ACUTE MECHANICAL LOADING

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In mammals, a multi-oscillator circadian system generates behavioural, metabolic and physiological ~24h rhythms, with tissue-specific physiological cues enabling local circadian phase adjustments. Emerging work has shown musculoskeletal tissue homeostasis and mechanical responses to be under circadian control. Nuclear factor erythroid 2-related factor 2 (NRF2), a master regulator of the antioxidant response, is a clock-controlled target in several peripheral tissues and also modifies circadian gene expression and rhythmicity. However, the role of NRF2 in mechanical loading-induced changes in musculoskeletal tissues has yet to be elucidated. Wild-type (WT) and *Nrf2* KO mice of young (3-6m) or old age (18-20m) harbouring a PER2::*luciferase* clock reporter were subjected to acute mechanical joint loading of the right leg (peak load 9N, 40 cycles of 10sec) during light phase whilst the contralateral (left) leg served as a non-loaded control. Musculoskeletal tissues were collected for analysis 4 hrs later. Real-time bioluminescence imaging of clock gene reporter activity, protein and mRNA levels of target markers, NRF2/ARE transactivation and genome-wide RNAseq analyses were undertaken. We show that acute mechanical loading in WT mice led to a decrease in gene expression of key members in the negative and auxiliary feedback loops of the molecular clock, associated with the phase-resetting of PER2::luc protein oscillations in the skeletal muscle and a knee joint. This was accompanied by a significant increase in the markers of oxidative burden as well as gene expression and protein abundance levels of antioxidant enzymes. Moreover, acute mechanical loading induced a significant activation of the redox-sensitive energy sensor, AMP-activated kinase (AMPK), known to be involved in molecular clock resetting. We thus examined whether the above acute mechanical responses were dependent on NRF2 activity. *Nrf2* KO mice showed an altered response to acute mechanical loading, characterized by blunted circadian resetting and antioxidant responses, and altered AMPK activation. Furthermore, dampened responses to acute mechanical signals were found in ageing WT mouse musculoskeletal tissues, whilst AMPK activator treatments in WT mice induced circadian resetting and antioxidant responses in an NRF2-dependent manner. In conclusion, these data demonstrate that AMPK/NRF2 axis is required for relaying acute mechanical signals to the musculoskeletal system by controlling redox-mediated phase resetting of musculoskeletal clocks and antioxidant protection, which have important implications in understanding biomechanical mechanisms involved in musculoskeletal tissue maintenance in health and with ageing.

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ROLE OF MITOCHONDRIA IN THE PHYSIOPATHOLOGY OF THE CARDIOMYOPATHY ASSOCIATED TO FRIEDREICH'S ATAXIA. STUDIES IN HUMAN iPS CELLS

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Friedreich's ataxia (FRDA) (OMIM #229300, ORPHA95) is a rare hereditary disease with a prevalence of 1/20,000 to 1/50,000 in the European population. It is classified as a hereditary peripheral neuropathy of a sensory type, with autosomal recessive inheritance. This disease is caused by the deficiency of a mitochondrial protein called frataxin. Lack of expression of this protein produces accumulation of iron, alterations in the biogenesis of iron-sulfur clusters, failures in complexes I, II and III of the respiratory chain and in the activity of the aconitase enzyme, and a reduction in the biosynthesis of the heme groups. As a consequence, finally, an overload of ROS derived from the Fenton reaction occurs. Together with the movement impairment, 60% of FRDA patients suffer cardiomyopathy, which is the most common cause of death in these patients and has no clear explanation of its physiopathological cause. Two iPSC cell lines from FRDA patients with cardiomyopathy) and a control line were differentiated to ventricular cardiomyocytes in our lab. Both FRDA cell lines showed changes in heartbeat parameters, such as heart rate and amplitude when compared to the control cell line. Also, calcium homeostasis measured by immunofluorescence showed important differences when compared to the control cell line. RT-PCR analyses of miRNAs related to myocardial function also showed clear differences, especially for miR-323-3p and miR-142-3p. Using EM, we found differences in the mitochondrial size, shape and in mitochondrial cristae organization. These results also correlate with changes in the cardiomyocytes cytoskeleton and in the structure of the sarcomeres using confocal microscopy techniques. Our results showed the correlation between mitochondrial changes and the impairment in ventricular cardiomyocytes activity derived from FRDA's iPS cells.

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ABERRANT MITOCHONDRIA-INFLAMMASOME CROSS-TALK IN RETT SYNDROME

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Rett syndrome (RTT), a devastating neurodevelopmental disorder, is caused in 95% of the cases by mutations in the X-chromosome-localized MECP2 gene. RTT manifests as a range of multisystem disturbances including altered lipid profile, subclinical inflammation, and overall OxInflammatory status in which mitochondrial dysfunction acts as central player. To decipher the molecular mechanisms underlying the pathophysiological manifestations affecting patients, we investigated whether mitochondria may play a role in the aberrant immune and oxidative responses of RTT. Recent findings from our and other labs unraveled several abnormalities in RTT mitochondria including atypical mitochondrial structure, deregulated expression of genes encoding oxidative phosphorylation factors and mitochondrial organization factors, impaired mitochondrial quality control, depressed energetic profile, and augmented mt-ROS production. In other brain diseases, mitochondrial dysfunction is a vital event during the activation of NLPR3 inflammasome, a multi-protein complex involved in innate immune response, that represents a common denominator in the crosstalk between inflammation and oxidative stress. Interestingly, using primary fibroblasts and lympho-monocytes isolated from RTT patients, we found a constitutive hyperactivation of NLRP3:ASC inflammasome associated with increased levels of nuclear p65 and ASC proteins, and pro-IL-1β mRNA, without the ability to further respond to the LPS + ATP stimuli. Furthermore, increased circulating levels of ASC, interleukin (IL)-18, and 1β were found in RTT individuals, thus corroborating the aforementioned cellular findings. In order to evaluate NLRP3 involvement in the transition from pre-symptomatic to symptomatic phase of RTT, we detected higher serum levels of IL-1β and IL-18 in symptomatic Het mice compared to WT. Of note, increased gene expression of Il-1b, Nlrp3, and ASC was observed in Het brains at the pre-symptomatic stage, suggesting a likely role of NLRP3 impairment in the early stages of the disease. Preliminary data showed that treatment with resveratrol, known to improve mitochondrial function, ameliorated the RTT mouse phenotype by restoring levels of some NLRP3-related components. Furthermore, mitochondrial dysfunction can result in ferroptosis, a form of cell death characterized by iron-dependent lipid peroxidation and accumulation of reactive oxygen species. After

treatment with two ferroptosis inducers, erastin (GPX4 inhibitor) or RSL3 (inhibitor of the cystine/glutamate antiporter), we found changes in GPx and GR activity, alteration in GPX4 protein levels and increased formation of 4HNE protein adducts. Mitochondrial ROS production and lipid peroxidation levels were higher in RTT after ferroptosis induction, while co-treatment with ferrostatin-1, a well-known inhibitor of ferroptosis, significantly prevented these processes. Interestingly, co-treatment with mito-TEMPO, a mitochondria-targeted superoxide dismutase mimetic, mitigated mitochondrial oxidative burden and prevented ferroptosis cell death in RTT cells. Overall, our results demonstrate the decisive role of mitochondrial dysfunction in RTT OxInflammation. Thus, we can speculate that exposure of RTT cells to any condition affecting the already compromised mitochondrial function could not only hyperactivate the inflammatory status but also precipitate ferroptosis cell death. Targeting mitochondria in RTT could represent a strategic coadjuvant therapy to improve the quality of life of the affected patients.

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MITOCHONDRIAL TRANSLATION IS THE PRIMARY DETERMINANT OF SECONDARY MITOCHONDRIAL COMPLEX I DEFICIENCIES

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Individual complexes of the mitochondrial oxidative phosphorylation system (OXPHOS) are not linked solely by their function; they also share dependencies at the maintenance/assembly level, where one complex depends on the presence of a different individual complex. Despite the relevance of this 'interdependence' behavior for mitochondrial diseases, its true nature remains elusive. To understand the mechanism that can explain this phenomenon, we examined the consequences of the aberration of different OXPHOS complexes in human cells. We demonstrate here that complete disruption of each of the OXPHOS complexes resulted in a perturbation in energy deficiency sensing pathways, including the integrated stress response (ISR) pathway. The secondary decrease of complex I (cI) level was triggered by both complex IV and complex V deficiency, and it was independent of ISR signaling. On the other hand, we identified the unifying mechanism behind cI downregulation in the downregulation of mitochondrial ribosomal proteins and, thus, mitochondrial translation. We conclude that the secondary cI defect is due to mitochondrial protein synthesis attenuation, while the responsible signaling pathways could differ based on the origin of the OXPHOS defect.

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NITRITE MITIGATES OXIDATIVE BURST IN ISCHEMIA/REPERFUSION IN BRAIN SLICES

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Nitrite is the typical byproduct of nitric oxide (•NO) autooxidation in biological systems. However, certain circumstances favor its reduction "back" to the signaling free radical, providing a non-enzymatic route for the synthesis of •NO. In pathophysiological conditions such as ischemia/reperfusion (I/R), where low oxygen availability limits nitric oxide synthase activity, nitrite reduction to •NO may allow protective modulation of mitochondrial oxidative metabolism and thus reduce the impact of I/R on brain tissue. In the current study, we used high-resolution respirometry to evaluate the effects of nitrite in an *in vitro* model I/R using hippocampal slices. We found that reoxygenation was accompanied by an increase in oxygen flux, a phenomenon that has been coined "oxidative burst". The amplitude of this "oxidative burst" was decreased by nitrite in a concentration-dependent manner. These results support the notion that nitrite mediates a decrease in the hyper-reduction of the electron transport system during ischemia, decreasing the accelerated oxygen consumption that characterizes the reoxygenation phase of I/R that has been associated with an increase in oxidant production. Additionally, a pilot in vivo study in which animals received a nitrate-rich diet as a strategy to increase circulating and tissue levels of nitrite also revealed that the "oxidative burst" was decreased in the nitrate-treated animals. These results may provide mechanistic support to the observation of a protective effect of nitrite in situations of brain ischemia.

