Plenary Lectures and Oral Presentations

O.1

Free radicals and adaptive homeostasis in biology, medicine, sport and nutrition

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Adaptive homeostasis is "the transient expansion or contraction of the homeostatic range for any given physiological parameter in response to exposure to sub-toxic, non-damaging, signalling molecules or events, or the removal or cessation of such molecules or events" (Davies K.J.A., *Molecular Aspects of Medicine* 49: 1–7, 2016). Adaptive homeostasis enables biological systems to make continuous short-term adjustments for optimal functioning despite ever-changing internal and external environments. Initiation of adaptation in response to appropriate metabolic signals allows organisms to expand their functional range, for example in the case of exercise- induced adaptation. In other instances, adaptive homeostasis allows organisms to successfully cope with normally toxic, stresses. These short-term responses are initiated following effective signals, including hypoxia, cold shock, heat shock, oxidative stress, caloric restriction, osmotic stress, mechanical stress, immune response, and even emotional stress. There is now substantial literature detailing a decline in adaptive homeostasis that, unfortunately, appears to manifest with ageing, especially in the last third of the lifespan. This lecture will present the hypothesis that one hallmark of the ageing process is a significant decline in adaptive homeostasis capacity. The mechanistic importance of diminished capacity for short-term (reversible) adaptive responses (both biochemical and signal transduction/gene expression-based) to changing internal and external conditions, for short-term survival and for lifespan and healthspan will be discussed. Studies of cultured mammalian cells, worms, flies, rodents, simians, apes, and even humans, all indicate declining adaptive homeostasis as a potential contributor to age-dependent senescence, increased risk of disease, and even mortality. Emerging work points to Nrf2-Keap1 signal transduction pathway inhibitors, including Bach1 and c-Myc, both of whose tissue concentrations increase with age, as possible major causes for age- dependent loss of adaptive homeostasis.

O.2

Treatment of neurological disorders and obesity related disorders by targeting mitochondrial dysfunction and oxidative stress with multi-ingredient supplements

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Impairments in mitochondrial structure and function are seen in neurological disorders, aging and metabolic disorders such as obesity and type 2 diabetes. Mitochondrial dysfunction can lead to a variety of cellular consequences including; increased oxidative stress, telomere shortening, reduce protein synthesis, inflammation, and cellular senescence. Single anti-oxidant trials have generally not been successful in several neurological disorders (i.e., Duchenne MD, Parkinson disease, ALS). Similarly, we have shown that high dose coenzyme Q10 was not associated with molecular or clinical benefits in patients with genetic mitochondrial disease. In contrast, we have used a multi-ingredient supplement (MIS, coenzyme Q10 + alpha lipoic acid + vitamin E + creatine monohydrate) approach targetting several of the final common pathways of cellular dysfunction, and shown significant biochemical benefits in mitochondrial disease patients. We have since used this "mitocentric" MIS approach to evaluate the potential clinical efficacy in pre-clinical models of obesity/fatty liver disease with very significant benefits. A seven ingredient MIS that was created from these pre-clinical studies was recently shown to reduce fat mass, preserve muscle mass and lower biomarkers of fatty liver disease in overweight and obese men and women. We have recently used this same mitocentric MIS approach to demonstrate efficacy in pre-clinical models of aging skin, sarcopenia and fertility.

O.3

Functional decline in low functional older adults: Role of iron dysregulation

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Preserving movement-related independence has become a clinical and public health priority. Perturbations in systemic, cellular and mitochondrial iron homeostasis, energy production and walking performance have not been investigated. Our work demonstrates that low-functioning (LF) older adults have a more rapid functional decline than those who are high-functioning (HF). The biological mechanisms that accelerate functional decline in LF older adults, however, remain poorly understood, and few therapies are available to slow rate of progression. As diverse as the etiologies of physical disability are, the mitochondria (Mt) appear to have a key role in the initial onset and progression of functional decline (1-3). A growing body of research indicates that perturbations in cellular iron transport and iron accumulation may contribute to increased oxidative damage to Mt DNA (mtDNA) and electron transport chain (ETC) proteins, together impinging on Mt function. Abnormalities in Mt iron homeostasis can cause disorders of skeletal muscle. Cellular iron import and export are critical for optimal cellular function and iron levels are modulated by the hormone hepcidin *via* binding and subsequent degradation of the iron export protein ferroportin (Fn) in the gut and skeletal muscle. Our preclinical work has shown that Fn levels in muscle decrease with age and there is an increase in intra-cellular iron accumulation (4). We observed iron acquisition (import) by skeletal myocytes through transferrin receptor (TfR1; main iron import protein) is markedly decreased, which is due to the accumulation of intracellular iron during aging (5). We also documented increased skeletal-muscle Mt iron stores in older animals, which markedly increased the susceptibility of Mt permeability transition pore (PTP) opening (6). In our human studies, we also found very similar changes in iron regulatory mechanisms with age systemically and in muscle (7, 8). Circulating levels of hepcidin (ng/mL) were markedly increased in the LF group (70 ± 34 mean ± standard de*via*tion (SD); (arbitrary units) compared to the HF group (39 ± 21) (age > 75 years) and young participants (17 ± 8) (7). Additionally, TfR1 protein expression had declined to very low levels in the LF group (0.06 ± 0.1) compared to the HF group (0.95 ± 0.6) and healthy young controls (2.1 ± 1.7) (7). Mitochondrial frataxin markedly declined in the LF participants. Recently we also showed an altered expression of markers of the autophagy/mitophagy-lysosomal system is related to deterioration of muscle function (9). The results from these studies will help to identify cellular targets for future studies to improve iron homeostasis and mitochondrial function, which should ultimately slow the rate of progression of functional decline in older adults.

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O.4

Muscle energetics and mitochondrial efficiency

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Exercise is a challenge to muscle energetics and limitations in energy processes are often related to fatigue. Formation of ATP during exercise occurs through both aerobic and anaerobic processes and the control and interplay between processes are complex. Mitochondria has a crucial role during prolonged exercise but studies of mitochondrial function in skeletal muscle have been hampered by technical difficulties in isolating mitochondria. Prof. Popinigis was able to overcome these difficulties and has presented some challenging work in this area.

Mitochondria are both a source and target of ROS and exercise is associated with increased formation of ROS. However, studies in isolated mitochondria demonstrate that mitochondrial ROS formation is low or absent at high rates of oxidative phosphorylation.

It is well known that endurance training can increase muscle mitochondrial volume. An increased muscle oxidative potential will primarily increase the lactate threshold i.e. the work rate that can be maintained without large increases in lactate formation. Another factor of importance for exercise performance, although less investigated, is mitochondrial efficiency i.e. amount of produced ATP per consumed oxygen (P/O ratio). Mitochondrial efficiency is dependent on substrate and temperature but also on the rate of oxidative phosphorylation. Mitochondrial efficiency is reduced at low rates of oxidative phosphorylation due to electron leak through the inner mitochondrial membrane. The main focus of this presentation will be on mitochondrial efficiency: factors of importance and relation to ROS.

O.5

Effect of exercise on the iron accumulation-possible role of Akt kinase

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Iron plays a pivotal role in numerous cellular processes, encompassing mitochondrial respiration, DNA synthesis, repair, vitamin D activation, etc. On the flip side, the potential for iron toxicity, arising from its propensity to catalyse the generation of reactive oxygen species, is well-established. From a biological standpoint, overt iron toxicity is elusive due to the rigorous regulation of its metabolism. Nevertheless, several studies have underscored that excessive iron accumulation in tissues elevates the risk of conditions such as diabetes, cancer, and cardiovascular events.

Thus, it is crucial to understand the mechanism of tissue iron accumulation and if exercise training can influence this process. We hypothesised that the impaired Akt-FOXO3a signalling pathway, characteristic of insulin-resistant tissues, triggers alterations in iron metabolism. Furthermore, exercise, known to enhance insulin sensitivity, might protect against tissue iron accumulation. Our *in vivo* and *in vitro* data clearly show that impairment of the Akt signalling pathway results in activation of FOXO3a, a transcriptional factor responsible for the upregulation of Ferritin. An increase in cellular ferritin consequently diminishes the labile iron pool, augmenting iron transport into cells and potentially initiating tissue iron accumulation. Research on animals and humans confirmed that exercise could induce changes in iron metabolism manifested by its lower tissue accumulation.

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O.6

Ketogenic diet and exercise: From molecular mechanisms to competition

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The ketogenic diet (KD), also historically known as protein sparing modified fast, was used for the first time as medical therapy by Dr. Wilder in 1921 as dietary approach to epilepsy in alternative to fasting. Although the composition of KD described in scientific literature vary, generally speaking, the proportion of macronutrient proposed initially by Peterman has been substantially maintained along the years with less than 20/30 g of carbohydrates or less than 5% of total daily energy intake from carbohydrate. Forty years after falling into disuse due to new antiepileptic drugs, in the late 1990s KD experienced a reemergence in the treatment of epilepsy and its interest expanded to other clinical conditions such as inflammation, metabolic syndrome, type 2 diabetes, PCOS, cancer and others not communicable diseases. Lately, KD has been proposed as a nutritional tool for sport and performance. Available data suggest that KD reduce fat mass in strength/power athletes without affecting performance or skeletal muscle mass; whilst results for endurance athletes are more conflicting, but most studies show a reduction of performance with an increase of energy cost. The role of the so-called keto adaptation and the wide inter-individual responses to KD are still on debate, e.g. many researchers advocate a role for microbiota in the individual response to KD. Moreover, the effects of KD on skeletal muscle response to exercise at molecular level is still not investigated. For these reasons, KD and athletic performance is still a promising and interesting field of research.

