23rd International Conference on

# Oxidative Stress Reduction, Redox Homeostasis & Antioxidants

# PARIS REDOX 2021

## ABSTRACTS BOOK

October 13-15, 2021

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# International Society of Antioxidants

## 23rd International Conference on

# Oxidative Stress Reduction,

# Redox Homeostasis and Antioxidants

**October 13-15, 2021**

**Interactive Online Conference**

**President of ISANH & Paris Redox 2021**

Prof. Harry van Goor

The global abstract book is referenced on the

Archives of International Society of Antioxidants in Health & Nutrition.



INTERNATIONAL SOCIETY OF ANTIOXIDANTS

# Welcome to Paris Redox 2021

We are pleased to welcome you to the organization of the 23rd International Conference on Oxidative Stress Reduction, Redox Homeostasis & Antioxidants "Paris Redox 2021" which will be held on October 13-15, 2021 as an Interactive Online Conference with Live Sessions.

This year, we will dedicate 2 sessions to Live Discussion and Networking with all other participants (i.e., attendees and speakers). You will have the chance to ask all your questions and get your answers directly during the sessions. Thanks to the platform of the conference, you can also see and access to all attendees, speakers (major, short oral and poster presentations), organizations and chairpersons' profiles. You can contact, chat, and fix an appointment with all attendees and speakers.

Paris Redox 2021 will surely make an eminent contribution to a better understanding of the reactive species-induced redox control in physiological conditions and various pathologies. This will lead to new therapeutic and disease-preventive agents. We therefore invite you to submit papers on reactive species related to health and disease ranging from fundamental and technical aspects to experimental and clinical diseases.

**Among the Topics which will be discussed during 3-days conference:**

- Redox 2021: Recent Advances & Perspectives
- Gut, Microbiome and Redox: Focus on Covid-19 Infections
- Brain, Neurodegenerative Diseases and Redox
- Ageing & Telomeres 2021: Advances and Perspective
- Redox Medicine: Innovations & Clinical Studies

All those interesting topics will be recorded and available in the platform. You will have access to them at any time, from the comfort of your home, for at least 1 month after the conference.

We would like to thank the members of the scientific committee and all invited speakers for their contribution. Their breadth of knowledge and expertise has helped make this conference as extraordinary as it is. We would like to particularly thank: Martin Bergö, Pedro Buc Calderon, Laurent Chatre, Carsten Culmsee, Marvin Edeas, Meng-Er Huang, Julie Lim, Carole Nicco, Oliver Nüsse, Kenneth Olson, Carole Peyssonnaux, Charareh Pourzand, Thierry Patrice, Jumana Saleh, and Miria Ricchetti.

Let us together make the 2021 Paris Redox meeting a resounding success.

All our warmest regards.

**Prof. Harry van Goor**
Department of Pathology and Medical Biology
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Groningen, the Netherlands

## *Thanks to All Our Speakers for their Valuable Contributions!*

# 23rd International Conference on

# Oxidative Stress Reduction, Redox Homeostasis and Antioxidants

## Two Live Interactive Sessions & Discussion

**October 13-15, 2021 - Online Interactive Congress**

## Paris Redox 2021 Speakers

**The Reactive Species Interactome: What's new ?**
**Laurent Chatre,** ISTCT, CNRS, Université de Caen-Normandie, France

**Maintenance of ER Homeostasis through Redox Regulation**
**Ryo Ushioda,** Kyoto Sangyo University, Japan

**Targeting Autophagy to Counteract Obesity-Associated Oxidative Stress**
**Federico Pietrocola,** Karolinska Institute, Sweden

**The Impact of Oxidative DNA Damage and Stress on Telomere Homeostasis**
**Patricia L. Opresko,** University of Pittsburgh Public Health & UPMC Hillman Cancer Center, USA

**New Methods to Evaluate Telomeres**
**Nedime Serakinci,** Turkish Republic of Northern Cyprus Presidency, Turkey

**Oxidative Stress as Key Player in Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) Infection**
**Livan Delgado Roche,** Laboratorios Liomont, Mexico

**Skeletal Muscle Redox Signaling in Rheumatoid Arthritis**
**Johanna T. Lanner,** Karolinska Institute, Sweden

**COVID-19: Immunopathology, Pathophysiological Mechanisms, and Treatment Options**
**Larissa Van Eijk,** University Medical Center Groningen, The Netherlands

**Redox Medecine & Ferroptosis**
**Brent Stockwell,** Columbia University, USA

**N-acetylcysteine (NAC) and Hydrogen Sulfide (H2S): A Convenient Rationale for Coronavirus Disease 2019 (COVID-19)?**
**Arno Bourgonje,** University Medical Center Groningen, The Netherlands

**Harnessing The UVA-Induced Redox Active Labile Iron Release to Improve the Efficiency of 5-Aminolevulinic Acid-Based Photodynamic Therapy (ALA-PDT) of Skin Cancer**
**Charareh Pourzand,** University of Bath, United Kingdom

**Raising the 'Good' Oxidants for Immune Protection**
**Ulla Knaus,** University College Dublin, Ireland

**Telomere Length and Cardiovascular Diseases**
**Nihal Inandiklioğlu,** Yozgat Bozok University, Turkey

**The Anti-inflammatory Effects of Nrf2 Activation**
**Albena T. Dinkova-Kostova,** Jacqui Wood Cancer Centre, United Kingdom

**Decreased Availability of Nitric Oxide and Hydrogen Sulfide is a Hallmark of COVID-19**
**Gopi Kolluru,** Louisiana State University Health Sciences Center-Shreveport, USA

**Peroxiredoxin Promotes Longevity and H2O2-Resistance in Yeast through Redox-Modulation of Protein Kinase A**
**Mikael Molin,** Chalmers University of Technology, Sweden

**Redox and Progeria: Advances and Perspective**
**Ricardo Villa-Bellosta,** University of Santiago de Compostela, Spain

**Targeting the BACH1-NRF2 Axis**
**Laureano de la Vega,** School of Medicine, University of Dundee, United Kingdom

**Beneficial and Detrimental Effects of Reactive Oxygen Species on Lifespan**
**Jeremy Van Raamsdonk,** McGill University, Montreal, Canada

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**Adam R Renslo,** University of California, USA

**Physiological Roles of 3-mercaptopyruvate sulfurtransferase in the Cardiovascular System**
**Andreas Papapetropoulos,** National and Kapodistrian University of Athens, Greece

**Cofilin1 Oxidation Links Oxidative Distress to Mitochondrial Demise and Neuronal Cell Death**
**Cartsen Culmsee,** Center for Mind, Brain and Behavior, Germany

**Redox-Modulating Agents in the Treatment of Viral Infections**
**Lucia Nencioni,** "Sapienza" University of Rome, Italy

**H2S Targets Mitochondrial Bioenergetics and Induces Metabolic Remodeling**
**Ruma Banerjee,** University of Michigan Medical School, USA

**Determinants of Telomere Length Across Human Tissues**
**Brandon L. Pierce,** The University of Chicago, USA

**Impact of dietary fructose on Brain Mitochondria and Oxidative Stress**
**Luisa Cigliano,** University of Naples Federico II, Italy

**Redox Regulation in Acetaminophen-Induced Acute Liver Damage**
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**Lutz Schomburg,** Institut für Exper. Endokrinologie, Germany

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**Akhilesh B. Reddy,** University of Pennsylvania, USA

**Potential Roles of Redox Dysregulation in the Development of Schizophrenia**
**Diana O. Perkins,** University of North Carolina, USA

**Targeting transcription factor NRF2 in Neurodegenerative Diseases**
**Antonio Cuadrado,** Universidad Autonoma de Madrid, Spain

**Red Blood Cells as a "Central Hub" for Sulfide Bioactivity: Scavenging, Metabolism, Transport, and Cross-Talk with Nitric Oxide**
**Miriam Margherita Cortese-Krott,** Heinrich Heine University Düsseldorf, Germany

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INTERNATIONAL SOCIETY OF ANTIOXIDANTS

# MAINTENANCE OF ER HOMEOSTASIS THROUGH DISULFIDE REDUCTASE

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Secretory and membrane proteins are folded in the ER and only correctly folded proteins are secreted through the Golgi apparatus. Although the misfolded proteins are retained in the ER for the further attempt of refolding by the aid of molecular chaperones, terminally misfolded proteins are discarded by a process known as ER-associated degradation (ERAD). We reported that disulfide reductase ERdj5 facilitates the ERAD through the reductase activity (Ushioda et al., ***Science*** 2008, Hagiwara et al., ***Mol. Cell*** 2011). These finding strongly suggested that Redox regulation by ERdj5 maintains Protein Quality Control in the ER. Recently, we showed the new role of ERdj5 as disulfide reductase in ER homeostasis. ERdj5 interacts with and promotes the activity of SERCA2b, a $Ca^{2+}$ influx pump on ER membrane (Ushioda et al., ***PNAS*** 2016). Thus, we proposed that protein, redox and calcium homeostasis in the ER have close cross-talks each other and ERdj5 has a crucial role in the maintenance of homeostasis in the ER. However, it is still unknown how ERdj5 acquires the electrons for reductase activity in the oxidative ER lumen.

# THE REACTIVE SPECIES INTERACTOME: WHAT'S NEW ?

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Why only studying one reactive species in your favorite field?
Why will happen if you look at other reactive species?
What will happen if you decide to include other reactive species?

From the concept of "stress" to oxidative stress, oxidative distress and eustress, and the reactive species Interactome, here will be highlighted the key points that ought to be considered. A united view linking ROS (oxygen species), RNS (nitrogen species), RSS (sulfur species), RCS (carbonyl species), and notable enzymes is presented. This overview is part of a review we have published in 2021, about the reactive species interactome (Malard et al., 2021).
Our united view aim to improve the understanding of the reactive species from identification to concentration and interactions, in physiology and pathology. A united view as an important landmark for redox evaluation, redox status, and redox medicine.

*References :*
*Malard E, Valable S, Bernaudin M, Pérès E, Chatre L. 2021. The Reactive Species Interactome in the brain. Antioxidants & Redox Signaling. doi: 10.1089/ars.2020.8238. Online ahead of print.*

# H2S TARGETS MITOCHONDRIAL BIOENERGETICS AND INDUCES METABOLIC REMODELING

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Toxic at high levels, H2S modulates a range of physiological processes. Biogenesis of H2S piggybacks on the transsulfuration pathway enzymes, while its clearance is catalyzed by a dedicated sulfide oxidation pathway housed in the mitochondrion.

Our studies are revealing that H2S signals by targeting mitochondrial bioenergetics, which fans out to other compartments via redox metabolite signaling. New developments in the H2S oxidation pathway and signaling via redox remodeling will be discussed.

# SKELETAL MUSCLE REDOX SIGNALING IN RHEUMATOID ARTHRITIS

Estela Santos-Alves ESTELA [1] , Carlos U MORADO [2] , Maarten M STEINZ [1] , Theresa MADER [1] , Baptiste JUDE [1] , Liu ZHENGYE [1] , Camilla I SVENSSON [2] , Eric Rullman[3] and **Johanna T LANNER** [1]

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Rheumatoid arthritis (RA) is a chronic inflammatory disorder that is characterized by synovial inflammation and pain. In addition, skeletal muscle dysfunction and weakness are common comorbidities that contributes to inability to work and reduced quality of life of patients afflicted with RA. Although, disease-modifying anti-rheumatic drugs (DMARDs) are efficient in retaining patients in a low state of disease activity, our and others previous studies have shown that they fail to improve body composition, muscle force and physical function. Loss in muscle mass cannot alone account for the muscle weakness induced by RA, but instead intramuscular dysfunction appears as a critical factor underlying the decreased force generating capacity for patients afflicted by arthritis. We recently showed that oxidative stress and associated oxidative post-translational modifications contribute to RA-induced muscle weakness in animal models of arthritis and patients with RA. Specifically we identified a subset of 3-nitrotyrosine (3-NT) and malondialdehyde adducts (MDA) on actin that reduced the contractile protein's capacity to contribute to force production1. However, it is still unclear how and which sources of reactive oxygen and nitrogen species (ROS/RNS) that are involved in the oxidative stress that drives the progression toward decreased muscle function in RA. Mitochondria are a known producer of ROS and essential for cellular energy synthesis and physical capacity. Analyses of our recent RNA-seq data of muscle (n=11 per group) indicated a reduced mitochondria health in patients with RA. To elucidate the role of mitochondria in RA-induced muscle weakness, we evoked arthritis by injecting complete Freud's adjuvant (CFA) in the knee or ankle joints of C57BL/6JRj and knock-in hNGFR100E mice. hNGFR100E mice have altered pain sensitivity, reducing the influence of pain-induced changes in locomotor activity and gait. Leg muscles from the leg afflicted with CFA resulted in reduced expression of mitochondria- and ROS-scavenger-related genes, which was present independent of locomotor activity and gait changes. These alterations were accompanied by ~20% lower mitochondrial density and size in skeletal muscle from CFA as compared with control animals. In conclusion, our data show that mitochondrial health is compromised in muscles from subjects afflicted with arthritis, which will be discussed in more detail in my talk.

*Reference:*
*1. Steinz, M.M., et al. Oxidative hotspots on actin promote skeletal muscle weakness in rheumatoid arthritis. JCI insight 5(2019).*

# RED BLOOD CELLS AS A "CENTRAL HUB" FOR SULFIDE BIOACTIVITY: SCAVENGING, METABOLISM, TRANSPORT, AND CROSS-TALK WITH NITRIC OXIDE

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Sulfide was revealed to be an endogenous signaling molecule regulating a plethora of cellular functions. It is involved in the regulation of fundamental processes, including blood pressure regulation, suspended animation, and metabolic activity of mitochondria, pain, and inflammation. The underlying biochemical pathways and pharmacological targets are still largely unidentified. Red blood cells (RBCs) are known as oxygen transporters and were proposed to contribute to cardiovascular homeostasis by regulating nitric oxide (NO) metabolism, also via interaction of hemoglobin with nitrite and NO itself. Interestingly, recent evidence indicates that RBCs may also play a central role in systemic sulfide metabolism and homeostasis, and, potentially, in the crosstalk with NO. Heme-containing proteins such as hemoglobin were shown to be targeted by both NO and sulfide. The talk aim at revising and discussing the potential impact of RBCs on systemic sulfide metabolism in the cardiovascular system.

Although the synthetic pathways and the reactivity of hemoglobin and other heme proteins with sulfide and NO are known, the in vivo role of RBCs in sulfide metabolism, physiology, pharmacology, and its pathophysiological implications have not been characterized so far.

To allow a better understanding of the role of RBCs in systemic sulfide metabolism and its cross-talk with NO, basic and translational science studies should be focused on dissecting the enzymatic and nonenzymatic sulfur metabolic pathways in RBCs in vivo and their impact on the cardiovascular system in animal models and clinical settings.