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NOVEL TARGETED VIOLOGEN FOR THE INDUCTION OF SUPEROXIDE PRODUCTION IN MITOCHONDRIA

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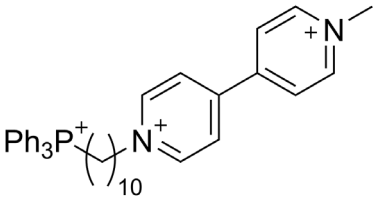
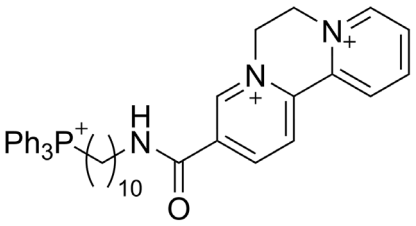
Mitochondrial production of $O_2^{\bullet-}$ and H_2O_2 has been implicated in redox signaling and in the pathogenesis of numerous diseases including cancer, neurodegeneration, and cardiovascular diseases. To understand the exact role of those species, new chemical biology tools for selective and efficient induction of mitochondrial superoxide production are needed. Here, we report the development of a new viologen-based redox cycling agent, mito-diquat (Mito-DQ), capable of inducing targeted mitochondrial $O_2^{\bullet-}$ production at significantly higher rates as compared to previously reported mito-paraquat (Mito-PQ), a widely used chemical tool to study mitochondria-dependent redox signaling[1,2].



Mito-PQ



Mito-DQ

Mito-DQ was synthesized by coupling a diquat (DQ) moiety to a mitochondria-targeting triphenylphosphonium cationic group via an alkyl linker[3]. To study the redox cycling activity of Mito-DQ in a cell-free system, xanthine oxidase (XO)-catalyzed oxidation of NADH as well as isolated bovine heart mitochondria were used as sources of $O_2^{\bullet-}$. Pulse radiolysis experiments were performed to characterize the radical species produced upon one-electron reduction of Mito-DQ and to determine the rate constant of its reaction with molecular oxygen to produce $O_2^{\bullet-}$. Induction of oxidant production in intact cells was studied using C2C12 myoblasts. Cellular production of $O_2^{\bullet-}$ was measured using high-performance liquid chromatography (HPLC) whereby hydroethidine probe oxidation to 2-hydroxyethidium was monitored. Stimulation of H_2O_2 production was measured by determining the rate of catalase-sensitive conversion of Amplex Red to resorufin, catalyzed by horseradish peroxidase. Our results indicate that Mito-DQ stimulates NADH oxidation, O_2 consumption, and $O_2^{\bullet-}$ production by NADH/XO system

in a dose-dependent manner (0.1-100 μM) and in isolated mitochondria. Mito-DQ-derived radical is stable in the absence of molecular oxygen, while decays within 200 μs in an air-equilibrated solution. Mito-DQ dose-dependently (1-100 μM) induced $O_2^{\bullet-}$ and H_2O_2 production in C2C12 cells under the conditions when no significant stimulation of oxidant production is observed for Mito-PQ. We conclude that Mito-DQ may be a useful chemical tool to study the role of mitochondrial $O_2^{\bullet-}$ production in model biological systems.

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MULTIMODAL IMAGING OF CELLULAR SENESCENCE – OXIDIZED LIPIDS AND ENZYMATIC ADAPTATIONS IN AGING SKIN AT THE SINGLE CELL LEVEL

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Changes in carbohydrate metabolism are a key feature of aging which also manifest in the epidermis. Furthermore, the synthesis and distribution of epidermal lipids changes with age. Both these parameters cannot be investigated with immunohistochemistry, as neither serves as useful epitope. We developed a multimodal analytical histocytometry approach combining modalities that localize lipids and enzymatic activities with immunofluorescent imaging of the skin to localize changes that are correlated with appearance of senescent cells. The activities of key metabolic enzymes were determined on tissue sections of aged and juvenile skin with a formazan-based assay. Lipids were localized and quantified using FTICR MALDI - mass spectrometric imaging. We correlated those modalities with immunofluorescent imaging and analyzed the intensities of the respective signals at single cell level, using Strataquest tissue cytometry. We analyzed skin from donors of young (< 30 y) versus advanced (> 67 y) ages and we investigated epidermal equivalent models containing labeled UV-damaged or senescent keratinocytes. Enzymatic activities displayed specific patterns across the stratifying epidermis, and had diverging trajectories in aging, with a marked decrease in suprabasal glucose-6-phosphate dehydrogenase (G6PD) activity. G6PD, the rate limiting enzyme of the pentose phosphate pathway was also identified as a rapid response pathway activated upon UV damage in the epidermis. The lipid molecular imaging identified differentiation- and age-related changes of polar lipids in skin biopsies and epidermal equivalents, and pro-senescent stress dependent reactive aldehydophospholipid species in the basal epidermal layers. While these methodologies are still in development, it is evident that correlative analytical imaging – with the aid of AI driven histocytometry – will continue to yield novel insights into skin and epidermal biology by localizing previously undetectable parameters within the epidermis in the context of aging.

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BIOIMAGING AND CHEMOGENETICS IN REDOX METABOLISM STUDIES

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A deep understanding of the roles of redox metabolites and pathways in physiology and pathology requires molecular tools that enable both visualization of these processes and their selective modulation. Over the last two decades, a number of genetically encoded fluorescent biosensors for key redox metabolites have been developed, allowing real-time detection in living systems of varying complexity. Recent developments in this area include the ultrasensitive probe HyPer7 and a new fluorogenic probe, HyPerFAST, which enables even more sensitive H_2O_2 detection across any chosen optical range, from blue to near-infrared. Complementary to imaging with biosensors, chemogenetics offers tunable substrate-dependent modulation of metabolic pathways, allowing the study of normal cell functioning and modeling dysfunctions caused by abnormal pathway activity and/or metabolite levels. We will present recent developments in this area that include insights on oxidative stress brought about by the use of D-amino acid oxidase (DAO) and intriguing details of the Warburg effect brought about by a new mitochondrial “booster”, Grubraw, based on bacterial D-amino acid dehydrogenase.

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EXPERIMENTAL DATA ON SQUAMOUS CELL CARCINOMA

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In recent decades, a global increase in the incidence of skin cancer, particularly squamous cell carcinoma (SCC), has been observed. To explore the pathogenesis and potential therapeutic approaches for this cancer type, *in vivo* studies employing various mouse models and ultraviolet (UV) light have been conducted. A comparative study on skin carcinogenesis across four hairless mouse models subjected to UV light exposure was initiated. The mouse strains utilized in this research were: SKH-hr1, SKH-hr2, SKH-hr2+ApoE, and immunodeficient Nude. Based on the various measured parameters, in contrast to the SKH-hr1, SKH-hr2+apoE and SKH-hr2 models were identified as the most appropriate. The bark extract of *Pinus maritima* (PBE) was examined for SCC preventive action. It was evaluated in two different experimental animal tumor models induced by ultraviolet radiation (UVR) and combination of UVR with 7,12-dimethylbenz[a]anthracene. A significant decrease in the number of animals bearing tumors, increase in viability and delayed appearance of tumors were observed. Through immunochemical analysis, the expression of P-glycoprotein, multi-drug resistance-associated protein (MRP), and glucose (GLUT-1) transporters in SCC, SCC adjacent area, and normal skin tissues were examined. It was revealed that all assessed transporters were expressed across all skin tissues; however, expression levels were notably higher in tumor and tumor-adjacent areas compared to normal tissues. Male and female hairless SKH-2 mice were exposed for 10 months to cigarette smoke (CS) and/or UV light after administration or not of French maritime pine bark extract (PBE) to study the SCC induction and possible protection by PBE. The results showed that UV and CS were harmful and act synergistically inducing SCC, whereas PBE seems to protect skin against SCC. Type 1 and 2 diabetic, and nondiabetic male mice were exposed to UV radiation for eight months. Remarkably, Type 1 diabetic mice did not develop squamous cell carcinoma or pigmented nevi, contrary to normal and Type 2 diabetic skin. Type 1 diabetic mice showed protection against oxidative stress.

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REDOX AND METABOLIC REPROGRAMMING OF BREAST CANCER CELLS AND ASSOCIATED ADIPOSE TISSUE - THE CORNERSTONES OF ADAPTIVE TUMOUR BEHAVIOUR

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A high proliferation rate and the malignancy of cancer cells are favoured by redox and metabolic plasticity, which is determined by the co-evolution of cancer cells with their host microenvironment. The tight functional connections between the mammary glands' epithelium and adipose tissue (AT) allow breast cancer cells to subjugate the AT and form a protumorigenic cancer-associated adipose tissue (CAAT). Our findings in luminal invasive ductal carcinomas in premenopausal women confirmed key cancer cell strategies - the Warburg effect, increased mitochondrial metabolism and redox adaptability, which are associated with a specific shift in the metabolic and redox phenotype of CAAT. Notably, the upregulated master redox-sensitive transcription factor Nrf2 appears to be responsible for the cancer cell-induced redox and metabolic shift of CAAT. We also investigated the role of Nrf2 in the metabolic co-evolution of cancer cells and CAAT during disease progression. Our results in the orthotopic breast cancer mouse model and in the co-culture of breast cancer cells with adipocytes confirmed the different spatiotemporal redox and metabolic properties of cancer cells and CAAT, established with respect to the Nrf2-coupled/uncoupled tumour microenvironment. The uncovered metabolic and redox strategies adopted by breast cancer cells according to CAAT properties and at different disease stages have helped to better understand the biology of the aggressive disease and to identify breast cancer vulnerabilities that could become therapeutic targets.

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REGULATION OF INTRACELLULAR CYSTINE REDUCTION AND PROTEIN CYSTEINYLATION

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The reduction of intracellular cystine to yield cysteine is critical for protein or glutathione synthesis and many other important biological processes, but its regulation is still unknown. We have shown that the thioredoxin-related protein of 14 kDa (TRP14) is the rate-limiting enzyme for intracellular cystine reduction. Upon TRP14 deficiency, cysteine synthesis through the transsulfuration pathway becomes the major source of cysteine in human cells, and knockout of both pathways is lethal in *C. elegans* subjected to proteotoxic stress. TRP14 can also reduce protein cysteinylation. However, paradoxically TRP14 knock-out mice were protected in acute pancreatitis through activation of Nrf2 and upregulation of the transsulfuration pathway, thus exhibiting less inflammatory infiltrate and edema. Therefore, TRP14 seems to be the enzyme principally responsible for intracellular cystine reduction, and it is also able to regulate protein cysteinylation together with thioredoxin 1.