O.7

Antioxidants and skeletal muscle fatigue

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Skeletal muscle's ability to regenerate and remodel in response to exercise is crucial for further contractile function. One of the challenges faced by working muscle is a rise in reactive oxygen/nitrogen species (RONS) content, which, under normal circumstances is balanced by the activity of oxidant scavengers. The literature review into exercise-induced oxidative stress and the role of antioxidants in sport was performed from 1995 to 2023 on Medline (PubMed) database. The analyzed data revealed that the overexpression of endogenous antioxidants impairs training adaptations in the muscle. These observations prove the double-faced role of RONS, which at low-to-moderate concentrations and/or after a transient surge (e.g. after blood-flow-restricted training) lead to the higher muscle mass, strength and performance. On the other hand, chronic excessive free radicals production primarily acts on myofibrillar proteins to reduce calcium release from sarcoplasmic reticulum, inhibit calcium sensitivity and depress force. Several studies have investigated the effects of a wide variety of antioxidant supplements, including vitamin C, vitamin E, β-carotene, coenzyme Q10, α-lipoic acid, N-acetylcysteine, resveratrol, glutathione, polyphenols, and molecular hydrogen on the rate of muscle fatigue. Collectively, there are still not enough evidence to unambiguously recommend the most effective antioxidant supplementation strategy. The inconsistencies between the results could be attributed to differences in the conditions of antioxidant intake (type, dose, duration, timing) and exercise volume. Additionally, inter-individual variability in redox state contributes to the greater improvement in exercise performance in individuals with lower antioxidant levels at rest, whereas, antioxidants may limit exercise performance in those with high antioxidant content at rest.

O.8

Impact of lifestyle on a 6CpG-epigenetic clock

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Background: Changes in DNA methylation along the life have been documented, and environmental exposures (i.e., diet, physical activity, and smoking) can accelerate or decelerate this process. The epigenetic clock estimates the biological age of an individual measuring the methylation pattern in specific areas of its genome. Recently, a new epigenetic clock based on 6 CpGs has been proposed, with high potential to become an accessible tool able to measure the epigenetic age (EA) of an individual. The aim of this study is to validate the 6 CpG epigenetic clock comparing it with other biomarkers of aging such as telomere length (TL) and methylation in the long interspersed nuclear elements (LINE-1). Moreover, the impact of life-style associated factors on these molecular marks has been evaluated.
Methods: 200 healthy participants having extreme dietary patterns (healthy vs western diet) were selected. Dietary intakes, body composition, physical activity level and smoking has been assessed. DNA extracted from whole blood was used to measure the 6CpG-EA, TL and LINE-1 methylation levels.
Results: 6CpG-EA was positively correlated with chronological age and negatively with TL and LINE-1 methylation. Despite no significant associations were detected with the overall diet quality (HEI), 6CpG-EA was correlated with dietary intakes of nutrients involved in the one-carbon (1C) metabolism, especially in the western diet group.
Conclusions: These outcomes support the 6CpG epigenetic clock as an accessible tool to estimate biological age, in accordance with other molecular markers of aging, and suggest that EA can be modulated by diet, especially through micronutrients involved in the 1C metabolism.

O.9

Gut microbiota–derived metabolite trimethylamine-N-oxide and insulin resistance developmen

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The metabolites produced by the gut microbiota can actively influence the health of the host (1). In recent years, there has been a focus on trimethylamine N-oxide (TMAO). Multiple studies have shown a positive association between TMAO and various diseases, including chronic kidney disease, endothelial injury, hypertension, atherosclerosis, heart failure, and neural degeneration (2). TMAO is also considered as a metabolite involved in the development of insulin resistance and diabetes (3). A recent meta-analysis, which examined 12 clinical studies, demonstrated a positive association between circulating TMAO levels and an increased risk of diabetes (4). According to the dose-response analysis, the prevalence of diabetes increases by 54% for every 5 μmol L-1 increase in plasma TMAO levels (4).
We have conducted studies to modulate TMAO formation, but elevated fasting plasma TMAO did not have an impact on circulating markers of insulin resistance. This suggests that additional stressors, such as oxidative stress or inflammatory response, may play a role in the development of insulin resistance and that diabetes can progress independently of the intestinal microbial production of TMAO.

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O.10

The need of exogenous antioxidants to support the endogenous antioxidants in cellular redox homeostasis maintenance; Conclusions from electrochemical assessments

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The exogenous, mostly food-derived, antioxidants are commonly perceived as additional line of support for endogenous antioxidant barrier in the situation of oxidative stress. However, oxidative stress may be expected to occur only in specific,
e.g. pathological situations, so currently the emphasis has shifted towards redox homeostasis maintenance. In the latter case, exogenous reducing compounds may be perceived as supporters of endogenous systems in neutralization of ROS that are generated during normal, physiological processes. In our recent research, we compared the antioxidant efficacy of exogenous and endogenous reducing compounds. Based on our results, it will be argued that the exogenous reducing agents are needed to take over the antioxidant barrier role to maintain cellular redox homeostasis and by this save endogenous antioxidants, so as they can play their other physiological roles.

O.11

Mitochondrial dysfunction underlies molecular mechanism of muscle disuse atrophy

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Skeletal muscle contraction maintains morphological and functional integrity, when IGF/Akt/mTOR suppresses FoxO-controlled protein degradation pathways such as the ubiquitin-proteolysis and autophagy/mitophagy. Muscle immobilization (IM) and inactivity activates protein degradation and imposes oxidative stress resulting in myocyte atrophy. Mitochondria play an important role in regulating both protein synthesis and degradation *via* a number of redox sensitive signaling pathways such as biogenesis primarily by PGC-1α, fusion and fission dynamics, mitophagy and apoptosis. During prolonged IM followed by remobilization, a downregulation of PGC-1α and increased mitochondrial oxidative damage can severely hamper mitochondrial inner membrane potential ($\psi 0$) and activate mitophagic process. Coupled with decreased mitochondrial biogenesis and increased fission protein and inflammatory cytokine production, mitochondria lose redox control and become more fragmented, which triggers further mitophagy and apoptosis. To support this "mitostasis theory of muscle atrophy", it has been demonstrated that PGC-1α overexpression *via* transgene or *in vivo* DNA transfection can successfully restore mitochondrial hemostasis and thus reduce proteolysis and ameliorate function. Understanding the mechanism governing mitostasis can potentially alle*via*te pathological tendency in muscle atrophy in cancer patients and has a significant implication in research on sarcopenia, the age-related muscle atrophy.

O.12

Exercise and epigenetical aging

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Epigenetic clocks can measure aging and predict the incidence of diseases and mortality. Higher levels of physical fitness are associated with a slower aging process and a healthier lifespan. We recently created DNAmFitAge, which contains physiological function-associated genes, and this new clock outperforms the existing DNA methylation based biomarkers and shows regular exercise is associated with younger biological age, better memory, and more protective blood serum levels. Based on these relationships DNAmFitAge could be an important biological marker of the quality of life. We also investigated the relationship between DNA methylation based aging clocks and the microbiome of the gut. Data revealed that in general, accelerated epigenetic aging can be linked to the abundance of pro-inflammatory and other pathogenic bacteria and decelerated epigenetic aging or high fitness level can be linked to the abundance of anti-inflammatory bacteria. Overall our data suggest that alterations in the microbiome can be associated with epigenetic age acceleration and physical fitness.

O.13

Exercise training decreases muscle nitrite content in rats

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Skeletal muscles are an important reservoir of nitric oxide (NO•) that is stored in the form of nitrite [NO_2^-] and nitrate [NO_3^-]. Nitrite, which can be reduced to NO• under hypoxic and acidotic conditions (nitrite bioactivation), is considered a physiologically relevant, direct source of bioactive NO•. Our results showed that the mammalian fast-twitch skeletal muscles possess greater potential to generate NO• *via* nitrite reduction than slow-twitch muscles and the heart. This property might be of special importance for fast skeletal muscles during strenuous exercise and/or hypoxia, since it might enhance their muscle blood flow *via* additional NO• provision (acidic/hypoxic vasodilation) and delay muscle fatigue (Majerczak *et al.* 2022). We also found that endurance training decreases basal nitrite in the locomotory muscles and in the heart, without changes in the basal [NO_3^-]. In the slow-twitch oxidative soleus muscle, the decrease in [NO_2^-] is present already after the first week of training. The underlying mechanisms of training-induced muscle NO_2^- decrease may involve an increase in the oxidative stress as well as metabolite changes related to an increased muscle anaerobic glycolytic activity contributing to: (i) direct chemical reduction of nitrite or (ii) activation of muscle nitrite reductases. It seems that nitrite bioactivation might be involved in the intensification of mitochondrial biogenesis in the locomotory muscles after endurance training (Majerczak *et al.* 2023, submitted).

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O.14

Effect of omega 3 fatty acids supplementation on blood antioxidant status, cardiac biomarkers, lipid profile and inflammatory mediators in endurance trained athletes

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Background: High-level athletes are subjected to intense training that may intensify inflammatory response and reduce the cardiovascular protection mechanisms of omega 3 polyunsaturated fatty acids (ω_3 PUFA). The objective of the study is to determine the blood prooxidant-antioxidant status, the erythrocyte content of fatty acids, the levels of cardiac damage markers (CKMB, hsTnT, H-FABP), and lipid profile in ultramarathon runners supplemented with ω_3 PUFA.
Methodology: The determination of biochemical markers was performed in athletes assigned to a placebo group and a supplemented group at rest and in response to running exercise before and after the ω_3 PUFA diet. The activity of superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT), glutathione reductase (GR), the concentration of reduced glutathione (GSH), the products of lipid peroxidation with thiobarbituric acid (MDA) and biochemical markers were assayed in all participants.
Results: There was a significant effect of the supplementation on the activity of SOD ($F=13.0$; $p<0.01$), GPX ($F=8.9$; $p<0.01$), GSH ($F=11.4$; $p<0.01$) and MDA ($F=46.1$; $p<0.001$)**.** Supplementation and exercise had an effect on the concentration of hsTnT ($F=3.9$; $p<0.015$), the activity of CKMB ($F=6.7$; $p<0.001$) and the concentration of inflammation mediators measured after the exercise tests.
The increased level of the oxidative defense markers was positively correlated with the ω_3 PUFA content in the erythrocyte membrane in the ultramarathon runners. Determination of the markers of an antioxidant balance in the blood may be useful in assessing the cardiovascular risk in the states of omega 3 acid deficiency in the body.

