



PHYSICIAN'S AND DENTISTS RESOURCE MANUAL FOR REDOX SIGNALING MOLECULES

REDOX DENTISTRY & THE SCIENCE OF REDOX SIGNALING MOLECULES



WWW.REDOXDENTISTRY.COM

Physician's and Dentist's Resource Manual for Redox Signaling Molecules

Redox Dentistry & the Science of Redox Signaling Molecules

Prepared by G. Lee Ostler DDS

No part of this curated collection shall be copied, reproduced or transmitted into different formats in any form or by any means. All information contained herein is publicly available on public domain sources and websites. No warranty liability whatsoever is assumed with respect to the use of the information contained herein. Although every precaution has been taken in the preparation of this work, the publisher and author assume no responsibility for errors or omissions. Neither is any liability assumed or taken for damages resulting from the use of the information contained herein. Users may store this work on their own computer and digital devices, print copies for personal use at will, and convey such or digital versions to other interested parties. Copyright © RedoxDentistry 2019. All rights reserved.

Table of Contents

Redox Signaling – Antioxidants, Nrf2, Glutathione	3
Oral Health.....	16
Aging	23
Asthma.....	25
Autism.....	25
Bowel & Colon Health.....	26
Cancer	26
Cardiovascular Health.....	29
Cellular Healing and Wound Repair	31
Cystic Fibrosis	33
Diabetes	33
Endocrine	35
Eye Health.....	36
Exercise.....	36
Hair	37
Fibromyalgia.....	38
Immune System	38
Mental Health	39
Mitochondria.....	40
Neurology / Nervous System	41
PEMF, Photobiomodulation, Low-level Light Therapy	43
Platelets, Platelet-rich Fibrin (PRF) and Redox Signaling.....	45
Skin	47
Wound Healing	48

Redox Signaling – Antioxidants, Nrf2, Glutathione

A Fluorescent Sensor for Imaging Reversible Redox Cycles in Living Cells. [Life in aerobic environments requires organisms to maintain strict control over their internal redox status. At the cellular level, aerobic respiration poses a particularly unique challenge for living systems, as the energy-releasing reduction of oxygen to water generates partially reduced reactive oxygen species (ROS) intermediates that can exert widely divergent physiological and/or pathological effects.¹ Unregulated production of ROS results in oxidative stress, and subsequent buildup of free radical damage to proteins, lipids, and nucleic acids is connected to serious human diseases where age is a risk factor.^{2,3} However, emerging evidence suggests that controlled production of one ROS in particular, hydrogen peroxide, can mediate cellular signal transduction through reversible oxidation and reduction of cysteine thiols and other redox-active groups.] Miller EW, Bian SX, et al. *J Am Chem Soc.* 2007 Mar 28; 129(12): 3458–3459. Published online 2007 Mar 3. doi: 10.1021/ja0668973. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2532511/>

A novel role for 12/15-lipoxygenase in regulating autophagy. [12/15-Lipoxygenase (LOX) enzymatically generates oxidized phospholipids in monocytes and macrophages. Herein, we show that cells deficient in 12/15-LOX contain defective mitochondria and numerous cytoplasmic vacuoles containing electron dense material, indicating defects in autophagy or membrane processing. However, both LC3 expression and lipidation were normal both basally and on chloroquine treatment. A LOX-derived oxidized phospholipid, 12-hydroxyeicosatetraenoic acid-phosphatidylethanolamine (12-HETE-PE) was found to be a preferred substrate for yeast Atg8 lipidation, versus native PE, while both native and oxidized PE were effective substrates for LC3 lipidation. Last, phospholipidomics demonstrated altered levels of several phospholipid classes. Thus, we show that oxidized phospholipids generated by 12/15-LOX can act as substrates for key proteins required for effective autophagy and that cells deficient in this enzyme show evidence of autophagic dysfunction. The data functionally link phospholipid oxidation with autophagy for the first time. ... In summary, this study demonstrates that deficiency in 12/15-LOX results in a lysosomal storage disorder phenotype, impacting on membrane processing, organelle clearance and autophagy in murine macrophages. The ability of oxidized phospholipids to act as LC3/Atg8 lipidation substrates links phospholipid oxidation, a key event in innate immunity and atherosclerosis with normal cellular processes required for cellular turnover and homeostasis.] Yoshinori Ohsumi, et al. <https://www.sciencedirect.com/science/article/pii/S2213231714001177>

A redox cycle within the cell cycle: ring in the old with the new. [In recent years, the intracellular oxidation–reduction (redox) state has gained increasing attention as a critical mediator of cell signaling, gene expression changes and proliferation. This review discusses the evidence for a redox cycle (i.e., fluctuation in the cellular redox state) regulating the cell cycle. The presence of redox-sensitive motifs (cysteine residues, metal co-factors in kinases and phosphatases) in several cell cycle regulatory proteins indicate periodic oscillations in intracellular redox state could play a central role in regulating progression from G0/G1 to S to G2 and M cell cycle phases. Fluctuations in the intracellular redox state during cell cycle progression could represent a fundamental mechanism linking oxidative metabolic processes to cell cycle regulatory processes. Proliferative disorders are central to a variety of human pathophysiological conditions thought to involve oxidative stress. Therefore, a more complete understanding of redox control of the cell cycle could provide a biochemical rationale for manipulating aberrant cell proliferation.] Menon SG, Goswami PC. *Oncogene* volume 26, pages 1101–1109 (22 February 2007). <https://www.nature.com/articles/1209895>