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BLOOD REDOX STATUS IN DIFFERENT HUMAN PATHOLOGIES

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The *in vivo* determination of oxidative stress always remains a great challenge. Our approach in Liège CHU consists of simultaneously measuring in blood samples four different kinds of biomarkers: enzymatic and non-enzymatic antioxidants, trace elements, markers of oxidative damage to lipids, and identification of sources leading to increased reactive oxygen species (ROS) production. All these biomarkers (n = 16) have been investigated in patients: 1) with Abdominal Aortic Aneurysm (AAA)[1] or operated for Thoracic Abdominal Dissection (TAD)[2], 2) suffering from Chronic Obstructive Pulmonary Disease (COPD)[3] or FacioScapuloHumeral Myopathy (FSHM)[4], 3) with COVID-19[5,6] and 4) with delirium[7]. When compared to our internal reference values, depletion in non-enzymatic antioxidants (vitamin C, β-carotene, vitamin C/vitamin E ratio, thiol proteins) and trace elements (zinc, selenium) was observed in the majority of these pathologies. By contrast, increased levels in glutathione peroxidase, copper/zinc ratio, lipid peroxides (ROOH), and myeloperoxidase are common in all these diseases.

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PREPARATION FOR OXIDATIVE STRESS: HISTORY, RECENT ADVANCES AND FUTURE DIRECTIONS

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Many animal species are remarkably resilient to the harmful conditions of hypoxia and reoxygenation, a phenomenon widely observed across many species and environmental settings. The ability to survive oxygen deprivation and reintroduction without significant cellular damage is partially attributed to the upregulation of antioxidants, a strategy termed “Preparation for Oxidative Stress” (POS). The concept of POS is that by producing more antioxidants under hypoxia animals would anticipate the eventual and potentially damaging reintroduction of oxygen. Historically, the specific mechanisms through which POS is activated remained elusive. Over the past decade, significant advancements have been made in understanding POS at a molecular level and in identifying its widespread in the animal kingdom. Notably, a detailed molecular mechanism for the activation of POS under conditions of low oxygen availability has been proposed, emphasizing the role of reactive oxygen species in modulating antioxidant response through redox-sensitive transcription factors. Furthermore, recent research has demonstrated the occurrence of POS in free-ranging animals under completely natural settings, confirming its ecological and physiological relevance. Despite recent advancements, some aspects of POS remain underexplored and should be prioritized in future research. These include the experimental validation of the mechanisms proposed to underlie POS and the assessment of the relevance of POS in multi-stressor scenarios, particularly to understand how organisms cope with combined stressors in fluctuating environments.

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EXERCISE-INDUCED SYSTEMIC RESPONSE: THE ROLE OF CIRCULATING EXTRACELLULAR VESICLES

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Regular physical exercise (PE) leads to a systemic adaptation to redox homeostasis perturbation, one of the hallmarks of exercise adaptation. Studies have shown that PE can alter the molecular composition of extracellular vesicles (EVs), impacting their ability to communicate with other cells and modulate physiological processes. EVs circulating in the body and secreted from various cell types, including skeletal muscle cells, contain various regulatory molecules and mediate intercellular communications and tissue cross-talk. Considering that the health-related benefits of a physically active lifestyle are partially driven by various bioactive molecules released into the circulation during exercise, collectively termed “exerkines”, there has been a rapidly growing interest in the role of EVs cargo as “carriers” in the multi-systemic, adaptive response to exercise. Indeed, a potential mechanism by which plasma EVs released during exercise impact ageing and diseases related to redox impairment is increased delivery of redox components, such as redox transcription factors and antioxidants. This presentation will offer a general overview of the biology of exercise-induced EVs and their putative role in health maintenance and disease prevention, with a focus on redox homeostasis control.

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REDOX REGULATION OF NEUROVASCULAR COUPLING BY NITRIC OXIDE TO IMPROVE COGNITION IN AGING AND NEURODEGENERATION

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The physicochemical properties of nitric oxide (NO) as an intercellular messenger, in particular the way it conveys information via volume signaling, translate into advantages of communication in the brain. This becomes apparent when considering neurovascular coupling (NVC), the tightly temporal and spatial functional communication between active neurons and local blood microvessels. That the brain is energetically expensive given its mass and that increased neuronal activity in a region of the brain is associated with a local increase in blood flow (CBF) has been known since the XIX century. In turn, the association between CBF dysregulation and cognitive decline has been consistently established in older adults (brain aging, neurodegenerative diseases, type II DM) and lab rodent models but the neurobiological links are poorly understood. I will discuss the notion that neuronal-derived NO is the key mediator of NVC in the hippocampus and that impairment of NVC is an early and likely causative event leading to cognitive decline. The premise is that by rescuing the functionality of NVC then cognitive enhancement should be observed. This will be experimentally supported on basis of a diet-driven redox mechanism, involving the interaction of nitrite with ascorbate released from active neurons. Data suggest that an operational NVC, allocating energy resources according to neuronal activity, is a most fundamental biochemical process that underlines biological organization to support cognition.

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MITOCHONDRIAL SIRTUIN 3 (SIRT3) IN AGEING: EXPLORING CELLULAR RESPONSES TO ETOPOSIDE-INDUCED DNA DAMAGE IN MALE AND FEMALE MOUSE EMBRYONIC FIBROBLASTS

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Ageing is a complex process characterised by the gradual deterioration of physiological functions and increased susceptibility to various age-related diseases. Mitochondrial dysfunction is an important factor contributing to ageing. Sirtuin 3 (Sirt3), a mitochondrial protein essential for energy homeostasis, plays a critical role in maintaining mitochondrial function, as loss of Sirt3 reduces energy and impairs cellular repair, which accelerates ageing. The aim of this study was to investigate the role of Sirt3 in male and female mouse embryonic fibroblasts (MEF) exposed to etoposide-induced DNA damage. We employed state-of-the-art genetic, molecular, and imaging technologies as well as metabolomic analyses to provide insights into the molecular mechanisms underlying these responses. We found that the loss of Sirt3 affected metabolic responses differently depending on sex: while male MEF showed minimal damage, possibly due to earlier stress adaptation, female MEF lacking Sirt3 were more vulnerable, suggesting that Sirt3 plays a critical role in enhancing their ability to withstand such challenges. By focusing on Sirt3 and sex-specific signalling pathways it modulates, this study has a potential for developing new strategies to combat diseases associated with DNA damage – a cornerstone of the ageing process.

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DIFFERENTIAL SPATIAL DISTRIBUTION OF SYNTHETIC NANO- AND MICRO-PARTICLES EXPLAINS THE EFFECTS ON CARDIOVASCULAR FUNCTION – IMPLICATIONS FOR AIR POLLUTION HEALTH EFFECTS

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Particulate matter (PM) air pollution presents a major environmental and public health challenge because of its non-uniform size distribution and chemical composition. Air quality regulations generally categorize particulate matter (PM) size into PM_{10}, $PM_{2.5}$, and ultrafine particles (UFPs) with aerodynamic diameters smaller than 10, 2.5, and 0.1 µm, respectively. We examined the differential impact of particle size per se on selected organ systems using a custom whole-body mouse exposure system using synthetic PM (SPM). The micrometer-sized SPM accumulated in the lungs as the primary entry organ, while ultrafine SPM showed less accumulation, implying a transition into circulation. Micro SPM-exposed mice exhibited inflammation and NADPH oxidase-derived oxidative stress in the lungs. Ultrafine SPM-exposed mice did not show oxidative stress in the lungs but rather at the brain, heart, and vasculature levels. Endothelial dysfunction and blood pressure increase were more pronounced in ultrafine SPM exposed mice, supported by increased endothelin-1 and decreased endothelial nitric oxide synthase expression, enhancing constriction and reducing vasodilation. To derive a preliminary estimate of the cardiovascular disease burden of UFPs in humans, we used new high-resolution exposure data at a global scale, and applied hazard ratios from an epidemiological cohort study. We derived a UFP-associated incidence of 419 (95% CI 78–712) thousand cardiovascular disease cases per year in the European Union and 5.6 (95% CI 1.1–9.3) million globally. This work provides novel insights into the different toxicological profiles of inhaled ultrafine particles and public health consequences of exposure, guiding future studies.

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FMP40 AMPYLASE REGULATES CELL SURVIVAL UPON OXIDATIVE STRESS BY CONTROLLING PRX1 AND TRX3 OXIDATION

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AMPylation (adenylation) is one of the post-translational protein modifications (PTM) leading to the diversification of protein functions and activity. With our collaborators, we discovered that the SelO family members of humans, yeast, and E. coli have AMPylase activity. The yeast SelO – Fmp40 – was identified in the proteome of the inter-membrane space of mitochondria. We have shown that Fmp40 is involved in the response of cells to hydrogen peroxide (H_2O_2) and menadione treatment: cells lacking the Fmp40 AMPylase grow sensitivity upon H_2O_2 and menadione treatment. *E. coli* SelO AMPylates glutaredoxin GrxA and the s-glutathionylation level of proteins is reduced in bacterial and yeast cells lacking SelO[1]. The objective of the study is to reveal the biological functions of Fmp40 in mitochondrial redox regulation. The decreased survival of *fmp40*Δ cells, observed in survival tests, depends on the oxidation of Trx3 upon oxidative stress. In contrast, we verified that *fmp40Δ* cells are resistant upon exposure to high concentrations of the hydrogen peroxide - phenotype dependent on the presence of the Glutaredoxin *Grx2, Thioredoxin Trx3, Peroxiredoxin Prx1,* Oxidation Resistance *Oxr1,* and Apoptotic inducing factor *Aif1* basing on qPCR analysis. We found multidimensional genetic interactions of *FMP40* with other known redox genes upon low or high oxidative stress. We revealed that Fmp40 AMPylates Prx1, Trx3, and Grx2 *in vitro* and it has a matrix-localized echo form. We discovered that Fmp40 is critical for the efficient reduction of Prx1 upon high oxidative stress. Moreover, Grx2 is involved in the Prx1 reduction directly and at the level of Trx3 reduction *in vivo.* Fmp40 regulates its function on Trx3 protein, most probably through Threonine66 which is AMPylated *in vivo.* In addition, Fmp40 is necessary to maintain the balance of cellular redox buffers GSH and NADPH. Overall Fmp40 regulates redox gene expression for efficient ROS neutralization and signaling which eventually determines the fate of cell survival upon oxidative stress.