O.15

The Last Dance of Prof. Jerzy Popiningis – from mitochondria and ROS to exercise-induced muscle protection

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The last experiment conducted with Prof. Jerzy Popininigis studied the impact of changes in mitochondria cholesterol concentrations on their function and oxidative stress. Lowering mitochondrial cholesterol levels was induced by methyl-beta-cyclodextrin (MbCD) in an *in vitro* model and prolonged swimming in rats (*in vivo* model). We observed that MbCD decreases the function, induces changes in the mitochondrial configuration state, and decreases the calcium chloride-induced swelling.
The exercise protocol caused oxidative stress in cardiac cells and increased phosphorylation of p66Shc without any alterations in Akt and extracellular signal-regulated kinases. Despite increased phosphorylation of p66Shc, no significant increase was observed in either mitochondrial H_2O_2 release or mitochondrial oxidative stress markers. Also, prolonged swimming did not affect anti-apoptotic (Bcl_2) and pro-apoptotic (Bax) protein levels. The exercise protocol also increased the caveolin-1 protein concentration in crude muscle mitochondria, which was related to reduced cholesterol levels and inhibited mitochondrial swelling. There were no changes in rat livers except for increased oxidative stress markers in mitochondria.
Conclusions: Lowering mitochondrial cholesterol through prolonged swimming appears to be a physiological phenomenon beneficial to skeletal muscle. This phenomenon may play an essential role in protecting skeletal muscles, especially in conditions of muscle pathology as in the case of amyotrophic lateral sclerosis.

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O.16

Life prolongation and skeletal muscle protection induced by swim training in Amyotrophic Lateral Sclerosis mice

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Amyotrophic lateral sclerosis (ALS), an incurable, chronic neurodegenerative disease, is phenotypically characterized by the loss of muscle tone, paresis, muscle atrophy, and spasticity. Among all therapies studied in the mice model of ALS, one of the best is swim training, which prolongs the lifespan. Still, the protective changes induced by swimming in skeletal muscle are poorly understood. Therefore, this study aimed to investigate possible mechanisms related to swim-induced prolongation of ALS mice life.
We used transgenic mice with the G93A hmSOD1 gene mutation in the present study. The swim training protocol started at the presymptomatic stage (70 days of age) and ended when the mice were 115. We examined the body and tibialis anterior muscle mass, muscle energy metabolism, oxidative stress parameters, and markers of MAMs (Caveolin-1 protein level and cholesterol content in crude mitochondrial fraction) in groups of mice divided according to disease progression and training status.
Median survivals were 126 days of age in the sedentary mice and 135.5 days in the swimming group of ALS mice. The progression of ALS, accompanied by body mass and skeletal muscle mass reduction, was related to lowering Caveolin-1 protein levels and accumulating cholesterol in a crude mitochondrial fraction. These changes were associated with aerobic and anaerobic energy metabolism dysfunction and higher oxidative stress. Swim training prolonged the lifespan of ALS mice and ameliorated the reduction of skeletal muscle mass, with accompanying changes in MAMs components. Swim training also maintained mitochondrial function and lowered oxidative stress.

O.17

Tea and other beverages: could the way they are prepared affect their nutraceutical properties?

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Tea (T), the beverage prepared from infusing leaves in water of the *Camellia sinensis* plant, is the most consumed non-alcoholic beverage in the world after water, highly appreciated due to its pleasant sensory properties, health properties and socio-cultural characteristics. Herbal teas (HT) are instead beverages made from steeping herbs, other than *Camellia sinensis* in water, and these herbal infusions have become increasingly popular among health-conscious consumers as caffeine-free, antioxidant- rich sources of hydration. The potential health benefits of T & HT are linked to their nutrient and phytochemical contents after brewing. The extraction of health- promoting phytocompounds is related with the final antioxidant capacity of the beverages, which in turn correlates with many parameters such as time/temperature of infusion, particle size, number of extractions, etc.. Therefore, variations in T & HT preparation are important to study in order to maximize the health properties of these beverages. Common household methods for brewing hot T & HT involve pouring boiling water over the dried leaves/herbs and allowing the mixture to sit for 5 minutes, while increasingly popular cold brewing techniques often use room temperature water and a 2-h steeping time.
The focus of my talk will be on the different studies that we have performed over the years, to discern how different brewing parameters may affect the nutraceutical properties of not only common teas from China and Africa, but also more recently of teas grown in Europe, as well as Rooibos (*Aspalathus linearis*) and *Crocus sativus* petals herbal teas.

O.18

Human muscle fatigue during exercise: the pioneering work by Henry Briggs

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Human skeletal muscles in healthy individuals are able to generate sufficient amount of force and power to perform various forms of daily locomotion but the level of sustainable power generating capabilities varies between individuals. The assessment of human exercise capacity is now a routine practice both in case of healthy individuals and patients since the level of physical capacity is a strong predictor of health. It's worth to mention that laboratory studies of human exercise capacity have a long history - dating back to pioneering work carried out before the end of the 20th century, resulting in development of a number of important concepts giving a solid background for today's exercise physiology. A great, but unfortunately forgotten concept of assessment of exercise capacity called "crest load" was presented by Henry Briggs (1920). Surprisingly, his paper work was very rarely cited in the literature. This is why in this lecture the Brigs' concept of the "crest load" an its relation to more modern concepts of humans exercise capacity such as the "lactate threshold" or the "critical power" will be presented (see, Zoladz & Grassi, 2020). Finally, the impact of exercise- induced disturbances in muscle metabolic stability on the muscle efficiency and muscle fatigue will be discussed (Grassi *et al.*, 2015).

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O.19

Mitochondria and oxidative stress, exploring the role of vitamin D: Implications for brain and skeletal muscle atrophy

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My scientific interest in mitochondria, free radicals, and vitamin D was encouraged by Prof. J. Popinigis, my mentor. The mitochondria play a pivotal role in ATP production *via* oxidative phosphorylation. However, dysfunctional mitochondria generate harmful ROS, which directly damage cellular components and activate cell death pathways. An in-depth understanding of mitochondrial dysfunction is crucial, as ROS overproduction fuels inflammation and contributes significantly to various pathologies. Vitamin D3, a fat-soluble, antioxidant, regulates various metabolic processes influencing human health mainly through VDR binding. The role of vitamin D and its mechanism of action are multidirectional and complex, concerning the regulation of the nervous, muscular, and immune systems, among others. Recently, our research group published data showing that vitamin D deficiency causes many deleterious changes. We found elevated markers of lipid and protein peroxidation, decreased protein content of PGC-1α, disruption of mitochondrial function, atrophy in skeletal muscle, and increased serum markers of inflammation in patients with low back pain. Further studies explored the effects of long-term DEXA treatment by reducing the serum concentration of 25(OH)D3, decreasing the body mass of rats, and inducing hippocampal atrophy. Moreover, a reduction in BDNF signaling in the hippocampus was associated with inhibited pAkt activity, decreased biogenesis, and reduced energy metabolism of mitochondria. Our findings reveal the complex relationship between vitamin D, mitochondria, and ROS, shedding light on their roles in human health and the potential for vitamin D3 treatment to mitigate the adverse effects caused by vitamin D deficiency.

O.20

Neuroprotective potential of the adaptation to exercise and cold

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Background: Neurodegeneration is a process associated mostly with senescence or neurodegenerative diseases, which are considered to be incurable. Besides a small percentage of genetic predisposition, two major contributing factors to neurodegeneration are oxidative stress and inflammation. Exercise is among the few therapeutic strategies studied for its neuroprotective effect. While exercise protocols have already been well described, cold exposition is a new strategy under consideration for its ameliorating effect on the progressive degeneration of neurons.
Objectives: The presentation aims to show the neuroprotective capabilities of the adaptation to cold exposition and exercise and to point out the possible risks of overstimulation.
Methodology: SOD1-G93A mice underwent swim training (five times per week for 30 min) in the presymptomatic stage of life (10th week). The spinal cord was analyzed for enzyme activities, oxidative stress, and autophagy markers.
Also, BABL/c mice were subjected to hypothermia therapy (4 weeks, five times per week) at four different levels of cooling (3 or 6 minutes in the 2 or 15 cm deep water, 4°C). Brown adipose tissue was analyzed to enzyme activities. The hippocampus was analyzed *via* WB and immunohistochemistry.
All mice underwent behavioral testing.
Results: Our data show that swim training triggers a neuroprotective effect on the spinal cord of SOD1-G93A mice. Swim training reduced oxidative stress, modified autophagy, and caused metabolic changes and mitochondria protection through the IGF-1 signaling pathway. Additionally, cold therapy on BALB/c mice positively influenced hippocampus but only at the proper stimulation.