Analysis of Nrf2-mediated transcriptional induction of antioxidant response in early embryos. [Nrf2 is a transcription factor that regulates the induction of an expansive set of antioxidant proteins that act in synergy to remove reactive oxygen species (ROS). We have recently discovered that Nrf2 signaling is involved in the induction of antioxidant response in ethanol-exposed embryos. We have also demonstrated that chemically induced transcriptional activation of Nrf2 and subsequent induction of a broad spectrum of antioxidant proteins can act as an endogenous protective system against ethanol-induced oxidative stress and apoptosis in early mouse embryos. This chapter describes the methods used to analyze Nrf2-mediated transcriptional induction of antioxidant response in mouse embryos. Data which have been generated by using these methods have also been included to provide examples of their usefulness in evaluating Nrf2-mediated antioxidant response in early embryos.] Chen SY. *Methods Mol Biol.* 2012;889:277-90. <https://www.ncbi.nlm.nih.gov/pubmed/22669671>

Antioxidant response elements: Discovery, classes, regulation and potential applications. [Exposure to antioxidants and xenobiotics triggers the expression of a myriad of genes encoding antioxidant proteins, detoxifying enzymes, and xenobiotic transporters to offer protection against oxidative stress. This articulated universal mechanism is regulated through the cis-acting elements in an array of Nrf2 target genes called antioxidant response elements (AREs), which play a critical role in redox homeostasis. Though the Keap1/Nrf2/ARE system involves many players, AREs hold the key in transcriptional regulation of cytoprotective genes. ARE-mediated reporter constructs have been widely used, including xenobiotics profiling and Nrf2 activator screening. The complexity of AREs is brought by the presence of other regulatory elements within the AREs. The diversity in the ARE sequences not only bring regulatory selectivity of diverse transcription factors, but also confer functional complexity in the Keap1/Nrf2/ARE pathway. The different transcription factors either homodimerize or heterodimerize to bind the AREs. Depending on the nature of

partners, they may activate or suppress the transcription. Attention is required for deeper mechanistic understanding of ARE-mediated gene regulation. The computational methods of identification and analysis of AREs are still in their infancy. Investigations are required to know whether epigenetics mechanism plays a role in the regulation of genes mediated through AREs. The polymorphisms in the AREs leading to oxidative stress related diseases are warranted. A thorough understanding of AREs will pave the way for the development of therapeutic agents against cancer, neurodegenerative, cardiovascular, metabolic and other diseases with oxidative stress.] Raghunath A, Sundarrai K, et al. *Redox Biol.* 2018 Jul;17:297-314. <https://www.ncbi.nlm.nih.gov/pubmed/29775961>

Antioxidants: Friend or foe? [Reactive oxygen species are the intermediates that are formed during the normal metabolic process which are effectively neutralized by the antioxidant system of the body. Any imbalance in this neutralization process causes oxidative stress which has been implicated as one of the cause in diseases such as Alzheimer's disease, cardiovascular disorders, cancer etc. Research has enabled the use of antioxidants as therapeutic agents in the treatment of various diseases. Literature also puts forth the negative effects of using antioxidants in the treatment of diseases. This review is a compilation of both the beneficial and detrimental effects of use of antioxidants in the treatment of diseases such as cancer, cardiovascular diseases, diabetes and oral diseases.] Sarangarajan R, Meera S, et al. *Asian Pacific Journal of Tropical Medicine*, Volume 10, Issue 12, December 2017, Pages 1111-1116. <https://www.sciencedirect.com/science/article/pii/S1995764517311999>

Autophagy, mitochondria and oxidative stress: cross-talk and redox signaling. [Reactive oxygen and nitrogen species change cellular responses through diverse mechanisms that are now being defined. At low levels, they are signalling molecules, and at high levels, they damage organelles, particularly the mitochondria. Oxidative damage and the associated mitochondrial dysfunction may result in energy depletion, accumulation of cytotoxic mediators and cell death. Understanding the interface between stress adaptation and cell death then is important for understanding redox biology and disease pathogenesis. Recent studies have found that one major sensor of redox signalling at this switch in cellular responses is autophagy. Autophagic activities are mediated by a complex molecular machinery including more than 30 Atg (AuTophagy-related) proteins and 50 lysosomal hydrolases. Autophagosomes form membrane structures, sequester damaged, oxidized or dysfunctional intracellular components and organelles, and direct them to the lysosomes for degradation. This autophagic process is the sole known mechanism for mitochondrial turnover. It has been speculated that dysfunction of autophagy may result in abnormal mitochondrial function and oxidative or nitrate stress. Emerging investigations have provided new understanding of how autophagy of mitochondria (also known as mitophagy) is controlled, and the impact of autophagic dysfunction on cellular oxidative stress. The present review highlights recent studies on redox signalling in the regulation of autophagy, in the context of the basic mechanisms of mitophagy. Furthermore, we discuss the impact of autophagy on mitochondrial function and accumulation of reactive species. This is particularly relevant to degenerative diseases in which oxidative stress occurs over time, and dysfunction in both the mitochondrial and autophagic pathways play a role]. Lee J, Giordano S, et al. *Biochem J.* 2012 Jan 15; 441(Pt 2): 523–540. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3258656/>

