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Redox Biology and Medicine
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An Affiliate Journal of the International Society for Free Radical Research

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CONTENTS

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SFRR-E Annual Meeting 2026 "Redox Biology, Environmental Exposure and Lifestyle"

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An Affiliate Journal of the International Society for Free Radical Research (SFRRI)

Aims and Scope

Free Radical Biology and Medicine is an international, interdisciplinary journal that publishes original contributions and reviews on a broad range of topics relating to redox biology, signaling, biological chemistry and medical implications of free radicals, reactive species, oxidants and antioxidants.

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SFRR-E Annual Meeting 2026

"Redox Biology, Environmental Exposure and Lifestyle"

01-06 June 2026, Mainz, Germany

Society for Free Radical Research Europe (SFRR-E)

Structure/Abbreviations:

1. Award lectures (AL)
2. Plenary lectures (PL)
3. Symposium lectures (SL)
4. Early Career Researcher Fellowship presentations (ECR)
5. Young Investigator Award presentations (YIA)
6. Sunrise Seminar lectures (SSL)
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1. Award Lectures

AL_01 | SFRR-E Annual Award Lecture
Neutrophils as drivers of cellular damage and inflammation in disease

Clare L. Hawkins
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Neutrophils play a critical role in innate immune defences and are rapidly recruited to inflammatory sites to clear infection. Myeloperoxidase (MPO) is an important antimicrobial protein, which is highly abundant in neutrophils. By catalysing the formation of hypochlorous acid (HOCl) and other oxidants, MPO rapidly kills phagocytosed pathogens. MPO is also critical to extracellular defensive mechanisms, via its release by degranulation and neutrophil extracellular traps (NETs). NETs contain MPO and other antimicrobial proteins, and are abundant in histones, which together kill pathogens. However, MPO and NETs can also induce damage to host tissue, which accelerates the development of disease. The mechanisms involved in these damaging reactions are complex and not completely understood. This presentation will focus on the mechanisms and consequences of the modification of proteins by MPO, particularly histones, which are involved in NET-induced tissue damage. Proteomic analysis of NETs revealed the presence of different post-translational modifications on histones. In addition to acetylation and citrullination, which are well characterised histone modifications on NETs, there was evidence for Tyr and Trp chlorination, Lys nitrile and aminoadipic semialdehyde formation, and Met oxidation. These oxidative modifications were particularly abundant in NETs from neutrophils stimulated with phorbol myristate acetate and were comparable to the pattern of modification observed on exposure of isolated histones to HOCl. HOCl can modulate histone reactivity with various cells including vascular smooth muscle cells, model macrophages and b-cells. In general, modification by HOCl decreases the extent of histone-induced cell death, but this can depend on the type of cell and environment, whereas there is a stimulatory or no effect on inflammatory and stress signaling. These findings provide new insight into cellular mechanisms involved in neutrophil related host cell damage, which could be valuable in the design of therapeutic strategies to combat inflammatory pathologies.

https://doi.org/10.1016/j.freeradbiomed.2026.05.018

AL_02 | SFRR-E Clinical Science Award Lecture
From nitric oxide biology to precision vascular medicine

Christian Heiss [1,2]
[1] *School of Medicine, University of Surrey, Guildford, U.K;* [2] *Vascular Department, Surrey and Sussex Healthcare NHS Trust, Redhill, U.K.*

This lecture will reflect on the importance of making vascular biology clinically relevant. Nitric oxide biology and endothelial function have provided fundamental insights into vascular homeostasis, injury and repair, and have deepened understanding of how ageing, smoking, diet, diabetes, physical inactivity and environmental exposures affect the vasculature. The challenge now is to translate this knowledge into clinical practice and use it to deliver precision vascular medicine. A clinically grounded view of vascular biology identifies the endothelium as the interface through which multiple exposures and risk factors converge. This perspective has informed work in vascular ageing, nutrition, peripheral artery disease and multimorbidity. The key question is no longer only what these mechanisms mean biologically, but how they can improve diagnosis, phenotyping, prevention and treatment in ways that are useful in daily care and allow more precise matching of intervention to individual patient need. Clinical academics are central to this effort, linking mechanistic insight to practical tools, service innovation, evidence generation and guideline development. The next opportunity for vascular biology lies in translation and implementation so that what has been learned from nitric oxide and endothelial biology leads to more personalised decisions, better pathways and better care for patients.

https://doi.org/10.1016/j.freeradbiomed.2026.05.019

AL_03 | SFRR-E Basic Science Award Lecture
Selenoproteins and reductive enzyme pathways in control of cell fate

Elias S.J. Arnér [1,2]
[1] *Division of Biochemistry, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden;* [2] *Department of Selenoprotein Research, National Institute of Oncology, Budapest, Hungary*

Selenoproteins are typically reductive enzymes utilizing selenocysteine (Sec, U) as the catalytic residue. Their intricate translation machineries necessitate genetic engineering for recombinant selenoprotein production at higher yields, which we employ using E. coli as expression host. Using this methodology, we recently discovered surprising examples of normally Cys-dependent enzymes becoming more efficient if expressed as Cys-to-Sec substituted artificial selenoproteins, which adds to the interesting questions regarding the evolutionary selection mechanisms for or against selenoproteins. In a cellular context, a wide range of intracellular signaling pathways are controlled by important selenoproteins, including thioredoxin reductases (TXNRD) and glutathione peroxidases (GPX). The development and use of inhibitors of these enzymes will thus trigger increased oxidative stress as well as perturbed signaling, the outcome of which will always be context dependent. Intriguingly, we recently found that signaling through tyrosine phosphorylation cascades, such as upon stimulation of the receptor for Epidermal Growth Factor (EGFR) is tightly controlled of the cytosolic thioredoxin system, dependent upon TXNRD1, as well as by glutaredoxins dependent upon glutathione (GSH), seemingly acting in parallel in control of protein tyrosine phosphatases such as PTP1B. These examples of how selenoproteins and reductive enzyme pathways control intracellular signaling,

ultimately determining cell fate, will be discussed.

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AL_04 | SFRR-E Leopold Flohé Award Lecture
Identification and targeting of mechanisms regulating membrane redox homeostasis

José P. Friedmann Angeli
Rudolf Virchow Center for Integrative and Translational Bioimaging, Julius-Maximilians University of Würzburg, Würzburg, Germany

Ferroptosis is an iron-dependent form of regulated necrotic cell death driven by phospholipid peroxidation. Although glutathione peroxidase 4 (GPX4) has long been considered the central defense against ferroptosis, recent work identified ferroptosis suppressor protein 1 (FSP1; formerly AIFM2) as a parallel, stand-alone protective system. FSP1 suppresses ferroptosis independently of GPX4 by using NAD(P)H to regenerate ubiquinone (CoQ10) to ubiquinol, which traps lipid peroxyl radicals and prevents membrane oxidative damage. This FSP1–CoQ10–NAD(P)H pathway has emerged as a key determinant of ferroptosis resistance in cancer cells and a promising therapeutic target. Building on this framework, we investigated mechanisms regulating FSP1 function and identified riboflavin (vitamin B2) metabolism as an important modulator of ferroptosis sensitivity. Riboflavin supports FSP1 stability and promotes the recycling of lipid-soluble antioxidants, thereby limiting phospholipid peroxidation. Conversely, pharmacological disruption of riboflavin metabolism, including treatment with the riboflavin antimetabolite roseoflavin, compromises FSP1 activity and sensitizes cancer cells to ferroptosis. These findings reveal a functional link between nutrient metabolism and ferroptosis defense and highlight the riboflavin–FSP1 antioxidant axis as a tractable vulnerability in cancer. In addition, we will present our initial efforts toward developing inhibitors targeting vitamin B2 metabolism to enhance ferroptotic cell death and which could be explored for ferroptosis-based cancer therapies.

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AL_05 | SFRR-E Catherine Pasquier Award Lecture
The unexpected role of sodium in mitochondrial redox biology

Pablo Hernansanz-Agustín
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In the last few years, mitochondrial Na^+ has emerged as an important player for cellular adaptation and bioenergetics. Previously, the role of Na^+ was confined to the co-maintenance of plasma membrane potential. Now, it has expanded, particularly in the mitochondria, after its discovery as a second messenger. During acute hypoxia, Na^+ enters in the mitochondrial matrix, interacts with phospholipids, regulating the inner mitochondrial membrane fluidity and reactive oxygen species (ROS) production by the mitochondrial electron transport chain. In addition, we have recently shown that, in normal conditions, Na^+ also have deep implications in bioenergetics. It forms a gradient across the inner mitochondrial membrane which accounts for up to half of the $\Delta\Psi mt$. This gradient is built up by the activity of the mitochondrial Na^+-specific Na^+/H^+ exchanger (NHE), which partially dissipates the H^+ gradient (ΔpH) to generate the Na^+ gradient (ΔNa^+). Interestingly, the molecular identity of this exchanger is complex I (CI). These roles of mitochondrial Na^+ have been recently found critical in cardiovascular disease outcome, cancer progression, neurodegenerative disease onset and altered immune response, pointing to the conserved nature of Na^+-dependent pathways. Now, our research focuses on the control of the Na^+-dependent for which we are finding ways to manipulate them from the atomic to the supramolecular level.

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2. Plenary Lectures

PL_01
Toxic exposures to transformative exposomics: towards holistic frameworks to understand cardiovascular health

Sanjay Rajagopalan
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Ambient environmental exposures are among the most consequential drivers of global cardiovascular morbidity and mortality, with a large share mediated through atherosclerotic cardiovascular disease (ASCVD). Mechanistic studies in humans and experimental models in air pollution as a prototypical and pervasive environmental exposure, have identified key pathways linking exposure to disease, including oxidative stress, systemic inflammation, autonomic imbalance, and translocation of particulate constituents. These upstream signals converge on downstream effectors such as endothelial dysfunction, thrombosis, vasoconstriction, and plaque instability, providing a biologically coherent framework for exposure-mediated atherogenesis. Despite these advances, conventional approaches have largely examined single exposures in isolation, limiting insight into the cumulative and interactive effects of the broader environmental milieu. This talk will highlight emerging paradigms in exposomics that aim to characterize the totality of environmental exposures across the life course and their integrated impact on cardiovascular health. Leveraging geospatial artificial intelligence, we present approaches to quantify the external exposome at high spatial and temporal resolution, linking air pollution, built environment features, and climate-related stressors to cardiovascular outcomes. Complementary causal inference frameworks are applied to disentangle complex exposure–response relationships and identify mechanistic pathways relevant to ASCVD. Together, these approaches signal a shift from reductionist models toward a systems-level understanding of cardiovascular risk. By integrating mechanistic biology with high-dimensional data science, exposomics provides a path toward more precise risk stratification and scalable prevention strategies that address both individual and population-level determinants of cardiovascular disease.

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PL_02
Traffic noise: the not-so-silent killer—How oxidative stress and inflammation drive cardiovascular disease

Thomas Münzel
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Traffic noise is a pervasive environmental exposure affecting hundreds of millions worldwide and is now recognized as a major contributor to cardiovascular, metabolic, and mental health disease burden. Transportation noise is dose-dependently associated with hypertension, coronary artery disease, stroke, heart failure, and diabetes, yet remains absent from current prevention guidelines. This keynote will place noise within the broader urban exposome, emphasizing its interaction with air pollution, heat, and socioeconomic stressors that collectively amplify cardiometabolic risk. A central mechanistic theme is the activation of brain–heart pathways, linking amygdala-driven stress responses to vascular inflammation and future cardiovascular events. The second part of the lecture will focus on redox biology: noise-induced oxidative stress, NADPH oxidase (NOX2) activation, endothelial dysfunction, eNOS uncoupling and thus nitric oxide depletion as unifying mechanisms across exposures. These insights support a paradigm shift, from viewing noise as a nuisance to recognizing it as a target for cardiovascular prevention, urban policy, and global health action.

https://doi.org/10.1016/j.freeradbiomed.2026.05.024

PL_03
What cysteine and methionine in mitochondrial proteins reveal about the rate-limiting redox step of the biological aging process

Bernd Moosmann
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The biological aging process has been widely suspected to be accelerated by free radical chain reactions. However, numerous attempts to modify the natural

course of aging in animals through the transgenic manipulation of antioxidant enzyme levels (affecting radical initiation) or the nutritional manipulation of antioxidant compound levels (affecting radical termination) have essentially failed. Comparative analysis of animal species with different longevities have recapitulated the absence of systematic changes in endogenous low-molecular weight antioxidants and antioxidant enzyme expression. However, they have yielded strong signals pointing to radical propagation as an optimized (i.e., suppressed) feature in long-lived species. Across phylogenetic groups, long-lived animals avoid protein cysteine and highly unsaturated fatty acids in the inner mitochondrial membrane, representing the catalyst and the substrate of radical propagation, respectively. Sharpening the distinction, the non-radical oxidant scavenger methionine is widely accumulated in mitochondrial membrane proteins, but this effect does not correlate with lifespan, but rather with aerobic metabolic rate. The derived hypothesis that radical propagation was central to the biological aging process was tested in vivo. Feeding of Drosophila melanogaster with simple hydrophobic thiols evoked a premature aging phenotype that was effectively indistinguishable from natural aging at the phenotypic, ultrastructural and transcriptomic level. Biochemical analysis of the animals indicated that chain-transfer catalysis through intramembrane thiyl radical formation caused the accelerated aging phenotype. It is concluded that the propagation step of lipid/membrane peroxidation is the rate-limiting redox step of aging.

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PL_04
Transcription factor NRF2: from redox control to disease intervention

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The transcription factor NRF2 has evolved from a redox-responsive regulator into a central hub of cellular homeostasis, orchestrating antioxidant, metabolic, proteostatic, and anti-inflammatory responses through the coordinated regulation of a broad cytoprotective gene network. Over the past decades, a systems medicine perspective has positioned NRF2 at the core of a network of mechanistically linked chronic conditions, including metabolic, neurodegenerative, cardiovascular, and autoimmune diseases. These diseases share processes such as oxidative stress, inflammation, and metabolic imbalance. Mechanistically, NRF2 activity is tightly controlled by KEAP1-dependent proteasomal degradation, together with complementary pathways such as GSK-3/β-TrCP, allowing integration of redox and signaling cues. This enables NRF2 to respond dynamically to environmental and endogenous stress while maintaining cellular resilience. Pharmacological targeting of the NRF2 pathway has shown therapeutic potential, as illustrated by clinically approved activators and an increasing number of modulators with different mechanisms of action. However, important challenges remain, including context-dependent effects, limited specificity, and the dual role of NRF2 in diseases such as cancer, where its hyperactivation may contribute to therapy resistance. Recent advances highlight the need to refine biomarker strategies, improve pharmacodynamic assessment, and develop network-based approaches to patient stratification. NRF2 also reflects a broader move away from single-target pharmacology toward multi-target cytoprotective strategies. In this lecture, we will discuss the evolution of NRF2 biology, its role in some disease networks, and the opportunities and limitations of its therapeutic modulation in the context of precision medicine for noncommunicable diseases.

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3. Symposium Lectures

Symposium I – Redox Changes and Oxidative Stress by Air Pollution: Contributions to the Redox Exposome

SL I_01
Air pollution exposure impairs alveolar epithelium repair through oxi-inflammatory pathways

Sofía Reynoso [1], Mariana Garcés [1], Lourdes Cáceres [1], Agustina Freire [1], Laura Caltana [2], Manuela Martinefski [3], Mayra El Zahr [1], Florencia Sarno [1], Timoteo Marchini [1], Valeria Tripodi [3], Pablo Evelson [1], Natalia Magnani [1,*]
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* Presenting author

Airborne particulate matter (PM) is a major environmental health threat associated with increased respiratory morbidity and mortality. Inhalation of urban air (UA) promotes oxi-inflammatory responses driven by the interplay between redox mechanisms and inflammation, potentially disrupting tissue repair processes. Here, we investigated whether oxi-inflammatory pathways initiated by chronic UA exposure impair alveolar epithelial repair following acute lung injury. BALB/c mice were exposed to filtered air (FA) or UA from Buenos Aires in exposure chambers for up to 8 weeks. UA exposure induced a pro-inflammatory and pro-oxidant environment, evidenced by increased IL-6 and decreased IL-10 levels in bronchoalveolar lavage (BAL), along with enhanced macrophage-derived nitric oxide and neutrophil superoxide production. To evaluate repair mechanisms, acute lung injury was induced by intratracheal instillation of hydrochloric acid after 8 weeks of exposure. Five days post-injury, UA-exposed mice exhibited redox homeostasis alterations, including increased mitochondrial reactive oxygen species production, NADPH oxidase activity, and mitochondrial dysfunction, as indicated by membrane depolarization and reduced oxygen consumption. Redox-sensitive signaling pathways were markedly altered. Although Nrf2 nuclear translocation was not observed at the evaluated time, the antioxidant system was impaired. In contrast, NF-κB activation was enhanced, as shown by increased nuclear translocation of the p65 subunit, leading to elevated TNF-α and IL-6 levels. These changes were associated with increased oxidative damage and BAL cell count and protein content, indicating barrier disruption. Histological and ultrastructural analyses revealed thickening of the alveolar wall and disruption of the alveolo-capillary barrier. Overall, UA exposure shifts lung redox status toward an oxidizing state and sustains inflammatory signaling, impairing resolution of injury and delaying alveolar epithelial repair. These findings highlight oxi-inflammatory pathways as key mediators of pollution-induced lung damage and potential targets for therapeutic intervention.

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SL I_02
Effects of particulate matter and noise on multiple organ systems – potential pharmacological interventions

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Exposure to particulate matter and environmental noise harms several organ systems at the same time, especially the lungs, heart, blood vessels and brain. These exposures trigger oxidative stress, inflammation and strong activation of the stress-hormone axis, which together drive high blood pressure, endothelial dysfunction and structural tissue injury. In experimental models using realistic urban particles and aircraft noise, combined exposure causes more severe damage than each stressor alone, supporting a synergistic effect on vascular and cerebral function. Very small (nano-sized) particles can cross the lung barrier, enter the bloodstream and reach distant vascular beds, where they promote vascular inflammation and microvascular dysfunction. These mechanistic insights open the door for targeted pharmacological interventions. Inhibition of NOX-2–dependent oxidative stress reduces vascular damage, lowers inflammatory signaling and improves endothelial function in exposed animals. In addition, cardiovascular drugs such as beta-blockers and statins can blunt noise-induced sympathetic activation, reduce NOX-2 expression and partially normalize antioxidant defenses in the heart, while $GABA_A$ receptor modulators like diazepam and selective serotonin reuptake inhibitors like citalopram, attenuate noise-induced elevations in blood pressure, heart rate, oxidative stress and inflammation in the heart and brain. Modulation of FOXO3-dependent stress responses and interference with Toll-like receptor and myeloid cell activation further limit organ injury in the heart, vasculature and brain. Overall, the available data suggest that environmental control measures to lower particulate matter and noise levels can be complemented by organ-protective pharmacological strategies targeting redox balance, neurohumoral activation and innate immune signaling in highly exposed or high-risk populations.

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SL I_03

Redox and inflammatory mechanisms linking PM2.5 exposure to impaired metabolism and thermogenesis

Timoteo Marchini
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Exposure to air pollution fine particulate matter ($PM_{2.5}$) accelerates the development of cardiovascular risk factors, including obesity. Alveolar macrophage $PM_{2.5}$ uptake orchestrates oxidative stress and inflammatory responses in the lungs and secondary target organs, such as adipose tissue, thereby promoting adiposity, impaired glucose homeostasis, and insulin resistance through mechanisms that remain incompletely understood. In this context, our research focuses on elucidating the crosstalk between macrophages and adipocytes following $PM_{2.5}$ exposure. In a real-life exposure model, C57BL/6 mice breathing polluted urban air (27 ± 8 μg/m^3 $PM_{2.5}$) for 16 weeks exhibited increased weight gain, impaired glucose homeostasis, and white adipose tissue (WAT) inflammation, together with altered metabolic and thermogenic gene expression in brown adipose tissue (BAT). In an acute exposure model, mice receiving $PM_{2.5}$ (1 mg/kg body weight) via intranasal instillation displayed a biphasic inflammatory response in the lungs, with neutrophils peaking at 6 hours and macrophages at 72 hours post-exposure, accompanied by increased pro-inflammatory gene expression and cytokine production. Bulk mRNA sequencing of sorted alveolar macrophages revealed a pro-inflammatory transcriptional signature and dysregulation of pathways related to superoxide anion metabolism. Metabolic cage analyses demonstrated significantly reduced heat production in $PM_{2.5}$-exposed mice, despite increased physical activity. To investigate macrophage-adipocyte interactions, we designed a Transwell co-culture system in which primary brown adipocytes were cultured in the lower compartment and bone marrow-derived macrophages (BMDMs) in the upper inserts, separated by a 0.4 μm pore membrane. Co-culture with $PM_{2.5}$-exposed BMDMs (100 μg/mL for 24 h) resulted in a significant reduction in metabolic and thermogenic gene expression in brown adipocytes, along with decreased glucose uptake, without affecting cell viability. Furthermore, Seahorse analysis revealed reduced UCP1-dependent respiration in brown adipocytes. Together, these findings provide novel insight into how redox and innate immune responses triggered by $PM_{2.5}$ exposure contribute to metabolic dysfunction and the development of cardiovascular risk factors.

https://doi.org/10.1016/j.freeradbiomed.2026.05.029

Symposium II – Exercise, Inflammation, and Redox Biology: Turning Stress into Adaptation

SL II_01

Role of redox signaling in skeletal muscle damage and adaptation to training in young and old populations

Maria Carmen Gómez Cabrera
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Redox signaling plays a central role in the regulation of skeletal muscle function, mediating both exercise-induced damage and the adaptive responses that follow. Reactive oxygen and nitrogen species (RONS), traditionally viewed as harmful byproducts of metabolism, are now recognized as essential signaling molecules that regulate muscle remodeling, mitochondrial biogenesis, and antioxidant defense systems. This dual role is particularly relevant in the context of aging, where redox homeostasis is altered and the balance between damage and adaptation becomes disrupted.

In young individuals, acute exercise induces a transient increase in RONS production that contributes to muscle damage but simultaneously activates signaling pathways involved in repair and adaptation, including upregulation of endogenous antioxidant systems and mitochondrial function. This hormetic response is crucial for improving muscle performance and resilience. In contrast, aging is associated with increased basal oxidative stress, impaired redox signaling, and reduced adaptive capacity. Older skeletal muscle often exhibits blunted responses to exercise-induced RONS, leading to diminished mitochondrial biogenesis, defective protein turnover, and impaired recovery.

Emerging evidence suggests that the qualitative aspects of redox signaling, such as localization, timing, and intensity, are critical determinants of whether RONS exert beneficial or detrimental effects. Furthermore, interventions such as exercise training can partially restore redox balance and improve adaptive responses in older populations, although these effects may be influenced by factors such as training modality, intensity, and nutritional status.

This presentation examines the role of redox signaling in skeletal muscle damage and adaptation across the lifespan, highlighting key molecular mechanisms and age-related differences. Understanding these processes is essential for designing targeted interventions to optimize muscle function and promote healthy aging.

https://doi.org/10.1016/j.freeradbiomed.2026.05.030

SL II_02

Unraveling molecular mechanisms of ROS and mitochondrial dysfunction in musculoskeletal impairments

Estela Santos Alves [1], Alexander van Deventer [1], Baptiste Jude [1], Emily Shorter [1,2], Jonathon Smith [3], Maarten M. Steinz [1], Thomas Gustafsson [4], Eric Rullman [4], Ferdinand von Walden [5], Camilla I. Svensson [1], Juleen R. Zierath [3], Jorge L. Ruas [1,6], Johanna T. Lanner [1,2,*]
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* Presenting author

Skeletal muscle weakness and fatigue are common features of many chronic diseases, including rheumatoid arthritis (RA), cardiovascular disease, and cancer. Yet their molecular drivers remain incompletely understood but are increasingly associated with alterations in mitochondrial function and redox homeostasis. While reactive oxygen species (ROS) are essential for physiological signaling, chronic inflammation can disrupt this balance and contribute to contractile and metabolic dysfunction. We have previously shown that RA patients, despite stable anti-rheumatic treatment and low disease activity, exhibit persistent muscle weakness, partly explained by oxidative modifications of the contractile machinery. Extending these observations, transcriptomic analyses of muscle biopsies from the same cohort revealed reduced expression of mitochondrial-associated genes, consistent with impaired oxidative capacity. These findings were recapitulated in a murine model of inflammatory arthritis (complete Freund's adjuvant, CFA), where mitochondrial content and size were reduced by ~40%, alongside a ~20% reduction in oxidative capacity. Importantly, similar mitochondrial impairments were observed in mice resistant to pain-induced reductions in physical activity, indicating that these alterations are driven by inflammation rather than disuse. At the molecular level, inflammatory conditions were associated with suppression of key regulators of mitochondrial function, including PGC-1α1, alongside broader perturbations in redox-sensitive signaling pathways, for example affecting transcriptional programs linked to mitochondrial biogenesis and oxidative metabolism. Collectively, our data support a model in which inflammation-driven disturbances in redox-sensitive signaling pathways contribute to mitochondrial dysfunction and impaired muscle function. Targeting the shift from adaptive to maladaptive redox signaling may represent a key strategy to preserve or restore muscle function in disease.

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SL II_03

Hydrogen peroxide as a stimulant of adaptations to physical activity in skeletal muscle and the potential facilitatory role of peroxiredoxins

Malcolm J. Jackson *, Robert Heaton, Samrajni Banerjee, Samantha A. Jones, Jonathan Temple, Kay Hemmal, Anne McArdle
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* Presenting author

Reactive oxygen species are generated by skeletal muscle during contractile activity and act to stimulate cellular adaptations to contractile activity including

mitochondrial biogenesis, stress responses, and metabolic regulation. Superoxide can potentially be generated through several pathways in contracting skeletal muscle, but most data now indicate that activation of NADPH oxidase 2 (NOX2) plays a key role. NOX2-generated superoxide is rapidly converted to H_2O_2 which acts to activate a series of adaptive signalling pathways. but a major challenge in elucidating detailed redox-regulated pathways in muscle has been the disparity between physiological intracellular H_2O_2 levels and the higher concentrations typically used in *in vitro* studies to activate redox-sensitive signalling pathways. This has led to development of the concept of redox relays, in which thiol peroxidases, particularly 2-Cys peroxiredoxins (Prdxs), act as intermediates by reacting with H_2O_2 and transferring oxidizing equivalents to downstream proteins. Our findings demonstrate that low levels of H_2O_2, or electrically-stimulated contractions rapidly oxidize Prdx1, Prdx2, and Prdx3 in mouse muscle fibres, supporting this hypothesis and subsequent transcriptomic analysis of human skeletal muscle myotubes in which Prdx2 was knocked down indicated that Prdx2 was essential for upregulating mitochondrial genes in response to H_2O_2 or contractions. These data are in accord with an intermediary role for Prdx is some redox-regulated pathways leading to adaptations to contractile activity in skeletal muscle, but the specific signalling pathways involved remain unclear.

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Symposium III – Lipid Peroxidation and Ferroptosis in Health and Disease

SL III_01
Lipid peroxidation as a marker of ferroptotic cell death: analytical and biochemical perspectives

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Maintaining cellular and organismal homeostasis requires robust quality control systems that monitor biomolecules such as DNA, proteins, and lipids. While DNA and protein quality control mechanisms are well characterized, lipid quality control remains comparatively poorly understood and represents an emerging area of research. Regulation of lipid homeostasis is essential for maintaining cellular physiology and enabling adaptation to environmental changes. Among the diverse stressors cells encounter, redox stress plays a particularly important role in shaping lipid metabolism. Lipids are highly susceptible to reactive oxygen species (ROS) due to the abundance of polyunsaturated fatty acyl chains, which are prone to oxidation. This process can compromise membrane integrity and ultimately trigger ferroptotic cell death. Despite its importance, the mechanisms underlying lipidome remodelling under pro-oxidative conditions remain insufficiently explored. Here, we present a comprehensive analytical framework that integrates tailored bioinformatic workflows with advanced analytical tools to enable sensitive detection and structural annotation of lipid peroxidation products in complex biological samples. Applying this approach across multiple ferroptosis models, including cultured cell lines, animal models, and human samples, we identified a distinct lipid peroxidation signature that reliably reports on ferroptosis induction and propagation. These findings provide new insight into lipidome remodelling during oxidative stress and establish a platform for systematic investigation of lipid quality control mechanisms in health and disease.

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SL III_02
Spatiotemporal monitoring of subcellular lipid peroxidation during ferroptosis enables the targeting of hotspots for intervention

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Lipid peroxidation (LPO), the radical chain reaction of lipids and molecular oxygen, drives ferroptosis. Although plasma membrane permeabilization is clearly the terminal event in ferroptosis, the steps leading to it remain nebulous. In particular, it remains unclear whether LPO at specific organelles play functional roles in ferroptosis execution – a knowledge gap that hinders the development of potent small molecule modulators of ferroptosis as therapeutics which could otherwise be directed to them. To help address this we have targeted STY-BODIPY, the oxidizable fluorophore used in the FENIX assay, to mitochondria, lysosomes, endoplasmic reticulum and the Golgi apparatus to monitor organelle-specific LPO in real time. Herein we report the synthesis of these probes, their reactivity to phospholipid-derived radicals in liposomal models of lipid bilayers and their co-localization with established commercial organelle markers in live cells. We demonstrate their application in multiplexed assays utilizing cell death markers for simultaneous monitoring of LPO and ferroptosis. Using high-throughput live cell imaging and ferroptosis inhibitors that modulate LPO in specific compartments, we show that lysosomes are key functional nodes wherein LPO occurs upstream of ferroptosis induced by GPX4 inactivation and can drive the process even when LPO is suppressed in the ER. Mitochondrial LPO, on the other hand, is dispensable for ferroptosis and occurs continuously independently thereof. Interestingly, the trends in probe oxidation can vary with ferroptosis inducer (i.e. GPX4 inactivation, GSH depletion, etc.), supporting a model with multiple mechanisms of ferroptosis sensitization and/or induction.

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PP I_D18
Lysosomal membrane targeting unlocks exceptional ferroptosis suppression

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Ferroptosis, a cell death modality driven by iron dependent lipid peroxidation, has been implicated in numerous pathologies, including neurodegeneration and ischemia reperfusion injury. Despite decades of research on the inhibition of lipid peroxidation by small molecules, no clinically validated therapies that selectively target ferroptosis are available. Phenoxazines are potent inhibitors of lipid peroxidation and ferroptosis. Here we show that directing phenoxazines to lysosomal membranes markedly enhances their anti-ferroptotic activity. A similar increase in the potency of phenolic and non-canonical inhibitors of lipid peroxidation suggests that lysosome targeting represents a general strategy for augmenting ferroptosis suppression. Molecular dynamics simulations indicate that lysosome targeted phenoxazines accumulate near the membrane water interface, in proximity to the unique reactive sites of the polyunsaturated lipids implicated in ferroptosis. A representative lysosome targeted phenoxazine afforded unprecedented protection from acute kidney failure and death resulting from inactivation of ferroptosis suppressor GPX4. Replacement of the lysosome targeting basic amine with an isosteric neutral moiety resulted in reduced efficacy, comparable to previously reported inhibitors. These findings identify subcellular localization and proximity to the membrane interface as determinants of ferroptosis suppression and support a functional role for lysosomes in ferroptotic cell death, while providing a bioavailable, brain-penetrant scaffold for probing ferroptosis in disease.

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Symposium IV – Lipid and Epilipid Signatures of Metabolic and Environmental Stress

SL IV_01
Lipidomic approaches to study the exposome in health and disease

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The exposome, encompassing the totality of environmental exposures across the

lifespan, is a major determinant of human health, yet remains challenging to quantify. Lipidomics, as a central component of metabolomics, provides a powerful framework to capture the biological imprint of environmental exposures by profiling endogenous metabolic responses. As an intermediate phenotype, the metabolome - and particularly the lipidome - integrates signals from chemical exposures, diet, microbiome, and host genetics, enabling mechanistic insights into exposure–disease relationships. In this presentation, I will discuss recent advances in lipidomic and multi-omics approaches to characterize the chemical exposome and its impact on disease. Our work demonstrates that environmental contaminants, including endocrine-disrupting chemicals such as PFAS, are associated with profound alterations in lipid and bile acid metabolism. In metabolic liver disease (MASLD), these exposures are linked to disrupted hepatic lipid pathways and bile acid homeostasis, with evidence for sex-specific effects. In type 1 diabetes, prenatal exposure to PFAS is associated with altered neonatal phospholipid profiles and increased risk of islet autoimmunity, suggesting early-life metabolic programming. Together, these findings highlight lipidomics as a key tool for decoding exposome - host interactions and for identifying mechanistic pathways linking environmental exposures to chronic disease risk.

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SL IV_02
Stored for the storm: phospholipids release anti-inflammatory nitro-lipids in endotoxemia

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Current paradigms in inflammatory lipid signaling primarily focus on enzymatically and chemically formed oxylipins, often overlooking the broader landscape of redox-derived bioactive lipids. Nitrated fatty acids (NO_2-FAs) represent a physiologically relevant class of anti-inflammatory mediators. However, the endogenous pathways governing their formation, mobilization, and temporal regulation during disease remain incompletely defined. This study investigates the formation of nitro-conjugated linoleic acid (NO_2-CLA) and its role as a transient, endogenous anti-inflammatory reserve that bridges dietary intake, redox chemistry, and immune signaling. We employed murine models and human subjects to quantify NO_2-FA levels during inflammatory stress. Mechanistic studies examined the links among dietary CLA intake, lipid nitration, tissue distribution, storage, and release. We specifically investigated the role of phospholipase A2 (PLA_2) in mobilizing NO_2-CLA from phospholipid reservoirs and assessed its impact on pro-inflammatory pathways and physiological parameters, including cytokine release and hypotension. We identified distinct dietary and membrane-derived pathways that drive the endogenous formation of NO_2-CLA. Our findings establish membrane phospholipids as previously unrecognized reservoirs for electrophilic NO_2-CLA, which are rapidly mobilized via PLA_2 activation during acute inflammatory events. Once released, NO_2-CLA acts as a potent signaling molecule that suppresses inflammatory cascades and competes with canonical pro-inflammatory mediators. Notably, this protective lipid pool is rapidly depleted during inflammatory stress in both murine and human models, revealing critical temporal aspects to its bioactivity. Early mobilization of NO_2-CLA was shown to function as a "brake" on pro-inflammatory signaling, significantly reducing systemic inflammatory responses. These findings redefine the generation and function of NO_2-FAs in vivo. By identifying a transient endogenous anti-inflammatory reserve that is depleted during disease, this work highlights a novel therapeutic opportunity: restoring NO_2-CLA levels through dietary supplementation or targeted mobilization to sustain inflammation resolution.

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SL IV_03
Algal lipids and epilipids as markers of environmental stress and beneficial bioactive compounds

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Microalgae represent a natural reservoir of bioactive lipids comprising polar lipids esterified to omega-3 polyunsaturated fatty acids (PUFA), such as glycolipids, phospholipids and betaine lipids. These long-chain fatty acids (FA) are sensitive to oxidation, particularly when microalgae are exposed to environmental variations that are perceived as stressors conditions that induce production of reactive oxygen species or activation of enzymatic oxidation. Oxidation of polar lipids can lead to the generation of oxygenated derivatives that have been described with interesting bioactive properties in animals. Although some works have explored their occurrence in plants, the presence of these modified lipids in microalgae remains poorly described mostly due to complications with their identification and analysis. Concomitantly, oxidation of lipids can lead to formation of oxylipins, which are lipid mediators that play critical roles in several organisms, but is still poorly described in microalgae, despite recent works providing evidence of the presence of bioactive oxylipins in microalgae species. This work explores the diversity of the profile of oxidized polar lipids five different microalgae species (Chlorella vulgaris, Chlorococcum amblystomatis, Scenedesmus obliquus, Nannochloropsis oceanica and Phaeodactylum tricornutum) using reverse-phase liquid chromatography mass spectrometry and of non-enzymatic oxylipins in Chlorella vulgaris grown under autotrophic and heterotrophic conditions. The identification of these modified lipids allows for a better understanding of the metabolic pathways governing the formation of these lipids as well as the valorization of microalgae biomasses as sources of bioactive oxidized lipids.

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Symposium V – Switches in the Balance: Redox Regulation of Kinase–Phosphatase Signalling

SL V_01
Cysteine redox switches in phosphotyrosine signalling

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Protein tyrosine phosphatases (PTPs) are cysteine-based enzymes and a classic paradigm for reversible redox control of signalling. Yet focusing solely on the catalytic cysteine underestimates the complexity of this regulation: PTPs contain multiple additional redox-sensitive cysteines whose functions remain largely unexplored. At the same time, protein tyrosine kinases are also redox-regulated, with oxidative cues capable of either enhancing or restraining kinase activity. This creates a dynamic and reciprocal layer of control over phosphotyrosine signalling, further expanded by the fact that core redox enzymes, including peroxiredoxins, are themselves regulated by phosphorylation. My laboratory is interested in how this molecular crosstalk shapes cellular decision-making, particularly in immunity and ageing. In this talk, I will highlight our work on pervanadate, a widely used PTP inhibitor that also exposes the sensitivity of signalling networks to oxidation, and on CD45, the abundant receptor PTP that acts as a key gatekeeper of immune signalling. We are dissecting how non-catalytic cysteines in CD45 tune its redox responsiveness and thereby influence T cell signalling output. Understanding these redox switches may reveal new ways to modulate T cell activation, including in the tumour microenvironment.

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SL V_02
Understanding oxidation-dependent regulation of protein kinases

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Protein kinases regulate virtually every aspect of cellular life through coordinated phosphorylation of protein substrates. Consequently, their activities are tightly and dynamically controlled through post-translational modifications, protein-protein interactions and subcellular localisation to ensure precise spatiotemporal organisation of signalling cascades. Here, we identify an additional regulatory layer controlling Aurora A kinase (AURA), a master regulator of mitosis, involving reversible oxidation of a conserved cysteine within the activation loop. Subsequently we demonstrated that this site represents an

evolutionarily conserved regulatory hotspot, with ~10% of the human kinome (including key members of the CAMK, AGC, and AGC-like families of kinases) possessing an equivalent cysteine, many of which we validate as being potently regulated by oxidation. Moreover, a growing number of protein kinases are regulated through a mechanistically diverse array of oxidation-dependent processes involving distinct or supplementary cysteine residues, underscoring the broader importance of redox regulation in shaping kinase signalling dynamics. These findings indicate an underappreciated integration of kinase and redox signalling. Here we present our ongoing efforts to dissect how oxidation-dependent molecular switches fine-tune kinase activity and signalling outputs under physiological and pathophysiological conditions, including for the understudied neuronal kinases, BRSK1 and BRSK2.

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SL V_03
Disulfide dependent PKARIα regulation as a novel redox sensing mechanism of vasodilation

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The regulatory RIα subunit of type I protein kinase A (PKARIα) can undergo oxidation to form an interprotein disulfide-linked homodimer, providing a molecular mechanism by which cellular oxidant signals interface with phosphorylation-dependent regulation. Although both PKA and oxidants are established modulators of vasodilation and blood pressure, it remains unclear whether redox sensing via PKARIα oxidation directly contributes to these physiological processes. Here, we identify PKARIα as a bona fide thiol-based redox sensor, the oxidation state of which is dynamic and critically regulates systemic arterial pressure by controlling oxidant-induced vasodilation. Mesenteric, carotid, penetrating cerebrovascular arteries, and aortae isolated from "redox-dead" Cys17Ser PKARIα knock-in mice (engineered to prevent disulfide-dimer formation) exhibited heightened vasoconstrictor sensitivity and markedly impaired vasodilation to oxidants. Importantly, vasodilation elicited by nitric oxide donors or cAMP elevation remained intact, demonstrating specificity for the oxidant-dependent pathway. In vivo, the pro-oxidant vasopressor angiotensin II increased vasoconstriction as expected, yet concomitantly promoted formation of vasodilatory disulfide-PKARIα and blunted the severity of the vascular response in ex vivo preparations. This redox-activation step limited hypertension progression and attenuated pressure-induced cardiac hypertrophy, establishing disulfide-PKARIα as an endogenous vasodilatory brake engaged during oxidant stress. Notably, despite clear vascular dysfunction ex vivo, Cys17Ser knock-in mice were normotensive. This unexpected phenotype was explained by adaptive reduction of sympathetic dominance, evidenced by a lower low-to-high frequency ratio in heart-rate variability analysis and lower norepinephrine levels in renal and cardiac tissues. Together, these findings provide strong mechanistic evidence that PKARIα oxidation couples oxidant signalling to arterial dilation and protects against pathological elevations in blood pressure. Therefore, PKARIα emerges as a redox-regulated kinase with translational potential, representing a promising thiol-based therapeutic target for the treatment or prevention of systemic hypertension.

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Symposium VI – Principles of Compartmentalized Redox Signalling across Kingdoms
SL VI_01
Abstract not submitted.
SL VI_02
Surfing on redox waves: ER peroxiporin AQP11, more than a channel

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* Presenting author

Until a short time ago, cells were considered relatively static entities, with organelles confined within a lipid bilayer that performed their well-defined functions. Nowadays, this view has changed dramatically, and the concept of dynamic homeostasis is arising, leading cells to resemble a patchwork of highly specialized microdomains. Redox signaling is no exception, and these domains have recently attracted considerable attention due to their emerging functional relevance. To enable redox signaling transmission through the movement of H_2O_2 across physically separated compartments, a family of protein channels known as peroxiporins (AQPs) facilitates its transport. The Endoplasmic Reticulum (ER) represents a privileged redox organelle, hosting key redox-active enzymes, including endoplasmic reticulum oxidoreductase 1α (Ero1α) and the peroxiporin AQP11. Using cell lines stably expressing the H_2O_2 -sensitive HyPer probe, we demonstrated that AQP11 conducts a constitutive H_2O_2 flux from the ER to the cytosol, thereby contributing to the maintenance of cellular redox homeostasis. Unexpectedly, we found that silencing the major ER oxidase Ero1α further increased the organelle's basal oxidative level. By adopting different strategies, we identify that the absence of Ero1α triggers the activation of complex III of the electron transport chain (ETC), leading to superoxide production, which is converted to H_2O_2 and delivered to the ER via AQP11. Thus, we postulate that the existence and the spatial organization of these different redox fluxes are fundamental to the establishment and maintenance of redox homeostasis and signaling.

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SL VI_03
Dose-dependent mitochondrial H_2O_2 signaling drives toxicity or stress adaptation and longevity in fission yeast

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Understanding how localized redox signals translate into cellular responses is critical for deciphering aging and cell fitness. Using genetic tools in fission yeast, we mapped the spatiotemporal distribution of hydrogen peroxide (H_2O_2) by coupling the ratiometric sensor HyPer7 with localized D-amino acid oxidase (Dao1) activity. We quantified a 2- to 5-fold H_2O_2 gradient across organelle membranes and confirmed that mitochondrial H_2O_2 effectively reaches the nucleus. We identified a signaling threshold: low-level mitochondrial H_2O_2 fluxes promote activation of antioxidant defenses increasing stress resistance and lifespan, whereas higher mitochondrial H_2O_2 disrupted mitochondrial morphology and respiration. This study establishes a quantitative framework for organelle-specific redox signaling, demonstrating that the site and intensity of H_2O_2 production are the determinants of cellular fate.

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4. Early Career Researcher Fellowship Presentations

ECR_01
Redox regulation of cancer stem cell heterogeneity in breast cancer patient-derived organoids

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Breast cancer is a highly heterogeneous disease composed of multiple tumour cell subpopulations, including cancer stem cells (CSCs), which contribute to tumour progression, therapy resistance, and relapse. Increasing evidence indicates that intracellular redox balance plays an important role in regulating tumour cell plasticity, yet the relationship between redox species production and CSC population dynamics remains poorly understood, particularly at the single-cell level. To investigate redox regulation in heterogeneous tumour systems, we combined breast cancer patient-derived organoids (PDOs) with compartment-specific genetically-encoded redox biosensors. Using cytofluorescence and imaging techniques, we identified distinct cell populations within BC PDOs. The composition and relative abundance of these populations differed between organoids derived from different patients, reflecting inter-tumour variability. Notably, CSC populations exhibited a redox state distinct from bulk tumour cells. To directly examine the role of hydrogen peroxide in tumour cell population dynamics, BC PDO lines were engineered to express the chemogenetic tool and biosensor DAAO-HyPer7, which enables controlled endogenous H_2O_2 production together with real-time monitoring. Single-cell analyses revealed heterogeneous H_2O_2 production among individual cells within organoids. Perturbation of intracellular redox metabolism using compounds that interfere with the production of redox species altered CSC population distribution. Similarly, induction of endogenous H_2O_2 production resulted in shifts in CSC populations, whereas treatment with exogenous H_2O_2 did not produce comparable effects. Together, these findings highlight the importance of intracellular redox dynamics and single-cell heterogeneity in regulating tumour cell states. The combination of PDOs and compartment-specific genetically-encoded redox biosensors provides a versatile platform to study how redox signalling contributes to tumour heterogeneity and may help identify redox-associated vulnerabilities in breast cancer.

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ECR_02
Advancing strategies to combat lipofuscin toxicity

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Lipofuscin is an oxidatively cross-linked aggregate composed of proteins, lipids, and metal ions that accumulates in post-mitotic tissues such as brain, muscle, and heart during ageing and is strongly associated with cell death and age-related diseases. We performed advanced mass spectrometric analysis of human heart lipofuscin to characterize the protein composition of lipofuscin and elucidate the mechanisms of its formation. By selectively removing interfering extracellular and loosely associated matrix using proteinase K digestion, we obtained the first proteomic characterization of the lipofuscin core. We identified 638 unique proteins, which were present in more than 50% of samples with at least two peptides. In contrast to previous data that attribute lipofuscin primarily to lysosomal or mitochondrial origins, our analyses revealed that lipofuscin core particles derive from diverse cellular sources including cytoplasm, mitochondria, nucleus, and lysosomes. Moreover, the most abundant proteins were cytoplasmic proteins. To explore potential strategies for mitigating the consequences of lipofuscin accumulation, we employed a neuronal cell model and systematically tested multiple interventions. Modulation of cellular uptake pathways, activation of the Nrf2-driven antioxidant response, inhibition of cathepsins to limit secondary damage following lysosomal membrane permeabilization, and suppression of pyroptotic cell death via caspase-1 inhibition were tested to reduce lipofuscin-associated cellular damage and cell death. Collectively, these findings redefine the molecular origins of lipofuscin and identify actionable pathways that may be targeted to alleviate lipofuscin-driven cytotoxicity during ageing and disease.

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5. Young Investigator Award Presentations

YIA I_01
Redox-targeted gene therapy for pharmacoresistant epilepsy

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Epilepsy affects more than 65 million individuals globally, and approximately one-third of patients remain pharmacoresistant, underscoring the urgent need for disease-modifying interventions beyond symptomatic seizure control. Accumulating evidence indicates that epileptogenesis is a redox-dependent process in which sustained oxidative stress promotes neuronal dysfunction, circuit reorganization, and neurodegeneration. We previously demonstrated that pharmacological activation of the antioxidant transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) confers neuroprotection and reduces seizure burden in post–status epilepticus models; however, systemic antioxidant approaches may interfere with physiological redox signaling and lack cellular specificity. We therefore hypothesized that selective, long-term activation of endogenous antioxidant programs within vulnerable neuronal populations would provide superior disease modification. Here, we developed a neuron-restricted AAV9 vector expressing Nrf2 under the CaMKIIα promoter, enabling sustained activation of intrinsic antioxidant pathways in excitatory hippocampal neurons. In vitro, CaMKIIα-Nrf2 significantly attenuated activity-dependent intracellular ROS accumulation and stabilized redox homeostasis, while in vivo administration resulted in neuron-specific Nrf2 induction and robust engagement of redox-regulatory networks. Using the kainic acid–induced status epilepticus model, we evaluated three clinically relevant paradigms: prophylactic delivery prior to insult, early intervention during epileptogenesis, and treatment after epilepsy establishment. Preventive and early post-insult expression markedly suppressed epileptogenesis, reduced spontaneous seizure frequency and severity, and preserved hippocampal integrity. Importantly, intervention in animals with established epilepsy resulted in long-term seizure suppression, demonstrating a unique disease-modifying potential. These findings demonstrate that cell-specific reinforcement of endogenous antioxidant capacity represents a mechanistically grounded, disease-modifying redox strategy with strong translational potential for pharmacoresistant epilepsy.

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YIA I_02
Proteomic analysis of mouse cardiomyocytes after hypoxia and hypoxia/reoxygenation

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Ischemia–reperfusion (IR) injury occurs when the blood flow to an organ is interrupted and then restored, and plays a key role in the pathogenesis of major cardiovascular diseases, such as heart attack and stroke. Multiple factors contribute to IR injury, particularly the excessive production of mitochondrial reactive oxygen species, which occurs predominantly during the reperfusion phase. In addition, mitochondrial dysfunction accelerates cardiac damage and promotes heart failure under ischemic conditions. Despite its clinical importance, the precise molecular mechanisms driving IR injury remain incompletely understood. To investigate how IR affects cellular redox responses and mitochondrial function, mouse cardiomyocytes were exposed to hypoxia or to hypoxia followed by reoxygenation. Protein expression changes were then examined using proteomic techniques. Following tryptic digestion, peptides in the samples were analyzed by liquid chromatography coupled to mass spectrometry (LC-MS/MS), and the data were analyzed using four different search engines against the Uniprot Mus musculus database. The results showed that a large number of proteins were differentially expressed in each condition, and functional analysis indicated that several of them were involved in metabolism, mitochondrial function and redox balance. To validate the observed changes in protein expression, selected proteins were quantified using targeted proteomics through Parallel Reaction Monitoring (PRM). The results showed that several subunits of

respiratory complexes I and IV were affected by oxygen concentration. Mitochondrial superoxide production was measured by fluorescence microscopy. Extracellular flux analyzer (Agilent Seahorse) measurements of the oxygen consumption rate and extracellular acidification rate showed adaptive changes in mitochondrial respiration and ATP generation following reoxygenation. Immunofluorescence analysis of the mitochondrial network showed a remodeling characterized by a more developed but unstable network. Our results indicate that hypoxia and hypoxia/reoxygenation alter protein expression, which in turn affects metabolism, mitochondrial function and dynamics, allowing cardiomyocytes to adapt to oxygen availability.

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YIA I_03

Modifications of the bacterial cell envelope by neutrophil-derived oxidants

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Neutrophils, specialized in phagocytosis orchestrate a coordinated attack on engulfed bacteria, including the highly toxic hypochlorous acid (HOCl) and HOCl-derived chloramines like N-chlorotaurine (NCT). In host-pathogen interactions, the bacterial envelope will naturally be the first target of these oxidants. Here, we examined occurrence of N-chloramines in the bacterial cell envelope, their biological activity, and impact on membrane integrity. Using a chemical probe for N-chloramines we demonstrated that HOCl forms N-chloramines with amino residues in some membrane lipids and lipopolysaccharides in E. coli cell envelopes. In contrast, NCT only showed poor chlorinating activity in vitro. N-chlorinated model membranes efficiently oxidized the redox-sensitive protein roGFP2, suggesting that cell envelope N-chloramines might disrupt cellular thiol homeostasis. While relatively stable in vitro, they are significantly less stable in living cells indicating both, active detoxification and reactivity with other cellular components. To study the effects of HOCl and NCT on membrane integrity, we used content leakage assays with model membranes and applied fluorescent probes for membrane permeability and potential measurements in E. coli cells. Bacteria actively take up taurine through ABC transporters (TauABC and SsuABC). To elucidate the role of taurine importers in NCT susceptibility, we monitored the growth of E. coli tauB and ssuB deletion mutants in the presence of NCT. Lack of TauB increased NCT resistance, suggesting that NCT toxicity depends on interaction with both, the bacterial cell envelope and cytosolic components. Overall, we propose that envelope N-chloramines are involved in modulating host immune cells and contribute to bacterial killing.

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YIA I_04

Kinetics of thioredoxin reductase 1 derivatization and associated cancer cell death by the small molecule inhibitor TRi-1

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Cancer cells' reliance on the cytosolic selenoprotein thioredoxin reductase 1 (TXNRD1) for sustained proliferation has led to the discovery and development of inhibitors such as auranofin and TRi-1, with pronounced anticancer effects and varying target specificity [1]. The effects of TRi-1 and auranofin are potentiated by their ability to create prooxidant TXNRD1-derived toxic enzyme forms known as SecTRAPs. However, the signaling programs they trigger are still poorly understood despite recent proteomics investigations [2], so we here present novel data regarding the mechanism of action (MoA) of TRi-1. TRi-1 promotes significant cytotoxicity in cancer cell lines upon short-term exposure (<1h), even if cell killing is not noted until days later. This is partially due to cell cycle arrest induction concomitant with a rapid irreversible decrease in CDK1/2 activity. Formation of covalently modified intracellular TXNRD1, with TRi-1 binding its selenolthiol active site, suggests rapid SecTRAP formation in the first hours following compound exposure. Our findings show that TRi-1, the most selective TXNRD1 inhibitor available, rapidly targets TXNRD1, and suggest a strong link between cell cycle regulation and SecTRAP formation as part of the anticancer effects of TXNRD1 inhibitors. Further MoA studies would significantly improve the development and therapeutic potential of this and other compounds targeting this selenoprotein.

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YIA I_05

***In-vivo* quantification of the Arabidopsis redox proteome under dynamic light conditions and its effect on the photosynthetic efficiency**

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Photosynthesis under natural conditions is highly dynamic, yet transitions from dark to light or low to high irradiance are characterized by a pronounced induction lag before maximal photosynthetic rates are achieved. This delay is thought to cause substantial losses in photosynthetic efficiency in fluctuating light environments. Redox regulation plays a central role in coordinating chloroplastic metabolism during these transitions, enabling rapid metabolic adjustment while preventing excessive accumulation of reactive oxygen species (ROS). However, the identity and dynamics of redox-regulated protein sites involved in photosynthetic induction remain poorly defined. To address this gap, we developed SPEAR, a novel redox proteomics approach that enables simultaneous identification of redox-sensitive cysteine residues and quantitative measurement of their oxidation state *in vivo*. Applying SPEAR to *Arabidopsis thaliana* exposed to dynamic light conditions, we identified 136 cysteine sites that undergo rapid redox changes within minutes of illumination in wild-type plants. These sites are distributed across key metabolic and photosynthetic pathways, highlighting extensive redox remodeling at the onset of photosynthesis. Using Arabidopsis mutants lacking 2-Cys peroxiredoxins (*2cpab*), we further uncovered a critical role for these enzymes in shaping early redox dynamics during photosynthetic induction. In parallel, we are investigating plants deficient in NADPH-dependent thioredoxin reductase C (*ntrc*), a central component of reductive redox signaling. Leveraging the quantitative protein abundance capability of SPEAR, we are currently assessing how loss of NTRC alters the proteome and the associated metabolic cost of disrupting redox regulation. Together, our findings provide a quantitative, site-specific view of redox dynamics during photosynthetic induction. These insights identify potential regulatory nodes that could be targeted—e.g., via genome editing—to reduce induction lag and enhance photosynthetic efficiency, contributing to strategies for crop improvement under fluctuating environmental conditions.

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YIA II_01

Melatonin counteracts Cd- and FFA-induced lipotoxicity in human hepatocytes: molecular insights from lipidomics

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Hepatocyte lipotoxicity is a key factor in the pathogenesis of non-alcoholic fatty liver disease (NAFLD), a leading cause of liver failure and transplantation worldwide. Persistent toxic pollutants, such as cadmium (Cd), have been extensively recognized as environmental risk factors for NAFLD. This study aims to study the effect of MLT in HepaRG cells, exposed to Cd toxicity, either alone or combined with free fatty acids (FFAs). Cd synergizes with FFAs to induce cellular steatosis and, among the FFAs tested, palmitic acid induced a more severe lipotoxic phenotype than oleic acid, as demonstrated by increased H_2O_2 production, lipid droplets and apoptotic cell death. MLT exerts a significant cytoprotective effect by counteracting these lipotoxicity-related hallmarks. Lipidomic analysis revealed a distinct molecular fingerprint induced by Cd and FFAs, as well as a specific lipid remodelling promoted by MLT. MLT reversed the Cd- and FFA-driven lipid alterations, restoring the lipidome in a specific manner dependent on the individual or combined treatments. Pathway analysis together with fatty acyl chain length and unsaturation profiling highlighted the central role of fatty acid metabolism in the MLT response. Within this context, stearoyl-CoA desaturase emerged as a key player and was subsequently validated by pharmacological inhibition and gene silencing experiments. These findings provide new insights into the cytoprotective mechanisms of MLT in Cd- and FFA-induced hepatocyte lipotoxicity, highlighting its therapeutic potential for treating NAFLD.