O.21

Genistein: Not only an antioxidant, but also a potential anti-neurodegenerative compound

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Genistein (trihydroxyisoflavone or 5, 7-dihydroxy-3-(4-hydroxyphenyl)-4H-1-benzopyran-4-one) is a natural compound, occurring in various plants while soy bean in especially rich in this substance. Genistein has various biochemical activities, including antioxidative action and anti-inflammatory function. Moreover, it resembles estrogen molecule, thus, it has been used in treatment of menopausal symptoms, acting as a phytoestrogen. However, studies from recent two decades suggest that genistein could be considered for treatment of lysosomal storage diseases (LSD), particularly mucopolysaccharidoses (MPS). This proposal was based on findings that this isoflavone can inhibit phosphorylation of epidermal growth factor receptor, thus, impairing the signal transduction mechanism that normally leads to activation of expression of genes coding for enzymes catalyzing production of glycosaminoglycans (GAGs). Studies conducted with animal models of MPS indicated that genistein application may result in decreased GAG storage in brain and can correct behavior of mice. Subsequently, preliminary clinical studies on efficacy of low doses of genistein in treatment of patients suffering from Sanfilippo disease (MPS III) were performed. More recent study demonstrated that genistein not only impaired efficiency of expression of genes coding for GAG synthetases, but also stimulated expression of most genes involved in lysosome biogenesis; the latter effect was caused by enhanced expression of the TFEB gene, which codes for a master regulator for lysosome biogenesis (the TFEB protein), and the translocation of the gene product from cytoplasm to the nucleus. Genistein was also reported to improve cell cycle in various MPS types, which is disturbed in affected cells. Finally, this isoflavone was shown to stimulate the autophagy process. This activity may be especially useful in treatment of neurodegenerative diseases caused by accumulation of various compounds, like improperly folded protein. Indeed, studies with cellular and animal models of Huntington disease and Alzheimer disease demonstrated that treatment with genistein leads to elimination of toxic protein aggregates, mutant huntingtin, and beta-amyloid and hyperphosphorylated tau protein, in cells used as models of theses diseases, respectively. Moreover, in animal models of these diseases, treatment with genistein caused complete correction of otherwise significantly disturbed behavior of mice and rats. Thus, one might suggest that genistein can be considered as an therapeutic option for various diseases, especially those caused for formation of abnormal aggregates in the central nervous system.

O.22

Mitochondrial dysfunction, oxidative stress and autophagy alterations in mice model of non-alcoholic fatty liver disease

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Excessive consumption of foods rich in saturated fats causes unhealthy fat accumulation in the liver, which is directly related to the increase in non-alcoholic fatty liver disease (NAFLD). NAFLD comprises a spectrum of metabolic states ranging from simple steatosis to non-alcoholic steatohepatitis (NASH), cirrhosis, and finally hepatocellular carcinoma as an ultimate consequence. This is predominantly meaningful in the context of chronic liver diseases, with NAFLD affecting approximately 25–30% of the worldwide population. Therefore, NAFLD is recognized as a severe liver pathology that is emerging as a major health concern. We hypothesize that the development of NAFLD can be modulated by autophagy, one of the major quality control systems for the removal of oxidized molecules and damaged organelles. Additionally, cellular machineries involved in the formation of reactive oxygen species (ROS) appear to be affected in NAFLD. Failure of autophagy may cause the accumulation of dysfunctional organelles, which contributes to the induction of oxidative damage to the liver in NAFLD progression. Moreover, the prospects for reversing NAFLD are considerably diminished once NASH is well established, but up to this point it responds to both lifestyle/diet and therapeutics strategies. Importantly, our findings prove that mitochondrial alterations and subsequent impairment are independent of an excessive mitochondrial ROS generation, which was found to be progressively diminished along with disease progression. Instead, increased peroxisomal abundance and peroxisomal fatty acid oxidation-related pathway suggest that peroxisomes may contribute to hepatic ROS generation and oxidative damage. We have shown, that this early non-alcoholic fatty liver stage is also associated with autophagic flux impairment. During the lecture, I will present the sequential events of mitochondrial alterations involved in NAFL progression and demonstrate that mitochondrial ROS are not one of the first hits that could cause NAFLD progression. Then, that the accumulation of damaged/dysfunctional organelles could instigate hepatocyte injuries and NAFLD progression.

Acknowledgements

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O.23

High density lipoprotein, the guardian angel of biomembranes

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Oxidative stress represents a key player in the pathogenesis of several human diseases and pathophysiological conditions. High-density lipoproteins (HDL) plasma levels are inversely correlated with the risk of atherosclerosis and other chronic diseases associated with oxidative stress. An increasing interest is devoted also to the effect of HDL on molecular mechanisms of cancer development. The protective effect of HDL has been mainly related to their role in the cholesterol reverse transport and to the modulation of intracellular cholesterol content and homeostasis. More recent studies demonstrate that circulating high density lipoprotein (HDL) exert pleiotropic functions. HDL behave as antioxidants due to their ability to inhibit oxidation of low- density lipoproteins (LDL) and biological membranes. Moreover they suppress inflammation and endothelial activation. Several lines of evidence suggest that the protective roles of HDL are at least partially related to apoproteins such as ApoA1 and to enzymes associated to HDL surface. Among HDL-enzymes, a key role is exerted by paraoxonase (PON1), a calcium-dependent enzyme. The protective role of HDL-PON is related to the ability to prevent the accumulation of oxidised lipids from oxidised lipoproteins (LDL and HDL) and biomembranes thereby inhibiting the atherogenic and inflammatory response induced by lipid peroxidation products.

O.24

Essential role of free radicals in the pathomechanism of acute pancreatitis

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Acute pancreatitis (AP) is a type of inflammatory disorder of the exocrine pancreas. Among gastrointestinal diseases acute pancreatitis is one of the most frequent cause of hospitalisation. In up to 20% of AP, there are serious complications with a mortality rate reported of up to 30%. Currently, there is no specific pharmacotherapy for AP underscoring the need for research that aids in the prevention and treatment of this disease. Autolysis of the pancreas is a hallmark of AP but molecular mechanism is not fully understood. In human disease as well as in experimental AP acinar cells die through apoptosis and necrosis but the trigger of necrosis remains incompletely understood. Our study demonstrated that infusion of tert-butyl hydroperoxide (tBuOOH), a free radical inducer, into the rat pancreatic duct, can induce a necrotizing type of AP.
A group of rats given carbon-centered radical scavenger – 4OH-TEMPO displayed significant prevention of both inflammatory markers and necrosis. Recently it was reported that tBuOOH induces ferroptosis in human and murine cell lines. AP seems to be pancreatic inflammation depending on ferroptosis. During the early period of experimental AP significant accumulation of lipide peroxide (LOOH) was observed because lipid peroxidation trigger ferroptotic mode of cell death, inhibition of lipid peroxidation during AP seems to be possible efficient therapeutic option.

O.25

Energy metabolism of cardiomyocyte in dyslipidemia and its effects on cardiac function

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Dyslipidaemia is a major risk factor for myocardial infarction. It is widely accepted that infarction is triggered by atherosclerosis and vascular occlusion that limits blood and oxygen supply to the heart cell. However, exact level of flow restriction that leads to infarction could be altered by many factors such as metabolic state of the cell. We focused our recent research on clarification whether metabolic alterations induced by dyslipidaemia in cardiomyocytes constitute a mechanisms that escalates myocardial injury.
In experiments with hypoxic exposure dyslipidemic mice presented profound changes in ECG ST-segment and troponin I leakage that was not observed in in controls. Cardiac proteomic pattern analysis revealed increased expression of mitochondrial proteins, including enzymes of fatty acid and branched-chain amino acid oxidation, accompanied by decreased expression of glycolytic enzymes in dyslipidemic *vs.* controls. These findings correlated with *in vivo* analysis, revealing a reduction in the entry of glucose and enhanced entry of leucine into the cardiac Krebs cycle. Plasma concentrations of branched chain amino acids were much lower in dyslipidemic mice as compared to controls. Surprisingly dyslipidemic mice performed much better on exercise tests reaching higher running speeds and distances than controls. Treatment with inhibitor of fatty acid oxidation Ranolazine reversed the oxidative metabolic shift in dyslipidemic mice and reduced cardiac damage induced by hypoxia. To address human relevance plasma samples form patients with familiar hypercholesterolemia we studied. This analysis revealed similar amino acid pattern with decreased concentrations of branched chain amino acids.
We suggest a novel mechanism for myocardial injury in dyslipidaemia that is consequent to increased reliance on oxidative metabolism in the heart. The alterations in the metabolic pattern that we identified constitute an adaptive mechanism that improves energy metabolism under normoxia. However, even in a mild normally well tolerated reduction of oxygen supply, this adaptation triggers myocardial injury and could account for increased infarction zone.

O.26

Protein tyrosine phosphatase PTP-1B an unusual nucleophile in physiology and pathology

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Protein tyrosine phosphatase PTP-1B is responsible for the regulation of tyrosine phosphorylation level.
Defining hallmark of PTP superfamily is strictly conserved active site sequence C(X)5R within the catalytic domain. Such sequence constitutes the phosphate-binding pocket of the enzyme existing as thiolate anion. Chemical modification of the thiol group of PTP-1B triggers an activation of receptor signalling that is mimicked by intercellular H_2O_2. H2O2 may relatively easily cross the cell membrane in response to insulin or epidermal growth factor stimulation. We decided to compare the impact of H_2O_2 on recombinant CD45 and PTP-1B. Treatment of CD45 and PTP-1B with different concentrations of H_2O_2: 5,10,20 [uM] induced the inactivation of both enzymes, but PTP-1B is considerably less sensitive. H2O2 may undergo conversion to more potent oxidant like peroxoacids being more inhibitory towards PTP-1. The computational study showed that active site of PTP-1B and CD45 is highly electropositive limiting access to catalytic cysteine residue suggesting electrostatic attraction of peroxoacid residue. Although the electrostatic potential on the surface of active sites is similar for CD45 and PTP-1B, structural surface of these phosphateses display many differences. The diverse region includes the topology of the second binding site contributing to the substrate specificity of PTP-1B. Small molecule modulators may bind in the allostelic binding site of PTP-1B from the active site. Allosteric inhibitors of PTP-1B block the mobility of catalytic WDP-loop stabilizing inactive conformation of PTP-1B.