Autophagy, mitochondria and oxidative stress: cross-talk and redox signaling. [Reactive oxygen and nitrogen species change cellular responses through diverse mechanisms that are now being defined. At low levels, they are signalling molecules, and at high levels, they damage organelles, particularly the mitochondria. Oxidative damage and the associated mitochondrial dysfunction may result in energy depletion, accumulation of cytotoxic mediators and cell death. Understanding the interface between stress adaptation and cell death then is important for understanding redox biology and disease pathogenesis. Recent studies have found that one major sensor of redox signalling at this switch in cellular responses is autophagy. Autophagic activities are mediated by a complex molecular machinery including more than 30 Atg (AuTophagy-related) proteins and 50 lysosomal hydrolases. Autophagosomes form membrane structures, sequester damaged, oxidized or dysfunctional intracellular components and organelles, and direct them to the lysosomes for degradation. This autophagic process is the sole known mechanism for mitochondrial turnover. It has been speculated that dysfunction of autophagy may result in abnormal mitochondrial function and oxidative or nitrate stress. Emerging investigations have provided new understanding of how autophagy of mitochondria (also known as mitophagy) is controlled, and the impact of autophagic dysfunction on cellular oxidative stress. The present review highlights recent studies on redox signalling in the regulation of autophagy, in the context of the basic mechanisms of mitophagy. Furthermore, we discuss the impact of autophagy on mitochondrial function and accumulation of reactive species. This is particularly relevant to degenerative diseases in which oxidative stress occurs over time, and dysfunction in both the mitochondrial and autophagic pathways play a role.] Lee, J, Giordano S, et al. *Biochem J.* 2012 Jan 15; 441(Pt 2): 523–540. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3258656/>

Autophagy signaling through reactive oxygen species. [Autophagy is a degradative pathway that involves delivery of cytoplasmic components, including proteins, organelles, and invaded microbes to the lysosome for digestion. Autophagy is implicated in the pathology of various human diseases. The association of autophagy to inflammatory bowel diseases is consistent with recent discoveries of its role in immunity. A complex of signaling pathways control the induction of autophagy in different cellular contexts. Reactive oxygen species (ROS) are highly reactive oxygen free radicals or non-radical molecules that are generated by multiple mechanisms in cells, with the nicotinamide adenine dinucleotide phosphate (NADPH) oxidases and mitochondria as major cellular sources. These ROS are important signaling molecules that regulate many signal-transduction pathways and play critical roles in cell survival, death, and immune defenses. ROS were recently shown to activate starvation-induced autophagy, antibacterial autophagy,

and autophagic cell death. Current findings implicate ROS in the regulation of autophagy through distinct mechanisms, depending on cell types and stimulation conditions. Conversely, autophagy can also suppress ROS production. Understanding the mechanisms behind ROS-induced autophagy will provide significant therapeutic implications for related diseases]. Huang J, Lam GY, et al. *Antioxid Redox Signal*. 2011 Jun;14(11):2215-31. <https://www.ncbi.nlm.nih.gov/pubmed/20874258>

Biological and physiological role of reactive oxygen species--the good, the bad and the ugly. [Reactive oxygen species (ROS) are chemically reactive molecules that are naturally produced within biological systems. Research has focused extensively on revealing the multi-faceted and complex roles that ROS play in living tissues. In regard to the good side of ROS, this article explores the effects of ROS on signalling, immune response and other physiological responses. To review the potentially bad side of ROS, we explain the consequences of high concentrations of molecules that lead to the disruption of redox homeostasis, which induces oxidative stress damaging intracellular components. The ugly effects of ROS can be observed in devastating cardiac, pulmonary, neurodegenerative and other disorders. Furthermore, this article covers the regulatory enzymes that mitigate the effects of ROS. Glutathione peroxidase, superoxide dismutase and catalase are discussed in particular detail. The current understanding of ROS is incomplete, and it is imperative that future research be performed to understand the implications of ROS in various therapeutic interventions.] Zuo L, Zhou T, et al. *Acta Physiol (Oxf)*. 2015 Jul;214(3):329-48. doi: 10.1111/apha.12515. <https://www.ncbi.nlm.nih.gov/pubmed/25912260/>