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FRIEND OR FOE: ASSOCIATION OF URIC ACID WITH OXIDATIVE STRESS IN CANINE HYPERADRENOCORTICISM

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Canine hyperadrenocorticism (HAC) or Cushing's syndrome is a multisystemic clinical condition caused by chronic exposure to elevated concentrations of glucocorticoids. It has been considered that oxidative stress is implicated in pathophysiology of HAC. The exact impact of uric acid (UA) on oxidative stress in hyperadrenocorticism remains unclear, given its ability to act as both an antioxidant and a pro-oxidant. In addition, increased UA levels are related to the development of hypertension, dyslipidemia, and type II diabetes in humans with HAC. For this purpose, we aimed to investigate the association of UA with the components of oxidative stress in dogs with HAC. This study included 12 dogs with newly diagnosed HAC and 12 healthy controls. The oxidative stress in serum samples was assessed by advanced oxidation protein product (AOPP) and thiobarbituric acid–reactive substances (TBARS), and antioxidative status by total antioxidant capacity (TAC), reduced glutathione (GSH) and paraoxonase-1 (PON-1). Uric acid was compared between two groups and correlated with oxidative stress parameters. The results showed that dogs with HAC exerted markedly higher level of UA compared to healthy controls ($p<0.001$). Additionally, higher levels of AOPP and TBARS ($p=0.001$; $p=0.043$) were observed in the HAC group, indicating oxidative damage compared to the controls. Among antioxidants, only GSH exhibited a difference between groups ($p=0.001$). Correlation analysis of UA revealed strong association with TBARS ($r=0.615$; $p=0.037$), which implies that UA is linked to an increase of oxidative stress in canine Cushing's syndrome. The results of this study indicate a possible pro-oxidant role of UA in dogs with HAC.

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MITOCHONDRIAL TARGETING AS A MEANS OF OVERCOMING CANCER DRUG RESISTANCE

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Our recent observations show that both resistant and stem-like cancer cells predominantly responsible for metastasis differ from chemotherapy-sensitive cells. We have shown bioinformatically and experimentally that mitochondria of such cells are much more prone to oxidative phosphorylation (OXPHOS) than radio- or chemotherapy-sensitive cancer cells from which they evolved during therapeutic interventions. Specifically, in triple-negative breast cancer models, we observed that such resistant cells exhibit higher mitochondrial membrane potential, higher OXPHOS and respiration, and increased resistance to oxidative stress, allowing them to survive chemo-radiotherapy. These findings of increased expression of OXPHOS-associated genes and proteins in chemoresistant cells and biopsies of relapsed tumors suggest an alternative druggable target. Our in vitro and in vivo (nude mice and *Artemia salina*) data suggest that certain antibiotics, inducers of mitochondrial dysfunction, create additive oxidative stress and can reduce the growth rate of tumors developed from resistant or stem-like cancer cells. Such repurposed drugs, selected from a chemical library, are also able to resensitize resistant tumors, allowing reuse of chemotherapeutic agents. In addition, their modification with a specific moiety (TPP) allows for increased delivery to mitochondria to reduce cytotoxic pressure on normal cells. Thus, research from our laboratory offers an alternative strategy for anticancer therapy of resistant tumors.

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BODY SIZE, BODY SHAPE AND BREAST CANCER RISK – METABOLIC AND REDOX LINK

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Variations in body size and shape might be linked to different biological processes that affect breast cancer risk. Epidemiological studies have confirmed that obesity, which is characterized by increased overall adiposity and assessed using body mass index (BMI), has direct relationship with the risk of breast cancer among postmenopausal women, and opposite relationship with the risk among premenopausal women ("obesity paradox"). In addition to BMI, anthropometric descriptors of body shape, like waist and hip circumference and waist-to-hip ratio are directly associated with both pre- and postmenopausal breast cancer risk. Excess adipose tissue, adipose tissue dysfunction, and adipose tissue-to-breast cancer crosstalk have important role in the initiation and progression of breast cancer due to the altered production of proinflammatory and proangiogenic mediators, growth factors, adipokines, and sex hormones, dysregulated insulin signaling pathway, as well as mitochondrial dysfunction and oxidative stress. Fat distribution pattern exerts an effect beyond the effect of overall obesity in relation to breast cancer development because of more adverse systemic metabolic effects related to visceral adiposity. Body height and its components have direct association with postmenopausal breast cancer risk. Increased risk of breast cancer in taller persons is probably due to increased levels of insulin-like growth factor (IGF-1), which is one of the major determinants of height, plays an important role in regulating breast stem cell number, and can affect cancer growth. Adult-attained height also reflects different aspects of maturation, including genetic, nutritional, and environmental factors. Assessment of changes in body height, mass, and distribution of adipose tissue throughout life is another important aspect of understanding the complex processes of metabolic reprogramming of energy pathways in breast cancer pathophysiology. Use of anthropometric descriptors of body size and shape can provide insight into underlying biological mechanisms, which is essential for developing targeted prevention and treatment strategies.

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IS REDOX-HYPERACTIVITY IN EXTREMOPHILIC MICROALGAE LINKED TO THEIR INCREASED METABOLIC BURDEN?

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The diverse uses of microalgae in ecological remediation, wastewater treatment, pharmaceutics, or food and biofuel production, have long kept these single-celled organisms in the spotlight. The focus of this study was on *Chlamydomonas acidophila* strain PM01, which thrives in acidic aquatic systems and is resistant to the presence of heavy metals in its environment. The redox metabolism of this microalga was assessed by its ability to reduce the EPR-active spin probe TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl), and compared to that of *Chlorella sorokiniana* strain CCAP 211/8K, a freshwater green microalga. The results showed that *C. acidophila* has a faster redox metabolic rate than *C. sorokiniana*, reducing 50% of TEMPO after 2.5, and 13 min, respectively. The addition of Mn^{2+} or Fe^{3+} to the culture medium of *C. acidophila* did not affect its reduction capacity, while it had a minor effect on *C. sorokiniana*. The faster rate in *C. acidophila* most likely represents the result of its adaptation to acidic environments. Namely, it has previously been suggested that acidophilic algae perform energy-demanding cellular processes in order to cope with the high pH gradient across the membrane. Moreover, the increased metabolic turnover requires an increased mitochondrial activity, resulting in a higher baseline production of superoxide and hydrogen-peroxide, subsequently compensated by an elevated baseline reduction capacity. Interestingly, the redox metabolic rate of *C. sorokiniana* was unaltered in suspensions that were kept in non-standard cultivation conditions (diurnal fluctuations of temperature and ambient lighting, absence of shaking) for five weeks. However, *C. acidophila* lost all of its reduction capacity in these conditions already after three days. These findings may be important when selecting the most appropriate microalgal strain for a specific application. Specifically, *C. acidophila* would likely be a good candidate for high-yield rapid production of endogenous products that are the result of its unique survival mechanism under extreme conditions.

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MITOCHONDRIAL TESTS THAT EXPOSE DISEASE CLUES AND LIFESTYLE EFFECTS

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The impairment of mitochondrial respiration, observed in neurodegenerative and cardiovascular disease, diabetes, cancer, and migraine headaches, has emerged as a biomarker of mitochondrial dysfunctions. Chronic fatigue, depression, and other behavior/mood disorders are also associated with mitochondrial malfunctioning, but so is our lifestyle! Our lab offers tests for insight into mitochondrial fitness, linking not only diseases but also behaviors and modern lifestyles that lead to health damage. Firstly, we focused on 88 (relatively) healthy volunteers, of which 32% were taking some medication (such as for high blood pressure or mood disorders), however, they considered themselves fit and healthy. The blood was drawn 3h before PBMC (peripheral blood mononuclear cells) isolation, followed by an immediate Seahorse XF Cell Mito Stress Test (Agilent) on the SeahorseXF96e instrument (Agilent). Parameters of mitochondrial respiration were carefully examined. There was a significant difference between BHI (bioenergetic health index), reserve capacity, coupling efficiency, and proton leak, between people who took medication for chronic but manageable comorbidities and completely healthy individuals. Later, in another group we examined the alterations in NAD^+ levels (by Q-NADMED Blood NAD^+ assay kit, NADMED) and mitochondrial respiration parameters in a binge-drinking session (consuming 10 or more units of alcohol in less than three days). The decrease in NAD^+ levels was positively correlated with the amount of alcohol consumed. Additionally, total NAD^+ levels positively correlated with the BHI. In another experiment, supplementation with niacin for 20 days, did not increase NAD^+ levels in (relatively) healthy individuals. Apart from mitochondrial respiration and NAD^+ levels, we focus on optimizing tests for mtDNA count and mitochondrial potential. All of these tests not only explore disease but also serve to monitor behaviors that lead to health damage or improvements.

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MIR-146A AND MIR-21 FROM PBMCS AND EXTRACELLULAR VESICLES IN GESTATIONAL DIABETES: A COMPARISON OF PAIRED SAMPLES FOR THE ANALYSIS OF POTENTIAL INDICATORS OF THE REDOX STATUS

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Dysregulation of the redox system and the interconnected low-level inflammation (LLI) act as a driving force of damaging mechanisms in gestational diabetes mellitus (GDM) and are strongly related to severe obstetric and neonatal complications of hyperglycaemic pregnancies. Major disturbances in microRNA-based mechanism accompany (glyco)oxidative stress ((g)OS), for which reason we hypothesized that microRNAs may serve as sensors and/or effectors of (g)OS/LLI in GDM and we chose candidates for GDM biomarker analysis among known (g)OS/LLI-associated microRNAs. The aim of the study was to analyze the properties of miR-146a-5p and miR-21-5p as redox status indicators in GDM, as well as to compare two different biological samples as sources of potentially relevant GDM biomarkers. miR-146a-5p and miR-21-5p were quantified by real-time polymerase chain reaction in peripheral blood mononuclear cells of patients with GDM and normoglycaemic pregnant controls (n=40 each), as well as in paired samples of extracellular vesicles (EVs) extracted from serum. Correlation analysis was conducted for the expression levels of tested microRNAs and the activities of glutathione reductase (GR), total superoxide dismutase (SOD), catalase (CAT), concentration of serum thiol groups and the level of Nrf2 mRNA. In both samples, tested microRNAs were upregulated in GDM group, with a more pronounced increase in expression in EVs, compared to peripheral blood mononuclear cells (PBMCs) (1.81 vs. 1.52 fold for miR-146a-5p and 1.98 vs. 1.58 fold for miR-21-5p). There was a significant positive correlation between the expression of miR-21-5p from PBMCs and Nrf2 in both GDM patients and controls, as well as a positive correlation with the activity of total SOD in GDM patients. On the other hand, miR-146a-5p from EVs demonstrated negative correlation with Nrf2 expression and the activity of total SOD. These data demonstrate the potential of (g)OS/LLI-related microRNAs miR-146a-5p and miR-21-5p to serve as indicators of GDM and the associated (g)OS-related changes.