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YIA II_02

Electrochemical on-demand generation of oxidant species on carbon electrodes in multiwell cell culture platforms

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Reactive species such as hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl) play key roles in redox regulation, oxidative stress, and inflammation. However, *in vitro* studies are often limited by the inability to maintain defined oxidant levels over time. Conventional bolus addition approaches lead to rapid concentration decay, poor temporal control, and limited reproducibility, restricting precise investigations of dose- and time-dependent cellular responses. Here, we present an electrochemical strategy for the controlled, *in situ* generation of oxidant species directly in cell culture media using carbon-based electrodes integrated into custom multiwell platforms (96-, 48-, and 24-well formats). By modulating the applied electrical input, oxidant production can be dynamically tuned, enabling programmable concentration profiles and sustained exposure over defined time windows. Cathodic operation enables continuous and adjustable H_2O_2 production via oxygen reduction. Anodic polarization in chloride-containing media provides access to reactive chlorine chemistry, enabling the electrochemical formation of HOCl. Importantly, carbon electrodes provide broad material flexibility, including 3D-printable composite filaments that enable rapid redesign of electrode geometries and scalable fabrication. We validate the electrochemical generation of both H_2O_2 and HOCl and assess their effects on cell viability, demonstrating species- and dose-dependent responses. This dual-mode, electrically programmable platform enables reproducible control over oxidant identity, concentration, and exposure dynamics, providing a versatile tool for studying oxidative stress mechanisms and redox-dependent cellular responses *in vitro*.

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YIA II_03

Sex-dependent effects of aging on NAD^+ metabolism and redox homeostasis in visceral adipose tissue

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It is well established that aging induces redox imbalance and oxidative stress in several tissues. However, the sex-dependent effects of aging on redox homeostasis in visceral adipose tissue (VAT) remain unclear. This study aimed to investigate how aging and sex influence redox regulation and senescence in VAT. Male and female mice aged 4–5 months (young) and 24 months (aged) were analyzed. Both sexes exhibited impaired glucose tolerance with aging. Regarding redox homeostasis, only aged males showed decreased catalase, superoxide dismutase (SOD), and glutathione peroxidase (GPx) activities compared with young males. No significant alterations in oxidative biomarkers were detected in either sex. Conversely, aged females presented reduced total and phosphorylated NRF2 levels, resulting in a lower pNRF2/NRF2 ratio, indicating sex-specific regulation of the antioxidant response. Despite these differences, both aged groups exhibited elevated p21 mRNA expression, with no significant variations in γH2AX, H2AX, or their ratio, suggesting comparable senescence signaling between sexes. Given the role of NAD^+ as a substrate for sirtuins that modulate redox balance, VAT NAD^+ levels were also examined. A significant reduction was observed only in aged males compared with young males, while females showed no age-related differences. This decline in aged males corresponded to elevated CD38 activity, whereas aged females displayed no CD38 changes but increased NAMPT expression, suggesting compensatory mechanisms of NAD^+ biosynthesis. To further investigate the link between NAD^+ and redox enzyme alterations in aged males, young and aged CD38 knockout (CD38KO) male mice were analyzed. CD38KO mice exhibited higher catalase, SOD, and GPx activities in both age groups compared with wild-type controls. Overall, aging induces sex-dependent NAD^+ depletion driven by increased CD38 activity, which is associated with reduced antioxidant defense in male mice. Despite sex differences in redox homeostasis, aging similarly increased senescence signaling in both sexes.

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YIA II_04

Stabilizing aminoferrocene-based reactive oxygen species catalysts through dialkylation to improve anticancer efficacy

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N-Monoalkylaminoferrocene (MAAF)-based prodrugs show strong anticancer activity against various cancer cell lines in the low micromolar range, as well as in vivo. They are selectively activated by reactive oxygen species (ROS), particularly H2O2, to release the corresponding MAAFs, a highly toxic hydroxy radical-generating catalyst, that pushes intracellular ROS levels beyond the tolerable limit for cancer cells. However, therapeutic potential of these compounds is limited by the short lifetime of the active MAAF catalyst under physiological conditions. Here, we report a strategy to enhance the stability and efficacy of aminoferrocene-based anticancer agents by transforming MAAFs into their corresponding N,N-dialkylaminoferrocenes (DAAFs). For this, a focused library of MAAFs and DAAFs with varying substituents was synthesized and characterized. Comparative stability studies revealed a pronounced improvement in stability upon dialkylation. Electrochemical analysis and cell-free DCFH-based ROS assays confirmed that DAAFs retain ROS-amplifying activity, while allowing fine-tuning of redox potential through structural modification. Cytotoxicity studies in various cancer and healthy cell lines demonstrated enhanced toxicity and selectivity for selected DAAF derivatives. Notably, one DAAF-based compound showed superior anticancer activity compared to its MAAF analogue, with IC50 values of 14.2 μM vs. 26.1 μM in A2780 ovarian

cancer cells, while exhibiting reduced toxicity toward non-malignant GM01379 lung fibroblasts (IC50 > 50.0 μM vs. 23.8 μM), identifying it as a promising leading structure. Overall, this work establishes dialkylation as an effective design principle to improve stability and selectivity of ROS-amplifying aminoferrocene-based anticancer drugs and provides a basis for further structure-activity relationship and mechanistic studies.

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YIA II_05

miR-199a inhibition ameliorates the amyotrophic lateral sclerosis phenotype in SOD1 mice by preserving neuromuscular junctions

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder characterized by progressive loss of motor neurons, muscle denervation and skeletal muscle atrophy. Increasing evidence indicates that skeletal muscle plays an active role in ALS pathogenesis, contributing to disease onset and progression. In line with this perspective, skeletal muscle has emerged as a promising therapeutic target for interventions aimed at slowing disease progression and preserving neuromuscular function in ALS. Using small RNA sequencing of skeletal muscle from ALS patients, we identified microRNA-199a (miR-199a) as a potential therapeutic target involved in the regulation of neuromuscular homeostasis and mitochondrial dynamics. In the SOD1-G93A mouse model of ALS, miR-199a expression was enriched in proximity to the neuromuscular junction (NMJ) as demonstrated by *in situ* hybridization, further supporting its functional relevance in maintaining neuromuscular homeostasis. Based on these findings, we hypothesized that inhibition of miR-199a could delay ALS progression. To test this hypothesis, antagomiR-199a, inhibitor of miR-199a, was administered intravenously to presymptomatic (postnatal day 40) male and female SOD1-G93A transgenic mice every two weeks until the symptomatic stage (postnatal day 80). Body weight and muscle force were monitored throughout the treatment, and histopathological analyses of skeletal muscle were performed at the experimental endpoint. To assess whether muscle-specific modulation of miR-199a affects motor neuron biology, RNA sequencing was conducted on quadriceps muscle and sciatic nerve from male mice, followed by validation with RT-qPCR and western blotting in both sexes. Antago miR-199a treatment preserved myofiber size, oxidative capacity, and neuromuscular innervation in both sexes, with minor sex-dependent differences. Transcriptomic analyses revealed that miR-199a inhibition modulated muscle signalling pathways associated with growth and atrophy, while activating NMJ-related pathways in the sciatic nerve. Altogether, these results identify miR-199a inhibition as a promising therapeutic approach to preserve muscle function in ALS and potentially other neuromuscular disorders.

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6. Sunrise Session Lectures

SSL I_01

Air pollution and redox biology: insights from animal studies

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Air pollution is the largest environmental risk factor for disease and premature death, with fine particulate matter ($PM_{2.5}$) identified as the major hazardous component. $PM_{2.5}$ arises predominantly from fossil fuel combustion and correlates with increased mortality from respiratory and cardiovascular diseases (CVD). In addition, $PM_{2.5}$ accelerates the development of CVD risk factors including hypertension, obesity and diabetes, at least in part through the interplay between oxidative stress and inflammation. To investigate the pathogenic mechanisms of $PM_{2.5}$ in animal models, intranasal or intratracheal instillation of $PM_{2.5}$ suspensions into anesthetized rodents is frequently used, due to its technical simplicity, dose handling, and reproducibility. However, $PM_{2.5}$ physicochemical characteristics may be altered during $PM_{2.5}$ preparation and delivery, and excessive upper airway irritation and nonrealistic exposure dosing and kinetics (usually ~1 mg/kg body weight for up to 48 h) are a major concern. In particular, intratracheal instillation bypasses the upper respiratory airways and normal particle clearance mechanisms. Moreover, instillation models are primarily suited to studying acute effects of $PM_{2.5}$. To evaluate chronic $PM_{2.5}$ effects, whole-body exposure chambers represent a more physiologically relevant approach. In these systems, collected $PM_{2.5}$ samples or polluted air are continuously delivered into specialized animal housing chambers at a controlled airflow (typically ~5 L/min), achieving concentrations of ~100 $\mu g/m^3$. Control animals are exposed in parallel under identical conditions using high-efficiency filtration systems to remove airborne pollutants. The ability to perform long-term exposures under biologically relevant conditions, combined with the use of transgenic and/or disease models, have significantly contributed to a better understanding of the *in vivo* biological effects of $PM_{2.5}$. In this sunrise seminar, we will discuss how these experimental models have contributed to advancing our understanding of the redox and inflammatory mechanisms triggered by $PM_{2.5}$ in the lung, systemically, and in secondary target organs such as the heart and adipose tissue.

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SSL I_02

From 2D to 3D, the evolution and impact of in vitro air pollution exposure models

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The effects of air pollution on human health generate great concern. worldwide. In their last report, the World Health Organization (WHO) estimates that 7 million premature deaths occur every year due to air pollution exposure, that are mainly attributable to stroke, ischemic heart disease, chronic obstructive pulmonary disease, lung and skin cancer. Besides the well demonstrated toxicity of air pollution gaseous components, such as NOx, SO2, O3, and CO, epidemiological studies indicate that particulate matter (PM) is a key component of polluted air and has been pointed out as the main responsible for the health comes that are associated with air pollution exposure. The transition from traditional two-dimensional to three-dimensional organoid systems marks a paradigm shift in air pollution exposure modeling. Although two-dimensional models have been instrumental in elucidating fundamental molecular and genetic mechanisms, they fail to accurately replicate the intricate three-dimensional architecture and dynamic microenvironment characteristic of vivo models. Here we outline how advanced organoid technologies can be a key relevant model showing our experience using lung and skin two and three-dimensional models of exposure to PM present in air pollution. Our findings contribute to the understanding of the mechanisms by which PM promotes inflammation and oxidative stress in lung and skin tissues.

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SSL I_03

Air pollution and oxidative damage in the lung epithelial lining fluid - insights from computational investigations

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The inhalation of particulate matter (PM) is correlated with enhanced morbidity and mortality, but the underlying chemical mechanisms are not yet well understood. Oxidative stress in the lung due to an excess presence of reactive oxygen species (ROS) is the leading hypothesis for the mechanism behind the adverse health effects. ROS, however, are already present and released in significant quantities in the human body. We use a multiphase chemical kinetics model of the human respiratory tract, KM-SUB-ELF, to quantify the effects of PM

on the production of ROS and oxidative damage in the epithelial lining fluid of the lung. The model aims at connecting laboratory investigations of air pollutant properties with the epidemiological evidence for air pollution health effects by providing quantitative, physiological metrics for air pollution toxicity. We find the total chemical ROS production of inhaled particles to be small in comparison to the various endogenous sources of ROS. In the model calculations, PM inflicts damage to biomolecules in the lungs mainly via the production of hydroxyl (OH) radicals. These radicals arise from conversion of peroxides through unimolecular decomposition and Fenton chemistry, suggesting transition metals and highly-oxygenated organic molecules as drivers of oxidative stress in the lungs. Further influencing factors are the water solubility of iron in the ambient aerosol and interactions with gaseous nitrogen oxides (NOx). These findings suggest that exposure-response curves may not only depend on particle mass, but also on local aerosol chemical composition, which would require adaptation in epidemiological frameworks for an accurate prediction of PM health effects. An experimentally-accessible metric that takes into account particle composition is the aerosol oxidative potential (OP). We present a chemical kinetic model of oxidative potential (KM-OP) and apply the model to field measurement data of PM composition and OP, obtaining good agreement for three different locations in Europe.

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SSL II_01
Publishing your first article: best practices for preparing and submitting a manuscript

Mark Gannon
Elsevier, Oxford, U.K.

Publishing your first research article can feel complex and at times, unclear, particularly for early career researchers navigating journal expectations for the first few times submitting their manuscript. This session aims to demystify the process, offering practical guidance on how to prepare, structure, and submit a manuscript with confidence. We will cover key elements of a strong paper: from structuring the manuscript, and selecting the right journal, to responding to reviewer comments and avoiding common pitfalls that lead to rejection. The talk will also provide insights into how editors and reviewers assess submissions, helping researchers better understand what makes a manuscript stand out. By the end of the session, attendees will have a clearer understanding for turning their research into a publishable article, along with actionable tips to improve their chances of success in the peer review process.

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SSL II_02
How to write a successful grant application: from early-career fellowships to EU consortia

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Securing competitive research funding is essential at every stage of an academic career. This presentation provides a brief overview of how to write successful grant applications, with a primary focus on early-career researchers (ECRs) while also covering mid-career and collaborative funding schemes. For ECRs, the focus is on Marie Skłodowska-Curie Actions (MSCA) Postdoctoral Fellowships, highlighting the importance of the candidate's development trajectory and the training and career development plan. For researchers transitioning to independence, key elements of ERC Starting and Consolidator Grant applications are discussed, including how to present a ground-breaking research vision, balance ambition with feasibility, and build a compelling CV. The session also briefly addresses EU collaborative consortium proposals (e.g., Horizon Europe Research and Innovation Actions), outlining essential responsibilities for both coordinators and partners. The presentation addresses common pitfalls and highlights features of successful proposals. The goal is to provide participants with practical strategies and considerations that may help strengthen their next application.

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SSL III_01
Looking beyond one's own nose: novel therapies based on noncoding RNAs

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This sunrise session aims to present an exceptional scientific career combining excellence in science with successful translation to therapeutic applications, bridging the gap between discovery and development and final take up by pharmaceutical industry. In the following, the session chairs lay-out the key features of this sunrise session.
Professor Thomas Thum, a physician scientist, entrepreneur and biotech leader, will be sharing his research focusing on the pathophysiological role of long noncoding RNAs (lncRNAs) in heart and lung diseases, with a particular emphasis on the development of RNA-based therapeutic strategies. His recent research is centered on long noncoding RNAs (lncRNAs) and circular RNAs (circRNAs), which have great potential as diagnostic and therapeutic targets for heart diseases and other conditions. He is a pioneer identifying microRNA-based therapy for the treatment of heart disease (Nature 2008), with landmark publications, including clinical studies on novel antisense therapy targeting microRNA-132 in patients with heart failure (Eur. Heart J. 2021) and preclinical development of a miR-132 inhibitor for heart failure treatment (Nat. Commun. 2020).
The translational potential of his research is demonstrated by more than 45 patents filed, several of which are in clinical development, and his work has led to the creation of Cardior Pharmaceuticals GmbH, a biotechnology company dedicated to RNA-based therapies for heart failure. In May 2024, Novo Nordisk acquired Cardior Pharmaceuticals for an amount of more than 1 billion euros.
These achievements would not have been possible without excellence in research. Thomas Thum has published over 500 scientific articles and was recognized with numerous awards, including the Paul Martini Prize in 2021, the Desmond Julian Prize in 2022, and the Sir Hans Krebs Prize in 2014.
With his lecture Thomas Thum will provide a guidance on how therapy-directed academic research can be translated towards promising clinical approaches, successful foundation of a pharmaceutical start-up and finally a high-value pharma company.

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7. Lunchtime Session Lectures and Poster

LS I_01
ECR activities at the SFRR-Europe Annual Meeting in Mainz 2026

Tim Baldensperger[1], José C. Casas Martinez[2], Vanesa Cepas-Lopez[3], Avilien Dard[4], Ufuk Ersoy[5], Sophie Hendrix[6], Paraskevi Kritsiligkou[7], Marin Kuntic[8], Veronica Lisi[9], Timoteo Marchini[10], Christina Mas-Bargues[11], Verónica Miguel Herranz[12], Chantalle Moulton[13], Konstantinos Papanikolaou[14], Anne Sophie Scheller[15], Alina Sigaeva[16], José Manuel Ugalde[17], Julia Vorhauser[18]
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Since its establishment in 2022, the Early Career Researchers (ECR) committee

of the SFRR-Europe has been dedicated to supporting young researchers through platforms for scientific training, dissemination, and professional networking. At the 2026 Annual Meeting in Mainz, the ECR Committee will host three events designed to foster community, mentorship, and career development among the next generation of redox biologists: (1) The ECR Networking event brings together young scientists attending the meeting through structured activities that promote teamwork and cooperation. By creating a welcoming and informal setting, this event enables ECRs to build connections with peers from across the redox biology community, making it especially valuable for those attending the conference alone or for the first time. (2) The Meet-the-Professors session bridges the gap between early career and senior members of our community. In a setting specifically designed to facilitate open exchange, ECRs have the opportunity to engage directly with established leaders in the field, introduce themselves, and discuss their science and career paths. (3) The Mentoring Lunch celebrates and sustains the connections formed through the ECR Mentoring Programme, which pairs young researchers with established scientists in a 1:1 mentoring relationship. This gathering brings together current and former mentors and mentees, strengthening the programme's alumni network and offering a space for ongoing support, exchange of experiences, and the formation of new mentoring relationships. Together with our regular ECR initiatives including monthly webinars, active dissemination in social media, and an editorial mentoring program, these events reflect the ECR committee's commitment to building a diverse, inclusive, and well-connected community of early career scientists within SFRR-Europe.

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LS II_01
Women in Science: Networking and Community Building

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This interactive session will focus on practical approaches to building meaningful professional networks and strengthening a sense of community within the redox field. We will explore how early-career and established researchers can effectively engage with scientific societies (e.g., SFRR-Europe, SFRRI) to support career development, visibility, and collaboration.
Participants will have the opportunity to share experiences and discuss challenges related to networking, mentorship, and inclusion. Guided discussion prompts will help facilitate conversations around building authentic connections, identifying opportunities within our societies, and creating supportive, inclusive peer communities across career stages.
The session will begin with brief introductions and informal discussion, followed by a more structured exchange of ideas, with the goal of fostering connections, sharing strategies, and developing actionable takeaways for long-term professional growth.

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LS III_01
Physiological oxygen levels: critical for high content screening of therapeutics in live cell culture models

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In vivo, epithelial cells, neurons, stem cells, cardiomyocytes and vascular endothelial and smooth muscle cells are exposed to physiological oxygen levels ranging from to ~2-13 kPa O_2, and yet the majority of studies with cell culture models maintain cells in standard CO_2 incubators gassed with atmospheric, hyperoxic O_2 levels (18 kPa O_2). Although the importance of studying cellular redox signaling under physiological O_2 levels is established, few studies have examined effects of adapting cells long-term (absence of HIF-1a stabilization) to defined O_2 levels (Keeley & Mann, Physiol. Reviews 2019;99:161-234; Sies et al., Nature Rev Mol. Cell Biol. 2022;23:499-515). Nitric oxide (NO) and NRF2 regulated redox signaling have primarily been studied in cells under atmospheric O_2 levels, and hence we characterized NO generation and NRF2 gene transcription in endothelial cells following 5d adaptation to hyperoxia (18 kPa) or physiological normoxia (5 kPa) using O_2 regulated Baker SCI-tive and invivoO_2 500 workstations. Activation of NRF2 and induction of glutathione-related genes was unaffected by changes in pericellular O_2 levels, whereas HO-1 and NQO1 expression induced by electrophiles or NO was attenuated under 5 kPa O_2 due to enhanced expression of the NRF2 repressor Bach1 (Chapple et al., FRBM 2016;92:152-62). Furthermore, a PP2A-mediated feedback mechanism regulates Ca^{2+}-dependent eNOS under 5 kPa O_2 (Keeley et al., FASEB J. 2017;31: 5172-5183), with NO bioavailability increased significantly under 5 kPa O_2 (Keeley et al., FASEB J. 2017;31: 5172-5183; Sevimli et al., Redox Biol. 2022;53:1023190; Altun et al., Free Radic Biol Med., 2024;221:89-97). Our recent studies in human brain microvascular endothelial cells adapted to 5 kPa O_2 provided the first evidence that 'physioxia' resets basal and NO stimulated K^+ channel activity (Yang et al., Redox Biol, 2026;89: 103981). Further insights on the importance of 'physioxia' is available at https://www.kcl.ac.uk/research/physiological-oxygen-laboratory. We encourage a paradigm shift in cell culture research to enhance the translation of high content screening of novel therapeutics *in vitro* to disease pathology and design of precision medicines.
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LS III_02
Adipose tissue oxygen levels matter: are we capturing true metabolic intra-organ cellular responses in standard in vitro models?

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The emerging concept of physioxia describes the physiological oxygen levels that tissues and cells experience *in vivo*, which are significantly lower than the atmospheric oxygen level (~21% O_2). In most metabolic tissues, physioxia ranges between 1–11% O_2, depending on the organ and microenvironment. I will discuss our current understanding of the metabolic tissue-specific effects of physioxia, focusing on adipose tissue, and where transition to hypoxia can lead to impairment of metabolism and consequent metabolic disease. I will present data on how modelling physioxia in vitro modulates critical responses in adipocyte progenitor cells. To model the physiological oxygen levels found in adipose tissue more closely, we cultured human and mouse preadipocytes at physioxic levels of 7% O_2 and monitored their differentiation capacity towards mature adipocytes in conjunction with their oxygen consumption (oxygen gradients) continuously, and non-invasively, using the Resipher system (Lucid Scientific, USA). This more physiological modelling may be particularly critical when comparing intra-organ metabolic interactions such as those in adipose-rich tumours, like breast cancer (BC). In obesity, adipose tissue expansion leads to local hypoxia (3-1%). Hypoxia is a common pathological feature of adipose tissue in obesity and tumours. We hypothesise that the hypoxic microenvironment shapes the metabolic activity of stroma (adipocytes) and breast cancer cells. We found that fatty acids and lactate from hypoxic adipocytes facilitated BC cell survival. Galloflavin, an inhibitor of lactate dehydrogenase (LDH)a, led to reduced lactate secretion in BC and reduced their survival. However, galloflavin was less effective in suppressing oxygen consumption in hypoxic BC cells. Under obesogenic conditions, galloflavin was not effective in reducing the size of BC spheroids in normoxia or hypoxia. This suggests that under hypoxic obesogenic environments, metabolic plasticity enables BC cells to bypass LDHA inhibition by switching from lactate-dependent glycolysis to adipocyte-derived fatty acid metabolism.

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LS IV_01
Reactive oxygen species in space: challenges for exploration and lessons for earth

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Skeletal muscle atrophy and weakness occur in many pathophysiological situations (e.g., disuse, ageing) and contribute to reduced mobility and lack of independence. Loss of protein homeostasis has been extensively reported as the final common event leading to loss of muscle mass, but, as with other tissues, the initiating signals leading to activation and progression of this loss of protein remain unknown. Reactive oxygen species (ROS) generation plays a crucial role in adaptive responses in skeletal muscle with more recent approaches revealing peroxiredoxins to be crucial in such adaptations, but dysregulation of such responses leads to muscle dysfunction. During a period of rest, changes in ROS signalling processes occur although how and when the transition occurs between normal 'rest' that is readily and rapidly reversed with normal loading compared with activation of degradative pathways in prolonged disuse has not been studied. Compelling evidence supports the potential association of mechanosensitive proteins with ROS signalling processes, including nNOS and the dystrophin associated proteins (DAG) complex at the plasma membrane and associations with mitochondria leading to activation of atrophic processes. Further evidence for such a core role for mitochondria signalling in disuse-induced atrophy comes from more recent studies of accelerated muscle atrophy in the unloaded environment of microgravity. This presentation will compare and contrast the newly emerging evidence for a disruption of ROS-mediated pathways in the development of muscle atrophy during ageing compared with the accelerated muscle wasting seen in microgravity with a focus on mechanosensing. Although focussing on skeletal muscle, such discussions have wider relevance for other tissues and will highlight the emerging opportunities for use of microgravity as an accelerated model of ageing with the re-emerging interest in sending humans into microgravity, the moon and mars.

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LS IV_02
Impact of COST Action CA20121 (BenBedPhar) on strengthening the SFRR community and advancing translational redox research

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The COST Action CA20121, "Bench to Bedside Transition for Pharmacological Regulation of NRF2 in Non-communicable Diseases (BenBedPhar)", has created a collaborative European network focused on the translational potential of NRF2 in redox biology. Together with its scientific work, one of its main achievements has been maintaining close and ongoing interaction with the Society for Free Radical Research (SFRR) community. BenBedPhar has contributed to SFRR activities by providing scientific communications and organizing satellite meetings since year 2022. These events have helped connect NRF2-related research with broader topics in redox biology, encouraging exchange between basic scientists, clinicians, and industry. They have also increased the visibility of translational redox research and supported new international collaborations. Supporting early-career researchers has been another important focus. BenBedPhar has taken part in the Spetses Summer School on Redox Biology and Medicine in 2025 and 2026, strengthening links between COST and SFRR training initiatives. In addition, the Action has organized several Training Schools covering key aspects of NRF2 biology, experimental models, drug discovery, and translation to clinical research. These activities have helped participants develop interdisciplinary skills and advance their careers. Beyond training, CA20121 has promoted short-term scientific missions, joint publications, and shared research resources, helping to build a connected and sustainable research community aligned with SFRR priorities. It has also encouraged inclusiveness by involving researchers from a wide range of COST countries. Overall, BenBedPhar has helped strengthen the connection between translational NRF2 research and the SFRR community. Through scientific events, training activities, and support for young researchers, it has contributed to increasing the reach and impact of redox biology within SFRR, while laying the groundwork for future collaborations.

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LS IV_03
How the cost actions EpilipidNET (CA19105) and COMULIS (CA17121) facilitated our skin biology research

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The philosophy of COST actions allows for diverse approaches for fostering networks of European researchers, and I and my laboratory had the honour of being tightly involved in two of such actions. Fascinating was that while we took on very diverse roles in the two actions, the result in having access to an excellent topic-oriented network afterwards was somewhat comparable. In EpiLipidNET, the Pan-European Network in Lipidomics and EpiLipidomics, I experienced the management tasks of being a workgroup leader and management team member. There, actively bringing together researchers in lipid biology and lipidomics and organizing their research collaboration and conferences on lipid signaling and its integration into web based, e.g., "wiki" approaches, was the energy well invested into networking. Completely different my and my lab's role in the action Correlated Multimodal Imaging in Life Sciences (COMULIS), where we took up the task to generate a model system that was subsequently analyzed by diverse analytical imaging methods. Both actions left us with still active follow up actions, learnings, including things that worked and things that do not work in a COST action, and the pleasure of having used European funds for the most valuable task – connecting young researchers to international networks, ideas and solutions for research excellence.

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LS IV_04
How to propose a competitive SFRR-E symposium

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The Society has been running meetings since 1982. These started out as biennial meetings but soon switched to annual events. A key feature of these meetings since their inception has been input from the members into the scientific program. This occurs via an annual call for symposia, with the submitted proposals then assessed by an international panel for possible inclusion into the program. Proposing a symposium, and then having it featured at the meeting is a great way of getting publicity and interest for your particular area of scientific interest – and also possibly getting increased recognition for your own work, your colleagues, and a great way of extending your scientific network. The Society has a well-established and highly-organized mechanism for proposing symposia and has clear guidelines as to how to construct and submit a symposium application. Although this is always a competitive process, the Society is keen to increase the variety of the science that appears at the annual meetings. As a consequence, this short presentation will outline the process and outline some tips and tricks to maximize the chances of getting your favorite topic area featured at a future SFRR-E meeting.

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8. Selected Oral Presentations

Selected Oral Presentations I – Redox Signaling & Molecular Biology

OP I_01
MAM proteomics identifies progressive ER–mitochondria signaling disfunction in Rett syndrome

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Rett syndrome (RTT) is a multisystem neurodevelopmental disorder in which mitochondrial dysfunction and chronic oxinflammation represent core pathogenic features. Increasing evidence implicates defective communication between the endoplasmic reticulum (ER) and mitochondria at mitochondria-associated ER membranes (MAMs), specialized contact sites coordinating calcium signaling, lipid metabolism, and mitochondrial bioenergetics. However, the contribution of MAM dysfunction to RTT pathogenesis remains largely unexplored. Here, we investigated the ER–mitochondria axis in brain of *Mecp2* heterozygous (HET) female mice, focusing on pre-symptomatic (HET1) and symptomatic (HET2) stages. Transmission electron microscopy revealed mitochondrial abnormalities, with reduced cristae density and swollen intracristal spaces in HET1 mice, progressing to severe cristae fragmentation and mitochondrial disorganization in HET2 animals. Quantitative proteomics of isolated MAMs uncovered profound, stage-dependent remodeling of the MAM proteome. Wild-type MAMs were enriched in proteins involved in oxidative phosphorylation, respiratory chain complex I assembly, mitochondrial protein import, and calcium-dependent metabolic regulation, consistent with their role as bioenergetic hubs. In contrast, heterozygous MAMs exhibited loss of mitochondrial identity, characterized by impaired calcium signaling, reduced mitochondrial protein import, and a shift toward lipid biosynthesis and stress-response pathways. Notably, the Complex I subunit NDUFB4 was completely absent in both HET stages, directly linking MeCP2 deficiency to respiratory chain destabilization. These findings were confirmed in *MECP2*-silenced human microglia and RTT patient-derived fibroblasts, which recapitulated MAM dysfunction, impaired calcium regulation, and increased lipid peroxidation. In conclusion, our data demonstrate that MAM dysfunction is an early and progressive event in RTT. MAMs transition from metabolic engines to stress-compensation interfaces, driving mitochondrial bioenergetic failure and disease progression.
Supported by: funds from the International Rett Syndrome Foundation (IRSF), Innovation Award Grant #3907 to A.P. and G.V. and by funds from project MNESYS (PE0000006) - A Multiscale integrated approach to the study of the nervous system in health and disease (DN. 1553 11.10.2022) to G.V.

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OP I_02

ER–mitochondria associated membranes (MAMs) dysfunction in Rett syndrome: Investigating calcium signaling alterations and potential therapeutic strategies

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Rett syndrome (RTT) is a severe neurodevelopmental disorder caused by mutations in the X-linked MECP2 gene, leading to significant neurological and metabolic impairments. Among the several cellular alterations, mitochondrial dysfunction has emerged as a key feature of RTT pathophysiology, although the underlying mechanisms remain to be clarified. We hypothesize that defects in calcium signaling, mediated by alterations in proteins controlling calcium exchange at the mitochondria-associated membranes (MAMs), contribute to mitochondrial dysfunction and redox imbalance in RTT. The calcium-regulatory complex formed by VDAC1, IP3R1, GRP75, and DJ-1 is essential for MAM integrity, enabling efficient Ca^{2+} transfer from the endoplasmic reticulum (ER) to mitochondria and supporting ATP production and redox signaling. Dysregulated calcium flux may therefore trigger excessive reactive oxygen species (ROS) generation, further aggravating mitochondrial impairment. Our data, obtained from dermal fibroblasts of RTT patients and brain tissues from female heterozygous $Mecp2^{+/-}$ mice, revealed significant alterations in gene and protein expression of key MAM-associated calcium regulators. We observed decreased VDAC1 protein abundance despite an increase in its mRNA expression, suggesting post-transcriptional instability likely driven by oxidative stress. This was accompanied by the concurrent upregulation of GRP75, DJ-1, and IP3R1. Building on these findings, we explored whether pharmacological modulation could restore proper MAM function. Treatment with resveratrol (25 μM, 24-72 h), a sirtuin-1 activator with mitochondrial protective properties, partially normalized the expression of MAM-associated proteins and improved mitochondrial function in RTT fibroblasts. Collectively, these results support the involvement of impaired ER–mitochondria calcium signaling in RTT and highlight resveratrol as a promising candidate to counteract MAM-related dysfunctions and oxidative imbalance, paving the way for new pharmacological approaches targeting aberrant mitochondrial–ER communication in RTT.

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OP I_03

Oxidative eustress regulates insulin signaling and promotes GLUT4-mediated glucose uptake in insulin-resistant skeletal muscle fibres

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Reactive oxygen and nitrogen species (RONS), at moderate levels—defined as oxidative eustress—act as second messengers in redox signalling rather than solely as damaging agents. In skeletal muscle, impaired insulin signalling and defective GLUT4 trafficking are central features of insulin resistance, contributing to obesity, type 2 diabetes, and ageing. Here, we investigated how oxidative eustress regulates insulin signalling and GLUT4-mediated glucose uptake in insulin resistant skeletal muscle.
Experiments were conducted in isolated skeletal muscle fibres from mice with high-fat diet (HFD)–induced insulin resistance. Glucose uptake was quantified using the fluorescent glucose analogue 2-NBDG, and GLUT4 trafficking was assessed by immunocytochemistry with a GFP-Myc–tagged GLUT4 construct. Controlled increases in intracellular hydrogen peroxide promoted GLUT4 translocation to the sarcolemma and significantly enhanced glucose uptake. Real-time imaging with HyPer7 biosensors revealed treatment-dependent changes in hydrogen peroxide levels in cytosolic and mitochondrial compartments, supporting its role as a compartmentalized redox signal.
In HFD-fed mice, low concentrations of hydrogen peroxide improved glucose handling and partially restored glucose uptake capacity. Previous studies from our group in C2C12 myotubes demonstrated that oxidative eustress enhances AMPK and AKT phosphorylation, identifying key signalling nodes linking redox modulation to GLUT4 mobilization. Collectively, these results demonstrate that oxidative eustress regulates insulin signalling pathways and promotes GLUT4 trafficking in insulin-resistant skeletal muscle. Our findings support a model in which RONS-mediated signalling acts as an alternative regulatory mechanism capable of enhancing glucose uptake when insulin action is impaired.

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OP I_04

Kynurenine aminotransferases salvage methionine via glutamine transamination in endothelial cells

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Kynurenine aminotransferases (KYAT) are redox sensitive pyridoxal phosphate-containing enzymes well known for the transamination of kynurenine to kynurenic acid in tryptophan metabolism. Interestingly, a single nucleotide variant (SNV) in the KYAT3 gene that decreases its expression in human arteries is significantly associated with an increase in plasma levels of imidazole lactate and 2-hydroxy-4(methylthio)butanoic acid (KMBA), the α-keto acids of histidine and methionine, respectively. The isoenzymes KYAT1 and KYAT3 can transaminate glutamine using α-keto acids as co-substrates to generate α-ketoglutaramate and replenish amino acids. Given that endothelial cells use glutamine at high rates the importance of KYAT1/3 for the endothelial metabolome was investigated here. KYAT1/3 double knockout endothelial cells were generated by CRISPR/cas9. Untargeted metabolomics (LC-MS/MS) revealed that methionine was the sixth most downregulated metabolite in KYAT1/3$^{-/-}$ as compared to non-targeted control cells (NTC). Targeted LC-MS/MS confirmed that methionine was indeed decreased whereas KMBA was increased, suggesting that endothelial KYAT1/3 are important for methionine salvage using glutamine. Functionally, deletion of KYAT1/3 decreased protein translation (SUnSET assay) and endothelial sprouting, altered genes related to cell cycle progression and induced endothelial senescence. Moreover, methionine deprivation decreased proliferation in both NTC and KYAT1/3$^{-/-}$ cells but upon supplementation of KMBA (100 μM) proliferation was rescued exclusively in NTC cells. Altogether, KYAT1/3 are key enzymes for methionine salvage and are important to maintain normal endothelial cell function.

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OP I_05
Selective engagement of nitric oxide signaling as a feedback regulator of hippocampal calcium dynamics

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Neuronal nitric oxide (NO) production is commonly viewed as a direct downstream consequence of intracellular calcium (Ca^{2+}) elevations. However, the precise functional relationship between Ca^{2+} activity and NO generation under biologically relevant conditions in intact neuronal networks remains insufficiently defined. The aim of this study was to establish a platform for simultaneous imaging of Ca^{2+} and NO dynamics in primary hippocampal neurons under near-physiological conditions and to investigate their functional interplay. To achieve this, we performed real-time live-cell imaging of NO and Ca^{2+} using the genetically encoded biosensors O-geNOps (NO) and jGCaMP8s (Ca^{2+}). Under controlled experimental conditions approximating physiological temperature and oxygen availability, we observed that NO production is not a universal consequence of ongoing Ca^{2+} signaling. Spontaneous, network-driven Ca^{2+} transients were largely uncoupled from detectable NO generation. In contrast, pronounced and sustained Ca^{2+} elevations induced by strong excitatory stimulation reliably triggered robust NO production. Furthermore, NO release exerted a feedback effect on neuronal activity by dampening spontaneous Ca^{2+} transients, revealing an inhibitory control mechanism that constrains excitability. Together, these findings position NO as a conditional modulator of neuronal signaling rather than a direct readout of all Ca^{2+} events. By directly resolving Ca^{2+}–NO interactions in living neurons under near-physiological conditions, this study provides a refined framework for understanding how NO signaling shapes neuronal activity.

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OP I_06
Mitochondrial NHE1 mediates ROS-dependent mPTP opening via hyperpolarization

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The Sodium/Hydrogen Exchanger (NHE1) is a key alkalinizing mechanism in the heart and becomes overactive in cardiac conditions such as diabetic cardiomyopathy. Although NHE1 is a membrane-bound exchanger, it has been detected in the mitochondria of rats, and its inhibition modulates mitochondrial Ca^{2+} handling. Our research group has found its overexpression in ventricular mitochondria from ob mice. Therefore, we aim to understand the consequences of this increased mitochondrial NHE1 expression. Δψm was measured with Rhodamine 123 and mPTP opening was assessed using the CRCassay with or without HOE 642 (a specific inhibition of NHE1) in isolated mitochondria from ob$^{-/-}$ mice ventricles. Mitochondrial Ca^{2+} content was determined using Fluo-3. HEK293T cells were transfected with a mitochondrial NHE1 overexpression plasmid. Δψm was quantified as JC-1 fluorescence and the mPTP opening was measured by calcein release (+/- HOE 642). Mitochondrial Ca^{2+} content was determined by a ratiometric Ca^{2+} indicator. ATP levels and mitochondrial ROS production were evaluated in both models using a commercial ATP assay kit and the H2DCFDA indicator, respectively. In isolated mitochondria, we observed increased sensitivity to mPTP opening and, surprisingly, lower Ca^{2+} content. These mitochondria also showed membrane hyperpolarization associated with augmented ROS production, which could explain the increased susceptibility to mPTP opening, and reduced ATP levels, indicating mitochondrial dysfunction. Similar findings were observed in the in vitro model. TEM analysis revealed smaller mitochondria in hearts from ob$^{-/-}$ mice, together with increased DRP1 expression in treated cells, suggesting enhanced mitochondrial fission in these cells. Notably, when mPTP opening was evaluated in the presence of HOE 642, results from ob$^{-/-}$ mitochondria became comparable to the control, establishing a link between mitochondrial NHE1 and mitochondrial dysfunction. Our results demonstrate a strong association between mitochondrial NHE1 overexpression and alterations in Δψm, Ca^{2+} content, ROS production and bioenergetics.

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Selected Oral Presentations II - Metabolism and Nutrition

OP II_01
Biliverdin reductase A and metabolic resilience in Alzheimer's disease

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Alzheimer's disease (AD) is increasingly recognized as a metabolic disorder, in which brain insulin resistance and mitochondrial dysfunction represent early pathogenic events. Biliverdin reductase A (BVRA), a pleiotropic protein with different roles as reductase, S/TS/Y kinase, scaffold and intracellular shuttle,

regulates insulin signaling and mitochondrial metabolism. Although reduced BVRA has been reported in obesity, type 2 diabetes, and AD, the mechanisms linking BVRA loss to impaired brain metabolic resilience remain to be addressed. We integrated experimental models and human biomarker approaches to define the metabolic consequences of BVRA loss across aging and AD. In wild-type and BVRA knockout mice, we assessed brain insulin signaling pathways, mitochondrial function (including respirometry-based profiling), biochemical readouts of neuronal bioenergetics, and cognitive outcomes under aging and metabolic stress conditions. In parallel, we quantified BVRA in neuronal-derived extracellular vesicles (nEVs) from Ctr subjects and AD patients and tested associations with cognitive performance and AD diagnosis. BVRA loss promoted defective insulin signaling, impaired pGSK3βS9 translocation into mitochondria, and reduced activity of mitochondrial respiratory complexes, converging on energy metabolism failure and exacerbated brain insulin resistance during aging and metabolic stress. In humans, reduced BVR-A levels in nEVs were significantly associated with cognitive decline and AD diagnosis. Collectively, these data support a role for BVRA as a molecular shuttle linking insulin signaling to mitochondrial function and cellular stress-response pathways. BVRA emerges as a critical mediator of brain metabolic resilience, whose loss accelerates insulin resistance, mitochondrial dysfunction, and cognitive deterioration. Targeting BVRA–dependent pathways may enable earlier diagnosis and more personalized therapeutic strategies in AD and related metabolic conditions.

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OP II_02

Age-dependent roles of Toll-like receptor 4 in islet inflammation and endocrine function

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With increasing age, the risk for diseases such as type 2 diabetes increases, characterized by impaired glucose metabolism, decreased glucose tolerance and disturbed insulin secretion. Pancreatic Langerhans islets are thereby regulators of glucose homeostasis, but undergo age-related structural and functional changes, including immune cell infiltration and fibrosis, potentially driven by chronic low-grade inflammation. Infiltrating immune cells, particularly macrophages and their cytokine release are central components of inflammatory signaling and closely linked to Toll-like receptor 4 (TLR4) pathways. TLR4 signaling is activated by bacterial lipopolysaccharides, which are known to promote inflammation and tissue damage in multiple organs. These observations suggest that TLR4 signaling may likewise contribute to age-related islet dysfunction and remodeling. Using two complementary approaches previously reported (Jelleschitz et al., Aging and Disease, 2025), we assessed age-dependent roles of TLR4 in pancreatic islets. First, aged C57BL/6J mice were treated with the TLR4 inhibitor TAK-242 for four months. This reduced insulitis, immune cell infiltration, and fibrosis in islets, while preserving insulin secretion. Second, we compared young and old myeloid-specific TLR4 knockout mice with their age-matched wildtype control. In contrast to pharmacological inhibition in old mice, lifelong myeloid-specific deletion of TLR4 altered islet composition and insulin secretion, and was associated with early signs of insulin resistance, highlighting the role of TLR4- dependent macrophage islet crosstalk at young age. Taken together, our results suggest, that while blocking TLR4 at higher age may reduce age-related changes by decreasing inflammation-related processes, an intact macrophage-islet interaction is required for normal β-cell function and islet development at young age. However, the mechanism behind the apparent dual role of TLR4 across age groups is not yet fully understood and requires further research.

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OP II_03

Nitrate-mediated redox communication: a novel inter-kingdom signaling pathway between gut microbiota and host epithelial cells during dysbiosis

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Humans are holobionts where gut microbiota ensure vital physiological functions. We propose a novel inter-Kingdom communication pathway based on microbial redox species production and host mucosal signaling. This interaction is modulated by dietary nitrate, a driver of NO synthesis in vivo, suggesting that nitrate-driven redox signaling is a biologically significant mechanism for maintaining host-microbiota homeostasis and preventing disease. We show that nitrate has a positive metabolic effect in a murine model of antibiotic-induced dysbiosis by regulating cecum morphology and preventing body weight loss ($p < 0.05$). Metagenomic analysis supports these observations, revealing that nitrate preserves Bacteroides genera, including bacteria involved in carbohydrate metabolism. The preservation of microbial diversity suggests that nitrate maintains a functional metabolic environment during dysbiosis. This metabolic stabilization is coupled with a distinct shift in the microbial "redox signature." We found that nitrate exerts a functional impact on the expression of microbial enzymes; notably, it preserves bacterial non-heme ferritin, which is involved in the generation of H_2O_2. This microbial-derived H_2O_2 acts as a paracrine signal that bridges the gap between the lumen and the mucosa likely activating the Nrf2 pathway in host epithelial cells. Reflecting this molecular crosstalk, the activation of the Nrf2/ARE pathway was assessed through the downstream expression of NQO1 and GCLM/GCLC. Preliminary results indicate that dysbiosis increases the expression of both proteins, while nitrate restores them to control levels. Furthermore, while antibiotic exposure decreases the expression of tight junction proteins, nitrate-mediated signaling correlates with the recovery of both occludin ($p < 0.05$) and claudin-5 ($p < 0.01$), reinforcing gut barrier integrity. Here, nitrate emerges as a regulator of microbiota-host interactions by enhancing bacterial metabolic performance, which reinforces gut barrier integrity and likely prevents leaky gut syndrome, offering a transformative redox-based approach for treating systemic disorders.

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OP II_04

Dietary nitrate as a modulator of host–microbiota redox interactions under antibiotic-induced dysbiosis

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The nitrate–nitrite–nitric oxide ($^{\cdot}$NO) pathway involves sequential reduction of dietary nitrate to nitrite by oral commensals and further conversion to $^{\cdot}$NO and other bioactive nitrogen oxides in the stomach and gut. This pathway supports $^{\cdot}$NO bioavailability and redox signaling, regulates cellular metabolism and mitochondrial efficiency, and may mitigate antibiotic-induced dysbiosis by preserving the *Bacteroides* genus, microbiome redox capacity, and epithelial barrier function. We investigated whether inorganic nitrate protects colonic mitochondrial oxidative phosphorylation (OxPhos), a key source of ATP required to sustain barrier integrity, and quantified S- and N-nitroso species during antibiotic-induced dysbiosis. Male Wistar rats were randomized into four groups (7 days): (1) control, (2) imipenem (1.25 μg/mL), (3) nitrate (10 mM), and (4) imipenem + nitrate (drinking water). Using chemiluminescence, nitrate supplementation significantly increased nitrite in the colonic mucosa, accompanied by higher plasma nitrite ($p = 0.0002$) and increased S- and N-nitroso compounds ($p = 0.05$). Co-treatment with imipenem partially blunted the rise in nitrite and nitroso species without altering nitrate levels, consistent with a requirement for microbiota- driven nitrate reduction and redox signaling. Mitochondrial O 2 consumption rate (OCR) was assessed in permeabilized colon biopsies using high-resolution respirometry (Oroboros O2k) with a SUIT

protocol. Nitrate increased maximal Complex I and II activities, resulting in higher ATP-linked respiration via Complex I ($p = 0.025$) and a trend toward increased mitochondrial spare capacity. Imipenem-induced dysbiosis reduced Complex IV activity ($p = 0.0015$); nitrate co-treatment restored this deficit. Dysbiosis also increased the CII/CIV ratio, which was normalized by nitrate intake. In conclusion, dietary nitrate, likely via microbiota-dependent formation of nitroso species, enhances colonic mitochondrial function under eubiosis, and mitigates mitochondrial impairment induced by antibiotic-induced dysbiosis. This may maintain adequate ATP synthesis needed to sustain barrier function. This reinforces the role of the nitrate–nitrite–·NO pathway in maintaining intestinal redox balance.

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OP II_05

Loss of Sirtuin 3 drives lipid remodeling and mitochondrial sensitivity to western diet-induced oxidative stress

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The Western diet (WD), characterized by high fat and sugar content and low fiber intake, is a major driver of obesity and metabolic syndrome in developed countries. WD imposes chronic nutrient overload, increasing mitochondrial fatty acid influx and placing sustained demand on oxidative metabolism. Such metabolic pressure predisposes cells to oxidative stress and adaptive metabolic rewiring. A key mediator of cellular adaptation to metabolic stress is Sirtuin 3 (Sirt3), a mitochondrial NAD^+-dependent deacetylase that regulates fatty acid oxidation, oxidative phosphorylation and antioxidant defenses. This study aimed to investigate the metabolic and mitochondrial response to WD-induced oxidative stress in primary murine hepatocytes, with a particular focus on the role of Sirt3. Primary hepatocytes isolated from male and female wild-type (WT) and Sirt3 knockout (KO) mice were exposed to a lipid mixture under high-glucose conditions for 6 hours to mimic WD-induced stress *in vitro*. Transcriptomic profiling was performed by RNA sequencing and metabolic alterations were assessed by LC-MS–based metabolomics. Selected protein expression was analyzed, while mitochondrial membrane potential was evaluated by flow cytometry. Mitochondrial ultrastructure was examined using transmission electron microscopy. Metabolomic analysis revealed depletion of PUFA-containing lysophospholipids in KO cells, indicating oxidative membrane damage and mitochondrial membrane remodeling. WD treatment reduced levels of sterol lipids, suggesting altered membrane properties under metabolic stress. Elevated malate levels in KO hepatocytes pointed to disruptions in the TCA cycle and impaired electron transport chain organization. Increased MitoTracker Deep Red fluorescence suggests mitochondrial remodeling and impaired mitophagy, while ETM analysis revealed mitochondrial membrane depolarization upon WD treatment in KO hepatocytes, particularly in males. Collectively, these data indicate that loss of Sirt3 leads to altered lipid composition and TCA cycle dysfunction in murine hepatocytes while WD treatment further exacerbates metabolic and oxidative stress resulting in mitochondrial remodeling and membrane depolarization.

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OP II_06

Early-life undernutrition modifies redox and inflammatory immune–cardiovascular responses to air particulate matter

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Early-life exposure to environmental stressors, such as air pollution-derived particulate matter (PM) and chronic undernutrition, may independently induce long-lasting alterations in immune and cardiovascular homeostasis. However, how their combined exposure modulates inflammation and redox balance remains insufficiently characterized. Objective: To evaluate alveolar macrophage (AM) response and its associated inflammatory alterations in cardiovascular tissues under subchronic exposure to Residual Oil Fly Ash (ROFA) in a Nutritional Growth Retardation (NGR) rat model. Male Wistar rats were fed ad libitum (Control) or a 20% restricted diet (NGR) from weaning and up to 4 weeks. Simultaneously, animals were intranasally instilled with ROFA (0.17 mg/kg) or the vehicle three times per week, generating four experimental groups: C, ROFA, NGR, NGR + ROFA. AM obtained by bronchoalveolar lavage were evaluated for metabolic activity, cytokine production, oxidative stress markers, and antioxidant level expression. Additionally, cytokine expression and CD68 positivity were evaluated in heart and aortic tissues. In Control animals, ROFA reduced AM metabolic activity ($p < 0.01$) and increased TNF-α secretion ($p < 0.01$). Interestingly, NGR-AMs showed a basal decrease in cell metabolism ($p < 0.05$) and increased TNF-α secretion ($p < 0.05$) compared to Control. Nevertheless, ROFA exposure in NGR-AMs did not induce further changes in mitochondrial metabolism or TNF-α secretion compared to the NGR group. Regarding oxidative stress markers, ROFA increased superoxide anion production ($p < 0.001$) and reduced Nrf2 expression ($p < 0.05$) in both dietary groups. At the cardiovascular level, ROFA increased IL-6 and IL-10 expression in the aorta ($p < 0.05$) and enhanced the presence of CD68-positive cells in the periaortic adipose tissue in both dietary groups. In the heart, ROFA augmented IL-6 while diminished IL-10 expression levels ($p < 0.001$) exclusively in Control animals. Early-life chronic undernutrition induces a proinflammatory, redox-imbalanced immune state with reduced adaptive capacity. Subchronic PM exposure triggers immune and cardiovascular responses in Control animals, whereas this inducible response is blunted by undernutrition, increasing susceptibility to PM-related cardiovascular dysfunction.

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Selected Oral Presentations III – Environmental Exposure and Lifestyle Risk Factors

OP III_01

Redox biology of wildfire smoke and impacts on human health

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Wildfire smoke is an escalating climate-driven exposure with growing cardiopulmonary and neurological burden. Yet the redox pathways by which complex smoke mixtures dysregulate innate immunity across the lung–brain axis remain incompletely defined. Aligning with EXPOHEALTH sessions on air pollution-driven oxidative stress and the redox exposome, we mapped redox-linked signalling and immune function in human myeloid cells exposed to bushfire smoke. Wildfire smoke extract (WFSE; burned Australian vegetation) was applied to donor-derived monocyte-derived microglia-like cells (MDMi) from young (<40 y) and aged (>60 y) cohorts. Bushfire smoke extracts from multiple plant fuels were also applied to THP-1 macrophages. Endpoints included nitrosative stress (nitrate/nitrite), antioxidant stress responses, MAPK/NF-κB signalling, cytokine secretion, and phagocytosis. In MDMi, WFSE elevated nitrate secretion at 24 h and remained increased through 96 h, indicating sustained nitrosative stress. WFSE significantly upregulated heme oxygenase-1 (HO-1) at 24 h, returning to baseline by 96 h. WFSE activated p38 and ERK1/2 and drove NF-κB p65 nuclear translocation by 24 h; p38 resolved by 96 h whereas ERK activation persisted. By 96 h, aged MDMi showed robust increases (e.g., TNF-α ~31-fold; IL-33 ~75-fold), with 8/10 cytokines elevated (TNF-α, IL-33, IFN-γ, IL-23, IL-10, IL-18, IFN-α2), compared with young MDMi. WFSE modestly reduced phagocytosis in young MDMi, with low baseline in aged donors. In macrophages, all bushfire

smoke extracts significantly reduced bacterial phagocytic capacity and promoted inflammasome-associated inflammation (increased cleaved IL-1β/caspase-1; elevated IL-1β and IL-8 secretion) alongside decreased NLRP3 protein. These findings provide potential redox targets for protecting firefighters and vulnerable communities during wildfire seasons. Together, these data identify wildfire smoke as a potent environmental redox stressor that links antioxidant and nitrosative responses with NF-κB/MAPK signalling to inflammatory and host-defence dysfunction, highlighting ageing as a key modifier of susceptibility.

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OP III_02

Long-term aircraft noise exposure induces neuroelectrophysiological remodeling and brain–heart axis oxidative stress in mice

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Noise pollution triggers stress responses and sleep disturbances, initiating a pathophysiological cascade characterized by hyperactivation of the amygdala, the hypothalamic-pituitary-adrenal axis, and the sympathetic nervous system. This cascade ultimately drives reorganization of neural oscillations and oxidative stress in the brain-heart axis. Male C57BL/6J mice were exposed to aircraft noise for 28 consecutive days. Non-invasive blood pressure was measured weekly. On day 28, EEG signals were recorded for spectral and correlation analyses. [^{18}F]FDG PET imaging was performed to assess cerebral glucose metabolism. Aortic vascular function was assessed by isometric tension assay, and reactive oxygen species (ROS) formation was quantified by DHE staining. Plasma norepinephrine levels were measured by HPLC with electrochemical detection (HPLC/ECD). Cortical and cardiac tissues were subjected to transcriptomic and proteomic profiling. Chronic aircraft noise exposure induced sustained hypertension in mice, accompanied by impaired aortic endothelial function and increased ROS formation. EEG spectral power was elevated in 4-10 Hz and >40 Hz bands, reflecting central fatigue and altered cognitive processing. Interregional correlations were reduced across delta, theta, and gamma bands, suggesting impaired network efficiency. Consistent with heightened stress signaling, [^{18}F]FDG PET demonstrated increased amygdala glucose uptake, and HPLC/ECD analysis showed an upward trend in plasma norepinephrine levels. Additionally, multiple mediators of inflammation and oxidative stress (both pro- and anti-) were upregulated in the brain-heart axis after noise exposure. This study provides direct evidence that chronic noise exposure induces maladaptive neuroelectrophysiological remodeling in mice via stress adaptation, implicating preferential disruption of stress-responsive circuits (e.g., amygdala-prefrontal axis). Molecular profiling reveals convergent redox-inflammatory effectors to neural and cardiovascular pathology.

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OP III_03

Synergistic effects of ultrafine particulate matter and noise in Alzheimer's disease and wild-type mouse models

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Ultrafine particulate matter (UFP) and aircraft noise (AN) are significant environmental stressors that contribute to the global burden of disease, such as respiratory, cardiovascular, neurodevelopmental, and neurodegenerative diseases. Among these, Alzheimer's disease (AD) is the most common neurodegenerative disorder, characterized by impaired memory and the accumulation of abnormal amyloid beta and phosphorylated tau proteins. However, how these two synergistic environmental stressors interact to affect brain health and contribute to the pathogenesis of AD remains unclear. This study aimed to investigate how inhaled UFPs and AN affect brain health and AD. We hypothesized that exposures impair neuronal function in healthy individuals and alter the neurobiological signature of AD. To test these hypotheses, a whole-body inhalation chamber using the mini combustion aerosol standard (miniCAST) burner and playback of recorded aircraft events from Düsseldorf Airport was used to simulate real-life exposure conditions. Mice expressing human APP and PSEN1 transgenes with a total of five AD-linked mutations (5xFAD) and wild-type littermates (C57BL/6) mice were randomly divided into 4 groups: clean air (control), freshly generated combustion-derived UFPs, AN, and a combination of UFP and AN. The exposures took place on weekdays for 4 hours each day, a total of9 days over the course of 2 weeks. Preliminary bulk RNA sequencing indicated alterations in expression of oxidative stress linked genes in the olfactory bulbs of exposed mice compared to the non-exposed group. Xenium spatial transcriptomics provided a map of gene expression in both wildtype and 5xFAD brain after UFP exposure. Furthermore, the effects of both stressors on the lung/heart-brain axis and AD pathological features are being assessed by multi-omics, biochemical, and histological analyses. The insights gained from this study enhance our understanding of the contribution of air pollution and noise to health, and their links to AD.