Cellular metabolic and autophagic pathways: traffic control by redox signaling. [It has been established that the key metabolic pathways of glycolysis and oxidative phosphorylation are intimately related to redox biology through control of cell signaling. Under physiological conditions glucose metabolism is linked to control of the NADH/NAD redox couple, as well as providing the major reductant, NADPH, for thiol-dependent antioxidant defenses. Retrograde signaling from the mitochondrion to the nucleus or cytosol controls cell growth and differentiation. Under pathological conditions mitochondria are targets for reactive oxygen and nitrogen species and are critical in controlling apoptotic cell death. At the interface of these metabolic pathways, the autophagy-lysosomal pathway functions to maintain mitochondrial quality and generally serves an important cytoprotective function. In this review we will discuss the autophagic response to reactive oxygen and nitrogen species that are generated from perturbations of cellular glucose metabolism and bioenergetic function.] Dodson M, Darley-Usmar V, et al. *Free Radic Biol Med*. 2013 Oct;63:207-21. <https://www.ncbi.nlm.nih.gov/pubmed/23702245>

Control of gene expression by redox potential and the requirement for chloroplast and mitochondrial genomes. [Recent experiments with bacteria have shown that light and oxygen can control gene expression through effects on oxidation-reduction potential. The term "redox sensor" is proposed as a general term for electron carriers that initiate control of gene expression upon oxidation or reduction. The term "redox response regulator" is proposed for DNA-binding proteins that modify gene expression as a result of the action of redox sensors. Redox sensors and redox response regulators may function together in feedback control of redox potential in photosynthesis and respiration, protecting the cell from damage caused by electrochemistry operating on inappropriate electron donors and acceptors. Chloroplast and mitochondrial redox sensors and redox response regulators, themselves encoded in the nucleus, may place expression of chloroplast and mitochondrial genes under redox regulatory control. This hypothesis offers an explanation for the persistence, in evolution, of chloroplast and mitochondrial genomes, and for the constancy of the subset of chloroplast and mitochondrial proteins encoded and synthesized within the organelle.] Allen JF. *J Theor Biol*. 1993 Dec 21;165(4):609-31. <https://www.ncbi.nlm.nih.gov/pubmed/8114509>

Decline in transcriptional activity of Nrf2 causes age-related loss of glutathione synthesis, which is reversible with lipoic acid. [Glutathione (GSH) significantly declines in the aging rat liver. Because GSH levels are partly a reflection of its synthetic capacity, we measured the levels and activity of gamma-glutamylcysteine ligase (GCL), the rate-controlling enzyme in GSH synthesis. With age, both the catalytic (GCLC) and modulatory (GCLM) subunits of GCL decreased by 47% and 52%, respectively ($P < 0.005$). Concomitant with lower subunit levels, GCL activity also declined by 53% ($P < 0.05$). Because nuclear factor erythroid2-related factor 2 (Nrf2) governs basal and inducible GCLC and GCLM expression by means of the antioxidant response element (ARE), we hypothesized that aging results in dysregulation of Nrf2-mediated GCL expression. We observed an approximately 50% age-related loss in total ($P < 0.001$) and nuclear ($P < 0.0001$) Nrf2 levels, which suggests attenuation in Nrf2-dependent gene transcription. By using gel-shift and supershift assays, a marked reduction in Nrf2/ARE binding in old vs. young rats was noted. To determine whether the constitutive loss of Nrf2 transcriptional activity also affects the inducible nature of Nrf2 nuclear translocation, old rats were treated with (R)-alpha-lipoic acid (LA; 40 mg/kg i.p. up to 48 h), a disulfide compound shown to induce Nrf2 activation in vitro and improve GSH levels in vivo. LA administration increased nuclear Nrf2 levels in old rats after 12 h. LA also induced Nrf2 binding to the ARE, and, consequently, higher GCLC levels and GCL activity were observed 24 h after LA injection. Thus, the age-related loss in GSH synthesis may be caused by dysregulation of ARE-mediated gene expression, but chemoprotective agents, like LA, can attenuate this loss.] Suh JH, Shenvi SV, et al. *Proc Natl Acad Sci U S A*. 2004 Mar 9;101(10):3381-6. <https://www.ncbi.nlm.nih.gov/pubmed/14985508>

Electrophiles modulate glutathione reductase activity via alkylation and upregulation of glutathione biosynthesis. [Cells evolved robust homeostatic mechanisms to protect against oxidation or alkylation by electrophilic species. Glutathione (GSH) is the

most abundant intracellular thiol, protects cellular components from oxidation and is maintained in a reduced state by glutathione reductase (GR). Nitro oleic acid (NO₂-OA) is an electrophilic fatty acid formed under digestive and inflammatory conditions that both reacts with GSH and induces its synthesis upon activation of Nrf2 signaling. The effects of NO₂-OA on intracellular GSH homeostasis were evaluated. In addition to upregulation of GSH biosynthesis, we observed that NO₂-OA increased intracellular GSSG in an oxidative stress-independent manner. NO₂-OA directly inhibited GR in vitro by covalent modification of the catalytic Cys61, with k_{on} of $(3.45 \pm 0.04) \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$, k_{off} of $(4.4 \pm 0.4) \times 10^{-4} \text{ s}^{-1}$, and K_{eq} of $(1.3 \pm 0.1) \times 10^{-7} \text{ M}$. Akin to NO₂-OA, the electrophilic Nrf2 activators bardoxolone-imidazole (CDDO-Im), bardoxolone-methyl (CDDO-Me) and dimethyl fumarate (DMF) also upregulated GSH biosynthesis while promoting GSSG accumulation, but without directly inhibiting GR activity. In vitro assays in which GR was treated with increasing GSH concentrations and GSH depletion experiments in cells revealed that GR activity is finely regulated via product inhibition, an observation further supported by theoretical (kinetic modeling of cellular GSSG:GSH levels) approaches. Together, these results describe two independent mechanisms by which electrophiles modulate the GSH/GSSG couple, and provide a novel conceptual framework to interpret experimentally determined values of GSH and GSSG.] Jobaggy S, Vitturi A, et al. *Redox Biol.* 2018 Nov 22;21:101050. doi: 10.1016/j.redox.2018. <https://www.ncbi.nlm.nih.gov/pubmed/30654300>