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VITAMIN MISUSE DURING THE COVID-19 PANDEMIC – SINGLE CENTER EXPERIENCE

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The global pandemic crisis affected almost every society and economy, challenged almost every health system worldwide. Above all, governments and non-governmental organizations had to fight the misinformation and conspiracy theories placed by the social and mass media. All of this had a profound impact on the public in terms of vaccine safety and the advantages of vitamin use in fighting the virus. This fear has opened doors to alternative medicines such as supplements (vitamins, minerals, herbal products, oils) that may have profound effects on the immune system. To determine the pattern of use of supplements during the pandemic in healthy individuals who tested negative for SARS-CoV-2. The 33 healthy individuals tested negative for SARS-CoV-2 in the pandemic period were included (Group 1). Total antioxidant power, iron-reducing (PAT), and plasma peroxides (d-ROMs) were measured using FRAS5 analytical photometric system and are reported in equivalents of ascorbic acid and H_2O_2, respectively. The oxidative stress index (OSI) was automatically calculated by the software. The obtained values were compared with 30 healthy individuals analyzed prior to the pandemic (Group 2). The mean values for oxidative stress parameters in Group 1 vs Group 2 were: d-ROMs 418 vs 266 U. Carr, PAT 3862 vs 2554 U. Carr, and OSI 111 vs 36. In all comparisons, a statistically significant difference was obtained ($p<0.05$, t-test). Individuals belonging to Group 1 had reported that they have consumed daily doses of Zinc (30 mg), Vitamin C (at least 1000 mg) and Vitamin D (at least 2000 IU) in a period of >1 month. Several of them have also used Isoprinosine, magnesium, and selenium. Uncontrolled intake of supplements can have a profound effect on the pro- and antioxidant balance resulting in interruption of the phycological balance and leading to increased oxidative stress index in otherwise healthy individuals.

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ADJUVANT TREATMENT WITH POLYPHENOLS REDUCES OXIDATIVE STRESS PARAMETERS IN IMATINIB TREATED PATIENTS WITH CHRONIC MYELOID LEUKEMIA

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Imatinib, a tyrosine kinase inhibitor (TKI) is used as a standard treatment in chronic myeloid leukemia (CML) patients. Increased levels of BCR-ABL1 expression in CML cells are associated with oxidative stress induction due to overproduction of reactive oxygen species (ROS) or by deficient antioxidant system, disease progression, and imatinib resistance. Current scientific research confirms that oxidative stress is involved in CML pathogenesis and response to TKI treatment. Moreover, recent findings suggest that the antioxidant properties of some natural compounds can provide benefits to patients with CML. To determine the effect of adjuvant treatment with polyphenols on the oxidative stress markers in imatinib-treated CML patients. 40 CML patients at the University Clinic of Hematology, Skopje, who received imatinib longer than 1 month were included in the study. 20 patients were additionally treated with Aronia melanocarpa extract and 20 patients received only imatinib (control group). Besides the regular clinical laboratory analysis for these patients, total antioxidant power (PAT) and plasma peroxides (d-ROMs) were measured at initial visit and after 21 and 42 days of treatment using FRAS5 analytical photometric system and the oxidative stress index (OSI) was automatically calculated. Oxidative stress parameters (d-ROM and OSI) were significantly higher at initial visit in both groups. In group of patients who received adjuvant polyphenols values for d-ROM and OSI were significantly lower after 21 and 42 days of treatment ($p<0.05$). Also, total antioxidant capacity (PAT) was significantly higher after 21 and 42 days of treatment initiation in comparison with the pretreatment values. In the control group, no significant differences were obtained between investigated parameters at any time of measurement. Adjuvant treatment with Aronia melanocarpa extract after 21 and 42 days led to significant reduction of oxidative stress parameters in patients with CML treated with imatinib.

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CHARACTERIZING THE BRAIN-HEART-VESSEL AXIS IN AIRCRAFT NOISE-INDUCED NEUROPSYCHIATRIC AND CARDIOVASCULAR COMPLICATIONS

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The World Health Organization (WHO) estimates that noise pollution leads to the loss of 1.6 million healthy life years annually in Western Europe alone, primarily due to night-time noise exposure which disrupts sleep and triggers stress responses. This study investigates the adverse health effects of aircraft noise on the brain-heart-vessel axis, combining cardiovascular and neuropsychiatric approaches. We aim to characterize the functional and biochemical consequences of both short-term and long-term noise exposure utilizing an established mouse model. Behavioural changes in exposed mice, including cognition, anxiety, depression, and social behaviour were assessed alongside cardiovascular parameters such as blood pressure, endothelial function tests, and analyses of oxidative stress and inflammation markers. Short-term noise exposure did not lead to any significant differences in the behaviour of the noise-exposed mice, whereas long-term noise-exposure leads to reduced social interaction and working memory as behavioural markers of depression. Functional cardiovascular parameters point to hypertension and impaired endothelial function in both short-term and long-term noise exposure, as well as oxidative stress and inflammation. These findings underscore previously reported cardiovascular impact of noise exposure while adding the suspected behavioural changes and metabolic markers of the affected brain-heart axis. The observed behavioural changes and cardiovascular impairments emphasize the complex interplay between environmental stressors and health, suggesting that long-term noise exposure can have profound effects on both mental and cardiovascular health. This study provides a comprehensive framework for future research aimed at reducing the adverse effects of noise pollution on the brain-heart-vessel axis.

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MEDITERRANEAN MUSSELS (*MYTILUS GALLOPROVINCIALIS*) UNDER SALINITY STRESS: EFFECTS ON ANTIOXIDANT CAPACITY

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Estuarine and intertidal bivalve mollusks frequently experience salinity fluctuations that may drive oxidative stress (OS) in the organism. Here we investigated OS markers and histopathological changes in gills and hemolymph of Mediterranean mussels *Mytilus galloprovincialis* acclimated to a wide range of salinities (6, 10, 14, 24, and 30 ‰). Mussels were captured at the shellfish farm with the salinity of 18% and then acclimated to hypo- and hypersaline conditions in the laboratory at the speed of 1.5±0.5‰ per day. Indicators of redox balance in hemocytes (intracellular reactive oxygen species (ROS) levels, DNA damage) and gills (thiobarbituric acid reactive substances (TBARS), protein carbonyls (PC), activity of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) were measured. The results revealed induction of OS in tissues and cells of mussels for both experimental increase and decrease salinity modeling. Hemocytes showed higher sensitivity to oxidative damage from salinity stress compared to gills, as DNA damage and elevated ROS levels were observed in all experimental groups except 14‰. A decrease in environmental salinity to 10 ‰ was likely within the physiological norm for mussels, as minor oxidative damage was noted. At a salinity of 6 ‰, the most significant signs of redox imbalance, including DNA damage, increased ROS production levels in hemocytes, and suppressed activity of SOD in gills were observed, along with elevated PC levels. An increase in environmental salinity up to 30 ‰ led to the enhancement of the activity of antioxidant enzymes in the gills, which may be attributed to the high capacity of the antioxidant system in this organ. The study provides new insights into the effects of salinity stress on the tissue and cellular redox balance of bivalves, which is crucial for better understanding the potential consequences of the global transformation of coastal ecosystems.

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RELATIONSHIP BETWEEN PLASMA/ERYTHROCYTES GLUTATHIONE RATIO AND HEALTH STATUS

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Considering the crucial antioxidant role of glutathione (GSH) in cells, its assessment is useful for both healthy populations and in different diseases. It is usually measured either in erythrocytes or in plasma, while it is unknown whether the distribution of GSH between these compartments depends on a presence of a disease, thus affecting the results. Therefore, our aim was to investigate the relationship between GSH in plasma and erythrocytes of healthy and diseased subjects. The study included 60 participants, 25 healthy subjects, and 35 patients with different diseases (cancer, heart failure, kidney diseases, chronic fatigue, sarcoidosis, Lyme disease). GSH levels were determined in plasma and erythrocytes using spectrophotometric method with Ellman's reagent. GSH plasma/erythrocytes ratio between two groups was compared by Mann-Whitney U test and the results are presented as median (interquartile range). The median value of plasma/erythrocytes ratio for healthy subjects was 3.79 (3.32-5.71), and for patients, it was 27.54 (1.53-54.76). This ratio was significantly higher in the group of patients compared to healthy participants (P=0.018). Our results indicate a redistribution of GSH from erythrocytes to plasma in the presence of different diseases. The fact that this preliminary study points out an association of health status with plasma/erythrocytes GSH ratio, regardless of the heterogeneity of a patient group, encourages further research in this direction.

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IMPACT OF SULFUR AMINO ACIDS SUPPLEMENTATION ON PERFORMANCE, OXIDATIVE STRESS, AND LIVER HISTOLOGY IN FEED-RESTRICTED INSHAS COCKERELS

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A 60-day experiment was conducted to investigate the impact of dietary sulfur amino acids (AA) on altered haemato-biochemical and redox parameters of Inshas cockerel chicks raised under restricted feeding. Male Inshas strain chickens were divided into five groups, each with five replicates of eight birds. The control group received the full National Research Council (NRC) requirements (100%). The other groups received diets meeting 90% of NRC requirements: 90% NRC; 90% NRC+Methionine; 90% NRC+Cysteine; and 90% NRC+both Methionine and Cysteine (AA-mix). AA supplementation improved growth performance compared to the control group. The birds that were given a combination of AA supplementation exhibited the highest body weight and carcass weight compared to other groups. AA supplementation improves blood physiological characteristics by reducing damage caused by feed restriction conditions. Serum parameters, including aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, alkaline phosphatase, gamma-glutamyl transpeptidase, and total protein concentration, showed decreased levels. Treatment with mixed AA maintained urea and uric acid concentrations at a level similar to the control group. Remarkably, a combination of AA treatments reduced the negative effects of feed restriction on young male chickens by enhancing the overall antioxidant capacity and activity of antioxidant enzymes in liver tissue (glutathione S-transferase, total superoxide dismutase, glutathione peroxidase, and amount of total glutathione), and decreased the malondialdehyde concentration. Feed restriction impacted liver histological structure, where hepatocytes were susceptible to feed restriction and included numerous cytoplasmic vacuoles, congested blood vesicles, lymphocytic infiltration, and pyknotic nuclei in treated cockerels. AA therapy restored most hepatic histological abnormalities. The findings suggest that AA supplementation significantly mitigated the adverse effects of feed restriction by improving haemato-biochemical parameters and hepatic redox status.