# PHYSIOLOGICAL ROLES OF 3-MERCAPTOPYRUVATE SULFURTRANSFERASE IN THE CARDIOVASCULAR SYSTEM

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Hydrogen sulfide is an important signaling molecule in the mammalian cells. H2S is enzymatically generated by 3-mercaptopyruvate sulfurtransferase (3MST), as well as by the transsulfuration pathway enzymes cystathionine γ-lyase (CSE) and cystathionine β-synthase (CBS). CSE, CBS and 3MST exhibit differences in their active site structure, cofactor and substrate preference, catalytic mechanism, mode of regulation, tissue and subcellular localization, and serve distinct biological roles in the cardiovascular and nervous systems. 3MST uses 3-mercaptopyruvate (3MP) as substrate to produce H2S, persulfides and polysulfides. 3MST generates H2S and pyruvate in a two-step reaction i.e., it transfers sulfur from 3MP to a cysteine 247 in its active site, yielding a 3MST persulfide and pyruvate. The persulfide then releases H2S after reacting with thiols or reduced thioredoxin or can be transferred to thiol protein or to small molecules. 3MST is expressed in vascular cells and cardiomyocytes and has been confirmed to be present in the heart, as well as in many blood vessels, but its function in the cardiovascular system remains enigmatic. During this presentation, the role of 3MST in vascular function, cardio protection and angiogenesis will be discussed.

*The research work was supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "First Call for H.F.R.I. Research Projects to support Faculty members and Researchers and the procurement of high-cost research equipment grant" (Project number: HFRI-FM17-886)*

# REDOX REGULATION IN ACETAMINOPHEN-INDUCED ACUTE LIVER DAMAGE

**Jun LU**[1], LI, Jing[1], Xin WEN[1], Xiaoyuan REN[2], Charareh POURZAND[3], Matthew D LLOYD[3], Arne HOLMGREN[2]

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Acetaminophen (APAP) is widely used for antipyretic and analgesic effects worldwide. However, its hepatotoxicity caused by intentional or unintentional overdose largely threatens people's health, even life.

We have found that APAP treatment affected mouse liver selenoprotein thioredoxin reductase (TrxR) activity and glutathione level in a dose- and time-dependent manner. Decrease of mouse liver TrxR activity and glutathione level was an early event, occurred concurrently with liver damage. The decreases of ratio of GSH/GSSG, TrxR activity and the increase of protein S-glutathionylation were correlated with the APAP-induced hepatotoxicity. In APAP-treated mice both mild deprivation or excess supplementation with selenium increased the severity of liver injury compared to that observed in mice with normal dietary selenium levels. In addition, knockout of *Glrx2*, encoding glutaredoxin 2 (Grx2), sensitized mice to hepatotoxicity induced by APAP. *Glrx2* depletion hindered GSH recovery after acetaminophen challenge, caused decreased GSH/GSSG ratio and subsequent protein thiols modification by GSH affecting the function of glutathionylated proteins. *Glrx2* knockout increased acetaminophen-induced hepatotoxicity by disrupting Trx and Grx system regulated redox microenvironment.

These findings indicated the critical role of Trx and GSH-Grx system in defense against acetaminophen caused liver injury and provided potential therapy target for acetaminophen poisoning.

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*2. Lu J, Holmgren A. The thioredoxin antioxidant system. Free Radical Biology and Medicine 66: 75-87, 2014*
*3. Hendrickson RG. What is the most appropriate dose of N-acetylcysteine after massive acetaminophen overdose? Clinical Toxicology 57: 686-691, 2019.*
*4. Jan Y-H, Heck DE, Dragomir A-C, Gardner CR, Laskin DL, Laskin JD. Acetaminophen Reactive Intermediates Target Hepatic Thioredoxin Reductase. Chemical Research in Toxicology 27: 882-894, 2014.*

# TARGETING TRANSCRIPTION FACTOR NRF2 IN NEURODEGENERATIVE DISEASES

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There is an urgent need to find a neuroprotective therapy for Alzheimer's disease. We are studying the relevance of homeostatic deviations that result from loss of activity of transcription factor NRF2, a crucial regulator of multiple stress responses. We have generated a mouse model that combines amyloidopathy and tauopathy with either wild type (AT-NRF2-WT) or NRF2 deficiency (AT-NRF2-KO). AT-NRF2-KO mice exhibit exacerbated deficits in spatial learning and memory and increased markers of proteinopathy, oxidative stress and neuroinflammation compared to AT-NRF2-WT-mice. The NRF2 activator dimethyl fumarate (DMF), which is the only approved drug targeting NRF2, and is being used for the treatment of remitting relapsing multiple sclerosis reduced glial and inflammatory markers and improved cognition and motor complications in the ATNRF2WT mice.

We are also analyzing redox and inflammatory biomarkers in blood of AD patients. We have analyzed the expression of 168 genes related to oxidative stress and inflammation in peripheral blood in a cohort of 40 non-demented controls vs. 40 matched early ADs.

We found a positive correlation between several NRF2 regulated and several NFκB regulated genes. This study demonstrates the relevance of normal homeostatic responses that decline with ageing, such as NRF2 activity, in the protection and biomarker monitoring of AD.

# POTENTIAL ROLES OF REDOX DYSREGULATION IN THE DEVELOPMENT OF SCHIZOPHRENIA

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Schizophrenia typically emerges in late adolescence and early adulthood with a lifetime prevalence of about 1%. Schizophrenia is typically chronic, disabling. Antipsychotic medications control psychosis symptoms but do not address other symptom domains such as impairments in cognition, motivation, and emotional responsivity.

In this presentation I will discuss converging evidence implicating redox dysregulation as a pathological mechanism driving the emergence of schizophrenia.

Schizophrenia is associated with biomarkers of oxidative stress, including elevations of oxidatively damaged lipids and proteins, mitochondrial abnormalities, and reductions in brain glutathione levels. Furthermore, major neuropathological findings associated with schizophrenia, especially impairments in GABA interneuron function, result from redox-related mechanisms.

In addition, redox dysregulation is a shared feature of divergent rodent schizophrenia models. Redox dysregulation in hypothesized to be an initial driver of schizophrenia neuropathology.

Thus, experimental medicine studies designed to test the hypothesized role of redox dysregulation in schizophrenia etiopathology may be most fruitful when focused on interventions at the prodromal and early stages of schizophrenia.

# IMPACT OF DIETARY FRUCTOSE ON BRAIN MITOCHONDRIA AND OXIDATIVE STRESS

**Luisa CIGLIANO[1],** Arianna MAZZOLI [1], Martina NAZZARO [1], Maria Stefania SPAGNUOLO [2], Cristina GATTO[1], Daniela Claudia MARESCA [1], Susanna IOSSA[1]

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**Introduction:** Youth is characterized by high consumption of processed foods and soft drinks rich in high-fructose corn syrup that can promote overweight and brain dysfunction. Our aim was to clarify brain alterations resulting from fructose consumption in juvenile age and whether these alterations can be rescued or persist after removing fructose from the diet.

**Material & Methods:** Young rats were first fed a fructose-rich diet for 3 weeks and then, to study the persistence or reversibility of sugar-induced brain changes, returned to control diet for further 3 weeks. Mitochondrial bioenergetics and oxidative status were evaluated in the hippocampus and frontal cortex, brain areas involved in learning and memory. Glucose transporter-5, fructose and uric acid levels, inflammation, as well as neurotrophins and markers of synaptic function were investigated.

**Results:** The fructose-rich diet induced mitochondrial dysfunction and oxidative stress, associated with inflammation and decreased markers of brain function. While most of the hippocampal changes were recovered by returning to a control diet, several alterations persisted in the frontal cortex.

**Conclusion:** These results highlight the risk arising from the increasing sugar consumption that must be strongly discouraged to prevent not only early dysfunction, but also persisting brain damage in the long term.

# COFILIN1 OXIDATION LINKS OXIDATIVE DISTRESS TO MITOCHONDRIAL DEMISE AND NEURONAL CELL DEATH

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Many cell death pathways, including apoptosis, regulated necrosis and ferroptosis are relevant for neuronal cell death and share common mechanisms such as the formation of reactive oxygen species (ROS) and mitochondrial damage. Here, we present the role of the actin-regulating protein cofilin1 in regulating mitochondrial pathways in oxidative neuronal death. Cofilin1 deletion in neuronal HT22 cells exerted increased mitochondrial resilience, assessed by quantification of mitochondrial ROS production, mitochondrial membrane potential and ATP levels. Further, cofilin1 deficient cells met their energy demand through enhanced glycolysis, whereas control cells were metabolically impaired when challenged ferroptosis. Further, cofilin1 was confirmed as a key player in glutamate-mediated excitotoxicity and associated mitochondrial damage in primary cortical neurons. Using isolated mitochondria and recombinant cofilin1, we provide a further link to toxicity-related mitochondrial impairment mediated by oxidized cofilin1. Our data revealed that detrimental impact of cofilin1 on mitochondria depends on oxidation of cysteine residues at positions 139 and 147.

Overall, our findings show that cofilin1 acts as a redox sensor in oxidative cell death pathways of ferroptosis, and also promotes glutamate excitotoxicity. Protective effects by cofilin1 inhibition are particularly attributed to preserved mitochondrial integrity and function. Thus, interfering with the oxidation and pathological activation of cofilin1 may offer an effective therapeutic strategy in neurodegenerative diseases.

# TARGETING THE BACH1-NRF2 AXIS

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**Introduction:** NRF2 is a transcription factor that controls a wide variety of genes encoding for antioxidant, detoxification and anti-inflammatory proteins. Although the main negative regulator of the NRF2 pathway is KEAP1, there are other factors involved. One of those factors is BACH1 (broad complex, tramtrack and bric à brac and cap'n'collar homology 1), a transcription factor that competes with NRF2 for its binding to the promoter of a subset of NRF2 target genes, being the potent antioxidant and anti-inflammatory enzyme HMOX1 the best characterised[1]. While KEAP1 inhibitors induce the expression of numerous cytoprotective genes, BACH1 inhibition will activate only a few, although they are extremely potent at inducing HMOX1. Additionally, BACH1 also activates genes involved in cancer metastasis (in a NRF2-independent manner)[2]. Based on this, BACH1 is a potential target against a variety of conditions linked to oxidative stress and inflammation, and also against cancer metastasis. Despite their therapeutic potential, only a few BACH1 inhibitors have been identified so far, and none has entered the clinical scenario yet.

**Results:** In this talk I will provide new data that challenge the actual NRF2-BACH1 model. I will compare the use of HMOX1 as a reporter for NRF2 activation or as a reporter for BACH1 inhibition and I will explain the cellular model we use to identify BACH1 inhibitors and how to differentiate them from KEAP1 inhibitors. Finally, I will present some recent data on the characterization of novel potent dual KEAP1/BACH1 inhibitors.

*1. J. Sun et al., 'Hemoprotein Bach1 regulates enhancer availability of heme oxygenase-1 gene', EMBO J., vol. 21, no. 19, pp. 5216–5224, Oct. 2002.*
*2. L. Lignitto et al., 'Nrf2 Activation Promotes Lung Cancer Metastasis by Inhibiting the Degradation of Bach1', Cell, vol. 178, no. 2, pp. 316-329.e18, Jul. 2019.*

# OXIDATIVE STATE OF PROTEIN DISULFIDE ISOMERASE REGULATES MITOCHONDRIAL FUNCTION

**Zhi-wei YE** [1], Jie ZHANG [1], Theodore L. MATHURAM [2],
Kennth D. TEW [1], Anna BLUMENTAL-PERRY [2], Danyelle M. TOWNSEND [3]

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**Rationale**: Cigarette Smoke (CS) is a major risk factor for Chronic Obstructive Pulmonary Disease (COPD). We have previously identified protein disulfide isomerase (PDI) as an endoplasmic reticulum resident target of CS-induced oxidation. Oxidized PDI has been demonstrated to persist in exposed cells, and its amounts to increase with the disease progression. This increase correlates with mitochondrial malfunction and apoptosis. To decipher if oxidized PDI contributes to pro-apoptotic redox signaling, we analyzed its function in a cellular exposure system.

**Methods**: MLE12 served as AT2-like cells and isolated mitochondrial from mouse liver were used as models for this study. PDI oxidation was induced by exposure of cigarette smoke extract (CSE). Cytotoxicity of CSE was conducted in PDI wild type and FLFL mutation, the cellular location of PDI were studied by immunofluorescent staining, and mitochondrial membrane potential was monitored by flow cytometry.

**Results**: CSE caused oxidation of PDI, especially S-glutathionylation. FLFL mutation significantly reduced the s-glutathionylation of PDI by CSE. We demonstrate that oxidized PDI comes in close proximity to mitochondria, where it causes cytochrome C release from mitochondrial, which in turn leads to caspase-3 mediated apoptosis. Rescue of mitochondrial function can be achieved in cells exposed to CS, transfected by PDI FLFL. PDI transfected cells showed more sensitive to CSE while FLFL mutation caused a similar effect to control.

**Conclusion**: CSE caused ER stress induces PDI oxidation, thus translates to mitochondrial, oxidized PDI induce cytochrome C release from mitochondrial to cytosol and cell death.

*Supported by NCRR P20RR024485 - COBRE in Oxidants, Redox Balance and Stress Signaling*

*References:*
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# MITOCHONDRIAL DYSFUNCTION IN A DROSOPHILA MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Proper neural function is essential for establishing neuronal structure, synapses, and the circuitry of the network as a whole. Deterioration of neural function is a hallmark of neurodegenerative diseases. One such disease, Amyotrophic Lateral Sclerosis (ALS) is a debilitating disorder stemming from a loss of neuron activity. Mutations in one of the many genes implicated in ALS, superoxide dismutase 1 (SOD1), have been shown to lead to aggregation of misfolded protein, cellular and molecular quality control errors, impaired axonal transport, mitochondrial dysfunction, and many other cellular pathologies. Mitochondrial damage has been noted in an array of age-related diseases and is commonly observed in cases of ALS. Here, we focus on investigating mitochondrial defects in a mutant dSod1G85R Drosophila ALS model.

Our results show that the dSod1G85R larvae have mislocalization of mitochondria, increased mitophagy, and changes in redox couples at different compartments of sensory neurons. We also observe a decrease in the number of mobile mitochondria in the axons of these neurons. Future investigation of mitochondrial dysfunction in the model and the ability of genetic suppressors to rescue ALS-phenotypes can help elucidate the mechanisms of disease pathogenesis and may be a step towards developing =therapeutics for battling neurodegenerative diseases.

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# MITOCHONDRIAL CONTACT SITE AND CRISTAE ORGANIZING SYSTEM IS IMPORTANT FOR THE REGULATION OF HEME SYNTHESIS AND REDOX CONTROL

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**Introduction**: Heme is an essential but inherently toxic redox cofactor required for a plethora of cellular processes in eukaryotes. The heme biosynthetic pathway is partitioned between the cytosol and mitochondria, with the first and final steps taking place in the latter. Although the pathway has been extensively studied, our understanding of how the relative rates of heme synthesis are regulated and which factors influence this process is limited.