Epigenetics and Immunometabolism in Diabetes and Aging. [Significance: A strong relationship between hyperglycemia, impaired insulin pathway, and cardiovascular disease in type 2 diabetes (T2D) is linked to oxidative stress and inflammation. Immunometabolic pathways link these pathogenic processes and pose important potential therapeutic targets. Recent Advances: The link between immunity and metabolism is bidirectional and includes the role of inflammation in the pathogenesis of metabolic disorders such as T2D, obesity, metabolic syndrome, and hypertension and the role of metabolic factors in regulation of immune cell functions. Low-grade inflammation, oxidative stress, balance between superoxide and nitric oxide, and the infiltration of macrophages, T cells, and B cells in insulin-sensitive tissues lead to metabolic impairment and accelerated aging. Critical Issues: Inflammatory infiltrate and altered immune cell phenotype precede development of metabolic disorders. Inflammatory changes are tightly linked to alterations in metabolic status and energy expenditure and are controlled by epigenetic mechanisms. Future Directions: A better comprehension of these mechanistic insights is of utmost importance to identify novel molecular targets. In this study, we describe a complex scenario of epigenetic changes and immunometabolism linking to diabetes and aging-associated vascular disease.] Guzik TJ, Cosentino F. *Antioxid. Redox Signal.* 29, 257–274. <https://www.liebertpub.com/doi/full/10.1089/ars.2017.7299>.

Exogenous antioxidants—Double-edged swords in cellular redox state; Health beneficial effects at physiologic doses versus deleterious effects at high doses. [The balance between oxidation and antioxidation is believed to be critical in maintaining healthy biological systems. Under physiological conditions, the human antioxidative defense system including e.g., superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione (GSH) and others, allows the elimination of excess reactive oxygen species (ROS) including, among others superoxide anions (O₂⁻), hydroxyl radicals (OH \cdot), alkoxyl radicals (RO \cdot) and peroxyradicals (ROO \cdot). However, our endogenous antioxidant defense systems are incomplete without exogenous originating reducing compounds such as vitamin C, vitamin E, carotenoids and polyphenols, playing an essential role in many antioxidant mechanisms in living organisms. Therefore, there is continuous demand for exogenous antioxidants in order to prevent oxidative stress, representing a disequilibrium redox state in favor of oxidation. However, high doses of isolated compounds may be toxic, owing to prooxidative effects at high concentrations or their potential to react with beneficial concentrations of ROS normally present at physiological conditions that are required for optimal cellular functioning. This review aims to examine the double-edged effects of dietary originating antioxidants with a focus on the most abundant compounds, especially polyphenols, vitamin C, vitamin E and carotenoids. Different approaches to enrich our body with exogenous antioxidants such as via synthetic antioxidants, diets rich in fruits and vegetables and taking supplements will be reviewed and experimental and epidemiological evidences discussed, highlighting that antioxidants at physiological doses are generally safe, exhibiting interesting health beneficial effects.] Bouayed J, Bohn T. *Oxid Med Cell Longev.* 2010 Jul-Aug; 3(4): 228–237. doi: 10.4161/oxim.3.4.12858. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2952083/>

Free radicals in the physiological control of cell function. [At high concentrations, free radicals and radical-derived, nonradical reactive species are hazardous for living organisms and damage all major cellular constituents. At moderate concentrations, however, nitric oxide (NO), superoxide anion, and related reactive oxygen species (ROS) play an important role as regulatory mediators in signaling processes. Many of the ROS-mediated responses actually protect the cells against oxidative stress and reestablish "redox homeostasis." Higher organisms, however, have evolved the use of NO and ROS also as signaling molecules for other physiological functions. These include regulation of vascular tone, monitoring of oxygen tension in the control of ventilation and erythropoietin production, and signal transduction from membrane receptors in various physiological processes. NO and ROS are typically generated in these cases by tightly regulated enzymes such as NO synthase (NOS) and NAD(P)H oxidase isoforms, respectively. In a given signaling protein, oxidative attack induces either a loss of function, a gain of function, or a switch to a different function. Excessive amounts of ROS may arise either from excessive stimulation of NAD(P)H oxidases or from less well-regulated sources such as the mitochondrial electron-transport chain. In mitochondria, ROS are generated as undesirable side products of the oxidative energy metabolism. An excessive and/or sustained increase in ROS production has been implicated in the pathogenesis of cancer, diabetes mellitus, atherosclerosis, neurodegenerative diseases, rheumatoid arthritis, ischemia/reperfusion injury, obstructive sleep apnea, and other diseases. In addition, free radicals have been implicated in the mechanism of senescence. That the process of aging may result, at

least in part, from radical-mediated oxidative damage was proposed more than 40 years ago by Harman (J Gerontol 11: 298-300, 1956). There is growing evidence that aging involves, in addition, progressive changes in free radical-mediated regulatory processes that result in altered gene expression.] Droge W. Physiol Rev. 2002 Jan;82(1):47-95. <https://www.ncbi.nlm.nih.gov/pubmed/11773609>