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OP III_04

Cardiovascular and metabolic acute effects of heated tobacco products in healthy smokers

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Tobacco smoking remains a major cardiovascular risk factor, contributing substantially to morbidity and premature mortality. Heated tobacco products (HTPs) are increasingly marketed as "reduced-harm" alternatives to combustible cigarettes, despite delivering pharmacologically active nicotine and aerosols containing reactive carbonyls and oxidant species. Independent human data on their acute redox and cardiovascular effects are limited. In this investigator-initiated, within-subject pre–post study, healthy adult smokers were supervised in their exposure to two consecutive HTP sticks (IQOS). Endothelial function was assessed by brachial artery flow-mediated dilation (FMD), together with hemodynamic parameters, venous blood gases, metabolic markers, and oxidative DNA damage measured by the comet assay. Acute HTP exposure significantly reduced FMD, indicating rapid endothelial dysfunction consistent with redox imbalance and impaired nitric oxide bioavailability. This was accompanied by increased systolic blood pressure and altered venous blood gases, including reduced oxygen tension and saturation and elevated carbon dioxide, suggesting impaired tissue oxygenation and oxidative metabolic stress. Changes in glucose and lactate levels indicated nicotine-driven sympathetic activation. Importantly, short-term HTP use induced significant oxidative DNA damage in peripheral blood cells, demonstrating acute genotoxic stress. These effects occurred after a single real-world exposure in otherwise healthy smokers. Collectively, the findings show that HTPs are not biologically inert and that even acute use disrupts vascular function, systemic metabolism, and genomic integrity, challenging claims of cardiovascular harm reduction and reinforcing the need for

regulatory vigilance.

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OP III_05

Redox and allostasis: coping with the challenges of overwintering in Antarctica

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As complex adaptive systems, humans maintain stability by navigating and responding to challenges/stressors through a dynamic process called allostasis. Both, 'fight-or-flight' responses and adaptation to chronic stress vary considerably between individuals depending on a multitude of intrinsic and extrinsic factors; when demand exceeds the body's resilience illness ensues. Redox processes underpin allostasis by serving as a "*lingua franca*" (common language) that enables efficient communication between levels of biological organisation. Yet, together, their role in adapting to isolated, confined, extreme (ICE) and hypoxic environments remain poorly understood. We sought to investigate whole-body redox regulation and allostatic load during prolonged ICE exposure in the MARS-C study supported by the European Space Agency. Thirteen healthy men (aged 27-54) resided at the Concordia Research Station, Antarctica (an Earth-based space analogue, 3233m above sea level) for 12-months, exposed to physical and psychological challenges. Physiological recordings (e.g. body composition, blood pressure) and serial collections of saliva, blood and 24-hour urine were performed before, during (at monthly intervals) and after overwintering at Concordia. Biomarkers of oxidative stress, nitric oxide and thiol status, reductive capacity and allostatic load, including cortisol, dehydroepiandrosterone, IL-6 along with inflammatory, cardio-vascular and metabolic markers, were measured using a combination of analytical techniques such as colorimetry, immunoturbidimetry, ELISA, ion chromatography-mass spectrometry and HPLC. Participants exposed to prolonged ICE and hypoxia elicited distinct changes in markers of redox balance and allostatic load. Notably, their responses to the shared environmental and psychosocial stressors varied substantially, with some readouts (e.g. glucose, uric acid) showing additional seasonal variation. The findings from this novel approach highlight that acclimatisation and adaptation to combined hypoxic and environmental stress is heterogenous and associated with redox regulation. This suggests redox regulation serves a central role in maintaining stability in demanding environments, such as Concordia.

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OP III_06

Skin fibroblasts' response to UVB irradiation: apoptosis, premature senescence or resistance?

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Ultraviolet B (UVB) radiation is a major contributor to skin photoaging. Although mainly absorbed by the epidermis, UVB photons managing to penetrate the upper dermis affect human dermal fibroblasts (HDFs), leading, among others, to a reduced number of HDFs and to the accumulation of senescent cells. We have shown in *in vitro* and *in vivo* studies that high UVB doses provoke apoptosis to HDFs and that JNKs/ATM-p53 activation and interplay are indispensable for the perpetuation of cellular defense towards UVB, while EGFR/Akt and Nrf2 are auxiliary anti-apoptotic machineries [Cell Death & Dis. (2022) 13: 647]. This cytotoxic effect depends on the anatomical site, the developmental stage and the differentiation status of the cells. On the other hand, repeated mild treatment with UVB radiation has been reported in the past to result in HDFs' premature senescence shortly after the end of the treatment period. However, a follow-up observation of UVB-treated cultures by our lab revealed the occurrence of mixed populations, containing both senescent cells and fibroblasts resisting senescence. "Resistant" fibroblasts were more resilient to a novel intense UVB radiation stimulus. RNA-seq analysis followed by functional experiments pinpointed ERCC6, encoding Cockayne syndrome group B (CSB) protein, as a key molecule conferring protection toward UVB-induced cytotoxicity and senescence, as CSB loss-of-expression rendered HDFs significantly more susceptible to UVB radiation. UVB-resistant HDFs remained normal and non-tumorigenic. Even though they formed a distinct population in-between young and senescent cells, resistant HDFs retained numerous tissue-impairing characteristics of the senescence-associated secretory phenotype, including increased matrix metalloprotease activity and promotion of epidermoid tumor xenografts [Aging Cell. 2025 Mar;24 (3):e14422]. Proteomic analysis has revealed numerous candidate effectors connected to the above-mentioned phenomenon. Collectively, UVB-induced apoptotic, resistant to senescence and senescent phenotypes of HDFs may all be characterized by undesirable traits regarding tissue homeostasis and subserve skin photodamage and photoaging.

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Selected Oral Presentations IV – Vascular Biology and Redox Biology-associated Modifications

OP IV_01

Telomeric noncoding RNA TERRA and its regulatory network in murine heart failure

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Heart failure (HF) remains a leading cause of mortality worldwide, exacerbated by age-related myocardial degeneration. Our prior work demonstrated that cardiomyocyte telomere shortening, a hallmark of myocardial aging, is driven by oxidative damage via NOX2 activity. Long non-coding telomeric repeat-containing RNA (TERRA), a key regulator of telomere homeostasis, may play a pivotal role in this process. In this study, we examined the contribution of myeloid NOX2 and TERRA dynamics in a neurohormonal HF mouse model using male mice (6–8 weeks old) subjected to unilateral nephrectomy, AngII infusion, and high-salt water for 6 weeks (ANS). Mice with myeloid-specific NOX2 knockout (LysM-NOX2-KO) were compared to littermate controls ($Nox2^{fl/fl}$ $LysM^{cre/wt}$ vs $Nox2^{fl/fl}$ $LysM^{wt/wt}$), as well as in wild-type C57BL/6J mice. Echocardiography showed preserved LVEF in Nox 2 KO mice (52% vs. 40%), despite similar elevated blood pressure. Telomere FISH confirmed shortening in both ANS groups. qPCR confirmed loss of NOX2 expression in KO hearts, alongside increased NOX4 and significantly elevated IL-6, suggesting compensatory proinflammatory remodelling. Importantly, TERRA transcripts from chromosomes 2, 18, and PAR regions were significantly downregulated in wild-type B6J mice following neurohormonal activation compared to Sham. These findings suggest that TERRA suppression is associated with stress-induced telomere dysfunction. KEGG enrichment analyses of downregulated genes revealed alterations in metabolic and circadian pathways (e.g., insulin signalling, cAMP, fatty acid degradation), while upregulated genes were enriched in PI3K-Akt, cytokine-cytokine receptor interaction, and ECM remodelling pathways—indicative of inflammatory and structural cardiac remodelling. Gene ontology analysis further highlighted dysregulation of contraction, ion transport, and cardiac conduction pathways. Collectively, these results underscore a

role for TERRA downregulation in the molecular cascade of HF. Myeloid NOX2 deficiency appears protective, potentially via IL-6-mediated signalling, independent of hemodynamic load. Our findings reveal a complex interplay between oxidative stress, inflammation, and telomeric regulation.

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OP IV_02

Hypochlorous acid-mediated modification diminishes the ability of histones to kill bacteria

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Neutrophils release extracellular traps (NETs) during inflammation as an important immune defence mechanism to clear infection. However, NETs are also implicated in the pathology of many diseases, particularly atherosclerosis and sepsis. NETs are composed of a DNA network containing histones and antimicrobial proteins like myeloperoxidase (MPO). MPO remains enzymatically active, generating hypochlorous acid (HOCl), a highly reactive oxidant that can modify proteins, including histones in NETs. Whether these oxidative histone modifications are generated *in vivo* during infection and affect the ability of histones to kill bacteria is currently unknown. To assess the extent of NET release induced by bacteria, primary neutrophils from healthy donors were stimulated with different types of bacteria. *S. aureus* and *E. coli* induced substantial NET release as indicated by Sytox Green staining of the DNA. We then examined the ability of histones to kill these bacteria and whether this is impacted by HOCl modification. It was shown that 2.5 μg/mL of an unfractionated histone preparation completely inhibits growth of *S. aureus*, while 325 μg/mL was necessary to inhibit *E. coli* growth. Modification of histones with HOCl (7.5-fold excess, 24 h) significantly decreased this toxicity to both types of bacteria. A quantitative multiple reaction monitoring (MRM) mass spectrometry method was established to detect specific histone H4 peptides containing methionine sulfoxide and/or 3-chlorotyrosine residues, HOCl-induced modifications observed on NETs in vitro. The quantification was linear over a large range (0.0625 ng/mL to 100 ng/mL) with a lower limit of detection of 0.125 ng/mL. Using this MRM method, we were able to detect oxidative histone modifications in samples from patients with infectious syndromes. This study is the first to show that histones are modified by HOCl in vivo during infection, which influences their ability to kill bacteria. This provides new insight into pathways that impact on inflammation in vivo.

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OP IV_03

Inhibiting coagulation factor XI improves cardiac dysfunction in ischemia/reperfusion injury in mice with excess neurohormonal activation

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Coagulation factor XI (FXI) has emerged as a promising antithrombotic target uncoupling thrombosis and hemostasis. Targeting FXI can attenuate angiotensin II (AngII) -induced vascular dysfunction, hypertension and renal damage, thus may be beneficial to prevent atherothrombotic events in high-risk patients. However, the potential role of FXI inhibition on cardiac ischemia reperfusion injury (IRI) in CVD caused by excess neurohormonal activation is still unknown. C57BL/6J male mice were treated with Ang II infusion, unilateral nephrectomy and high-salt diet (AngII + +) for 4 weeks to model excess neurohormonal activation. FXI antisense oligonucleotide (FXI ASO) or scrambled ASO was injected. Left anterior descending was temporarily ligated for 45 minutes, followed by the reperfusion. After 72 hours, cardiac function was analyzed by high frequency ultrasound, real-time PCR. FXI ASO administration reduced hypertension in the AngII + + operated group compared to control. In cardiac IRI, the inhibition of FXI resulted in better LV ejection fraction and less LV mass and significantly improved endothelial dysfunction compared to mice injected with scrambled ASO and did this to a greater extent in AngII + + than in sham mice. Furthermore, the expression levels of adhesion molecules and the expression of pro-inflammatory targets, such as Vcam-1, CCL2, IL-6, and IL-1β, were significantly decreased in the ischemic myocardium of FXI-depleted mice in the AngII + + following cardiac IRI group compared to scramble ASO with IRI group. Our results suggest that the inhibition of FXI synthesis by ASO treatment offers cardiovascular protection in cardiac IRI in mice with excess neurohormonal activation via anti-inflammatory effects.

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OP IV_04

Proteomic analysis of oxidative modifications on metabolic proteins in human atherosclerotic plaques and control arteries

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Cardiovascular disease is a major cause of mortality and morbidity. A prime cause is atherosclerosis and plaque formation at sites along the arterial tree. Considerable evidence links atherosclerosis with chronic inflammation and leukocyte (neutrophil, macrophage) activation. We hypothesized that this would result in oxidant-mediated damage to arterial proteins. In this study human carotid artery plaques and control (superior thyroid) tissue from 84 subjects were analyzed by LC-MS/MS for the presence of protein post-translational modifications (PTMs) generated by redox reactions. Of 7150 proteins detected across all samples, 2724 carried at least one PTM, with these including mono- and di- oxidation at methionine, tryptophan, histidine and tyrosine, conversion of cysteine to cysteic acid, and chlorination and nitration of tyrosine and tryptophan. Higher levels of PTMs were detected on proteins from plaques compared to control artery tissue, but similar types of modifications were detected. Higher levels of PTMs were detected in plaques classified as unstable compared to stable, consistent with PTMs being associated with plaque instability. The levels of multiple PTMs correlate with the concentration of the heme protein myeloperoxidase, a neutrophil-derived species, implicating this enzyme and its oxidants in protein damage, though direct causality has not been established except for chlorination. Large numbers of PTMs were detected on key metabolic proteins including those involved in glycolysis/gluconeogenesis, the pentose phosphate pathway, the tricarboxylic acid cycle and oxidative phosphorylation. Particularly high levels were detected on glycolytic enzymes, including glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and Complex V subunits consistent with perturbations to cellular metabolism in atherosclerotic plaques.

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OP IV_05
Mitochondrial remodelling as a pivotal redox-sensitive mediator of breast cancer-adipose tissue crosstalk

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The dynamic interplay between breast cancer cells and cancer-associated adipose tissue (CAAT), as the principal constituent of their tumour microenvironment (TME), is orchestrated through the synchronised modulation of key metabolic pathways and altered redox signalling. Even though in recent years mitochondria have been recognised as integral to the establishment and maintenance of the malignant phenotype, the context dependence and molecular mechanisms underlying their reprogramming remain insufficiently defined. To elucidate how mitochondrial morphology and function are shaped in relation to malignancy, we conducted a study on female patients with benign and malignant breast tumours. Metabolomic analysis revealed distinct changes in mitochondrial-associated metabolites in both malignant tumours and CAAT compared to their benign counterparts. Moreover, confocal microscopy analysis showed increased mitochondrial turnover in these tissues, with mitochondrial fission taking precedence over mitochondrial fusion. We further investigated whether mitochondrial remodelling in breast cancer depends on the redox signalling within the TME. To this end, we employed an orthotopic breast cancer model in wild-type (WT) and Nrf2 knockout (Nrf2KO) mice in order to examine the effects of impaired Nrf2 activation in the TME as one of the key regulators of redox-metabolic integration. Additionally, we analysed different time points of early tumour growth (10, 50, and 200 mg tumours) to monitor redox-sensitive, time-dependent mitochondrial remodelling during tumour progression. Breast tumours from Nrf2KO mice displayed significantly altered mitochondrial populations in regard to their functional activation, morphological remodelling, key metabolic pathways and ROS production, particularly at the inital time point of early tumour growth (10 mg tumours). Collectively, our data underscore the significance of mitochondrial morphological and functional remodelling for the malignant phenotype of breast cancer and identify Nrf2-mediated redox signalling in the TME as a relevant target point for breast cancer therapy.
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OP IV_06
One protein, two enzymatic chemistries: cryptic redox catalysis in a plant glycolytic enzyme

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The classical “One Gene, One Enzyme” principle has long shaped molecular biology, yet many proteins are now recognized as having “moonlighting” functions, performing additional roles beyond their canonical reactions. While non-enzymatic moonlighting functions are increasingly reported, dual catalytic activities within a single protein remain rare, and the boundary between metabolism and redox regulation is still largely drawn along protein family lines. We found a “two-in-one” enzyme in *Arabidopsis* that retains its canonical glycolytic activity while also catalyzing a glutathione-dependent redox reaction typically associated with dedicated redoxins. Complementary evidence from *in vitro* enzymatic assays, non-reducing immunoblotting, and mass spectrometry supports a glutathione-dependent catalytic activity that is mechanistically separable from glycolysis, suggesting that redox chemistry can be embedded within a classical metabolic scaffold. Sequence- and structure-guided analyses revealed a conserved glutathione-interaction motif and pointed to a candidate catalytic residue underlying the redox activity. Site-directed mutagenesis of this residue abolished the intrinsic glutathione-dependent redox catalysis *in vitro* while preserving glycolytic activity, thereby uncoupling the two functions. Notably, the sequence signature associated with the redox activity is widely conserved among land plants, consistent with an evolutionarily broad integration of redox capacity into central metabolism. Taken together, these findings challenge fold- and homology-based assumptions about which proteins can perform glutathione-dependent redox chemistry and suggest a route by which plants may couple redox state to core metabolism.

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9. Flash Talks - also presented as posters (PP)
Flash Talks I – Redox biology of human diseases

FT I_01/PP II_B03
AMPK as a key mediator of cardioprotection in doxorubicin-induced cardiotoxicity

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In clinical practice, the anthracycline doxorubicin (DOX) remains one of the most essential chemotherapeutic agents in the treatment of diverse tumor types. Unfortunately, the clinical application of DOX is limited by various side effects, cardiotoxicity in particular. This project aims to investigate the mechanisms underlying doxorubicin-induced heart failure, focusing on oxidative stress, inflammatory processes, and cardiac functional parameters, with a particular emphasis on the role of 5'-adenosine monophosphate-activated protein kinase (AMPK) and sex differences in an animal model. We used a mouse model of chronic low-dose DOX administration (cumulative dose of 20mg/kg) to induce heart failure in male and female animals. Females showed preserved cardiac function, whereas males displayed reduced systolic function and increased oxidative stress and inflammation, effects mediated by the AMPK pathway. Supplementary pharmacological activation of AMPK via AICAR (20 mg/kg/d for 7 days) in males attenuated DOX-associated cardiotoxicity. We observed improved cardiac function, measured by echocardiography, decreased superoxide formation in the heart, as shown by HPLC analysis, and a lowered oxidative burst in blood. Additionally, we saw reduced levels of markers of oxidative stress and inflammation (e.g., malondialdehyde and IL-6). In contrast, monocyte-specific AMPK depletion exacerbated myocardial damage in females, specifically worsening cardiac function, increasing reactive oxygen species (ROS) formation in the heart, and elevating oxidative burst in blood. Levels of oxidative stress and inflammation markers were also elevated. Anthracyclines, including doxorubicin, have been widely used for decades as effective cancer therapies. Nevertheless, their cardiotoxic effects, particularly the risk of heart failure, remain a significant clinical concern. Our findings suggest that female sex and the AMPK pathway mediate cardioprotective effects. Understanding the gender-specific differences and implementing protective therapeutic approaches (e.g., modulation of AMPK) in doxorubicin-induced heart failure has the potential to refine individualized treatment and thus enhance overall outcomes in cancer therapy.

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FT I_02/PP II_B02
Oxidized insulin alters glucose signaling and has immunogenic potential

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Hypochlorous acid, hydrogen peroxide, and other reactive species characterize oxidative cellular stress and chronic inflammation and function as signaling

molecules, for example, for the recruitment and activation of immune cells. Conversely, reactive species can induce non-enzymatic oxidative protein modifications (oxPTMs) that can alter the functionality or immunogenicity of proteins. Using insulin, a regulator of glucose homeostasis, the protein was oxidized with hydrogen peroxide or in multi-species environments generated by cold physical gas plasmas. Different species compositions modified insulin (oxIns) with individual oxPTM patterns, which were identified by mass spectrometric analysis. The oxIns variants showed heterogeneous effects on glucose recognition and uptake, as well as on metabolic activity and proliferation in cell lines from five different human tissues. Furthermore, the gene expression of GLUT4, Nrf2, and HMOX1 was modulated and correlated with the expression of Akt and Erk for all oxIns variants. In an immunological context, antigen-presenting THP-1-NF-κB reporter cells showed no effect of hydrogen peroxide-rich plasma or hydrogen peroxide on NF-κB signaling, whereas other oxIns variants reduced NF-κB signaling. Importantly, NκB correlated with cell size, expression of the surface marker MHC-II, IL-18 secretion, and Akt signaling. These data provide evidence for dose-dependent effects of enriched reactive species in a multispecies environment on protein modifications and their cell biological and immunological consequences.

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[2] Clemen, R. et al., Redox Biology 2024 77:103372
[3] Clemen, R., et al., Vaccines 2022 10:1814

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FT I_03/PP I_B03

Mutations of glial fibrillary acidic protein associated with Alexander disease increase susceptibility to protein modification and network disruption by oxidants

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The type III intermediate filament protein glial fibrillary acidic protein (GFAP) plays a key role in astrocyte and brain homeostasis, and its mutations can result in the severe and rare neurodegenerative Alexander's disease (AxD). Previous work links AxD pathogenesis to oxidative and electrophilic stress, and notably, many AxD GFAP mutations introduce nucleophilic residues, particularly cysteine. We investigated how these GFAP mutations affect thiol redox regulation and filament organization using astrocytic cell models. Expression of AxD-mutants (Arg239Cys, Arg79Cys and Glu373Lys), increased formation of disulfide-bonded oligomers, and augmented sensitivity to oxidative and electrophilic stress. These changes correlate with abnormal GFAP network organization, enhanced aggregation, and morphologically-distinct remodeling in response to hydrogen peroxide, hypochlorous acid and peroxynitrous acid. In contrast to wild-type GFAP, oxidative challenges induced persistent alterations in mutant GFAP filament architecture. Detailed characterization of the alterations elicited by the presence of a second Cys was performed in cells expressing the GFAP Arg239Cys, which unveiled impairment of mitochondrial and lysosomal function and increased lysosomal fragility, suggesting a feed-forward loop between GFAP oxidation, cytoskeletal disruption and oxidative stress. Complementary high-resolution LC-MS/MS studies of recombinant oxidant-exposed wild-type and GFAP variants showed that the AxD mutations enhance the susceptibility of Cys residues, including the mutation-introduced Cys, to reversible and irreversible oxidative modifications, such us intermolecular (protein-protein) disulfide bond and oxy-acid formation, indicating a direct effect of the mutations on protein vulnerability to modification. Together, these data show that AxD-associated GFAP mutations favor reversible and irreversible oxidative protein modifications, both in cells and in vitro, potentially contributing to impaired protein filament assembly and more severe responses to oxidative stress. This heightened vulnerability to oxidative and electrophilic stress may contribute decisively to astrocyte dysfunction and the progression of Alexander disease.

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FT I_04/PP I_B02

Uric acid– driven redox modulation in endothelial cells: Insights from a novel biosensor

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Uric acid (UA) has paradoxical anti- and pro-oxidant effects. Some authors suggest its pro-oxidant action is indirectly mediated by Nox activation. Our group showed UA acts as a pro-oxidant via heme protein oxidation, generating urate radical and urate hydroperoxide (HOOU). UA is linked to endothelial cell (EC) dysfunction and cardiovascular disease. This study investigated whether UA's pro-oxidant effects result from HOOU/lipid hydroperoxides or activation of ROS-generating enzymes producing superoxide ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2), and examined redox signaling between endothelial and immune cells. We used a novel GFP-based biosensor, PxIII-roGFP2, to detect organic peroxides and compared its reactivity. Second-order rate constants were $2.7 \pm 0.29 \times 10^6$, $4.6 \pm 0.42 \times 10^7$, and $1.5 \times 10^5 \pm 4.0 \times 10^{-4}$ M^{-1} s^{-1} for HOOU, linoleic acid hydroperoxide, and H_2O_2, confirming higher selectivity for organic peroxides. HUVECs were transiently transfected with mitochondrial or total PxIII-roGFP2, or Hyper7, to monitor organic hydroperoxides and H_2O_2 in real time. Confocal microscopy showed that 2 h UA treatment increased PxIII-roGFP2 oxidation, likely by stimulating organic hydroperoxide production in HUVECs. Hyper7 oxidation was not detected, indicating no measurable H_2O_2 production. The MitoSOX assay indicated no $O_2^{\bullet-}$ formation. Organic hydroperoxides act as signaling or cytotoxic molecules; therefore, UA's effects on endothelial–immune cross-talk were assessed after 48 h. Proteins modified by lipid peroxidation and GPx4 and ICAM-1 expression were analyzed by Western blot in HUVECs. UA, in the presence of macrophages, increased all markers. UA induced redox imbalance and pro-inflammatory effects even at physiological levels, highlighting the importance of regulating UA in cardiovascular disease and supporting new therapeutic strategies.

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FT I_05/PP II_B01

Copper oxide nanoparticles restore redox-regulated autophagic homeostasis via tfeb signaling in colistin-induced acute kidney injury

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Colistin is a last-line antibiotic for multidrug-resistant infections but frequently induces acute kidney injury (AKI) driven by oxidative stress and intracellular stress signaling. Although autophagy is generally considered cytoprotective, excessive or dysregulated activation may contribute to renal tubular injury. The redox-dependent mechanisms underlying maladaptive autophagy in colistin-induced AKI remain incompletely defined. We investigated whether copper oxide nanoparticles (CuO NPs) could therapeutically restore redox-autophagy homeostasis in this model. HK-2 cells and a murine model of colistin-induced AKI were treated with CuO NPs. Oxidative stress and autophagic flux were evaluated using intracellular ROS assays and LC3-II/p62 turnover analysis. TFEB signaling regulation was assessed using pharmacologic and molecular approaches. Renal function and histologic injury were analyzed in vivo. Colistin exposure induced severe redox imbalance accompanied by excessive autophagic activation and lysosomal stress. CuO NP treatment significantly reduced oxidative stress and restored balanced autophagic flux. Mechanistically, CuO NPs reactivated Akt/GSK3β signaling and suppressed aberrant TFEB activation, preventing lysosomal overactivation and re-establishing intracellular stress homeostasis. In vivo, CuO NP administration significantly improved renal function and attenuated tubular injury. These findings identify redox-driven autophagic dysregulation as a central pathogenic mechanism in colistin-induced AKI. CuO nanoparticles confer renoprotection by restoring TFEB-regulated autophagic

homeostasis, highlighting nanomedicine-based targeting of redox-autophagy signaling as a promising therapeutic strategy for drug-induced kidney injury.

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FT I_06/PP I_D11

Ageing alters cysteine oxidation–mediated redox signalling in skeletal muscle: Integrative omics and AI-based structural predictions

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Ageing is associated with loss of skeletal muscle mass and strength (sarcopenia) and disrupted redox homeostasis. Short-term metabolic processes are controlled by allosteric effectors and/or post-translational modifications, including oxidative modifications of cysteine residues. Redox signalling is essential for muscle adaptation, yet the mechanisms by which ageing disrupts cysteine-based regulation are poorly defined. The drivers of site-specific reactivity and signalling specificity in aged muscle remain unknown. Here, we interrogated the OxiMouse dataset [1] to map age-dependent cysteine oxidation in skeletal muscle and, using state of the art AI, simulate oxidative modifications at key residues to predict structural and functional consequences. Ageing was found to remodel the redox landscape through selective oxidation of discrete cysteine residues, often in a site-specific manner, even within the same protein. This supports a model in which ageing drives pathway-targeted modulation of protein function rather than a uniform, global oxidative shift. Moreover, age-related cysteine oxidation is not randomly distributed but appears to target interconnected protein networks involved in mitochondrial metabolic pathways, muscle structure and function, and proteostasis, indicating a coordinated remodelling in redox signalling as a hallmark of skeletal muscle ageing. To connect these proteomic signatures to mechanisms, we used AlphaFold3 to simulate progressive cysteine oxidation (sulfenic/sulfinic/sulfonic states) and predict structural outcomes for individual proteins. This approach recapitulated established redox-dependent regulation, such as allosteric weakening of KEAP1–NRF2 DLG-motif engagement following oxidation of KEAP1 Cys151. The same strategy was then applied to prioritise functionally important cysteines identified in our dataset, exemplified by the predicted destabilisation of SOD1 metal coordination and dimer stability when key disulfide/oxidation states are perturbed. These results support a model in which skeletal muscle ageing drives selective, rewiring of cysteine-based redox signalling networks and demonstrate how integrating deep redox proteomics with AI-based structural simulation can prioritise key oxidation-sensitive cysteines as mechanistic nodes and potential intervention targets for sarcopenia.

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Flash Talks II – Inflammation and Redox-regulated Adaptations

FT II_01/PP II_B09

Non-canonical ferroptosis inhibitor orchestrates metabolic, redox, and iron-regulatory adaptations

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Acute and chronic liver diseases remain a major global health burden, driven in large part by excessive hepatocyte death. Ferroptosis, an iron-dependent form of programmed cell death triggered by lipid peroxidation, is increasingly recognized as a key driver of liver pathology. Here, we show that a potent small-molecule ferroptosis inhibitor identified in our lab ($EC_{50} = 150$ nM) orchestrates a coordinated network of metabolic, redox, and iron-regulatory adaptations that protect hepatocytes from ferroptotic death. RNA sequencing identified pyruvate dehydrogenase kinase 4 (PDK4) as a candidate gene of interest, and functional knockdown experiments revealed that it is essential for mediating resistance to ferroptosis. Mechanistically, inhibitor treatment induces PDK4 upregulation and an increase in the Coenzyme Q10 reduced-to-oxidized ratio ($CoQ10H_2$/CoQ10), reflecting a metabolic shift from glucose-derived pyruvate oxidation toward fatty acid catabolism that supports redox buffering. Meanwhile, hepatocytes establish a high-turnover iron state, with upregulated transferrin receptor (TFRC), ferritin (FTH), and nuclear receptor coactivator 4 (NCOA4), allowing control of iron uptake, storage, and mobilization alongside suppression of lipid peroxidation. Collectively, these redox, metabolic, and iron-regulatory changes prevent ferroptotic death, providing a mechanistic framework for hepatocyte protection and a rationale for therapeutic strategies targeting ferroptosis.

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FT II_02/PP I_C04

Mitofusin 2 deficiency causes pancreatic fibrosis after inducing acute pancreatitis in mice

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Mitochondrial dynamics regulate organelle quality control, homeostasis, and function. Specifically, mitochondrial fusion optimizes bioenergetic efficiency and acts as a compensatory mechanism against oxidative stress. In contrast, mitochondrial fission acts as a crucial quality control mechanism by generating smaller organelles that may be subsequently removed through mitophagy. GTPases Mitofusin 1 and 2 (MFN1/2) and optic atrophy 1 (OPA1) mediate fusion of outer and inner membranes, respectively, whereas fission is mediated by the dynamin-related GTPase (DRP1). Our results show that MFN2 protein levels decreased 24h after cerulein-induced acute pancreatitis induction, whereas MFN1 remained unchanged. To study the role of MFN2 in acute pancreatitis, we generated acinar cell-specific Mfn2 knock-out mice (MFN2 KO). In these mice, plasma amylase activity did not increase following induction of pancreatitis, suggesting a restrained exocrine response despite pancreatitis induction. High-resolution respirometry using the Oroboros system shows that acute pancreatitis markedly diminished the respiratory capacity of pancreatic mitochondria through mitochondrial complexes I and II in wild-type mice. However, MFN2 KO mice exhibited lower mitochondrial respiration than their wild type littermates under basal conditions and exhibited no further reduction with acute pancreatitis. Histological analysis via Masson's trichrome staining revealed prominent collagen deposition in MFN2 KO mice at 24h after pancreatitis induction. This structural remodeling was consistent with an upregulation of the mRNA expression of redox-sensitive cytokine Tgf-β1 and also of the α-SMA protein, suggesting an accelerated fibrogenic response. In conclusion, our findings show that MFN2 deficiency promotes an unusually early onset of pancreatic fibrosis, directly linking mitochondrial dynamics to pancreatic fibrogenesis.

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FT II_03/PP I_D10

Coq10 supplementation prevents the inhibition of myogenic differentiation in injured c2c12 murine myoblasts

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Recent scientific research shows that oxidative stress and mitochondria can act

as potential regulators of skeletal muscle myogenesis process, which underlies the repair and regeneration mechanisms of muscle tissue. Its impairment can lead to loss of skeletal muscle mass and strength, known as sarcopenia, and consequently to the leakage of its functionality and performance, accelerating the aging process. Preservation of mitochondrial integrity and functionality in differentiating myoblasts, is necessary to complete the myogenic process during myotube maturation. Coenzyme Q_{10} (CoQ_{10}), is an endogenous and lipophilic mitochondrial nutrient, with antioxidant and bioenergetic roles. Transported by plasma lipoproteins, CoQ_{10} is involved in ATP production, as an electron carrier inside the mitochondria electron respiratory chain, and in the protection from oxidative damage to biological membranes, by regeneration of vitamin E and vitamin C. The aim of this study was to investigate the role of ubiquinol to prevent the inhibition of myogenic differentiation in oxidatively injured skeletal muscle C_2C_{12} murine myoblasts. In order to identify CoQ_{10} bioavailability, cellular uptake was first evaluated by performing a dose-response curve by incubating cells for 24 h with 0.5, 1, 2.5, 5, 10 μg/mL of both ubiquinone and ubiquinol. C_2C_{12} cell cultures were treated at 70% confluence with the lowest concentration of ubiquinol (0,5 μg/mL) for 24 hours until 100% confluence. The cells were induced for differentiation and, after 24 hours, were treated for 1 hour with 650 μM of H_2O_2. After 24 and 72 h from exposure, analysis about oxidative damage, mitochondrial dynamic, morphometric evaluations and functionality were performed. Exogenous ubiquinol prevented hydrogen peroxide-induced oxidative damage, preserving the activity of key regulators of myoblast differentiation into myotubes, as confirmed by confocal imaging and contractility analysis. These findings support a potential role for ubiquinol in maintaining skeletal muscle function by sustaining cellular differentiation processes, in particular during aging.

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FT II_04/PP I_C02
Methylation analysis of sepsis patients reveals intrinsic differences at admission

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Sepsis is a "life-threatening organ dysfunction caused by a dysregulated host response to infection". A subset of these patients experience prolonged ICU stays and may develop "Persistent Inflammation and Catabolic Syndrome" (PICS). They have higher rates of readmission and reinfection, with increased probability of morbidity and mortality in the subsequent months and years. However, the heterogeneity of the patient hinders the administration of the correct treatment. The use of novel biomarkers is key to diagnose and classify during the initial hours of admission, decreasing the risk of adverse outcomes. In this work, we focus on establishing epigenetic biomarkers that can accurately predict higher risk patients due to intrinsic methylation patterns, as well as possible modifications of these markers due to sepsis that can increase the probability of future complications. We obtained PBMCs from peripheral blood from sepsis patients admitted to the ICU of the Clinical University Hospital of Valencia. Sample was collected during the first 24h of admission, after 14 days of admission, and at 6 months and 12 months after first admission to the ICU. Using the DNA methylation array EPIC850K, we first studied the differences between non-PICS patients and PICS patients at admission, as well as differences after a prolonged ICU stay. Differentially methylated genes and promoters were selected for further validation by pyrosequencing, such as the VARS2 promoter. VARS2 is a valyl-tRNA synthetase that is essential for mitochondrial translation, with its dysregulation having an impact on redox metabolism and mitochondrial fatty acid oxidation. Pyrosequencing of an independent cohort revealed that at admission PICS patients show hypermethylation in VARS2 promoter in relation to non-PICS patients. Furthermore, this difference is maintained at 14 days, 6 months, and 12 months after admission. Determining and characterizing these differences could aid in the adequate diagnosis and monitoring of the sepsis patient.

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FT II_05/PP I_C05
Targeting IL-2R nitration to enhance Treg cell-based immunotherapy

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Redox balance is crucial in the pathogenesis of multiple sclerosis (MS). The peroxynitrite in CNS is a major cause of nerve damage. Regulatory T cell (Treg)-based immunotherapy has emerged as a promising strategy to restore immune tolerance in MS; however, the redox-dependent mechanisms that limit Treg stability and therapeutic efficacy remain poorly understood. However, the mechanism of regulating peripheral peroxynitrite to affect Treg-mediated immune regulation has not been fully defined. Herein, we tested the hypothesis that peripheral peroxynitrite could lead the nitration of interleukin-2 receptor (IL-2R) to reduce Treg population and functions and aggravate MS pathology. We found that the increases of 3-nitrotyrosine and IL-2R nitration in Treg were coincided with disease severity in experimental autoimmune encephalomyelitis (EAE) mice. Mechanistically, peroxynitrite induced IL-2R nitration, reduced periphery Treg expansion and functions, and increased Teff infiltration in the CNS, aggravating demyelination and neurological deficit in the EAE/MS pathology. Those changes were abolished by peroxynitrite decomposition catalyst treatment. Those results suggest that targeting IL-2R nitration in Tregs could be a promising therapeutic strategy to modulate CNS immune homeostasis in the EAE/MS pathology. Notably, adoptive transfer of autologous Tregs pretreated with the peroxynitrite catalyst significantly reduced Th17 cell populations in both peripheral lymph nodes and the lumbar spinal cord of recipient EAE mice, and markedly alleviated neuropathology. Together, our results indicate that targeting peripheral IL-2R nitration represents a mechanistically grounded strategy to optimize Treg-based cell therapy for the treatment of MS.

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FT II_06/PP I_D09
Resistance training preserves functional capacity in aged mice: interactions with harmol and piceid supplementation

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Mitochondrial dysfunction is associated with clinical biomarkers of sarcopenia and frailty, making it a promising therapeutic target for mitohormetic strategies aimed at preserving functional capacity during aging. Mitohormetic agents induce mild-reversible stress, activating adaptive responses improving mitochondrial function and resilience. We aimed to evaluate whether three mitohormetic strategies: harmol (H) or piceid (P) supplementation and/or resistance training (T), could prevent or delay functional and mitochondrial decline in old mice. Old male C57BL/6 mice ($n = 63$; 20.3 ± 0.1 months) underwent a 6-week intervention consisting of resistance training and/or oral harmol or piceid supplementation (100 mg/kg body weight). Six experimental groups were included: sedentary-water (SW), trained-water (TW), sedentary-piceid (SP), trained-piceid (TP), sedentary-harmol (SH), and trained-harmol (TH). Functional performance was assessed before and after the intervention. Mitochondrial function was evaluated in fresh permeabilized skeletal muscle fibers and in

isolated frozen mitochondria. Additional measurements included mitochondrial membrane potential (ΔΨm) by flow cytometry, peroxide production in a mitochondria-enriched fraction, citrate synthase activity, and plasma levels of 8-hydroxydeoxyguanosine (8-OHdG) and malondialdehyde (MDA) as markers of oxidative damage.VO_2max declined with age in all groups; however, this decline was attenuated in trained mice compared with sedentary animals, indicating a protective effect of resistance training and, to a lesser extent, harmol supplementation. No significant differences were observed in mitochondrial respiration through complexes I, II, IV, I+II, maximal, or basal respiration, nor in citrate synthase activity, ΔΨm, peroxide production, or MDA levels. Plasma 8-OHdG levels were significantly lower in the TH group compared with most other groups. In isolated mitochondria from frozen muscle, differences were observed in complex IV activity among trained groups, with distinct responses to harmol and piceid. Resistance training primarily drove functional benefits in old mice, while harmol may modestly affect mitochondrial complex IV activity and oxidative DNA damage.

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Flash Talks III – Brain Function & Neurodegeneration and Ischemia-Reperfusion

FT III_01/PP I_D01
Proteome profile of Alzheimer-like phenotypes in the brain of young and old individuals with Down Syndrome: focus on BACH1/NRF2 axis

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Down syndrome (DS) stands out as the most prevalent genetic contributor to intellectual disability, marked by the presence of an extra copy of chromosome 21. Notably, individuals with DS exhibit significant neuropathological changes for a diagnosis of Alzheimer's disease (AD) typically by the age of 50ys. To search and identify biomarkers crucial for detecting and understanding the mechanisms involved in DS neuropathology, we delved into the analysis of protein expression on post-mortem brain samples. We evaluated the frontal cortex of post-mortem brain samples from patients with DS both before and after the onset of AD pathology (DSAD), in comparison with age-matched healthy patients (HD Young and Old). Employing a comprehensive label-free shotgun proteomics approach, we sought to gain a deeper understanding of the intricate protein profiles associated with DS and its progression into DSAD. Further, in order to better decipher relevant changes outlined by proteomics, coupled in vitro analysis on primary cortical neurons isolated by a tg mouse model of DS (Ts2Cje) have been employed. Collected results have been analyzed using different databases and analysis software to understand relevant pathways, networks, and function associated with experimental data. These observations reveal a genotype-associated alteration in protein expression profile that further progresses with aging. Notably, the affected signaling pathways encompasses energy-related processes, synaptic transmission, and stress response. Particularly, in the comparison between DS and age-matched healthy individuals, we discern that genotype plays a pivotal role in driving mitochondrial dysfunction, impairing insulin signaling and oxidative phosphorylation, and inducing aberrant autophagy and Nrf2-mediated antioxidant response. This latter emerged as a robust signature of the aging trajectory of DS individuals, by aberrantly modulating the redox phenotype, ferroptosis cascade and autophagy system, that act in concert to drive neuronal senescence and degeneration. This study contributes to shedding light on the intricate relationship among gene-protein-phenotype by showing how extensive protein remodelling, caused by aneuploidy, dynamically occurs through life in DS subjects, resulting in significant modulation and severity of phenotypes.

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FT III_02/PP I_C09
Defective PI3K/Akt–FoxO3a–mediated stress adaptation in Rett syndrome

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Rett syndrome (RTT) is a severe neurodevelopmental disorder caused by MECP2 mutations, characterized, among others, by systemic redox imbalance and oxinflammatory features. Increasing evidence indicates that defective adaptive responses to oxidative stress are critical drivers of disease progression. Within this framework, the transcription factor Forkhead box O3 (FoxO3a) has emerged as a key regulator of cellular stress resilience, coordinating antioxidant defenses, autophagy and cell survival programs, although its contribution to RTT pathophysiology has not yet been characterized. This study examines whether an aberrant adaptative response driven by the PI3K/Akt–FoxO3a axis could contribute to abnormal redox homeostasis typical of RTT. To evaluate this signaling dynamics, control and RTT fibroblasts were subjected to 16 h serum deprivation followed by 30 minutes challenge with H_2O_2 (100 μM) to model oxidative stress. AKT and FOXO3A mRNA levels were quantified by qPCR, and total and phosphorylated Akt and FoxO3a were assessed by immunoblotting. Preliminary data shows a significant upregulation of AKT and FOXO3A mRNA in control fibroblasts following H_2O_2 treatment, while RTT fibroblasts exhibit an attenuated and non-significant increase in gene expression after the oxidant insult. At the protein level, total AKT significantly decreases in H_2O_2-treated RTT cells compared to their untreated condition with its phosphorylated form showing a similar trend. Moreover, total FoxO3a protein levels tend to decrease in RTT fibroblasts after oxidative challenge relative to their untreated condition. Ongoing immunofluorescence analyses aim to define how oxidative stress reshapes the subcellular compartmentalization of FoxO3a and its phosphorylated form in RTT fibroblasts, while further gene expression studies will assess downstream targets of this axis. Altogether, these preliminary findings suggest that a dysfunctional Akt–FoxO3a axis could play a role in RTT-associated redox imbalance and "oxinflammatory" features, highlighting this stress-response pathway as a promising target for novel therapeutic strategies in RTT.

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FT III_03/PP I_B01
Dietary nitrate drives nitrite signaling to restrain complex I reverse electron transfer after ischemia–reperfusion

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Ischemia–reperfusion contributes to neuronal injury in stroke and related disorders. Reperfusion drives rapid redox imbalance and oxidant generation, yet therapies that blunt these early events remain limited. Inorganic nitrite (from NO oxidation or dietary nitrate) can be reduced to NO under hypoxia/acidosis and may remodel mitochondrial electron flow. We tested whether nitrite restrains the reoxygenation-associated response in hippocampal tissue and identified the mitochondrial mechanism involved.
Oxidative phosphorylation (OxPhos) was assessed by high-resolution respirometry in saponin-permeabilized rat hippocampal slices subjected to an anoxia/reoxygenation protocol (A/R). During NADH-linked (Complex I) OxPhos, reoxygenation increased oxygen flux to 115.0 ± 2.9% of the pre-anoxia baseline versus time-matched no A/R slices ($p = 0.0005$). This respiratory surge, consistent with enhanced oxidant-generating conditions, was prevented by nitrite (10 μM; 99.3 ± 5.0%). With succinate-supported respiration (Complex II-

linked OxPhos), reoxygenation similarly increased flux (101.6 ± 2.5% control vs 115.2 ± 1.2% A/R), and nitrite did not prevent this increase (A/R + nitrite: 111.5 ± 3.3%). Addition of ascorbate abolished nitrite's protection, consistent with nitrosation-dependent mechanisms. Blocking Complex I reverse electron transport (RET) with rotenone during succinate-supported respiration eliminated the surge (102.8 ± 2.2% control; 104.0 ± 1.8% A/R), identifying Complex I RET as a major contributor and the primary locus of nitrite action.

Dietary nitrate supplementation (10 mM, 1 week) increased plasma nitrite (0.4 ± 0.1 to 1.6 ± 0.2 μM; $p = 0.0002$) and showed an upward trend in hippocampal mitochondrial nitrite (0.11 ± 0.02 to 0.17 ± 0.06 nmol/mg protein; p=0.3) measured using gas-phase chemiluminescence. Nitrate reduced the reoxygenation-associated change from 108.8 ± 1.8% (control) to 79.2 ± 6.8%, and broad-spectrum antibiotics abolished these effects, supporting a microbiota requirement for nitrate-to-nitrite conversion.

Nitrite limits reoxygenation-associated mitochondrial stress by suppressing Complex I RET via nitrosation-dependent signaling, and dietary nitrate engages this pathway in a microbiota-dependent manner.

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FT III_04/PP I_D03

APPL2 deletion promotes neurogenesis and functional recovery after ischemic stroke via regulation of mitochondrial dynamics and function

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Ischemic stroke remains a leading cause of long-term disability, and enhancing neurogenesis represents a key strategy for improving post-stroke functional recovery. Adaptor protein containing phosphotyrosine interaction, PH domain, and leucine zipper 2 (APPL2) is known to participate in diverse cellular signalling pathways; however, its specific role in post-stroke neurogenesis and mitochondrial regulation has not been elucidated. In this study, we investigated the function of APPL2 in neural regeneration and mitochondrial dynamics following ischemic stroke using brain-specific APPL2 conditional knockout (cKO) mice. APPL2 cKO mice were generated by crossing Nestin-Cre mice with APPL2-floxed mice. Under basal conditions, APPL2 cKO mice exhibited significantly enhanced cognitive function, motor coordination, and reduced anxiety-like behaviour, as assessed by Morris water maze, novel object recognition, rotarod, and open field tests. At 28 days after middle cerebral artery occlusion (MCAO), APPL2 cKO mice demonstrated markedly improved long-term functional recovery across all behavioural paradigms, accompanied by increased proliferation of neural stem/progenitor cells and accelerated neuronal differentiation in the hippocampal dentate gyrus. To explore the underlying mechanisms, C17.2 neural stem cells (NSCs) were subjected to oxygen-glucose deprivation (OGD) followed by induced differentiation. APPL2 knockout significantly accelerated neuronal differentiation in OGD-exposed NSCs. This effect was associated with enhanced mitochondrial function, as evidenced by increased mitochondrial membrane potential, elevated mitochondrial superoxide levels, and greater mitochondrial mass. Moreover, APPL2-deficient NSCs maintained a fused, network-like mitochondrial morphology, whereas control cells exhibited mitochondrial fragmentation. Collectively, these findings identify APPL2 as a negative regulator of neurogenesis and mitochondrial homeostasis. Deletion of APPL2 confers neuroprotection and promotes post-stroke functional recovery, at least in part, through the optimization of mitochondrial dynamics and function. Thus, APPL2 may represent a novel therapeutic target for ischemic stroke.

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FT III_05/PP I_D02

Role of the mitochondrial sodium/calcium exchanger NCLX in ferroptosis, cell viability and brain injury after ischemic stroke

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Ischemia-reperfusion (IR) injury, as can happen in ischemic stroke with recanalization, causes oxidative stress and iron accumulation, critical processes that can trigger cell death by ferroptosis. In this study, we evaluated whether NCLX inhibitors modulate molecular pathways of ferroptosis and cell death, both in cellular models and in a murine model of cerebral ischemia-reperfusion. In human neuroblastoma SK-N-DZ cells, IR induced lipid peroxidation, reactive oxygen species (ROS) production, an increase in free iron levels and an upregulation of transferrin receptor (TfR1) and iron regulatory protein 1 (IRP-1), suggesting an adaptive regulation of iron uptake in response to oxidative stress. Pharmacological inhibition of NCLX, as well as treatment with the ferroptosis inhibitor ferrostatin-1 (Fer-1), reduced ROS, lipid peroxidation and free iron levels. However, regarding cell viability, neither in SK-N-DZ cells nor in primary neurons did we observe any improvement upon treatment with NCLX inhibitors; indeed, in primary cortical neurons, viability was further decreased compared to vehicle-treated cells, indicating a cell-type–dependent vulnerability under IR conditions. In mice subjected to transient middle cerebral artery occlusion (tMCAO) as a model of cerebral ischemia-reperfusion, treatment with the NCLX inhibitor ITH-12575 significantly decreased infarct volume and brain iron accumulation in the ischemic hemisphere, reducing neuronal TfR1 upregulation in the peri-infarct area, consistent with a partial attenuation of ferroptosis-related pathways. Nonetheless, ITH-12575–treated animals showed increased brain edema, higher mortality and elevated expression of inflammatory and blood–brain barrier disruption markers, including IL-1β and MMP-9. Overall, these findings highlight that NCLX inhibition can beneficially modulate iron homeostasis and molecular hallmarks of ferroptosis after ischemia-reperfusion but also reveal important safety concerns related to neuronal survival and neuroinflammatory responses that must be carefully addressed before considering this strategy as a therapeutic avenue for ischemic stroke.

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FT III_06/PP I_B04

Toll-like receptor 2 impacts small intestinal villus capillarization through epithelial dual oxidase 2

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The commensal microbiota critically shapes postnatal intestinal development and vascular remodeling. In the small intestine, capillary network formation within villi is essential for nutrient absorption and depends on an epithelial–to-endothelial crosstalk. Redox signaling represents a key interface in this process. The NADPH oxidase Dual oxidase-2 (DUOX2) exerts microbicidal activity through reactive oxygen species (ROS) generation and regulates microbiota–host interactions, while Toll-like receptor 2 (TLR2) modulates epithelial renewal and angiogenesis. However, their contribution to redox-dependent vascular remodeling in the small intestine is incompletely understood. Here, we explored the role of epithelial TLR2 and DUOX2 in small intestinal villus vascularization. Expression of gut epithelial DUOX2 was significantly increased in mice deficient in epithelial TLR2 expression. While both, global and epithelial-specific Duox2-deficient mice showed a reduction in mucosal capillary networks, epithelial-specific Tlr2-deficient mice showed enhanced gut mucosal vascularization as compared to wildtype controls. Loss of DUOX2 activity resulted in an altered microbial community structure in the small intestine, as

determined by 16S rRNA gene sequencing. Under inflammatory conditions, in the dextran sulfate sodium (DSS) model of acute intestinal inflammation and in biopsies from the inflamed terminal ileum of Crohn's disease patients, Duox2 expression and villus vascularization were markedly increased. Collectively, our findings identify epithelial DUOX2 as a redox-active regulator of microbiota-driven epithelial–to-endothelial crosstalk that controls villus vascularization. Moreover, epithelial TLR2 emerges as a modifier of small intestinal vascular remodeling, at least in part through regulation of DUOX2. These data highlight a redox-dependent mechanism linking innate immune signaling and microbial colonization to intestinal vascular morphogenesis.

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Flash Talks IV – Environmental and Pharmaceutical Exposures

FT IV_01/PP I_A01
Co-delivery of ibuprofen and Curcumin in nebulized polymeric micelles to optimize household air pollution adverse effects

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It has been reported that the exposure to indoor particulate matter (PM) impairs redox metabolism and promotes inflammation, which might aggravate respiratory diseases. Lung epithelial cells are suggested to play a central role in this scenario, since they produce inflammatory and oxidative stress mediators following PM uptake. In the present work we aimed to study the pathways leading to redox metabolism alterations and NLRP3 inflammasome activation in A549 cells, EpiAlveolar 3D tissue and Balb/c mice exposed to indoor dust (ID). Additionally, this project explores a nano-pharmaceutical approach to enhance the efficacy of anti-inflammatory and antioxidant drugs, using curcumin (Cur) and ibuprofen (Ibu). When A549 cells were exposed to ID, intracellular redox status and oxidative damage to lipids measured as 4-HNE protein adducts were observed. Additionally, dose-and time-dependent NFkB nuclear translocation, NLRP3-inflammosome activation and IL-1β levels were increased after ID exposure. On the other hand, EpiAlveolar 3D tissue exposed to ID showed an impairment in barrier integrity and an increase in HO-1 and 4-HNE signal. In addition, colocalization of both inflammasome components, NLRP3 and ASC, were also found as well as IL-1β levels. When nanomicelles pretreatment was performed, A549 cells showed intracellular redox status values and IL-1β levels similar to control group. The protective pathway seems to be involved with Nrf2 nuclear translocation and further HO-1 increase. On *in vivo* studies, lung redox metabolism alterations due to ID on Balb-c mice were reverted with a previous inhalation of nanomicelles, suggesting a protective effect. Finally, biodistribution studies with radiolabeled nanomicelles using ^{99m}Tc, demonstrated the successful delivery of micelles into the lungs by intranasal instillation. Our findings contribute to the understanding of the mechanisms by which ID promotes inflammation and oxidative stress in lung tissues and, overall, our nanoformulations stand as promising nanotechnological platforms to optimize lung therapy.

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FT IV_02/PP I_A02
Oral administration of nano- and microplastics disrupts redox homeostasis and spindle organization in mouse oocytes: enhancement by low-dose cadmium and limited protection by melatonin

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Nano- and microplastics (NMPs) are emerging environmental contaminants increasingly recognized as potential inducers of oxidative stress and reproductive toxicity. Their bioaccumulation and interaction with hazardous metals such as cadmium (Cd) may exacerbate reactive oxygen species (ROS) generation, thereby disrupting redox homeostasis and compromising oocyte quality. However, the in vivo redox-mediated effects of exposure to different-sized NMPs on the female germline remain poorly defined. This study investigated the impact of 35-day oral administration of 40 or 200 nm polystyrene NMPs, alone or combined with a low, non-toxic dose of Cd, on the quality of in vivo-derived mouse oocytes, and evaluated the protective potential of the antioxidant melatonin. Young CD1 female mice received 5 μg/mL NMPs in drinking water. Experimental groups included NMPs alone, 40 nm NMPs + Cd (13.5 μg/L), and corresponding groups co-treated with melatonin (10 μM). Following superovulation (PMSG/hCG), ovulated oocytes were collected for analysis. Total ROS levels were assessed by CellROX staining, while meiotic spindle organization, chromosome alignment, and SIRT1 expression were evaluated by immunofluorescence. Although NMP exposure did not affect the number of mature oocytes retrieved, all treatments induced significant spindle abnormalities, including altered spindle length and pole morphology, which were not prevented by melatonin. Exposure to 200 nm NMPs significantly increased total ROS levels, an effect reversed by melatonin. Both 200 nm NMPs and 40 nm NMP + Cd upregulated SIRT1 expression, suggesting the activation of a redox-sensitive adaptive response that persisted despite antioxidant supplementation. In conclusion, chronic oral NMP exposure disrupts meiotic architecture and redox balance in vivo, triggering SIRT1-mediated stress adaptation. Co-exposure to non-toxic dose of Cd further potentiates oocyte alterations, highlighting a synergistic vulnerability to combined environmental contaminants. While melatonin attenuates ROS overproduction, it does not fully rescue structural meiotic defects. Ongoing F1 and F2 studies will determine potential multi- and transgenerational redox and reproductive consequences.