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UNCOUPLING PROTEIN 1 EXPRESSION IN LIPOMA TISSUE AND LIPOMA-DERIVED STEM CELLS

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Mechanisms and factors that lead to the formation of lipomas, benign tumors of adipose tissue, are still insufficiently elucidated. Mesenchymal stem cells (MSCs) isolated from lipomas have some similar characteristics to MSCs isolated from white adipose tissue but differ at the molecular level and in their differentiation potential. Considering histological appearance of lipomas, it is not clear to what extent lipomas share common characteristics with other adipose tissue type, brown adipose tissue. Therefore, the aim of this study was to examine the level of uncoupling protein 1 (UCP1), a marker of brown adipose tissue, expression in lipoma tissue as well as in MSCs isolated from lipomas, i.e. lipoma-derived mesenchymal stem cells (LDSCs). LDSCs were grown in standard cell culture conditions and subjected to adipogenic differentiation. UCP1 expression was examined at the RNA level, using Real-Time PCR, and at the protein level, using immunohistochemistry and immunogold staining. Expression of UCP1 in lipoma tissue and LDSCs was compared with the expression of UCP1 in subcutaneous white adipose tissue (scWAT) and adipose-derived mesenchymal stem cells (ADSCs) grown and differentiated in the same cell culture conditions. Differences were observed in UCP1 expression at both RNA and protein levels in lipomas compared to scWAT directing the future research towards the potential of browning mechanisms of adipose tissue involved in lipoma tissue formation.

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IMPACT OF HYPOTHYROIDISM ON CuZnSOD AND MnSOD DURING SPERMATOGENESIS IN RATS

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Thyroid hormones play an important role in both testis development and spermatogenesis. While hypothyroidism has been known to generally induce metabolic suppression, lower respiration rate, and reduce free radical formation, recent studies reported an increased production of reactive oxygen species (ROS). First line of antioxidant defense in testes is comprised of two isoforms of superoxide dismutase (SOD), CuZnSOD and MnSOD differently localised in cell. This study aimed to investigate the effects of hypothyroidism on the expression, localisation, and activity of these two SOD isoforms during spermatogenesis. Hypothyroidism was induced in two-month-old male Wistar rats by 0.04% methimazole in drinking water for 7, 15, and 21 days, while euthyroid control group drank tap water. CuZnSOD protein expression was decreased after 15 and 21 days while its activity was decreased by 40% in all examined time points of methimazole treatment in comparison to euthyroid control. At the same time, neither MnSOD protein expression nor its activity was changed by treatment. However, cell and stage-specific CuZnSOD and MnSOD immunoexpression in the rat testes were changed in hypothyroidism and may contribute to the altered spermatic characteristics. Our results suggest that changes in CuZnSOD and MnSOD expression play role in redox disbalance leading to hypothyroidism-induced maturation arrest of spermatogenesis.

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EFFECTS OF CHRONIC COLD EXPOSURE ON ANTIOXIDANT DEFENSE IN BROWN ADIPOSE TISSUE AND LIVER OF AGED RATS

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Aging is a natural process characterized by a decline in organic structure-function and an increase in mortality over time. While many exogenous and endogenous factors contribute to aging, the long-term effects of low environmental temperature have been poorly described. To address this, our study compared 24-month-old male Mill Hill hybrid hooded rats raised at a standard temperature of 22±1°C with age-matched rats that were kept in a cold room (4±1°C) from the age of 6 to 24 months. 3- and 6-month-old rats raised at 22±1°C were included as room temperature controls. We examined two metabolically active organs, interscapular brown adipose tissue (iBAT) and liver. It was found that 24-month-old rats chronically exposed to cold exhibit increased food consumption, which may be attributed to a higher metabolic demand. Chronic exposure of aged rats to low environmental temperature led to an increase in iBAT relative mass, total glutathione (GSH) content, and antioxidant defense (AD) enzyme activity: CuZn superoxide dismutase, Mn superoxide dismutase, catalase, glutathione peroxidase, and thioredoxin reductase. Respirometric analysis further demonstrated an increase in mitochondrial uncoupling in iBAT in 24-month-old rats kept at 4±1°C. Conversely, there was no change of the same parameters in the liver, which maintained consistent AD enzyme activity and GSH content across all experimental groups. Our study confirms that iBAT of aged rats remains responsive to stimulation by low environmental temperature, supporting thermogenic processes through uncoupling and a robust increase in the AD system. These results highlight tissue-specific effects of chronic cold exposure on aged rats underlying acclimation-driven physiological changes.

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REDOX METABOLIC CHANGES IN TUMOR AND ASSOCIATED ADIPOSE TISSUE OF COLON CANCER PATIENTS

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Colorectal cancer presents a significant global health challenge, with a high mortality rate. It is the third most commonly diagnosed cancer and is therefore a major cause for concern. The development of colorectal cancer is multifaceted, involving a combination of genetic predispositions and lifestyle factors. The redox and metabolic states may influence the intricate process of colon cancer development. To gain a deeper understanding of the redox-metabolic profiles associated with colon cancer, a human study was conducted. In biopsies from patients with colon cancer, the antioxidant status: copper, zinc superoxide dismutase (CuZnSOD), manganese superoxide dismutase (MnSOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutamate-cysteine ligase (GCL), thioredoxin (Trx) and lactate metabolism were examined in tumor and unaffected colon tissue (remote 15-20 cm) as well as in adipose tissue: proximal (near the tumour tissue), distal (remote 6 cm) and unaffected (remote over 6 cm). The protein levels of CuZnSOD, MnSOD, GSH-Px, and Trx are higher in the tumor tissue compared to the unaffected colon tissue. In addition, the expression of the lactate dehydrogenase (LDH) A isoform, the total activity of LDH and the lactate concentration are higher in transformed tumor tissue than in normal colon tissue. In addition, the lactate concentration is highest in the proximal part and decreases radically in the adipose tissue with increasing distance from the tumor. On the other hand, the protein expression of CuZnSOD, CAT, GSH-Px and GCL shows an opposite profile. The redox profile and lactate concentration clearly indicate a redox metabolic interaction between tumor and adipose tissue in shaping the malignant phenotype in human colorectal cancer.

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THE ROLE OF NRF2-DEPENDENT METABOLIC REPROGRAMMING OF BROWN ADIPOSE TISSUE IN ORTHOTOPIC BREAST CANCER MODEL

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Breast cancer is characterized by specific metabolic changes that support tumorigenesis, highlighting the emerging appreciation of cancer as a metabolic disease. These metabolic changes are simultaneous with redox reprogramming with nuclear factor erythroid 2-related factor 2 (Nrf2) representing their master integrator. Given that interscapular brown adipose tissue (IBAT) influences whole-body metabolism, our goal was to investigate the redox-metabolic crosstalk between the tumor and the host at the systemic level by exploring Nrf2-driven metabolic changes that occur in IBAT in the orthotopic model of breast cancer in wild-type (WT) and mice lacking functional Nrf2 (Nrf2KO). We analyzed the protein expression of key enzymes involved in glucose and lipid metabolism in control groups and at different points during tumor growth (10 mg, 50 mg, 100 mg, 200 mg, and 400 mg). In both WT and Nrf2KO mice, the results indicated a transient induction of hexokinase 2 expression during the early phase of tumor growth (<100 mg). Accordingly, pyruvate dehydrogenase expression followed the same profile. In Nrf2KO mice, a general decline in glyceraldehyde-3-phosphate dehydrogenase, phosphofructokinase-1, and glucose-6-phosphate dehydrogenase expression was detected during the late phase of tumor growth (>100 mg). Since no changes in WT mice occurred, these findings are considered Nrf2-dependent. Concomitantly, a decrease in protein expression of fatty acid synthase and acetyl-CoA carboxylase in Nrf2KO mice was observed. These observations correspond to decreased levels of 5'-AMP-activated protein kinase and hypoxia-inducible factor 1 during the late-phase (>100 mg) of tumor growth in Nrf2KO mice which suggests their involvement in transcriptional regulation. Our results revealed that IBAT metabolism responds to tumor growth and underscored that this communication is Nrf2-dependent giving implications for further understanding of breast cancer in the light of systemic metabolic disease.

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APPLICATION OF FREE RADICAL SCAVENGERS IN HUMAN LUNG CANCER CELLS IRRADIATED WITH PHOTONS AND CARBON IONS

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Ionising radiation damages DNA directly, or indirectly, causing water radiolysis and formation of free radicals. Indirect irradiation effects could be diminished in the presence of free radical scavengers, such as dimethyl sulfoxide (DMSO). Such conditions would allow the evaluation of direct radiation effects and provide a better understanding of cellular response to irradiation-induced damages. The goal of this study was to investigate the effects of low (γ-rays) and high linear energy transfer (LET) radiation (carbon ions) in non-small lung cancer cells HTB177. Cells were pre-treated with DMSO and irradiated with ^{60}Co γ-rays and 62 MeV/u carbon ions, with doses ranging from 1-5 Gy. Results obtained by clonogenic survival and γ-H2AX foci assay showed that DMSO increased cell survival and decreased number of DNA damages, which points to radioprotective effect of DMSO. The contribution of direct and indirect radiation effects was estimated by the degree of protection (DP) in presence of DMSO. The values of DP rose in a concentration-dependent manner in all irradiated samples. In cells irradiated with γ-rays, 35% of damages were caused directly, while 65% of lesions could be attributed to indirect radiation actions. In presence of carbon ions, contribution of direct effects was 49%, while 51% of damage resulted from indirect radiation effects, showing that free radicals attain an important role in both low and high LET irradiations. The obtained results showed that DMSO can be used as a free radical scavenger to examine the direct and indirect effects on human cancer cells. The numerical Monte Carlo simulations allow modelling of direct and indirect irradiation actions in cancer cells with photons and hadrons. Therefore, this data will be used for validation and further improvement of numerical simulations in comparison to the data collected on different cell lines and irradiation energies, with the goal to improve therapeutic protocols.