**Materials & Methods**: We used quantitative proteomic, biochemical and functional genetic analyses to investigate molecular organization and catalytic features of the terminal heme biosynthetic enzyme, ferrochelatase (Hem15) in yeast genetic models.

**Results**: Our study reveal a dynamic association between Hem15 and the mitochondrial contact site and cristae organizing system (MICOS). MICOS deficiency negatively impacts Hem15 activity and results in accumulation of toxic heme precursors, manifesting in oxidative damage. Restoring intermembrane connectivity in MICOS-deficient cells using an engineered tether protein mitigates these toxic effects.

**Conclusion**: Our data provide new insights into how heme biosynthetic machinery is organized, linking mitochondrial architecture-organizing factors to heme and redox homeostasis. We posit that MICOS-shaped membrane architecture is critical for supplying substrates/products in heme biosynthesis, and - if compromised - creates higher oxidative burden upon heme synthesis amplification.

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# ORGANISMAL AND CELLULAR STRESS RESPONSES TO DISRUPTION OF MITOCHONDRIAL LONP1 PROTEASE

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**Introduction:** Mitochondrial LonP1 protease is an essential component of protein quality control mechanisms in all species. Beyond maintaining mitochondrial proteostasis in cells, LonP1 is implicated in several regulatory processes, whereas its inhibition leads to cellular senescence[1]. LonP1 is also upregulated in a variety of human tumors and therefore represents a potential target for cancer therapy[2]. However, the role of LonP1 in organismal stress response and aging or the signaling cascades induced in response to LonP1 deficiency in an organism and within cancer cells has not been established so far.

**Materiel & Methods:** Using genetic and pharmacological inhibition approaches against LonP1 in the nematode *Caenorhabditis elegans* and in human cancer cells, we analyzed the expression of a number of genes involved in stress-responsive signaling pathways, and we performed survival assays under different environmental insults.

**Results:** Loss of LonP1 reduced lifespan, increased ROS accumulation and induced mitochondrial dysfunction in *C. elegans*. Accordingly, a retrograde transcriptional response that involves both the mitochondrial and cytoplasmic unfolded protein response pathways are activated in both experimental systems, with distinct consequences on stress response.

**Conclusion:** In the cells, LonP1 elicits adaptive cytoprotective mechanisms that can inhibit cancer cell survival but diversely modulate organismal stress response and aging.

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# RESTORING OF REDOX HOMEOSTASIS, METABOLIC ADAPTION, AND LONGEVITY: IMPLICATIONS FOR UNDERSTANDING THE EFFECTS OF CIMICIFUGA RACEMOSA EXTRACT ZE 450 ON CELLULAR RESILIENCE TO OXIDATIVE STRESS

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Climacteric complaints are caused by hormonal changes in the female body.
Extracts prepared from the rhizome of Cimicifuga racemosa have been shown to effectively treat menopausal complaints such as hot flushes. Recent studies revealed Cimicifuga racemosa extract Ze 450 also plays a role in regulating energy metabolism [1]; among others by the activation of AMPK a key modulator of fatty acid and glucose metabolism. In particular, Ze 450 exerted a metabolic shift from mitochondrial oxidative phosphorylation to glycolysis and prevented neuronal cells from oxidative damage [2].

The aim of this study was to investigate the molecular mechanisms underlying the metabolic role of mitochondria under conditions of oxidative stress. We investigated whether Ze 450 targets the respiratory chain function of isolated cortical mitochondria directly, thereby increasing resilience of neuronal cells against oxidative cell death, induced by erastin in vitro, and in the nematode C. elegans exposed to mitochondrial poisons in vivo. The effects of Ze 450 on mitochondrial respiration and its protection against oxidative cell death were further compared to metformin and estrogen receptor stimulation. High-resolution respirometry and extracellular flux analysis revealed that Ze 450 mediated a direct effect on mitochondria by enhancing the metabolic shift towards glycolysis, and this was associated with cMyc and HIF1α regulation. These effects of Ze 450 were mediated independently of estrogen receptor activation and distinct from effects exerted by metformin. Furthermore, Ze 450 increased survival of C. elegans challenged with mitochondrial toxins.

**These findings** shed light on metabolic mechanisms promoted through a metabolic shift to glycolysis via direct effects on mitochondria, which enhances neuronal resilience against ferroptosis in vitro and promotes longevity in vivo.

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# DIABETES-MEDIATED IL-17A-IL-17R SIGNALING IN PHOTORECEPTORS ENHANCE OXIDATIVE STRESS AND THE ONSET OF DIABETIC RETINOPATHY

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**Introduction**: Diabetes-induced inflammation elicits oxidative stress and vascular leakage in the retina, which leads to the onset of diabetic retinopathy [1]. Diabetic retinopathy is a microvascular disease of the retina and the leading causes of blindness in the working-age population worldwide [2]. Previous studies have established a role for photoreceptors in retinal inflammation and oxidative stress [3]. Here we identified constitutive expression of the IL-17A receptor (IL-17R) and the Act1 adaptor molecule on photoreceptors, which enhance reactive oxygen species (ROS) production.

**Materials & Methods**: Oxidative stress and retinal pathology were examined in Streptozotocin (STZ)-induced diabetic C57BL/6, IL17A-/-, and IL17RC-/- mice 2- and 8-months after diabetes was confirmed. While mechanistic studies were performed in primary murine photoreceptor cells and a photoreceptor cell line.

**Results**: In vivo, retinal inflammation and ROS production was ablated in diabetic IL-17A-/- and IL-17RC-/- mice. Ex vivo, IL-17A enhanced ROS production, which was significantly decreased in siAct1 and siTRAF4 photoreceptor cells. Retinal endothelial cell death and vascular impairment was halted in the absence of IL-17A, IL-17R, Act1, TRAF4, and ROS.

**Conclusion**: Diabetes-mediated IL-17A induce photoreceptors to produce ROS in an IL-17R-Act1-TRAF4-dependent manner, which leads to retinal endothelial cell death, capillary degeneration, and the onset of diabetic retinopathy.

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# IMPACT OF REPETITIVE GAS PLASMA STRESS ON HEAD AND NECK CANCER CELLS

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**Introduction**: To overcome the resistance development and resulting tumor relapse as the currently biggest problem in cancer therapy, we studied the adaptive responses of head and neck tumor cells upon repeated exposure to medical gas plasma-induced oxidative stress. Identification of adaptation-associated genes could abet to improve the efficacy and outcomes of different tumor therapeutic treatments.

**Material & Methods**: A novel in vitro cell culture model was established, wherefore malignant cells were exposed to gas plasma once per week over two months. Flow cytometry, colorimetric assays, confocal laser scanning microscopy and microarray analysis allowed the identification of cell physiological and molecular changes as well as adaptation-relevant signalling pathways over time.

**Results**: After a short period of increased plasma sensitivity, cancer cells adapted to the repetitive stress and showed augmented susceptibility accompanied by an altered cellular phenotype. Additionally, we compared the plasma response of the adapted resistant phenotype to the wild type in vivo and evaluated their resistance characteristics.

**Conclusion**: The initial good response of the cancer cells upon the repetitive plasma exposure was subsequently followed by resistance development. Genes that correlated with this adaptation process are involved in pro-angiogenic and pro-tumorigenic pathways. Further investigations are needed to validate the vital role of potential target genes.

# FERROPTOSIS AMONG CANCER CELL DEATH MODALITIES INDUCED BY PHOTODYNAMIC TREATMENT

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Photodynamic treatment (PDT) can trigger various cell death modalities depending on metabolic peculiarities of cells, subcellular localization of photosensitizer (PS), and intensity of PDT. Since cancer cells often acquire resistance to apoptosis, the PDT ability to induce alternative cell death modalities, such as ferroptosis, is of high interest.

In the present work we tested the peculiarities of oxidative stress and death induced in cells of several cancer lines by hydrophilic and lipophilic tetrapyrrole PSs.

Using genetically encoded H2O2-sensitive sensor, we showed that PDT induces massive secondary production of peroxide which starts after the irradiation is completed. In case of lysosome-localized hydrophilic Photosens, cytoplasmic accumulation of peroxide precedes that in mitochondria [1]. Cell death with this PS is accompanied with activation of lipid peroxidation superior to that of other treatment options. Protective action of inhibitors of both apoptosis and ferroptosis [2], and presence of cells died with or without caspase proteolytic activity support our hypothesis that Photosens but not membranotropic PSs can induce mixed type cell death with features of apoptosis and ferroptosis.

We believe that ferroptosis induction in PDT is a powerful alternative strategy for increasing cancer therapy efficiency [3], especially given the immunogenic nature of ferroptosis [4].

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# THE ROLE OF CRISPR/CAS9-MEDIATED DRP1 KNOCKOUT IN FERROPTOSIS

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Mitochondria are highly dynamic organelles, undergoing constant fission and fusion in order to maintain mitochondrial integrity and function. Neurodegenerative diseases have been attributed to impaired mitochondrial dynamics in numerous studies [1]. In conditions of oxidative stress, the activation of GTPase Dynamin-related Protein 1 (Drp1) leads to excessive mitochondrial fragmentation [2]. Pharmacological inhibition of Drp1 preserved mitochondrial morphology and function, leading to enhanced cellular resilience against oxidative stress [2]. Recent studies indicate an emerging role of mitochondria in ferroptosis through the modulation of iron, Glutathione Peroxidase 4 (GPX4) and lipid peroxidation [3, 4], however, the role of Drp1 in ferroptosis has not been clarified.

In this study, we investigated the effect of Drp1 knockout in neuronal HT22 cells challenged with ferroptosis inducers by inhibition of the Xc-Antiporter with erastin as well as inhibition of GPX4 with RSL3. Fluorescence-activated cell sorting (FACS) measurements were conducted after respective cell labelling to detect cell death (Annexin V/Propidium iodide), mitochondrial membrane potential (TMRE) and mitochondrial superoxide formation (MitoSox). Mitochondrial morphology was assessed by fluorescent microscopy and ImageJ software after staining the cells with MitoTracker Deep Red, and Seahorse XF Analyzer was used for detecting mitochondrial respiration and glycolytic activity.
Stable knockout of Drp1 was achieved in immortalized mouse hippocampal HT22 cells using CRISPR/Cas9 technology and Western blot analysis verified complete decline of Drp1 protein levels in different Drp1 KO colonies. Analysis of mitochondrial morphology revealed significantly enlarged mitochondria in Drp1 KO cell lines in comparison to wildtype HT22 cells under standard culture conditions, confirming reduced mitochondrial fission. Further, Drp1 KO significantly abolished cell death as well as loss of mitochondrial membrane potential induced by erastin or RSL3. Mitochondrial ROS formation and mitochondrial respiration were preserved in Drp1 KO cells despite incubation with erastin. In comparison, the effects of Drp1 KO in preserving mitochondrial ROS formation and mitochondrial respiration were less pronounced in RSL3-mediated ferroptosis.

The findings from this study suggest that excessive mitochondrial fission mediated by Drp1 is a key process to execute cell death in ferroptosis. This process can be impeded by the knockout of Drp1. Further investigation is needed to elucidate the detrimental impact of Drp1 in mitochondrial pathways of ferroptosis as well as mechanisms underlying cellular resilience against ferroptosis in the Drp1 KO cell lines.

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# DEFEAT CANCER WITH OXIDATIVE STRESS GENERATED BY PHOTODYNAMIC THERAPY

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**Introduction**: Intense oxidation at tumor sites might help to defeat cancer. A most convenient way to produce localized oxidative stress is to use photodynamic therapy (PDT). It combines light and a pro-drug, which in the presence of oxygen, generates ROS. The generated oxidative stress exerts cytotoxic effects on the tumor microenvironment along with systemic anti-tumor immunity1. Major advances in the field have been achieved with redaporfin, a bacteriochlorin with intense absorption in the NIR and immunostimulatory properties.

**Material & Methods**: Mice bearing tumors were submitted to redaporfin-PDT. Cured mice were rechallenged with live cancer cells and blood samples were analysed regarding different immune populations. The main hallmarks of immunogenic cell death (ICD) were assessed by standard techniques and vaccination experiments based on PDT-treated cancer cells were conducted.

**Results**: Redaporfin-PDT mediated high cure rates in different mouse models of cancer with abscopal inhibition of metastases. The weakened anti-cancer effects observed in immunodeficient mice, or when CD8+T cells were depleted, confirmed the immunostimulatory properties of redaporfin-PDT. Cancer cells treated with redaporfin-PDT exhibit the main IDC hallmarks along with an effective vaccination outcome2-4.

**Conclusion**: Redaporfin-PDT is a promising treatment for cancer. It is in phase I/II clinical trials for head and neck cancer with promising results including when used with immunotherapy5.

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# DISSECTING NRF2 RESPONSES TO ENDOGENOUS AND EXOGENOUS OXIDATIVE STRESS USING REPORTER MICE

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NRF2 signaling has been implicated in a myriad of physiological (e.g. cell fate and determination) and pathological (e.g. cancer, neurodegeneration, environmental exposure) processes. However, how to measure the dyamics of these responses in vivo and at cellular resolution remains a major challenge. We have exploited a Hmox1 reporter line that makes it possible to obtain this information in mice to study the role of NRF2 responses in human diseases (e.g. accelerated ageing) and toxicology (e.g. environmental pollutants). Using a T2A technology, we expressed multiple complementary reporter molecules from the murine Hmox1 locus, including firefly luciferase, to allow long-term, non-invasive imaging of Hmox1 expression, and β-galactosidase for high-resolution mapping of expression patterns post-mortem. Further backcrossing of Hmox1 reporter mice with NRF2-null mice allowed us to study the genetics of NRF2-dependent responses in vivo. We also validated these models pharmacologically by exposure to NRF2 activating agents (e.g. TBE-31) or enviromental toxicants (e.g. inorganic arsenic, diesel exhaust components) in the presence or absence of candidate drugs (e.g. anti-inflammatory agents, antioxidants). We further used these reporters as biomakers of pathogenesis progression in human disease models where oxidative stress and NRF2 responses play an importan role (i.e. Progeria).
In summary, we propose the broad application of these models to study the role of oxidative stress in the pathogenesis of disease and its prevention.

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# OVEREXPRESSION OF SKELETAL MUSCLE NRF2 PROTECTS AGAINST AGING-ASSOCIATED DYSFUNCTION IN SKELETAL MUSCLE AND HEART

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**Introduction**: Nrf2 is a master transcription factor governing hundreds of genes involved in anti-oxidation, anti-inflammation, detoxification, and metabolism1. Skeletal muscle (SkM) deletion of Keap1 upregulates over 100 cytoprotective proteins and enhances exercise capacity in adult mice2. We hypothesize that Keap1 KO protects against aging-associated myopathy in SkM and in the myocardium.