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FT IV_03/PP I_A03
Resveratrol counteracts redox imbalance and mitochondrial dysfunctions induced by nano and microplastics (NMPs) in human granulosa cells

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The widespread presence of nano- and microplastics (NMPs) in the environment has raised growing concerns about their impact on human health; however their effects on female reproduction remain largely unexplored. Investigating how NMPs affect ovarian cells is particularly relevant, as these particles can disrupt redox balance and compromise cellular functions. This study aimed to elucidate the direct effects of NMPs on human granulosa cell (hGC) function, focusing on cell survival, oxidative stress, and mitochondrial activity, and to evaluate the potential protective role of the natural antioxidant Resveratrol. The hGC line KGN was exposed to NMPs of differentsizes (40, 70, 100, 200 nm) at concentrations ranging from 5 to 1000 μg/mL for 24–168 h, with or without 5 μM Resveratrol. NMP internalization was assessed by confocal microscopy and live imaging (Nanolive, MediaSystem Lab). Cell viability, apoptosis, and mitochondrial ROS were evaluated using CellCountingKit-8, TUNEL assay, and MitoSOX Red, respectively. Mitochondrial bioenergetics was analyzed using the Mito-Stress kit (SeahorseXFe96, Agilent), and ATP production was quantified with the CellTiter-Glo ATP assay kit (Promega). Oxidative DNA damage was measured by 8-OHdG immunofluorescence. Protein expression of key-regulators of cell cycle progression (p21, p53) and antioxidant response (catalase, SOD1, SOD2, SIRT1, SIRT3), as well as 4-HNE, was analyzed by Western blotting. Our findings revealed that NMP exposure impaired cell cycle progression and survival in a time-dependent manner. All NMP sizes induced oxidative imbalance and cellular injury, with smaller NMPs causing more pronounced mitochondrial dysfunctions and bioenergetic alterations. In response, hGCs activated SIRT1-mediated adaptive mechanisms to enhance antioxidant defenses. Resveratrol supplementation mitigated NMP-induced oxidative damage and mitochondrial stress. In conclusion, NMP toxicity in ovarian cells is closely linked to disrupted redox homeostasis and impaired mitochondrial function. The protective effects of Resveratrol underscore the potential of antioxidant-based strategies to

counteract NMP-related threats to female reproductive health.

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FT IV_04/PP I_C01

New therapeutic strategies for cytokine storm-associated diseases using redox nanoparticles

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Hemophagocytic lymphohistiocytosis (HLH) is a fatal hyperinflammatory disease characterized by clinical manifestations resulting from a cytokine storm, including severe cytopenia and splenomegaly. Current treatment strategies frequently fail to cure HLH, and many patients die due to the disease or the complications of its treatment. Hence, safer and more effective therapies are strongly desired. Thus, we focused on redox nanoparticles (RNP), which are pH-sensitive and disintegrate under acidic conditions, effectively scavenging reactive oxygen species (ROS) at inflamed sites. Given the bidirectional relationship between ROS and inflammation, we evaluated whether RNP could exert therapeutic effects in a mouse model of HLH. HLH was induced in C57BL/6 mice by intraperitoneally injecting CpG and an IL-10 receptor-blocking antibody (αIL-10R) on days 0, 2, 4, and 7. RNP or vehicle was subcutaneously administered on the same days. Mice were humanely euthanized on day 8 or 9, and blood and spleen samples were collected for subsequent analyses. Treatment with RNP significantly suppressed body weight loss and improved clinical scores of HLH, leading to prolonged survival compared with the vehicle group. Cytopenia was ameliorated in the RNP-treated mice compared with the vehicle-treated group, although splenomegaly was not improved by the RNP treatment. Compared with naive mice, HLH-induced mice treated with vehicle exhibited increased frequencies of monocytes, macrophages, and neutrophils in splenocytes. RNP significantly reduced the frequencies of macrophages and neutrophils. Serum levels of multiple pro-inflammatory cytokines and chemokines, such as IFN-γ, TNF-α, monocyte chemoattractant protein-1 (MCP-1), IL-12(p70), and IL-6, were markedly increased in response to CpG/αIL-10R injections. RNP significantly reduced the levels of TNF-α, MCP-1, and IL-6. These findings demonstrate that RNP could efficiently alleviate HLH and provide insights into potential therapeutic strategies for other cytokine storm-mediated diseases. We are planning to conduct further analyses, including RNA sequencing of splenic immune cells.

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FT IV_05/PP I_C03

Hepatic supersulfides attenuate acetaminophen-induced liver injury via enhanced detoxification and anti-inflammatory mechanisms

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Acetaminophen (APAP) is a widely used antipyretic and analgesic agent; however, overdose can lead to hepatotoxicity and, in severe cases, acute liver failure. Development of therapeutics that mitigate APAP-induced liver injury is essential to prevent progression to hepatic failure. Upon overdose, APAP is metabolized in the liver to the highly reactive electrophile *N*-acetyl-*p*-benzoquinone imine (NAPQI), which induces hepatocellular damage. Glutathione (GSH), a key intracellular nucleophile, exists partially in a modified form as glutathione persulfide (GSSH), which exhibits enhanced nucleophilicity and functions as a supersulfide. While detoxification of NAPQI via GSH conjugation is well established, the role of GSSH in NAPQI detoxification has remained unknown. In this study, we investigated the protective role of hepatic supersulfides against APAP-induced liver injury using a murine model. Utilizing a newly developed tandem mass spectrometry technique, we demonstrated that supersulfides form conjugates with NAPQI, which are subsequently excreted in the urine. Moreover, administration of supersulfide donors, such as *N*-acetylcysteine (NAC) tetrasulfide and thioglucose tetrasulfide, elevated hepatic supersulfide levels and significantly attenuated APAP-induced liver injury. Notably, the protective effects of these donors surpassed those of conventional NAC treatment. Our findings suggest that the hepatoprotective effects of supersulfide donors involve not only enhanced detoxification of NQPOI, thereby reducing hepatocellular damage, but also suppression of inflammation. These results highlight the therapeutic potential of targeting hepatic supersulfides in the treatment of APAP overdose.

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FT IV_06/PP II_D01

Oxygen-dependent effects of thermal treatment on (poly)phenol composition and antiglycation mechanisms in chilean blackcurrant (*Ribes cucullatum*) extracts

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(Poly)phenols are natural antioxidants found in foods, which have been associated with health benefits. Their ability to inhibit the formation of advanced glycation end products (AGEs) has been attributed to multiple mechanisms, including scavenging of reactive oxygen species and dicarbonyl compounds. However, limited information is available regarding how thermal treatment (TT) under different oxidation conditions affects (poly)phenol composition and, consequently, their antiglycation activity and protein protection mechanisms. In this work, changes in chemical composition and antiglycation mechanisms of (poly)phenol-enriched extracts (PEE) from Chilean blackcurrant (*Ribes cucullatum*) were evaluated after exposure to different temperatures (4°C and 90°C) at two oxygen condition (0% and 20% O_2) in buffer pH 7.4. (Poly)phenolic composition and redox properties were characterized by HPLC-DAD-MS and cyclic voltammetry, respectively. Antiglycation activity was assessed by incubating bovine serum albumin (BSA) with glucose and different concentrations of PEE and determining IC50 values of fluorescence inhibition (ex=325/em=440). Samples exposed to 20% O_2 were more oxidized than those exposed to 0% O_2, and this promoted the isomerization and glycosylation of phenolic compounds when the PEE was thermally treated. These changes enhanced antioxidant capacity (DPPH and ORAC assays), increased methylglyoxal-trapping ability, and decreased the levels of carbonyl protein formation in thermally treated PEE. In contrast, PEE thermally treated at 90°C under 0% O_2 showed lower IC_{50} values for fluorescent AGEs compared with samples treated at 20% O_2. HPLC-DAD analysis after incubation with BSA with PEE for 40h, showed that PEE incubated at 0% O_2 possesses a higher association with BSA when compared with PEE incubated at 20% O_2, indicating different interaction association of (poly)phenols to BSA, which could likely affect the protein glycation. Overall, these results indicate that TT of (poly)phenols from *Ribes cucullatum* increases the antioxidant and antiglycation activities, compared without TT, highlighting the relevance of oxidation during thermal treatment in modulating antiglycation efficiency.

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10. Poster Presentations
Poster Presentations I Group A – Environmental Exposure

PP I_A01/FT IV_01
This abstract is published under FT IV_01.
PP I_A02/FT IV_02
This abstract is published under FT IV_02.
PP I_A03/FT IV_03
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PP I_A04
Redox imbalance mediates nano- and microplastic-induced meiotic dysfunction in mouse oocytes: protective effects of N-acetylcysteine

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Nano- and microplastics (NMPs) are widely distributed in the environment and are gaining attention for their potential impact on human health. Their presence in reproductive tissues has been reported, but their direct effects on female germ cells remain very poorly investigated.
The present study aimed to determine whether NMPs can penetrate mouse oocytes, to evaluate their effects on meiotic progression and redox homeostasis *in vitro*, and to test whether antioxidant supplementation with N-acetylcysteine (NAC) can mitigate NMP-induced alterations. Cumulus-oocyte complexes (COCs) were collected from PMSG-primed young CD1-mice and exposed to 5-50 μg/ml NMPs of different sizes (40 nm; 200 nm) during 16 h *in vitro* maturation (IVM). The uptake of fluorescent NMPs was assessed by confocal and STED super-resolution microscopy. After IVM, oocytes were isolated from cumulus cells (CCs), and the percentage reaching metaphase II (MII) was recorded. Gene expression was analysed by real-time TaqMan PCR. Mitochondrial and total ROS were evaluated by MitoSOX and CellROX staining, respectively. Spindle morphology and SIRT1 protein levels were assessed by immunofluorescence. Antioxidant intervention was implemented by supplementing the IVM medium with 250 μM N-acetylcysteine (NAC). Our data demonstrated that all NMPs were internalized by cumulus cells, whereas only 40 nm particles penetrated the oocyte. Exposure to 40 nm NMPs significantly reduced the MII rate and induced spindle abnormalities. Oocytes exposed to 200 nm NMPs exhibited increased mitochondrial and total ROS levels. 40 nm NMPs decreased Sirt1 and Sod2 transcripts while increasing SIRT1 protein expression. NAC supplementation significantly improved maturation rates in NMP-treated groups, reduced total ROS levels in 200 nm NMP-exposed oocytes, and restored SIRT1 protein levels in 40 nm NMP-treated oocytes. These findings indicate a size-dependent detrimental effect of NMPs on oocyte competence, identify oxidative stress as a key mediator of toxicity, and support the protective effects of NAC supplementation.

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PP I_A05
NLRP3 inflammasome as a new player in microplastic cutaneous toxicity

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Pollution is one of the main threats to cutaneous tissues. In the last decade, microplastics (MPs) and nanoplastics (NPs), defined as plastic particles smaller than 5 mm and 100 nm respectively, have emerged as ubiquitous contaminants in air, water, and soil, with potential human exposure not only via ingestion and inhalation, but also via dermal contact. Increasing evidence suggests that both micro and nano plastics (MNPs) can interact with skin barrier and induce oxidative stress and inflammatory responses. In this context, cutaneous inflammasome activation has been suggested to be particularly susceptible to out-doors challenges. The inflammasome is a cytosolic multiprotein complex of the innate immune system that plays a central role in sensing environmental danger signals and triggering inflammatory responses. Several inflammasome subtypes have been described in the skin, among which NLRP1 and NLRP3 are the most relevant. In particular, NLRP3 is highly responsive to exogenous stressors and has been shown to be activated in multiple pollution-target organs, including the lung and gastrointestinal tract. Despite the growing evidence linking environmental pollutants to inflammasome activation, data on the effects of microplastics exposure on inflammasome signaling in human skin are currently lacking. Based on this rationale, we are investigating whether MPs exposure could affect inflammasome activation in human skin using different cutaneous models (2D, 3D and skin explants). Concentrations of MPs that did not impair cellular viability were first identified, followed by analysis of the skin inflammasome pathway. Preliminary results obtained through immunochemical and immuno-cytochemical analyses indicate that MPs exposure can modulate inflammasome signaling, specifically inducing activation of the NLRP3 pathway. Overall, these findings provide novel evidence supporting the potential skin toxicity of MP and lay the groundwork for future studies exploring the inflammatory and toxicological effects of nano-sized microplastics on skin health.

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PP I_A06
Urban air pollution exposure induced region-specific modulation of the glutathione cycle in the central nervous system

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Air pollution is increasingly recognized as a significant risk factor for neurodevelopmental and neurodegenerative disorders. Airborne particulate matter present in urban air (UA) alters redox homeostasis and promotes neuroinflammation, yet the underlying redox mechanisms remain unclear. We investigated whether UA exposure alters the central nervous system (CNS) antioxidant defense system, focusing on the glutathione (GSH) cycle across distinct brain regions. Eight-week-old BALB/c mice were exposed to filtered air (FA) or urban air (UA) in whole-body exposure chambers in Buenos Aires for 4 weeks. We compared brain cortex (Cx), olfactory bulb (OB), and cerebellum (Cer) to identify region-specific redox alterations. We first assessed the redox status evaluated by the GSH/GSSG ratio. UA exposure significantly decreased the GSH/GSSG ratio in Cx and OB ($p < 0.05$). In Cx, this reduction was driven by increased GSSG levels, whereas in OB it resulted from decreased GSH content ($p < 0.05$). In contrast, an increase in GSH/GSSG ratio ($p < 0.05$) was observed in Cer due to a decrease in GSSG concentration ($p < 0.01$). Regarding the GSH redox cycle enzymes analyzed, UA-exposed mice presented increased GPx activity in Cx ($p < 0.05$), no differences in BO and reduced GPx activity in Cer ($p < 0.05$), while GR activity increased in Cx and OB ($p < 0.05$) and remained unchanged in Cer. Despite region-specific modulation of the GSH cycle, all areas exhibited increased protein carbonyl content levels following UA inhalation ($p < 0.001$ Cx and OB; $p < 0.05$ Cer). These findings suggest that UA exposure affects GSH metabolism in an area-specific manner, promoting oxidative damage that might play a key role in the neurotoxicity mechanisms and CNS functional impairments associated with air pollution.

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PP I_A07

Cellular responses to traffic-related ultrafine particles and the role of NRF2 signaling as a mitigation strategy against toxicological effects

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Air pollution is a worldwide problem with severe health impacts, including toxic cellular effects. Traffic emissions are the primary source of the finest particulate matter (PM), the ultrafine particles (UFP; diameter ≤100 nm), which are hazardous due to their small size, allowing them to cross bodily barriers and reach the central nervous system. Various signaling pathways regulate cellular redox homeostasis. Nuclear factor erythroid 2-related factor 2 (NRF2) is a key transcription factor in cellular antioxidant and anti-inflammatory responses. As UFP exposure can lead to toxicity, inflammation and oxidative stress, new means to mitigate adverse cellular effects are needed. While UFPs can activate NRF2 as a defense mechanism, the protective response is in many cases insufficient. Therefore, pre-activation of NRF2 through pharmacological approaches could potentially reduce the UFP-induced adverse cellular effects. However, the connection between air pollution and NRF2 activation remains limited, especially in the upper respiratory tract, in which cells of the olfactory cleft mucosa (OM) located in the rooftop of the nasal cavity are exposed to airborne environmental stressors. To address this, this study investigates how UFPs derived from different sources affect NRF2 signaling using air-liquid interface (ALI) and submerged cultures of primary human OM cells. NRF2 activity will be assessed by NRF2 transcription factor activity assay, while gene and protein expression of key target genes will be evaluated by real-time qPCR and Western blot. To evaluate the ability of NRF2 induction to limit toxicity, DNA damage, oxidative stress and inflammation, cells will be pretreated with NRF2 inducers such as sulforaphane (SFN), dimethyl fumarate (DMF) or curcumin at various doses and timepoints before UFP exposure. In conclusion, this study aims to assess the potential of NRF2 activation as a strategy to reduce UFP-induced cellular effects and to further define the role of NRF2 in cellular responses to UFP exposure.

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PP I_A08

Investigation of the cellular and molecular effects of emerging airborne pollutants in a unique human cell model

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Humans are constantly exposed to harmful airborne substances, including emerging pollutants such as ultrafine particles (UFPs) and micro-and nanoplastics (MNPs). Through inhaled air, particles enter the nasal cavity and contact the olfactory mucosa (OM), specialized tissue lining the roof of the cavity. This tissue represents a potential entry route of particles into the brain through the olfactory pathway. Here we use a unique primary cell model of the human OM to investigate the cellular and molecular mechanisms involved in exposure to emerging airborne pollutants. Cells were exposed to UFPs and MNPs at various concentrations and timepoints, after which toxicity, barrier permeability assays and omics approaches were used to understand particle effects at the human OM. Dysregulation of redox signalling, oxidative stress and dysfunction of mitochondria were observed, alongside size- and composition specific effects of airborne particles in OM cells. These data reveal new exposure targets that can be used to alleviate adverse effects of airborne emerging pollutants at the nose-brain interface.

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PP I_A09

Cardiometabolic biomarker screening in subchronic aircraft noise exposure in NZO mouse strain

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Epidemiological evidence indicates a positive association between long-term traffic noise exposure and increased cardiometabolic risk, particularly the development of type two diabetes mellitus (T2DM). Individuals with adverse lifestyle factors – such as smoking and obesity – appear to be especially susceptible to the detrimental metabolic effects of environmental noise. However, the mechanistic pathways underlying this interaction and compounding effect remains insufficiently defined. This study investigates the impact of subchronic aircraft noise exposure on metabolic dysregulation and aims to identify early biomarkers of cardiometabolic impairment. Male and female New Zealand Obese mice, a well-established model of obesity-associated metabolic dysfunction, were continuously exposed to aircraft noise at a mean sound pressure level of 72 dB(A) for four weeks. Animals were stratified into two age groups and were fed standard diet. Postmortem analyses assessed oxidative stress, metabolic parameters, inflammatory & stress-related signaling pathways. In addition, cardiovascular function was evaluated using electrically stimulated isolated cardiomyocytes, isometric tension recording, non-invasive blood pressure measurement and high-resolution respirometry. Noise exposure resulted in elevated blood pressure, endothelial dysfunction and attenuated body weight gain across all age and sex groups compared with controls. Plasma analyses revealed a trend toward higher hypothalamic-pituitary-adrenal axis activity and altered circulating metabolic marker levels in both sexes. Oxidative stress, as envisaged by dihydroethidium staining, MDA, 3NT and 4HNE, was observed by noise providing a potential explanation for the impaired metabolic pathways and prediabetic/preobese phenotype. Collectively, these findings suggest that subchronic aircraft noise exposure promotes cardiometabolic dysregulation, potentially mediated through activation of stress-response pathway. This supports the previous observation that environmental noise could explain higher hazardous risk for development and progression of diabetes. Ongoing analyses aim to clarify sex-specific responses in this metabolically susceptible animal model and to identify predictive circulating biomarkers for noise-induced metabolic dysfunction.

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PP I_A10

Preadiposity and prediabetes biomarker screening in subchronic aircraft noise exposure mouse models

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Chronic traffic noise exposure is associated with cardiovascular outcomes, including hypertension, stroke, heart failure, and arrhythmias. The World Health Organization estimates that ~100 million people in Western Europe are exposed to harmful noise levels, resulting in more than 392,000 lost healthy life years annually. Emerging evidence links environmental noise to metabolic disorders such as obesity and type 2 diabetes mellitus, yet the underlying mechanisms remain poorly defined. This study aims to establish an animal model to investigate early metabolic alterations induced by subchronic noise exposure and to identify biomarkers of preadiposity and prediabetes. Using a noise exposure protocol by the group of Andreas Daiber, twelve-week-old male and female C57BL/6J wild-type mice were continuously exposed to aircraft noise for four weeks. Blood pressure and body weight were monitored weekly. Postmortem analyses assessed oxidative stress, metabolic parameters, inflammatory and stress-related responses in cardiovascular and metabolic tissues. Noise-exposed animals exhibited a sustained elevation in blood pressure, pronounced endothelial dysfunction, and attenuated body weight gain compared to controls. Cardiac tissue in noise exposed male mice showed decreased maximal mitochondrial respiration and elevated inflammatory marker expression. Plasma analyses indicated trends toward increased hypothalamic–pituitary–adrenal (HPA) axis activity and altered metabolic markers in both sexes. Histological analyses revealed no significant changes in cardiomyocyte size or myocardial collagen deposition. Functional assessment of isolated cardiomyocytes demonstrated prolonged contraction times in females, with no functional alterations in males. Collectively, these findings indicate that subchronic aircraft noise exposure induces physiological stress and early metabolic alterations, supporting the hypothesis that environmental noise contributes to prediabetic changes through oxidative and inflammatory mechanisms. Ongoing analyses aim to characterize sex-specific responses and to identify circulating biomarkers predictive of noise-induced
metabolic dysfunction. This work provides a foundation for future studies examining the impact of environmental noise in diabetic and metabolically challenged animal models.

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PP I_A11

Acute and chronic aircraft noise exposure induces differential behavioral, circuit, and synaptic adaptations in mice with a link to adverse redox processes

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Aircraft noise is a significant source of environmental pollution. Aviation noise is characterised by high-intensity peaks that can disrupt sleep and increase stress and anxiety levels. Acute exposure to aircraft noise activates the amygdala and hippocampus, triggers the hypothalamic–pituitary–adrenal (HPA) axis, and stimulates the sympathetic nervous system. Chronic noise exposure results in sustained HPA activation, thereby leading to maladaptive physiological effects. However, the mechanisms underlying noise-induced behavioral alterations at the circuit and synaptic levels remain unclear. The present study intends to investigate the effects of acute and chronic exposure to aircraft noise on behavior, neuronal activation, and postsynaptic organization in mice. C57BL/6j mice were exposed to aircraft noise for either 4 days (acute) or 28 days (chronic). Behavioral assessments demonstrated that chronic noise exposure resulted in increased locomotion, reduced working memory, and decreased sociability. Phenotypic changes were also associated with neuroinflammatory and cerebral oxidative stress markers (e.g., elevated ROS formation and NADPH oxidase expression levels). Neuronal activity was quantified using c-Fos immunohistochemistry. Chronic noise exposure increased cortical activity but reduced striatal and hippocampal activity. To look at synaptic changes, postsynaptic density fractions from the cortex, striatum, and hippocampus were isolated, and synaptic proteins were analysed by western blotting. Short-term noise exposure caused widespread changes in synapses, with higher levels of the scaffold proteins Homer1 and Shank3 in all three regions and increased GFAP in the cortex, indicating early astrocyte involvement at synapses. In contrast, long-term exposure led to changes in specific regions. These results show that aircraft-related noise pollution can induce temporary changes in synapses, characterized by early scaffold remodeling and astrocytic involvement, followed by chronic circuit-specific synaptic reorganization with a potential role of adverse redox processes. This study provides mechanistic insights into how aircraft-related noise pollution can alter behavioural outcomes.

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PP I_A12

The impact of aircraft noise exposure on the therapeutic efficacy of empagliflozin in an animal model of obesity

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Obesity and type 2 diabetes mellitus (T2DM) remain leading contributors to global morbidity and mortality. Sodium-glucose co-transporter 2 inhibitors (SGLT2i), such as empagliflozin, have emerged as innovative therapies for T2DM and offer additional cardioprotective effects. This study aims to determine whether simultaneous exposure to aircraft noise modulates the therapeutic effectiveness of empagliflozin in an established experimental model of obesity. To induce obesity, C57BL/6JRj mice were administered a Western-style high-fat diet for a duration of 20 weeks. Empagliflozin treatment (10 mg/kg/day) was initiated via drinking water after 14 weeks. To assess the impact of aircraft noise, mice were additionally exposed to aircraft noise during the final four days of the treatment period. Exposure to aircraft noise alone significantly disrupted glucose metabolism, elevated blood pressure, impaired endothelial function and increased vascular oxidative stress and inflammation. Subsequent six-week empagliflozin therapy improved glycemic control and ameliorated vascular and oxidative parameters in high-fat diet-fed animals. However, these beneficial effects were diminished or absent in animals subjected to concurrent aircraft noise exposure. SGLT2i, such as empagliflozin, have gained widespread application in clinical settings beyond type 2 diabetes mellitus (T2DM) therapy. Our findings reveal that concomitant exposure to aircraft noise diminishes the protective efficacy of SGLT2 inhibitors in an obese murine model. Given advances in personalized medicine, elucidating the interplay of multiple risk factors, particularly environmental stressors, is essential to optimize therapeutic outcomes and enhance protection for susceptible populations.

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PP I_A13

Molecular-level understanding of aerosol oxidative potential and its health effects

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Exposure to ambient air pollution is a major risk factor for human health however, the physiological effects of particulate matter (PM) still remain unclear. Oxidative stress due to excess formation of reactive oxygen species (ROS) in the lungs is a leading hypothesis for the molecular mechanism behind adverse health effects of PM. However, ROS are continuously produced in biological systems as part of tightly regulated physiological processes, making it challenging to distinguish homeostatic redox regulation from harmful oxidative damage. Here, we apply and compare two kinetic modeling frameworks to bridge this gap. The KM-OP model quantifies the oxidative potential (OP) of fine particulate matter using cell-free assays and simulates redox-driven depletion of

antioxidants, and the KM-SUB-ELF model, which is a multiphase chemical kinetics model of the human respiratory tract which explicitly accounts for gas- and particle-phase pollutant deposition, mass transport and chemical interactions within the epithelial lining fluid (ELF). KM-OP quantifies the effects of PM on the production of ROS and consumption of antioxidants, such as ascorbic acid (AA) and dithiothreitol (DTT), while KM-SUB-ELF quantifies the effects of particulate pollutants on the production of ROS and oxidative damage in the lung. We evaluate whether oxidative potential correlates with oxidative stress in the lungs by comparing model predictions of OP with those of nitrotyrosine, a biomarker of oxidative stress. Our results show a significant positive correlation between OP and nitrotyrosine levels, indicating that cell-free oxidative potential can capture in vivo oxidative damage. These findings support oxidative potential as a useful exposure metric. Our model results provide a basis for further investigation of different metrics of the potential toxicity of PM.

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PP I_A14

Sex effects of menthol in atherosclerosis in e-cigarette-exposed mice

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The effects of vaping and individual aerosolized e-liquid constituents on atherosclerosis, vascular aging, and gut microbiome remodeling remain poorly characterized. We aimed to determine the contributions of e-cigarette aerosol components to vascular senescence and atherosclerosis in ApoE$^{-/-}$ mice. Male and female atherosclerotic ApoE$^{-/-}$ mice were exposed to e-liquid constituents (vehicle, vehicle plus nicotine, vehicle plus nicotine plus menthol) for 48 minutes per day, 5 days per week for 4 months, with vascular pathology assessed in vivo. Exposure to all tested e-liquid formulations, including vehicle, nicotine-containing, and menthol-containing aerosols, increased atherosclerosis in both male and female mice, with the most robust effects observed in the nicotine-containing formulation and in the descending aorta. Females exhibited more sensitivity to e-liquid exposure, with greater plaque accumulation in both the aortic arch and descending aorta. Notably, the addition of menthol was associated with a reduction in plaque burden compared with nicotine alone in both sexes. These findings demonstrate that individual e-liquid aerosol components increase atherosclerosis, with nicotine producing the most pronounced pro-atherogenic effects and menthol reducing these effects, without eliminating overall atherosclerotic risk.

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PP I_A15

Impact of shisha smoke exposure on health effects in mice – comparison of coal heating and electric heating

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Shisha smoking has recently gained popularity in Europe. It is estimated that about 10 percent of youth in Europe smoked shisha during the past 30 days, which accounts for millions of people. Numerous studies demonstrated that coal-heated shisha smoking leads to increased oxidative stress and inflammation in multiple organ systems and endothelial dysfunction. The aim of this study was to compare cardiovascular health effects of coal-heated shisha smoke (high carbon monoxide (CO) and particular matter level) and electric-heated shisha smoke exposure (no CO and lower particular matter level). We performed a 3-day exposure (4x30 min/d) in a C57BL/6J mouse model to evaluate endothelial function, blood pressure, oxidative stress, and mitochondrial function. Both exposures increased ROS production in cross-sections of retinal and cerebral arterioles measured by dihydroethidium staining, while only coal-based protocol increased ROS production in lung. High-resolution respirometry showed that only coal-heated shisha increased mitochondrial maximum respiratory capacity in heart homogenates. RNA-seq of lung and heart tissue revealed that only coal-heated shisha exposure changed expression of genes participating in iron and heme metabolism, which could be explained by CO effects. No significant changes were observed in systolic and diastolic blood pressure. Only coal-based protocol increased pulse pressure. Both coal- and electric-heated shisha exposure significantly impaired endothelial function, demonstrated by rightward shift of acetylcholine relaxation curves in isolated aortic sections, with more pronounced effect after coal-heated shisha exposure. RNA-seq of aorta of coal-heated shisha-exposed mice revealed changes suggesting vascular smooth muscle cell dysfunction and consistent with angiotensin II effects, which could explain endothelial dysfunction after both exposures. Our current results demonstrate that coal-heated shisha is more detrimental for the cardiovascular system compared to electric-heated shisha. However, electric-heated shisha exposure can also negatively affect the cardiovascular system. Further research is necessary to better clarify the underlying mechanisms responsible for the observed differences.

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PP I_A16

Preventing UV induced skin damage with a marine world based new technology

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Ultraviolet (UV) radiation represents a major environmental stressor for the skin, promoting the generation of reactive oxygen species (ROS) that drives oxidative stress, inflammation, and structural damage within the epidermis. Chronic UV exposure compromises epidermal barrier integrity, accelerates extracellular matrix degradation, and activates oncogenic pathways implicated in photo carcinogenesis, including melanoma. Natural strategies capable of preserving barrier function and limiting UV-induced damage are therefore of considerable interest. Marine red algae such as Gracilaria sp. produce a wide range of bioactive compounds, including mycosporine-like amino acids, sulfated polysaccharides, carotenoids, and phenolic molecules, which are known for their UV-absorbing, antioxidants, and anti-inflammatory properties. In this study, an aqueous extract of Gracilaria sp. was applied topically to ex vivo human skin explants prior to UV irradiation to evaluate its protective potential. The treatment significantly increased the expression of filaggrin, a key marker of epidermal differentiation and barrier integrity, both under basal conditions and following UV exposure, eLectively counteracting the UV-induced reduction observed in untreated samples. Oxidative damage was significantly attenuated, as demonstrated by a significant decrease in 4-hydroxy-2-nonenal (4-HNE) levels at 3 and 24 hours after treatment, indicating sustained protection against lipid peroxidation. Immunofluorescence analyses showed rapid nuclear translocation of nuclear factor erythroid 2–related factor 2 (NRF2) within one hour of application, regardless of UV exposure, suggesting early activation of antioxidant defense mechanisms. Interestingly, the expression of the downstream target of NRF2, heme oxygenase-1 (HMOX-1), was reduced following treatment, likely reflecting a lower intracellular oxidative burden due to the intrinsic antioxidant activity of the extract. In parallel, UV-induced upregulation of Tyrosinase and Tyrosinase-Related Protein 1 (TYRP1) was inhibited, indicating a modulation of melanogenic responses. Overall, these results demonstrate that Gracilaria sp. extract provides multifaceted skin protection by strengthening barrier function, reducing oxidative stress, and modulating cellular stress response pathways.

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PP I_A17

Skin impairment of realistic photo pollutant Polycyclic Aromatic Hydrocarbons and ozone exposure in *vitro/vivo* study

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Human skin is submitted to many environmental stressors from outside as tropospheric ozone pollution, from outside and inside with the deleterious synergy between Polycyclic Aromatic Hydrocarbons (PAHs) contained in particle matter and sun exposure. Understanding the consequences on skin both in vitro and in vivo is crucial to adapt specific skin care solutions. In this study we compared at transcriptomic level on the same reconstructed skin model the impacts of i) chronic exposures to realistic doses of ozone (3 exposures at 0.1ppm) and ii) photo pollution via a combination of PAHs Benzopyrene exposures (15 nM in the medium mimicking systemic contamination) and three long UVA exposures at 7,5 J/cm2. Gene expression analysis revealed in both cases an induction of the inflammatory cytokines IL1β, IL6 and an imbalance in differentiation pathway genes. 8 isoprostane lipid damage were also detected. Specifically, photo pollution stress induced oxidative response target, and down regulated filaggrin processing and corneocytes lipid gene. Those data were validated at protein and histological levels with immunostaining, Natural Moisturizing Factor and parakeratoses quantifications. Regarding specific chronic ozone impacts, gene modulations were related to detoxication, certain intracellular pathway and desquamation. Oxidative impacts of ozone on lipids and modification on corneodesmosin and SPRR3 were respectively confirmed by quantification of aldehydes, carbonylated forms, and immunostaining. In vivo, cross-sectional study in Chinese urban population have shown among other impaired skin barrier in individuals living in polluted city. Furthermore, a clinical study with 6 x 0,3 ppm ozone for 2 weeks revealed markers of inflammation (IL6), oxidized forms of aldehydes and methionine sulfoxide in correlation with our vitro data. Our results show that diverse environmental exposures such as ozone, PAH with UVA can trigger skin inflammation, barrier impairment and oxidative stress which, in turn, could contribute towards rising incidence of atopic dermatitis in polluted urban environments.

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PP I_A18

Lens as a target of oxidative damage after chronic exposure to urban air pollution

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Particulate matter (PM), a major component of air pollution, has been associated with harmful ocular effects, in which oxidative stress is considered a central mechanism underlying its pathophysiology. Within the eye, the antioxidant system of the lens contributes to preserving the redox homeostasis of adjacent ocular tissues. This study aimed to assess the redox status of mouse lenses following exposure to urban air. Male Balb/c mice, 8 weeks old, were exposed to urban air (UA, n=18) or filtered air (FA, n=18) in exposure chambers located in the city of Buenos Aires (average PM level: 15.2 ± 5.3 μg/m^3). The animals were exposed for 12 weeks (CICUAL-REDEC-2025-578-E-UBA-DCT_FFYB). The animals were sacrificed, the eyes were enucleated, and the lenses were separated and lysed. Antioxidant enzymes, reduced (GSH) and oxidized (GSSG) glutathione levels, protein oxidation and protein conformation using circular dichroism (CD) and 1-anilinonaphthalene-8-sulfonic acid (ANS) fluorescence study were evaluated. Glutathione Peroxidase (GPx) activity increased (63%, $p<0.05$) in UA group whereas no major differences in superoxide dismutase (SOD) activity were observed. Glutathione reductase (GR) activity decreased (40%, $p < 0.05$) along with the GSH/GSSG ratio (62%, $p < 0.05$) in UA compared to FA. Although, protein oxidation significantly increased (113%, $p < 0.05$), SDS–PAGE revealed no changes in protein molecular mass, and CD/ANS studies showed no alterations in protein conformation. These findings indicate that exposure to urban air pollution modifies the redox balance of the lens, potentially compromising the antioxidant protection of surrounding ocular tissues. Nevertheless, although a shift toward a more pro-oxidant environment was observed, no significant alterations in protein conformation were detected. The association between protein oxidation and the GSH/GSSG ratio suggests that the lens glutathione pool may play a protective role against oxidative protein damage. Increased protein oxidation could ultimately contribute to lens opacification, representing a possible mechanism involved in cataract formation.

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Poster Presentations I Group B – Vascular Biology

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PP I_B05

Hypoxia modulates the synthesis and post-translational modification of collagens generated by human coronary artery smooth muscle cells

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Hypoxia has been suggested to play a central role in the development of atherosclerotic plaques and other diseases by modulating cell metabolism. We hypothesized that hypoxia might modulate the protein complement of vascular smooth muscle cells of the artery wall and drive phenotypic changes. Here we report a detailed liquid chromatography-mass spectrometry (LC-MS/MS) study of changes induced in human coronary artery smooth muscle cells (HCASMC) on transfer from an atmosphere of 20 to 1% O_2 (hypoxia) for 1 and 7 days. Exposure to 1% O_2 resulted in marked changes in the HCASMC proteome with 755 proteins (of 5,842 total; ~13%) altered in abundance after 1 day, and 2874 (1234 up, 1640 down; ~49%) after 7 days. Significant changes in specific collagens, collagen-modifying hydroxylases and cross-linking enzymes (lysyl oxidases and related species, prolyl and lysyl hydroxylases), were detected with most of these upregulated by hypoxia. These data are consistent with significant extracellular matrix (ECM) remodeling under hypoxia and increased collagen synthesis, processing, secretion and incorporation into ECM. Analysis of protein post-translational modifications (hydroxylations) indicates enhanced hydroxylation of multiple collagen peptides, particularly within the helical coil regions. Immunoblotting and ELISA data support these ECM changes. Comparison with proteomic data obtained from advanced symptomatic human carotid artery atherosclerotic plaques and control artery samples, has provided evidence for a similar pattern of changes, particularly with regard to collagen-modifying and processing enzymes, consistent with the presence of hypoxic regions within human plaques. Together, these data indicate that hypoxia drives collagen-focused ECM remodeling.

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PP I_B06
Physiological oxygen levels reset K^+ channel activity in human vascular endothelial cells

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Human vascular endothelial cells play a critical role in vascular homeostasis and their function is influenced by oxygen tension, which varies in a spatial-temporal manner across tissues. In this study, we investigated how adapting human brain microvascular endothelial cells (hCMEC/D3) to physiological oxygen tension (5 kPa O_2) alters their basal and nitric oxide (NO)-stimulated potassium (K^+) channel activity. Whole-cell K^+ currents were recorded using the Nanion Port-a-Patch system housed within an oxygen-controlled workstation. hCMEC/D3 cells displayed exclusively outwardly rectifying K^+ currents. Compared with standard culture under 18 kPa O_2, long-term adaptation to 5 kPa O_2 resulted in significantly larger basal outward currents (+60 mV: 0.598 ± 0.058 nA) and NO-enhanced currents (+60 mV: 0.795 ± 0.075 nA). Acute application of the NO donor NOC-7 (400 μM) potentiated outward currents only in cells adapted to 5 kPa O_2 (40 mV: $P = 0.0053$; 60 mV: $P < 0.0001$). In contrast, NO had no significant effect on cells maintained at 18 kPa O_2. Pharmacological isolation of BK, IK and SK channel components using TEA, TRAM-34 and apamin revealed marked oxygen-dependent differences in channel contribution. Cells cultured at 5 kPa O_2 exhibited a significantly greater proportion of blocker-sensitive currents (82%) than those at 18 kPa O_2 (44%). At 5 kPa O_2, sequential inhibition substantially reduced current amplitudes at 50 mV, whereas effects were smaller and often non-significant under hyperoxic conditions. Protein expression of KCa1.1, KCa3.1 and KCa2.3 channels remained unchanged between oxygen conditions, indicating that altered currents were due to functional modulation rather than expression changes. These findings demonstrate that physiological oxygen tension profoundly shapes endothelial electrophysiological phenotype, enhancing K^+ channel activity and NO responsiveness. This modulation has important implications for vascular tone regulation and highlights the need for physiologically relevant oxygen levels in endothelial models used for drug discovery and translational research.
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PP I_B07
Effect of microplastic tire abrasion of endothelial angiogenic capacity

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Urbanization and the increasing number of vehicles have resulted in sustained emissions of traffic-related contaminants. In particular, tire and road wear particles in road runoff have raised concern due to their potential to contribute to long-term human exposure. Cardiovascular disease is the leading cause of death world-wide and atherosclerosis, inflammatory cell activation as well as failure of angiogenesis contribute to this. We hypothesize that road runoff impacts on the function of endothelial cells, which are critical to maintain vascular health. Road runoff samples were collected over the course of two years on the A4 federal highway in Aachen (Germany), covering seasonal and meteorological variability. The samples were filtered and organic extracts were produced from the filter residues. The freeze-dried samples were first sterilized by gamma irradiation and then extracted via Ultrasound-Assisted Extraction. In vitro, the pure particulate filter residues, the organic extracts, and the particles washed after extraction were examined on their effect on human umbilical vein endothelial cells (HUVEC). The following aspects were studied: proliferation, apoptosis assays and gene expression as determined by quantitative PCR, focusing on markers for oxidative stress, immune responses, and endothelial-associated genes. Road runoff induced concentration-dependent effects on apoptosis and proliferation of endothelial cells. In addition, proinflammatory transcriptional responses were detected (CD31, IL-6, ANGPTN2), indicating activation of stress- and immune-associated signaling pathways. The most pronounced effects occurred in particle-containing fractions in comparison to the particle free organic extracts (IC_{50} = 2.1 mg/ml versus IC_{50} = 11.2 mg/ml), suggesting that particle-mediated processes contribute significantly to the induction of endothelial dysregulation. The results will contribute to a better understanding of the human toxicological relevance of traffic-related runoff and provide insights into the processes through which complex environmental mixtures can influence endothelial functions. The project is funded by the German Research Foundation as part of the Cardio-Pulmonary Institute.

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PP I_B08
Endothelial cytochrome P450 reductase-derived cholesterol limits angiogenesis

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The cytochrome P450 reductase (POR)/CYP51 monooxygenase is a redox system important for sterol synthesis. Cholesterol is a key membrane constituent involved in signaling. Cellular cholesterol is determined by uptake and de novo synthesis. High circulating cholesterol is linked to cardiovascular disease, but the role of endogenous cholesterol synthesis in endothelial function, in contrast, is unknown and was studied here. To inhibit cholesterol synthesis in endothelial cells, POR and CYP51A1 CRISPR knockout was performed in human aortic endothelial cells (HAEC) and human umbilical vein endothelial cells (HUVEC). Furthermore, an endothelial-specific tamoxifen-inducible POR knockout mouse ($ecPOR^{-/-}$) was generated. Knockout of POR and CYP51A1 in HAEC led to accumulation of the CYP51A1 substrate lanosterol, whereas its product desmosterol was reduced. Functionally, loss of endogenous cholesterol synthesis was linked to increased angiogenic sprouting in HUVEC. Similarly, endothelial sprouting from aortic-segments was increased in $ecPOR^{-/-}$ compared to control mice. Importantly, increased angiogenesis was also observed *in vivo* in the retina of $ecPOR^{-/-}$ as compared to control mice. Cellular cholesterol levels are sensed by SREBP2 (sterol regulatory element-binding protein 2), and indeed, SREBP2 activation was increased after deletion of POR in cultured cells as well as *in vivo* (*en face* aorta). Overexpression of active, cleaved nuclear SREBP2 increased angiogenesis similarly to the deletion of POR and CYP51A1. RNA-seq of $POR^{-/-}$ HAEC showed significant upregulation of cholesterol-related genes LRP1, SC5D, and HMGCS1 as well as those of the angiogenic PI3K/AKT-pathway such as ANGPT2 and JAK3. qPCR analysis after SREBP2 and POR/SREBP2 double knockout showed that induction of both cholesterol and angiogenesis pathways is SREBP2-dependent and suppressed in the double knockout, as demonstrated by genes such as HMGCS1 and ANGPT2. Together, these findings show that inhibition of endothelial cholesterol synthesis activates SREBP2 to drive pro-angiogenic gene expression, identifying the POR/CYP51A1–SREBP2 axis as a key regulator of angiogenesis.

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PP I_B09
Purine metabolism shows a biphasic response to oxidative stress in endothelial cells

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Reactive oxygen species (ROS) are short-lived molecules that induce transient cellular responses, which allow them to function as signaling messengers. Their effects can be mediated through metabolic enzymes whose activity depend on redox sensitive prosthetic, such as heme, or thiols, which are important for enzyme structure and activity. The dynamics of metabolic responses to oxidative stress is not yet fully characterized. To address this, we challenged human umbilical vein endothelial cells (HUVEC) with hydrogen peroxide (H_2O_2, 300 μM) in a time course manner (3, 10, 30, 90, 270 and 900 minutes) and performed untargeted metabolomics using LC-MS/MS. Among 387 confidently identified metabolites, those of purine metabolism exhibited the most pronounced alterations. There was a rapid increase, after 10 minutes of exposure to H2O2, in AMP, GMP, IMP, adenosine, inosine, and guanosine, hallmarks of ATP/GTP turnover and energetic reprogramming. This was followed by an accumulation phase at 90 minutes, where AICA ribonucleotide (AICAR) presented an 82-fold increase that subsided at later time points. Targeted metabolite quantification conducted using other oxidative stress inducers including oxidized LDL (Ox-LDL), lysophosphatidylcholine (LPC) and PKC inhibitors, elicited a similar response. Our findings suggest a blockage within purine metabolism with an early use of metabolites associated with energy usage and a late response in the de novo purine biosynthesis pathway. The increase in AICAR
suggests the enzyme ATIC (5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase) to be a redox-sensitive control node in purine flux under oxidative stress.

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PP I_B10
Vascular and erythrocyte effects of quercetin in spontaneously hypertensive rats

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Quercetin, a natural antioxidant, is being investigated for its endothelial- and cardio-protective effects, with potential benefits in hypertension, long COVID, and other cardiometabolic disorders associated with endothelial dysfunction. This study examined the effects of quercetin on endothelial and erythrocyte function and systolic blood pressure (BP) in normotensive and spontaneously hypertensive (SHR) rats. Twelve-week-old male Wistar and SHR rats were treated orally with quercetin (20 mg/kg/day) for 6 weeks, while controls received vehicle. Systolic BP (tail-cuff method) was monitored. Vascular reactivity (wire myography, organ bath) of the femoral and small mesenteric arteries, and aorta was assessed, evaluating endothelium-dependent vasorelaxation and vasoconstrictive responses to noradrenaline. Erythrocyte deformability (ektacytometry), nitric oxide (NO) production (DAF fluorescence), and intracellular free radical levels (flow cytometry) were also assessed. Quercetin had no effect on the heart-to-tibia length ratio in either Wistar or SHR rats and did not reduce BP in either group after six weeks of treatment (Control Wistar: 113 ± 2 mm Hg vs. Wistar-quercetin: 112 ± 2 mm Hg, $p > 0.05$; Control SHR: 162 ± 3 mm Hg vs. SHR-quercetin: 167 ± 3 mm Hg, $p > 0.05$). In SHR, femoral and mesenteric arteries exhibited increased contractile responses to noradrenaline, which were not modified by quercetin, and quercetin had no effect on endothelium-dependent relaxation in either Wistar or SHR rats. However, quercetin induced a slow, concentration-dependent vasorelaxation in the aorta, fully abolishing maximal arterial contraction at 10^{-4} mol/l. Quercetin also did not alter the reduced erythrocyte NO production and deformability, as well as increased free radical levels observed in SHRs.

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PP I_B11
Dimethyl fumarate reduces blood pressure and alters vascular function in Western diet-fed rats

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A Western diet (WD) can promote redox imbalance and contribute to cardiometabolic diseases (CMDs). Nuclear factor erythroid 2-related factor 2 (NRF2) is a central regulator of antioxidant defense and a key molecular link in CMDs. We evaluated the effects of WD (Altromin, Germany) and the NRF2 activator dimethyl fumarate (DMF) on blood pressure, femoral arterial reactivity, and blood markers of liver function. Male, seven-week-old borderline hypertensive rats (BHR) were assigned for 9 weeks to WD, DMF (20 mg/kg/day in 0.25% DMSO in drinking water), or combined WD + DMF treatment. Control and DMF-only rats received the standard diet (Altromin C-1090) and drank vehicle (0.25% DMSO) in drinking water. WD significantly increased systolic blood pressure, whereas DMF significantly reduced it in both C-1090- and WD-fed rats. WD increased the relative weights of the left heart ventricle and liver; however, DMF decreased only the relative liver weight in the WD + DMF group. WD elevated alanine aminotransferase and aspartate aminotransferase, reduced high-density lipoprotein cholesterol (HDL-C), without the alterations in total cholesterol levels. WD also increased hepatic thiobarbituric acid–reactive substances (TBARS) and the atherogenic index of plasma (AIP; log[triacylglycerols/HDL-C]). In WD-fed rats, DMF reduced triacylglycerol levels and improved AIP, but failed to lower hepatic TBARS. Femoral artery function was assessed by wire myography. WD reduced acetylcholine-induced relaxation and elevated noradrenaline (NA)- and serotonin-induced contraction, while DMF blunted these effects. In addition, DMF attenuated constriction elicited by depolarising high-K+ solution. Collectively, DMF may represent a therapeutic tool to attenuate WD-induced vascular alteration-associated hypertension as well as hypertriglyceridemia in genetically prehypertensive rats. However, DMF did not protect against WD-induced hepatic oxidative damage.
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PP I_B12
Lipid A adjuvant ameliorates doxorubicin-induced mitochondrial dysfunction

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Vaccine adjuvants are used to reinforce vaccine efficacy by enhancing immune responses. Their application has expanded beyond traditional infectious disease prevention to include the development of novel vaccines for treating cancer and neurological disorders. Although vaccination is associated with cardiovascular adverse events, the effects of adjuvants on the heart remain insufficiently understood. Furthermore, chemotherapy, known to induce oxidative stress, may also reduce vaccine efficacy, potentially limiting vaccination timing in cancer patients. Considering the broad applications of adjuvants, optimizing their safety profile represents a promising strategy to address this issue. Because the heart is rich in mitochondria involved in generating oxidative stress, this study evaluated adjuvant effects using cardiac mitochondrial respiratory function as a primary indicator. Neonatal rat cardiomyocytes (NRCMs) were treated with five

types of vaccine adjuvants, including clinically used formulations: Alum, Sigma Adjuvant System, MF59, AS03, and Lipid A. NRCMs were co-treated with the anticancer drug Doxorubicin (DOX) and the adjuvant. Treatment with all adjuvants except Lipid A resulted in no significant changes in cell viability or oxygen consumption rate (OCR). In contrast, Lipid A-treated cardiomyocytes exhibited significantly increased OCR and hyperpolarized mitochondrial membrane potential. Moreover, Lipid A substantially attenuated the reduction in cardiomyocyte OCR and contraction velocity induced by DOX. These protective effects were not observed following treatment with TAK-242, a selective Toll-like Receptor 4 (TLR4) inhibitor. Furthermore, DOX administration reduced antibody titer compared to controls, but this suppression was restored by Lipid A treatment. These findings demonstrate that no adverse effects on the myocardium were observed with the adjuvant used. Furthermore, unlike the other four adjuvants tested, it was revealed that Lipid A was found to enhance myocardial mitochondrial function via TLR4 signaling, yielding systemic beneficial effects such as improvement in anticancer drug-induced cardiac dysfunction and antibody deficiency.

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PP I_B13

Endothelial cuproptosis drives atherosclerosis via a butyrylation-dependent STAT1–SLC31A1 axis

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Cardiovascular diseases remain the leading cause of mortality worldwide, with atherosclerosis as their primary pathological basis. Endothelial injury is a hallmark initiating event and a critical driver of atherosclerotic progression. Cuproptosis, a newly identified form of regulated cell death induced by intracellular copper overload, has been scarcely investigated in atherosclerosis. This study aimed to determine whether endothelial cuproptosis occurs in atherosclerosis and to elucidate its regulatory mechanisms and therapeutic relevance. Atherosclerosis-prone mouse models were used to evaluate cuproptosis-associated features in the aortic endothelium. The impact of copper chelation using tetrathiomolybdate (TTM) on atherosclerotic progression was assessed *in vivo*. Mechanistic studies were performed in endothelial cells using transcriptomic analysis, metabolomics, and LC–MS/MS–based proteomics to identify key regulatory factors and post-translational modifications. Typical features of cuproptosis were observed in the aortic endothelial layer of atherosclerotic mice. Copper chelation with TTM significantly attenuated atherosclerotic lesion development. We further identified aberrant upregulation of the copper transporter SLC31A1 in endothelial cells as a major contributor to intracellular copper overload. STAT1 was identified as a key transcriptional regulator of SLC31A1. Integrated metabolomic and proteomic analyses revealed that STAT1 butyrylation plays an important role in regulating SLC31A1 expression and endothelial cuproptosis. Notably, sodium butyrate supplementation alleviated atherosclerotic progression in mice, accompanied by increased STAT1 butyrylation levels. These findings identify endothelial cuproptosis as a previously unrecognized mechanism in atherosclerosis and reveal a STAT1–SLC31A1 axis regulated by STAT1 butyrylation, highlighting copper homeostasis and metabolic regulation as potential therapeutic targets.

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PP I_B14

Redox modulation of cerebrovascular dynamics and vascular remodeling by dietary inorganic nitrate in type 2 diabetes

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Type 2 diabetes (T2D) is increasingly recognized as a risk factor for diabetic cognitive impairment, a condition closely linked to early neurovascular dysfunction and progressive vascular remodeling. Converging evidence indicates that reduced nitric oxide (NO) bioavailability and redox imbalance play a central role in this process, compromising neurovascular coupling (NVC), disrupting cerebral blood flow (CBF) regulation, and promoting maladaptive vascular adaptations. Dietary inorganic nitrate has gained attention as a strategy to enhance NO bioavailability via the nitrate–nitrite–NO pathway and to influence redox-sensitive mechanisms, representing an attractive non-pharmacological approach to improve cerebrovascular structure and function in T2D. Expanding on our previous evidence that inorganic nitrate restores NO-dependent hippocampal NVC and spatial working memory in GK rats, we further investigated its impact on cerebrovascular regulatory dynamics and diabetes-associated vascular structural adaptations. In 4-month-old GK rats, 12 weeks of nitrate supplementation significantly improved cortical NVC responses in the somatosensory cortex without altering the diabetic metabolic phenotype, reinforcing a glycemia-independent mechanism. Nitrate supplementation also promoted normalization of baseline CBF oscillatory patterns. At the structural level, nitrate attenuated diabetes-induced remodeling of the retinal microvascular network, characterized by increased vascular area, branching complexity, and total vessel length. Similar protective trends were observed at the systemic level in the aorta, occurring in parallel with reduced NADPH oxidase-dependent oxidative stress. Ongoing analyses are exploring additional redox-sensitive and vascular integrity markers in the brain, including antioxidant defense enzymes and regulators of angiogenic signaling and endothelial junctional organization, to further delineate the molecular framework underlying nitrate-mediated vascular protection. Collectively, these findings support the concept that inorganic nitrate modulates cerebrovascular regulation and vascular remodeling in T2D through coordinated effects on NO bioavailability and redox homeostasis, with potential relevance for diabetes-associated cognitive dysfunction.

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PP I_B15

Properties of erythrocyte hemoglobin in stroke and Alzheimer disease

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Systemic disorders such as cerebral ischemia and Alzheimer disease (AD) are accompanied by alterations in brain's blood supply, with hypoxia and anemia acting as risk factors. Erythrocyte dysfunction may represent a hallmark of pathology development and a potential diagnostic marker. We have focused on oxygen-binding hemoglobin properties using the surface-enhanced Raman Spectroscopy (SERS). SERS on gold nanoparticles selectively enhances the hemoglobin Raman signal from the erythrocytes' submembrane region, hence at the symptomatic stage of AD for 5xFAD mice we revealed significant increase of non-functional methemoglobin (metHb): 40% of all recorded spectra. While oxyhemoglobin's oxygen affinity significantly decreases, due to relatively more probable heme B dome conformation which we estimated spectrally. The weakening of the oxygen-binding properties of the submembrane hemoglobin correlates with increased lipid peroxidation of the erythrocyte membrane and increased content of free hemoglobin in blood plasma. Thus, metHb initiates membrane oxidative processes that induce erythrocytes' apoptosis, which in turn may enhance neurodegeneration due to the development of oxidative stress upon Fe^{3+} release. Notably the defined hemoglobin properties' adjustments correlate with the classic biochemical sign of AD, the accumulation of tau protein in the brain. Blood oxygenation also decreases during reperfusion in rat models with the middle cerebral artery occlusion, presumably as a result of the capillaries' endothelium violation, so that
erythrocytes contact with brain tissues and deoxygenate, inducing delayed burst in H_2O_2 production that we detected in vivo by the H_2O_2-sensitive biosensor HyPer7. In contrast during permanent ischemia the increase of hemoglobin oxygen affinity is an adaptive antioxidant mechanism in conditions of electron-overloaded mitochondrial respiratory chain and an increased risk of H_2O_2 production. We believe that SERS is a useful tool for assessing the oxygen-binding properties of hemoglobin, which are sensitive to both physiological and pathological conditions.

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PP I_B16

Sex-dependent role of AMPK in the development of endothelial dysfunction in metabolic syndrome

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AMP-activated protein kinase (AMPK) is a key regulator of vascular metabolism and is essential for maintaining endothelial function. Endothelial dysfunction (ED), caused by reduced nitric oxide (NO) bioavailability due to oxidative stress and inflammation, is a major complication of metabolic syndrome (MetS) and increases cardiovascular risk. Different vascular beds, including large conduit arteries, resistance vessels, and peripheral arteries, vary in susceptibility to metabolic stress and may respond differently to AMPK modulation. This study examined the role of AMPK in endothelial function across multiple vascular beds in MetS, with a focus on sex-dependent effects. MetS was induced in male and female Wistar Kyoto rats by a high-fat diet (HFD, 10 weeks). AMPK activity was inhibited using Compound C (1.5 mg/kg, twice weekly for 4 weeks). Endothelial function was assessed in the aorta, small mesenteric, and femoral arteries. In males, HFD caused pronounced ED in the aorta with reduced acetylcholine (Ach)-induced relaxation, worsened by AMPK inhibition. Combined HFD and AMPK inhibition increased Nos3 mRNA but reduced eNOS protein, suggesting post-transcriptional regulation. Similar impairments occurred in mesenteric and femoral arteries, affecting NO-dependent and NO-independent relaxation and enhancing EDCF-mediated contraction under NOS inhibition. In females, HFD alone did not cause overt ED. However, AMPK inhibition during HFD exposure induced ED, particularly affecting NO-dependent relaxation in the aorta and femoral artery. Maximal relaxation in females was slightly reduced by HFD alone in the aorta, indicating early endothelial changes. These findings show that AMPK is crucial for endothelial function in multiple vascular beds during MetS and exhibits sex-dependent effects. Males are highly susceptible to HFD-induced ED, whereas females are relatively protected unless AMPK is inhibited. Targeting AMPK may offer a therapeutic approach to reduce vascular complications in MetS.