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MITIGATION OF $PM_{2.5}$-INDUCED CARDIOVASCULAR DAMAGE BY STATINS AND ACE INHIBITORS

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Particulate matter (PM) is well recognized as the major contributor to the air pollution disease burden. Presently, the data pointing to the direct effects of PM on the cardiovascular health are numerous, but the mitigation strategies are still at the level of reduction of exposure. In the present study, we used a mouse model of real-life $PM_{2.5}$ exposure treated with either a statin (atorvastatin) or an ACE inhibitor (captopril) in order to observe the potentially protective effects of cardiovascular drug treatment on the underlying mechanisms of detrimental, $PM_{2.5}$-induced, cardiovascular effects. Captopril treatment mitigated the $PM_{2.5}$-induced blood pressure while both drugs reduced selected markers of oxidative stress in the vasculature and heart. Both drugs were successful in mitigating the vascular oxidative stress by reducing the activation of the NADPH oxidase enzyme. In addition, both drugs were able to reverse the $PM_{2.5}$-induced increase in vascular endothelin-1. The treatment also reduced the level of 3-NT positive proteins in the lung and mitigated the effects on dysregulated eNOS expression. Drugs did not mitigate the inflammatory response in the lung and in circulation with only captopril reducing the pulmonary IL-6, but not CD68 expression. In summary, ACE inhibitors can potentially mitigate the effects of $PM_{2.5}$ on the vascular function and oxidative stress by lowering blood pressure and statins have a known antioxidant effect, e.g. via inhibition of NADPH oxidase. Our present data provide novel insights into possible mitigation strategies for $PM_{2.5}$-induced cardiovascular disease. Since statins and ACE inhibitors represent first-line therapies for cardiovascular disease, CVD patients, e.g. with coronary artery disease, ischemic heart disease, and hypertension representing highly vulnerable groups for air pollution health effects, may benefit from pre-established therapies with these drugs to prevent additive cardiovascular damage by $PM_{2.5}$ exposure.

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DIFFERENT DEGREES OF OXIDATION CAUSE DIFFERENT CELL TRANSFORMATIONS AND FORMATION OF MICROPARTICLES

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Oxidative stress (OS) has a significant impact on the lifespan and physical fitness of living organisms. It is commonly associated with ageing and can lead to changes in the functionality of red blood cells (RBCs). The precise mechanisms underlying these changes are not fully understood. Unlike mammals, avian RBCs have a nucleus and functional mitochondria that regulate the cellular response to oxidative stress. In this study, we examined the effects of OS on red blood cells from adult female quail (*Coturnix japonica*, n=12). We used flow cytometry to analyze the formation of OS-induced microparticles and RBC transformation. We also evaluated band 3 clustering and phosphatidylserine externalization at the cell surface using eosin-5-maleimide and Annexin-V fluorescent probes, respectively. In addition, we analyzed band 3 clustering using confocal microscopy. We used a laser diffraction-based method to analyze cell deformability, and we characterized hemoglobin species spectrophotometrically. We found that OS caused band 3 clustering, microparticle formation, and phosphatidylserine release onto the cell membrane. The microparticles formed under the influence of oxidants differed from those formed under the influence of A23187 (calcium ionophore). The rate of microparticle formation and the onset of osmotic rigidity depended on the oxidant concentration. Erythrocyte-derived microparticles contained hemoglobin oxidized to hemichrome (HbChr). Overall, these findings demonstrate that avian erythrocytes undergo different processes during oxidative stress, depending on the level of oxidation. These differences are due to variations in cellular transformations and the formation of different types of microparticles.

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EFFECT OF SUCCINATE DEHYDROGENASE DEFICIENCY ON MITOCHONDRIAL FUNCTION

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Succinate dehydrogenase (SDH) connects the tricarboxylic acid (TCA) cycle and the respiratory chain. Mutations in SDH subunits have been associated with tumorigenesis and mitochondrial disease. In this project, we focused on subunit A of SDH (SDHA), primarily associated with inherited mitochondrial disease, and investigated the consequences of its loss or re-expression of mutant variants in HEK cells (SDHA KO). Lack of SDHA led to a downregulation of all SDH subunits and a secondary downregulation of the majority of mitochondrial complex I and IV subunits. Cellular respiratory capacity was severely decreased in the model, SDH-dependent respiration completely abolished and complex I-dependent respiration attenuated, reflecting the downregulation of respiratory chain complexes in general. Finally, the NAD^+/NADH ratio was increased in SDHA KO, indicating complex rearrangement of the TCA. It resulted in higher glycolytic activity and lipid accumulation.

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COMBINED EFFECTS OF GLYPHOSATE-BASED PESTICIDES AND ELEVATED TEMPERATURE ON OXIDATIVE STRESS PARAMETERS AND ACETYLCHOLINESTERASE ACTIVITY OF BALKAN CRESTED NEWT (*TRITURUS IVANBURESCHI*) LARVAE

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Amphibians are the most threatened vertebrate class. Exposure to pesticides and climate change are considered pivotal factors in the global decline of their populations. Glyphosate-based formulations are the most widely used herbicides, but increasing evidence of their harmful effects, including oxidative stress in exposed organisms, has sparked a heated debate. Current climate prediction models assume a global temperature rise of 3 °C to 5 °C in the coming decades. In poikilotherms, any changes in ambient temperature are directly translated into changes in physiological and biochemical processes. Furthermore, elevated temperatures could intensify the toxic effects of pesticides present in the environment. The aim of this study was to examine the effects of low, environmentally realistic concentrations of glyphosate-based herbicides (30 µg/L active ingredient) and elevated temperature (optimal t_1=19°C and increased t_2=23°C) on glutathione content (GSH), antioxidant enzyme activities (SOD, CAT, GSH-Px, GR and GST), activity of acetylcholinesterase (AChE) and levels of oxidative damage (TBARS - lipid peroxidation and PC - protein carbonylation) in larvae of the Balkan crested newt (*T. ivanbureschi*). Our findings revealed that glyphosate had a significant effect on the activity of all antioxidative enzymes, with the exception of SOD. Herbicide and elevated temperature led to a significant increase in the activities of CAT, GSH-Px, GST, and GR, as well as GSH concentration. This response of the antioxidative defense system prevented oxidative damage to lipids and proteins. Glyphosate exhibited a neurotoxic effect by inhibiting AChE only at elevated temperatures, while no significant change occurred at the optimal temperature. The findings suggest the importance of examining the potentially harmful effects of glyphosate in different ecological contexts, such as an increase in average temperatures by several degrees predicted by future climate scenarios.

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EFFECTS OF ARTIFICIAL LIGHT ON OXIDATIVE STRESS PARAMETERS IN AMPHIBIANS: A CASE STUDY OF *HYLA ARBOREA*

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Human activity and rapid urbanization created disturbance factors that drastically changed natural habitats. The introduction of artificial light at night changed natural light-dark regimes affecting a range of biological processes. Disruption of circadian rhythm is linked with changes in endocrine and neurobiological systems that control hormonal regulation, food intake, metabolism, reproduction, and behavior of animals. Oxidative stress was suggested as a possible mechanism through which artificial light could affect an organism's physiology and health. We examined the oxidative status of tree frog (*Hyla arborea*) under two artificial night light intensities 20 lux and 90 lux. Artificial light affects the antioxidant system of both larval and juvenile stages. Larvae had higher activity for glutathione peroxidase only for 90 lux, while greater lipid damage was observed in individuals under both light regimes compared to control. Juvenile individuals showed boosted antioxidant response seen through higher activities of superoxide dismutase, catalase, and glutathione peroxidase. Finally, development under artificial light led to higher levels of protein damage in juveniles. Artificial light at night acts primarily through direct effects and can persist across life stages. Overall results point out that exposure to artificial light alters physiological traits in amphibians, such as oxidative status that could have various consequences on individuals in natural populations.

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NEUROPEPTIDE Y DISRUPTS REDOX BALANCE IN HUMAN EXTRAVILLOUS TROPHOBLASTS

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Human extravillous trophoblasts play a key role in implantation, placentation, and successful pregnancy outcomes due to their ability to migrate and invade through the uterine spiral arteries. Abnormalities in the trophoblasts' migratory and invasive abilities may result in insufficient remodeling of the uterine spiral arteries. This leads to the development of preeclampsia, a syndrome in pregnancy. Neuropeptide Y (NPY) is a sympathetic neurotransmitter that plays a significant role in the adaptive stress response as well as in the regulation of body energy balance. This study was designed with the aim of investigating whether preeclampsia is associated with NPY-induced disruption of trophoblast migration and redox balance. For this purpose, the concentration of NPY was determined in the plasma of 20 healthy and 20 preeclamptic pregnant women. The obtained results demonstrated that in preeclampsia, the concentration of NPY is significantly lower (190 pg/mL) than in a healthy pregnancy (260 pg/mL). After that, NPY in concentrations of 190 pg/mL and 260 pg/mL was used to treat the human extravillous trophoblast cell line HTR-8/SVneo for ten weeks. The effect of NPY on trophoblast proliferation was determined by counting cells during each passage. After the end of the treatment, the effect of NPY on migration and intracellular concentrations of superoxide anion radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and nitric oxide (NO) were examined. The obtained results show that NPY induces changes in trophoblast proliferation and reduces their migration at both applied doses. In addition, both doses of NPY induce a decrease in intracellular concentrations of $O_2^{\bullet-}$, H_2O_2, and NO. The NPY concentration of 190 pg/mL significantly decreased $O_2^{\bullet-}$ in trophoblasts in comparison to the concentration of 260 pg/mL. This study demonstrates that NPY affects the migration and redox balance regulation of trophoblasts. It also disrupts the trophoblast redox balance at a level characteristic of preeclamptic pregnancy.

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FATTY ACID PROFILES DIFFER BETWEEN HEALTHY AND MULTIPLE SCLEROSIS-DIAGNOSED ADULTS

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Multiple sclerosis (MS) is a chronic, immune-mediated inflammatory disease of the central nervous system (CNS). Relapsing-remitting multiple sclerosis (RRMS) is the most common clinical subtype of MS. MS is characterized by demyelination and myelin is mainly composed of lipids. Lipids play many roles in the CNS including signaling, structural support, mediating inflammation, and membrane biogenesis. Omega-3 polyunsaturated fatty acids (PUFA) are central to maintaining health and they are present in a wide array of tissues with broad functions including the active component of phospholipid cell membranes and substrate for molecular signaling pathways. This study aimed to evaluate fatty acids (FA) profiles of patients with RRMS (n=30) compared to healthy people (n=20). Analysis of total lipids was performed from erythrocyte samples. The total lipid extracts from erythrocytes were prepared by adding chloroform/methanol (2:1, *v*/*v*) mixture containing butylated hydroxytoluene (0.05% BHT weight/volume). FA methyl esters were prepared by transmethylation with 3N HCl in methanol. FA profiles were determined by gas chromatography (GC). The content of individual FA was expressed as a percentage of the total FA. Results of this study revealed that total saturated fatty acids (SFA) are significantly higher in MS patients compared to controls. While total PUFAs, total n-3 PUFAs, and omega-3 index are statistically lower in MS patients. The n-6/n-3 ratio is significantly higher in MS patients compared to controls. Also, the AA/EPA ratio is significantly lower in the control group compared to MS patients. Conversely, the EPA/AA index is significantly reduced in MS patients. Omega-3 lipids, which have a protective role by preserving the blood-brain barrier, are significantly reduced in the erythrocytes of patients with MS. Increased n-3 PUFA and decreased SFA intake could counteract inflammation, energy storage and utilization imbalance and, overall state in patients with MS.