Poster Session

P.1

Clinical evaluation of RESCOVIN® syrup: Assessing its antioxidant properties in respiratory health

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Research background: Respiratory health is of paramount importance, and the search for effective supplements to boost it is ongoing. Previous studies have shown antioxidant, immunomodulatory, and potentially antiviral effects of plants rich in polyphenols such as *Lonicera caerulea* var. *kamtschatica Sevast.* (LCK), *Aronia melanocarpa* (AM), and *Echinacea purpurea* (L.) *Moench* (EP).
Objectives: This study aimed to evaluate the antioxidant properties of RESCOVIN® and their potential impact on respiratory health.
Methodology: A cross-over, placebo-controlled clinical trial was conducted with 29 participants. The study group initially received a placebo for 60 days, followed by a 60-day wash-out period, and subsequently, RESCOVIN®. Oxidative stress markers, such as isoprostane and SOD activity, were assessed before and after a supplementation period. Additionally, respiratory symptoms and inflammation indicators were monitored.
Results: The results showed a significant improvement in antioxidant markers after the RESCOVIN™ treatment. Moreover, participants during the RESCOVIN® treatment reported fewer respiratory symptoms and reduced inflammation level. The findings suggest that RESCOVIN® supplementation can effectively combat oxidative stress, contributing to improved respiratory health. These results support the potential of RESCOVIN® as a valuable addition to respiratory wellness strategies.

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P.2

AP119 as the potential radioprotector against ionizing radiation due to its strong antioxidant and anti-inflammatory properties – *in vitro* and *in vivo* studies

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Research background: Radiotherapy is one of the most powerful methods in the treatment of cancer. However, ionizing radiation used in the therapy causes damage to surrounding healthy tissues. The harmful effects of ionizing radiation are associated among others with oxidative stress and inflammatory response.
Objectives: The aims of this study were to evaluate the antioxidative and anti- inflammatory properties of AP119, a polyphenol-rich composition based on natural standardized extracts, as a potential radioprotector.
Methodology: In the *in vitro* study, Hek293T and hPBMC cells were exposed to oxidative stress conditions after pre-treatment with AP119. *In vivo* experiments were conduced on BALB/c mice daily supplemented with AP119 for 7 days, followed by a single dose of 6 Gy radiation. After an additional 4 days of supplementation, the mice were sacrificed, and the tissues were collected. The levels of biomarkers related to oxidative stress and inflammation were assessed by qPCR, flow cytometry, ELISA and Griess method. Lipid peroxidation was measured by MDA concentration.
Results: AP119 demonstrated antioxidative and anti-inflammatory properties in both *in vitro* and *in vivo* studies. Compared to the positive control, lower expression level of biomarkers involved in the oxidative stress and inflammatory response, such as *Sod2, Hmox1, Nrf2, Rela, Il1b* was observed. Additionally, AP119 decreased lipid peroxidation in the tissues and ROS level in hPBMCs. Furthemore, AP119 decreased cytokine levels of IL-2 and IFNγ in *in vitro* model. AP119 holds promise as a potential radioprotector against ionizing radiation.

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P.3

Antioxidant properties of RESCOVIN®: A comprehensive evaluation

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Research background: RESCOVIN®, a polyphenol-rich blend comprised of extracts from chokeberry (*Aronia melanocarpa*), honeyberry (*Lonicera caerulea* var. *kamtschatica Sevast.*), and purple coneflower (*Echinacea purpurea*), has gained attention for its potential as a natural antioxidant.
Objectives: This study is a comprehensive assessment of the antioxidant properties of RESCOVIN® through a series of *in vitro* experiments.
Methodology: The effect of RESCOVIN® blend on LPS-induced inflammation and oxidative stress in A549 cells and a mucociliary tissue (EpiAirway™) 3D model was assessed. To examine antioxidant defense mechanisms, we measured the expression of key antioxidant genes, including CAT, HMOX1, SOD1, and SOD2. Additionally, we determined superoxide dismutase (SOD) activity.
Results: Our study revealed that RESCOVIN®, demonstrates remarkable antioxidant effects. In A549 cells, RESCOVIN® significantly upregulated antioxidant genes, particularly CAT, HMOX1, SOD1, and SOD2. This increase in gene expression correlated with a substantial elevation in SOD activity, indicating enhanced cellular antioxidant defenses. However, in EpiAirway™ tissues, we observed a distinct response characterized by the downregulation of antioxidant genes. Its ability to enhance antioxidant gene expression and SOD activity in A549 cells suggests its potential in reducing oxidative stress-related damage and strengthening cellular defense mechanisms. The diverse response observed in EpiAirway® tissues emphasizes the complexity of its effects and calls for further research to elucidate the intricate mechanisms underlying RESCOVIN®'s antioxidant properties and its potential applications in oxidative stress-related conditions.

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P.4

Oxidative stress status and autophagy alterations in patients with non-alcoholic fatty liver disease

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Despite decades of research, the pathogenesis of non-alcoholic fatty liver disease (NAFLD) is still not completely understood, with oxidative stress and impaired autophagy constituting two interrelated processes that could contribute to disease progression. Therefore, in this study, we aimed to evaluate the oxidative stress and autophagic profile in liver biopsies from NAFLD patients.
Liver samples were obtained intraoperatively in a cohort of 16 obese patients scheduled for bariatric surgery. Biopsies from 5 patients without NAFLD served as controls. NAFLD staging was performed histopathologically. The parameters assessing hepatic oxidative stress and autophagy markers were examined by Western blot. Correlations between the levels of investigated proteins and disease stage were determined by Spearman's rank correlation test.
We found no significant changes in the oxidative stress profile of hepatic tissue of NAFLD patients, except for the decreased level of carbonylated proteins. Decreased protein carbonylation together with significant correlations between the thioredoxin system and obesity-related clinical parameters suggest alterations in the thiol-redox signaling. Among autophagic proteins, we found a significant increase in the level of optineurin in patients with the most severe disease phenotype. The levels of optineurin as well as the p-p62/p62 ratio were significantly correlated with the disease stage.
Altogether, these data do not seem to support the presence of oxidative stress at different NAFLD stages. On the other hand, both optineurin and p62 serve as selective autophagy adaptors, and their varying levels along NAFLD severity suggest that disrupted autophagic processes may play a significant role in the progression of this condition.

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P.5

Iron status determined changes in health measures induced by Nordic Walking with time restricted eating in older adults

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Background and Aims: The excessive accrual of iron within the organism is linked to an augmented susceptibility to various pathologies and potentially contributes to the progression of biological senescence. Routine physical exercise has demonstrated the capacity to mitigate age-associated iron accumulation; however, the precise underlying mechanisms remain partially elucidated.
Trial design and methods: In the present investigation, we endeavored to enhance comprehension of the interplay among physical activity, iron accrual, and the pathogenesis of diseases. To this end, we assessed the impact of a 12-week training regimen featuring Nordic walking (NW) paired with time-restricted eating (TRE) in a cohort of 24 elderly individuals (mean age: 70.3±7.68 years) exhibiting disparate baseline ferritin levels, categorized as either low (<75 ng/ml; LF) or high (≥75 ng/ml; HF).
Results: Following the 12-week NW+TRE intervention, a statistically significant reduction in ferritin levels was observed among all participants [Δ=–16.88 ng/ml, CI=(28.85, –4.91), p=0.01]. Furthermore, we detected substantial distinctions between the LF and HF cohorts in terms of the decrease in serum ferritin levels [LF: Δ=–6.05 ng/ml, CI=(–13.98;1.89); HF: Δ=–29.68 ng/ml, CI=(–53.98; –5.38); p=0.04]. Additionally, NW+TRE resulted in a reduction in HbA1c levels [Δ=–2.92 mmol/mol, CI=(–2.89; –1.6), p<0.01]. Importantly, an analysis of HbA1c and ferritin levels unveiled that more substantial alterations in HbA1c levels were significantly linked with a greater reduction in stored iron levels (p=0.04, ES=0.17, F=4.59).
Conclusion: In conclusion, the effects of the 12-week NW+TRE intervention on serum ferritin levels are modulated by the initial body iron reserves. Furthermore, more pronounced alterations in HbA1c levels were observed in individuals who exhibited a more substantial decrease in serum ferritin.

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P.6

Study of inhibitory properties of oxovanadium(IV) and dioxovanadium(V) complexes against PTP1B phosphatase in correlation to cytotoxicity against breast cancer cell lines

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Among the proteins that could be the focus of targeted therapies, there's the protein tyrosine phosphatase PTP1B. The reversible oxidation process serves as a universal mechanism to control the activity of protein tyrosine phosphatases. The oxidation process induces conformational changes in the structure of the enzyme's active site, preventing substrate binding. Vanadium complex compounds have attracted the interest of researchers because of their reported anti-diabetic, anti-inflammatory and anti-tumor properties. Their cytotoxic mechanism is largely attributed to the generation of reactive oxygen species, which are produced in cells in Fenton-like reactions.
In the presented study we investigated oxovanadium(IV) and dioxovanadium(V) complexes as potential PTP1B inhibitors with anticancer activity against breast cancer cell lines. We used recombinant PTP1B enzyme to test their inhibitory effects. To determine the mechanism of inactivation, we performed computer simulations. To determine the relationship between inhibitory properties and cytotoxicity, we evaluated the *via*bility of the MCF-7 and MDA-MB-231 breast cancer cell lines by comparing them with untransformed HaCaT cells. In addition, our goal was to investigate whether encapsulation of the most potent PTP1B inhibitors among those tested in lipid nanoparticles could enhance their cytotoxic effects against the triple-negative breast cancer cell line MDA-MB-231.
Our study showed a relationship between PTP1B phosphatase activity and cytotoxic results. The efficacy of the compounds depended on their structure, physicochemical properties, and cellular delivery approach. These findings have potential implications for the identification and development of targeted PTP1B inhibitors as an innovative therapeutic strategy to aid or prevent breast cancer development.