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PP I_B17

Protective role of AMPK during metabolic syndrome development: differences between males and females

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AMP-activated protein kinase (AMPK) is a key metabolic regulator that plays an essential role in maintaining vascular homeostasis. Reduced nitric oxide bioavailability caused by oxidative stress and inflammation is a hallmark of endothelial dysfunction and represents a major complication of metabolic syndrome (MetS), contributing significantly to cardiovascular morbidity and mortality. This study investigated the protective role of AMPK in MetS and potential sex differences. MetS was induced by a high-fat diet (HFD, 10 weeks) in male and female Wistar Kyoto rats. The role of AMPK was assessed using its inhibitor, Compound C (1.5 mg/kg, twice weekly for 4 weeks). Male rats with MetS exhibited increased body weight gain, adiposity, and impaired metabolic regulation. Aortic endothelial dysfunction was associated with elevated reactive oxygen species production and enhanced inflammatory markers (iNOS, COX2, TNFα). Under metabolic stress, AMPK inhibition further aggravated endothelial dysfunction, accompanied by metabolic dysregulation, oxidative stress, and inflammation. In females, preserved AMPK activity was associated with a protective effect against MetS-induced cardiovascular damage, likely mediated by estrogen signaling. In contrast, AMPK inhibition abolished this protection and promoted endothelial dysfunction and cardiovascular impairment. These findings suggest that AMPK is crucial for maintaining endothelial function during MetS progression in both sexes. In females, AMPK appears to interact with estrogen signaling pathways, enhancing vascular protection. Targeting AMPK activation may therefore represent a promising strategy to reduce cardiovascular risk in MetS, particularly in individuals with pre-existing cardiometabolic disorders.

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PP I_B18

Extracellular quantum sensing in bioreactor-grown yeast cells using fluorescent nanodiamonds

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Fluorescent nanodiamonds (FNDs) are nanoparticles that have been successfully applied to intracellular quantum sensing for the detection of free radicals, which play a key role in numerous cellular processes. Owing to their resistance to photobleaching, FNDs enable real-time monitoring of cellular health through

quantitative measurements of free radical levels. FNDs host a crystalline defect known as the negatively charged nitrogen–vacancy (NV^-) center, formed when a nitrogen atom substitutes for a carbon atom adjacent to a lattice vacancy. These NV centers exhibit magnetic properties and spin-dependent luminescence, allowing optical signal readout. Quantum sensing is performed using diamond magnetometry via T_1 relaxometry, which relies on optical pumping of the NV center into a bright ground state using a 532 nm laser pulse. The relaxation time of this state is directly correlated with the local concentration of radical species. The primary objective of this project is to establish a protocol for employing extracellular FNDs as biosensors to evaluate the physiological state of bioreactor-grown *Saccharomyces cerevisiae* cultures. Nanoparticle internalization in yeast is challenging due to the presence of a thick cell wall, and cell wall–disrupting methods are often time-consuming and costly. Therefore, an extracellular sensing strategy was adopted to assess free radical production under stress conditions. To mimic a bioreactor failure, yeast cultures incubated with nanodiamonds were first measured at 30°C and subsequently exposed to elevated temperature of 50°C. Preliminary analysis of the T_1-relaxometry data revealed a distinction between the two temperature conditions. A reduction in T_1 relaxation times indicated an increased concentration of free radicals in the vicinity of the FNDs induced by thermal stress of cells. This work demonstrates the potential of nanodiamond-based quantum sensing as a powerful tool for monitoring the physiological state of bioreactor-grown yeast cultures without cell wall treatment.

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Poster Presentations I Group C – Inflammation and Immunity

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PP I_C06
Neuroimmune regulation of hyperoxia-induced lung injury via the cholinergic anti-inflammatory pathway

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Patients requiring mechanical ventilation with supplemental oxygen, particularly during severe respiratory failure such as that observed in COVID-19, frequently develop persistent cognitive impairment. This condition is well documented among survivors of intensive care unit (ICU) stays. Emerging evidence suggests that the relationship between brain and lung injury is bidirectional. Cognitive impairment has been associated with increased susceptibility to lung injury and pulmonary infections, highlighting the importance of neuroimmune communication pathways in regulating pulmonary health and disease. The discovery of the vagus nerve–mediated cholinergic anti-inflammatory reflex, which suppresses inflammatory responses through activation of α7 nicotinic acetylcholine receptors (α7nAChR) on macrophages, provides a potential mechanistic link between neural regulation and pulmonary inflammation. To explore the relevance of this pathway in the context of COVID-19 and respiratory failure, we conducted a meta-analysis of more than 100,000 COVID-19 patients across 71 studies to examine the relationship between nicotine exposure from tobacco use and COVID-19. Surprisingly, only 10.1% of COVID-19 patients were smokers, substantially lower than the global smoking prevalence of 21.9%, and comparisons within individual studies consistently revealed a lower proportion of smokers among COVID-19 patients relative to their regional populations. We further tested whether activation of the cholinergic anti-inflammatory pathway via α7nAChR signaling could mitigate hyperoxia-induced inflammatory lung injury, a clinically relevant model of supplemental oxygen therapy. Administration of nicotine after the onset of hyperoxia significantly attenuated lung injury and improved survival in mice, which associated with markedly reduced levels of the damage-associated molecular pattern HMGB1 in both airway fluids and systemic circulation. Nicotine also suppressed hyperoxia-induced HMGB1 release in cultured macrophages while preserving macrophage functions. Together, these findings highlight the importance of neuroimmune signaling in regulating pulmonary inflammation and suggest that targeting the vagus nerve–mediated cholinergic anti-inflammatory pathway may represent novel strategies to provide lung protection during severe respiratory failure.

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PP I_C07
Mitochondrial dysfunction in AR42J pancreatic acinar cells during endotoxemia

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Sepsis and endotoxemia represent systemic inflammatory syndromes driven primarily by the host response to infection. Molecular mechanisms within the exocrine pancreas cells have gained attention, as these cells represent an early and important target during endotoxemia and sepsis. Their high metabolic demand, dependence on mitochondrial function, and sensitivity to inflammatory stress position acinar cells as a relevant model for studying how systemic inflammation translates into dysfunction at the cellular level in the pancreas. Our aim was to analyze the cellular oxidative and nitrosative status and mitochondrial function in a cellular model of pancreatic endotoxemia. AR42J rat pancreatic acinar cells were treated with serum obtained from rats previously injected ip with 8 mg/kg LPS or vehicle (control). This unique experimental model highlights the physiopathological effects of systemic inflammation in a cellular model. Mitochondrial function by measuring ATP production, mitochondrial membrane potential, and mitochondrial mass were assessed. In addition, ROS production and NO levels were analyzed. H_2O_2 (H_2O_2 control value: 0.030±0.001 nmol/min mg protein) levels increased from 2 to 24 hours after serum-LPS-treatment, while NO production increases at 6h. Mitochondrial membrane potential was found decreased from 2 hours, while the mitochondrial ATP production rate was found decreased from 6 hours (control value: 3.3±0.3 nmol/min mg protein). Interestingly, mitochondrial function shows control values at 24h. Our results show that in this pancreatic cell model of endotoxemia, mitochondrial function becomes impaired in association with elevated NO levels. This model approach provides a valuable complement to vivo studies and may help to further elucidate the molecular mechanisms activated in the pancreas during endotoxemia and sepsis.

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PP I_C08
Electrophilic natural products and NRF2 activators exert bidirectional control over pro-inflammatory gene expression

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Electrophilic natural products and synthetic drugs are generally viewed as anti-inflammatory agents that act by inhibiting the NFκB pathway. Alongside Nrf2-dependent activation of antioxidant defenses, this anti-inflammatory activity is thought to underlie many of their health benefits. However, our data indicate that the concept of dietary and pharmacological electrophiles (EPhs) functioning solely as anti-inflammatory compounds is overly simplistic. In our experiments, cultured cells were treated with combinations of electrophilic compounds and TNFα or IL-1β, followed by expression analysis of cytokines, chemokines, and adhesion-molecules. While EPhs suppressed several NFκB-dependent genes induced by TNFα or IL-1β, they failed to inhibit—or even upregulated—a subset of strongly pro-inflammatory genes, including IL-8, COX-2, CXCL1, CXCL2, and CXCL3. This effect was observed in endothelial cells, monocytes, fibroblasts, and keratinocytes. Induction of these genes was blocked by p38 MAPK inhibitors, and increased mRNA stability was also detected. These findings suggest that EPhs do not uniformly suppress cytokine-driven inflammation but instead reshape the inflammatory gene profile. This raises the possibility that pharmacologically counteracting the pro-inflammatory arm of EPh action—such as through inhibition of p38 MAPK or its downstream targets—could enhance their overall anti-inflammatory potential in therapeutic or dietary contexts.

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PP I_C10

Targeting NF-κB/Nrf2 crosstalk restores redox–inflammatory signaling in Rett Syndrome

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Rett syndrome (RTT) is a neurodevelopmental disorder caused by mutations in the X-linked MECP2 gene and is characterized by chronic oxidative stress and persistent subclinical inflammation. NF-κB and Nrf2 are central regulators of inflammatory and antioxidant responses, respectively, and their coordinated crosstalk is essential for maintaining redox homeostasis. Whether this regulatory interplay is functionally altered in RTT remains unclear. The aim of this study was to investigate the status of NF-κB/Nrf2 signaling and their crosstalk in primary dermal fibroblasts derived from RTT patients and healthy controls (CTR), under basal conditions and following exposure to lipopolysaccharide (LPS), alone or in combination with sulforaphane (SFN), and the NF-κB inhibitor BAY-11-7082, used as pathway-specific modulators. Nuclear localization of NF-κB p65 and Nrf2, target gene expression, and selected post-translational regulatory components were analyzed. Under basal conditions, RTT fibroblasts exhibited increased nuclear NF-κB p65 and elevated acetylated NF-κB levels, whereas Nrf2 activation appeared blunted, consistent with a persistent OxInflammatory state. This profile was associated with increased CBP/p300 levels without a compensatory upregulation of SIRT1, suggesting altered acetylation–deacetylation dynamics favoring sustained NF-κB activity. Upon LPS stimulation, CTR cells showed coordinated activation of both NF-κB and Nrf2 pathways with appropriate downstream gene induction. In contrast, RTT fibroblasts failed to induce a physiological response, supporting the presence of dysfunctional pathway crosstalk. Combined treatments significantly reduced IL6 and CXCL8 expression and increased HMOX1 transcription and HO-1 protein levels in RTT fibroblasts. Collectively, these data suggest that dysregulation of NF-κB/Nrf2 crosstalk may represent a disease-related feature of RTT, contributing to its persistent OxInflammatory profile. Targeted modulation of this axis warrants further investigation as a potential therapeutic strategy.

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PP I_C11

Mitochondrial dysfunction and ox inflammatory signatures across Rett Syndrome progression: integrating in vitro and ex vivo evidence

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Rett syndrome (RTT), also known as MECP2-related disorder, is a severe neurodevelopmental condition that predominantly affects girls and is primarily associated with mutations in the MECP2 gene. After an initial phase of apparently normal development, affected children experience a gradual decline in cognitive and motor abilities. While the genetic basis of RTT is well established, the mechanisms driving disease progression remain incompletely understood. Increasing evidence, however, points to a role for mitochondrial dysfunction, oxidative stress, and inflammation. In this study, we investigated mitochondrial alterations occurring during RTT progression in Mecp2 knockout mice, using both in vitro and ex vivo approaches. Primary cortical neurons were analyzed across distinct stages of maturation (DIV6, DIV12, and DIV18), corresponding to the presymptomatic, transitional, and symptomatic phases of RTT. Mitochondrial morphology and network organization were assessed through immunofluorescence microscopy and quantitative morphometric analysis. To complement these findings, ex vivo analyses were performed on cortical sections from Mecp2 knockout mice at postnatal days P0 and P49. Mitochondrial organization in brain tissue was examined using immunofluorescence imaging, while RT-PCR and Western blot assays quantified key regulators of mitochondrial biogenesis and dynamics, including TFAM, PPARGC1A, MFN1, MFN2, OPA1, DRP1, and FIS1 and MID49. Our results reveal a progressive emergence of mitochondrial abnormalities in MeCP2-deficient neurons, with early disruptions in network organization and more marked structural defects at later maturation stages. These observations are further supported by ex vivo data from P49 cortical tissue, which show pronounced mitochondrial structural alterations compared to early postnatal development (P0). In conclusions, we showed that mitochondrial abnormalities are critical for RTT progression and may contribute to the promotion of oxidative stress and inflammation. Future studies will expand on these results using induced pluripotent stem cells (iPSCs) derived from RTT patients and differentiated into neurons to study mitochondrial abnormalities.

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PP I_C12

Site-selective tyrosine modifications and cross-linking of the grass pollen allergen Phl p 5a by peroxynitrite

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Reactive oxygen and nitrogen species generated during oxidative stress chemically modify proteins, altering protein structure and function. Peroxynitrite is particularly relevant in this context as it is strongly linked to inflammatory diseases and environmental stress responses. Its decomposition yields nitrogen dioxide, carbonate and hydroxyl radicals that can initiate oxidative modification of tyrosine residues, giving rise to nitration, hydroxylation, and covalent dityrosine cross-links. These radical-mediated processes are increasingly recognized as a molecular link between environmental oxidative stress and adverse health outcomes, yet which tyrosine residues are preferentially modified within

proteins remains insufficiently understood. We investigate peroxynitrite-induced tyrosine modifications using the major grass pollen allergen Phl p 5a as a protein model relevant to airborne exposure. Bottom-up LC–MS/MS combined with SEC-HPLC was employed to resolve site-specific tyrosine nitration, hydroxylation, and covalent tyrosine cross-link formation across a range of peroxynitrite-to-protein ratios. Nitration reached a maximum extent of ~ 60% at the highest investigated peroxynitrite-to-protein ratio (5/1), involving all tyrosine residues. Tyrosine nitration exhibited asymptotic dose–response behavior, consistent with preferential modification of a subset of tyrosine residues. In contrast, hydroxylation remained limited (~ 1.8%), affected 7 residues and showed near linear dose–response behavior. Multiple tyrosine cross-links were detected, with highest abundance at 3/1 peroxynitrite-to-protein ratio. Site-resolved analysis indicates that atom-specific solvent accessibility, local environment, and structural flexibility influence the susceptibility of tyrosine residues to modification and the resulting modification patterns. By linking peroxynitrite exposure to selective protein modification patterns, this work provides insight into how oxidative stress may shape protein modification, contributing to the molecular understanding relevant to human health.

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PP I_C13

Detection and effects of microbial *N*-chloramines and thiol oxidation during host pathogen interaction

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In humans, neutrophils represent the initial line of defense against bacterial pathogens. Invading bacteria are killed by neutrophils by a process called phagocytosis, during which bacteria are exposed to oxidative substances. One of these substances is the highly toxic hypochlorous acid (HOCl), which is generated by Myeloperoxidase (MPO) in the phagolysosome. In the phagolysosome HOCl can react to the even more toxic taurine-*N*-chloramine (TauCl) or monochloramine (NH_2Cl). Both are involved in pathogen clearance. In Proteins, HOCl, TauCl and NH_2Cl primarily oxidizes thiol groups but can also react with free amines to form protein *N*-chloramines. However, which of these three chlorinating species reacts to which extent, and how these reactions contribute to bacterial killing remains unknown. To better understand the specific roles of different chlorinating species, we compared their reactivity in inducing *N*-chlorination and thiol oxidation. Our data demonstrate that HOCl, TauCl, and NH_2Cl exhibit distinct and sometimes opposing chlorination and oxidation efficiencies toward thiols and lysines. While TauCl and NH_2Cl exhibit higher toxicity to *E. coli* compared to HOCl, TauCl induces lower intracellular thiol oxidation and produces hardly any intracellular *N*-chloramines. Kinetic analyses *in vitro* further reveal that HOCl reacts fastest with roGFP2 protein thiols, followed by NH_2Cl and TauCl. Despite the clear effect of *N*-chlorination on protein function, there are currently no strategies to study the extent of *N*-chloramines *in vivo*. We have recently shown that protein *N*-chlorination can be detected *in vitro* using the DANSyl derivative DANSyl sulfinic acid ($DANSO_2H$). To advance insights into the role of *N*-chlorination in host-pathogen interactions, we aim to harness this chemistry in combination with isotopic labeling to establish a robust, quantifiable labeling protocol to detect *N*-chloramines *in vitro* and *in vivo*. The labeling degree was quantified through in-gel fluorescence measurements of the DANSyl products and confirmed by mass spectrometry analysis.

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PP I_C14

Nitric oxide regulates innate immune memory in alveolar macrophages

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Trained immunity is a functional state of the innate immune response, which is characterized by long-term epigenetic and reprogramming of the innate immune system. This enables a fast and strong response to future challenges. The production of nitric oxide (NO) is part of the response to pathogen attack, but less is known, if these molecules are essential for establishing innate immune memory. We are investigating redox-signaling in trained immunity in alveolar macrophages using mouse MH-S cell line. We demonstrated that treatment with flagellin is inducing innate immune training. This results, in comparison to non-trained cells, in enhanced expression of immune marker genes, such as *TNF* and *NOS2*, after treatment with a second stimulus (LPS). Both, flagellin and LPS treatment, are inducing NO production. Interestingly, inhibition of flagellin-induced NO production with the NO synthase inhibitor L-NMMA significantly reduced the flagellin-induced training effect. In contrast, treatment with the NO synthase inhibitor as first stimulus is also inducing innate immune training. RNA-seq analysis revealed that L-NMMA is up-regulating and training genes involved in mitochondria organization, oxidative phosphorylation and ribosome biogenesis. Since mitochondria play a pivotal role in establishing innate immune memory, the regulatory function of NO in trained immunity seems to be the coordination of mitochondrial processes. Overall, a detailed understanding of redox-mechanisms in trained immunity might allow us to develop immunotherapies to promote trained immunity on one side and to treat excessive or defective trained immunity on the other side.

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PP I_C15

Immunoproteasome inhibition reduces inflammation driven oxidative protein damage in diabetic wound healing

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Chronic diabetic wounds are characterized by sustained inflammation and elevated reactive oxygen species (ROS) production, leading to impaired tissue repair and oxidative protein damage. While proteostatic dysfunction has been implicated in wound pathology, growing evidence suggests that inflammation-driven ROS generation is a primary source of oxidative damage in chronic wounds. The immunoproteasome, a specialized proteasome variant predominantly expressed in inflammatory cells, regulates pro-inflammatory signaling; however, its role in linking inflammation to oxidative protein damage during diabetic wound healing remains unclear. The aim of this study was to determine whether immunoproteasome activity couples inflammatory signaling to ROS-mediated protein oxidation in chronic diabetic wounds. Animals were treated with the selective immunoproteasome inhibitor ONX 0914 (10 mg/kg). Wound healing was assessed macroscopically using standardized digital planimetry and histologically by hematoxylin and eosin and Masson's trichrome staining. Oxidative stress parameters, including superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), malondialdehyde (MDA), and protein carbonylation, were quantified by ELISA. Pro-inflammatory cytokines IL-1β, IL-6, and TNF-α were analyzed by western blot ($p<0.05$). Immunoproteasome inhibition significantly accelerated wound closure and improved histological healing. ONX 0914 treatment markedly reduced IL-1β, IL-6, and TNF-α expression and was associated with decreased lipid peroxidation and protein carbonyl levels, indicating attenuation of oxidative protein damage. Antioxidant defense markers

were significantly higher compared to untreated diabetic controls. These findings demonstrate that, in diabetic wounds, oxidative protein damage is primarily driven by inflammation-associated ROS rather than impaired clearance of oxidized proteins. By uncoupling inflammatory signaling from oxidative stress, immunoproteasome inhibition emerges as a mechanistically novel strategy to improve the redox and inflammatory microenvironment in chronic diabetic wound healing. This work provides new insight into the crosstalk between immunoproteasome activity, inflammation, and oxidative protein damage in chronic diabetic wound healing.

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PP I_C16

Olive oil-derived hydroxytyrosol counteracts oxysterol-induced neuroinflammation in Alzheimer's disease through activation of the SIRT1 pathway

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Cholesterol oxidation products, known as oxysterols, are among the main contributors to Alzheimer's disease (AD), as they play a critical role in promoting neuroinflammation. Given the lack of effective therapeutic options for AD, increasing attention has been directed toward preventive strategies. Growing experimental evidence indicates that adherence to the Mediterranean diet is associated with a reduced risk of dementia. Notably, olive oil contains hydroxytyrosol (HXT), a potent polyphenol widely recognized as a bioactive compound capable of improving cognitive function. However, limited information is currently available regarding the anti-inflammatory effects of HXT in the context of AD. The present study aimed to evaluate the ability of HXT to prevent neuroinflammation induced by an oxysterol mixture whose composition reflects the levels previously quantified in brain samples from patients with severe AD, using human neuroblastoma SK-N-BE cells as an in vitro model. We also investigated the involvement of sirtuin 1 (SIRT1)-dependent anti-inflammatory pathways underlying the effects of HXT. Our results showed that oxysterol exposure significantly increased the expression and production of the inflammatory mediators IL-1β, IL-6, IL-8, TNFα, IFNγ, and MCP-1. This pro-inflammatory response was markedly attenuated by HXT pre-treatment. Furthermore, oxysterols were found to trigger neuroinflammation through TLR4-mediated NFκB activation. HXT treatment induced SIRT1 mRNA overexpression, increased protein levels, and enhanced enzymatic activity. Through SIRT1 activation, HXT inhibited oxysterol-induced TLR4 upregulation and the subsequent nuclear translocation of NFκB p65. Importantly, the anti-inflammatory effects of HXT were abolished in the presence of sirtinol, a specific SIRT1 inhibitor. Overall, these findings demonstrate that HXT mitigates oxysterol-induced neuroinflammation via modulation of the SIRT1/TLR4/NFκB pathway, supporting its potential as a nutraceutical strategy to prevent neuroinflammation and, consequently, neurodegeneration in AD.

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PP I_C17

Mechanisms of N-Chloramine reduction in Escherichia coli during host-pathogen interaction

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During the innate immune response, neutrophils generate highly reactive oxygen species (ROS), including hypochlorous acid (HOCl), via the enzyme myeloperoxidase. Beyond thiol oxidation, HOCl reacts with primary amines on lysine residues to form *N*-chloramines and can also modify arginine side chains, leading to *N*-chlorinated proteins. While bacterial systems that repair oxidized thiols are well characterized, significantly less is known about the mechanisms involved in *N*-chloramine reduction. In the cellular environment, both HOCl and *N*-chloramines are highly reactive and can disrupt proteostasis, impair protein function, and ultimately affect bacterial fitness. Therefore, the efficient neutralization and repair of these modifications are critical for survival during host-induced oxidative stress. This study aimed to identify cellular pathways involved in *N*-chloramine reduction, focusing on the glutathione (GSH) and thioredoxin (Trx) systems in *Escherichia coli*. Protein *N*-chlorination was monitored *in vivo* using the fluorescent probe DANSyl sulfinic acid ($DANSO_2H$), enabling time-resolved quantification in wild type and KEIO deletion strains lacking components of redox systems. *In vitro*, an NADPH-coupled thioredoxin assay was used to assess enzymatic *N*-chloramine reduction activity. Samples were further analyzed by SDS-PAGE to visualize changes in *N*-chloramine levels over time. Our *in vivo* data suggest that GSH acts as an important chemical buffer, as a GSH lacking mutant showed increased initial chlorination and delayed recovery compared to wild type. This phenotype could be complemented by exogenous GSH uptake. Additionally, *in vitro* assays indicate that the Trx system contributes to *N*-chloramine reduction through an enzymatic mechanism, supported by TrxA-dependent NADPH consumption and a decrease in *N*-chloramine signal. Together, these findings suggest that both systems may play distinct roles in *N*-chloramine reduction, supporting proteostasis and bacterial fitness under host-induced stress.

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PP I_C18

Development of oxidized glutathione based skin-permeable gels to prevent postoperative adhesions

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Post-operative adhesions are pathological connections binding organs or tissues across a virtual space, which occur in 79–93% of patients following a major abdominal or pelvic procedure. These are a leading cause of postoperative complications, including intestinal obstruction. The most common preventive approach for post-operative abdominal adhesions is currently the implantation or administration of biomaterial barrier products, which are effective in only half of the patients and have limitations in their practicability. Therefore, there is a great need for a more effective and more widely applicable preventative treatment for postoperative abdominal adhesions. We have previously reported that peritoneal resident-macrophages accumulate on fibrin clots and function as a physical barrier during postoperative adhesion formation. Recently, we also found that oxidized glutathione (GSSG) exhibits anti-inflammatory and tissue-repairing properties. To establish a minimally invasive therapeutic approach, we developed a skin-permeable gel encapsulating GSSG using S/O (solid-in-oil) technology. As we expected, the skin-permeable GSSG gels prevented post-operative abdominal adhesions in the mouse model and increased glutathione level in the peritoneal cavity cells, indicating improved intracellular redox state of peritoneal resident-macrophages. Furthermore, the skin-permeable GSSG gel treatment reduced pericardial adhesion formation and improved cardiac dysfunction after myocardial infarction. Notably, flow cytometric analysis showed a significant reduction of chronic inflammatory cells in the pleural cavity. These findings suggest that the newly developed oxidized glutathione gel not only inhibits postoperative adhesion formation but may also ameliorate chronic heart failure. The skin-permeable immunomodulatory strategy represents a promising and minimally invasive therapeutic approach for post-operative adhesion prevention and its related complications.

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Poster Presentations I Group D – Brain Function & Neurodegeneration and Ageing

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PP I_D04
Peripheral blood biomarkers RCAN1, Clusterin, RAGE, and malondialdehyde for early diagnosis and progression of Alzheimer's Disease

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Alzheimer's disease (AD) is strongly linked to oxidative stress, redox imbalance, and chronic inflammation, processes influenced by environmental exposures and lifestyle factors. Identifying peripheral redox-related biomarkers could provide minimally invasive tools for early detection and risk stratification. We evaluated four candidates involved in redox signaling and oxidative damage pathways: malondialdehyde (MDA, a lipid peroxidation marker), RAGE (receptor for advanced glycation end products), RCAN1 (a stress-responsive regulator of calcineurin), and Clusterin (a chaperone linked to oxidative and inflammatory stress). Participants were selected from the Vallecas Project (Spain). Longitudinal analyses included 52 individuals with paired baseline and 5-year follow-up samples. Cross-sectional analyses included 83 subjects classified as cognitively healthy controls (CTL), Mild-cognitive impairement (MCI), or Alzheimer's Disease (AD). Biomarkers were quantified in plasma/serum by ELISA (Clusterin, RCAN1, RAGE) or UPLC (MDA). A multivariable predictive model for AD diagnosis was developed using penalized logistic regression incorporating biomarkers, age, sex, and APOE ε4 genotype, with repeated stratified 7-fold cross-validation. Longitudinally, only Clusterin levels decreased in the AD progression group. Cross-sectionally, Clusterin, RCAN1, and MDA levels were significantly reduced in AD compared with controls and MCI subjects after multiple-comparison correction. Moreover, individuals who later converted to MCI exhibited higher baseline MDA and lower RAGE levels, suggesting early redox dysregulation preceding clinical decline. The predictive model achieved a mean cross-validated accuracy of ~92% and an AUC of 0.95 (95% CI: 0.94–0.96), demonstrating strong discriminative performance. Peripheral biomarkers linked to oxidative stress and redox signaling show dynamic alterations during cognitive decline and may capture early biochemical changes preceding clinical symptoms. These findings support the role of systemic redox dysregulation in AD pathogenesis and highlight the potential of blood-based redox biomarkers as accessible tools for early detection and precision prevention.

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PP I_D05
Physiological oxygen determines ion channel activities: role of physioxia in understanding vascular insufficiency

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Nearly all cell-based studies rely on atmospheric oxygen (~18kPa) as experimental condition, even though most tissues *in vivo*, including the brain, experience only ~4–8kPa oxygen, defined as physioxia. This systematic mismatch has influenced decades of experimental design and skewed our understanding of neuronal physiology. Emerging evidence indicates that cellular responses and proteome profiles differ significantly under atmospheric O_2 compared with *in vivo*-like conditions (~4–8kPa, physioxia). To characterise the effects of physiological O_2 on neuronal function we differentiated and cultured mouse neuroblastoma N2a cells at physiological (5kPa) and hyperxoxic (18kPa) followed by short-term hypoxic insults (~2kPa). Neurophysiological parameters were assessed under all oxygen conditions. Culture Preparation: N2a were cultured in DMEM + GlutaMAX and differentiated (1% FBS and 20μM of retinoic acid [RA] or 7-10 days) under 18kPa and subjected to reduced serum and RA for 7-10 days prior to any experimentation. Physioxia and hypoxia were achieved by culturing cells for 4-5days at 5kPa and 24hrs at 2kPa O_2, respectively. Electrophysiological recordings were made using a Nanion Port-a-Patch (Nanion Technologies) or MultiClamp 700B amplifier and HEKA Patchmaster and Clampex 11.2 (Molecular Devices) software. Immunocytochemistry and proliferation assays were performed at various timepoints.
Statistics: Unpaired Student's t-test and ANOVA were used where appropriate with $p<0.05$ considered significant. Results: Physioxia did not affect cell proliferation but increased the proportion of NeuN-positive cells and enhanced axonal length. Electrophysiological recordings revealed increased voltage-gated and TEA-sensitive potassium as well as increased voltage-gated sodium currents under physioxia, without changes in half-activation voltages ($V_{1/2}$). Neuronal excitability as measured by current-evoked action potential firing was unaffected under physioxia. Passive membrane properties were affected under physioxia culture conditions, resulting in lower membrane time-constants (t_m) and membrane resistance (R_m) but unchanged cell capacitance (C_m). Our data provide new evidence that physioxia alters neuronal phenotypes compared with hyperoxic culture conditions.
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PP I_D06
Exploring the neuroprotective potential of a red wine polyphenolic extract in rotenone-based cellular and mouse models of Parkinson's disease

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Parkinson's disease (PD) is a progressive neurodegenerative disorder and one of the fastest-growing neurological conditions. It is characterized by degeneration of dopaminergic neurons in the substantia nigra pars compacta that leads to striatal dopamine depletion and motor impairment. Although PD aetiology remains unclear, it is believed that PD arises from a complex interplay between endogenous and environmental factors that converge on interconnected mechanisms of neurodegeneration, including mitochondrial dysfunction, nitroxidative stress and neuroinflammation. Emerging evidence further implicates intestinal dysbiosis and gut–brain axis dysfunction in PD pathogenesis. PD remains incurable, highlighting the urgent need to develop effective strategies to prevent and/or limit dopaminergic neurodegeneration. Notably, epidemiological and preclinical findings suggest a potential neuroprotective role for dietary polyphenols. However, the underlying mechanisms remain largely elusive. Increasing evidence indicates that polyphenols may exert their beneficial effects through modulation of key redox signalling cascades and gene transcription, while also influencing the composition and activity of the gut microbiota. The present study aimed to explore the neuroprotective potential of a non-alcoholic polyphenolic extract obtained from a Portuguese red wine (RWE) in cellular and mouse models of PD based on rotenone-induced mitochondrial dysfunction and redox imbalance. SH-SY5Y cells were challenged with rotenone in the presence or absence of different RWE concentrations, and cell viability was assessed. C57BL/6 mice were subjected to intraperitoneal injections of low-dose rotenone, with or without RWE supplementation. Sensorimotor performance was evaluated through behavioural tests, and faecal samples were collected for microbiota analysis. RWE significantly prevented rotenone-induced cytotoxicity in SH-SY5Y cells in a concentration-dependent manner. In vivo, RWE supplementation

improved motor performance in rotenone-exposed mice. Taken together, these preliminary results support the neuroprotective potential of RWE in experimental models of PD and are consistent with modulation of redox-associated neurodegenerative mechanisms.
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PP I_D07
Modulation-specific effects of telecommunication radiation on metabolic activity and gene expression in neuronal-like cells

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Wireless technologies produce widespread electromagnetic radiation, and the safety of these emissions are being tested continuously. Multiple studies suggest that low-power, non-ionizing telecommunications radiation is suggested to influence cellular redox balance. We hypothesised that exposure to low-power telecommunication signals will alter cellular mitochondrial function and gene expression in neuronal cells without significantly impacting overall metabolic viability. Methods: Neuroblastoma cell line, SHSY5Y cells were differentiated to neuronal like cells before exposing them to three radiation conditions: 5G phone mixed signals, sine wave or 256-QAM waveform. Cell viability was performed by Cell Titre blue assay. Mitochondrial function was measured by mitochondrial membrane permeability assays and the Seahorse analyser. Transcriptomics analysis was performed to investigate gene expression. Results and discussion: In the viability assay, only the 256-QAM signal appeared to directly lower metabolic viability. The Seahorse Mito Stress test mitochondrial performance was not impacted by radiation conditions. The intensity and nature of gene expression appear to be strongly associated with the specific type of radiation used, highlighting the importance of signal parameters when considering the health risks posed by low-power telecommunication signals. The relevance of these findings is indicated by the modulation of genes and pathways linked to neurodevelopment, stress signalling and inflammation, outlining a mechanistic framework for understanding how low-power telecommunication signals may affect neuronal cells.

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PP I_D08
The PNPO–PLP pathway is required for neonatal survival and cellular redox homeostasis

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Pyridoxamine 5′-phosphate oxidase (PNPO) is known as an oxygen-dependent enzyme that generates pyridoxal 5′-phosphate (PLP), an active form of vitamin B6 that supports multiple metabolic processes. However, physiological roles of PNPO in mammalian development remain unclear. Here, we examined the functional significance of PNPO using systemic Pnpo$^{-/-}$ mice and cellular models. Pnpo$^{-/-}$ mice exhibited perinatal lethality shortly after birth. Maternal supplementation with PLP or pyridoxal (PL), a dephosphorylated form of PLP, significantly extended neonatal survival, demonstrating that PNPO-dependent PLP supply is essential for early postnatal viability. To clarify molecular mechanisms underlying the lethality, we established *Pnpo*$^{-/-}$ mouse embryonic fibroblasts (MEFs). *Pnpo*$^{-/-}$ MEFs showed extensive cell death and impaired proliferation, which were fully rescued by PL supplementation, indicating critical roles of PNPO-PLP pathway in cell growth and survival. Intriguingly, treatment with a ferroptosis inhibitor rescued the cell death observed in *Pnpo*$^{-/-}$ MEFs but failed to restore their proliferative capacity. These findings indicate that lipid peroxidation underlies the cell death caused by PNPO deficiency, whereas the impaired proliferation likely reflects broader roles of PLP in metabolic pathways beyond its anti-ferroptotic function. Because PLP is required for selenocysteine synthesis, we examined expression levels of GPX4, an anti-ferroptotic selenoprotein, and found remarkable decrease of GPX4 in *Pnpo*$^{-/-}$ MEFs. Consistently, GPX4 protein level was also decreased in the hearts of *Pnpo*$^{-/-}$ neonates, implying that cardiac dysfunction due to elevated lipid peroxidation may be one of the causes of *Pnpo*$^{-/-}$ neonate lethality. Given the previously described role of the PNPO–PLP pathway in oxygen-responsive supersulfide synthesis, our current results suggest that this pathway functions as a central hub linking oxygen availability to antioxidant defense through coordinated regulation of selenoproteins in addition to supersulfides.

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PP I_D12
Comparative analysis of oxidative stress markers and erythrocyte functional properties in healthy children and young adults

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Previous studies on oxidative stress during aging have primarily focused on individuals aged 60 years and older, while considerably less attention has been paid to another important phase of development – adolescence. Erythrocytes are highly sensitive to oxidative changes, and their properties decline with age as oxidative status alters. This study investigated changes in markers of lipid peroxidation (TBARS), protein oxidation (AOPP), the ratio of reduced to oxidized glutathione (GSH/GSSG), and a marker of antioxidant capacity (FRAP) in erythrocytes from healthy children (mean age 4.7 years) and young adults (mean age 22.2 years) of both sexes. Additionally, erythrocyte deformability (filtration methods, ektacytometry), nitric oxide production (DAF fluorescence), and Na,K-ATPase activity (enzyme assay) were examined. Assessment of oxidative stress markers revealed significant age-dependent differences: AOPP levels were higher in young adults, whereas the GSH/GSSG ratio was higher in children. No differences were observed between groups in TBARS or FRAP. Although Na,K-ATPase activity was lower in young adults than in children. This was accompanied by increased mean cell volume (84.58 ± 3.55 fl in children *vs.* 90.03 ± 4.44 fl in young adults, $p < 0.01$) and reduced osmotic resistance. Nitric oxide production was lower in young adults (20 177 ± 434 a.u. in children vs. 12 418 ± 234 a.u. in young adults, $p < 0.0001$), while erythrocyte deformability remained unchanged (84.17 ± 0.84% in children vs. 84.17 ± 1.10% in young adults, $p > 0.05$). In conclusion, oxidative stress markers and erythrocyte properties change not only in old age but already in early adulthood. The age-related reduction in Na,K-ATPase functionality contributes, at least partially, to the decreased osmotic resistance and increased erythrocyte volume during growth. These changes may contribute to the decline in erythrocyte deformability later in life. The findings demonstrate that measurable erythrocyte alterations occur early in life, even in healthy individuals.
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PP I_D13
From scents to senescence: SELENBP1 as a regulator of redox signaling and endothelial aging

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Human selenium-binding protein 1 (SELENBP1) exhibits methanethiol oxidase (MTO) activity, converting volatile sulfur compounds such as methanethiol into hydrogen sulfide (H_2S) and hydrogen peroxide (H_2O_2). These redox-active signaling molecules link sulfur metabolism to cellular redox signaling pathways and are critically involved in aging and aging-associated diseases. Previously, the *C. elegans* ortholog SEMO-1 has been implicated in pro-aging effects. However, the role of MTOs in the regulation of aging, particularly in humans, remains poorly understood. To address this, we investigated the impact of SELENBP1 on aging in primary human umbilical vein endothelial cells (HUVECs). SELENBP1 was the only H_2S-producing enzyme substantially downregulated during both replicative and stress-induced senescence: protein levels and MTO activity of SELENBP1 were substantially diminished in senescent cells. SELENBP1 deficiency following siRNA treatment upregulated the expression of senescence markers, including senescence-associated β-galactosidase activity, and increased angiogenic capacity. Intracellular and mitochondrial levels of reactive oxygen species were not affected by SELENBP1 knockdown. However, both knockdown and overexpression of SELENBP1 altered the expression of proteins involved in H_2S metabolism as well as oxidative and ER stress-related proteins. These findings indicate a functional role of SELENBP1 as a regulator of senescence-associated processes, including angiogenesis and redox regulation, in human endothelial cells.

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PP I_D14

Feeding skin cellular regeneration: the importance of 1c metabolism and the potential of 5-MTHF to counteract UV-induced damage

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One-carbon metabolism is essential for DNA synthesis, epigenetic regulation, and the production of amino acids and lipids. The biologically active form of folate, 5-methyltetrahydrofolate (5-MTHF), plays a central role in cell growth, tissue repair and, in the skin, supports fibroblasts activity, collagen synthesis, and cell regeneration. Solar radiation reduces skin folate levels, particularly in aging skin, impairing repair mechanisms. Despite its importance in nutrition, the regenerative and anti-photoaging potential of folate in skincare remains largely unexplored. Most studies have focused on folic acid, a synthetic form of vitamin B9 that requires intracellular conversion to 5-MTHF and presents limitations due to low solubility, photo-instability, and metabolic activation requirements. In contrast, newly developed reduced folate salts, which are bioequivalent to active folate, show improved stability, enhanced skin penetration and antioxidant properties. Human dermal fibroblasts (HDF) were cultured to 80-90% confluence and exposed to a sublethal UVA dose identified through a preliminary time-course study. Two experimental setups were used: pre-treatment, where cells were incubated with 5-MTHF (Quatrefolic®) 24 hours before irradiation, and post-treatment, where cells were irradiated first and then treated with 5-MTHF for up to 72 hours. Supplementation with 5-MTHF showed dose-dependent cellular uptake, remaining stable for up to 48 hours. Moreover, 5-MTHF significantly reduced UVA-induced cytosolic and mitochondrial reactive oxygen species, particularly at higher concentrations, and enhanced wound healing, as demonstrated by improved closure rates in scratch assays. Both pre- and post-treatment with 5-MTHF reduced DNA damage, as confirmed by comet assay analysis at all tested concentrations. The impact of 5-MTHF in the remodelling of dermal structural components is also being studied.Overall, these findings highlight the potential of 5-MTHF as a protective and regenerative agent for skin health, supporting its application in cosmetic and therapeutic strategies, although further investigation is required to confirm these outcomes.

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PP I_D15

MeCP2 deficiency promotes mitochondrial alterations and inflammaging-related dysfunctions in ovaries: potential implications for neurodevelopmental disorders

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MECP2 is an X-linked gene, encoding an ubiquitous epigenetic regulator, functioning both as a repressor or an activator of gene transcription, and being involved in chromatin compaction. MECP2 gene dosage is critical, as its deficiency leads to Rett syndrome, and the duplication of its gene locus results in MECP2 duplication syndrome, which are two rare neurodevelopmental disorders. Although MeCP2 has been extensively investigated in the central nervous system, increasing evidence indicates its relevance in peripheral tissues, associated with chronic perturbation of redox homeostasis [1]. Importantly, brain and ovaries are engaged in continuous bidirectional communication: on one side through the hypothalamic–pituitary–gonadal axis, and on the other through ovarian hormones modulating brain functions, like memory, mood, and behaviour. Recently, a possible role played by this epigenetic reader in reproductive function and oocyte maturation has been described, although current data remain conflicting. The aim of this project is to investigate how MeCP2 deficiency could affect ovarian functionality and whether this alteration might be linked to premature (inflamm)aging of the tissue. To this end, ovaries from Mecp2$^{+/-}$ female mice [B6.129P2(C)-Mecp2$^{tm1.1Bird/J}$] (12- and 24-week-old mice) and wild-type littermates were collected. RNA-seq analysis of ovarian tissue revealed that, already at the pre-symptomatic stage (12 weeks), MeCP2 deficiency induces deregulation of extracellular matrix–receptor interaction and hormonal signalling pathways, suggesting early remodelling of the ovarian microenvironment. At 24 weeks, more pronounced alterations were observed, involving inflammatory signalling, mitochondrial oxidative phosphorylation, and ovarian steroidogenesis. These molecular changes were associated with inflammasome activation, depletion of the follicular reserve, and the emergence of an aging-like phenotype. Overall, our findings identify MeCP2 as a possible regulator of ovarian homeostasis and indicate that its deficiency promotes mitochondrial dysfunction and chronic inflammation, driving premature ovarian inflammaging. Furthermore, considering the brain-ovary axis, this condition may contribute/exacerbate neuronal symptoms in MeCP2-related pathologies.

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PP I_D16

The methanethiol oxidase SEMO-1 modulates lifespan and oxidative stress resistance in C. elegans in a substrate-dependent manner

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Methanethiol is a volatile sulfur compound naturally occurring in food. In humans, it is predominantly generated by intestinal bacteria through methionine metabolism and physiologically degraded by selenium-binding protein 1 (SELENBP1), which functions as a methanethiol oxidase (MTO), catalyzing the conversion of methanethiol into H_2S, H_2O_2, and formaldehyde. We previously demonstrated that the C. elegans ortholog of SELENBP1, SEMO-1, acts as a pro-aging factor under standard laboratory conditions, as SEMO-1 deficiency

extends lifespan and enhances resistance to paraquat-induced oxidative stress. However, these effects were observed in the absence of exogenous methanethiol sources, and it was unclear to what extent SEMO-1 constitutes a defense mechanism against methanethiol. Therefore, we investigated whether the longevity and stress-resistance phenotype of SEMO-1 knockout nematodes persists under methanethiol exposure and whether SEMO-1 influences methanethiol sensing. Methanethiol exposure was achieved by feeding nematodes L-methionine γ-lyase (MGL)-overexpressing E. coli. In contrast to standard laboratory conditions, methanethiol exposure abolished the enhanced stress resistance and longevity of SEMO-1 knockout animals. Rather, viability of SEMO-1-deficient worms was lower under these conditions than that of control animals. Behavioral assays revealed no difference between SEMO-1 knockout and wild-type nematodes in sensing MGL-overexpressing bacteria, whereas both groups preferred control bacteria. Moreover, SEMO-1 expression was not altered upon exposure to methanethiol. The role of SEMO-1 in methanethiol defense and in regulating longevity and stress resistance appears to be independent of each other, as paraquat exposure caused an upregulation of SEMO-1 expression, but not of MTO activity; this effect persisted in the presence of Methanethiol. Together, these findings demonstrate that the longevity and stress-resistance phenotype of SEMO-1 deficiency is strictly context-dependent and lost upon methanethiol exposure. The role of SEMO-1 in regulating aging and oxidative stress resistance is therefore critically influenced by environmental methanethiol availability.

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PP I_D17
Replicative ageing in human keratinocytes links cell cycle dysregulation with enhanced oxidative reactivity

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Skin integrity declines with age, in part due to dysfunction of keratinocytes, the primary cells of the epidermis. Two major features of skin ageing are cellular senescence and increased oxidative stress. While both processes are well established, the way they interact within keratinocytes and how this interaction contributes to overall skin ageing remains unclear. To study how cell cycle regulation and oxidative stress responses are linked in replicatively aged normal human keratinocytes (NHKs). Early- and late-passage NHKs were analyzed for expression of senescence-associated markers p16 INK4a , p21 Cip1 , and p53 using Western blotting and immunofluorescence. Oxidative stress responses were evaluated by measuring ultra-weak photon emission (UPE). Late-passage NHKs demonstrated significant upregulation of p16 INK4a and p21 Cip1 , accompanied by reduced p53 expression, indicative of a senescence-associated phenotype. Upon oxidative stimulation, these cells exhibited a markedly increased UPE signal compared to early-passage cells, reflecting enhanced oxidative reactivity. Replicative ageing in keratinocytes is associated with impaired cell cycle regulation and increased susceptibility to ROS-mediated damage. This coupling may contribute to compromised skin barrier function, delayed wound healing, and accelerated visible ageing. Targeting the interaction between senescence pathways and oxidative stress responses may offer novel strategies to preserve skin function during ageing.

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Poster Presentations II Group A – Redox Chemistry, Redox Signaling and Molecular Biology

PP II_A01
Coenzyme A biology under oxidative stress

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Coenzyme A (CoA) is an essential cofactor present in all living cells. CoA and its thioesters play critical roles in diverse biochemical processes, including cellular metabolism, signal transduction, regulation of gene expression, and the antioxidant response. Dysregulation of CoA biosynthesis in animal models and inborn mutations in human genes involved in the CoA biosynthetic pathway have been associated with neurodegeneration and cardiomyopathy. The discovery that CoA exhibits an antioxidant function, mediated by its covalent attachment to proteins (termed CoAlation) in response to oxidative stress, has paved the way for a new area of research. Progress in this emerging field of research has been driven by the development of innovative tools and methodologies, including: (a) anti-CoA mAb, which specifically recognize CoA in ELISA, WB, IP and IHC; (b) a robust mass spectrometry-based methodology for the identification of CoAlated proteins; and (c) efficient *in vitro* CoAlation and deCoAlation assays. They have been employed to demonstrate that protein CoAlation is a reversible and widespread post-translational modification induced by oxidizing agents and metabolic stress in cells, tissues and model organisms. To date, we have identified more than 2300 CoAlated proteins and showed that CoAlation modulates the activity and subcellular localization of modified proteins. It can also protect oxidized cysteine residues from over-oxidation and induce significant conformational changes. Based on these findings, we propose that under physiological conditions CoA functions as a key metabolic cofactor but acts as an antioxidant in cellular response to oxidative or metabolic stress. Recent advances on this emerging topic of redox regulation will be presented with the focus on enzymes implicated in regulation of the CoAlation/deCoAlation cycle.

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PP II_A02
Detection and partial purification of mammalian CoA-dependent thiol transferase

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Protein CoAlation is a universal, redox-dependent post-translational modification involving the conjugation of coenzyme A (CoA) to cysteine thiols. This modification alters protein function and is implicated in contexts from Alzheimer's disease pathology to bacterial hibernation, underscoring its broad biological significance. Despite this, the enzymes directly regulate protein CoAlation remains unknown, limiting functional *in vivo* studies. To address this, we initiated an activity-guided purification to isolate the putative CoA-transferase. A biochemical assay utilizing 1-chloro-2,4-dinitrobenzene (CDNB) was employed to screen for and track the CoA transferase activity. Starting from mouse tissue lysates, CoA-transferase activity was successively enriched through ammonium sulphate precipitation, hydrophobic interaction chromatography, anion-exchange chromatography, and size-exclusion chromatography, achieving approximately 100-fold purification. Following mass spectrometry of active fractions, candidate proteins were further prioritized using the open-source machine learning algorithm (Deep Molecule-ESP) yielding a shortlist of promising candidates. This work establishes a purification strategy and yields a focused candidate list, providing a critical foundation for the molecular identification of the mammalian CoA-transferase and future studies into CoAlation's role in redox biology.

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PP II_A03
Orthovanadate – a redox switch for superoxide?

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Superoxide radical anion ($O_2^{\bullet-}$) is a form of reactive oxygen species formed upon a single electron transfer to molecular oxygen. Its increased production has been implicated in numerous pathologies, while its role in redox signaling is mostly attributed to its ability to intercept nitric oxide and to serve as a precursor of hydrogen peroxide. One of the most challenging aspects in deciphering the role

of superoxide in redox biology and pathologic processes is its sensitive and specific detection. It has been demonstrated that orthovanadate, widely used as a phosphatase inhibitor, sensitizes the chemiluminescent probe L-012 to superoxide, enabling sensitive detection of $O_2^{\bullet-}$ in cells and blood vessels expressing NADPH oxidases. We hypothesized that the chemistry behind the L-012 chemiluminescence enhancement by orthovanadate can be extended to other redox probes sensitive to one-electron oxidation, with various detection modalities. Here, we report the results of our studies on the effect of orthovanadate on superoxide-induced oxidation of Amplex Red, a probe widely used in combination with peroxidase to detect hydrogen peroxide. We used chemical, enzymatic, and cellular sources of $O_2^{\bullet-}$ and observed that while Amplex Red is not oxidized by superoxide alone, in the presence of orthovanadate it undergoes conversion to red fluorescent resorufin in all three tested models of $O_2^{\bullet-}$ generation. We conclude that orthovanadate may act as a molecular switch converting a weakly oxidizing superoxide into a strongly oxidizing intermediate, which we tentatively attribute to a peroxyl radical-type adduct. This chemistry may be important both for development of new assays for superoxide detection and for better understanding of the biological chemistry and mechanisms of toxicity of orthovanadate.

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[2] Haigh et al., Antioxidants 2023, 12:1689.

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PP II_A04

Hetero-oligomerization drives structural plasticity of eukaryotic peroxiredoxins

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Peroxiredoxins (Prxs) are central regulators of cellular redox homeostasis, acting not only as highly efficient peroxide scavengers but also as modulators of redox signaling and stress adaptation. In eukaryotic cells, multiple Prx1/AhpC-type isoforms often coexist within the same compartment; however, their functional interplay has remained largely unexplored, as they have long been assumed to assemble exclusively into homo-oligomeric complexes. We identify hetero-oligomerization as a conserved and inducible feature of eukaryotic Prx1-type peroxiredoxins. Distinct isoforms co-assemble into hetero-dimers and hetero-decamers with a wide range of subunit stoichiometries. In yeast, oxidative stress triggers Tsa1–Tsa2 hetero-decamer formation, and incorporation of sub-stoichiometric Tsa2 subunits markedly stabilizes the decameric state. This substoichiometric effect supports a highly cooperative mechanism by which minor isoforms can remodel decamer architecture and functional properties. Hetero-oligomerization is not restricted to yeast but extends to human (PRDX1–PRDX2), plant (BAS1A–BAS1B), and kinetoplastid (LiPRX1–LiPRX2) systems, where mixed assemblies are catalytically competent and reshape dimer–decamer equilibria. Given that oligomeric state can influence reactivity, hyperoxidation susceptibility, and chaperone function, isoform mixing introduces an additional regulatory layer to peroxiredoxin biology. These findings overturn the long-standing homo-oligomer-only paradigm and establish hetero-oligomerization as an evolutionarily conserved mechanism that expands structural plasticity and tunes redox enzyme behavior across eukaryotic cells.

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PP II_A05

Copper-induced methanethiol oxidase activity of selenium-binding protein in Caenorhabditis elegans

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The Caenorhabditis elegans ortholog of the human selenium-binding protein 1 (SELENBP1), SEMO-1, has been identified as a methanethiol oxidase (MTO), which catalyzes the conversion of methanethiol to hydrogen sulfide (H_2S), hydrogen peroxide (H_2O_2) and formaldehyde. Previous in vitro-studies using recombinant SEMO-1 demonstrated copper-dependent enzymatic activity that was largely independent of selenite availability. However, the regulation of SEMO-1 by copper and selenium in vivo has not yet been fully characterized. To address this, the effects of modulating copper and selenite availability on SEMO-1 expression and enzymatic MTO activity in vivo were examined in C. elegans. SEMO-1 expression was analyzed by immunoblotting and qRT-PCR, and enzymatic activity was determined using a coupled assay based on in situ methanethiol generation followed by detection of methanethiol-derived H2S. Exposure to copper increased SEMO-1 protein levels, whereas copper chelation reduced them in a dose-dependent manner. This chelation-induced decrease was not reflected at semo-1 mRNA level. The observed changes in protein expression were accompanied by corresponding differences in enzymatic activity, supporting the previously demonstrated copper-dependence of SEMO-1. In contrast, selenite exposure markedly increased SEMO-1 protein levels without affecting overall MTO activity. Combined copper and selenite treatment increased SEMO-1 protein abundance and was associated with elevated enzymatic activity. When enzymatic activity was analyzed using equal amounts of SEMO-1, no further increase in activity was observed upon copper exposure. These findings indicate that SEMO-1, under standard nematode culture conditions, is largely copper-saturated in vivo. These data also suggest that SEMO-1 protein abundance and enzymatic activity can be uncoupled through regulating copper availability.

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[1] Philipp et al., BioFactors 48:699-706 (2022)
[2] Philipp et al., Redox Biol. 65:102807 (2023)

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PP II_A06

Oxygen dependence of hydrogen peroxide production in isolated mitochondria and permeabilized cells

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Reactive oxygen species ROS play vital roles in cell signalling but the magnitude of physiological ROS production and control by intracellular oxygen concentration remain controversial topics. The concept of *reductive stress* claims that mitochondrial ROS production increases under hypoxia, whereas *hyperoxic* oxidative stress represents ROS oxyconformance with higher ROS production at increasing O_2 concentrations. To address this discrepancy, we applied high-resolution respirometry to measure simultaneously respiration and H_2O_2 production (Amplex UltraRed^{TM} assay) in different mitochondrial preparations, varying oxygen concentrations from intracellular tissue normoxia (low O_2: 20-60 μM in the medium) to environmental normoxia at air saturation (high O_2: ~200 μM). We compared (*1*) mitochondria isolated from mouse brain and heart, (*2*) pure and crude preparations of mitochondria isolated from liver hepatoma cell lines, and (*3*) permeabilized liver hepatoma cells and human fibroblasts. At low respiratory rates in the leak state (no added ADP), when mitochondrial membrane potential and protonmotive force are high and O_2 flux compensates mainly for the proton leak, H_2O_2 flux was high at air saturation. In all mitochondrial preparations, H_2O_2 flux increased from anoxia to high O_2, whereas O_2 flux remained constant from low O_2 to air saturation. H_2O_2/O_2 flux ratios were ~0.02 at air saturation and declined to < 0.01 at intracellular tissue normoxia. Our results highlight the importance of controlling oxygen concentrations in measurements of ROS production in mitochondrial preparations. High O_2 near air saturation leads to artificially elevated hyperoxic stress compared to physiological intracellular O_2 concentrations. Simultaneous measurements of respiration and H_2O_2 production at physiological normoxia suggest that mitochondrial ROS production represents a smaller fraction of O_2 consumption than proposed in the current literature.