**Materiel & Methods**: Experiments were carried out in 43 male SkM-Keap1flox/flox mice assigned to 4 groups: young WT (11), young Keap1 KO (12), aging WT (11), and aging Keap1 KO (9). At 5 months in young mice and 25 months in aged mice, a treadmill running test was done and echocardiography to evaluate myocardial function.

**Results**: Aging WT displayed significantly shorter running distance than young WT, which was improved in aging KO mice. Aging WT exhibited lower ejection fraction (EF), longer isovolumic relaxation time (IVRT), and higher myocardial performance index (MPI) than young WT. Aging-associated cardiac dysfunction was partially alleviated in aging KO.

**Conclusion**: These data suggest that chronic activation of SkM Nrf2 not only attenuates aging-associated SkM dysfunction but also improves cardiac aging parameters. The later effects, we speculate, are mediated by transference of Nrf2-upregulated cytoprotective proteins from the Keap1-deficient SkM to the myocardium through SkM-derived EVs3.

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# CYTOTOXICITY AND OXIDATIVE STRESS OF AMITRAZ AND ITS METABOLITES IN HEPG2 CELLS

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**Introduction**: Bee products,as honey, have become popular products due to the health-promoting effects. Amitraz is an acaricide widely used to protect honeybee against Varroa destructor mites. Amitraz is hydrolyzed to N-(2,4-dimethylphenyl)formamide (DMF) and 2,4-dimethylaniline (DMA). Amitraz can produce subchronic effects that are difficult to quantify.

**Material & Methods**: Amitraz, DMF and DMA were exposed to HepG2 cells to determine the cytotoxic effect by the MTT and PC assays. Oxidation mechanisms, such as lipid peroxidation (LPO) by TBARS method and reactive oxygen species (ROS) by fluorescein probe, were evaluated. Finally, a screening of toxicants in honey based on liquid chromatography/quadrupole time-of-flight mass-spectrometry (LC/Q-TOF-MS) was carried out.

**Results**: Amitraz and its metabolites showed a concentration-dependent reduction in HepG2 cell viability by both assays. Amitraz demonstrated higher cytotoxic than its metabolites. Amitraz increased the production of MDA at all concentrations tested, whilst DMF and DMA only increased MDA production at the highest ones. DMF and DMA showed the highest ROS production after 750 µM exposure until 3.27 and 2.3 folds, respectively. However, amitraz did not show significant increase of ROS production in HepG2 cells.

**Conclusion**: ROS and LPO induced by amitraz and its metabolites are mechanisms implicated in HepG2 cell damage. Acknowledgement: PID2020-115871RB-I00.

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# OXIDATIVE STRESS

# IN MENOPAUSAL WOMEN WITH INSOMNIA

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**Introduction**: 16–42% premenopausal and 35−60% postmenopausal women have a sleep disorders [1]. It is highly possible, that main consequences of sleep loss may be oxidative stress [2-4]. The aim of this research is an assessment of oxidative stress parameters levels in peri- and postmenopausal women with insomnia.

**Materials and Methods**: 158 menopausal women divided into perimenopausal (n=57, mean age – 49.24±2.84 years) and postmenopausal (n=101, mean age – 56.25±4.91 years) groups were examined. Each group was divided into control (without insomnia) and main group (with insomnia). The program of the study included the following methods: questionnaire, general medical examination, gynecological examination, spectrophotometer, enzyme immunoassay and statistical. Exclusion criteria: artificial menopause; hormone replacement therapy; diabetes; cancer; exacerbation of chronic diseases. Advanced oxidation protein products (AOPP), thiobarbituric acid reactants (TBARs), 8-hydroxy-2'-deoxyguanosine (8-OHdG) and advanced glycation end products (AGEs) levels were determined in plasma and serum blood.

**Results**: In perimenopause AOPP, 8-OHdG and AGEs levels were higher in women with insomnia as compared to control ($p<0.05$). Postmenopausal women with insomnia had a higher TBARs and 8-OHdG levels as compared to control ($p<0.05$). Moreover, insomniac postmenopausal patients had a higher AOPP and 8-OHdG levels and a lower TBARs and AGEs levels as compared to perimenopausal ones ($p<0.05$).

**Conclusion**: Our results indicate the development of oxidative stress in menopausal women with insomnia.

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# OXIDATIVE STRESS MARKERS: NEW PROSPECTIVE IN INFLAMMATORY BOWEL DISEASES

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The OxIBDiet project (NCT04513015) investigates the pathogenetic mechanisms of inflammatory bowel disease (IBD) in children and adults with 2 aims: 1. to characterize the oxidative status of IBD subjects and 2. to evaluate the effects of a dietary treatment high in antioxidants.

Forty children (age 12 ± 4) and twenty-eight adults (age 33.7 ± 20.4) with IBD and a group of age and gender matched healthy controls have been enrolled so far. Oxidative stress parameters were assessed in blood samples by analysing intracellular ROS levels; total antioxidant capacity and antioxidant enzymes activity (CAT, SOD, GSTs, GPXs, GR).

The results showed a different response between the group of children and adults, indicating a better capacity to compensate a chronic inflammatory state established by the disease in children compared to adults. For example, IBD children showed a significant increase in the enzymatic activity of GPXs and CAT at the intracellular level and a significant increase of SOD in plasma to indicate an active response to counteract a state of oxidative stress that is undetectable in adults. In conclusion, our data show a condition of redox imbalance caused by IBD leading to a more adaptive response in children than in adults suggesting an involvement of oxidative stress in IBD pathogenesis.

*Supported by This research was supported by "Ministero della Salute" grants n RF-2018- 12366976*

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# STUDY OF OXIDATIVE STRESS UNDER THE TRUE OXYGENATION OF THE SKIN, PHYSIOXIA

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Oxygen partial pressure plays a significant role in skin tissue homeostasis. Oxygen is the precursor of reactive oxygen species (ROS) and ROS generation, when exceeding the skin natural antioxidant capacities, induces an oxidative stress described as the main cause of skin photoaging.
Usually, in-vitro assays are performed at atmospheric oxygen levels (~18% O2 in incubators), called normoxia. However, the physiological level in the skin varies from 1.5 to 7%, a condition called physioxia (1, 2). Here we compare the oxidative stress between those two conditions.

To assess oxidative stress, we quantified ROS production, and evaluated the antioxidant capacities (SODs, Catalase and GPX4), both at RNA and protein level, in keratinocytes according to oxygen level.

Our results showed a higher resistance against oxidative stress of keratinocytes grown in physioxia compared to normoxia. The transcriptional profile of antioxidants is different in physioxia and we demonstrated the overexpression of catalase which could be responsible for this resistance.
This is the first study that makes the link between oxygen levels and the status of oxidative stress in skin cells.
**Our results demonstrated** the importance of oxygen level in keratinocytes culture that will allow a better understanding of the physiological mechanisms of the skin

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# REVERTING MITOCHONDRIAL IMPAIRMENT IN SKIN FIBROBLASTS FROM PARKINSON'S DISEASE PATIENTS BY USING A MITOCHONDRIA-TARGETED ANTIOXIDANT ANTIOXCIN4

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**Introduction**: Parkinson's Disease (PD) is the most common neurodegenerative movement disorder [1]. It is currently accepted that mitochondrial dysfunction and oxidative stress have been implicated in the early stages of the disease [1]. Driven by the lack of efficient pharmacological therapies, we hypothesized that a modified dietary-based mitochondriotropic antioxidant therapy could revert PD-associated mitochondrial dysfunction.

**Material & Methods**: Human skin fibroblasts from sporadic PD patients (HFsPD) and their sex- and age-matched controls were treated with a maximal non-lethal concentration of the mitochondria-targeted dietary antioxidant, AntiOxCIN4, during 48 h, and then exposed to a glucose-free/glutamine/pyruvate medium (OXPHOS medium) for more 24 h period. By using standard methods, ROS, mitochondrial function, oxygen consumption and antioxidant defense system-related pathways were analyzed.

**Results**: Our results demonstrated that AntiOxCIN4 decreased oxidative stress in HFsPD to values similar to healthy donors. Moreover, AntiOxCIN4 enhanced cellular responses to oxidative stress by improving the cellular redox state in HFsPD. In addition, HFsPD treated with AntiOxCIN4 displayed increased maximal respiration rate and improved metabolic activity, switching the metabolic phenotype of HFsPD becoming more similar to their sex- and age-matched controls.

**Conclusion**: The data validate the AntiOxCIN4 as a potential drug to normalize metabolic and mitochondrial metabolism in PD.

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# VITAMIN K2: A PROMISING ANTIOXIDATIVE REGIMEN FOR NEURODEGENERATIVE DISEASE PREVENTION

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**Introduction**: Till now, only five drugs have been approved by the FDA, USA for the treatment of Alzheimer's disease. Current studies are being focused on the screening of bioactive compounds for their effect on preventing Aβ formation, oxidative stress reduction and targeting organelle dysfunction to prevent AD in early stages (1,2).

**Materials & Methods**: Human neuroblastoma SH-SY5Y cells were treated with streptozotocin and menadione in a dose-dependent manner, followed by the post-treatment of vitamin K2. Modulating effects of vitamin K2 on cell viability, Lactate Dehydrogenase release, reactive oxygen species (ROS), mitochondrial membrane potential, ER stress marker (CHOP), an indicator of unfolded protein response (UPR) p-IRE1α, GSK3α/β, total tau and Aβ 42 were studied (3).

**Results**: Vitamin K2 significantly reduced neuronal cell death by inhibiting cytotoxicity, ROS levels and retained mitochondrial membrane potential. It significantly decreased the expression of CHOP protein along with the levels and the nuclear localization of p-IRE1 α. In addition, it downregulated the expression of GSK3 α/β and decreased the total tau protein, with a diminutive effect on secreted Aβ 42.

**Conclusion**: Vitamin K2 alleviated mitochondrial damage, ER stress and tauopathy mediated neuronal cell death, highlighting its role as a novel antioxidative therapeutics targeting related cellular processes.

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# DYNAMIC OF TELOMERE LENGTH CHANGE IN TWO GROUPS OF PATIENTS (ACUTE MYOCARDIAL INFARCTION AND HAEMODIALYSIS PATIENTS) AND RELATION WITH REDOX STATUS

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**Introduction:** Different pathological processes induce telomere shortening, which is not irreversible; enzyme telomerase catalyses the reaction of telomere lengthening. Aim of this investigation was to test leukocyte telomeres' length (LTL) change upon two acute event: acute myocardial infarction (AMI) and haemodialysis session (HDS) and also to test its relation with redox status change in patients' blood.

**Material & Methods:** Peripheral blood leukocytes' telomere length (LTL), telomerase activity along with concentrations of selected parameters of redox status (advanced oxidation protein products-AOPP, ischemia modified albumin-IMA, total oxidative status-TOS, malondialdehyde-MDA, total sulphydril groups-SHG, so as superoxide-dismutase-SOD and paraoxonase 1 (PON1)) were measured in two patients' groups: AMI (n=93) and end stage renal disease patients on HD treatment (n=130).

**Results:** LTL of AMI patients after the pPCI procedure were significantly increased (1.16 (0.92-1.35) vs. 1.29 (1.08-1.61), p<0.001), while telomerase activity remain similar at the both study points. LTL in haemodialysis patients before and after HDS slightly increased but without statistical significance: 0.92 (0.70 – 1.14) vs. 0.95 (0.70-1.23), p=0.209. LTL in both patients group were in correlation with several redox status parameters.

**Conclusion**: LTL is dependent on chronic pathological processes, acute disease exacerbation and treatments, and by redox status activation in different pathological conditions.

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# INVESTIGATION OF PREMATURE CELLULAR SENESCENCE IN PRE-ECLAMPSIA AND INTRAUTERINE GROWTH RESTRICTION

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**Aim**: To investigate both the senescence associated secretory phenotype (SASP) in maternal blood and placental markers of cellular senescence in adverse pregnancy outcomes.

**Methods**: Maternal plasma and term placental samples were taken at term gestation from nulliparous women with pre-eclampsia (n=13), intra-uterine growth restriction (n=13) and age-matched healthy pregnancies (n=20). SASP panel of cytokines were evaluated on maternal plasma through multiplex ELISA assay. Placental absolute telomere length and gene analysis was performed by RTqPCR. Statistical analysis was performed using Stata 14.2 and GraphPad Prism 8®.

**Result**: Plasma IL-6 levels in pre-eclampsia were significantly increased when compared to controls (0.54 pg/ml ± 0.271 v 0.3 pg/ml ± 0.102; p=0.017). Adjusting for variables, IUGR was associated with 0.254kbp (95% CI: -4.064, 4.573) longer and pre-eclampsia was associated with 3.246kbp (95% CI: -1.026, 7.519) longer telomere length compared to controls, results were not statistically significant. There was a significant increase in placental gene expression of senescence markers, CHEK1, CCNB1, IGFBP5 and PTEN gene level in pre-eclampsia (p>0.05) when compared to controls and ID1 was significantly reduced (p>0.05) in intra-uterine growth restriction when compared to controls.

**Conclusion**: Elevated maternal IL6 and placental expression of biomarkers of senescence suggest that both altered inflammatory and redox signalling may modulate placental ageing in pre-eclampsia but not intrauterine growth restriction.

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# VALPROIC ACID MODULATES REDOX AND NITRIC OXIDE STATUS IN MOUSE EMBRYOS

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The teratogenic effects exhibited by valproic acid (VPA) at the molecular level are not well known.[1] Previously, it was demonstrated that suppression of nitric oxide (NO) synthesis leads to improvement of the teratogenic effects of VPA, whereas VPA- induced neural tube defects were reduced in a dose-dependent manner due to the increased NO signal by sildenafil induction.[2] In this study, an evaluation was carried out for the first time to assess the No synthesis modulation by VPA in mouse embryos during early organogenesis. On gestation day 8, ICR-CD1 mice were provided 600 mg/kg of VPA. Embryos were collected eight and 24 h later. NO synthase (NOS) isoform expression and the modulation of their molecular mechanisms were analyzed. As the main results, embryonic in utero exposure to VPA governed time-dependent change of NOS isoforms expression, with a decrease in constitutive NOS (cNOS) expression and activity and an increased expression and activity of inducible NOS (iNOS). In VPA- exposed embryos, we observed a redox system imbalance with an increase of tyrosine nitration and S-nitrosylation (biomarkers of NO-mediated post-translational modifications) associated with an endogenous antioxidant system reduced. The data suggest that VPA acts as a NO depleting agent, confirming it as a teratogenic- effector signal.

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# ADVANCED OXIDATION PROTEIN PRODUCTS (AOPP) AND CORONARY CALCIFICATIONS IN CHRONIC HEMODIALYSIS PATIENTS

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**Introduction**: Oxidative stress is considered to be an important factor to Cardiovascular morbidity in hemodialysis patients.The aim of this research is to establish the link between the levels of (AOPP) in the hemodialysis patients and coronary calcifications.