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P.7

Cotoneaster hissaricus and *Alchemilla acutiloba* as representatives of the *Rosaceae* family with significant antioxidant potential

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Since the action of free radicals is closely linked to numerous diseases of the organism, searching for novel therapeutic substances with antioxidant activity is of great significance. To this purpose, scientists are increasingly turning to plants used traditionally on which information in the literature is insufficient. *Cotoneaster hissaricus* and *Alchemilla acutiloba* have common aspects, i.e. they are used in traditional medicine, and belong to the *Rosaceae* family. A review of the literature shows that representatives of the *Rosaceae* family are abundant in polyphenolic compounds, the presence of which contributes to their significant antioxidant activity.
The main objective of our study was to determine the antiradical properties of so far untested extracts in order to use them in future in health-promoting treatments. Three methods based on chemical models were employed, namely: (DPPH•) free radical scavenging activity, ABTS•+ radical scavenging assays and metal chelating activity (CHEL).
The study indicated that the most active fractions in terms of antioxidant capacity obtained from *Alchemilla acutiloba* are: ethyl acetate and butanol fractions of 60% methanolic aerial parts extract as well as butanol fraction from the roots. Fractions obtained from the leaves of *Cotoneaster hissaricus* with promising antioxidant properties are: ethyl acetate and diethyl ether fraction from methanol– acetone–water leaves (3:1:1,v/v/v; 3×100 mL) extract.
Discussed extracts deserve further research and more detailed analysis, clarifying their mechanism of action. The obtained results provide a basis for further studies regarding the bioactivities and biological activities of representatives of the *Rosaceae* family.

P.8

A comprehensive study of AP029's antioxidant properties in diabetes

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Research background: Type 2 diabetes is a growing health concern with associated risks. The management of diabetes and its complications is of utmost importance. Dietary polyphenols have gained attention for their potential to regulate blood glucose levels and mitigate diabetes-related complications.
Objectives: The objectives of this study were to assess the antioxidant properties of AP029 and its constituents with focus on diabetic molecular mechanisms.
Methodology: The ORAC assay determined the ability of the samples, including both individual components and the AP029 composition, to neutralize oxygen radicals, while the DPPH assay assessed the radical scavenging activity of AP029 constituents. Changes in ROS levels in PBMC were monitored as a marker of antioxidant activity. Male db/db (BKS(D)-Leprdb/JOrlRj) mice, received daily oral administration of 0.9% NaCl solution/metformin/AP029/AP029+metformin for 6 weeks in the *in vivo* study.
Results: The series of ORAC and DPPH experiments conducted on individual components of AP029 as well as AP029 demonstrated potent antioxidant activity. The application of AP029 constituents leads to a reduction in ROS levels within PBMC. In the context of antioxidant activity, our *in vivo* mouse model demonstrated that the administration of AP029 and metformin resulted in a decrease in glucose and insulin levels. We observed increased levels of SIRT1, and SOD2, indicative of augmented antioxidant defenses. Conversely, there was a reduction in the expression of pp65 in the liver, suggesting a potential attenuation of inflammatory.

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P.9

The effect of acute sodium bicarbonate ingestion on inflammatory blood markers and heart rate in CrossFit-trained females

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Background: The study aimed to examine the effect of sodium bicarbonate (SB) ingestion on selected inflammatory blood markers and heart rate (HR) during high- intensity functional exercise.
Methods: Fourteen CrossFit-trained females (age: 29±5 years, body mass: 64.7±7.5 kg) completed the study protocol. Participants ingested either 0.35 g·kgFat-Free Mass-1 of SB or placebo (PLA) 120 min pre- exercise in a randomized double-blind cross-over design. The battery of exercise tests consisted of two bouts of 30-s Wingate Test interspaced with discipline-specific exercises (3 min of Wall Ball Shots and 3 min of Burpees). HR was measured continuously throughout (Polar Team, Finland) and time spent at various zones of exercise intensities was analyzed. Blood samples for the evaluation of interleukin 10 (IL-10) and tumor necrosis factor alfa (TNF-α) were collected at REST and post-exercise after 45 min of RECOVERY (REC). A control (CTRL) evaluation without any supplementation was also performed.
Results: There were no differences in IL-10 or TNF-α concentration at REST between study conditions. However, after SB ingestion, IL-10 and TNF-α at REC was significantly lower compared to PLA and CTRL. Moreover, SB ingestion resulted in significantly lower TNF-α at REC compared to REST. SB intake did not alter the time spent in different exercise intensities as evaluated by HR monitoring.
Conclusions: Extracellular buffering support through sodium bicarbonate ingestion may have beneficial effects on mitigation of exercise-induced disturbances of pro-/anti-inflammatory status of the body.

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Poster Contest for Young Scientists

PYS1

Selective estrogen' derivatives and hydrogen peroxide as potential indicators of neurodegeneration

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Estrogens regulate several physiological processes, including brain cell development and differentiation. 2-methoxestradiol (2-ME2), a 17-estradiol (E2) metabolite, has been shown to have anticancer properties both *in vivo* and *in vitro*. The research aimed to establish if 2-ME2 is a possible neurodegenerative agent in both a tissue culture model and a clinical setting.

2-ME2 activity was measured in an *in vitro* Parkinson's disease (PD) model based on the neuroblastoma SH-SY5Y cell line. The flow cytometry, confocal microscopy and western blot findings indicated that 2-ME2 caused nitro-oxidative stress and regulated heat shock proteins (HSP), resulting in DNA strand breakage and apoptosis.

Next, the clinical significance of 2-ME2's neurotoxic action in a PD *in vitro* model was explored.

The LC-MS/MS methodology was utilised to measure estrogens and their derivatives, particularly hydroxy and methoxyestrogens, in the blood of PD patients, while the stopped-flow method was employed to quantify hydrogen peroxide (H_2O_2) levels. Methoxyestrogens and H_2O_2 levels in patients' blood were higher than in control participants, whereas hydoxyestrogens were lower. Based on the above, we propose that measuring plasma levels of methoxyestrogens and H_2O_2 might be a potential PD biomarker.

Acknowledgements

The study reported is the subject of a pending patent application, No. P.441360, titled "The use of hydrogen peroxide and 17-estradiol and its metabolites as biomarkers in the diagnosis of neurodegenerative diseases." The study was funded by 01-10023 by the Medical University of Gdansk, Poland.

PYS2

Effect of combined therapy of vitamin D3 and sodium butyrate on neuroprotection in an experimental model of ischemic stroke, the role of mitochondria

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Ischemic stroke (IS) remains a global cause of death and disability. Previous studies have underscored the neuroprotective effects of vitamin D3 (VD) and sodium butyrate (SB) regarding IS. Nevertheless, the role of mitochondria in this critical effect remains elusive, mostly in older. Recent studies have shown the potential benefits of SB and VD, although this promising therapeutic approach remains largely unexplored. The goal was to determine the neuroprotective effects of combined VD and SB treatment within the first hour after IS in older rats. Moreover, we aimed to explore the efficacy of combined therapy with monotherapy of these compounds and investigate their impact on mitochondrial metabolism.

44 Wistar rats aged 18-20 months were randomly assigned to five groups: sham, control-stroke, vitD-stroke, SB-stroke, and vitD&SB-stroke. Following stroke induction using endothelin-1 for the first hour, treatments were administered. Blood and brain samples at 2, 24 hrs., and the fifth day after IS were collected.

We found that combined therapy of VD and SB had a protective effect on anchoring protein kinase-1 and vitamin D receptor protein contents. Moreover, Fluoro-jade labeling revealed fewer degenerated neurons in groups treated with VD and SB. The reduction of α-ketoglutarate dehydrogenase content in the ischemic core compared to the intact hemisphere was found. A single dose of VD administrated within one hour after IS was sufficient to increase 25(OH) D3 concentration in blood through five days. Our results suggest that combined therapy of VD and SB within the first hour after IS may have promising potential for neuroprotection.

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PYS3

The effect of Colostrum Bovinum supplementation on selected adaptation and nutritional status indices in trained athletes

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Colostrum Bovinum (COL) is a substance produced naturally by the mammary glands of cows for 24–72 h after calving. Evidence suggests that COL may have many clinical or therapeutic applications in humans, especially in physically trained population. In view of the content of biologically active compounds, like nutrients, immune and growth factors, COL supplementation may contribute to body adaptations and nutritional status of athletes. Twenty-five endurance- trained male participants (triathletes n=14, swimmers n=9) completed double-blind placebo (PLA)-controlled cross-over study aimed at examining the effect of COL supplementation on adaptation/muscle damage (AMD) and nutritional status (NS) indices at rest. Study participants were supplemented with 25 g per day of COL and PLA (milk protein) in a randomly assigned cross-over manner for 12 weeks with 4 weeks of wash-out period between treatments. The study protocol consisted of 4 visits (before and after) COL/PLA supplementation. Venous blood was taken at rest, after standardized meal and used to assess AMD (alanine aminotransferase, aspartate aminotransferase, creatine kinase, creatinine, lactate dehydrogenase, urea) and NS (albumin, glucose, total protein) indices. There were no differences in any of the selected biochemical markers between COL and PLA treatments. As a result of the ingestion of both – COL and PLA, athletes were able to train equally during the 12-weeks period. COL and high- quality milk protein (PLA) supplementations seem to equally affect selected biomarkers of adaptation and nutritional status of athletes.