Glutathione and immune function. [The immune system works best if the lymphoid cells have a delicately balanced intermediate level of glutathione. Even moderate changes in the intracellular glutathione level have profound effects on lymphocyte functions. Certain functions, such as the DNA synthetic response, are exquisitely sensitive to reactive oxygen intermediates and, therefore, are favoured by high levels of the antioxidant glutathione. Certain signal pathways, in contrast, are enhanced by oxidative conditions and favoured by low intracellular glutathione levels. The available evidence suggests that the lymphocytes from healthy human subjects have, on average, an optimal glutathione level. There is no indication that immunological functions such as resistance to infection or the response to vaccination may be enhanced in healthy human subjects by administration of glutathione or its precursor amino acid cysteine. However, immunological functions in diseases that are associated with a cysteine and glutathione deficiency may be significantly enhanced and potentially restored by cysteine supplementation. This factor has been studied most extensively in the case of human immunodeficiency virus (HIV)-infected patients who were found to experience, on average, a massive loss of S equivalent to a net loss of approximately 4 g cysteine/d. Two randomized placebo-controlled trials have shown that treatment of HIV-infected patients with N-acetyl-cysteine caused in both cases a significant increase in all immunological functions under test, including an almost complete restoration of natural killer cell activity. It remains to be tested whether cysteine supplementation may be useful also in other diseases and conditions that are associated with a low mean plasma cysteine level and impaired immunological functions.] Droge W, Breitkreutz R. Proc Nutr Soc. 2000 Nov;59(4):595-600. <https://www.ncbi.nlm.nih.gov/pubmed/11115795>

How mitochondria produce reactive oxygen species. [The production of ROS (reactive oxygen species) by mammalian mitochondria is important because it underlies oxidative damage in many pathologies and contributes to retrograde redox signalling from the organelle to the cytosol and nucleus. Superoxide ($O_2^{\bullet-}$) is the proximal mitochondrial ROS, and in the present review I outline the principles that govern $O_2^{\bullet-}$ production within the matrix of mammalian mitochondria. The flux of $O_2^{\bullet-}$ is related to the concentration of potential electron donors, the local concentration of O_2 and the second-order rate constants for the reactions between them. Two modes of operation by isolated mitochondria result in significant $O_2^{\bullet-}$ production, predominantly from complex I: (i) when the mitochondria are not making ATP and consequently have a high Δp (protonmotive force) and a reduced CoQ (coenzyme Q) pool; and (ii) when there is a high NADH/NAD⁺ ratio in the mitochondrial matrix. For mitochondria that are actively making ATP, and consequently have a lower Δp and NADH/NAD⁺ ratio, the extent of $O_2^{\bullet-}$ production is far lower. The generation of $O_2^{\bullet-}$ within the mitochondrial matrix depends critically on Δp , the NADH/NAD⁺ and CoQH₂/CoQ ratios and the local O_2 concentration, which are all highly variable and difficult to measure in vivo. Consequently, it is not possible to estimate $O_2^{\bullet-}$ generation by mitochondria in vivo from $O_2^{\bullet-}$ -production rates by isolated mitochondria, and such extrapolations in the literature are misleading. Even so, the description outlined here facilitates the understanding of factors that favour mitochondrial ROS production. There is a clear need to develop better methods to measure mitochondrial $O_2^{\bullet-}$ and H₂O₂ formation in vivo, as uncertainty about these values hampers studies on the role of mitochondrial ROS in pathological oxidative damage and redox signaling.] Murphy MP. Biochem J. 2009 Jan 1; 417(Pt 1): 1–13. Published online 2008 Dec 12. doi: [10.1042/BJ20081386]. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2605959/>