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PP II_A07

Dual promoter architecture of NFE2L2 underlies isoform-specific translational control of NRF2

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Transcription factor NRF2 (encoded by the *NFE2L2* gene) is a central signalling hub that coordinates antioxidant, detoxification, anti-inflammatory, and metabolic programmes to maintain cellular homeostasis. It plays a critical role in degenerative diseases, chronic inflammation, and cancer. *NFE2L2* is transcribed from two promoters: the canonical promoter (P1) and an alternative promoter (P2), generating transcripts that differ exclusively in exon 1. Exon 1 of P1-derived transcripts contains the AUG start codon, whereas the considerably longer exon 1 of P2-derived transcripts forms an extended 5′ untranslated region (5′UTR). In humans, the major P1-derived transcript encodes canonical NRF2 isoform 1, while the predominant P2-derived transcript encodes isoform 2, which is 16 amino acids shorter at the N-terminus. Although both isoforms are expressed at the mRNA level across human tissues, the existence of isoform 2 at the protein level and the functional significance of alternative promoter usage have remained unresolved. Using isoform-specific siRNA, we provide the first direct evidence of endogenous NRF2 isoform 2 protein expression in human cells and demonstrate that it activates canonical NRF2 target genes. Both isoforms are similarly unstable under basal conditions due to KEAP1-mediated degradation. Despite comparable mRNA levels in lung cells, P1- and P2-derived transcripts differ in translation efficiency: isoform 1 is actively translated under basal conditions, whereas isoform 2 is subject to an additional layer of translational control. The alternative 5′UTR of P2 transcripts contains evolutionarily conserved motifs with putative regulatory activity, providing a mechanistic basis for promoter-dependent translational tuning. Collectively, our findings identify an evolutionarily conserved transcriptional–translational circuit that expands the regulatory logic of NRF2 and reveals previously unrecognised complexity in its promoter-dependent expression.

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PP II_A08

Hypoxia-driven temozolomide resistance in glioblastoma is mediated by NRF2 and PGC-1α signaling

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Hypoxia promotes chemoresistance in glioblastoma (GBM), in part by activating NRF2-dependent stress defenses and mitochondrial adaptation programs. NRF2 also intersects with mitochondrial metabolism, and emerging evidence links NRF2 activity to PGC-1a–associated transcriptional programs that support oxidative metabolism and therapy tolerance. This study examined the role of NRF2 in hypoxia-associated resistance to temozolomide (TMZ) in U87MG cells and assessed whether pharmacologic suppression of NRF2 pathway output with brusatol enhances TMZ sensitivity under hypoxia. U87MG cells were cultured under normoxia or hypoxia (1%O_2) and treated with TMZ, brusatol, or the combination. Cell viability assays were used to determine IC_{50} values and combination index (CI). HIF-1a expression, EGFR phosphorylation, and NRF2 nuclear accumulation were analyzed by western blot. Antioxidant genes, growth-related signaling genes, and PGC-1a downstream mitochondrial targets were quantified by real-time PCR. PGC-1a nuclear localization and oxidative DNA damage (8-hydroxy-2′-deoxyguanosine; 8-OHdG) were assessed by immunofluorescence. Hypoxia increased the TMZ IC_{50}, accompanied by elevated HIF-1a expression and NRF2 nuclear accumulation. CI analysis indicated synergistic cytotoxicity for the TMZ–brusatol combination. Hypoxia upregulated antioxidant genes (GCLM, GPX, GST, HO-1, NQO1, SOD1) and growth-related signaling genes (NF-kB, TGFb, EGF, EGFR), and increased PGC-1a activity with higher expression of its downstream targets SIRT3, CYCS, and ATP5F1B. Brusatol co-treatment suppressed antioxidant gene induction, reduced EGFR phosphorylation, attenuated the PGC-1a–linked mitochondrial transcriptional program, and increased 8-OHdG accumulation. Pharmacologic suppression of NRF2 pathway output with brusatol enhances TMZ efficacy under hypoxia, coincident with reduced HIF-1a–linked EGFR signaling and concurrent disruption of redox control and PGC-1a–mediated mitochondrial adaptation programs implicated in hypoxia-associated TMZ tolerance in GBM.

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PP II_A09

Stimulator of sGC attenuates pro-oxidant activity in the model of chronic heart failure caused by pressure and volume overload

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Redox imbalance, characterized by increased oxidative stress and reduced antioxidant capacity, contributes to development and progression of heart failure (HF). Excess reactive oxygen species impair cellular signaling, promote myocardial remodeling, and lead to cardiac dysfunction. Recently, attention has been directed to the cardioprotective signalling pathway of nitric oxide (NO), soluble guanylate cyclase (sGC) and cyclic guanosine monophosphate (cGMP), which may be dysregulated under conditions of HF. The aim of our study was to examine the efficacy of sGC stimulator in attenuating pro-oxidant activity in the model of HF and hypertension. To evaluate protective effects of sGC stimulator *BAY41-8543* (3mg/kg/day, 30 weeks) alone or combined with ACE inhibitor *Trandolapril* (0.25mg/kg/day, 30 weeks), we used 10-weeks old male hypertensive Ren-2 transgenic rats (TGR) with volume overload caused by aortocaval fistula (ACF) (n = 27). Male TGR (n=16) and normotensive HanSD rats (n = 17) were used as control groups. The sGC stimulator caused a reduction in mortality of rats. TGR rats treated with sGC stimulator exhibited a significant increase in key antioxidant proteins - SOD1, CH10, ACSF2, NDUS1, DHE3, GSTM2 and PCCA. We also detected a significant increase in levels of MMP2, total and phosphorylated connexin-43, and PKCε, implicated in remodeling of extracellular matrix and intercellular communication, in the left ventricle of TGR rats. Our results support the hypothesis that sGC stimulators might be suitable for combating HF, particularly through their antioxidant properties, but due to adverse effect on fibrosis, further studies are needed to optimize treatment strategies.

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PP II_A10

Oxidative modification of human glucose 6-phosphate dehydrogenase induced by peroxynitrite affects enzyme activity in a bicarbonate-dependent manner

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Glucose-6-phosphate dehydrogenase (G6PDH) catalyzes the rate-limiting step of the pentose phosphate pathway, in which glucose-6-phosphate is oxidized to 6-phosphogluconolactone with concomitant reduction of $NADP^+$ to NADPH, a key cofactor for the redox homeostasis of cells. Despite the critical role of G6PDH in cell function and survival, the oxidation of the human isoform (*h*G6PDH),

triggered by peroxynitrite ($ONOO^-$), a well-established biological oxidant, has not been studied. We hypothesized that $ONOO^-$ exposure would induce modifications to *h*G6PDH at specific amino acids and affect catalytic activity, which would be modulated by bicarbonate ($NaHCO_3$). *h*G6PDH was expressed in *E. coli* BL21 and purified by Nickel-affinity chromatography and exposed to $ONOO^-$ in the absence or presence of 25mM $NaHCO_3$. Enzyme activity was determined by quantifying NADPH formation at 340 nm. SDS-PAGE, circular dichroism (CD), western blot (for carbonyls), fluorescence arising from ANS binding, and liquid chromatography with mass detection (LC-MS) were employed to characterize $ONOO^-$-mediated modifications. The exposure of *h*GPDH to 10- and 40-fold molar excess of $ONOO^-$ induced a loss of enzyme activity of 43% and 66%, respectively, with this reduced in the presence of $NaHCO_3$, to 22% and 38%, respectively. Minimal changes in ANS binding and the CD spectra were observed. New reducible crosslinks were detected (by SDS-PAGE) mainly in the absence of $NaHCO_3$. Oxidative modifications were detected by LC-MS which involves modifications at specific residues (W509, M212 and M450) with the extent of these changes decreased by $NaHCO_3$. These results demonstrate that NADPH generation by *h*G6PDH is significantly modulated by $ONOO^-$, with this explained by modifications at specific amino acids through processes that do not affect the secondary structure of the protein or its hydrophobic pockets. In the presence of $NaHCO_3$, a different pattern of reactivity is observed, explaining the decreased loss of enzyme activity induced by $ONOO^-/NaHCO_3$.

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PP II_A11

Nanodiamond quantum sensing of free radicals in chinese hamster ovary cells

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In the biopharmaceutical industry, bioreactor batch failures are highly common and extremely costly occurrences, threatening economical and environmental sustainability of biomaterial processes. Chinese hamster ovary (CHO) cells, the primary model for recombinant protein production, are increasingly cultured at high densities to meet market demands, which impose increased metabolic and oxygen requirements. These intensified conditions elevate cellular oxidative stress and waste accumulation, potentially compromising cell viability, productivity, and product quality. Effective control of cellular stress is therefore critical for maintaining robust and efficient bioprocesses. Free radicals are key indicators of cellular stress. However, their transient and highly reactive nature complicates their detection, using traditional stress sensing techniques, which often lack spatial resolution and are prone to predictive inaccuracies. To address this, we apply quantum sensing with fluorescent nanodiamonds (FNDs) containing NV centers to enable detection of magnetic noise from free radicals in living cells. NV centers emit stable red fluorescence that responds sensitively to local magnetic fluctuations, enabling real-time, nanoscale detection of free radicals. In this study, we monitor free radical dynamics and their relationship to cellular stress, using high spatio-temporal resolution diamond-based quantum sensing. This approach elucidates stress-related mechanisms affecting CHO cell performance, and establishes a foundation for developing early-detection markers of CHO cellular stress. This research paves the way forward for a sensor that supports real-time assessment of bioreactor health, and offers a potential tool to prevent bioreactor crashes and provide reliable biomarkers for the product yield and quality.

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PP II_A12

Identification of 4-hydroxynonenal adducted proteins in T cells

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A major consequence of oxidative stress is increased lipid peroxidation and the generation of reactive aldehydes including 4-hydroxynonenal (4HNE). This compound, and related species, are reactive molecules as they are α,β-unsaturated aldehydes which react rapidly with nucleophiles including Cys residues on proteins via Michael adduction. These reactions are rapid, and result in dysfunctional proteins which play a role in both signaling and multiple pathologies including neurodegenerative and cardiovascular diseases. Whilst previous studies have identified small numbers of 4HNE-modified proteins, we hypothesized that this might be the 'tip of the iceberg'. Here we have used click-chemistry and LC-MS-based proteomics to allow unbiased assessment of protein adduction. Treatment with Jurkat T cells with 1-30 μM 4HNE (with an attached alkyne tag) allowed identification of up to 5850 adducted proteins and their interaction partners. 2700 proteins were identified at the lowest concentration, with this reaching a maximum of 5500 proteins at 10 μM 4HNE. Some of the identifications are known targets (eg VDAC2, GAPDH, TXN) but many novel targets have been unveiled, including ADAR and CDK6, with the precise Cys that is adducted being determined. The concentration-dependent experiments indicate that some proteins are particularly susceptible, though whether this is due solely to Cys reactivity, or also abundance, remains to be determined. Complementary fluorescence imaging experiments have provided data on cellular sites. Extension to other α,β-unsaturated aldehydes (e.g., fumarates, prostaglandin J2) indicates some common targets, but also differential targeting, localization and numbers of affected proteins. These data highlight the importance of Cys as a 4HNE target, with the sites and targets being concentration dependent. Large numbers of novel modified species have been identified, thereby providing new insights into the effects of lipid peroxidation-derived aldehydes (and drug species such as fumarates), and contributes to a better understanding of protein adduction in pathology and drug treatment.

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PP II_A13

An in silico multi-scale molecular modeling and in vitro analysis of α-tocopherol and α-tocopheryl phosphate interactions with the Nox2 complex

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α-Tocopherol is traditionally defined as an antioxidant through its free hydroxyl group; however, growing evidence indicates that its biological effects extend beyond radical scavenging. α-Tocopheryl phosphate (α-TP), a phosphorylated physiological derivative of α-tocopherol, lacks classical antioxidant activity yet displays enhanced biological potency, suggesting distinct regulatory mechanisms. This study aimed to compare α-tocopherol and α-TP by in silico analysis of their interactions with the NOX2 complex, followed by experimental validation of their effects on superoxide production. An in silico multi-scale molecular modeling approach was employed as the primary investigative strategy. Because the complete structure of the assembled NOX2 complex is not available, a ternary structural model integrating key regulatory domains of p22phox, p47phox, and p67phox was constructed and refined through molecular dynamics simulations. Induced-fit docking and interaction analyses revealed distinct interaction patterns between α-tocopherol and α-TP with regulatory regions of the NOX2 complex, indicating that phosphorylation alters binding behavior and may promote non-antioxidant regulatory effects. To validate the computational predictions, complementary in vitro analyses were performed using phorbol 12-myristate 13-acetate–stimulated human umbilical vein endothelial cells as a model of NOX2 activation. Intracellular reactive oxygen species generation was assessed using the DCFH-DA fluorescence assay, while superoxide specificity was confirmed using superoxide dismutase–sensitive cytochrome c reduction. Changes in NOX2 gene expression were evaluated by quantitative real-time PCR, and pharmacological inhibition was applied to verify NADPH oxidase dependency. Cell viability assays confirmed the absence of cytotoxic effects. While α-tocopherol showed limited effects under these conditions, α-TP consistently modulated NOX2-related responses, supporting a regulatory mechanism that does not rely on classical antioxidant activity.

Overall, this combined in silico–in vitro study supports emerging non-antioxidant roles of vitamin E derivatives and highlights α-TP as a potential modulator of NOX2-mediated redox signaling.
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PP II_A14
PRDX-2 coordinates redox and ER stress signalling to support mitochondrial–ER communication during exercise

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Mitochondria and the endoplasmic reticulum (ER) are central regulators of cellular homeostasis, communicating through specialised mitochondrial–ER contact sites (MERCS) that enable metabolic exchange and signal integration. These contact sites are highly dynamic and respond rapidly to cellular stress. In skeletal muscle, exercise-induced contraction elevates reactive oxygen species (ROS) production and protein-folding demand, leading to activation of stress-response pathways and enhanced organelle coupling. Despite this, the molecular mechanisms governing MERCS regulation remain poorly understood. Here, we examined mitochondrial remodelling and MERCS dynamics following ROS generation using in vitro systems and a Caenorhabditis elegans exercise model. We show that mild oxidative stress promotes MERCS assembly, supporting mitochondrial network remodelling and improved physiological performance in C. elegans. In contrast, these adaptive responses were markedly compromised in prdx-2 mutant animals, which display elevated basal ER stress, altered redox homeostasis and a fragmented mitochondrial network. Mechanistically, our data demonstrate that exercise-induced adaptation requires PRDX-2 activity and signalling through the PEK-1 branch of the UPRER. In addition, PRDX-2 was found to operate within a shared regulatory network alongside key UPR-associated transcription factors. Collectively, these findings identify PRDX-2 as a pivotal coordinator of mitochondrial–ER communication, linking redox control with adaptive UPR signalling to drive mitochondrial remodelling and sustain physiological function in response to exercise.

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PP II_A15
Disruption of ER homeostasis by selenoproteins-induced reductive stress

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The endoplasmic reticulum (ER) is a critical organelle responsible for the oxidative folding of membrane and secretory proteins, and its lumen is maintained in an oxidizing redox state to support this process. When this environment shifts toward a more reducing state, oxidative protein folding is impaired, leading to the accumulation of misfolded proteins and ER stress. Excessive ER stress can trigger cell death and has been implicated in the pathogenesis of neurodegenerative diseases and diabetes. Although potent pharmacological agents have been widely used to induce ER stress, the impact of reductive stress under physiological conditions on ER function remains poorly understood. Selenoproteins, which contain the essential trace element selenium, play important roles in antioxidant defense. However, their overexpression can excessively enhance cellular antioxidant capacity, thereby inducing reductive stress and contributing to the worsening of diabetes [1]. Despite this connection, the effect of excess selenoproteins-induced reductive stress on the ER redox environment and its function has not yet been clarified. In this study, we investigated the effects of selenoproteins-induced reductive stress on the ER redox environment and proteostasis. Our findings suggest that excessive selenoproteins production disrupts ER homeostasis. These results provide new insights into the mechanisms by which excess selenoproteins may contribute to diabetes and may inform the development of future therapeutic strategies.
[1] Oo et al., *Cell Reports*, 2022

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PP II_A16
Maintenance of ER homeostasis through protein persulfidation

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The endoplasmic reticulum (ER) is a major site of oxidative protein folding. Within the ER lumen, disulfide oxidoreductases, such as protein disulfide isomerase (PDI), catalyze the formation of disulfide bonds. During this process, electrons are transferred to molecular oxygen, generating hydrogen peroxide (H_2O_2), a reactive oxygen species (ROS). While H_2O_2 is reduced by peroxiredoxin 4 (Prx4) and glutathione peroxidase 7 (Gpx7) to maintain redox balance in the ER, excessive H_2O_2 can leak into the cytosol, leading to oxidative stress. Previously, we identified ERp18 as a catalase-like enzyme that decomposes H_2O_2 in the ER lumen [1]. ERp18 binds zinc ions (Zn^{2+}) via a Zn^{2+}-binding motif, His-Xxx-Xxx-Xxx-Cys-Xxx-Xxx-Cys (HXXXCXXC), and forms a trimer that acquires catalase-like activity. However, the mechanisms underlying Zn^{2+} binding and dissociation, as well as the regulation of ERp18 function, remain unclear. Recently, persulfidation has been shown to regulate the function of Zn^{2+}-binding proteins [2]. These findings suggest that ERp18 undergoes persulfidation, leading to functional alterations and contributing to a novel redox regulatory mechanism in the ER. Notably, ERp18 has been implicated in aging in *Caenorhabditis elegans*, where it promotes lifespan extension. Therefore, persulfidation of ERp18 may provide novel insights into the molecular mechanisms underlying aging and ER homeostasis.
[1] Tsutsumi et al., *Cell Rep.* 2024
[2] Li et al., *RSC Chem.* Biol., 2024

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PP II_A17
Exploring the role of retinal cryptochromes and the radical pair mechanism in avian navigation

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Birds orient and navigate over long distances by extracting directional information from the Earth's magnetic field. The orientation is thought to involve a radical pair-based mechanism which requires a light-sensitive receptor molecule. The likely candidates include cryptochromes, a class of blue-light sensitive flavoproteins. It is, however, not clear which specific cryptochrome(s) could be the primary magnetoreceptor. Cryptochrome 4 (Cry4) can bind FAD and, in European robins (*Erithacus rubecula*), is localized to the outer segments of double cone and long-wavelength single cone photoreceptors. These properties are important for the function of the proposed radical pair-based mechanism. The existing monoclonal antibody against avian Cry4, however, only works in robin tissue. For the investigation of Cry4 localization in other light-migratory bird

species, reliably working antibodies were lacking. Our aim was to generate a polyclonal antibody that reliably detects Cry4 in different bird species. We immunized a rabbit with the full-length sequence of blackcap (*Sylvia atricapilla*) and quail (*Coturnix coturnix*) Cry4 and performed affinity purification of the resulting serum. We validated the antibodies in QNR/K2 cells transfected with the European robin Cry4 sequence. We performed immunocytochemical stainings with each antibody candidate in combination with the monoclonal Cry4 marker 3C2. Besides non-transfected QNR/K2 cells showing no staining signal from either antibody, all cells showed a signal from both antibodies simultaneously. This finding suggests strong specificity of the newly developed antibodies. For further validation, we performed Western blots of lysate from transfected QNR/K2 cells and detected single characteristic bands at the expected molecular mass of Cry4 for each antibody. Our future steps include using the validated antibodies to characterize and compare Cry4 distribution in situ in the retina of multiple night-migratory bird species.
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PP II_A18
Identifying the enzymes of the coalation/decoalation cycle

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Protein CoAlation is a recently identified oxidative post-translational modification (PTM) that protects cysteine residues from irreversible overoxidation through the formation of mixed disulfide bonds with coenzyme A (CoA). In addition to its protective role, protein CoAlation has been shown to regulate protein activity and influence conformational state. Initial studies in primary rat cardiomyocytes demonstrated that protein CoAlation is strongly induced under oxidative stress following treatment with various oxidizing agents. Importantly, removal of these oxidants reverses CoAlation, highlighting its dynamic and reversible nature as a redox-regulated PTM. Furthermore, proteomic analyses indicate that protein CoAlation targets a distinct subset of proteins compared to S-glutathionylation. Despite these advances, the enzymatic mechanisms regulating protein CoAlation remain largely uncharacterized. Although CoA disulfide reductase (CoADR), an enzyme responsible for reducing CoA disulfide dimers, was identified over two decades ago, the enzymes mediating the transfer of CoA to protein thiols and its subsequent removal from proteins and low-molecular-weight dimers have not yet been defined. This project aims to identify enzymes involved in regulating protein CoAlation using the Nebraska Transposon Mutant Library (NTML), a comprehensive transposon mutant collection targeting non-essential genes in the Staphylococcus aureus USA300 genome. Preliminary results show that induction of the CoADR mutant strain with diamide results in a global decrease in protein CoAlation compared to the wild-type strain. Notably, 2–3 proteins exhibit markedly increased CoAlation and will be identified by mass spectrometry. By screening disrupted strains, we seek to uncover proteins with enzymatic activity involved in the CoAlation and de-CoAlation process. This project is in collaboration with Professor Alex O'Neill at the University of Leeds.

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Poster Presentations II Group B – Redox Biology of Human Diseases

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PP II_B04
Sex specific mechanisms of oxidative stress, inflammation, and cardiac dysfunction in doxorubicin-induced cardiotoxicity

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The anthracycline doxorubicin (DOX) remains a clinically important chemotherapeutic agent and is widely used in the treatment of various tumor entities; however, its clinical application is limited by several adverse effects, most notably cardiotoxicity. This study investigates the mechanisms underlying doxorubicin-induced cardiotoxicity in an experimental animal model, focusing on oxidative stress, inflammatory pathways, and alterations in cardiac function, with particular emphasis on sex-specific differences. A mouse model of chronic low-dose doxorubicin (DOX) administration was used to induce cardiotoxicity (5 mg/kg per injection; cumulative dose 20 mg/kg over 4 weeks). Cardiac function was assessed by echocardiography after the final dose. Reactive oxygen species (ROS), inflammatory markers, and gene expression profiles were analyzed. DOX treatment impaired diastolic function in female mice and systolic function in male mice. These alterations were accompanied by increased oxidative stress and inflammation (e.g., IL-6), which were more pronounced in males. In females, diastolic dysfunction was specifically associated with enhanced mitochondrial superoxide production, as determined by HPLC-based MitoSOX analysis. Anthracycline-based chemotherapy, such as doxorubicin, remains a central component of cancer treatment but is associated with a substantial risk of cardiotoxicity. In this study, female sex was associated with less severe functional impairment and lower levels of oxidative stress in a model of doxorubicin-induced cardiotoxicity. Further investigation of sex-specific mechanisms may improve risk stratification and lead to more individualized therapeutic approaches.

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PP II_B05
The role of autophagy in doxorubicin-induced toxicity

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Distinguishing acute from chronic drug effects is essential, as these responses can differentially influence signalling pathways and determine whether cells activate adaptive mechanisms or undergo death. Doxorubicin (DOX), an anthracycline widely used in chemotherapy, induces cytotoxicity through multiple mechanisms, including reactive oxygen species (ROS) generation, DNA damage, and topoisomerase II inhibition. Oxidative stress is a major contributor to DOX toxicity and its dose-limiting side effects; therefore, antioxidants have been explored as modulators of oxidative stress and stress-responsive signalling. Excessive oxidative stress promotes accumulation of damaged cellular components, activating autophagy, a catabolic process crucial for cellular homeostasis. Although autophagy is protective, its dysregulation may drive death. This study aimed to evaluate the effects of the antioxidant sulforaphane (SFN) and quercetin (QCT) on DOX-induced responses in HEK293 cells, with a focus on autophagy-related signalling. HEK293 cells were preincubated with SFN/QCT for 2 hours prior to DOX exposure and subsequently treated with DOX for 3 or 22 hours. Cell viability was assessed using the MTT assay. Protein expression was analysed by Western blotting, and intracellular ROS levels were measured using the fluorescent probe H2DCFDA. Results: Short-term treatment with SFN and QCT activated autophagic and pro-survival signalling, as evidenced by increased P-Beclin-1, LC3A/B, and P-Akt. In contrast, prolonged DOX exposure impaired autophagic flux and induced apoptosis. Antioxidant treatment preserved LC3A/B levels and markedly reduced apoptosis after 24 hours, while P-Akt signalling remained unaffected. SFN and QCT modulate the cellular response to DOX in a time-dependent manner. Short-term effects involve activation of Akt-dependent survival signalling and protective autophagy, whereas long-term antioxidant treatment preserves functional autophagic flux and reduces apoptosis independently of Akt signalling. These findings highlight the protective potential of antioxidants in mitigating DOX-induced oxidative stress.
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PP II_B06

Txnip signaling in diabetic cardiomyopathy: exploring novel therapeutic avenues

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Diabetes is a metabolic disorder characterized by impaired insulin secretion or action, resulting in chronic hyperglycemia that affects multiple organs. Among its major comorbidities is diabetic cardiomyopathy, a secondary condition that alters cardiac function and morphology and exhibits pronounced sex-specific differences in susceptibility. Indeed, our in vivo RNA sequencing analyses revealed a decline in the expression of cardiac functional genes in female murine diabetic hearts, accompanied by a marked upregulation of genes associated with oxidative stress. Notably, we further observed increased expression of thioredoxin-interacting protein (Txnip), a key regulator of redox homeostasis that inhibits thioredoxin (TRX) and has been extensively studied in the context of diabetes and metabolic disorders. In vitro, high-glucose conditions were confirmed to induce Txnip gene upregulation, protein translocation, and co-localization with TRX1 in coronary artery vascular smooth muscle cells (VSMCs), which are a critical factor for vascular wall stability and adaptability and serve as a primary interface through which any cardiac complications may be initiated. Using compartment-specific HyPer7 analyses, we demonstrated that these changes were associated with a reduction in mitochondrial—but not cytosolic—antioxidant capacity, indicative of a pro-oxidative intracellular environment. Furthermore, high-glucose exposure dysregulated several cell type-specific functional signaling pathways, as reflected by altered expression of marker genes linked to fibrosis, calcification, and migration potential of the VSMCs. Collectively, these findings highlight the mitoROS/Txnip axis as a promising therapeutic target for diabetic cardiomyopathy. We aim to test new combinatorial approaches using mitochondrial-targeted antioxidants, such as Mitoquinone, together with the Txnip inhibitor SRI-37330, in order to mitigate the deleterious effects of hyperglycemia on the female heart.
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PP II_B07

Enhanced oxygen efficiency and cardiometabolic adaptability after concurrent training in hypertrophic cardiomyopathy

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Hypertrophic cardiomyopathy (HCM) is characterized by impaired oxygen utilization, reduced energetic efficiency, and limited cardiovascular adaptability. Exercise is a potent stimulus capable of inducing coordinated cardiac and skeletal muscle adaptations that enhance oxygen utilization and metabolic homeostasis. To determine whether supervised high-intensity concurrent resistance and endurance training improves oxygen efficiency and post-exercise recovery in HCM patients. Ten clinically stable HCM patients completed a 12-week individualised concurrent training program. Resistance training was performed at 55–70% of estimated 1RM, with 2–3 min inter-set rest, completing ~60–80% of maximal repetitions. Endurance training comprised one weekly continuous session at 5–10 beats·min^{-1} above VT1 and one fartlek-style session alternating 2–6 min bouts at 5–10 beats·min^{-1} below VT2 with 1–2 min active recovery at VT1. High-intensity interval duration progressed from 14 to 25 min (≈2:1 work-to-recovery ratio). Anthropometry, echocardiography, and cardiopulmonary exercise testing were performed pre- and post-intervention. Fat mass decreased (−1.9 kg; 95% CI −3.0 to −0.7; $p < 0.01$) and skeletal muscle mass increased (+1.6 kg; 0.6 to 2.5; $p < 0.01$). Maximal wall thickness declined by −0.8 mm (−1.6 to −0.04; $p < 0.05$) without adverse remodeling. VO_2max increased by +2.6 ml·kg^{-1}·min^{-1} (1.4 to 3.8; $p < 0.05$) and VO_2 at VT2 by +2.2 ml·kg^{-1}·min^{-1} (0.5 to 3.9; $p < 0.05$). O_2-pulse increased across intensities ($p<0.05$), and energetic efficiency improved up to 90% of VO_2max, peaking at VT2 (+3.4%; 1.5 to 5.4; $p < 0.01$). VO_2 recovery accelerated, with reductions of 9, 9, and 24 s in time to 12.5%, 25%, and 50% decline ($p < 0.05$). Intervention enhances oxygen efficiency, cardiovascular adaptability, and recovery kinetics in HCM, supporting structured exercise as a adjuvant clinical strategy.
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PP II_B08

Effects of molecular hydrogen on the pathophysiology of heart failure

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Heart failure (HF) is a progressively developing condition in which the heart gradually loses its ability to pump blood effectively, thereby failing to provide an adequate supply of oxygen to organs and tissues. Molecular hydrogen (H_2) is a gas with antioxidant and anti-inflammatory effect that due to its small size can diffuse through biological membranes and neutralize the most aggressive free radicals directly within cells. In our study we suspect that administration of H_2 to rats with HF may reduce the likelihood of cellular and tissue damage. In this work, we evaluated H_2 and vitamin C administration to six-month-old Wistar rats with isoproterenol (ISO)-induced HF. HF was induced via daily subcutaneous injections of ISO (50 mg/kg) for five consecutive days. Thereafter, the group with H_2 inhaled 4 % H_2 in air for 30 minutes each day, while the vitamin C group received vitamin C (1.5 mg/L) in drinking water ad libitum for six weeks. Blood plasma was analyzed for key biochemical markers (lipids, renal and hepatic function, glucose, LDH). Left ventricular tissue was harvested to assess pro-inflammatory cytokines interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α), and nuclear factor kappa B (NF-κB) by Western blotting. H_2 administration also reduced the expression of pro-inflammatory proteins TNF-α and NF-κB, while simultaneously increasing the expression of anti-inflammatory protein IL-10. H_2 administration also improved oxidative stress parameters in ISO-treated animals. Both administered treatments contributed to the normalization of biochemical parameters following ISO administration; however, H_2 consistently demonstrated a stronger normalizing effect. Based on these results, we conclude that H_2 inhalation reduced inflammation, oxidative stress, and improved selected biochemical parameters compared with the ISO group. These findings highlight the therapeutic potential of H_2 and support its further investigation as an adjunctive therapy for HF.
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PP II_B10
Targeting azo initiators to mitochondria - a novel approach to study the role of mitochondrial lipid peroxidation in ferroptosis

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Ferroptosis is a non-apoptotic form of regulated cell death driven by the accumulation of lipid reactive oxygen species (ROS) resulting from the interaction of iron, oxygen, and oxidizable phospholipids. Recent preclinical studies indicate that ferroptosis represents a promising therapeutic target for pancreatic ductal adenocarcinoma (PDAC). Despite its therapeutic potential, the mechanisms by which lipid peroxidation is initiated, propagated, and regulated, how it ultimately leads to ferroptotic cell death, and how sensitivity to ferroptosis is modulated by signaling and metabolic pathways, are not fully understood. Distinguishing ferroptosis from other forms of cell death requires the identification of specific molecular intermediates and events. Moreover, although various inducers and inhibitors of ferroptosis have been reported, the precise mechanisms and metabolic pathways through which these compounds act remain to be fully characterized. Determining the reactivity profiles of ferroptosis inducers is therefore essential for understanding the redox and metabolic mechanisms of ferroptotic cell death, accurately interpreting results, and guiding the rational design of agents for both mechanistic studies and potential clinical applications. Here, we report the design, synthesis, and comprehensive characterization of a mitochondria-targeted azo initiator (Mito-Azo) as a chemical tool to study the role of mitochondrial lipid peroxidation in the induction of ferroptosis in human PDAC cells. Thermal- and light-induced decomposition of Mito-Azo was confirmed to result in a controlled free radical generation. Cellular responses were evaluated by assessing cell viability and real-time proliferation, and the contribution of distinct cell death pathways was examined using selective inhibitors. Our preliminary results indicate that the developed mitochondria-targeted azo initiator potently inhibits PDAC cell proliferation and induces ferroptotic cell death. We propose that the development of well-characterized azo initiators designed to induce compartment-specific lipid peroxidation represents a promising strategy for studying ferroptosis-related cellular mechanisms, and may provide a foundation for future therapeutic strategies in cancer.
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PP II_B11
The hormetic effect of 7-ketocholesterol in preventing ferroptosis

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Ferroptosis is an iron-dependent form of cell death characterized by lipid peroxidation, which plays a significant role in liver and neurodegenerative disease progression. 7-ketocholesterol (7KC) is the most common product of the reaction between cholesterol and oxygen radicals and is the predominant concentrated oxysterol found in the blood. At elevated concentrations, 7KC is a powerful inducer of oxidative stress, inflammation, and cellular degeneration, which are common features of many chronic diseases. Currently, the role of low concentrations of 7KC in ferroptosis is yet to be elucidated. This study aimed to investigate the impact of 7KC on hepatocytes and neuronal cells ferroptosis. AML12 hepatocytes were exposed to 20μM Erastin for induction of ferroptosis. To assess the effect of 7KC, cells were treated with ≥20μM of 7KC and cell death was evaluated. Gene expression was assessed by RNA sequencing and real-time PCR. HT4 hypocampal neuronal cells were also treated with glutamate to induce ferroptosis in the presence and absence of 7KC. 7KC protected cells from ferroptosis induced by either Erastin or RSL3 in hepatocytes or by glutamate in neuronal cells. At higher concentrations, 7KC was toxic indicating hormetic effect of this compound. In hepatocytes, 7KC reduced malondialdehyde levels and prevented Erastin-induced decrease in polyunsaturated fatty acids. Additionally, 7KC inhibited the cholesterol synthesis pathway, while Erastin enhanced it. The addition of 7KC also led to the suppression of the enzyme 7-dehydrocholesterol reductase, which metabolizes 7-dehydrocholesterol (7-DHC) to cholesterol. At similar low concentrations, 7KC was a superior anti-ferroptotic molecule to 7-DHC. Finally, Erastin also increased hepatocyte steatosis while 7KC prevented such lipid accumulation. These results suggest that 7KC at low nontoxic concentrations may protect against ferroptosis and lipid peroxidation while higher concentrations are toxic.

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PP II_B12
Protective effects of cysteine analogs against methylglyoxal-Induced cytotoxicity in human dermal fibroblasts and retinal pigment epithelial cells

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Methylglyoxal (MGO), a highly reactive dicarbonyl compound, is known to induce cellular dysfunction and apoptosis through oxidative stress and the formation of advanced glycation end products. In this study, we investigated the protective effects of cysteine and its analogs against MGO-induced cytotoxicity in human dermal fibroblasts (HDFs) and retinal pigment epithelial (RPE) cells. Cell viability was evaluated following exposure to MGO or glyoxal, with or without treatment with various cysteine (Cys) analogs, including cysteine, N-acetylcysteine (NAC), cysteinamide (C-NH_2), S-allyl cysteine (SAC), cysteine ethyl ester (CEE), and reference compounds such as aminoguanidine (AGD) and pyridoxamine (PYM). MGO induced a dose-dependent reduction in cell viability in both HDFs and RPE cells. Several cysteine analogs significantly attenuated MGO-induced cytotoxicity, with Cys, C-NH_2, and PYM showing particularly strong protective effects. In scratch wound-healing assays, MGO markedly impaired cell migration, whereas treatment with Cys or C-NH_2 significantly restored wound closure in both cell types. Furthermore, Western blot analysis revealed that MGO exposure increased the expression of pro-apoptotic markers, including Bax and cleaved caspase-3 and -9, while decreasing the expression of the anti-apoptotic protein Bcl-2. These apoptotic changes were substantially reversed by treatment with Cys and C-NH_2. Taken together, these findings demonstrate that cysteine analogs, especially Cys and C-NH_2, effectively protect HDFs and RPE cells from MGO-induced cytotoxicity by improving cell viability, promoting wound healing, and modulating apoptosis-related signaling pathways. These compounds may have therapeutic potential for preventing MGO-related cellular damage in skin.

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PP II_B13
Evaluation of the mechanisms by which Nrf2 activation inhibits lung fibrosis

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Our working hypothesis is that Nrf2 antagonizes lung fibrogenesis by limiting

oxidative stress, a key factor that promotes TGF-β signaling, a central driver of fibrosis. Conversely, TGF-β signaling may suppress Nrf2 activity to sustain oxidative stress conditions that favour fibrogenesis. This project therefore aims to determine whether a reciprocal antagonism exists between Nrf2 and TGF-β signaling and, if so, to define its physiological consequences in lung fibrosis and evaluate how this interaction may be therapeutically exploited. IMR-90 human lung fibroblasts cultured in DMEM supplemented with 20% FBS were used as an experimental model. Nrf2 activity was manipulated using both genetic (siRNA-mediated knockdown) and pharmacological approaches (RTA-408 treatment). The resulting effects on the mRNA and protein expression of TGF-β–inducible genes were assessed by RT-qPCR and western blotting, respectively. Additional methodologies employed in this study included molecular cloning, luciferase reporter assays, RNA-seq, miRNA screening, kinome profiling, and colony formation assays. RNA-seq analysis, supported by RT-qPCR validation, demonstrated that Nrf2 knockdown in IMR-90 cells increases the expression of TGF-β target genes, whereas genetic or pharmacological derepression of Nrf2 (via Keap1 knockdown or RTA-408 treatment) suppresses their expression. Conversely, TGF-β treatment downregulates Nrf2 target genes, with marked selectivity for members of the aldo-keto reductase family. Luciferase reporter assays and kinome profiling further indicate that modulation of Nrf2 significantly influences both canonical and non-canonical TGF-β signaling pathways. In addition, miRNA screening suggests that Nrf2 may regulate antifibrotic microRNAs that are themselves transcriptionally repressed by TGF-β. These findings support the existence of reciprocal antagonism between Nrf2 and TGF-β signaling in human lung fibroblasts. Ongoing studies will validate the RNA-seq and kinome profiling datasets, determine the physiological consequences of Nrf2-TGF-β crosstalk, and investigate the possibility that Nrf2 regulates the expression of antifibrotic microRNAs.

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PP II_B14

Oxidized-LDL induces metabolic alterations in retinal pigment epithelial cells

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Age-related macular degeneration (AMD) is a degenerative retinal disease that leads to central vision loss and visual field distortion. Currently, effective therapeutic options for AMD remain limited. In recent years, mitochondrial dysfunction has been identified as a key contributor to AMD progression. Mitochondrial damage disrupts the balance of intracellular metabolic pathways, including oxidative phosphorylation (OXPHOS) and glycolysis. Oxidized low-density lipoprotein (ox-LDL) is a major component of drusen that accumulates in the retinas of patients with AMD and may contribute to retinal pigment epithelium (RPE) dysfunction. In this study, we investigated whether ox-LDL induces metabolic alterations in RPE cells, a key site of AMD pathology. Human induced pluripotent stem cell–derived RPE cells (iPSC-RPE) were matured through long-term culture. The cells were treated with ox-LDL on days 1 and 4 and analyzed on day 7. Mitochondrial fatty acid β-oxidation (FAO) activity was evaluated using a fluorescent probe FAOBlue. ox-LDL treatment altered FAO activity, consistent with altered mRNA expression levels of several FAO-related genes. Oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were measured as indicators of OXPHOS activity and glycolytic activity, respectively. ox-LDL treatment reduced OCR and increased ECAR in RPE cells, indicating suppression of OXPHOS and a compensatory shift toward glycolysis. Furthermore, mitochondrial ROS (MitoROS) levels were elevated following ox-LDL treatment, and pretreatment with the MitoROS scavenger MitoTEMPO suppressed the ox-LDL–induced metabolic alterations in RPE cells. These findings suggest that MitoROS generated during ox-LDL exposure drive metabolic reprogramming in RPE cells, which may contribute to AMD pathogenesis.

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PP II_B15

miR-379-3p regulates mitochondrial homeostasis and innate inflammation response

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Cancer cachexia is a multifactorial wasting syndrome in which mitochondrial dysfunction and systemic inflammation drive skeletal muscle loss. miR-379-3p is significantly downregulated during early cachexia across multiple cachexia models, with reduced expression associated with poor survival; however, its mechanistic role in redox–inflammatory crosstalk remains unclear. Here, we investigated how miR-379-3p regulates mitochondrial stress and downstream inflammatory signalling, focusing on the link between mitochondrial DNA (mtDNA) release and activation of the cGAS–IFIT1 axis. Cachectic muscle exhibits increased mitochondrial reactive oxygen species (mtROS) and loss of mitochondrial integrity, accompanied by the cytosolic release of mitochondrial DNA (mtDNA). This mitochondrial stress signal activates the cGAS–IFIT1 inflammatory axis, linking redox imbalance to interferon-driven responses. We found that this response is sex-dependent, with male muscle displaying predominant mitochondrial dysfunction, while females exhibit a stronger interferon signature. Mechanistically, we identify the purinergic receptor P2RY6 as a direct target of miR-379-3p. Restoration of miR-379-3p attenuates mtROS accumulation, suppresses cGAS–IFIT1 signalling, and improves calcium handling pathways. Together, these findings position miR-379-3p as a key regulator of mitochondrial homeostasis and innate immune activation in cancer cachexia. Targeting the miR-379-3p–P2RY6 axis represents a novel therapeutic strategy to mitigate early mitochondrial dysfunction and inflammation-driven muscle wasting.

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PP II_B16

Association of appendicular skeletal muscle mass and strength with BDNF, irisin, and redox balance in geriatric patients

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Impaired muscle–brain signalling and oxidative stress may play key roles in age-related loss of muscle mass and strength. Irisin and brain-derived neurotrophic factor (BDNF) are myokines involved in muscle metabolism and neuromuscular function, while total oxidant status (TOS), total antioxidant status (TAS), and the oxidative stress index (OSI) reflect systemic redox balance and may help explain muscle deterioration in older adults. Aims: To evaluate associations of skeletal muscle mass and muscle strength with circulating irisin, BDNF, and systemic redox balance in geriatric patients. Appendicular skeletal muscle mass (ASM) was assessed by bioelectrical impedance analysis; low muscle mass was defined as ASM < 20 kg in men and < 15 kg in women. Muscle strength was evaluated using handgrip strength (HGS), with low HGS defined as < 27 kg in men and < 16 kg in women. Serum irisin, BDNF, TOS, and TAS were measured, and OSI was calculated. Associations were examined using correlation and logistic regression analyses. The study included 133 hospitalized geriatric patients (74.4% women; mean age 79.1 ± 7.3 years). ASM was negatively correlated with irisin ($p = 0.006$), the irisin/BDNF ratio ($p = 0.001$), and TOS ($p = 0.01$), and positively

correlated with BDNF ($p = 0.04$); these associations were not significant after sex stratification. No significant correlations were found between HGS and the studied biomarkers. The risk of low muscle mass increased with TAS (OR = 1.005; 95% CI: 1.00–1.01; $p = 0.046$), while low HGS was associated with higher OSI (OR = 1.59; 95% CI: 1.11–2.29; $p = 0.01$). Muscle mass in geriatric patients is associated with myokine signaling and redox balance, whereas muscle strength is more strongly related to oxidative stress, suggesting a role of disrupted muscle–brain communication and redox homeostasis in sarcopenia and dynapenia.

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PP II_B17

Serum lipid peroxide levels and their associations with muscle mass, muscle strength, and body fat indices in older adults

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Aging is associated with increased production of reactive oxygen species and oxidative stress, contributing to declines in skeletal muscle mass and strength and leading to sarcopenia and functional impairment. Lipid peroxides (LPO), as markers of free radical–induced lipid peroxidation, may provide insight into the role of oxidative stress in muscle deterioration and frailty in older adults. Aims: This study aimed to assess the association between serum lipid peroxide (LPO) levels and skeletal muscle mass, muscle strength, and body fat–related indices in hospitalized geriatric patients. The study included patients hospitalized in a geriatric ward. Body composition was assessed using bioelectrical impedance analysis and included skeletal muscle mass (SMM), SMM standardized to height squared (skeletal muscle index, SMI) and to body weight (relative SMM; SMM/weight), total fat mass (FM), body fat percentage (PBF), and the fat mass to fat-free mass ratio (FM/FFM). Muscle strength was evaluated using handgrip strength (HGS). Serum LPO was measured by ELISA. Associations between variables were analyzed using correlation analysis. A total of 131 participants were included (mean age 79.1 ± 7.4 years; 74.0% women). The median serum LPO concentration was 12.22 ng/mL (IQR: 8.88–15.70). Serum LPO levels were significantly correlated with relative skeletal muscle mass (SMM/weight; $p = 0.009$), body fat percentage (PBF; $p = 0.04$), and the FM/FFM ratio ($p = 0.04$). No significant associations were observed between LPO and age, the number of chronic diseases, absolute SMM, SMI, or handgrip strength, the latter analyzed separately in men and women. In hospitalized geriatric patients, higher lipid peroxide levels were associated with lower relative skeletal muscle mass and unfavorable body fat indices, but not with muscle strength. These findings suggest that free radical–mediated oxidative stress may primarily affect body composition rather than muscle function in older adults.

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PP II_B18

Regulation of DJ-1 by CoAlation in Parkinson's Disease

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Coenzyme A (CoA) is an essential cofactor for cellular metabolism, gene expression regulation, and the biosynthesis of major cellular macromolecules. Recently, we found CoA can bind to cysteine residues via disulfide bond, this modification is termed CoAlation. CoAlation may play a protective role by shielding redox-sensitive cysteine residues during cellular responses to oxidative and metabolic stress. Disruptions in CoA synthesis and reduction of CoA level is known to cause neurodegeneration, namely Pantothenate kinase-associated neurodegeneration. Given the central role of oxidative stress in neurodegenerative conditions, CoA may exert neuroprotective effects in disorders such as Parkinson's Disease (PD). In the context of PD, the protein DJ-1 acts as an oxidative stress sensor, promoting antioxidant responses, cell survival, and suppressing apoptosis upon C106 oxidation. However, the oxidative stress in PD may over-oxidise C106 and its other two cysteine (C46 and C53), thereby inactivating DJ-1. We have found DJ-1 is CoAlated under oxidative stress at C46 and C53 using LC-MS analysis. This may protect DJ-1's cysteine from irreversible oxidation and/or modulate its function. Our in vitro and in vivo studies confirm DJ-1 CoAlation, and ongoing work is investigating its impact on DJ-1's structure and neuroprotective functions. These findings provide novel insights into the protective role of CoAlation in oxidative stress and its potential therapeutic implications for neurodegenerative diseases.

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PP II_B19

Endothelial NADPH oxidase 5 (NOX5) exacerbates renal damage induced by type II diabetes

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Type II diabetes is characterized by chronic hyperglycemia and is associated with microvascular complications such as diabetic nephropathy. Although its pathogenesis is not fully understood, oxidative stress has emerged as a key contributor to disease development. Chronic hyperglycemia increases reactive oxygen species (ROS) production, in part through the activation of NADPH oxidase 5 (NOX5). NOX5-derived ROS promote inflammation, fibrosis, and endothelial dysfunction, leading to renal structural and functional damage. This study aims to determine whether endothelial NOX5 overexpression contributes to renal pathology under diabetic conditions. Diabetes was induced by a 12-week high-fat, high-fructose diet (HF–HFD) in adult mice with endothelial-specific human NOX5 overexpression (Nox5Cre) and in their Cre control littermates. Metabolic parameters (body weight, plasma glucose, and intraperitoneal glucose tolerance), renal function (urinary albumin and plasma cystatin C), glomerular structure (renal corpuscle, glomerular, and Bowman's space area), fibrosis, and ROS production were assessed. All analyses were performed in the presence or absence of the antioxidant N-acetylcysteine (NAC). Under a normal diet, Cre mice of both sexes gained more weight than Nox5Cre mice. Under HF–HFD, males of both genotypes gained weight similarly, while female Nox5Cre mice gained less weight than female Cre mice. HF-HFD increased blood glucose independently of genotype and affected kidney function differently by sex and genotype: in males, it elevated urinary albumin and plasma cystatin C in Cre mice, whereas in Nox5Cre mice only plasma cystatin C was increased; in females, diabetes reduced urinary albumin and increased plasma cystatin C, particularly in Nox5Cre mice. Diabetes also induced glomerular hypertrophy, Bowman's space enlargement, and renal fibrosis, effects exacerbated in NOX5-Cre mice. ROS levels were higher in Nox5Cre mice at baseline and further increased by HF-HFD, while antioxidant treatment with N-acetylcysteine mitigated metabolic alterations, renal dysfunction, structural damage, fibrosis, and ROS production. These findings indicate that diabetes impairs kidney function, particularly the glomerular filtration barrier, through structural and fibrotic alterations, which are exacerbated by endothelial NOX5 overexpression. Antioxidant treatment with NAC may mitigate these pathological changes.

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Poster Presentations II Group C – Cancer and Pharmacology & Toxicology

PP II_C01
Nrf2 activation sensitizes cancer cells to quinone-based Hsp90 inhibitors

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NF-E2 p45-related factor 2 (Nrf2) is an inducible transcription factor controlling the cellular response to stress, commonly experienced by cancer cells in various forms. Heat shock protein 90 (Hsp90) is an important chaperone which ensures correct protein folding. Proteins in cancer cells are often expressed with mutations, rendering them unstable without the chaperone function of Hsp90. Inhibitors of Hsp90 are in development as anticancer therapeutics. Nrf2 hyperactivity has been reported in several types of cancer. Nrf2 activation results in increased levels of NAD(P)H:quinone oxidoreductase 1 (NQO1), which reduces quinone-based Hsp90 inhibitors (pro-drugs) to more potent hydroquinones (actual drugs). The main aim of this project was to determine whether the quinone-based Hsp90 inhibitors 17-17-N-allylamino-17-demethoxygeldanamycin (17-AAG) and 17-dimethylaminoethylamino-17-demethoxygeldanamycin (17-DMAG) would be synthetically lethal with Nrf2 activation. We found the levels of the Nrf2 target NQO1 to be higher in tumour versus normal brain tissue in a mouse model of glioma. Pharmacological activation of Nrf2 by omaveloxolone (a drug used in patients with Friedreich's ataxia), and the consequent induction of NQO1, increased the cytotoxicity of 17-DMAG and 17-AAG. This occurred despite a common limitation of Hsp90 inhibition, the activation of the cytoprotective heat shock response, regulated by transcription factor heat shock factor 1 (Hsf1). The limitation of the heat shock response activation was further confirmed by the finding that Hsf1-knockout cells were more sensitive to 17-DMAG than their wild-type counterparts. Unexpectedly, the Hsp90 inhibitors decreased the basal and induced levels of NQO1. Pharmacological Nrf2 activation was greater in Hsf1-knockout cells, whereas pharmacological activation of Hsf1 was more pronounced in Nrf2-knockout cells, suggesting competition between the transcription factors. Together, these results suggest Nrf2 activation is a viable strategy for enhancing the cytotoxicity of quinone-based Hsp90 inhibitors, as well as emphasising complex interactions between the cellular stress responses that should be addressed in future studies.

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PP II_C02
Brown adipose tissue undergoes Nrf2-driven redox reprogramming in orthotopic breast cancer mouse model

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Brown adipose tissue (BAT) is a key regulator of systemic metabolism. Its function is closely linked to redox homeostasis, which responds to systemic challenges in cancer. Hence, we aimed to investigate the role of nuclear factor erythroid 2-related factor 2 (Nrf2) as a regulator of redox-metabolic reprogramming in BAT during tumour growth. Using an orthotopic model of breast cancer in wild-type (WT) and mice lacking functional Nrf2 (Nrf2KO), we analysed protein expression and/or activity of key proteins involved in redox regulation in BAT at different stages of early tumour growth (10-400 mg). Our results indicate increased uncoupling protein 1 (UCP1) immunoexpression and protein levels after tumour induction, with higher levels in Nrf2KO than in WT naïve mice. However, in >100 mg tumours, UCP1 expression was reduced in Nrf2KO mice, indicating that UCP1-mediated redox regulation was not sustained. Notably, tumour growth affected glutathione (GSH)-dependent antioxidant defence in Nrf2KO mice. Namely, GSH levels were elevated in Nrf2KO naïve mice but markedly decreased following tumour induction. Glutathione peroxidase 1 (GSH-Px1) protein levels declined, while GSH-Px4 was induced after tumour induction in Nrf2KO mice. Regarding additional H_2O_2-eliminating enzymes, catalase (CAT) protein expression increased with tumour growth in both WT and Nrf2KO mice. Peroxiredoxin 3 protein expression decreased in WT mice but was strongly induced in Nrf2KO mice during tumour growth. In contrast, thioredoxin protein levels declined in both WT and Nrf2KO mice, more prominently in Nrf2KO mice. Moreover, CuZn superoxide dismutase (CuZnSOD) protein levels declined in both WT and Nrf2KO mice, whereas MnSOD levels decreased during later tumour growth in Nrf2KO mice only. Nevertheless, GSH-Px, CAT, and SOD activities remained largely unchanged, indicating additional post-transcriptional regulation. Our findings identify Nrf2 as an important regulator of BAT redox homeostasis, shaping its adaptive response and potential systemic impact on breast cancer progression.

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PP II_C03
Nrf2-dependent changes in FAS and NADPH-producing enzymes in cancer-associated adipose tissue during in vivo breast tumor growth

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Tumors metabolically co-opt surrounding adipose tissue (referred to as cancer-associated adipose tissue, CAAT) to support growth, but the molecular mechanisms underlying this process remain unclear. In human breast cancer, we previously showed that metabolically altered adipose tissue increased expression of Nuclear factor erythroid 2-related factor 2 (Nrf2), one of the important metabolic regulators, suggesting that Nrf2 may play an important role in the breast cancer-CAAT metabolic crosstalk. The goal of this study was to investigate whether/how Nrf2 influences the expression of FAS and NADPH-producing enzymes in CAAT during breast tumor progression. To define Nrf2-dependent patterns of metabolic adaptation in CAAT, we measured protein levels of fatty acid synthase (FAS), glucose-6-phosphate dehydrogenase (G6PDH), malic enzyme 1 (ME1), isocitrate dehydrogenases 1 and 2 (IDH1/2), and mitochondrial nicotinamide nucleotide transhydrogenase (NNT) in CAAT from three tumor growth stages (10, 50, 200 mg) using an orthotopic breast cancer model in wild-type (WT) and Nrf2 knockout (Nrf2KO) mice. FAS expression increased with tumor growth in WT CAAT, but remained at control levels in Nrf2KO CAAT. Baseline G6PDH expression was approximately 55% lower in Nrf2KO mice and declined with tumor progression in both genotypes. In WT CAAT, NNT levels remained stable, while IDH1/2 and ME1 expression decreased by approximately 30% and 50%, respectively, with increasing tumor size. In contrast, in Nrf2KO CAAT, IDH1/2 and ME1 expression progressively increased during tumor growth. These results show that tumor-associated FAS expression in CAAT coincides with distinct patterns of Nrf2-dependent NADPH enzymes. Overall, in Nrf2KO CAAT, alternative NADPH-generating pathways appear to compensate for reduced canonical NADPH production, sustaining CAAT lipid synthesis during tumor progression. These results highlight Nrf2-dependent metabolic adaptations in CAAT as potential novel, non-invasive therapeutic targets for breast cancer.

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PP II_C04

NOX2-mediated mitochondrial dysfunction unveils a therapeutic window for ferroptosis induction in acute myeloid leukaemia

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Acute myeloid leukemia (AML) is a heterogeneous hematological malignancy that requires improved therapeutic strategies. Our group focuses on NADPH oxidases (NOXs), critical enzymes in redox regulation. Previous studies demonstrated that both pharmacological inhibition and deletion of NOX2 in an AML model cell line led to a significant GSH depletion and impaired mitochondrial respiration. Building on these findings, this study explores the role of NOXs in mitochondrial dynamics and evaluates their therapeutic potential. We found that NOX2 knockout AML cells exhibit reduced mitochondrial area and Feret diameter. In line with this, cytometry and lipidomic analyses revealed a significant reduction in cardiolipin, a mitochondria-specific phospholipid essential for proper bioenergetic function. These data indicate that NOX2 deletion may impair mitochondrial morphology and dynamics. Furthermore, NOX2 deficiency increases VDAC abundance, a family of voltage-dependent anion channels recently associated with iron accumulation and ferroptosis in other pathological contexts. Consistent with these observations, NOX inhibition increases the liable iron pool in AML cell lines. The elevated iron levels, together with the previously described GSH reduction following NOX inhibition, both hallmarks of ferroptosis, prompted us to investigate the mitochondrial alterations and therapeutic potential of inducing ferroptosis with sulfasalazine while inhibiting NOX activity with APX-115 across multiple AML cell lines. The pharmacological combination reduced OPA1L/OPA1S ratio and increased VDAC levels, indicating a stronger impact on mitochondria than the individual drugs. Notably, the combination synergistically decreased proliferation in multiple AML cell lines and in patient-derived samples. Additionally, preliminary *in vivo* studies indicate that pre-treating leukemic granulocyte-macrophage progenitors with APX-115 and sulfasalazine improves survival compared to monotherapies. Together, these findings provide novel insights into the role of NOXs in mitochondrial dynamics and underscore the therapeutic potential of simultaneously inhibiting these enzymes and inducing ferroptosis in AML.