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PYS4

Blood TMAO and TMA levels as a potential new markers of post-exercise muscle damage

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Background: Eccentric exercise induced muscle damage (eeimd) causes the loss of muscle strength and muscle soreness, changes in oxidative stress, inflammation and muscle damage markers. The role of tmao and tma in eeimd remains unclear.
Objectives: the aim of the study was to determine selected markers in male subjects who completed eeimd and to compare the obtained results with plasma and urine tmao and tma concentrations as potential new markers of muscle damage.
Methodology: Eighteen males, aged between 19 and 25 years old were included in this study. All of the participants finished one of two proposed eeimd protocols: drop jump test or cyclus2 eccentric ergometer test. The levels of following factors of oxidative stress (-sh groups, carbonyl groups), inflammation (leukocytes, nlr, plr, sii, tnf-α), muscle damage (ck, mb), as well as tmao and tma levels were measured at six time points: pre-, post, 2h, 24h, 48h and 72h after eeimd . The knee extensor peak torque (kept) was assessed using the biodex system dynamometer at four time points: pre-, 24h, 48h and 72h after eeimd.
Results: There were no significant changes in oxidative stress markers and tnf-α level, whereases increased levels of leukocytes, nlr, plr, sii were observed after eeimd. Moreover there were higher levels of muscle damage markers, with a parallel decrease of kept. We found strong correlations between plasma pre-tmao and pre-tma with mb levels measured 2h after exercise tests that can be an interesting starting point for the interpretation and further research.

PYS5

The effect of the UV light on keratinocyte *via*bility

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Although ultraviolet nail lamps are becoming more popular, their safety still remains debatable. They change aggregation state because of the presence of photoinitiators. Photoinitiators absorb UVA radiation from manicure lamps and then dissolve, leaving free radicals, which initiate the polymerization process.
Two types of lamps are currently used in beauty salons: light emitting diode (LED) lamps and UV nail lamps consisting of either fluorescent bulbs. UV lamps, curing the product in 2–4 minutes, emit UV radiation from 300 nm to 410 nm, with a peak emission at 375 nm, while LED lamps, curing the product in 30–60 seconds, emit light with a peak wavelength of 385 nm and a wavelength range of 375 to 425 nm. Many sources indicate that during the procedure, the hands are radiated for around 3-6 minutes, and the sessions are usually repeated by clients every two or three weeks. Therefore, the are serious concern regarding DNA damage induced by nail exposure to UV machines, as well as their possible role in skin carcinogenesis. In UV-irradiated samples, elevated levels of reactive oxygen species (ROS) are found, which is consistent with 8-oxo-dG damage and mitochondrial dysfunction.
Based on the above data, there is a strong need to conduct research on the effects of radiation from nail dryers on skin cells. We propose a study that aims to investigate the survival of human keratinocytes irradiated by UV nail drying equipment in direct response to previously released literature data.

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Medical University of Gdansk, ST-46

PYS6

Polyphenols-rich extracts of blueberries (*Vaccinium* L.) protect red blood cells against oxidative stress

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Three ecotypes of blueberries (*Vaccinium* L.), including wild bilberries, highbush, and lowbush blueberries, have been known to be rich in polyphenols, which possess antioxidant and healthy features. The purpose of our research was to investigate the protective effect of polyphenol-rich extracts of the three blueberry species' fruits on red blood cell membranes against oxidative stress.
To determine the extracts' polyphenolic compositions, we used the UPLC-ESI- MS method. To verify the antioxidant properties of each extract, oxidative damage of red blood cells' membranes was induced by AAPH and UVC. The hemolytic activity of extracts and their impact on osmotic resistance were measured spectrophotometrically, as well as the shapes of erythrocytes were also assessed microscopically. The effects of extracts on the packing arrangement and fluidity of the lipids of erythrocyte membranes were studied fluorometrically.
The results showed that each of the blueberry extracts is a rich source of polyphenols, especially from the group of anthocyanins. Extracts did not induce hemolysis nor affect the osmotic resistance of erythrocytes. What is most important, extracts protect the erythrocytes' membrane against induced oxidative damage. Polyphenols in blueberries did not affect the fluidity of the membrane, but they localized in the surface area of the erythrocyte, where they can protect membranes from the free radicals in the blood. The cell shape changes also confirmed the above arrangements.
Our research results demonstrate that the polyphenols in blueberry extracts can protect blood cells against oxidative stress and suggest that they can be safely used as a dietary supplement.

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PYS7

The impact of vitamin D3 supplementation on mitochondrial energy metabolism and neurogenesis in rats' hippocampus in chronic cold water immersion

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Cold exposure is recognized as a potent stressor that can have adverse effects on cognitive function. The physiological role of vitamin D (VD) and its mechanism of action are multidirectional and complex, involving the regulation of mitochondrial biogenesis and function, neurotrophin level, and neuroprotection. Our previous studies have demonstrated that prolonged exposure to cold leads to an elevation in corticosterone secretion into the bloodstream in rodents. Some data even hint at a potential link to adaptive mechanisms that may positively or negatively impact neurogenesis and hippocampal function. This study aimed to investigate the effects of vitamin D3 supplementation on mitochondria energy metabolism, neurogenesis, and specific proteins associated with neuronal stability in the rat hippocampus under conditions of chronic cold exposure.
The study was conducted on male Wistar rats (n=24), which were divided into four groups: control (CON; n=6), sham stress conditions (SHM; n=6), cold water immersion + placebo (CWI; n=6), and cold water immersion + 600 IU/kg/day VD (CWI+VD; n=6). The hippocampus was dissected, frozen, and stored at –80°C for further analysis.
We found reduced hippocampal weight in both CWI groups. Additionally, in the CWI+VD group, we noted elevated citrate synthase activity alongside decreased content of pro-brain-derived neurotrophic factor, insulin growth factor, and phosphorylated serine/threonine kinase. The CWI group exhibited increased content of RNA binding motif protein 3 and neurofilament light protein.
Preliminary results suggest that VD treatment has no impact on the rat hippocampus and may interact synergistically with the effects induced by chronic cold immersion.

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PYS8

Edible flowers collected in Poland as a potential source of natural antioxidants

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Balanced diet based on plant products, especially those rich in antioxidants is one of the strategies to prevent and support the treatment of non-communicable diseases, such as: type II diabetes, inflammation, neurodegenerative diseases, coronary diseases, hypertension and atherosclerosis. In last decades, research for antioxidant activity has focused mainly on fruits, vegetables and herbs. Recently, with increasing consumer awareness and interest in healthy and natural foods, the popularity of flowers as a food product is growing. The unique composition of phytochemicals with primarily antioxidant activity contributes to the anti-inflammatory, hepatoprotective, neuroprotective, anticancer and antidiabetic effects of edible flowers.
In this study, the ethanolic (70%, v/v) extracts from 15 edible flowers from Asteraceae, Caryophyllaceae, Rosaceae, Papilionaceae and Plumbaginaceae family collected in the Northern Poland were used. Most of the flowers analyzed in this study have not been examined in this respect until now. The total antioxidant potential of flowers was determined by spectrophotometric methods using the following reagents: ABTS, DPPH and Folin-Ciocalteu, while antioxidant profiles were prepared by high- performance thin-layer chromatography with detection with the DPPH reagent (HPTLC-DPPH). Total antioxidant capacity of the samples were expressed as the Trolox equivalent antioxidant capacity (TEAC) index.
HPTLC profiles of antioxidants obtaied for flower extracts, as well as the results of *in vitro* tests suggest that edible flowers may be a rich source of antioxidants. The highest total antioxidant activity was observed for Rosa rugosa extract. The results of the analyzes presented in this study may become a contribution to further research in this direction.

PYS9

The landscape of proteins representing antioxidant defense system in fibroblasts derived from patients suffering from Mitochondrial Membrane Protein-Associated Neurodegeneration (MPAN) subtype of Neurodegeneration with Brain Iron Accumulation (NBIA)

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Introduction: Neurodegeneration with Brain Iron Accumulation (NBIA) is a rare neurodegenerative disease characterized by extensive iron accumulation in the brain. One of NBIA subtypes – MPAN (mitochondrial membrane protein-associated neurodegeneration), caused by the mutation in C19orf12 gene encoding mitochondrial protein, is linked to multiple abnormalities in cellular metabolism. Very often, disruption of the energy metabolism may lead to an increase in reactive oxygen species production. This, in turn, when not balanced by the antioxidant systems, may lead to oxidative stress and oxidative damage. Oxidative stress underlies or accompany several pathologies and may exacerbate the patients' condition. Therefore, our aim was to characterize the status of enzymatic oxidative stress-response system in the MPAN patients' fibroblasts in order to identify alterations in the level of antioxidant enzymes which could be linked to the clinical phenotype of MPAN patients.

Materials and methods: Primary skin fibroblasts derived from 11 MPAN patients and 4 healthy donors were cultured in standard or OXPHOS promoting conditions. The level of antioxidant enzymes and proteins involved in redox homeostasis regulation, the scale of oxidative damages were evaluated by mass spectrometry and western blot respectively. Analysis, clustering and heat maps generation was performed in GraphPad Prism.

Results: Fibroblasts derived from MPAN patients cultured in OXPHOS promoting condition, had significantly higher level of mitochondrial superoxide than controls when compared to cells grown in the presence of glucose. Despite the same level of carbonylated proteins in control and patients fibroblasts (cultured in glycolysis supporting condition), the level of 4-HNE tended to be higher in fibroblasts harboring mutation in C19orf12 gene. Mass spectrometry analysis revealed differences in the profiles of antioxidant enzymes of MPAN fibroblasts and healthy controls especially when the cells were forced to rely on mitochondrial respiration. Moreover, the lack of significant manifestation of the oxidative damages to proteins in MPAN fibroblasts could be explained by alterations in the level of proteins involved in the degradation of the oxidatively damaged proteins.

Conclusions: Metabolic alterations in primary fibroblasts derived from MPAN patients are accompanied by the changes in the levels of antioxidant enzymes. These alterations, together with an increased ROS levels may suggest the presence of the oxidative stress, which in turn may contribute to MPAN clinical phenotype in NBIA patients.