Mitochondrial generation of superoxide and hydrogen peroxide as the source of mitochondrial redox signaling. [This review examines the generation of reactive oxygen species by mammalian mitochondria, and the status of different sites of production in redox signaling and pathology. Eleven distinct mitochondrial sites associated with substrate oxidation and oxidative phosphorylation leak electrons to oxygen to produce superoxide or hydrogen peroxide: oxoacid dehydrogenase complexes that feed electrons to NAD⁺; respiratory complexes I and III, and dehydrogenases, including complex II, that use ubiquinone as acceptor. The topologies, capacities, and substrate dependences of each site have recently clarified. Complex III and mitochondrial glycerol 3-phosphate dehydrogenase generate superoxide to the external side of the mitochondrial inner membrane as well as the matrix, the other sites generate superoxide and/or hydrogen peroxide exclusively in the matrix. These different site-specific topologies are important for redox signaling. The net rate of superoxide or hydrogen peroxide generation depends on the substrates present and the antioxidant systems active in the matrix and cytosol. The rate at each site can now be measured in complex substrate mixtures. In skeletal muscle mitochondria in media mimicking muscle cytosol at rest, four sites dominate, two in complex I and one each in complexes II and III. Specific suppressors of two sites have been identified, the outer ubiquinone-binding site in complex III (site IIIQ_o) and the site in complex I active during reverse electron transport (site IQ). These suppressors prevent superoxide/hydrogen peroxide production from a specific site without affecting oxidative phosphorylation, making them excellent tools to investigate the status of the sites in redox signaling, and to suppress the sites to prevent pathologies. They allow the cellular roles of mitochondrial superoxide/hydrogen peroxide production to be investigated without catastrophic confounding bioenergetic effects. They show that sites IIIQ_o and IQ are active in cells and have important roles in redox signaling (e.g. hypoxic signaling and ER-stress) and in causing oxidative damage in a variety of biological contexts.] Brand MD. Free Radic Biol Med. 2016 Nov;100:14-31. doi: 10.1016/j. <https://www.ncbi.nlm.nih.gov/pubmed/27085844>

Mitochondrial redox signalling at a glance. [Redox signaling occurs when a biological system alters in response to a change in the level of a particular reactive oxygen species (ROS) or the shift in redox state of a responsive group such as a dithiol–disulphide couple (D'Autreaux and Toledano, 2007; Finkel, 2011; Fourquet et al., 2008; Janssen-Heininger et al., 2008; Rhee, 2006). Although ROS are best known as damaging agents in pathology, a more nuanced view has developed. It is now clear that some ROS, such as hydrogen peroxide (H₂O₂), can act as messengers both in the extracellular environment and within cells (D'Autreaux and Toledano, 2007; Fourquet et al., 2008; Janssen-Heininger et al., 2008; Rhee, 2006). Mitochondria seem to be an important redox signalling node, partly because of the flux of the ROS superoxide (O₂^{•-}) generated by the respiratory chain and other core metabolic machineries within mitochondria (Balaban et al., 2005; Finkel, 2011; Murphy, 2009a). In addition, the mitochondrial matrix is central to metabolism, as oxidative phosphorylation, the citric acid cycle, fatty acid oxidation, the urea cycle and the biosynthesis of iron sulphur centres and haem take place there. Furthermore, mitochondria have key roles in apoptosis, calcium homeostasis and oxygen sensing (Duchen, 2004; Murphy, 2009a; Murphy, 2009b). Consequently, mitochondria are at the core of many biological processes, and redox signals to and from this organelle help to integrate mitochondrial function with that of the cell and organism. In this Cell Science at a Glance article we outline how mitochondrial redox signals are produced and modulated, the mechanisms by which redox signals can alter mitochondrial function and the experimental procedures available to assess this.] Collins Y, Chouchani ET, et al. *J Cell Sci* 2012 125: 801-806; doi: 10.1242/jcs.098475. <http://jcs.biologists.org/content/125/4/801>

Molecular and cellular responses to oxidative stress and changes in oxidation-reduction imbalance in the intestine. [Recently, it has become increasingly apparent that oxidants, in addition to being agents of cytotoxicity, can play an important role in mediating specific cell responses and expression of genes involved in degenerative pathophysiological states, such as inflammation and cancer. In particular, nuclear transcription factor kappaB (NF-kappaB), a multisubunit transcription factor, has been implicated in the transcriptional up-regulation of inflammatory genes in response to oxidants or changes in cellular oxidation-reduction status. This paper provides an overview of the cellular responses to oxidative stress and oxidation-reduction imbalance and the role of NF-kappaB in these responses and summarizes the current strategies used to study NF-kappaB activation and nuclear translocation, particularly in relation to dietary oxidant-mediated pathophysiology of the intestine.] Aw TY. *Am J Clin Nutr.* 1999 Oct;70(4):557-65. <https://www.ncbi.nlm.nih.gov/pubmed/10500026>

Molecular Inflammation as an Underlying Mechanism of the Aging Process and Age-related Diseases. [Aging is a biological process characterized by time-dependent functional declines that are influenced by changes in redox status and by oxidative stress-induced inflammatory reactions. An organism's pro-inflammatory status may underlie the aging process and age-related diseases. In this review, we explore the molecular basis of low-grade, unresolved, subclinical inflammation as a major risk factor for exacerbating the aging process and age-related diseases. We focus on the redox-sensitive transcription factors, NF-κB and FOXO, which play essential roles in the expression of pro-inflammatory mediators and anti-oxidant enzymes, respectively. Major players in molecular inflammation are discussed with respect to the age-related up-regulation of pro-inflammatory cytokines and adhesion molecules, cyclo-oxygenase-2, lipoxygenase, and inducible nitric oxide synthase. The molecular inflammation hypothesis proposed by our laboratory is briefly described to give further molecular insights into the intricate interplay among redox balance, pro-inflammatory gene activation, and chronic age-related inflammatory diseases. The final section discusses calorie restriction as an aging-retarding intervention that also exhibits extraordinarily effective anti-inflammatory activity by modulating GSH redox, NF-κB, SIRT1, PPARs, and FOXOs.]. Chung HY, Lee EK, et al. *Journal of Dental Research*, Volume: 90 issue: 7, page(s): 830-840. <https://journals.sagepub.com/doi/abs/10.1177/0022034510387794>.