**Materials and Methods**: study grouping 67hemodialysis patients,all patients underwent a Coro-CT scan to assess coronary calcifications according to the Agatston coronary calcium score.We divided the patients into three groups according to calcification score, we measured the levels of AOPP and we studied the correlation between the rate of AOPP at the level of the different groups.

**Results**:T he mean age of the patients is 55±12.5years,the mean AOPP level is 82.82±12µmol/l,we divided the patients into 3 groups according to the agtston coronary calcium score,we found 27% of the patients had a low score<100,48% had an average sore101-400 and 25% had a severe score>400.We found that the rate of AOPP worsened with increasing calcification score,patients with a low score had a rate of AOPP53.22±42µmol/l,the mean score had an AOPP level 82.45±7µmol/l and patients with a severe calcification score had an AOPP level108.3 ±34µmol/l, there is a postive correlation between the rate of coronary calcifications and the rate of AOPP r=0.544,p=0.0001.

**Conclusion**: AOPP may be predictive of cardiovascular complications in hemodialysis patients.

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23rd International Conference on

# Oxidative Stress Reduction Redox Homeostasis and Antioxidants

## Abstracts of Day 2

## October 15 - 2021

INTERNATIONAL SOCIETY OF ANTIOXIDANTS

# COVID-19: IMMUNOPATHOLOGY, PATHOPHYSIOLOGICAL MECHANISMS, AND TREATMENT OPTIONS

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Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), continues to spread globally despite the implementation of vaccines and preventive health measures. Although most COVID-19 cases are characterized by a mild, self-limiting disease course, a considerable subset of patients develop a more severe condition, varying from pneumonia and acute respiratory distress syndrome (ARDS) to multi-organ failure (MOF).

Progression of COVID-19 is thought to occur as a result of a complex interplay between multiple pathophysiological mechanisms, all of which may orchestrate SARS-CoV-2 infection and contribute to organ-specific tissue damage.

Pathological findings in tissue specimens of patients with COVID-19 provide valuable information with regard to our understanding of pathophysiology. Excessive inflammation is a hallmark of COVID-19, which is thought to interplay with oxidative stress in causing pulmonary and systemic injury. Understanding the complex pathogenesis of COVID-19 is relevant as this broadens our understanding on the possibilities for therapeutical intervention.

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# OXIDATIVE STRESS AS KEY PLAYER IN SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS (SARS-COV-2) INFECTION

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The emergence of viral respiratory pathogens with pandemic potential, such as severe acute respiratory syndrome coronavirus (SARS-CoV-2), the pathogenic agent of Covid-19, represent a serious health problem worldwide.

Respiratory viral infections are, in general, associated with cytokine production, inflammation, cell death, and other pathophysiological processes, which could be link with a redox imbalance or oxidative stress.

These phenomena are substantially increased during aging, a population at high mortality risk by Covid-19. Severity and mortality risk of SARS-CoV-2 infection or Covid-19 disease have been associated with the age and/or comorbidities, including cardiovascular diseases and diabetes.

The aim of the present work was to contribute to understand the potential link between oxidative stress and SARS-CoV-2 infection.

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# DECREASED AVAILABILITY OF NITRIC OXIDE AND HYDROGEN SULFIDE IS A HALLMARK OF COVID-19

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**Introduction**: Recent outbreak Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been affecting millions of people globally. Coronavirus disease 2019 (COVID-19) is increasingly associated with cardiovascular complications requiring hospitalizations. However, the mechanisms underlying these complications remain unknown.

**Methods**: We assessed regulators of key cardiovascular functions, Nitric oxide (NO) and hydrogen sulfide (H2S) in COVID-19 patients. H2S and NO levels were compared between cases and controls in the entire study population and subgroups based on age, race, sex, risk factors and severity outcome of COVID-19 infection. Multivariable regression analysis was performed to identify the effects of traditional determinants of gasotransmitters on NO and H2S levels in the patients with COVID-19 infection.

**Results**: Significantly reduced NO and H2S levels were observed in both Caucasian and African American COVID-19 patients compared to healthy controls. Receiver-operating characteristic analysis of NO and H2S metabolites in the study population showed free sulfide levels to be highly predictive of COVID-19 infection based on reduced availability.

**Conclusion**: These observations provide the first insight into the role of NO and H2S in COVID-19 infection, where their low availability may be a result of reduced synthesis secondary to endotheliosis, or increased consumption from scavenging of reactive oxygen species.

# PREDICTION OF SURVIVAL ODDS IN COVID-19 BY ZINC, AGE AND SELENOPROTEIN P AS COMPOSITE BIOMARKER

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The current coronavirus disease (COVID-19) pandemic is caused by SARS-CoV-2 infections and challenges our society and the health system dramatically. Following infection, the immune system reacts on several levels including the biosynthesis and secretion of proinflammatory cytokines. One consequence of the inflammation is an adaptation of liver, undergoing an acute phase response. In combination with the disease-dependent hypoxia, trace element status is affects and in particular circulating zinc (Zn) and selenium (Se) concentrations decrease with disease severity. For Zn there is no established protein biomarker in blood, whereas circulating Se is controlled by the Se transport protein selenoprotein P (SELENOP), which is synthesized mainly by hepatocytes and serves both as Se supply protein and as biomarker of Se status.

We have analyzed serum samples from patients with COVID-19 and compared survival odds and mortality risk in relation to trace element concentrations. Both Se and Zn declined with COVID-19 severity, and low serum concentrations were associated with poor prognosis (1). This interrelationship originally observed with adult German patients was reliably replicated in a study conducted in Gent, Belgium, where we observed the relevance of comorbidities for trace element dependent survival (2). A particularly precise prediction model was developed based on the serum concentrations of the Se transporter SELENOP along with Zn and age (3). These data stimulated the idea of supplementing severely diseased patients with Se and Zn. Such an intervention was conducted by colleagues at the University Hospital Wuerzburg and analyzed for trace elements and biomarkers of Se status. The results highlighted SELENOP as a most informative parameter reflecting supplementation success (4). We conclude that the drastically declining concentrations of Se and Zn during COVID-19 cause a dysfunctional immune system, with potential relevance for long-term sequelae of the infectious disease, i.e., increased risk for autoimmune disease and long-COVID (5). It appears prudent not to become exposed to SARS-CoV-2 with a poor Se or Zn status, but rather be prepared by sufficiently high intake, in order to avoid the danger of dropping into severe deficiency during disease.

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*Conflict of Interest: LS holds shares in selenOmed GmbH, Berlin, Germany.*

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# N-ACETYLCYSTEINE (NAC) AND HYDROGEN SULFIDE (H2S): A CONVENIENT RATIONALE FOR CORONAVIRUS DISEASE 2019 (COVID-19)?

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Hydrogen sulfide (H2S) is one of the three main gasotransmitters that are endogenously produced in humans and are protective against oxidative stress. Recent findings from studies focusing on coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has shifted our attention to a potentially modulatory role of H2S in this viral respiratory disease. H2S may be of potential importance since this gasotransmitter has been shown to be protective against lung damage through its antiviral, antioxidant, and anti-inflammatory actions. Furthermore, many COVID-19 cases have been described demonstrating remarkable clinical improvement upon administration of high doses of N-acetylcysteine (NAC). NAC is a renowned pharmacological antioxidant substance acting as a source of cysteine, thereby promoting endogenous glutathione (GSH) biosynthesis as well as the generation of sulfane sulfur species when desulfurated to H2S. Combining H2S physiology and currently available knowledge of COVID-19, H2S can be hypothesized to target three main vulnerabilities of the SARS-CoV-2 virus: (1) cell entry through interfering with functional host receptors, (2) viral replication through acting on RNA-dependent RNA polymerase (RdRp), and (3) the escalation of inflammation to a potentially lethal hyperinflammatory cytokine storm (toll-like receptor 4 [TLR4] pathway and NLR family pyrin domain containing 3 [NLRP3] inflammasome). Dissecting the breakdown of NAC reveals the possibility of increasing endogenous H2S levels, which may provide a convenient rationale for the application of H2S-targeted therapeutics. Should we aim for the application of H2S-targeted therapeutics in COVID-19? Clinical trials are currently on their way to demonstrate the potential effectiveness and applicability of NAC supplementation in the context of COVID-19.

# NEW METHODS TO EVALUATE TELOMERES: APPLICATION OF U-STELA FOR ACCURATE MEASUREMENT OF EXTREMELY SHORT-TELOMERES

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During aging, telomeres shorten due to cell turnover. This gradual shortening is replication driven and does not necessarily explain the presence of ultra-short telomeres. Ultra-short telomeres are observed when there is a sudden shortening in telomeres not related with cell division and may arise from breaks in telomeres due to oxidative damage and replication slippage.

Universal STELA is an accurate method for evaluation of extreme-short telomeres. Compared to golden standard well known TRF assay, that measures mean telomere length, U-STELA is developed to overcome several problems detecting abrupt telomere shortening in a single chromosome out of 92 chromosome ends same time. The novel approach in U-STELA is to anneal a linker or telorette to the G rich 3' overhang of the telomere which is a product of restriction digestion after DNA isolation. Telorette enables stable PCR of telomeric regions without template slippage ensuring successful completion of PCR.

Telomere science showed that single or a small group of ultra-short telomeres are more influential in senescence associated disease progression rather than shortening that reflected as average telomere length, therefore it is important to identify the presence and load of ultra-short telomeres in diseases.

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# THE IMPACT OF OXIDATIVE DNA DAMAGE AND STRESS ON TELOMERE HOMEOSTASIS

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Telomeres are protective nucleoprotein-DNA caps at chromosome ends that profoundly influence genome stability and aging. Studies have shown that oxidative stress is associated with accelerated telomere shortening and dysfunction, and that telomeric TTAGGG repeats are highly susceptible to damage from reactive oxygen species[1].

To directly test how oxidative damage impacts telomeres, we used a chemoptogenetic tool to selectively produce the common lesion 8-oxoguanine (8oxoG) exclusively at telomeres. HeLa cancer cells were largely unaffected by a single induction of telomeric 8oxoG. However, chronic production of telomeric 8oxoG over time impaired cell growth, and induced telomere shortening and losses, chromosome fusions, chromatid bridges and micronuclei, which were exacerbated by repair deficiency[2]. In contrast, a single induction of telomeric 8oxoG in non-disease human fibroblast and epithelial cells was sufficient to trigger multiple hallmarks of p53-depedent cellular senescence and growth arrest. Telomeric 8oxoG enriched for markers of telomere dysfunction in replicating, but not non-replicating cells. Damage failed to shorten telomeres, but rather produced fragile sites and mitotic DNA synthesis at telomeres, indicative of impaired replication[3].

Our studies reveal that chronic oxidative telomere damage drives genomic instability in cancer cells, but acute telomeric 8oxoG drives rapid premature senescence in non-disease cells by promoting telomere fragility.

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# BENEFICIAL AND DETRIMENTAL EFFECTS OF REACTIVE OXYGEN SPECIES ON LIFESPAN

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Despite decades of research dedicated to understanding aging, the mechanisms underlying the aging process remain incompletely understood. The widely-accepted free radical theory of aging (FRTA) proposes that the accumulation of oxidative damage caused by reactive oxygen species (ROS) is one of the primary causes of aging. To define the relationship between ROS and aging, there have been two main approaches: comparative studies that measure outcomes related to ROS across species with different lifespans, and experimental studies that modulate ROS levels within a single species and measure the resulting effect on lifespan.

Comparative studies have shown that levels of ROS and oxidative damage are inversely correlated with lifespan. While these studies in general support the FRTA, this type of experiment can only demonstrate correlation, not causation. Experimental studies involving the manipulation of ROS levels in model organisms have generally shown that interventions that increase ROS tend to decrease lifespan, while interventions that decrease ROS tend to increase lifespan. However, there are also multiple examples in which the opposite is observed: increasing ROS levels results in extended longevity and decreasing ROS levels results in shortened lifespan.

Overall, the data suggest that the relationship between ROS and lifespan is complex, and that ROS can have both beneficial or detrimental effects on longevity depending on the species and conditions.

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# TELOMERE LENGTH AND CARDIOVASCULAR DISEASES

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Telomeres are specialized non-coding DNA repeat sequences (TTAGGG) found at the ends of chromosomes. Telomere lengths progressively shorten with each cell division.

There are many studies on telomere length and cardiovascular diseases risk in the literature. Some studies have shown that shorter telomere length is a risk factor for heart diseases such as coronary heart disease, myocardial infarction, hypertension, and heart failure. Oxidative stress, which is shown as one of the main mechanisms underlying cardiovascular diseases, increases the rate of telomere shortening and causes cellular aging. The accumulation of telomere-related DNA damage induced by increased oxidative stress during aging has been shown to promote aging of cardiomyocytes.

Most studies to date have used blood samples for telomere length measurements. Our current understanding of the role of telomere length in cardiovascular disease is based on measurements in blood leukocytes. Fewer studies have examined the distribution of TL between different human tissues. In the future, tissue- and cell-specific assessments of telomere length and more studies with large populations are needed to clarify the role of telomere length in predicting cardiovascular disease risks.

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# DETERMINANTS OF TELOMERE LENGTH ACROSS HUMAN TISSUES

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Telomeres are protective caps at the ends of DNA molecules found in all human cells. Telomeres consist of proteins that bind the end of the DNA sequence, preventing degradation and damage. Shortening of the DNA component of telomeres occurs as humans age, a likely underlying cause of the age-related decline of the human body functions.

Our understanding of how and why telomere length varies across individuals, and the health consequences of this variation, is based almost entirely on studies of telomeres in blood cells. For most human organs prone to disease, we lack basic knowledge regarding TL variability in human populations. This study represents an unprecedented view of telomere length variability in human tissues, reporting telomere length data for 6,391 tissue samples representing >20 different tissue types and 962 unique tissue donors. We describe how telomere length varies within and among humans, including clear differences in telomere length among tissue types. This work confirms that (1) telomeres in blood cells are an imperfect proxy for telomere length in many other disease-relevant tissues, most of which are not accessible for human studies, and (2) telomeres shorten with age in most human tissues, but a varying rates. This manuscript also describes numerous biological insights that point to new research directions. For example, genomes of African Ancestry have longer telomeres, providing motivation to study the role of TL in health disparities and evolution.

The major breakthrough provided in this paper is a significant expansion of data and knowledge regarding telomeres variability in humans (from few tissue types to many). This work is of broad interest to researchers studying aging and its connection to human health and clinicians treating patients with telomere biology disorders. This freely available data resource will promote unique lines of research across numerous scientific disciplines.

# REDOX CLOCKWORKS: THE IMPORTANCE OF TIME

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Dr. Reddy confirms that redox processes appear to be integral to the proper function of molecular circadian clocks in both nucleated non-nucleated cells. Daily oscillation of redox processes and connected metabolic pathways necessitates the consideration of time of day not only when planning lab experiments but also in the clinic.