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PP II_C05

Redox proteomic analysis of pathways induced by glutathione and thioredoxin system inhibition in leukemia cells

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Limited therapeutic options and frequent drug resistance remain major obstacles in high-risk myelodysplastic syndrome and acute myeloid leukemia (MDS/AML). Leukemia cells undergo metabolic reprogramming that enhances antioxidant defenses and reshapes redox signaling. These processes are largely mediated by site-specific cysteine modifications that regulate differentiation, proliferation, and apoptosis, thereby promoting leukemic survival. We hypothesized that antioxidant systems represent a targetable vulnerability in MDS/AML. OCI-M2 cells were treated with buthionine sulfoximine (BSO), an inhibitor of glutathione (GSH) synthesis, and auranofin (AUR), which blocks thioredoxin (TXN) regeneration. Global proteomic and redox proteomic changes were analyzed by LC/MS. Proliferation was monitored in real time using IncuCyte, and flow cytometry was used to assess intracellular GSH, ROS, apoptosis, and lipid peroxidation. Inhibition of GSH and TXN systems suppressed proliferation and reshaped both the proteome and redox landscape. GSH depletion induced relatively modest global proteomic changes but significantly altered cysteine oxidation (at least by 6 %) in proteins controlling apoptosis (31 oxidized) and mitotic progression (30 reduced). In contrast, TXN inhibition triggered a pronounced stress response, including upregulation of HMOX1, HSP70 family members, and both pro- and anti-apoptotic regulators. Notably, ferroptosis suppressors (CRYAB, CLU, AKR1B1) were among the most increased proteins. AUR decreased oxidation of 302 cysteine-containing peptides across 258 unique proteins linked to lipid metabolism and ferroptosis protection, including AKR1B1. Importantly, ferrostatin-1 rescued AUR-induced cell death, with a more pronounced effect in therapy-resistant cells. Together, these findings demonstrate that targeting GSH and TXN systems activates distinct redox-dependent cell death programs in MDS/AML. Additionally, TXN inhibition reveals a ferroptosis-associated vulnerability that is particularly more prominent in treatment-resistant leukemia cells.

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PP II_C06

KRAS G12D hyperactivation promotes proliferation and migration in cholangiocarcinoma and increases dependence on G6PD-mediated NADPH metabolism

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Cholangiocarcinoma (CCA) frequently harbors activating KRAS mutations, with KRAS G12D representing a predominant oncogenic variant. Oncogenic KRAS drives metabolic reprogramming to sustain rapid proliferation and anabolic growth. Glucose-6-phosphate dehydrogenase (G6PD), the rate-limiting enzyme of the oxidative pentose phosphate pathway, is a principal source of cytosolic NADPH, which supports reductive biosynthesis and antioxidant defense against reactive oxygen species (ROS). Whether KRAS G12D hyperactivation increases reliance on G6PD-mediated NADPH metabolism in CCA remains incompletely defined. To determine whether KRAS G12D enhances proliferative and migratory capacity in CCA cells and confers increased vulnerability to pharmacologic G6PD inhibition. Methods: Doxycycline-inducible KRAS G12D expression was established in SISP-K01, KKU-068, and KKU-138 CCA cell lines. Proliferation was monitored using live-cell imaging, migration by wound healing assays, and cell cycle distribution by flow cytometry. Cells were treated with the G6PD inhibitors dehydroepiandrosterone and 6-aminonicotinamide, and proliferative, migratory, and cell cycle responses were evaluated. KRAS G12D induction significantly increased proliferation and accelerated wound closure compared with controls. Cell cycle analysis demonstrated relative G1 shortening and S-phase enrichment. Pharmacologic G6PD inhibition markedly suppressed proliferation and migration, with KRAS-induced cells exhibiting greater sensitivity. G6PD inhibition also induced G2/M accumulation, suggesting impaired metabolic support for cell cycle progression. Studies are ongoing to define the contribution of NADPH disruption to oxidative stress in KRAS-hyperactivated cells. KRAS G12D hyperactivation promotes aggressive cellular behavior while increasing dependence on G6PD-mediated NADPH metabolism, identifying G6PD as a potential metabolic vulnerability in KRAS-driven CCA.

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PP II_C07

microRNAs regulate cancer cachexia through mitochondrial stress and interferon response

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Cancer cachexia is a highly prevalent wasting syndrome in cancer patients. Inflammation is a hallmark of symptomatic cachexia, however early stages of cachexia are not well understood. In mouse models of cachexia, using multi-omics approaches, we characterised changes in mitochondrial proteins, and elevated levels of cGAS and PPIF, suggesting mitochondrial stress, already at

early stages of cachexia. Concurrently, we demonstrated activation of the interferon response, likely associated with mitochondrial stress. Multiple genes involved in interferon response and mtDNA release pathways were regulated by several microRNAs, including miR-26a-5p and miR-379-3p. miR-26a-5p and miR-379-3p were downregulated in muscle in multiple models of cancer cachexia. Their low levels were associated with poor survival of patients with lung cancer. Restoring miR-26a-5p led to improved mitochondrial homeostasis *in vitro* and miR-379-3p prevented loss of muscle mass and function *in vivo*. Together, our data suggest an important link between mitochondrial damage with mtDNA release and elevated interferon response in cancer cachexia, and a key role for coordinated regulation of these processes by microRNAs.

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PP II_C08

From carbonyl to thiocarbonyl: impact of thionation of flavones on their anti-cancer and redox activity

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Flavones such as chrysin and baicalein are recognized for their anticancer properties, yet their clinical utility is frequently hampered by poor bioavailability. Structural modification through thionation has emerged as a potential strategy to enhance the cytotoxic efficacy of these compounds. The objective of this study was to synthesize 4-thio analogs of chrysin and baicalein and investigate their anticancer activity and molecular mechanisms in breast cancer cells with varying receptor statuses. Thioflavones were synthesized from parent compounds using Lawesson's reagent. Biological activity was evaluated in MCF-7 (ER+), MDA-MB-231 (TNBC), and SK-BR-3 (HER2+) breast cancer lines. Experimental methods included MTT assays for metabolic activity, flow cytometry for apoptosis (Annexin V/7-AAD) and superoxide production (DHE), and imaging cytometry for markers of the Unfolded Protein Response (UPR), specifically p-IRE1, ATF6, and GRP78. Additionally, activity was assessed in 3D spheroid models and doxorubicin-induced senescent cells. At a concentration of 1 μM, thiochrysin significantly increased apoptotic cell death and superoxide levels in MCF-7 and SK-BR-3 cells compared to unmodified chrysin. In MCF-7 cells, thiochrysin-induced oxidative stress triggered the UPR, as indicated by the activation of IRE1 and ATF6 pathways and the upregulation of the chaperone GRP78. Thiochrysin also effectively reduced the viability of 3D spheroids and targeted drug-resistant senescent populations. Thiobaicalein, while active, demonstrated lower selectivity and higher toxicity toward normal MCF-10F cells. Thionation of chrysin significantly enhances its pro-apoptotic potential through a mechanism involving oxidative stress and ER stress-mediated UPR activation. These findings suggest that thiochrysin represents a promising candidate for overcoming drug resistance in breast cancer therapy.

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[1] Witkowski et al., Apoptosis 31, 114 (2026)

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PP II_C09

Converting free radical kinetics into a functional metabolic pK in metastatic breast cancer cells using quantum sensing

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Quantifying intracellular free radical dynamics in live cancer cells remains a major challenge in redox biology and pharmacological profiling. Conventional ROS probes and endpoint binding assays lack temporal resolution and do not directly capture functional metabolic consequences of compound exposure. Here, we present a translational methodology using quantum sensing in the Quantum Nuova™ platform to convert real-time free radical kinetics into a quantitative metabolic pK, benchmarked against classical binding-derived pK values. Experiments were performed in the metastatic breast cancer cell line MDA-MB-231, a mitochondria-driven system characterized by elevated oxidative metabolism. Quantum Nuova™ employs fluorescent nanodiamonds (FND) and relaxometry to detect free radical-induced magnetic noise and monitor longitudinal relaxation dynamics (T1) in live cells at subcellular location non-destructively and without exogenous probes. Cells were cultured in DMEM high glucose and incubated overnight with 1μg/ml FNDs previously functionalized with VDAC2, an specific antibody to target mitochondria, and measure the localized drug action at subcellular level, and following exposure to a panel of mitochondria-targeting anticancer drug compounds at seven different incremental concentration from 25 nM to 12 μM, incubated for 3 hours, dose-dependent free radical curves were recorded in real time. From these kinetic profiles, we derived a metabolic pK parameter representing the functional midpoint of redox modulation under physiological conditions. Across compounds, metabolic pK values demonstrated strong concordance with binding assay pK values, validating quantitative alignment. Importantly, the free radical-derived metric revealed transient oxidative bursts and adaptive mitochondrial responses not observable in endpoint assays. These results position free radical kinetics as a quantifiable pharmacodynamic parameter and introduce metabolic pK as a bridge between molecular binding and cellular metabolic response for drug potency assays base on free radical dynamics, opening new opportunities for redox-informed drug discovery.

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PP II_C10

Oxidative and metabolic profile of human red blood cells in the presence of bisphenol B

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Bisphenols (BPs) are chemicals that are widely used as monomers in the production of plastics to improve their flexibility and strength. However, errors in polymerization processes or degradation phenomena can trigger the migration of BPs from packaging to food and beverages. Exposure to these compounds poses a critical risk to human health, as BPs are classified as endocrine disruptors and potent oxidizing agents. Although the literature extensively describes the action of BPs on different cell lines, their impact on red blood cells (RBCs) remains poorly studied. The present study evaluates the action of bisphenol B (BPB) on RBCs by analyzing their impact on the membrane (hemolysis and morphology), on the oxidative state (methemoglobin, lipid peroxidation, thiol groups, superoxide dismutase and catalase activity), on the metabolic framework (anion flux kinetics, phosphatase activity, ATP levels) and on caspase 3 activity. Our results show that after 2 hours of incubation BPB (from 5.0 to 50.0 nM) causes a slight increase in hemolysis percentage at the highest concentrations, while after 24 hours all the concentrations induce a significant increase in membrane damage and slight changes in cell morphology. After 2 hours of incubation, low concentrations of BPB (5.0 nM) cause oxidation of thiol groups, lipid peroxidation, increased catalytic activity of superoxide dismutase and catalase, and activation of caspase 3, indicating a decisive trend towards the worsening of the cellular oxidative picture. This adverse condition may be counteracted by the increase in kinetics of the anion flow through the band 3 protein, which partially could explain the resistance to hemolysis recorded after few hours of incubation with BPB. Even if this fails to counteract the cell death triggered by the caspase activation and which for longer incubation times, inexorably leads the cell to lysis.

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PP II_C11

Chemogenetic modulation of H_2O_2 to overcome multidrug resistance in glioblastoma using photoactivated chemotherapy

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Multidrug resistance (MDR) is a major obstacle in the treatment of glioblastoma and is closely associated with altered redox homeostasis. Hypoxia-driven metabolic adaptation leads to sustained elevation of reactive oxygen species (ROS), which supports tumor cell survival and therapeutic escape. While moderate ROS levels promote proliferation and stress adaptation, excessive oxidative stress can overwhelm cellular antioxidant capacity and induce cytotoxicity. However, the redox thresholds that define this transition from tolerance to vulnerability in glioblastoma remain poorly characterized.This project investigates how compartment-specific hydrogen peroxide (H_2O_2) signaling influences MDR by combining chemogenetic control of ROS with PhotoActivated ChemoTherapy (PACT), an oxygen-independent treatment modality based on light-activated ruthenium complexes. Recombinant D-amino acid oxidase (DAAO) is used to generate tunable H_2O_2 fluxes within defined subcellular compartments, while real-time ROS dynamics are quantified using genetically encoded fluorescent indicators (GEFIs). These controlled oxidative stresses are combined with PACT targeting nicotinamide phosphoribosyltransferase (NAMPT), a key regulator of the NAD^+/NADH balance and cellular metabolic resilience. By correlating local H_2O_2 accumulation with PACT response, redox alterations in the glioblastoma cell line U87MG are used to determine resistance or susceptibility, as reflected by changes in thiol oxidation, glutathione turnover, and mitochondrial redox imbalance. The project establishes organelle-targeted chemogenetic H_2O_2 platforms, maps antioxidant and thiol-dependent adaptive responses, and validates redox-driven sensitization mechanisms in 3D glioblastoma models. Conducted within a Marie Skłodowska-Curie Postdoctoral Fellowship, this work provides the first mechanistic integration of chemogenetics and PACT, offering a redox-centered strategy to overcome MDR in highly resistant tumors.

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PP II_C12

Dual redox activity of rose extract modulates metabolism and proliferation in multiple myeloma cells

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Cancer cells, including multiple myeloma cells, exhibit elevated basal oxidative stress compared with normal cells. This altered redox state may create a therapeutic vulnerability, as further increases in reactive oxygen species (ROS) can selectively impair tumor cell survival. The aim of this study was to investigate the effects of rose extract on redox balance, cellular metabolism, and proliferation in multiple myeloma cells. Methods: Multiple myeloma cells were treated with rose extract and analyzed for changes in oxidative stress and metabolic activity. Hydrogen peroxide (H_2O_2) production was assessed as a marker of ROS generation. Cellular ATP and NADH levels were measured to evaluate metabolic alterations. Cell proliferation was analyzed following treatment with rose extract. To determine whether H_2O_2 mediates the observed effects, catalase an enzyme that decomposes hydrogen peroxide into water and oxygen was added to selected experimental conditions. Treatment with rose extract increased hydrogen peroxide production, further elevating oxidative stress in myeloma cells. This pro-oxidant effect was accompanied by a decrease in intracellular ATP and NADH levels, indicating disruption of cellular metabolic activity. Consistent with these metabolic changes, rose extract significantly inhibited myeloma cell proliferation. Importantly, the addition of catalase reversed the metabolic effects, restoring ATP levels and partially rescuing cell proliferation, indicating that hydrogen peroxide plays a central role in mediating the observed responses. Our findings suggest that the antiproliferative effect of rose extract in multiple myeloma cells is mediated by increased oxidative stress driven by hydrogen peroxide production. Targeting the elevated oxidative stress characteristic of cancer cells may represent a promising strategy to selectively impair tumor cell viability while sparing normal cells, which typically maintain lower basal ROS levels.

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PP II_C13

Glutathione transferase omega 1-1 and cancer resistance against Topotecan: new insights into the pathogenetic mechanism

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Glutathione transferase omega 1-1 (GSTO1-1) is a member of the glutathione transferase superfamily with both reductase and thioltransferase activity. GSTO1-1 is highly expressed in a number of neoplasms, but its functions and polymorphisms have not yet been thoroughly investigated. Previous results obtained in our laboratories have demonstrated that GSTO1-1 modulates the cellular response against cisplatin and arsenic trioxide suggesting the existence of general, GSTO1-1-dependent, resistance mechanisms. Topotecan is an antitumor drug used for the treatment of ovarian cancer, small cell lung cancer and cervical cancer. Topotecan is a topoisomerase I-inhibitor, i.e. a drug with a mechanism of action other than cisplatin and arsenic trioxide. The aim of this study was thus to investigate the possible role(s) of GSTO1-1 in the resistance against Topotecan. Two cell lines with different levels of GSTO1-1 were obtained from the cervical carcinoma derived HeLa cell line, i.e., the HeLa GSTO1-1 stable transfected (HeLaGSTO1 +) cell line and the HeLa CRISPR/Cas 9 ko (HeLaGSTO1-) cell line. Cells were used to test the toxicity of Topotecan (Alamar), the levels of apoptosis (Hoechst staining), MAPKs activation (immunoblot), GSTO1-1 localization (IHF) and cell migration (scratch assay). The survival of HeLaGSTO1 + cells was higher at all concentrations and time points selected, suggesting that GSTO1-1 expression can efficiently protect cells against Topotecan toxicity. Indeed, a transient nuclear translocation of GSTO1-1, the activation of survival pathways (Akt and ERK1/2) and a lower number of apoptotic cells were detectable in the more resistant HeLaGSTO1 + cells. Finally, the scratch assay revealed a greater migratory capacity for HeLaGSTO1 + cells. Our results suggest that GSTO1-1-modulated pathways may confer increased resistance against Topotecan to tumor cells. These results confirm that GSTO1-1 may play a role in cancer resistance against anticancer drugs through pro-survival pathways going beyond substrate specificity and classical detoxification reactions.

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PP II_C14

Quetiapine-induced myeloperoxidase-dependent neutrophil extracellular trap formation in in dimethylformamide-differentiated HL-60 cells

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NETosis is a specialized form of programmed cell death in which neutrophils release chromatin decorated with granular proteins to form neutrophil extracellular traps (NETs) that contribute to host defense. Myeloperoxidase (MPO) plays a central role in canonical NETosis through the generation of reactive oxidants. However, the effects of clinically used drugs that interact with MPO on NET formation remain poorly understood. Quetiapine, an atypical antipsychotic, undergoes MPO-mediated oxidation and has been associated with rare but serious immune-related adverse effects, including neutropenia and inflammatory reactions. Recently, dysregulated NET formation has emerged as a potential contributor to drug-induced immune toxicity. Aim of the study was to

determine whether quetiapine induces NET formation through MPO-dependent mechanisms. HL-60 cells were differentiated with 70 mM dimethylformamide (DMF) to generate neutrophil-like cells. The differentiation was evaluated by detecting CD11b expression using flow cytometry, and the ability of the differentiated cells to generate superoxide anion was assessed by nitroblue tetrazolium (NBT) reduction. The superoxide anion production following quetiapine exposure was also quantified using the NBT assay. MPO peroxidation and chlorination activities were assessed using guaiacol-based peroxidase assays and taurine chloramine assays, respectively. NET formation was quantified using Sytox Green-based plate reader assay and visualized by fluorescence microscopy. Liquid chromatography-mass spectrometry (LC-MS) was employed to quantify quetiapine metabolites generated by MPO using DMF-differentiated HL-60 cells and isolated NET (MPO-DNA complex). Quetiapine induced concentration-dependent NET formation in DMF-differentiated HL-60 cells. Quetiapine significantly modulated MPO activity and increased superoxide anion production, and inhibition of MPO significantly reduced quetiapine-induced NET formation. LCMS analysis confirmed that MPO within NET structures oxidized quetiapine to form N-desalkyl quetiapine. Quetiapine induced NET formation through an MPO-dependent oxidative mechanism. These findings identify NETosis as a potential contributor to quetiapine-associated immune toxicity and underscore the importance of evaluating MPO-driven NET formation in drug safety assessment.

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PP II_C15

Time- and matrix-dependent variation of redox biomarkers following an experimental systemic corticosteroid administration in dogs

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Corticosteroids are known to influence oxidative stress and antioxidant capacity, but their dynamic effects across different biological matrices remain poorly defined. This study aimed to characterize time- and dose-dependent redox responses to systemic corticosteroid administration using the dog as an experimental model. Animals were assigned to control, 1 mg/kg, or 5 mg/kg corticosteroid groups and sampled at 0, 2, 4, 8, 12, 24, 48, and 72 h post-corticosteroid subcutaneous administration. Redox biomarkers were measured in saliva, serum, and erythrocyte lysates. Longitudinal and multivariate analyses were applied to assess time and dose effects, and matrix-specific responses. Marked matrix- and time-specific effects were observed. Saliva showed early alterations, with significant changes detected as early as 2 h post-treatment, particularly in the 1 mg/kg group. In contrast, serum biomarkers exhibited more pronounced variation at intermediate time points (between 4 and 12 h), while erythrocyte lysates displayed delayed but stronger responses at the highest dose (5 mg/kg). For example, Trolox equivalent antioxidant capacity (TEAC) levels in saliva decreased transiently, while advanced oxidation protein products (AOPP) levels increased significantly in the same matrix. Serum paraoxonase-1 (PON-1) showed dose- and time-dependent changes. These findings show that redox biomarker responses vary by matrix and time. TEAC, AOPP, and PON-1 in canine saliva and serum responded quickly, reflecting early changes in antioxidant capacity, oxidative damage, and enzyme activity. In contrast, erythrocyte lysates exhibited slower, dose-dependent changes, particularly at the highest corticosteroid concentration. This highlights the value of combining different sample types and time points to better understand redox changes following systemic corticosteroid exposure.

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PP II_C16

Coenzyme Q deprivation and side effects induced by Simvastatin on early differentiating and mature C2C12 murine skeletal muscle myotubes

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A large body of evidence shows that statins reduce mortality and morbidity from cardiovascular diseases in patients with elevated LDL cholesterol levels. However, other studies report adverse effects such as myopathy, ranging from mild myalgia to severe rhabdomyolysis. The mechanisms underlying statin-associated muscle symptoms (SAMS) are not yet fully understood. Impaired muscle regeneration may partly explain these symptoms, and coenzyme Q is one of the factors that may play a key role in this process. Several studies have demonstrated that the toxic effects of statins can compromise mitochondrial function and reduce the expression of markers of cell proliferation, differentiation, and fusion, thereby impairing myoblast differentiation. However, many in vitro studies use statin concentrations that exceed the physiological levels found in the blood and tissues of patients treated with statins, limiting their relevance for understanding the pleiotropic effects of statins in vivo. In this context, the aim of the present study was to further investigate the role of statin-induced coenzyme Q depletion in both differentiated and early differentiating myoblasts derived from murine skeletal muscle cells (C2C12) using an acute and relatively high doses simvastatin treatment. Specifically, we assessed the reduction of Coenzyme Q biosynthesis, a reduction of ATP levels, an increased mitochondrial reactive species of oxygen and an increased lipid peroxidation. In parallel, we observed partial alterations in morphology through confocal microscopic analysis and in the differentiation process. Finally, we observed a decrease in the contractile capacity of myotubes subjected to treatment with electrical pulsed stimulation (EPS). We are now translating the protocol and results to a chronic in vitro model based on low-dose simvastatin exposure, designed to better mimic the conditions to which patients are normally exposed and for assessing the role of coenzyme Q depletion using 4-p-nitrobenzoic acid treatment, a selective coenzyme Q inhibitor, and a coenzyme Q supplementation.

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PP II_C17

Pharmacology and systemic bioavailability of tocotrienol-rich fraction in healthy individuals and women with pre-operative breast cancer

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Tocotrienols are natural bioactives and part of vitamin E family making them powerful antioxidants. Many pre-clinical and clinical studies have established the potent nature of tocotrienols in conferring beneficial health effects such as cardioprotective, anti-inflammatory, cholesterol lowering and immune enhancing properties. Although been tested in many cancer studies with remarkable findings, the Maximum Tolerated Dose (MTD) of tocotrienols in breast cancer which is the most lethal form of cancer affecting women worldwide is yet to be established. Therefore, we first developed a plasma detection method and validated in healthy participants. This was a single-dose, randomized, crossover study with a one-week washout period separating the two treatment periods. Healthy volunteers were given four 200 mg Tocotrienol-Rich Fraction (TRF) capsules and blood samples were taken during 24 hours on both periods. Next, we designed a dose-escalation study to establish the MTD of TRF in pre-operative breast cancer patients and establish the pharmacokinetic profile of TRF in these patients. Patients were given dosage of 400 mg/day, 800 mg/day to 1600 mg /day for two weeks prior to their surgery. Pharmacology profiling analysis was carried out on Day 1 with blood samples taken at several time points over 12 hours. Plasma tocotrienol concentrations were determined using HPLC method. Both studies showed no significant changes in the pre- and post-study liver and renal functions, haematological status and coagulation profile.

T_{max} was achieved at 5 hours. Biphasic absorption pattern indicating enterohepatic recirculation was observed. In MTD study, there was a similar trend of increase in C_{max} and AUC for all 3 isomers from 400 mg to 800 mg TRF and a drop at 1600 mg TRF. ANOVA results for C_{max} and AUC for all 3 doses and isomers did not show any significant difference with $p > 0.05$. Alpha and gamma tocotrienols showed higher bioavailability in comparison with delta tocotrienol and it is also a reflection of the lower concentration of delta tocotrienol in the capsules. The Wilcoxon Signed Rank Test for T_{max} did not show any significant difference between the dosing levels for all three isomers ($p > 0.05$). Oral supplementation of TRF is shown to be safe and tolerable in both healthy population and patients undergoing surgery.

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PP II_C18
Identification of novel oxidized phospholipids that activate platelet-activating factor receptor using HPLC fractionation and comprehensive LC-MS/MS analysis

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Platelet-activating factor receptor (PAFR) mediates inflammatory and immune responses. In addition to platelet-activating factor (PAF), several oxidized phosphatidylcholines (oxPCs) also act as PAFR ligands. In biological systems, lipid peroxidation generates structurally diverse oxPC species, including hydroperoxides, hydroxides, epoxides, aldehydes, and carboxylic acids. Thus, we hypothesized that additional oxPC species functioning as PAFR activators remain to be identified. We previously developed a comprehensive LC-MS/MS-based method for oxPC analysis. In this study, we applied this method to identify novel oxPCs capable of activating PAFR. Major phosphatidylcholines (PCs) containing polyunsaturated fatty acids were oxidized *in vitro*. The oxPC mixtures were then fractionated by preparative reverse-phase HPLC. Each fraction was analyzed for oxPC species using LC-MS/MS and evaluated for PAFR activation using TGFα shedding, Ca^{2+} mobilization, NanoBiT-based Gq dissociation, and β-arrestin recruitment assays in HEK293 and CHO cells. Site-directed mutagenesis (His248Trp) was performed to assess ligand–receptor interactions. PAFR-mediated signaling was further examined in mouse peritoneal macrophages. Among the oxPC fractions, we identified 1-palmitoyl-2-(4′-oxo-butanoyl)-*sn*-glycero-3-phosphocholine (POBPC) as a major PAFR-activating species. POBPC activated PAFR in a dose-dependent manner and induced Gq activation, intracellular Ca^{2+} mobilization, and β-arrestin recruitment. The His248Trp mutation significantly attenuated POBPC-induced receptor activation, indicating that POBPC shares a binding site with PAF. In primary macrophages, POBPC triggered Ca^{2+} elevation and ERK phosphorylation, which were inhibited by a PAFR antagonist. Using HPLC fractionation combined with LC-MS/MS and functional assays, we identified POBPC as a novel ligand for PAFR. POBPC activates both G protein– and β-arrestin–mediated signaling and induces downstream responses in macrophages. These findings expand the repertoire of bioactive oxPCs and provide new insights into the role of oxidized phospholipids in PAFR-mediated inflammatory signaling.

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PP II_C19
Modeling tumor hypoxia in standard laboratory settings with an engineered biomaterial-based cell culture device

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Hypoxia is a well-recognized hallmark of cancer: approximately 90% of solid tumors develop and proliferate within oxygen-deprived microenvironments. Cellular responses to low oxygen are primarily mediated by hypoxia-inducible factors (HIFs), which orchestrate transcriptional and metabolic reprogramming that promotes tumor cell survival, migration, and invasiveness, ultimately compromising therapeutic efficacy. Nonetheless, *in vitro* anticancer drug screenings are still largely conducted in room air (~19% O_2), a hyperoxic setting that distorts gene expression and metabolic behaviour, reducing the pathophysiological relevance of preclinical data. This work presents the application of the *OxygenControl plate*, an innovative multiwell device that incorporates a functional biomaterial to precisely regulate oxygen concentration *in vitro*, with no requirement for hypoxia workstations, chambers, or specialized incubators. The *OxygenControl plate* was produced by depositing a functional oxygen-modulating matrix onto the bottom of standard multiwell plates. Dissolved oxygen levels and spatial distribution in the culture medium were characterised by scanning electrochemical microscopy (SECM) using a three-electrode setup comprising a 10 µm platinum microelectrode (working), a platinum wire (counter), and a silver quasi-reference electrode. MCF-7 and A549 cancer cell lines were cultured at 1%, 3%, and 5% O_2, and HIF stabilization was assessed by ELISA and Western blotting. Target oxygen concentrations were reached within minutes and maintained stable for up to 5 days. SECM confirmed uniform O_2 distribution across all tested well formats. Cell growth curves demonstrated device biocompatibility, while Western blotting and ELISA revealed time-dependent HIF stabilization at controlled oxygen levels. The *OxygenControl plate* represents a practical and user-friendly solution for generating physiologically relevant hypoxic microenvironments *in vitro*, offering a valuable tool for cancer research and drug screening applications.

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Poster Presentations II Group D – Metabolism and Nutrition

PP II_D01/FT IV_06
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PP II_D02
Loss of mitochondrial plasticity negatively impacts on HCC development in the context of MASLD

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Liver steatosis is a common condition that can progress to Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD) and eventually lead to Hepatocellular Carcinoma (HCC), but the mechanisms behind this progression remain unclear. Given the association between MASLD and mitochondrial oxidative stress, we aimed to evaluate the role of impaired mitochondrial function in MASLD progression to HCC. We compared disease progression between WT and PGC-1α KO mice, as PGC-1α deficiency models mitochondrial dysfunction and loss of metabolic flexibility. Mice were fed normal or high-fat diets (HFD) and treated or not with teratogens (T) for 24 weeks. In parallel, we used a FaO cell line model to reproduce these conditions through treatments with palmitic acid and hydrogen peroxide, and by modifying energy substrates with glucose or galactose media. Weight gain follow up showed that animals treated with HFD + T gained less weight that single treated animals, and the effect was significantly more marked in KO mice. Histology analysis of liver samples showed that liver fat accumulation was lower in KO mice than in WTs but more importantly HFD + T mice had lower levels of fat accumulation than HFD mice and again the effect was higher in KO mice. Disease development was confirmed by evaluation of tumor burden, that was significantly higher in KO mice than in WT mice. Enhanced tumor burden in double treated KO mice was not associated to higher proliferation rates, nor higher levels of fibrosis, but with reduced cell death rates, despite increased oxidative stress levels related to failure to induce antioxidant and detoxification systems in KO mice. In agreement with these findings, FaO cells exposed to palmitic acid and H_2O_2 showed a marked decrease in viability and an increase in caspase activity, effects that were more pronounced under galactose culture conditions, confirming their dependence on mitochondrial oxidative metabolism. Moreover, oxidative stress induction was

accompanied by a blunted NRF2 response, indicating an impaired antioxidant adaptation that mirrors the in vivo phenotype of PGC-1α deficiency. These results indicate that mitochondrial dysfunction and PGC-1α deficiency promote HCC by increasing oxidative stress and cell death pathways.

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PP II_D03

Dietary cyanidin-3-O-glucoside reshapes redox homeostasis and steroidogenic signalling in healthy leydig cells

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Anthocyanins are widely recognized as dietary antioxidants, yet their impact on testicular redox signalling and endocrine function under non-stressed conditions remains incompletely understood. This study investigated how cyanidin-3-O-glucoside (C3G) shapes oxidative stress, mitochondrial function, metabolism, and steroidogenesis in murine TM3 Leydig cells under basal conditions. Cells were exposed to increasing C3G concentrations (0-50.00 μM), and cell viability, redox status, mitochondrial polarization, steroidogenic output, and exometabolomic profiles were evaluated using standard colorimetric, fluorescence, immunochemical, molecular, and ^{1}H-NMR approaches. Redox status was characterised by intracellular ROS quantification together with multiple markers of oxidative damage, including lipid peroxidation, protein nitration, and protein carbonylation, to provide an integrated assessment of oxidative stress. C3G elicited a non-linear, dose-dependent redox response. At low concentrations (0.50.5.00 μM), C3G reduced intracellular ROS and oxidative damage, preserved mitochondrial membrane potential and maintained mitochondrial DNA content. In this range, C3G selectively attenuated androstenedione production despite maintained or increased expression of key steroidogenic actors, in association with reduced global PKA-dependent phosphorylation, suggesting interference with phosphorylation-dependent activation of StAR and related steroidogenic substrates. Low C3G concentrations supported a proliferative metabolic phenotype, with enhanced substrate consumption and lactate release consistent with increased metabolic activity and cell growth. In contrast, 50.00 μM C3G triggered mitochondrial depolarization, altered mitochondrial biogenesis markers, Sirt1 downregulation, and LDH-defined cytotoxicity, indicative of redox imbalance and mitochondrial distress. Overall, C3G defines a concentration window in healthy Leydig cells in which dietary-relevant anthocyanin exposure couples redox protection and metabolic activation with a selective down-tuning of androgen production via putative interference with PKA-dependent steroidogenic signalling. These findings highlight the dose- and context-dependent nature of anthocyanin actions on redox homeostasis and endocrine function and underline the need to carefully consider anthocyanin-rich dietary exposures when assessing redox-related influences on the male reproductive system.

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PP II_D04

Communication between skeletal muscle and adipose tissue: extracellular vesicles as modulators of lipid metabolism and potential regulators of adipocytes phenotype

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Exercise training induces metabolic adaptations in skeletal muscle and white adipose tissue (WAT), partly mediated by secreted factors, including myokines, adipokines, and extracellular vesicles (EVs). Contracting skeletal muscle releases EVs, that can act as endocrine mediators by delivering bioactive cargo to target cells. Although skeletal muscle-derived EVs (SkM-EVs) contribute to muscle homeostasis, their role in modulating adipose tissue function and metabolism remains poorly understood. This study investigates the effects of SkM-EVs released by C2C12 myotubes during physiological contraction on adipogenesis and metabolic features of 3T3-L1 adipocytes in vitro. The objectives are to: (i) assess morphological and functional changes during adipocyte differentiation following SkM-EVs exposure; (ii) define the molecular mechanisms involved; and (iii) explore whether SkM-EVs influence lipid droplet–mitochondria interactions. EVs were isolated from the conditioned medium of C2C12 myotubes by ultracentrifugation and administered to 3T3-L1 cells throughout the differentiation process. Cytotoxicity assays confirmed the safety of the selected EV concentrations used. SkM-EV treatment resulted in reduced intracellular lipid accumulation and alterations in lipid droplet distribution, as demonstrated by Oil Red O staining and quantification. Gene expression analysis by RT-PCR at multiple differentiation stages revealed transcriptional changes between CTRL and EV treated groups. Ongoing functional imaging studies will clarify whether these effects are associated with changes in lipid droplet–mitochondria interactions. In parallel, experimental conditions are being optimized to measure metabolic parameters, including dissolved oxygen (DO) and oxygen consumption rate (OCR), using the Cytena S-NEST bioreactor. Overall, these preliminary findings support a role for SkM-EVs as modulators of adipocyte metabolism, highlighting a potential mechanism of muscle–adipose tissue crosstalk relevant to exercise-induced metabolic benefits.

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PP II_D05

A new conception of the existence of the Closed 9-Stepped Cycle of Proton Conductance

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We propose the Closed 9-Stepped Cycle of Proton Conductance (C9SCPC) as an integrated bioenergetic framework unifying mitochondrial metabolism, erythrocyte buffering, and respiratory gas exchange into a loss-free, cyclic system. The cycle is established in two main stages: 1. Equation correction: The classic overall oxidation ($C_6H_{12}O_6 + 6O_2 \rightarrow$ Energy $+ 6H_2O + 6CO_2$) is reformulated to account for donor substrates, ADP + Pi, O_2, intermembrane H^+, and membrane redox potential control, yielding ATP, heat, CO_2, metabolic water, and matrix H^+; 2. Stage mapping: Parameters of the corrected equation are organized into nine sequential, interconnected stages covering mitochondrial events, blood/serum processes, lung exchange, and tissue oxygen release. The C9SCPC theory extends Mitchell's chemiosmosis and Boyer's binding-change mechanism to a complete, closed, loss-free proton–electron cycle: Stages 1–5: Proton and electron separation from food substrates and electron transport chain activity; Stages 6–7: Proton logistics bridge where mitochondrial CO_2 and metabolic water are converted to H^+ and HCO_3^- via carbonic anhydrase, coupled to hemoglobin histidine buffering; Stage 8: Lung oxygen uptake and Haldane effect-driven adjustments (Hamburger shift) facilitate O_2 loading and H^+ transport; Stage 9: Tissue oxygen release governed by the Bohr effect, returning electrophilic oxygen to mitochondria, closing the proton–electron loop. The transition from Stage 9 → Stage 1 is highlighted as the key mechanism maintaining a closed, intensifying cycle. This architecture introduces a system-level proton conservation principle, bridging conceptual gaps between mitochondrial bioenergetics and whole-body gas transport.

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PP II_D06

Sirtuin 3 controls sex-specific metabolic flexibility in white adipose tissue under Western diet-induced stress

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The global rise in obesity has placed white adipose tissue (WAT) at the center of metabolic research, as its dysfunction contributes to insulin resistance, metabolic syndrome and related chronic diseases. The Western diet (WD), high in fat and sugar, drives obesity by imposing chronic nutrient overload on WAT, increasing fatty acid flux and metabolic pressure on its limited oxidative capacity, thereby promoting oxidative stress. White adipocytes store energy as triglycerides yet possess relatively low mitochondrial density, rendering them vulnerable to metabolic overload. Sirtuin 3 (Sirt3), a mitochondrial NAD^+-dependent deacetylase, regulates fatty acid oxidation, oxidative phosphorylation and antioxidant defenses and is therefore critical for metabolic adaptation. This study investigated sex-specific metabolic responses to WD-induced oxidative stress in gonadal (gWAT) and subcutaneous (sWAT) WAT, with a focus on Sirt3. Eight-week-old wild-type (WT) and Sirt3 knockout (KO) mice were fed WD for four weeks. Plasma non-esterified fatty acids (NEFA), adipocyte morphology, and expression of selected metabolic and antioxidant proteins were analyzed. In gWAT, female mice exhibited larger adipocytes and increased fatty acid synthase (FASN) expression, consistent with enhanced *de novo* lipogenesis. In this depot, Sirt3 acted as a negative regulator of lipogenesis, as its loss reduced lipid storage while increasing β-oxidation upon WD. WD-fed males showed elevated plasma NEFA levels, particularly in WT mice, indicating increased lipid release from WAT. Notably, Sirt3 deficiency lowered NEFA concentrations alongside increased HADHB expression, suggesting enhanced mitochondrial fatty acid oxidation rather than systemic lipid spillover. In sWAT, loss of Sirt3 in WD-fed males resulted in smaller adipocytes. In females, WD reduced Acc1 expression, indicating suppressed *de novo* lipogenesis, whereas Sirt3 deficiency downregulated Atgl and AcSOD2, suggesting impaired lipolysis and reduced mitochondrial antioxidant capacity, potentially exacerbating oxidative stress. These findings identify Sirt3 as a key regulator of sex- and depot-specific oxidative metabolic flexibility in WAT under WD-induced stress.
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PP II_D07
Loss of Sirtuin 3 impairs AMPK–Acc1 signaling and promotes lipid accumulation in female adipocytes

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Obesity is a global health problem, with more than 890 million obese adults worldwide. Sex differences in obesity-related diseases show that premenopausal women often have a more favourable metabolic profile than men. Adipose tissue is central to energy homeostasis, and defective adipogenesis leads to adipose dysfunction under metabolic stress. Sirt3 is a mitochondrial NAD + -dependent deacetylase that regulates fatty acid oxidation, oxidative phosphorylation, and antioxidant defence. Reduced Sirt3 activity has been linked to insulin resistance and metabolic disease. However, the specific role of Sirt3 in adipogenic differentiation remains unclear. Here we aimed to determine how Sirt3 and sex affect adipogenic differentiation efficiency and characteristics in MEF-derived adipocyte cells. To investigate this, we used sex-stratified Sirt3 WT and KO MEFs differentiated into adipocyte-like cells, and assessed lipid accumulation by Oil Red O staining, confocal microscopy with PLIN1/DAPI, lipid droplet morphometry, gene expression of adipogenic markers, metabolomic analysis and immunoblotting of lipid metabolic regulators. Results show that while all groups formed adipocyte-like cells, Sirt3 loss increased lipid accumulation, most prominently in female KO adipocytes, which also exhibited the highest lipid droplet number and largest lipid droplet size, and the highest expression of adipogenic markers, favouring a lipogenic profile with reduced lipolytic capacity. Given the strongest phenotype in females, we used bortezomib (BTZ), a proteasome inhibitor that suppresses de novo lipogenesis to test whether Sirt3 loss disrupts this anti-lipogenic response. Mechanistically, BTZ activated AMPK–Acc1 signaling in WT cells, whereas Sirt3 KO adipocytes failed to phosphorylate AMPK. Similarly, AICAR significantly increased AMPK phosphorylation in WT cells, but this effect was absent in the Sirt3 KO genotype. These data indicate that Sirt3 is required for intact AMPK–Acc1 anti-lipogenic control during lipogenesis; Sirt3 loss promotes a female-biased lipogenic phenotype and renders adipocytes highly susceptible to BTZ-induced lipid accumulation, consistent with impaired AMPK–Acc1 regulation.
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PP II_D08
Oxidative and structural insights into palmitate-induced SERCA1a inhibition

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Type 2 diabetes mellitus (T2DM) is associated with elevated glucose and increased circulating free fatty acids, predominantly palmitate. Palmitate is a key mediator of lipotoxicity that disrupts Ca^{2+} homeostasis and has been reported to reduce sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) activity in multiple cell types. However, the molecular events linking fatty acid exposure to SERCA structural changes and functional inhibition remain poorly understood. Current evidence suggests that palmitate induces ER/SR dysfunction by disrupting global Ca^{2+} handling, inducing ER stress, and reducing ATPase activity and/or expression. However, oxidative modifications and conformational alterations of SERCA1a—such as protein carbonyl formation, thiol oxidation, lipid peroxidation, and structural changes—have been studied primarily under oxidative stress conditions rather than T2DM-relevant palmitate-driven lipotoxic stress. SERCA1a activity and ATP/Ca^{2+} kinetic parameters were measured in isolated SR vesicles using an NADH-coupled ATPase assay. Protein carbonyls were quantified using an ELISA carbonyl kit, and lipid peroxidation was expressed as % C11-$BODIPY^{581/591}$ oxidation. Conformational changes were monitored by FITC fluorescence in the ATP-binding cytosolic region and by intrinsic tryptophan fluorescence together with the NCD-4 probe in the transmembrane/ Ca^{2+}-binding region. Results indicate that palmitate decreases SERCA1a activity in a concentration-dependent manner (IC_{50} = 0.62 mM), accompanied by a marked alteration in ATP affinity, suggesting complex inhibition. This functional impairment was accompanied by increased protein carbonylation and lipid peroxidation, elevated cysteine accessibility, and conformational changes in both the cytosolic and transmembrane regions of SERCA1a. Collectively, these findings indicate that palmitate inhibits SERCA1a through combined oxidative damage and structural destabilization of both ATP- and Ca^{2+}-binding regions, suggesting that lipotoxic injury compromises SERCA1a function by concurrently impairing catalytic capacity and structural integrity.
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PP II_D09
Targeted sarco/endoplasmic reticulum Ca^{2+}-ATPase activation reverses pump impairment under diabetogenic conditions

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Disruption of endoplasmic reticulum (ER) Ca^{2+} homeostasis is a key contributor to β-cell dysfunction under diabetogenic stress. Reactive metabolites such as methylglyoxal (MGX), elevated during hyperglycemia, promote protein carbonylation, thiol oxidation, and advanced glycation end-products, impairing the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA), the primary pump maintaining ER Ca^{2+} stores. Similarly, lipotoxic exposure to palmitate (PAL), elevated in type 2 diabetes, suppresses SERCA activity and promote ER stress,

insulin signaling defects, and β-cell apoptosis. These insults converge on Ca^{2+} dysregulation as a shared mechanism linking gluco- and lipotoxicity. Although pharmacological SERCA activation has been proposed to restore ER Ca balance, it remains unclear whether protection results from direct pump stimulation or from secondary antioxidant effects. Here, we evaluated a series of novel allosteric SERCA activators under MGX- and PAL-induced stress. SERCA1a ATPase activity was quantified using an NADH-coupled enzyme assay. Antioxidant capacity was assessed by ABTS and DPPH radical scavenging assays. Inhibition of lipid peroxidation was measured in sarcoplasmic reticulum vesicles labeled with C11-BODIPY 581/591 after initiation with 2,2'-azobis(2-amidinopropane) dihydrochloride. The compounds increased SERCA activity and restored pump function following glucolipotoxic stress. Only a subset showed modest antioxidant properties, and recovery of SERCA function correlated with activation potency rather than radical scavenging or lipid peroxidation inhibition. These data indicate that direct allosteric stimulation of SERCA is sufficient to counter diabetogenic stress–induced dysfunction, independently of redox modulation. This work supports targeted SERCA activation as a promising approach to correcting ER Ca^{2+} dysregulation under diabetogenic stress and highlights its potential relevance for preserving β-cell function in type 2 diabetes.
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PP II_D10

Dietary copper deficiency impairs the capability for systemic methanethiol oxidation in mice

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The essential trace element copper (Cu) serves as cofactor in several enzymes involved in metabolism and redox homeostasis. Recently, selenium-binding protein 1 (SELENBP1), originally identified through binding selenium (Se) at supraphysiological doses, was added to the list of cuproenzymes. We showed that the capability of bacterially produced recombinant human SELENBP1 to act as methanethiol oxidase (MTO) – catalyzing the conversion of the volatile and toxic sulfur compound methanethiol to hydrogen sulfide, hydrogen peroxide and formaldehyde – strictly depends on the presence of Cu rather than Se. The capacity for SELENBP1-mediated detoxification of methanethiol, a product of the metabolism of some gut microbiota, is usually high given the very low methanethiol levels measured in breath and body fluids of healthy individuals. To clarify the role of Cu and Se for the systemic enzymatic degradation of methanethiol, we analyzed SELENBP1 protein levels and MTO activity in liver, colon and blood samples obtained from mice fed a diet deficient in Cu or Se, as compared to mice with adequate dietary supply of trace elements. Dietary Cu deficiency resulted in diminished MTO activity, as displayed by methanethiol-derived production of hydrogen sulfide, in liver, colon and erythrocytes of both male and female mice. In contrast, dietary Se deficiency did not affect the *ex vivo* MTO activity. Hepatic SELENBP1 levels were regulated in a sex-specific manner: Cu- and Se-deficient diets increased SELENBP1 levels in males, whereas in females a decrease was observed as a consequence of Cu-deficiency. In erythrocytes, SELENBP1 levels and MTO activity were decreased under Cu-deficient conditions in both sexes. Taken together, we found that dietary Cu deficiency results in impaired capability of mice to oxidize methanethiol.
[1] Philipp et al., Redox Biol. 2023, 65: 102807.

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PP II_D11

Beetroot juice nitrate-rich enhances nitrate metabolism and endothelial function in response to isometric exercise in males under androgenic anabolic steroid abuse

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Androgenic anabolic steroid (AAS) abuse has demonstrated detrimental effects on cardiovascular health. Inorganic nitrate (NO_3^-) has been demonstrated to improve nitric oxide homeostasis and restore endothelial function, but its effects on men undergoing AAS abuse are unclear. A double-blind and placebo-controlled crossover trial has been completed to investigate the effects of beetroot juice rich or NO_3^- depleted. Thirteen young males [mean (SD) age: 31 y (9); BMI: 30 kg/m^2 (4): total free testosterone: 1118.0 ng/dL (495.2)] undergoing AAS abuse were included in this trial. Following a 12-h fasting period, with a 7-day interval, they completed two random morning laboratory visits two hours after: 1) ingestion of 140 ml beetroot juice (NO_3^- 12.8 mmol); or 2) 140 ml of beetroot juice NO_3^-- depleted (NO_3^- 0.38 mmol). Unstimulated saliva samples were collected at rest, followed by flow-mediated dilation (FMD) before and after the isometric resistance (handgrip) test. Supplementation with NO_3^--rich beetroot juice effectively increased salivary NO_3^- (40.59 μM [95%CI: 34.99 to 46.19], $p < 0.001$) and NO_3^- (3.08 μM [95%CI: 1.26 to 4.90], $p = 0.002$) concentrations compared with placebo, confirming protocol compliance. Endothelial function (FMD) was significantly greater in the NO_3^- group compared with placebo under both baseline (2.37% [95%CI: 0.31 to 4.40], $p = 0.02$) and post-exercise conditions (2.57% [95%CI: 0.54 to 4.60], $p = 0.01$). These findings demonstrated that dietary NO_3^- supplementation enhanced endothelial function independently of exercise. Comparisons between protocols (NO_3^- vs placebo) to the isometric strength test (handgrip) performance did not reveal changes (0.02 kgf [95%CI: -9.74 to 9.71, $p = 0.99$). In conclusion, this study revealed that dietary NO_3^- enhanced NO metabolism and modestly improved endothelial function associated with AAS abuse.
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[2] Benjamim et al., Free Radic Biol. Med. 2024, 211: 12-23.
[3] Benjamim et al., Free Radic Biol Med. 2024, 215:25-36.

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PP II_D12

The preventive influence of beetroot juice on cardiovascular effects of heated tobacco products

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Tobacco smoking remains a major cardiovascular risk factor, contributing substantially to morbidity and premature mortality. Recently, alternative products such as e-cigarettes and heated tobacco products (HTP) have emerged, yet their cardiovascular effects are not fully elucidated. This study aimed to investigate the effects of preventive beetroot consumption under conditions of HTP use. Cardiovascular and metabolic acute effects were examined in healthy smokers. We assessed flow-mediated dilatation (FMD) as well as vital and blood gas parameters in healthy smokers acutely exposed to HTP. Based on a placebo-controlled crossover trial we investigated the effect of preemptive beetroot juice ingestion. Acute HTP exposure significantly reduced FMD, indicative of impaired endothelial function and elevated cardiovascular risk comparable to conventional cigarette smoking. Some changes were also observed in vital parameters or blood gases. Preventive administration of beetroot juice did mitigate these effects. Despite tobacco industry claims alternative tobacco products as safer or smoking cessation aids, current evidence regarding their acute and long-term cardiovascular effects remains inadequate. Given their rising popularity, notably among adolescents and young adults, further comprehensive research is imperative. Findings from this study indicate that acute exposure to heated tobacco products induced vascular dysfunction, corresponding to heightened cardiovascular risk. However, the prophylactic administration of beetroot juice

partially ameliorated these alterations.
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PP II_D13

Effects of vitamin C supplementation on individualized oxidative stress and inflammation responses: A set of n-of-1 trials

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Personalized antioxidant supplementation is often promoted as a superior strategy to optimize redox balance and inflammatory status. However, the assumed advantage of personalization cannot be empirically supported unless meaningful inter-individual variability in treatment response exists. If responses are broadly similar and the mean effect is acceptable, "average medicine" may be sufficient for most individuals. In this context, we examined short-term vitamin C supplementation effects and the presence of true between-individual response heterogeneity in healthy young men with vitamin C insufficiency using an aggregated series of replicate n-of-1 trials. Eight recreationally active males completed four vitamin C (1 g, oral) and four placebo administrations, delivered once weekly in randomized paired cycles after a controlled low-vitamin C dietary run-in to establish insufficiency. Plasma vitamin C and inflammatory biomarkers (interleukin-6 and tumor necrosis factor-α) were assessed following each administration (i.e., 8 observations per participant). Within-participant linear mixed-effects models and across-participant meta-analytic synthesis were used to estimate replicate-averaged treatment effects and quantify person-by-treatment interaction, while accounting for cycle-to-cycle within-person fluctuations. Vitamin C supplementation increased plasma vitamin C (mean +20.6 μmol/L, 95% CI 16.8 to 24.5), though the mean change did not clearly exceed a minimum clinically important difference (i.e., 23 μmol/L; MCID). Supplementation also reduced interleukin-6 (−1.2 pg/mL, 95% CI −1.7 to −0.7) and tumor necrosis factor-α (−0.5 pg/mL, 95% CI −0.9 to −0.2). Evidence for participant-by-treatment interaction was imprecise and not statistically significant, while shrinkage-adjusted individual effects converged strongly toward the group mean, indicating that apparent "response differences" were largely explained by within-person cycle-to-cycle variability rather than stable inter-individual heterogeneity. These findings challenge inflated claims of personalized superiority and support status-guided, average supplementation strategies when the average effect is consistent across individuals and exceeds a relevant MCID.
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PP II_D14

Dry and liquid marination with rooibos extracts protects pork patties from lipid oxidation and maintains oxidative balance during refrigerated storage and after cooking by different methods

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Rooibos (*Aspalathus linearis*) is an herbal infusion native to South Africa, with a unique polyphenolic profile and strong antioxidant potential, largely attributed to *Aspalathin*. Depending on processing, rooibos is available as fermented or unfermented, the latter retaining higher polyphenolic content and antioxidant activity. While extensively studied in beverages, its role in controlling oxidative processes in meat systems remains poorly explored. This study evaluated the capacity of fermented and unfermented rooibos extracts, applied via dry and liquid marinades, to modulate free radical–induced lipid peroxidation and redox imbalance in pork loin patties during refrigerated storage (0–13 days at 4 °C) and following different cooking methods (pan, microwave, sous-vide, and oven). Total polyphenols of the extracts were quantified using the Folin-Ciocalteu assay, and their polyphenolic profiles were determined by HPLC analysis. Oxidative deterioration of the patties during storage and after cooking was assessed by measuring peroxide value and malondialdehyde (TBARS) as markers of lipid oxidation, while Coenzyme Q10 redox status was evaluated to monitor redox homeostasis. Sensory analysis was also performed to assess consumer acceptability. Both rooibos extracts significantly limited lipid peroxidation during storage and after cooking, with unfermented rooibos consistently showing superior antioxidant efficacy. Dry marination provided stronger protection during cold storage, whereas liquid marination more effectively reduced cooking-induced peroxide and MDA formation and preserved Coenzyme Q10 redox balance. Among cooking methods, pan and sous-vide cooking induced a lower oxidative burden, while oven and microwave cooking generated higher oxidative stress and benefited most from rooibos-mediated protection. Moreover, sensory evaluation indicated high consumer acceptability of rooibos-marinated meat. Overall, rooibos polyphenols effectively attenuate free radical–mediated lipid oxidation and preserve redox homeostasis in meat systems, enhancing nutritional quality and sensory attributes, while contributing to dietary antioxidant intake and supporting oxidative stress management in consumers.

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PP II_D15

***In vitro* and *in vivo* investigation of antioxidant potential of Varthemia iphionoides methanolic extract on rat brain tissue**

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The pharmaceutical sector makes substantial use of food and medicinal plants, both of which have a major role to play in promoting health. These plants' significant medicinal potential is attributed to their secondary metabolites, which include phenolic compounds which perform anti-inflammatory, anti-hemolytic, antioxidant effects and proteolytic activity. The purpose of this work is to examine the phenolic and protein content of the *V. iphionoides* methanolic extract, as well as its anti-inflammatory, anti-hemolytic, and antioxidant properties. Additionally, it is being investigated for its proteolytic action and possible effects on some crucial therapeutic enzymes. The extract of *V. iphionoides* in methanol showed anti-inflammatory effectiveness of 90.67%, anti-hemolytic activity of 92%, and DPPH scavenging capacity of 64%. The total protein content was 10.239 mg/ml and the total phenolic content was 2.03 mg GAE/g. Fourteen phenolic compounds were found using LC-MS/MS analysis, with 3-O-methylquercetin having the greatest concentration (111 μg/mg). The extract has decreasing effect on lipid peroxidation process by measuring MDA levels in all Rat groups treated with the extract and increasing effects on antioxidant enzymes compared to control group. Additionally, the extract demonstrated proteolytic activity, and has an inhibitory action against trypsin and an enhancement activity against chymotrypsin and papain enzymes. Phenolic compounds are among the biomolecules found in the methanolic extract of *V. iphionoides*. Significant biological activities are also present in it, and they are linked to the phenolic content. Additionally, it exhibits proteolytic action, inhibits the activity of the trypsin enzyme, and increases the activity of the papain and chymotrypsin enzymes, which are therapeutic enzymes.