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THE ROLE OF MACROPHAGE MIGRATION INHIBITORY FACTOR IN LIVER INFLAMMATION, OXIDATIVE STRESS, AND APOPTOSIS IN MICE ON A FRUCTOSE DIET

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Macrophage migration inhibitory factor (MIF) is a pleiotropic cytokine that plays an essential role in the inflammatory response and various other biological effects such as activation of apoptosis and oxidative stress. Fructose-enriched diets have previously been associated with the development of low-grade inflammation leading to metabolic stress. The aim of the present study was to investigate the combined effects of deletion of the *Mif* gene and a 9-week 20% fructose-enriched diet on metabolic inflammation, apoptosis, and oxidative stress in the liver of wild-type (WT) and *Mif* knockout ($MIF^{-/-}$) male C57Bl/6J mice. We analyzed liver histology and expression of pro-inflammatory genes: Tumor necrosis factor (TNF), interleukin 1β (IL-1β), and IL-6. Antioxidant activity was estimated by the protein levels of antioxidant enzymes catalase (CAT), superoxide dismutase (SOD1), mitochondrial MnSOD (SOD2), glutathione reductase (GR) and glutathione peroxidase (GPX). The results showed that antioxidant protection was activated in the liver of MIF-deficient mice. Increased hepatic expression of the cytokines IL-6 and IL-1β was observed in the same animals. Histologic analysis confirmed the presence of apoptosis, inflammation, enlarged Kupffer cells, and regenerative changes, such as binucleated hepatocytes, anisonucleosis, and anisocytosis. In addition, confluent and focal necrosis was observed in the liver of $MIF^{-/-}$ mice, which was even more pronounced in the animals consuming fructose. In conclusion, MIF may play a protective role in metabolic stress, as inflammation, oxidative stress, apoptotic and necrotic changes occur in the liver in its absence.

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THE ASSOCIATION OF TUMOR SIZE AND THE PRESENCE OF LYMPH NODE METASTASES IN BREAST CANCER PATIENTS

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Breast cancer is one of the most common malignant diseases in women worldwide. Since the involvement of axillary lymph node metastases is related to the poor prognosis of these patients, the objective of our study was to investigate the association between breast tumor size and the presence of axillary lymph node metastases. Our research was performed at the Institute of Oncology of Vojvodina in Sremska Kamenica. The study consisted of 72 women diagnosed with breast cancer aged between 29 and 84 years (average age: 59.04±10.87 years) whose breast tumor was surgically removed at the Institute of Oncology of Vojvodina. Patients who received preoperative chemo- or radiotherapy were excluded from the study. The data concerning breast tumor size and the presence of axillary lymph nodes in these women was obtained from the reports of Department of Pathoanatomical Diagnostics of the Institute of Oncology of Vojvodina. The results of our study indicated to positive, statistically significant moderate correlation between the size of breast tumor and the presence of axillary lymph node metastases ($r=0.32$, $p=0.01$). Receiver operating curve (ROC) analysis notified that cut-off value of breast tumor size for the presence of axillary lymph node metastasis was 22.5 mm (AUC=0.70, $p=0.01$). In our investigation, women with breast tumor size of 22.5 mm or larger were predisposed to the presence of axillary lymph node metastases.

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TUMOR SIZE AS THE BEST PREDICTOR FOR THE PRESENCE OF BREAST CANCER METASTASES IN AXILLARY LYMPH NODES

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The metastasis of breast cancer to the axillary lymph nodes represents a crucial aspect of disease progression and prognostic evaluation. The presence of metastases in the axillary lymph nodes is a key indicator that breast cancer is in an advanced stage, which can influence the therapeutic approach and the patient's prognosis. For this reason, we conducted a study aimed at examining the factors that contribute to the presence of metastases in lymph nodes in our female population. This research represents a prospective study conducted at the Institute of Oncology of Vojvodina in Sremska Kamenica. The study included 72 female participants diagnosed with breast cancer who underwent surgery at the Institute of Oncology of Vojvodina and had not received preoperative chemotherapy or radiation therapy. Initially, anamnestic data were collected from the participants, followed by a pathohistological analysis of the tumor tissue samples, including immunohistochemical analysis. We examined the influence of age, tumor size, activity of estrogen, progesterone, and HER2 receptors (human epidermal growth factor receptor-2) in tumors, as well as the occurrence of menarche and breastfeeding duration, on the presence of metastases in axillary lymph nodes. The results of binary logistic regression showed that the only significant predictor for the presence of metastases in axillary lymph nodes was tumor size ($p=0.01$, Wald=6.57, and Exp(B)=1.11), while the other examined predictors were not statistically significant ($p>0.05$). In our study population, the size of the breast cancer was crucial for the presence of metastases in the axillary lymph nodes.

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PROGNOSTIC POTENTIAL OF LEUKOCYTE TELOMERE LENGTH AND PARAOXONASE 1 ACTIVITY IN SMALL CELL LUNG CANCER

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Small cell lung cancer (SCLC) is the leading cause of cancer-related deaths worldwide and is characterized by rapid growth, early metastasis, and high mortality rates. This study investigated the prognostic potential of leukocyte telomere length (LTL) and paraoxonase 1 (PON1) activity in 60 SCLC patients treated with a cisplatin/etoposide (PE) regimen. Patients were observed at baseline, after 2 cycles, and after 4 cycles of chemotherapy. The primary objective was to evaluate the prognostic potential of these biomarkers for patient survival. LTL was measured from isolated genomic DNA using real-time quantitative polymerase chain reaction (RTq-PCR), while PON1 activity was determined using a spectrophotometric method. A Kaplan-Meier survival analysis was performed with cut-off values below the 25th percentile for LTL and PON1 activity to determine their prognostic power for overall survival. The analysis revealed that both LTL and PON1 are significant predictors of patient survival, suggesting that patients with levels below the 25th percentile have a higher risk of death (Log Rank = 3.956, p = 0.047; Log Rank = 3.834, p = 0.050, respectively). Telomeres, the protective caps at the ends of chromosomes, shorten with each cell division and reflect cell aging and genomic stability. Shorter telomere lengths in leukocytes have been associated with a poorer prognosis and lower survival rates in SCLC patients. Similarly, reduced PON1 activity is associated with increased oxidative stress, which contributes to cancer progression and poorer clinical outcomes. Monitoring PON1 activity could help in assessing patient prognosis and adjusting treatment strategies. These findings suggest that LTL and PON1 activity have significant prognostic value in SCLC and serve as useful indicators for identifying high-risk patients and guiding treatment decisions to improve outcomes.

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PREDICTIVE VALUE OF TOTAL OXIDANT STATUS AND TOTAL ANTIOXIDANT STATUS IN NON-ALCOHOLIC FATTY LIVER DISEASE

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According to global epidemiology, non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease, affecting about a quarter of the adult population worldwide. NAFLD is characterised by the accumulation of triglycerides in hepatocytes (steatosis), which can progress to non-alcoholic steatohepatitis, a more severe form of NAFLD. Oxidative stress is closely linked to the disease progression due to the activation of inflammatory pathways. The aim of this study was to identify markers of redox status that could predict the risk of developing steatosis. The study included 179 participants who underwent ultrasound examination at University Medical Centers Zemun and Zvezdara. Participants were divided into two groups: 119 patients with steatosis and 60 apparently healthy controls (control group, CG). Biochemical markers as well as markers of redox status: total oxidant status (TOS) and total antioxidant status (TAS) were determined in serum spectrophotometrically on biochemical analysers. Univariate and multivariate binary logistic regression analyses were used to test the predictions of TOS and TAS for NAFLD. Patients had higher body mass index (P<0.001), glucose (P<0.001), uric acid (P<0.001), TOS (P=0.007), and TAS (P<0.001) levels compared to CG. Univariate binary regression analysis revealed significant predictive capability of TOS and TAS for NAFLD demonstrated by the following ORs: 1.104 (1.020-1.195) (P=0.014) and 1.003 (1.001-1.004) (P<0.001), respectively. After applying multivariate binary logistic regression analyses (adjustments were made for sex and BMI), TOS and TAS kept independent significant predictive capability for NAFLD, as demonstrated by the following ORs: 1.098 (1.009-1.195) (P=0.030) and 1.002 (1.000-1.003) (P=0.026), respectively. TOS and TAS are positively associated with the risk of developing NAFLD, independent of sex and BMI. Both markers are elevated, probably because increased oxidative activity requires a stronger antioxidant defence response, which should be confirmed by a follow-up study including more participants.

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ARTIFICIAL LIGHT DISRUPTS NATURAL DAY/NIGHT VARIATION IN ANTIOXIDANT SYSTEM OF TREE FROG (*HYLA ARBOREA*)

Marko D Prokić[1]*, Jelena P Gavrić[1], Svetlana G Despotović[1], Branka R Gavrilović[1], Tijana B Radovanović[1], Ana Kijanović[2], Marko Mirč[2], Tanja Vukov[2], Nevena Đukić[3], Nataša Tomašević Kolarov[2], Tamara G Petrović[1]

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One of the adaptive features that organisms developed throughout their long natural history is the ability to change the activity of cells, tissues, and organs on a daily, 24-hour, basis. These cyclical changes are synchronized to the external environment through a light-dark regime and internal circadian clock. Daily recurring environmental changes are followed by variations in animal behavior and physiology, which include oscillations in neuroendocrine, metabolic, cardiovascular, and immune functions. By transforming the circadian periodicity of day, artificial light from anthropogenic sources might interfere with organisms leading to a disturbance in hormone levels and physiological stress. In this study, we investigated daily natural variations in the antioxidant system and the effects of artificial light on the redox balance in larvae of tree frogs. We compared antioxidant parameters in tadpoles from the natural day/night cycle (control) with ones exposed to artificial light at night (treatment). The antioxidant response was measured at four time points during 24h (morning, day, evening, and night). Our results showed that only GR activity did not display day/night changes nor was affected by night illumination. For GSH-Px and GST we reported changes in activity at different times of day that were in the same manner for both treatment and control. The highest values were in the morning compared to the other time points. Variation during 24h was also observed for SOD, CAT and GSH. However, exposure to night light affected the pattern and intensity of these parameters compared to the control group. Overall our study suggests that daily differences in metabolic activity can result in variations in the antioxidant system and that the presence of artificial light affects these changes. The disrupted natural rhythm of the antioxidant response may further reflect on other physiological processes and lead to a state of oxidative stress.

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