# PEROXIREDOXIN PROMOTES LONGEVITY AND H2O2-RESISTANCE IN YEAST THROUGH REDOX-MODULATION OF PROTEIN KINASE A

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Peroxiredoxins are hydrogen peroxide scavenging enzymes that also carry out signaling and chaperone functions. We have shown that these enigmatic cysteine-dependent enzymes play a key role in regulating the rate at which organisms age (Molin, 2021; Nystrom et al., 2012). Furthermore, they appear to play a key role in the regulation of tumorigenesis, a process that displays a markedly increased incidence with age. In yeast, the major cytosolic typical 2-cysteine peroxiredoxin, Tsa1 is required both for slowing down aging and for promoting resistance to H2O2 upon caloric restriction (Molin et al., 2011). In addition, mild Tsa1 overexpression extends yeast life-span in calorie replete conditions (Hanzen et al., 2016) suggesting that peroxiredoxin functions are intimately connected to the aging process. However, the mechanistic details of their involvement in both these cellular roles are still largely missing. We have recently been able to show that Tsa1 impacts on hydrogen peroxide resistance and life-span not by scavenging hydrogen peroxide, but rather by repressing the nutrient signaling Ras-cAMP-PKA pathway at the level of the protein kinase A (PKA) enzyme (Roger et al., 2020). In addition, since mildly increased hydrogen peroxide levels increases yeast life-span in a Tsa1-dependent manner (Goulev et al., 2017), these data point to roles of peroxiredoxins in hydrogen peroxide signaling, but not scavenging, to be critical for slowing down aging and for increasing the resistance to hydrogen peroxide. Furthermore, our data collectively point to that both hydrogen peroxide and peroxiredoxin are sufficient for mitohormetic life-span extension in yeast.

I will discuss our ongoing efforts to elucidate mechanisms by which peroxiredoxins stimulate hydrogen peroxide signaling. Using cysteine sulfenylation-specific probes we found that Tsa1 stimulates the modification of cysteines in the PKA catalytic subunit by H2O2 suggesting that Tsa1 is essential for PKA redox regulation. Through mass-spectrometric characterization we found that a significant proportion of the PKA catalytic subunits are glutathionylated on two cysteine residues, but we were unable to observe signs of disulfide bonds on non-reducing gels. Nevertheless, redox modification of the conserved Cys243 is sufficient to inhibit the phosphorylation of a conserved threonine (Thr241) in the kinase activation loop and enzyme activity, and preventing Thr241 phosphorylation is able to overcome the H2O2 sensitivity of Tsa1-deficient cells. Our results will be discussed in terms of a model of aging where nutrient signaling pathways constitute hubs integrating information from multiple aging-related conduits, including a peroxiredoxin-dependent response to H2O2. The data furthermore show a mechanism by which peroxiredoxins can regulate signaling through the conserved signaling protein kinase PKA in a redox-dependent manner, largely independent on the second-messenger cAMP. The kinase activation loop cysteine is conserved in many eukaryotic protein kinases, potentially more than one-tenth including many human kinases, suggesting that this redox- and peroxiredoxin-dependent mechanism of kinase regulation may be conserved in many species.

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# REDOX AND PROGERIA: ADVANCES AND PERSPECTIVE

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**Introduction:** Hutchinson–Gilford progeria syndrome (HGPS) is an extremely rare, sporadic genetic disorder that is characterized by premature aging and accelerated cardiovascular disease progression, including that of vascular calcification1. Most HGPS patients carry a de novo none inherited autosomal dominant heterozygous mutation of the LMNA gene (p.G608G in humans; p.G609G in mice). This mutation activates a cryptic splice donor site, which causes synthesis of a lamin A mutant that disrupts nuclear membrane architecture and induces multiple cellular defects, including abnormal gene transcription, signal transduction, and DNA damage2,3. HGPS patients die at a mean age of 13–14 years (a mean of ~38 weeks old in LmnaG609G/+ mice), typically because of a cardiovascular event4.

The cellular balance between oxidation and reduction reactions (redox state) plays a critical role in aging5. Thus, both increases in mitochondrial ROS and a deterioration in antioxidant status stimulate aging. Genes associated with cytoprotective defense systems, including those encoding proteins involved in glutathione (GSH) production and GSH/NADPH regeneration, are regulated by nuclear factor erythroid 2-related factor 2 (NRF2), indicating that NRF2 regulates the redox state5. Thus, loss of NRF2 function is key piece to many pathologic conditions, including progeria, aging and age-related diseases6.

**Results**: A mouse model of premature aging (LmnaG609G/+ mice) showed an increased level of mitochondrial reactive oxygen species (ROS), and a reduced basal antioxidant capacity, including loss of the NADPH-coupled glutathione redox system. LmnaG609G/+ mice also exhibited reduced mitochondrial ATP synthesis secondary to ROS-induced mitochondrial dysfunction7. Treatment of LmnaG609G/+ vascular smooth muscle cells with magnesium-enriched medium improved the intracellular ATP level, enhanced the antioxidant capacity, and thereby reduced mitochondrial ROS production7. Moreover, treatment of LmnaG609G/+ mice with dietary magnesium improved the proton pumps (complexes I, III, and IV), stimulated extramitochondrial NADH oxidation and enhanced the coupled mitochondrial membrane potential, and thereby increased H+-coupled mitochondrial NADPH and ATP synthesis, which is necessary for cellular energy supply and survival7. Consistently, magnesium treatment reduced calcification of vascular smooth muscle cells in vitro and in vivo and improved the longevity of mice7. This antioxidant property of magnesium may be beneficial in children with HGPS.

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# RAISING THE 'GOOD' OXIDANTS FOR IMMUNE PROTECTION

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Redox medicine is a therapeutic concept that aims to modify the levels of reactive oxygen species (ROS) and secondary reaction products for health benefit. In immunity oxidants have always been considered a double-edged sword, beneficial in antimicrobial host defense and damaging in inflammatory disease. In the last decade, a more nuanced view of ROS as essential component of many basic cell functions, and a distinction between different types of oxygen species, their quantity, location, and microenvironment took hold. It is now apparent that ROS deficiency at mucosal barriers and in innate immune cells leaves the host not only susceptible to infections but can also perpetuate inflammation and delay tissue restitution. These new insights call for considering oxidant enhancing prophylactic and therapeutic interventions, an avenue still underdeveloped. This strategy will require attention to redox dynamics in complex cellular systems, integration of the overall spatiotemporal cellular environment and precise target validation to yield a novel class of effective and safe therapeutics. Here, I will provide examples of beneficial ROS in immune homeostasis, infection, and acute inflammatory disease, and address emerging therapeutic strategies for ROS augmentation to induce and strengthen protective host immunity.

# REDOX-MODULATING AGENTS IN THE TREATMENT OF VIRAL INFECTIONS

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**Introduction:** Different viruses, including influenza (IV), cause oxidative stress, that activates redox-sensitive intracellular pathways important in the regulation of specific steps of virus life-cycle and in the host response. Several redox-modulating compounds have been found to interfere with viral replication, e.g. a GSH-derivative (GSH-C4), by inactivating Protein Disulfide Isomerase, blocked IV-Hemagglutinin maturation and consequently replication[1]. GSH-C4 also improved the immune response of aged IV-infected mice[2]. We recently studied the Glucose-6-phosphate dehydrogenase (G6PD), an enzyme involved in glucose metabolism and in GSH regeneration. G6PD is under the Nrf2-mediated antioxidant response, that may be improved by a pro-GSH (I-152) molecule[5].

**Material & Methods:** Redox parameters, enzyme activity were measured using colorimetric assays. Protein expression by Western blot and immunofluorescence.

**Results:** IV-infection caused a strong reduction of G6PD expression and activity as well as Nrf2-mediated pathway. In infected G6PD-silenced cells, GSH levels were further decreased compared to scramble cells, indicating that infection causes a GSH depletion in part by impairing G6PD. I-152 treatment induced an increase in G6PD and Nrf2 expression inhibiting viral replication.

**Conclusion:** Targeting specific cell pathways with redox-modulating compounds may open the way to novel antiviral strategies to fight different viruses, including SARS-CoV2.

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# REDOX MEDICINE AND FERROPTOSIS

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Prof. Brent R. Stockwell will discuss the mechanisms and therapeutic relevance of ferroptosis, which is a form of cell death driven by peroxidation of phospholipids containing polyunsaturated fatty acyl moieties. He will describe studies on a patient-derived variant of GPX4, an enzyme that is a primary defense against this form of cell death.

# HARNESSING THE UVA-INDUCED REDOX ACTIVE LABILE IRON RELEASE TO IMPROVE THE EFFICIENCY OF 5-AMINOLEVULINIC ACID-BASED PHOTODYNAMIC THERAPY (ALA-PDT) OF SKIN CANCER

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**Introduction**: Topical ALA-PDT of skin cancer is based on ALA-mediated accumulation of protoporphyrin IX (PpIX), which upon exposure to high-intensity visible light, can catalyze the generation of ROS, resulting in cell death.[1] To improve the efficiency of ALA-PDT, we used UVA as the light source to decrease considerably the duration of phototherapy, as UVA is absorbed more efficiently by PpIX and is significantly more cytotoxic than conventional lights.[2] Furthermore UVA can increase the intracellular levels of harmful labile iron (LI),which in turn is a major contributor to cell death by acting as a catalyst for oxidative cell damage.[3,4]

**Methods**: We adopted both indoor and outdoor UVA-based light fractionation ALA-PDT protocol as our optimization strategy, using HaCaT keratinocytes as a model. The cytotoxicity MTT and clone forming assays corroborated with spectrofluorimeter-based measurements of ALA-mediated PpIX accumulation[2] and UVA-mediated LI release.[3]

**Results**: Exploiting the rapid release of LI following application of short pulses of low UVA doses (1-2.5 kJ/m$^2$) to ALA-treated cells rather than a continuous visible light source, sensitized HaCaT cells to subsequent UVA doses leading to maximum photo-killing. **Conclusion**: This study highlights the potential of UVA-based light fractionation to improve the topical ALA-PDT protocols for actinic keratosis and superficial non-melanoma skin cancer.

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## THE ANTI-INFLAMMATORY ACTIVITY OF TRANSCRIPTION FACTOR NRF2

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**Introduction**: Transcription factor Nrf2 and its main negative regulator Keap1 are at the interface of redox and intermediary metabolism. Nrf2 activation by small molecules that target Keap1 (termed inducers) provides protection via the transcriptional regulation of large networks of cytoprotective proteins. Additionally, pharmacologic Nrf2 activation linearly correlates with inhibition of pro-inflammatory responses.

**Materials and Methods**: Studies in cellular and mouse models, as well as in human subjects, were conducted using genetic or pharmacologic means of Nrf2 activation to assess its cytoprotective and anti-inflammatory effects. These studies were recently complemented by high-resolution quantitative proteomics coupled with metabolomic analyses in bone marrow-derived macrophage (BMDM) cells from wild-type-, Nrf2-knockout and Keap1-knockdown mice.

**Results**: Pharmacologic or genetic Nrf2 activation protected against solar-simulated ultraviolet (SSUV) radiation-mediated inflammation and skin carcinogenesis in mice, and the Nrf2 activator sulforaphane reduced skin photodamage in humans. Nrf2 disruption significantly affected the proteome and metabolome of unstimulated and lipopolysaccharide-stimulated BMDM cells, with alterations in redox, carbohydrate and lipid metabolism, and innate immunity. The Nrf2 activator, 4-octyl itaconate (4-OI) remodeled the inflammatory macrophage proteome, increasing redox and suppressing anti-viral immune effectors in Nrf2-dependent manner.

**Conclusion**: Nrf2 activation potentiates macrophage responses to LPS whilst attenuating pro-inflammatory signaling.

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# TARGETING AUTOPHAGY TO COUNTERACT OBESITY-ASSOCIATED OXIDATIVE STRESS

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The pharmacological targeting of polyamine metabolism is currently under the spotlight for its potential in the prevention and treatment of several age-associated disorders. We previously demonstrated that autophagy-deficient mice are more sensitive to develop metabolic syndromes in response to dietary unbalance. We found that the exogenous supplementation of the autophagy inducer spermidine is sufficient to alleviate signs of metabolic dysfunction in mouse models of HFD-induced obesity. Based on the principle of chemical similarity, we have identified triethylenetetramine dihydrochloride (TETA), an FDA-approved spermidine analogue, as a promising candidate for use in human studies.

Here, we report the finding that TETA, a copper-chelator agent that can be safely administered to patients for the long-term treatment of Wilson disease, exerts therapeutic benefits in animals challenged with hypercaloric dietary regimens. TETA reduced obesity induced by high-fat diet, excessive sucrose intake, or leptin deficiency, as it reduced glucose intolerance and hepatosteatosis, but induced autophagy.

Mechanistically, these effects did not involve the depletion of copper from plasma or internal organs. Rather, the TETA effects relied on the activation of an energy-consuming polyamine catabolism, secondary to the stabilization of spermidine/spermine N1-acetyltransferase-1 (SAT1) by TETA, resulting in enhanced enzymatic activity of SAT. All the positive effects of TETA on high-fat diet-induced metabolic syndrome were lost in SAT1-deficient mice.

Altogether, these results suggest novel health-promoting effects of TETA that might be taken advantage of for the prevention or treatment of obesity.

# FERROUS IRON-DEPENDENT PHARMACOLOGY

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KRAS mutations drive a quarter of cancer mortality and most are undruggable. Several inhibitors of the MAPK pathway are FDA approved but poorly tolerated at the doses needed to adequately extinguish RAS/RAF/MAPK signaling in the tumor cell.

We found that oncogenic KRAS signaling induced ferrous iron (Fe2+) accumulation early in and throughout mutant KRAS-mediated transformation. To exploit this metabolic change, we synthesized an iron-activatable MEK inhibitor prodrug using an iron(II) sensor inspired by clinical stage antimalarial endoperoxides.
The caged MEK inhibitor achieved potent MAPK blockade in tumor cells while sparing normal cells and tissues.

Cell killing by this compound was mediated by MEK inhibition as predicted and did not induce ROS production or ferroptosis. Iron-caging allowed sustainable, effective treatment of tumor-bearing animals, with tumor-selective drug activation producing superior systemic tolerability.

Ferrous iron accumulation is an exploitable feature of KRAS transformation and a novel iron(II)-activatable MEK inhibitor holds promise for improving treatment of KRAS-driven solid tumors.

23rd International Conference on

# Oxidative Stress Reduction Redox Homeostasis and Antioxidants

## Abstracts for Poster Presentation

## *(in alphabetic order)*

INTERNATIONAL SOCIETY OF ANTIOXIDANTS

# ASSESSMENT OF SYSTEMIC AND PLACENTAL OXIDATIVE STRESS: A PILOT STUDY IN PREECLAMPSIA

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**Introduction:** Preeclampsia is a severe complication of pregnancy with both maternal and fetal adverse outcomes. Albeit local and systemic oxidative stress have been incriminated as underlying pathomechanisms, data in the literature are controversial. The present study was purported to assess the oxidative stress in plasma and placental tissue in pregnancies complicated by preeclampsia.