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PP II_D16

Cyanidin-3-O-glucoside modulates mitochondrial redox responses and protects against endothelial lipotoxicity in vitro and in high-fat diet–induced obese mice

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Chronic consumption of high-fat diets (HFDs) disrupts lipid homeostasis, leading to lipid accumulation and lipotoxicity. In endothelial cells, lipid overload promotes endothelial dysfunction, an early event in atherosclerosis by increasing reactive oxygen species (ROS), impairing mitochondrial integrity, and disrupting cellular redox balance. To determine whether cyanidin-3-O-glucoside (C3G) (i) improves HFD-induced dyslipidemia in mice and (ii) protects endothelial cells from palmitate-induced lipotoxic injury, focusing on lipid accumulation, ROS burden, and mitochondrial stress-adaptive signaling (including peroxisome proliferator-activated receptor gamma coactivator 1α; PGC-1α) relevant to obesity-associated endothelial dysfunction. Male C57BL/6 mice were fed a 60% HFD to induce obesity and then treated with C3G. Body weight and energy efficiency were monitored, serum total cholesterol, triglycerides, HDL-C, and LDL-C were quantified, and aortic tissues were collected to assess mitochondrial oxidative stress and redox-signaling markers. In vitro, EA.hy926 endothelial cells were exposed to palmitic acid to induce lipotoxic stress and subsequently treated with C3G. Endpoints included cell viability, intracellular ROS, lipid accumulation, and PGC-1α nuclear localization assessed by imaging-based analysis, supported by biochemical and gene-expression assays. HFD feeding increased body weight, energy efficiency, and circulating lipids (total cholesterol, triglycerides, LDL-C), whereas C3G treatment improved these metabolic parameters. In endothelial cells, palmitic acid induced lipotoxic injury, evidenced by reduced viability, increased ROS production, and greater lipid accumulation. Palmitic acid also increased nuclear localization of PGC-1α, consistent with a compensatory mitochondrial biogenesis response to oxidative stress. HFD induces obesity-associated dyslipidemia, and palmitic acid triggers endothelial lipotoxic oxidative stress with activation of PGC-1α–linked stress-adaptive signaling. C3G improves circulating lipid profiles in HFD-fed mice and mitigates key lipotoxic/oxidative stress features in endothelial cells, supporting its potential to counter obesity-related endothelial dysfunction and downstream metabolic vascular risk through regulation of redox–mitochondrial responses.

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PP II_D17

Deciphering the consequences of transient drought on the grain proteome of wheat

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Drought stress frequency and intensity have increased significantly globally due to climate change, which has been particularly detrimental to crop productivity, including that of the widely cultivated bread wheat (*Triticum aestivum*). Stress during the reproductive stage has the most potent effect on yield decline. Herein, we evaluated the impact of moderate drought during flowering on grain quality across two contrasting cultivars–the sensitive cultivar *Chyhyrynka* and the tolerant cultivar *Sofiia Kyivska*. Proteins were isolated using a single-step detergent-assisted extraction method, digested with trypsin, and analyzed by liquid chromatography-tandem mass spectrometry. We quantified 5,433 proteins in mature grains and revealed 728 differentially abundant proteins across genotypes and drought treatment. According to the principal component analysis, genotype contributed more to the variance in protein accumulation than drought treatment, with a distinct grouping of tolerant and sensitive cultivars. The protein profiling revealed that drought stress alters the protein profiles of wheat grains, with sensitive cultivars increasing seed storage proteins and stress proteins, and tolerant cultivars boosting metabolic and stress proteins, indicating distinct molecular strategies for drought adaptation. Notably, the total grain yield declined significantly in the sensitive genotype. Next, we will focus on redox proteome alterations in flag leaves under water shortage and subsequent recovery at the reproductive stage, as this is the most critical for yield formation. The discoveries will reveal molecular markers and pathways essential for developing drought-resilient wheat, thereby improving crop yield in the face of progressing climate change.

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PP II_D18

Clinical translation of nanostructured lipid carrier (NLC) encapsulating vitamin E tocotrienols: Phase 1 bioavailability / bioequivalence study

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Vitamin E is a lipid-soluble essential micronutrient composed of 8 naturally occurring congeners (α, *β*, ɣ, δ-tocopherols and tocotrienols). The absorption of vitamin E generally parallels with dietary fat, but quantitative differences were observed to be lower for tocotrienols (T3) ranging between 8 to 25% (1). We aim to develop T3-encapsulated nanostructured lipid carrier (NLC) formulation suitable as nutraceutical ingredients to be used in health supplements and functional food (2). For clinical translation, the objective of this study is to investigate the bioavailability and bioequivalence of T3-encapsulated NLC (T3-NLC) in a Phase 1 clinical trial. Process scale-up using ultrasonication method and spray-drying was studied using quality by design method. Optimized T3-NLC was incorporated into a functional beverage at 100-300% recommended dietary allowances of vitamin E per serving. Oral bioavailability study of T3-NLC was evaluated against non-formulated T3 capsules in healthy volunteers. In-line ultrasonication required energy input above 200Ws/g to produce particles at 142±10nm for volume up to 20L, with 2 recirculation cycles. Beverages containing T3-NLC remained stable at particle size < 200 nm over storage of 6 months at 25°C. In bioavailability study, the relative ratio for maximum concentration (C_{max}) of α, ɣ and δ-tocotrienols were found to be 170, 236 and 211 respectively. In conclusion, T3-NLC presented particle size < 200nm and remained stable after process scale-up to 20L using in-line ultrasonication. In a bioequivalence study involving 23 healthy volunteers, T3-NLC showed superior bioavailability of tocotrienols compared to unformulated T3.

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1. EXPOHEALTH Premeeting Symposium Lectures

EH I_01

The exposome concept

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There is general consensus that environmental pollution and non-chemical stressors contribute to the incidence and prevalence of chronic non-communicable disease (e.g., cardiovascular, metabolic and mental). Clinical and epidemiological studies support that air pollution and traffic noise are associated with a higher risk for cardiovascular disease and significantly contribute to overall mortality. Various large scale studies and expert commissions have identified air pollution as the leading health risk factor in the physical environment, followed by water and soil pollution with heavy metals, pesticides, other chemicals and occupational exposures, however neglecting the non-chemical environmental health risk factors: mental stress, light exposure, climatic changes and traffic noise. Especially for traffic noise-related health effects there are numerous clinical and epidemiological studies reporting significant impact on cardiovascular disease, however, without mentioning traffic noise as a risk factor in actual guidelines of clinical societies or health action plans. In this respect, the "exposome" provides a comprehensive description of lifelong exposure history.

My presentation provides an in-depth overview on the health effects of the external exposome, with emphasis on air pollution and traffic noise. The role of environmental exposures for redox changes and oxidative damage and their impact on the exposome will be discussed in full detail. In addition, we summarize how the exposome contributes to non-communicable disease and thereby to the global burden of disease.

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EH I_02
Epidemiological Studies on the Association between Air pollution and Childhood Cancer: Methodological challenges in Exposure Assessment

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Epidemiological evidence linking outdoor air pollution exposure to childhood malignancies remains heterogeneous and inconclusive. This study critically examines methodological challenges in exposure assessment, a primary source of uncertainty in air pollution epidemiology, particularly when integrating heterogeneous data sources for long-term exposure characterization. We performed a targeted scoping review to identify critical methodological gaps in observational studies of outdoor air pollution and childhood cancer, focusing on: (1) exposure metric selection (e.g., residential proximity vs. spatiotemporal models), (2) critical exposure windows (prenatal/postnatal), and (3) multi-pollutant complexity. Subsequently, we quantified concordance between two international concentration datasets EXPANSE and ACAG using Germany-wide $PM_{2.5}$ estimates. Exposure misclassification due to metric heterogeneity represents a key limitation across studies. In the comparison of the EXPANSE and ACAG concentration datasets, every sampling scheme produced high concordance and the two models yielded identically spatiotemporal patterns: elevated concentrations in western and southern Germany, and a pronounced national decline over the observation period. So far, investigations on air pollution and childhood cancer have yielded results thar still require confirmation. Furthermore, several methodological challenges have emerged in this field, underscoring the need for high-quality exposure data. Our findings indicate that ACAG and EXPANSE generate highly comparable $PM_{2.5}$ estimates across Germany. An ongoing epidemiological study is evaluating whether both datasets yield consistent risk estimates.

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EH I_03
Air pollution health impact and redox considerations

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Air pollution is a major contributor to morbidity and mortality, causing over 8 million premature deaths annually. Cardiorespiratory diseases account for the vast majority of premature mortality. However, over the last decade, epidemiological studies have found associations between air pollution exposure and conditions of all major organ systems. These associations are supported by a large literature revealing a complex network of interacting mechanisms underlying the links between various air pollutants and diseases. Notably, inflammation and oxidative stress are hallmarks of air pollution exposure, which exacerbate the progression of disease at multiple stages across mechanistic pathways. This short presentation will give an overview of the how the redox properties of air pollution promote oxidative stress. It will consider the relevance of particulate matter oxidative potential and how this feeds into cellular sources of free radicals, signal transduction and pathophysiological processes in the lung, cardiovascular system, and other organs. It will consider what this means for individual susceptibility to air pollution and potential therapeutic strategies to reduce the harmful actions of this important environmental risk factor.

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EH I_04
Noise pollution health impact and redox considerations

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Environmental noise is a biologically active stressor that induces cardiovascular injury through well-defined molecular pathways. Building on the emerging framework summarized in the recent AHA Scientific Statement, this presentation focuses on mechanistic insights derived from translational human and experimental models. Controlled human field studies demonstrate that even short-term nocturnal aircraft or railway noise exposure impairs endothelial function, increases catecholamines, and induces oxidative stress, with rapid reversibility by antioxidant interventions such as vitamin C. Complementary animal models reveal a central role of NADPH oxidase (NOX2)-driven reactive oxygen species formation, eNOS uncoupling, and activation of inflammatory pathways including NF-κB and TGF-β signaling. Noise further amplifies cardiometabolic injury in models of hypertension, diabetes, and myocardial infarction, indicating priming effects. Pharmacological modulation targeting adrenergic signaling, AMPK and NRF2 pathways as well as alpha and beta-receptor blockade effectively attenuates these responses. These data establish noise as a causal redox-driven vascular stressor and provide a mechanistic basis for targeted therapeutic and preventive strategies.

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EH I_05
Cardiovascular damage by noise and/or PM (UFP) in preclinical models – lung/brain-heart axis

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Particulate matter (especially ultrafine particles) and environmental noise are now recognized as major non-traditional cardiovascular risk factors that promote hypertension, vascular dysfunction and adverse cardiac events. In preclinical models, noise and particulate matter trigger cardiovascular damage via tightly linked lung and brain pathways that converge on the heart and the vasculature. Noise exposure activates central stress circuits, causing sympathetic overactivity, surges in blood pressure and heart rate, and widespread endothelial dysfunction in both large arteries and peripheral circulation. Inhaled particles, in turn, deposit in the lung, induce marked pulmonary oxidative stress and inflammation, and partly translocate into the bloodstream, where they directly injure the vascular wall and microcirculation. When noise and particulate matter act together, these effects reinforce each other through certain mechanisms, leading to stronger redox imbalance, inflammatory activation and structural injury along the lung/brain–heart axis than with either insult alone. Across these models, a common pattern emerges: upregulation of NOX-2–dependent reactive oxygen species formation, disturbed antioxidant defenses and activation of innate immune signaling in the lung, brain and heart. Our preclinical data point out which pathways are individually regulated and which ones are additively regulated by both stressors, in order to provide a better mechanistic understanding and potential points of intervention.

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EH I_06
Cerebral damage by noise and/or PM (UFP) in preclinical models – implications for Alzheimer's disease

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Exposure to ultrafine particles (UFP) and noise pollution are significant environmental stressors associated with a wide range of negative health impacts, including neurodegeneration. Alzheimer's disease (AD) is the most common

neurodegenerative disorder characterized by impaired memory and the brain accumulation of abnormal amyloid beta and phosphorylated tau proteins. Up to 40 % of dementia cases are attributed to environmental factors, however, the exact mechanisms of how UFP and noise exposure affect brain health and contribute to the pathogenesis of AD remains unclear. This study aimed to investigate the effects of UFP and aircraft noise (AN) exposure in the context of AD. Transgenic mice modelling AD (5xFAD) and their wildtype (WT) littermates were randomly divided into 4 groups: clean air (control), UFP, AN, and a combination of UFP + AN. Mice were exposed to a playback of recorded aircraft events from Dusseldorf Airport, Germany and UFPs were generated using a mini combustion aerosol standard (miniCAST) burner in whole-body inhalation chambers. 10x Genomics Xenium spatial transcriptomics provided a map of gene expression in both WT and 5xFAD brain after UFP exposure. Metabolon untargeted metabolomics generated data on over 1000 plasma metabolites in exposed mice. The insights gained from this study enhance understanding of the contribution of air and noise pollution to health and their link to AD.

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EH I_07

Kinetic computational model for lung damage by particulate matter

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Air pollution is a major risk factor for public health and epidemiological studies identify fine particulate matter (PM2.5), ozone (O_3), and nitrogen oxides (NOx) as the most hazardous pollutants. Oxidative stress in the lung epithelium due to an excess presence of reactive oxygen species (ROS) is the leading hypothesis for the mechanism behind the adverse health effects of air pollution. Accordingly, measurements of the production of ROS by air pollutants is an increasingly popular means to quantify their toxicity and several cellular and acellular assays for the so-called oxidative potential (OP) of PM2.5 have been developed. ROS, however, are a heterogeneous group of several radical and non-radical species, which are already present and released in significant quantities in the human body. Furthermore, numerous defense mechanisms for oxidative stress protect tissues through enzymatic and antioxidant reactions, which is often not considered during measurements of OP. Here, we use a multiphase chemical kinetics model of the human respiratory tract, KM-SUB-ELF, to quantify and compare the effects of gas-phase and particulate pollutants on the production of ROS and oxidative damage in the epithelial lining fluid of the lung. The model aims at bridging the gap between laboratory investigations of air pollutant properties and the epidemiological evidence for air pollution health effects by providing quantitative, physiological metrics for air pollution toxicity. Our findings suggest that the adverse health effects may not be primarily related to direct chemical production of ROS and the so-called oxidative potential of PM2.5, but rather to the conversion of peroxides into more reactive species such as the hydroxyl radical, or the stimulation of biological ROS production. The analysis highlights remaining uncertainties in the relevant physical, chemical and biological parameters, suggesting a critical reassessment of current paradigms in elucidating and mitigating the health effects of different types of air pollutants.

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EH II_01

Non-optimal temperatures and health impact with focus on cardiovascular diseases

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Ambient temperature is an established environmental factor influencing cardiovascular morbidity and mortality. Numerous epidemiological studies describe a non-linear association between temperature and cardiovascular outcomes, with increased risks observed during both heat and cold exposure. Although recent attention has focused on heat-related health effects, cold temperatures continue to account for a considerable share of temperature-associated cardiovascular events in many regions. Several physiological pathways have been proposed to explain these associations. Exposure to high temperatures may lead to dehydration, reduced plasma volume, and electrolyte disturbances, which can affect cardiovascular stability and increase susceptibility to arrhythmias and thrombotic events. In contrast, cold exposure is linked to peripheral vasoconstriction, elevations in blood pressure, increased sympathetic activity, and inflammatory responses, all of which may contribute to the onset of acute cardiovascular conditions, including myocardial infarction and stroke. In addition, interactions with air pollution and behavioral adaptations may modify these effects. The impact of non-optimal temperatures is not evenly distributed across populations. Older individuals, patients with pre-existing cardiovascular disease, and those with limited adaptive capacity appear to be at higher risk. Environmental factors such as urban heat islands may further influence exposure patterns. This presentation will summarize current epidemiological findings and discuss potential biological mechanisms underlying temperature-related cardiovascular effects. It will also address expected changes in exposure patterns in the context of climate change and consider implications for prevention and public health.

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EH II_02

Computational modelling of climate hazards and interaction with air pollution

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Anthropogenic emissions alter atmospheric composition and therefore the climate, with implications for air pollution- and climate-related human health. Outdoor atmospheric risk factors, such as air pollution and non-optimal temperature, are a major concern for health, and they are subject to change in the future under different climate change and socioeconomic scenarios. We show the necessity to use numerical global climate model to investigate these important risk factors. Furthermore, we use model outputs from the recent Intergovernmental Panel on Climate Change multi-institution simulations to assess future changes in mortality attributable to long-term exposure to both non-optimal temperature and air pollution. We show that, even under a moderate scenario (SSP2-4.5), end-of-century mortality could quadruple from present-day values to around 30 (confidence level 95%:12-53) million/year, potentially reaching 44 million/year in a more pessimistic scenario (SSP5-8.5). While pollution-related mortality is projected to increase five-fold by the end of the century in a moderate scenario, temperature-related mortality will experience a seven-fold rise, making non-optimal temperature exposure more important than air pollution as health risk factor for at least 20% of the world's population. Population aging emerges as the primary driver of increased mortality, countering efforts to improve air quality and mitigate climate change. These findings underscore the urgency not only to improve air quality but also to simultaneously implement more effective climate change policies to prevent significant loss of lives in the future.

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EH II_03

Source-specific air pollution pathways to physiological and psychophysical health

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Air pollution affects human health through multiple pathways, extending beyond cardiopulmonary outcomes to include psychological stress, emotional

well-being, and risk perception. Taking $PM_{2.5}$ pollution in North China as a case study, we examine how emissions from different sources are translated into outdoor air pollution, indoor exposure, physiological health risks, and psychophysical responses. Residential fuel combustion is shown to contribute substantially to indoor $PM_{2.5}$ exposure and premature mortality, particularly through household energy use and winter heating, whereas nonresidential emissions have stronger influence on ambient $PM_{2.5}$ pollution and related psychophysical impacts, including worry, stress, depression, and irritation. By integrating emission inventories, atmospheric chemical transport modeling, indoor exposure assessment, and health impact evaluation, we provide a pathway-based perspective on how source-specific air pollution control can reduce exposure, lower premature mortality, and improve subjective well-being, highlighting the need for future air quality management to consider not only ambient concentration reductions but also exposure environments, pollution perception, and health-relevant intervention pathways.

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EH II_04
Redox-metabolic reprogramming by cold temperatures

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Cold exposure is a physiologically relevant environmental factor that elicits systemic adaptive responses to maintain thermal and metabolic homeostasis. Beyond its established role in adaptive thermogenesis, growing evidence indicates that cold exposure acts as a hormetic stressor, promoting tissue-specific redox and metabolic reprogramming across multiple tissues. Both acute and chronic cold exposure stimulate mitochondrial biogenesis and oxidative metabolism, particularly in brown and beige adipose tissue and skeletal muscle. This metabolic activation is accompanied by transient increases in reactive oxygen species (ROS) production, which serve as essential signaling molecules. Cold-induced ROS activate redox-sensitive transcriptional pathways, including Nrf2 and PGC-1α, leading to enhanced antioxidant defense, improved mitochondrial quality control, and increased metabolic flexibility. Upregulation of endogenous antioxidant systems, such as superoxide dismutases, glutathione peroxidases, and catalase, contributes to establishing a new redox steady state characterized by greater resilience to oxidative and metabolic stress. In adipose tissue, this redox remodeling supports thermogenic programming, lipid mobilization, and improved insulin sensitivity, while in skeletal muscle it enhances oxidative capacity and endurance related adaptations. In the cardiovascular system, controlled, moderate cold exposure induces adaptive vascular and myocardial responses linked to redox signaling and metabolic switching, whereas excessive or poorly tolerated exposure may provoke adverse hemodynamic and redox effects. Overall, when appropriately applied, cold exposure may represent a non-pharmacological strategy to modulate systemic metabolism and redox homeostasis beneficially. Investigating cold exposure-induced redox signaling in metabolically and disease-relevant tissues in human metabolism (primarily muscle and white fat) may improve our understanding and elucidate novel pathways regulating metabolic disorders.

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EH II_05
Hibernation: Molecular remodeling sustains life in the cold

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Life on earth has been adapting to extreme environmental conditions for billions of years, developing extra-ordinary molecular mechanisms for survival. Hibernation is one of the most sophisticated, evolutionarily conserved survival strategies at low temperatures, when food reserves are limited. Specific, poorly defined stimuli, dictated by the circadian rhythm, direct the molecular mechanisms of metabolic reprogramming, including preparation (prehibernation), entry (torpor) and awakening (posthibernation) from hibernation. Deep hibernation (torpor) is characterized by strong metabolic depression (more than 95%), body temperature close to ambient (about 0°C); heart rate drops from 350-400 beats to only 5-10, and breathing from >40 cycles per minute to less than one. What's more, biosynthetic, transcription- and translation-related processes are suppressed, and catabolic ones are favored. Complete molecular reprogramming is synchronized at the systemic level, with characteristic tissue specificities. Its knowledge is important for the understanding of numerous pathological conditions in human medicine (obesity, metabolic syndrome, diabetes, ischemia/reperfusion, hypothermia, transplantation, aging, cancer...), with a new imperative of application in space travel. This is a review of the molecular redox mechanisms of hibernation that underlie the beauty of survival under extreme environmental conditions.

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EH II_06
Measuring the toxicity of chemicals

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Adverse Outcome Pathways (AOPs) provide a mechanistic framework linking molecular initiating events to adverse health outcomes via defined key events (KEs) and key event relationships (KERs). In vitro models are increasingly used to generate human-relevant data within this context; however, experimental conditions critically determine assay performance and the interpretability of AOP-aligned findings. Using the LUHMES model of human dopaminergic neurons, we evaluated selected factors that modulate cellular responses to toxic insults. Metabolic substrate availability markedly influenced cellular phenotype, as glucose-supported glycolysis masked mitochondrial liabilities, whereas galactose-driven oxidative phosphorylation enhanced sensitivity to mitochondrial stress. Iron availability further regulated susceptibility to ferroptosis, with elevated iron promoting lipid peroxidation and sensitization, while iron restriction conferred relative resistance. In addition, cellular context played a decisive role: neuronal monocultures displayed increased vulnerability to oxidative stressors, whereas astrocyte co-culture provided neuroprotection, likely via glutathione-dependent mechanisms; conversely, inflammatory activation of glial cells exacerbated neuronal sensitivity. Collectively, these parameters significantly shape KE magnitude and downstream interpretation, with direct implications for hazard identification and potency assessment. Standardization and transparent reporting of experimental conditions are therefore essential to enhance reproducibility and to support the integration of in vitro data into AOP-informed toxicological and biomedical research.

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EH III_01
Aging and Environmental Factors: Effects of Nutrition

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Aging is inevitable associated with metabolic alterations. Redox balance disturbances and severe oxidation are often leading to irreversible protein modification. If not degraded in time, such protein material accumulates. Such accumulated protein aggregates – also referred to as lipofuscin – have a number of metabolism-modulating effects, often enhancing the aging process itself. It is well established that postmitotic cells are especially prone to accumulate lipofuscin, among those neurons, skeletal muscle and cardiomyocytes and also ß-cells. Although this is known for some time, the metabolic and functional consequences of nutrition and the metabolic state on this aspect of the aging process is hitherto not well understood. Aging processes seem to be enhanced in the context of obesity, increasing the risk of morbidity and mortality related to cardiovascular disease. Therefore, studies on responses of the heart that arise from prolonged obesity and the specific influence of biological age remain somewhat elusive. We compared the effects of diet-induced obesity on metabolism in the heart (with the postmitotic cardiomyocytes) and the postmitotic skeletal muscle. Although, cardiac metabolism is somewhat adaptive, distinct maladaptive remodeling processes may contribute to the development of cardiac dysfunction. Therefore, we analyzed the metabolic, structural, and functional properties of the heart in addition to the diet-induced model in the genetic model

of NZO mice. Cardiac function was assessed by echocardiography, plasma metabolite profiles were analyzed, and cardiac proteomes were quantified by mass spectrometry and used in a a kinetic model of cardiac central metabolism (CARDIOKIN1). In conclusion, young mice were protected against diet-induced cardiac dysfunction, whereas such a diet in aged mice led to heart failure and impaired physical performance. However, dietary stress might have distinct metabolic and functional effects on different metabolic genetic background.

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EH III_02
Mitigation of environmental exposure risk factors by physical exercise

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Physical exercise is increasingly recognized as an important modifier of health risks associated with both environmental exposures and medical treatments. Epidemiological evidence shows that regular physical activity provides substantial cardiovascular, metabolic, and cognitive benefits, even in the presence of air pollution. Although exercise increases ventilation and thus pollutant uptake, most studies indicate that, at low-to-moderate pollution levels, the overall benefits outweigh the risks, while these advantages may be reduced under conditions of extreme exposure. Exercise enhances antioxidant capacity, improves endothelial function, and reduces systemic inflammation, thereby partially counteracting pollution-related damage. In the context of heat stress, repeated exercise promotes heat acclimation, improving thermoregulation, expanding plasma volume, and stabilizing cardiovascular responses, which together lower the risk of heat-related illness. In oncology, exercise serves as a valuable adjunct to treatment by mitigating adverse effects of therapies such as Doxorubicin and Trastuzumab. It reduces cancer-related fatigue, preserves functional capacity, and improves quality of life. Notably, exercise also provides cardioprotection by attenuating declines in cardiac function, reducing oxidative stress, and limiting inflammation and myocardial damage associated with cardiotoxic treatments. These effects are partly mediated by the exercise-induced secretome, which includes proteins and non-coding RNAs (ncRNAs), often packaged within extracellular vesicles (EVs). As mediators of intercellular communication, EVs influence both healthy and tumor tissue behavior, as well as treatment response. This presentation will examine the interplay between environmental exposures and physical activity, and will highlight recent findings from our group showing that exercise modulates EV cargo to enhance cardiomyocyte resistance to oxidative stress, suppress tumor aggressiveness, and improve chemotherapy efficacy, supporting its role as a non-invasive adjunct in cancer care.

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EH III_03
Particulate matter induces tissue OxInflammation: From mechanism to prevention

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The mechanisms through which particulate matter (PM) exposure drives adverse health effects are not yet fully understood. Available evidence suggests that PM affects interface organs—including the eyes, lungs, gastrointestinal tract, and skin—and that such disruptions may contribute to systemic outcomes. Simultaneously, particles may enter the body through inhalation or ingestion (e.g., via contaminated food), resulting in changes to distant organs such as the heart. Taken together, these findings support the notion that PM exposure begins as a localized tissue event with the potential to propagate systemically. A deeper mechanistic understanding of PM toxicity is essential for developing targeted preventive strategies and for uncovering determinants of interindividual susceptibility. In vitro and animal model studies have implicated multiple, mutually non-exclusive pathways, encompassing both direct effects of particle constituents and indirect effects driven by proinflammatory signaling. It has been suggested that PM-induced local inflammation triggers a systemic inflammatory response marked by endothelial activation and increased oxidative and nitrosative stress—processes that likely account for the elevated morbidity and mortality consistently observed in populations exposed to polluted environments. Collectively, the body of evidence highlights the need to reduce ambient PM concentrations in order to limit exposure at primary interaction sites—the respiratory tract, ocular surface, gastrointestinal tract, and skin—and to prevent downstream health consequences, including respiratory dysfunction, exposure-related eye disease, inflammatory skin conditions, gastrointestinal disturbances, and systemic effects such as cardiovascular disease. At the individual level, air quality monitoring tools can support risk reduction by guiding behavioral adjustments. Minimizing exposure remains one of the most effective strategies for reducing the population-level burden of PM-related disease.

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EH III_04
Targeting inflammatory and oxidative pathways to prevent particulate matter-induced lung damage

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Air pollution, particularly airborne particulate matter (PM), is a leading global health risk, strongly associated with cardiopulmonary morbidity and mortality. PM exposure disrupts airway and vascular homeostasis by triggering oxidative stress and persistent inflammation, ultimately compromising the integrity of the alveolar–capillary barrier and promoting maladaptive tissue remodeling. Despite growing epidemiological evidence, effective therapeutic strategies to counteract PM-induced damage remain limited. Therefore, assessment of the molecular pathways underlying PM-driven cardiopulmonary injury, with a focus on actionable therapeutic targets became important. PM exposure induces excessive production of reactive oxygen species, overwhelming endogenous antioxidant defenses and activating redox-sensitive signaling cascades, including NF-κB, MAPK, and NLRP3 inflammasome pathways. These events drive the release of pro-inflammatory mediators, enhance endothelial dysfunction, and exacerbate epithelial barrier breakdown. We highlight emerging intervention strategies aimed at restoring redox balance and dampening local and systemic inflammation. These include pharmacological antioxidants, NRF2 pathway activators, inhibitors of inflammasome activation, and cytokine-targeted therapies. In addition, modulation of epithelial repair processes and barrier function represents a promising avenue to preserve tissue integrity. Importantly, combinatorial approaches targeting both oxidative and inflammatory axes may offer superior protection against PM-induced adverse health effects. Advancing our understanding of these interconnected pathways is critical for the development of targeted therapies and preventive strategies. Such approaches hold significant potential to mitigate the burden of air pollution-related cardiopulmonary diseases and improve public health outcomes in increasingly polluted environments.

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EH III_05
Inflammasome involvement in skin response to pollution exposure

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As the most apparent organ subjected to external environmental stressors, the impact of pollution on the skin has been extensively researched over the past few decades. While ultraviolet (UV) radiation is recognized as the most harmful stressor that our skin encounters on a daily basis, various elements of tropospheric pollution have also been demonstrated to influence skin health and its functions. Notably, agents such as ozone, particulate matter, cigarette smoke, and micro/nano plastics have been identified as factors that can alter skin homeostasis. Furthermore, the interplay among various pollutants has garnered significant attention in current research. Although the molecular mechanisms by which pollutant exposure results in cutaneous pathological manifestations, it has been noticed that an inflammatory status is a common denominator of all those skin conditions. For this reason, recently, the activation of a cytosolic

multiprotein complex involved in inflammatory responses (the inflammasome) that could promote the maturation of proinflammatory cytokines interleukin-1β and interleukin-18 has been hypothesized to play a key role in pollution-induced skin damage. Our data were able to show cutaneous inflammasome as a novel target of pollutant exposure and the eventual usage of inflammasome inhibitor as new technologies to counteract pollution-induced skin damage. Possibly, the ability to inhibit the inflammasome activation could prevent cutaneous inflammaging and ameliorate the health and appearance of the skin.

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EH III_06
Interventions against and measurement of skin aging

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Prolonged tissue residence of senescent cells is a candidate driving force of redox-stress and age-related changes that impair tissue function. Especially the human skin which is exposed to extrinsic stressors exhibits structural and functional alterations that impair its defenses at advanced age. We utilized automated tissue cytometry to correlate morphological, biochemical and metabolic transitions in aging epidermis with a senescent phenotype of the residing cells. Using tissue zymography of enzyme activities at key metabolic nodes in young and aged human epidermis *in situ* we identified age-related and senescence associated increase in lactate dehydrogenase (LDH) activity at cellular resolution. Conversely, we found an age-related systemic reduction in Glucose-6-phosphate dehydrogenase (G6PD) activity. The activities were compatible with buildup of pentose phosphate pathway metabolites, abatement of lactate and altered transporter expression in senescent keratinocytes (KC), and we could corroborate the findings in organotypic models containing labeled senescent KC. Furthermore, we identified an outside-in gradient of G6PD activity in young skin which is likely connected with a previously described antioxidant protective gradient, and which is lost in aging. Our results suggest that sparse senescent cells drive both, local and systemic metabolic reprogramming in the aging epidermis. Finally, we identified that an extract of Camellia. Ssp. can temporarily induce G6PD activity in the epidermis and yield a short term restoration of the juvenile metabolic configuration.

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EH IV_01
Of Sensing, Adaptation and Resilience: Coping with hypobaric hypoxia and psychosocial stress

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All living systems including humans have a need to constantly interact (while co-evolving) with their environment to ensure they remain fit for purpose as conditions change. This requires continuous sensing and adaptation of the cellular metabolic machinery to distinguish acute mild challenges from chronic major stressors (such as noise and air pollution) and possible existential threats and make appropriate adjustments accordingly. Food intake and its processing by the gut and oral microbiome ensures provision with essential building blocks and energy while nutrition provides the foundation for our understanding as to how metabolic flexibility is achieved and maintained. The latter is essential to cope with multiple stressors and balance this against other demands such as maintenance and repair. While these capabilities remain important across the entire life course, they are particularly critical in early development and during growth. This presentation will focus on the molecular interactions of small reactive oxygen, nitrogen and sulfur species (ROS, RNS, RSS) and their biological sensors at cellular boundaries and explore how these different elements are integrated across levels of regulation, from subcellular to higher-level physiological functions. These interactions have been conceptualised in the form of the 'reactive species interactome' and the 'redox interactome' frameworks. However, these have not yet been harmonised with challenges arising from sociocultural interactions which requires integration with the concept of interoception and the salutogenesis framework. This presentation will provide a brief overview of how alterations in environmental oxygen levels are sensed and responded to and how this translates into changes in whole-body redox state. A more comprehensive capture of such responses may not only be helpful to characterise differences in responses to social and environmental stressors between individuals but also allow quantification of the reserve capacity to deal with stress (personalised redox phenotyping).

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EH IV_02
Increasing oxidative stress resistance in the brain by NRF2 activation

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Ischemic stroke is the second most common cause of death worldwide, yet screening of cytoprotective drugs or compounds in vitro or in rodent models of ischemic stroke have failed to translate clinically. We previously reported that dietary sulforaphane (SFN), known to activate phase II and antioxidant enzymes via the KEAP1-NFR2 defense pathway [1], affords neurovascular protection and improves neurological and behavioral outcomes following cerebral ischemia-reperfusion injury [2]. In a model of stroke, we established that SFN upregulates NRF2/HO-1 and improves the non-reflow phenomenon. In brain microvascular endothelial cells (bEnd.3), a nanoparticle phosphorescence probe (MitoXpress-Intra) confirmed that cells adapted to 5kPa O_2 recapitulate in vivo O_2 (3.6 kPa). GSH levels were significantly higher in cells maintained under 18 compared to 5kPa O_2. Adapting cells to 5kPa O_2 attenuated induction of HO-1 and GCLM in response to SFN [3], consistent with our previous findings in HUVEC [4]. Acute hypoxia-reoxygenation led to a rapid burst of reactive oxygen species, which was significantly reduced by pretreatment with PEG-SOD or pretreatment with SFN, and interestingly increased intracellular metal profiles assessed by LA-ICP-MS. We recently reported that adapting human brain endothelial cells (hCMEC/D3) to 5kPa O_2 resets both basal and NO stimulated K^+ channel activity [5], which would affect the membrane potential brain endothelium. We encourage a paradigm shift in the design of in vitro experiments to recapitulate physiological O_2 levels encountered in vivo, with the aim of enhancing translation of cell physiology to animal models and humans [6].

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2. G-ReXS Postmeeting Symposium Lectures

GR I_01
Supersulfides in skeletal muscle homeostasis

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Supersulfides are recently recognized biomolecules characterized by sulfur catenation, with established antioxidant and anti-inflammatory properties. The primary enzymatically generated supersulfide, cysteine hydropersulfide (CysSSH), is produced in part by the cytosolic cysteinyl-tRNA synthetase CARS1. To investigate the in vivo role of CARS1-dependent CysSSH synthesis, we generated a skeletal muscle–specific mutant mouse (Cars1-MKO) in which canonical aminoacylation activity is preserved while supersulfide-producing activity is selectively attenuated. Compared with control littermates, Cars1-MKO mice exhibited post-weaning growth retardation and notable alterations in skeletal muscle, the detailed characterization of which is currently underway.

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GR I_02
Cystine C-S bond cleavage fuels cysteine production under disulfide reductase deficiency

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All organisms have thioredoxin reductase (TR) or glutathione reductase (GR), the only enzymes that use NADPH to reduce cytosolic disulfides into thiols, thereby powering deoxyribonucleotide biosynthesis, elimination of oxidants, oxidative damage-repair, and reduction of the disulfide nutrient cystine into the thiol amino acid cysteine. Hence, TR/GR-null bacteria or yeast are inviable yet remarkably, mice with TR/GR-null livers thrive, in part by synthesizing life-sustaining cysteine via alternative pathways that evolved in metazoans. Although TR/GR-null livers generate some of their cysteine via the serine-transsulfuration pathway, we here show that most cysteine in TR/GR-null livers comes from a pathway in which pyridoxal phosphate-dependent cleavage of a carbon-sulfur bond in cystine generates cysteine-persulfide, which decomposes nonenzymatically into cysteine. This potent yet previously unrecognized pathway is regulated by cellular levels of sulfur-metabolites and represents a potent cytoprotective response that might be induced in most mammalian cells under conditions that chronically elevate cytosolic cystine levels.
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GR I_03
Radical-induced protein persulfidation protects proteins against radical damage

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Protein per- and polysulfidation is widespread across all domains of life, yet it remains difficult to study, and its biological functions are still poorly understood. Here, we present a new method for the robust quantification of global protein persulfidation in biological samples. With this approach, we confirm that 0.5–5% of all protein thiols are per- or polysulfidated, depending on the organism and cell type. We further dissect the contributions of individual enzymes and metabolites to endogenous protein persulfidation and find that free radicals rapidly trigger its increase. Mechanistically, we identify an enzyme that links radical sensing to persulfide production. We then show that protein persulfidation shields proteins from radical-induced carbonylation, thereby helping cells withstand prolonged radical stress.

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GR I_04
Carbohydrate-based sulfide donors

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Hydrogen sulfide (H_2S) and its related reactive sulfur species are important regulatory molecules in redox biology. These species have shown many physiological effects, ranging from anti-inflammation to antioxidation. To advance their research, donor compounds that can slowly release sulfur species in biological conditions while producing minimum bioactive byproducts are needed. In this presentation, I will report on our research on developing carbohydrate-based donor compounds, which include thiosaccharides, thioglucose-based polysulfides and persulfides, as well as selenoglucose derivatives. We have found that 1) glucose oxidase can catalyze the oxidation of thioglucose to form both H_2S and H_2O_2, which in turn promotes protein persulfidation; 2) thioglucose and other thiosaccharides are slow-releasing H_2S donors; 3) thioglocose tetrasulfide is an effective H_2S donor, which can also serve as a persulfide precursor; and 4) glucose-diselenide derivatives are H_2S scavengers and potential H_2Se donors. I will present data to show these compounds' release profiles and mechanisms. Their applications in several biological models will also be discussed.

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GR I_05
Development of reactive sulfur species-producing enzyme inhibitors by fluorescent probe-based screening

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For detailed studies of the physiological functions of reactive sulfur species (RSS), including H_2S and super sulfurs, RSS-producing enzyme inhibitors are indispensable tools. To develop these inhibitors, high-throughput screening (HTS) utilizing fluorescent probes is a highly useful methodology. We previously designed and synthesized HSip-1 (Hydrogen Sulfide imaging probe-1), an H_2S-selective fluorescent probe based on a macroazacyclic copper ion (II) complex (Sasakura et al., J. Am. Chem. Soc. 2011, 133:18003-18005), as well as SSip-1 (Sulfane Sulfur imaging probe-1), a reversible off/on fluorescent probe for sulfane sulfur. By applying HSip-1 to an HTS of a chemical library containing over 160,000 compounds from The University of Tokyo, Drug Discovery Initiative (DDI), we identified selective inhibitors for 3-mercaptopyruvate sulfurtransferase (3MST) (Hanaoka et al., Sci. Rep. 2017, 7:40227) and cystathionine γ-lyase (CSE) (Echizen et al., Sci. Rep. 2023, 13:16456). More recently, we further designed and synthesized red and green fluorescent probes for H_2S specifically optimized for HTS. These probes exhibit low background fluorescence even in assay systems with high concentrations of thiol substrates. Using these probes, we performed HTS on chemical libraries of 32,000 and 214,000 compounds (The University of Tokyo, DDI) to target cystathionine β-synthase (CBS) and Thiosulfate sulfurtransferase (TST), respectively. Through these screenings, we successfully identified highly selective CBS and TST inhibitors that demonstrate high selectivity over other RSS-producing enzymes, such as CSE and 3MST.

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GR II_01
Membranes and thiols – will they ever become friends again in the presence of oxygen?

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Aerobic and even anaerobic life are characterized by a bewildering multiplicity of structural building blocks and soluble molecules that can be broadly circumscribed as antioxidant defenses. In the current paper, a retro-analytic approach is pursued to make the intriguing richness of antioxidant defenses in modern life more intelligible. It concludes that the branching spectrum of modern antioxidant defenses can be traced back to two primordial events that required ad hoc biological responses, which yet turned problematic under rising oxygen concentrations during later phases of evolution. Those two events were: (i) a temperature drop of approximately 40°C, demanding major adjustments to lipid bilayer structure, especially entailing fatty acid unsaturation, and (ii) the loss of the surrounding metal sulfide environment during the escape of early life from its presumed hydrothermal birthplace, making a direct anchoring of catalytic FeS clusters via genetically encoded cysteine necessary. Both ad hoc answers, membrane unsaturation and genetically encoded cysteine, turned increasingly detrimental with increasing ambient oxygen concentrations, resulting in multiple countermeasures of variable universality, for example, glutathione, new genetically encoded amino acids (methionine, tryptophan, selenocysteine) and the development of a prolific secondary metabolism based on the shikimate pathway. The outlined scenario is supported by the observed general evolutionary patterns of changing redox reactivity over time (as exemplified by changing HOMO-LUMO gaps) and the pronouncedly deviant and unique behavior of the amino acid cysteine under selective conditions, e.g., in mitochondria or in archaea dwelling under harsh chemical conditions.

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GR II_02
Abstract not submitted

GR II_03
Decoding the RNA Modification Atlas under Oxidative Stress

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RNA modifications have emerged as critical regulators of gene expression and cellular adaptation, adding an important layer of post-transcriptional control to stress-responsive pathways. Although oxidative stress is known to profoundly influence RNA metabolism and cell fate, the epitranscriptomic features associated with this process remain insufficiently characterized. In particular, how mitochondrial oxidative stress shapes the RNA modification landscape is still poorly understood. In this study, we investigated the RNA modification atlas under oxidative stress and identified a distinct modification signature specifically associated with mitochondrial oxidative stress. Our analysis revealed that this signature is not merely a global consequence of cellular damage, but rather reflects a selective and coordinated remodeling of the epitranscriptomic landscape. By integrating RNA modification profiling with transcriptomic analyses, we found that these changes are linked to stress-responsive gene expression programs, including pathways related to mitochondrial function, redox homeostasis, and cellular adaptation.

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GR III_01
Spatiotemporal monitoring of subcellular lipid peroxidation during ferroptosis

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Lipid peroxidation (LPO), the radical chain reaction of lipids and molecular oxygen, drives ferroptosis. Although plasma membrane permeabilization is clearly the terminal event in ferroptosis, the steps leading to it remain nebulous. In particular, it remains unclear whether LPO at specific organelles play functional roles in ferroptosis execution – a knowledge gap that hinders the development of potent small molecule modulators of ferroptosis as therapeutics which could otherwise be directed to them. To help address this we have targeted STY-BODIPY, the oxidizable fluorophore used in the FENIX assay, to mitochondria, lysosomes, endoplasmic reticulum and the Golgi apparatus to monitor organelle-specific LPO in real time. Herein we report the synthesis of these probes, their reactivity to phospholipid-derived radicals in liposomal models of lipid bilayers and their co-localization with established commercial organelle markers in live cells. We demonstrate their application in multiplexed assays utilizing cell death markers for simultaneous monitoring of LPO and ferroptosis. Using high-throughput live cell imaging and ferroptosis inhibitors that modulate LPO in specific compartments, we show that lysosomes are key functional nodes wherein LPO occurs upstream of ferroptosis induced by GPX4 inactivation and can drive the process even when LPO is suppressed in the ER. Mitochondrial LPO, on the other hand, is dispensable for ferroptosis and occurs continuously independently thereof. Interestingly, the trends in probe oxidation can vary with ferroptosis inducer (i.e., GPX4 inactivation, GSH depletion, etc.), supporting a model with multiple mechanisms of ferroptosis sensitization and/or induction.

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GR III_02
Lysosomal lipid peroxidation triggers ferroptosis induction

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Ferroptosis is a regulated cell death modality driven by iron-dependent lipid peroxidation (LPO) and has gained attention as a potential target in cancer therapy. Although considerable progress has been made in elucidating how LPO is initiated and controlled, uncertainties remain about where within the cell LPO predominantly occurs and how iron contributes to this process. By employing a fluorescent probe that we established for detecting lipid-derived radical species—critical intermediates in ferroptosis—we found that LPO within lysosomes induces iron release and promotes the propagation of LPO throughout the cell via enhanced lysosomal membrane permeabilization (LMP). In contrast, cell lines that are relatively resistant to ferroptosis showed little or no evidence of LMP. Collectively, these observations indicate that lysosomes play a central role in the initiation of ferroptosis.

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GR III_03
Mitochondria orchestrate the balance between Coenzyme Q and cholesterol synthesis in cancer cells

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Coenzyme Q (CoQ) and cholesterol are both synthesized from the same isoprenoid precursors but in different compartments, mitochondria and ER respectively, providing a clear example of compartmentalized metabolism. However, how these pathways are coordinated and regulated remains unclear. Our data reveal a mitochondria-centered metabolic hierarchy in cancer cells. When isoprenoids are limited, mitochondria prioritize CoQ synthesis to preserve mitochondrial respiration. When precursors are abundant, excess substrates are diverted toward cholesterol synthesis or CoQ export, while CoQ levels in mitochondria remain stable.
These findings uncover a novel layer of inter-organelle metabolic control, showing how mitochondria integrate precursor availability and biosynthetic demand to orchestrate cellular lipid allocation. By defining the principles that balance CoQ and cholesterol synthesis, our work provides fundamental insights into metabolic hierarchy, organelle communication, and cellular resilience, with broad implications for cancer metabolism and mitochondrial biology.

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GR III_04
Ferroptosis and the metastatic microenvironment

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Metastasis is the major cause of cancer-related morbidity and mortality, yet the mechanisms that enable cancer cells to survive during dissemination remain incompletely understood. Our work has identified ferroptosis, an iron-dependent form of lipid peroxidation-driven cell death, as a key barrier to metastatic progression. We previously showed that cancer cells are highly vulnerable to ferroptosis in the bloodstream, whereas lymph protects metastasizing cells from ferroptosis. In recent work, we find that lymph node metastatic cells undergo a shift in ferroptosis protection mechanisms. In the hypoxic lymphatic niche, glutamate-cysteine ligase and glutathione levels are reduced, while GPX4 undergoes proteasomal degradation. As a result, melanoma cells in lymph nodes become dependent on ferroptosis suppressor protein 1 (FSP1), which we found localizes to perinuclear lysosomes. This shift in ferroptosis dependency creates a therapeutic vulnerability: FSP1 inhibitors effectively suppress melanoma growth in lymph nodes but not in subcutaneous tumors. These findings show that the lymphatic microenvironment actively shapes ferroptosis resistance during metastasis and suggest that sensitizing cancer cells in lymph, either by increasing lipid oxidative stress or by targeting adaptive ferroptosis defense pathways such as FSP1, represents a new strategy to inhibit metastatic progression.

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GR IV_01
Targeting ferroptosis in lung cancer

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Pre-clinical and clinical studies have demonstrated how dietary antioxidants or mutations activating antioxidant metabolism promote cancer, highlighting a central role for reactive oxygen species (ROS) in tumorigenesis. However, it is unclear if ROS ultimately increases to a point of cell death. Emerging evidence indicates that cancer cells are preferentially susceptible to ferroptosis, a form of cell death that is triggered by elevation of lipid ROS. Despite broad enthusiasm about harnessing ferroptosis as a novel anti-cancer strategy, it remains unknown whether ferroptosis is a barrier to tumorigenesis and how this can be leveraged therapeutically. Combining CRISPR/Cas9 somatic editing with genetically-engineered mouse models of, we aim to identify mechanisms of ferroptosis suppression in lung adenocarcinoma.

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GR IV_02
Biomarker and therapeutic considerations for persulfides and polysuflides in sickle cell disease

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Sickle cell disease (SCD) is an inherited red blood cell (RBC) disorder with patients suffering lifelong injuries to critical organs, recurring episodes of pain and an overall shortening of lifespan. RBCs from SCD patients are more susceptible to hemolysis underpinning stress induced vaso-occlusive crisis leading to tissue ischemia, pain and inflammation. Moreover, thiol antioxidant systems including peroxiredoxin-2 activity are compromised in SCD. Polysulfides have been shown to confer protection in various diseases via anti-oxidant and anti-inflammatory mechanisms. The role of RBCs in the metabolism of persulfide and polysulfide species has also been reported, but how this may change in SCD and whether polysulfides could confer therapeutic benefit in SCD is not known. In this presentation, we present preliminary data characterizing persulfide and polysulfide levels in RBCs and plasma from SCD patients in crisis, and in a humanized (human hemoglobin expressing) mouse model of WT (HbAA) or SCD (HbSS). We also present preliminary data assessing the potential therapeutic effects of GSSSG towards pain and lung injury end points in SCD.

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GR IV_03
Genetically-encoded biosensors as tools to address neglected redox diseases

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The role of persulfides in cellular pathophysiology is increasingly recognised. This is largely driven by mass spectrometry-based methods for identifying and quantifying persulfidated proteins and low-molecular-weight species, and by chemical dyes such as SSP4 for detection in living cells. However, these approaches have significant limitations: mass spectrometry precludes dynamic measurements and is vulnerable to artifacts from alkylating blocking agents, while available chemical dyes lack compartment specificity and are irreversible. To address these shortcomings, PersIc (Persulfide Indicator), a genetically encoded fluorescent biosensor was developed. PersIc is ratiometric, reversible, and has a high dynamic range, enabling real-time persulfide measurements in living cells with compartment-specific resolution. Crucially, unlike two previously published biosensors, PersIc has a defined specificity and is selective for hydropersulfides over polysulfides and H_2S. By integrating mass spectrometry data, kinetic measurements, and predicted protein structures, I will present a mechanistic model that likely explains this selectivity. To illustrate the biological utility of PersIc, I will focus on facioscapulohumeral muscular dystrophy (FSHD) — one of the most prevalent yet neglected muscular dystrophies, with no curative treatment. FSHD is driven by aberrant derepression of DUX4, an embryonic transcription factor that triggers reverse electron transfer, generating H_2O_2 and ultimately causing muscle cell death. Using PersIc alongside complementary methods, I assess the role of persulfides in FSHD pathogenesis and discuss their therapeutic potential in this debilitating disease.

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GR IV_04
Zn^{2+}-mediated supersulfide formation prevents age-related cardiac fibrosis

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A growing body of evidence has suggested that sulfur-containing biomolecular species composed of multiple concatenated sulfur atoms, collectively termed supersulfides, are abundantly present in mammalian cells and play pivotal roles in maintaining cellular homeostasis. These include regulation of mitochondrial energy metabolism, membrane potential formation, protein and organelle quality control, and stress response/adaptation. In the murine heart, we have demonstrated that specific supersulfides exert cardioprotective effects against ischemia–reperfusion injury and chronic heart failure (CHF) after myocardial infarction. Supersulfides also suppress cardiac remodeling, particularly interstitial fibrosis, suggesting their therapeutic potential for fibrotic diseases beyond the heart. Clinically, reduced circulating zinc ion (Zn^{2+}) levels strongly correlate with the severity of CHF, and Zn^{2+} supplementation has been reported to improve CHF patient prognosis. However, the relationship between Zn^{2+} and supersulfide biology remains largely unexplored. We have recently shown that the transient receptor potential canonical 6 (TRPC6) channel, a receptor-operated cation channel, enhances cardiac contractility via local Zn^{2+} influx. Although TRPC6 is more abundantly expressed in cardiac fibroblasts than in cardiomyocytes, its functional role in fibroblasts remains unclear. We investigated whether TRPC6-dependent Zn^{2+} influx negatively regulates cardiac fibrosis through modulation of sulfur metabolism using a pharmacological TRPC6 activator (PPZ2) in mice. PPZ2 administration markedly suppressed cardiac interstitial fibrosis induced by chronic β-adrenergic stimulation as well as age-associated cardiac fibrosis. In contrast, these antifibrotic effects were abolished in mutant mice lacking Zn^{2+} permeability of the TRPC6 channel. Mechanistically, PPZ2 treatment increased the formation of supersulfides in the heart and enhanced antifibrotic signaling mediated by Zn^{2+}-dependent protein Cys disulfide bond formation. Collectively, our findings suggest that activation of the TRPC6–Zn^{2+}–supersulfide axis exerts potent antifibrotic effects and highlight its potential as a therapeutic target for preventing age-related organ stiffening and treating heart failure.

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GR V_01
Innate immune regulation targeting supersulfide metabolism

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Cysteine is an amino acid having thiol as a functional group, and plays an essential role in maintaining the structure of proteins through disulfide bonds. It also acts as an antioxidant, and enzyme activity center within cells. In recent years, cysteine persulfide and polysulfides, in which sulfur atoms are conjugated (sulfur catenation) to the cysteine thiol group, are abundantly present in cells as forms of glutathione and protein persulfides and polysulfides, and they have a wide variety of biological effects such as extremely strong antioxidant activity, anti-inflammatory effects, regulation of immune function, and control of energy metabolism. These diverse biological functions are not found in the original cysteine thiols, or are much stronger, so it is called "supersulfides", meaning that they have functions that exceed those of sulfides. Currently, the metabolism and functional regulation of supersulfides are attracting a great deal of attention worldwide as targets for new drug discovery and diagnosis. In this session, I would like to discuss regulatory roles of supersulfides on innate responses, and their possible applications to the treatment of infectious diseases.

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GR V_02
Reactive sulfur species in hypoxia across biology

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Hypoxia profoundly reshapes cellular redox chemistry, yet most models remain centred on reactive oxygen species. In this talk, Reactive Sulfur Species (RSS) – including persulfides and polysulfides – are shown to be the dominant oxidative and signalling agents under low-oxygen conditions across biology. Drawing on data from bacteria, cancer cells and mammalian systems, hypoxia-driven sulfur metabolism generates chemically potent RSS that selectively oxidise protein thiols, rewire signalling pathways and alter cellular fate. I will discuss emerging evidence that persulfides act as kinetically privileged oxidants, and that elemental sulfur represents an overlooked but biologically relevant product of hypoxic metabolism. Together, these findings motivate a reassessment of redox biology under hypoxia, positioning sulfur chemistry as a conserved and central driver of redox regulation from microbes to mammals.

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GR V_03
The fate of circulating per/polysulfides in hypoxia and hyperoxia

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As Life emerged on Earth oxygen was scarce while sulfur (and metals) were abundant. Sulfur is particularly well suited to support electron exchange reactions, exemplified by its critical role in mitochondrial function. The transition from an essentially anaerobic to an aerobic world during the 'Great Oxidation Event' must have posed a formidable threat to the functioning of the sulfur-rich regulatory circuitry that underpins metabolic flexibility. Sulfane sulfur species including hydropersulfides and polysulfides have been found in virtually all life forms across kingdoms and proposed to serve fundamental functions in higher organisms. While considerable effort has been spent in studying their biological role within cells, much less is known about their fate in the extracellular environment. We sought to investigate how circulating concentrations of sulfane sulfur in humans change in response to oxygen concentrations. This presentation will compare results from studies of healthy human volunteers exposed to either progressive hypobaric hypoxia during ascent to high altitude (Caudwell Xtreme Everest expedition) and in an environmental chamber (Duke Chamber Study) or hyperoxia with patients undergoing cancer resection surgery administered either 30, 55 or 80% oxygen (PULSE-Ox study) during general anesthesia. Concentrations of thiosulfate and sulfate in blood plasma were quantified using IC-MS while those of the sulfane sulfur pool were measured by fluorimetry using SSP4. Our results revealed a gradual decline in steady-state concentrations of thiosulfate and sulfate in hypoxia while opposite trends were observed in patients breathing supplementary oxygen. In contrast, circulating sulfane sulfur species concentrations remained relatively constant. In conclusion, bodily sulfur production scales with exposure to environmental oxygen, and circulating sulfane sulfur may serve as a buffer enabling to achieve redox balance at the whole-body level. The extent to which circulating sulfane sulfur levels are modifiable with specific interventions affecting resilience, such as nutrition and/or physical activity, remains to be determined.

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Symposium III – Lipid Peroxidation and Ferroptosis in Health and Disease

SL III_03
Evolutionarily conserved cyclo-octasulfur prevents ferroptosis in mammals

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Elemental sulfur (cyclo-octasulfur; S_8) is an evolutionarily ancient metabolite. It is well known to be utilized by bacteria, archaea and algae, as well as, to lesser extent, by plants. However, its generation, storage, or utilization in animals has remained unknown until now. Here we demonstrate the existence and functional role of S_8 metabolism in mammalian cells. We find that S_8 accumulates at millimolar concentrations within mitochondrial membranes and in lipid droplets in both mouse and human cells. Furthermore, we identify lipid droplet-associated nitric oxide synthase as a previously unrecognized source of S_8. Notably, endogenous levels of S_8 are markedly elevated in cancerous versus normal breast adipose tissue. Functionally, we show that accumulation of S_8 in lipid droplets limits lipid peroxidation, thereby protecting cells from ferroptosis. Therapeutic injections of solubilized S_8 prevents lipid peroxidation in a mouse model of osteoarthritis. In summary, our findings reveal a previously unrecognized mammalian S_8 pool that serves as a defense mechanism against oxidative membrane damage, thus providing a potential ability of cancer cells to evade ferroptosis.

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