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PYS10

The impact of vitamin D3 and dimethyl fumarate on neuroprotection in a model of alzheimer's disease induced by streptozotocin

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Several therapeutic strategies for the treatment of Alzheimer's disease (AD) have been proposed. However, they have not demonstrated sufficient effectiveness and only offer a slight reduction of symptoms. Vitamin D (VITD) and dimethyl fumarate (DMF) have shown anti-inflammatory and antioxidant properties, making them potential candidates for AD treatment. Our study aimed to evaluate the therapeutic potential of combined therapy involving VITD and DMF in comparison to the monotherapy of these compounds for the treatment of AD symptoms.
We used 40 Wistar rats aged between 3-4 months divided into five groups: STZ + CTR (n=8) induced AD symptoms without treatment, VEH + CTR (n=8) non-AD controls without treatment, STZ + VITD (n=8) induced AD symptoms treated with vitamin D3, STZ + DMF (n=8) induced AD symptoms treated with DMF, and STZ + VITD + DMF (n=8) for induced AD symptoms treated with both vitamin D3 and DMF. We assessed cell nuclei counts using immunofluorescence, measured selected protein content using WB, and analyzed vitamin D metabolites.
In the CA4 region of the hippocampus, cell nuclei count was significantly preserved in the therapy groups. Notably, the combined VITD and DMF therapy showed a significant reduction in p-TAU protein content in the examined tissue. Furthermore, the measurement of vitamin D metabolites revealed an increased demand for its active form in both groups receiving vitamin D.
Our preliminary studies highlight the potential therapeutic efficacy of investigated in ameliorating neurodegenerative processes. Additionally, the complexity of vitamin D's role, as indicated by metabolite measurements, requires further exploration.

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PYS11

Cytotoxic evaluation of functionalized gold nanoparticles for mitochondrial disruption and ROS-mediated anticancer effects

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Background and Purpose: Gold and silver nanoparticles are widely explored in cancer nanomedicine due to their finely tunable surface functionalities. This study explores the potential of functionalized gold nanoparticles as a drug delivery platform in cancer therapy. Specifically, we focus on their ability to induce cellular damage through mitochondrial disruption and the generation of reactive oxygen species (ROS). Additionally, we investigate their potential for localized antitumor effects using near- infrared radiation (NIR).
Materials and Methods: We synthesized and characterized three types of gold nanoparticles: one spherical and two gold nanorods. These nanoparticles were functionalized with Doxorubicin-loaded polyethylene glycol (PEG) and linked with polyethyleneimine (PEI). We assessed ultrastructural changes and cellular uptake of the nanoparticles using transmission electron microscopy (TEM). Cytotoxicity was evaluated through mitochondrial dehydrogenase activity (MTT) assay conducted on breast cancer cells (MDA-MB-231 and MCF-7). NIR was performed for the nanorods. Oxidative damage was quantified through ROS assays and flow cytometric analysis, while changes in mitochondrial membrane potential were measured using the fluorescent dye JC-10.
Results and discussion: Our findings indicate that the nanocomposites exhibit cytotoxicity against breast cancer cells (MDA-MB-231 and MCF-7). TEM imaging revealed severe mitochondrial damage associated with spherical nanomaterials. Notably, the spherical nanocomposite demonstrated selective cytotoxicity against cancer cells compared to non-cancer cells (HEK-293).
Conclusion: This study highlights the potential of gold nanoparticles loaded with doxorubicin-PEG conjugates, linked *via* PEI, as a promising approach for anticancer therapy. These nanoparticles have shown efficacy in inducing mitochondrial dysfunction, a key factor in their anticancer activity.

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PYS12

The effect of different training and acute exercise on blood irisin and BDNF concentration among older adults

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Physical activity is one of the best methods of preventing age-related diseases through releasing into the bloodstream proteins named exerkines. Exerkines are involved in glucose metabolism, regulating muscle performance, and counteracting sarcopenia. Among many exerkines, brain-derived neurotrophic factor (BNDF) and irisin are recently the most investigated.
This study aimed to assess the changes in BDNF and irisin in response to acute exercise and regular training and its conjunction with elders' physical performance and insulin resistance.
The effect of exercise was assessed on adults aged 58 ± 3.6 years, assigned into two groups – highly trained and untrained. The effect of the regular training was evaluated among elders aged 71.3±5.5 years assigned into three groups: folk–dance (DG), balance training (BG), and control group (CG) performing training 3 times a week for 12 weeks.
The results indicated a tendency in irisin change after a single bout of exercise: a slight decrease (18.4%) in the untrained and an increase (12.5%) in the trained group. In turn, BDNF significantly decreased only in the trained group ($p=0.02$). After the training protocol, significant improvement in physical performance (timed up and go, $p= 0.0006$ for BG and $p= 0.0039$ for DG and 6 minutes walk, $p=0.0001$ for BG and DG) and in DG amelioration of insulin resistance was observed (HOMA-IR, $p=0.023$, HOMA-%S, $p=0.020$, QUICKI, $p=0.032$). These positive changes were accompanied by a decrease in BDNF ($p=0.0002$ for BG, $p=0.0025$ for DG) and an increase in irisin ($p=0.029$ for BG and $p=0.022$ for DG).

PYS13

The effect of a meatless, ketogenic restrictive diet on body composition, body mass and strength capacity in healthy adults

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Background: The relationship between ketogenic diet (KD), body composition and strength capacity seem to have not been fully investigated. The aim of the study was to assess whether a KD rich in omega-3 polyunsaturated fatty acids would influence body weight, body composition and strength capacity.
Materials and Methods: Fourteen healthy participants were recruited with inclusion criteria – non-smoker, not abusing alcohol, not subjected to physical exercise for at least 48 hours before examination. Each participant received a 3-meal nutritional plan – 500 kcal reduction based on the Mifflin-St Jeor Equation and PAL. PRE and POST body composition was measured by DEXA and strength tests on the Biodex System 4 Pro dynamometer. The test comprised an alternating maximum 4 s knee extensors and flexors isometric contraction, with a 20 s pause between each trial. The procedure was repeated three times. The highest values were used for statistical analysis.
Results: After 2-week of meatless KD, a significant decrease in body mass, body fat, visceral fat and free fat mass was recorded. Moreover, there were no significant changes in the percentage of FFM (%FFM) as well as in the strength of knee flexors and extensors.
Conclusions: The KD significantly affects the loss of body weight, body fat, visceral adipose tissue with simultaneous loss of lean body mass. However, after 2-week intervention, no significant differences in skeletal muscle strength were observed. The results of these studies may be particularly useful for rapid weight reduction in athletes limited by weight categories.

PYS14

The effect of high-intensity interval exercise on executive performance and prefrontal cortex activation among elderlies – a fNIRS study

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Background: Cognitive decline poses a significant threat to the independence and quality of life of the elderlies. Aging is intrinsically linked to the deterioration of cognitive functions. However, a growing body of evidence suggests that regular exercise can offer substantial benefits in improving cognition. High-intensity interval exercise (HIE) has particularly shown promise in enhancing muscle as well as cognitive functions particularly among younger individuals.
Aim: The purpose of the present study was to assess acute effect of HIE on executive function focusing on underlying neural substrates in older adults.
Methods: The study involves sixteen elderlies. The main experiment consisted of two sessions, control (CTL) and high-intensity interval exercise (HIE) separated by at least one week. Each trial was conducted in a randomized, counterbalanced manner, with half of participants starting with the HIE session (Fig. XX). The HIE protocol consists of eight 60 s cycling bouts at ~90% HRmax intensity and 30 s resting (Fig. xx). Participants performed the Trial Making Test (TMT-A and TMT-B) before and after exercise bouts or control. Cortical activation has been measured applying functional Near-Infrared Spectroscopy (fNIRS).
Results: HIE contributed to a significant, shorter execution time in TMT-B test. Moreover, an increased prefrontal activation (DLPFC and MFG) has been observed following acute bout of HIE.
Conclusions: The results suggest that the proposed HIE protocol can effectively enhance executive functions among older adults. This cognitive performance enhancement may be attributed to increased activation in cortical areas crucial for cognitive functioning.

PYS15

Metabolic alterations, manifestation of the oxidative stress and changes in the proteomic profile of fibroblasts derived from patients suffering from MPAN subtype of Neurodegeneration with Brain Iron Accumulation – an intimate triangle explaining their clinical phenotype

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Introduction: Neurodegeneration with Brain Iron Accumulation (NBIA) is a rare disease with diverse clinical symptoms, characterized by the deposition of the iron in the brain. Among several NBIA subtypes, especially mitochondrial membrane protein-associated neurodegeneration (MPAN) caused by mutation in *C19orf12* gene is recently investigated due to the fact, that the molecular mechanism underlying MPAN is still not fully understood. The goal of our research is to characterize metabolic alterations and correlate with the clinical phenotype of MPAN patients.
Materials and methods: Fibroblast of 11 MPAN patients and 4 healthy donors were used. ROS levels were examined with the use of MitoSOX, DHE and CM-H2DCFDA probes. Mitochondrial function was evaluated by the mt□□ and OCR measurements. Correlations between cells growth and functional parameters were determined by PCA.
Results: Fibroblasts derived from MPAN patients revealed impaired mitochondrial functions as well as increased ROS levels. These alterations were much better visible under conditions favoring mitochondrial metabolism. Patients' fibroblasts exhibited slower proliferation without any signs of cell death. Interestingly, altered proliferation positively correlated with an affected mitochondrial respiration and increased mitochondrial and cytosolic superoxide levels. Interestingly, decreased level of H_2O_2 in patients' fibroblasts has been observed in comparison to the healthy controls.
Conclusions: In fibroblasts of MPAN patients we have found alterations in the cellular and mitochondrial metabolism as well as the presence of the oxidative stress. The scale and direction of observed alterations positively correlate with the severity of the disease of individual MPAN patients.

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