**Material & Methods:** In order to assess the systemic oxidative stress, participants (n=20) were divided into: preeclamptic (n=10) and healthy pregnancies (n=10). For the study of placental oxidative stress, 19 subjects were included in the preeclamptic group (11 patients, with mild and severe preeclampsia)and the healthy pregnancies (n=8). Systemic oxidative stress was assessed in plasma using the Diacron equipment, measuring both reactive oxygen metabolites and plasma antioxidant capacity. Placental samples (central and peripheral) were collected stored at -80; cryosections were incubated with dihydroethidium and analyzed in confocal microscopy.

**Results**: High levels of systemic oxidative stress were found in both healthy and preeclamptic pregnancies; however, the antioxidant capacity in plasma was significantly increased in the preeclamptic group. The severe forms of preeclampsia had higher oxidative stress in both regions of the placentas as compared to either healthy pregnancy or mild preeclampsia.

**Conclusion:** In this pilot group, an increase in local, placental oxidative stress and the systemic antioxidant capacity of plasma were found in the preeclamptic pregnancies.

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# NEUROPROTECTIVE ACTIVITY AND ABSENCE OF IN VITRO AND IN VIVO TOXICITY OF BORAGE FLOWERS

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**Introduction:** The flowers of *Borago officinalis* L. (Boraginaceae), commonly known as borage, are edible, and their use is widespread as a culinary ingredient. Scientific literature on this part of borage is scant; For this reason, studies are necessary to evaluate its impact on human health, especially due to the toxic potential of the alkaloids present in this species. The objective of this work is to study the potential antioxidant and neuroprotective effect of fresh borage flower extract as well as its absence of toxicity.

**Material and Methods:** The extract was obtained by percolation with ethanol. The antioxidant activity was evaluated in vitro through the ORAC method. For the evaluation of the neuroprotective potential, the Neuro-2a cell line and the model organism Caenorhabditis elegans were used. For the cytoprotective capacity, the effect on viability was determined after inducing a state of stress with hydrogen peroxide for 1 hour. In C. elegans, the paralysis test was performed using strain CL4176. Analysis of the time to develop paralysis was analysed using Kaplan-Meier survival curves.

**Results:** In the ORAC test, a good oxygen radical absorption capacity was observed, 0.54 ± 0.03 µmol TE/mg extract. The evaluation of the protective activity in the cell line in Neuro-2a was carried out by comparing the viability of cells exposed to H2O2 treated with the extract or without treatment, observing a significant improvement in viability at all doses tested. In the paralysis test in C. elegans CL4176, the survival curves of the nematodes treated with extract were significantly different from the control nematodes by delaying the moment in which paralysis occurred ($p \leq 0.0001$), finding a relationship dose-response between the different concentrations studied.

**Conclusion:** The results obtained demonstrate the absence of toxicity of the extract obtained from the borage flowers as well as the potential to be used as functional foods.

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# ANTIOXIDANT ACTIVITY OF YELLOW PEA PROTEIN ISOLATE NUTRALYS® S85F

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In recent years, the demand for "all natural" products is increasing because of the increasing limitations on the use of synthetic antioxidants and enhanced public awareness of health issues. Many natural food components like oils and oilseeds, proteins and protein hydrolysates, fruits and vegetables, oat and rice bran, spices, herbs and tea have antioxidant properties. Natural antioxidants from these food components provide oxidative stability to the food product. The antioxidant activity of proteins is mainly due to interactions between their ability to inactivate reactive oxygen species, chelate pro-oxidative transition metals, scavenge free radicals, and reduction of hydroperoxides.

The objective of this study was to verify the antioxidant potential of Pea Protein Isolate 'NUTRALYS® S85F' through different in vitro tests:

- Total antioxidant radical scavenging activity (orac assay);
- Superoxide radical scavenging activity (nbt assay);
- Lipid peroxidation inhibition assay (mda kit);
- Hydroxyl radical scavenging activity (hrs assay);
- Nitric oxide scavenging ability.

NUTRALYS® S85F clearly demonstrates intrinsic antioxidant activities as can be observed from its scavenging activity toward the peroxyl radicals (ORAC), scavenger of the superoxide radicals (NBT), hydroxyl radical scavenging (HRS), prevention of lipid oxidation (MDA) and nitric oxide scavenging (NO) capabilities. Based on our results, NUTRALYS® S85F will provide oxidative stability to food products and health benefits to the consumer.

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# ND-13, A JD-1 DERIVED PEPTIDE, AS A NOVEL PHARMACOLOGICAL APPROACH TO PREVENT RENAL INFLAMMASOME ACTIVATION UNDER PRO-OXIDANT CONDITIONS

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**Introduction:** Inflammasome is a crucial regulator of renal inflammation which is a key factor in the pathogenesis of renal diseases. DJ-1 is a renal protein with antioxidant and anti-inflammatory properties. ND-13 is a peptide consists of 13 highly conserved as from the DJ-1 sequence. In this study, we determined the capacity of ND-13 and Nrf2 pathway to attenuate inflammasome activation.

**Materiel & Methods:** Mouse bone marrow macrophages were treated with Bardoxolone and Nrf2 inducer and ND-13 for 24 hours.

**Results:** The IL-1β concentration in the medium increased by NLRP3 inflammasome stimulation by LPS/ATP, and decreased in macrophages pre-treated with Bardoxolone (65.07±26%, n=4, $P<0.05$) but not pre-treated with ND-13. This data was confirmed by Bardoxolone concentration-response curve. Additionally, in presence of $H_2O_2$ (100nM), ND-13 significantly decreased IL-1β release after NLRP3 activation (88.6±1.2%, n=4, $P<0.05$). DJ-1 expression induced by the D2R agonist quinpirole, does not increase Nrf2 activity in normal conditions, however, Nrf2 activity increased by $H_2O_2$ and in a greater extent in the presence of D2R agonist (+330±82%, $P<0.05$) in human proximal tubular cells.

**Conclusion:** All these data point out that DJ-1/Nrf2 pathway stimulation is a promising approach to decrease immune cells inflammasome activation, and ND-13 could be a new approach to attenuate inflammation in renal diseases.

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# ANALYSIS OF THE DIFFERENTIAL ACTIVITY AND EXPRESSION PROFILE OF ANTIOXIDANT PEROXIDASE ENZYMES IN CYTOSOLIC AND MEMBRANE FRACTIONS OF ERYTHROCYTES FROM PREPUBERTAL OBESE CHILDREN

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**Introduction**: Erythrocytes obese children, especially with linked insulin resistance (IR), exhibit exacerbated oxidative stress characterized by a depletion of the endogenous antioxidant system, in particular cytosolic catalase. However, the contribution and importance of other antioxidant defense enzymes involved in the detoxification and protection of the erythrocyte redox state has not yet been studied.

**Material and Methods**: We evaluated the role of erythrocyte antioxidant catalase, glutathione peroxidase and peroxiredoxin as study parameters, using a childhood obesity-related study model. Our objective was to examine changes in terms of antioxidant expression levels (immunoblotting) and catalitic activity (colorimetric methods) between different fractions that integrate the erythrocyte (cytosolic and membrane), and between the different study groups composed of 6 control and 12 prepubertal obese children (OB), 6 of them with IR.

**Results**: We detected the existence of all three peroxidase enzymes in both fractions, emphasizing a reduced presence of CAT and Prdx2 and a raised content of GPx1 in membrane fraction from OB, predominantly with IR. Interestingly, we found that GPx and Prdx activity was considerably decreased in erythrocytes membrane from OB with IR, with no remarkable differences in catalase activity.

**Conclusion**: Our preliminary data highlights a marked presence of NADPH-dependent peroxidase in the erythrocyte membrane, with restricted catalytic activity in metabolically unhealthy obese children.

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# INFLAMMAGING OF YOUNG PEOPLE ANTIOXIDANTS DIET THERAPY SPORT

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My curiosity for young people 9-35 years old with several blemishes visible against the background of Chronic Inflammation, suffering from extremely long-term traumatic gory stressful situations.Anamnesis: weight loss/cachectic thinness, asthenia, hypotonia and muscle hypotrophy, abuse of doping, cannabis, smoking, nervousness, hypothyroidism, excessive sweating , headaches, changes in vision, hypertension, skin laxity, insomnia, problems concentrating in school, Metabolic Syndrome, polysintomatic Chronic Oxidative Stress and Post Traumatic Syndromes with risk of developing negative influence or genetic expression. Manifestation of chronic diseases such as cardiovascular and cancer, polycystic ovary, infertility, suicides.As a pathology and Aging Marchers useful metal proteins.Relevant anamnestic data of patients: exposure to stress factors ( Chronicle Oxidative Stress, Environmental,psicofisical trauma in extremely traumatic cruel gory decisions for children -removal from mothers,various cruel injuries,viral or bacterial infections, allergies, Aging,ecc. Calculated B.M.I., Waist circumference. Calculate caloric requirements for ideal weight and greater needs for growth,basal metabolism 60-70%,thermogenic power of food 7-15%,stress,physical activity 20-30%,viral and bacterial diseases, chronic dehydration and mineral loss. In the blood test included the following Marchers of aging: Zinc-albumin; Cooper-ceruloplasmin; Cuprum-Alpha 2 macroglobulin, Selenium;cortisolo,Interleuchina-1,6,cholesterol,triglycerides,FSH,TRN,FT3,FT4,HTG,protein electrophoresis;blood glucose,homocysteine,fibrinogen,non-protein nitrogen,urune nitrogen,counting free radicals e ecc. RMN of thebrain,hormone secretion,evalutation Ophthalmologist,Cardiologist. I set up combined programm for any patient.Drug therapies for hypothyroidism and hypertension ecc. for improve genetic influence. Tissues re-compacting treatment to process youthful elastic re-composition an revascularisation.Diet Therapy:Essential poliamino acids,vitamins,minerals.Antioxidants- resveratrol of grapes,lowers inflammatory response by inibit

*Supported by my private practice , 32 years*

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# PLASMA ACTIVITY OF ANTIOXIDANT ENZYMES INDUCED BY MAXIMAL EXERCISE IN YOUNG MEN OF VARIOUS BODY COMPOSITION

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**Introduction**: It was investigated whether maximal exercise induces changes in blood antioxidant enzyme activity of young healthy men and whether these changes vary along with body composition.

**Material & Methods**: Healthy subjects selected from 1,549 volunteers, aged 18-30, were assigned to groups: CON (n=13, average lean body mass - LBM and percentage of body fat - %BF; values between the 40th-60th percentile); HBF (n=13, average LBM and high %BF - above 80th percentile); HLBM (n=16, high LBM and average %BF). A graded maximal exercise test was performed until exhaustion. The activity of plasma superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) were measured before and after maximal exercise. Changes in plasma volume were considered for the results obtained after exercise.

**Results**: In the CON and HLBM groups, there were no significant changes in the plasma activity of SOD, CAT or GPx after maximal exercise. In the HBF group, plasma SOD activity decreased while CAT activity increased significantly.

**Conclusion**: Maximal exercise induces changes in the activity of antioxidant enzymes among young healthy men depending on body composition. Increased fat mass promotes post-exercise oxidative stress.

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# TRANSLOCATION OF CHLOROPLAST NPR1 TO THE NUCLEUS IN RETROGRADE SIGNALING IN RESPONSE TO ABIOTIC STRESS

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Chloroplasts play a pivotal role in biotic and abiotic stress responses, accompanying changes in the cell reduction/oxidation (redox) state. This study showed that the transient accumulation of nonexpressor of pathogenesis-related genes 1 (NPR1) protein, a redox-sensitive transcription coactivator, was translocated from chloroplasts to the nucleus in a redox-dependent manner under salinity stress. Immunoblotting and fluorescence image analysis showed that chloroplast-targeted NPR1-GFP fused with cTP (chloroplast transit peptide from RbcS) was migrated into the nucleus during the responses to salt stress. Chloroplast functionality was essential for retrograde translocation, in which the stomules and cytoplasmic vesicles participated. Treatments with H2O2 and an ethylene precursor enhanced this retrograde translocation. Compared to each wild-type plant, retrograde signaling-related gene expression was severely impaired in the npr1-1 Arabidopsis mutant, but enhanced transiently in the NPR1-Ox transgenic tobacco line. Therefore, NPR1 might be a retrograde signaling hub that improves a plant's adaptability to changing environments. Plastid-to-nucleus retrograde signaling crucially contributes to normal growth and development in plants. For adjustment of cellular metabolism under adverse environmental conditions, particularly in photosynthetically active leaf cells, chloroplast NPR1 may be an emergency device, after which it functions as a retrograde communicator for the protective machinery from chloroplasts to the nucleus in a redox-mediated manner.

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# CORRELATION BETWEEN OXIDATIVE STRESS MARKERS AND CYTOKINE PROFILE IN PATIENTS WITH SEVERE COVID-19

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**Introduction**: SARS-CoV-2 infection is characterized by a pro-inflammatory state and excessive cytokine production, which are accompanied by alteration of antioxidant defense. The study was performed in order to investigate the correlation between a set of cytokines and oxidative stress markers in severe COVID-19 patients.

**Methods**: A total of 11 cytokines (including chemokines and growth factors) were analyzed in 14 patients with severe COVID-19 by using High Sensitivity Evidence Investigator™ Biochip Array technology. FRAS5 analytical photometric system was applied for the measurement of oxidative stress parameters (d-ROM, PAT, OS index).

**Results**: A positive and significant correlation was detected between all cytokines and the investigated oxidative stress markers, except a negative correlation between IL-10 and the total antioxidant capacity, PAT. The correlation was not significant between OSI and IL-8 ($r = 0.3762$, $p = 0.8552$) and between d-ROM and VEGF ($r = 0.2156$, $p = 0.999$). IL-6 demonstrated strongest correlation with all of the oxidative stress markers: d-ROM ($r = 0.9725$, $p = 0.0001$), PAT ($r = 0.5000$, $p = 0.0001$) and OSI ($r = 0.9593$, $p = 0.012$). Similar behaviour was detected between IFN-γ and d-ROM ($r = 0.4006$, $p = 0.0001$), PAT ($r = 0.6030$, $p = 0.0001$) and OSI ($r = 0.4298$, $p = 0.012$).

**Conclusion**: This study shows that selected cytokines from the tested panel correlate with oxidative stress, suggesting that oxidative stress measurement by a fast and inexpensive photometric method could be a useful tool to help physicians provide timely and early interventions in severe COVID-19 patients.

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## METFORMIN MITIGATES MONOAMINE OXIDASE-RELATED CARDIAC OXIDATIVE STRESS IN RATS WITH DIET-INDUCED OBESITY

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**Introduction:** Obesity, the most severe pandemic of our century, has been unequivocally associated with cardiovascular oxidative stress. Monoamineoxidases (MAOs) with 2 isoforms, MAO-A and B, are mitochondrial enzymes that constantly generate reactive oxygen species (ROS) in hearts and vessels.

**Material and Methods:** The present study performed in a rat experimental model of high calorie junk food (HCJF) diet-induced obesity was double-aimed: to assess the role of MAO in ROS generation and whether metformin is able to mitigate it. After 24 weeks of HCJF, MAO expression (immune fluorescenceand qRT-PCR) and ROS production (spectrophotometry – FOX assay,and immunefluorescence – DHE staining). Experiments were performed in the presence *vs* absence of MAO inhibitors (clorgyline and selegiline, 10 μM) and metformin (10 microM, 12 h).

**Results:** Obesity elicited an increased oxidative stress in the hearts of obese *vs* non-obese rats, an effectt hat has been significantly mitigated by metformin (10 microM, 12 h). Both MAO isoforms are present in the rat heart with increased expression in the obese animals. In vitro incubation with Metformin decreased MAO-A and B expression of in the diseased hearts.

**Conclusion:** In the rat model of diet-induced obesity, MAO elicited cardiac oxidative stress that was mitigated with metformin.

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# METABOLIC PROFILES OF ASTROCYTES AND NEURONS IN NORMOXIC AND HYPOXIC CONDITIONS

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**Introduction**: In conventional cell culture, so-called normoxic conditions, cells are grown in around 19% O2, 5% CO2. However, the average O2 level available to the cells in our bodies is much less. As cellular metabolism is dependent on changes in O2 tension, the level of O2 in cell culture conditions is a factor to be considered when creating in vitro cell models for studying metabolism.

**Methods**: PC12 and C6 cells were cultured in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin. Cells were grown in 19% O2, 10% O2, and 1% O2 for 5-days. The bioenergetic profile of the cells was measured using high-resolution respirometry (Oxygraph-2k).

**Results**: Rat neurons (PC12) and astrocytes (C6) have distinct metabolic profiles, with neurons being more oxidative than astrocytes. Astrocytes' respiration is less responsive to different oxygen conditions, while PC12 metabolic profiles are oxygen sensitive.

**Conclusion**: Oxygen tension affects the metabolic profiles of cells in culture. Furthermore, astrocytes and neurons respond differently to varying oxygen concentrations. Therefore, it is paramount to establish a baseline metabolic profile for each cell/condition used in further studies.

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# LACK OF CORRELATION BETWEEN SUPEROXIDE DISMUTASE, GLUTATHIONE PEROXIDASE AND INSULIN RESISTANCE IN PATIENTS WITH CHRONIC HEPATITIS

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**Introduction**: We investigate the association of superoxide dismutase (SOD) and glutathione peroxidase (GPx) with insulin resistance parameters in patients with chronic hepatitis.

**Materials and Methods**: 26 patients with chronic hepatitis were studied. Fasting serum glucose, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides (Mindray BS 200e, China) were analyzed. SOD (Bender MedSystems, Austria), GPx-1 (Bio Vendor, Czech Republic) and insulin (Nova Tec, Germany) were determined using ELISA methods. The relationship between SOD, GPx and parameters of insulin resistance was analyzed.

**Results**: SOD and GPx mean levels were 25.46 ± 1.72 ng/ml and 2.19 ± 0.58 ng/ml, respectively. Significant correlation was not found between SOD and total cholesterol (r = - 0.045), LDL-cholesterol (r = - 0.099), HDL-cholesterol (r = 0.194), triglycerides (rho = 0.197), glucose (rho = 0.026), insulin (rho =0.354), HOMA-IR (rho = 0.323), P > 0.05. No correlation between GPx and triglycerides (rho = - 0.187), glucose (rho = - 0.217), insulin (rho = - 0.273), HOMA-IR (rho = - 0.211), total cholesterol (r = 0.035), HDL-cholesterol (r = 0.172), LDL-cholesterol (r = 0.040), P > 0.05 was found.

**Conclusion**: No correlation between SOD and GPx and studied metabolic parameters was found. Other indicators of insulin resistance probably correlate with SOD and GPx.

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# LACTATE SUPPLEMENTATION MITIGATES OBESITY IN HIGH-FAT DIET-FED MICE

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**Introduction**: In the last two centuries, lactate was considered only a byproduct of anaerobic metabolism causing muscle pain after physical exercise. However, the last decade of research revealed that it has a number of signaling and metabolic functions mediated by its specific G protein-coupled receptor, GPR81, and by mitochondrial retrograde signaling.

**Material & Methods**: We prepared a formula releasing lactate in the gut and examined its effect on the adipose tissue of a high-fat diet (HFD) fed C57BL6/J mice.

**Results**: Three weeks of daily supplementation with 2 % formulated lactate in the diet resulted in a significant decrease in body weight compared to HFD only (56±4 g versus 61±2 g, $p<0.03$). Interestingly, no change in the amount of feed consumed by mice was observed (3.2±0.4 g versus 3.4±0.2 g, $p<0.03$). While fat tissues shrank significantly (4.0±0.5 g versus 5.2±0.5 g, $p<0.03$ for visceral fat; 4.4±0.5 g versus 4.8±0.5 g, $p<0.03$ for subcutaneous fat), however, the weight of heart, kidney or gastrocnemius muscle did not change. In parallel, a drop in insulin and leptin levels was observed in lactate+HFD fed mice compared to HFD only (26±8 ng.ml-1 versus 33±17 ng.ml-1, $p<0.03$ for leptin; 580±310 pg.ml-1 versus 1080±380 pg.ml-1, $p<0.03$ for insulin). No signs of pathological changes in the liver and gastrointestinal tract of lactate-fed mice were observed.

**Conclusion**: In conclusion, the lactate-releasing formula decreases the body weight in HFD-fed mice, which was caused by a reduction of adipose tissue. The exact mechanism will be further investigated.

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# BLOOD PLASMA'S PROTECTIVE ABILITY AGAINST THE DEGRADATION OF S-NITROSOGLUTATHIONE UNDER THE INFLUENCE OF AIR-POLLUTION-DERIVED METAL IONS IN PATIENTS WITH EXACERBATION OF HEART FAILURE AND CORONARY ARTERY DISEASE

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**Introduction**: Aqueous soluble inorganic components of airborne particulate matter containing redox-active transition metal ions may affect the stability of S-nitrosothiols and disrupt the balance in the homeostasis of nitric oxide. Disturbance in nitric oxide (NO) bioavailability is thought to be one of the key factors common to cardiovascular disease. Uncontrolled elevation of the NO concentration may exacerbate inflammation through the damage of the endothelial cells, mediated by superoxide anion and hydroxyl radical, local vasoconstriction, and increased vascular resistance as well as aggregation and growth of platelets.

**Material & Methods**: Blood plasma's protective ability against the decomposition of S-nitrosoglutathione (GSNO) under the influence of aqueous PM extract among patients with exacerbation of heart failure and coronary artery disease was studied and compared with a group of healthy volunteers.

**Result**s: In the environment of CVD patients' plasma, NO release from GSNO was facilitated compared to the plasma of healthy controls, and the addition of ascorbic acid boosted this process. The correlation between the concentration of NO released and -SH level in blood plasma revealed that the amount of free thiol groups is one of the crucial factors in GSNO decomposition.

**Conclusion**: Redox-active inorganic fraction of urban PM should be considered as one of the environmental factors leading to the progression of cardiovascular disease via uncontrolled GSNO decomposition.
Wądołek A. acknowledges the fellowship with the project POWR.03.02.00-00-I013/16

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# SCOPOLETIN AND UMBELLIFERONE PROTECT HEPATOCYTES AGAINST PALMITAT AND BILE ACID-INDUCED CELL DEATH BY REDUCING ENDOPLASMIC RETICULUM STRESS AND OXIDATIVE STRESS

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**Background**: The number of patients with non-alcoholic fatty liver disease (NAFLD) is rapidly increasing due to the growing epidemic of obesity. Non-alcoholic steatohepatitis (NASH), the inflammatory stage of NAFLD, is characterized by lipid accumulation in hepatocytes, chronic inflammation and hepatocyte cell death. Scopoletin and umbelliferone are coumarin-like molecules and have antioxidant, anti-cancer and anti-inflammatory effects. Cytoprotective effects of these compounds have not been described in hepatocytes and the mechanisms of the beneficial effects of scopoletin and umbelliferone are unknown.

**Aim**: to investigate whether scopoletin and/or umbelliferone protect hepatocytes against palmitate-induced cell death. For comparison, we also tested the cytoprotective effect of scopoletin and umbelliferone against bile acid-induced cell death. Methods: Primary rat hepatocytes were exposed to palmitate (1 mmol/L) or the hydrophobic bile acid glycochenodeoxycholic acid (GCDCA; 50 µmol/L). Apoptosis was assessed by caspase-3 activity assay, necrosis by Sytox green assay, mRNA levels by qPCR, protein levels by Western blot and production of reactive oxygen species (ROS) by fluorescence assay.

**Results**: Both scopoletin and umbelliferone protected against palmitate and GCDCA-induced cell death. Both palmitate and GCDCA induced the expression of ER stress markers. Scopoletin and umbelliferone decreased palmitate- and GCDCA-induced expression of ER stress markers, phosphorylation of the cell death signaling intermediate JNK as well as ROS production.

**Conclusion**: Scopoletin and umbelliferone protect against palmitate and bile acid-induced cell death of hepatocytes by inhibition of ER stress and ROS generation and decreasing phosphorylation of JNK. Scopoletin and umbelliferone may be considered as a therapeutic modality for the treatment of NAFLD.

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# INHIBITION OF MITOCHONDRIAL METABOLISM LEADS TO SELECTIVE ERADICATION OF CELLS ADAPTED TO ACIDIC MICROENVIRONMENT

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**Introduction**: Metabolic transformation of cancer cells leads to the accumulation of lactate and significant acidification in the tumor microenvironment.

**Materials & Methods**: Several human cell lines (PaTu-8902, HeLa, HepG2, MRC-5) were cultured in media resembling lactate levels and acidosis in the tumor microenvironment. They were treated with oxidative stress-triggering therapeutics, and their metabolism was analyzed.

**Results**: Here, we report that cancer cells adapted to acidosis are significantly more sensitive to oxidative damage induced by hydrogen peroxide, high-dose ascorbate, and photodynamic therapy. Higher lactate concentrations abrogate the sensitization. Mechanistically, acidosis leads to a drop in antioxidant capacity caused by a compromised supply of nicotinamide adenine dinucleotide phosphate (NADPH) derived from glucose metabolism. However, lactate metabolism in the Krebs cycle restores NADPH supply and antioxidant capacity. CPI-613 (devimistat), an anticancer drug candidate, selectively eradicates the cells adapted to acidosis through inhibition of the Krebs cycle and induction of oxidative stress while completely abrogating the protective effect of lactate. Simultaneous cell treatment with tetracycline, an inhibitor of the mitochondrial proteosynthesis, further enhances the cytotoxic effect of CPI-613 under acidosis and in tumor spheroids.

**Conclusion**: We suggest considering tumor acidosis as the Achilles heel of cancer enabling selective therapeutic induction of lethal oxidative stress.

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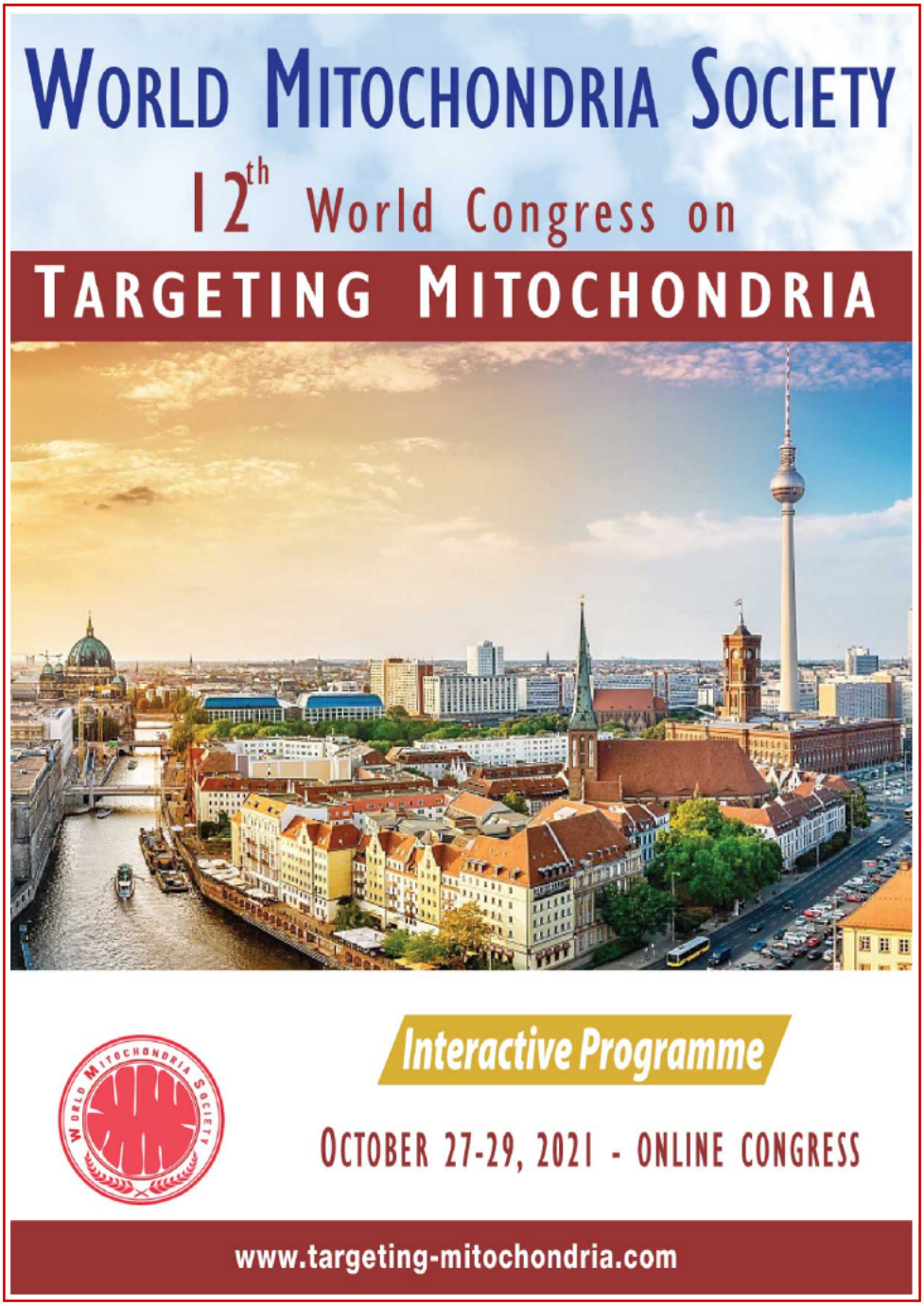


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