Oxidative stress and autophagy: the clash between damage and metabolic needs. [Autophagy is a catabolic process aimed at recycling cellular components and damaged organelles in response to diverse conditions of stress, such as nutrient deprivation, viral infection and genotoxic stress. A growing amount of evidence in recent years argues for oxidative stress acting as the converging point of these stimuli, with reactive oxygen species (ROS) and reactive nitrogen species (RNS) being among the main intracellular signal transducers sustaining autophagy. This review aims at providing novel insight into the regulatory pathways of autophagy in response to glucose and amino acid deprivation, as well as their tight interconnection with metabolic networks and redox homeostasis. The role of oxidative and nitrosative stress in autophagy is also discussed in the light of its being harmful for both cellular biomolecules and signal mediator through reversible posttranslational modifications of thiol-containing proteins. The redox-independent relationship between autophagy and antioxidant response, occurring through the p62/Keap1/Nrf2 pathway, is also addressed in order to provide a wide perspective upon the interconnection between autophagy and oxidative stress. Herein, we also attempt to afford an overview of the complex crosstalk between autophagy and DNA damage response (DDR), focusing on the main pathways activated upon ROS and RNS overproduction. Along these lines, the direct and indirect role of autophagy in DDR is dissected in depth.] Filomeni G, DeZio D, et al. *Cell Death and Differentiation* volume 22, pages 377–388 (2015). <https://www.nature.com/articles/cdd2014150>

Oxidative Stress, Radiation-Adaptive Responses, and Aging. [Organisms living in an aerobic environment were forced to evolve effective cellular strategies to detoxify reactive oxygen species. Besides diverse antioxidant enzymes and compounds, DNA repair enzymes, and disassembly systems, which remove damaged proteins, regulation systems that control transcription, translation, and

activation have also been developed. The adaptive responses, especially those to radiation, are defensive regulation mechanisms by which oxidative stress (conditioning irradiation) elicits a response against damage because of subsequent stress (challenging irradiation). Although many researchers have investigated these molecular mechanisms, they remain obscure because of their complex signaling pathways and the involvement of various proteins. This article reviews the factors concerned with radiation-adaptive response, the signaling pathways activated by conditioning irradiation, and the effects of aging on radiation-adaptive response. The proteomics approach is also introduced, which is a useful method for studying stress response in cells.] Miura Y. *Journal of Radiation Research* 45(3):357-72 · October 2004. *Journal of Radiation Research* 45(3):357-72 · October 2004 https://www.researchgate.net/publication/8116191_Oxidative_Stress_Radiation-Adaptive_Responses_and_Aging

Oxidative Stress: A Unifying Mechanism for Cell Damage Induced by Noise, (Water-Pipe) Smoking, and Emotional Stress-Therapeutic Strategies Targeting Redox Imbalance. [SIGNIFICANCE: Modern technologies have eased our lives but these conveniences can impact our lifestyles in destructive ways. Noise pollution, mental stresses, and smoking (as a stress-relieving solution) are some environmental hazards that affect our well-being and healthcare budgets. Scrutinizing their pathophysiology could lead to solutions to reduce their harmful effects. Recent Advances: Oxidative stress plays an important role in initiating local and systemic inflammation after noise pollution, mental stress, and smoking. Lipid peroxidation and release of lysolipid by-products, disturbance in activation and function of nuclear factor erythroid 2-related factor 2 (Nrf2), induction of stress hormones and their secondary effects on intracellular kinases, and dysregulation of intracellular Ca²⁺ can all potentially trigger other vicious cycles. Recent clinical data suggest that boosting the antioxidant system through nonpharmacological measures, for example, lifestyle changes that include exercise have benefits that cannot easily be achieved with pharmacological interventions alone. CRITICAL ISSUES: Indiscriminate manipulation of the cellular redox network could lead to a new series of ailments. An ideal approach requires meticulous scrutiny of redox balance mechanisms for individual pathologies so as to create new treatment strategies that target key pathways while minimizing side effects. FUTURE DIRECTIONS: Extrapolating our understanding of redox balance to other debilitating conditions such as diabetes and the metabolic syndrome could potentially lead to devising a unifying therapeutic strategy.] Golbidi S, Li H, et al. *Antioxid. Redox Signal.* 28, 741-759. <https://www.ncbi.nlm.nih.gov/pubmed/29212347>

Oxidative stress and chronic inflammation in osteoarthritis: can NRF2 counteract these partners in crime? [Osteoarthritis (OA) is an age-related joint degenerative disease associated with pain, joint deformity, and disability. The disease starts with cartilage damage but then progressively involves subchondral bone, causing an imbalance between osteoclast-driven bone resorption and osteoblast-driven remodeling. Here, we summarize the data for the role of oxidative stress and inflammation in OA pathology and discuss how these two processes are integrated during OA progression, as well as their contribution to abnormalities in cartilage/bone metabolism and integrity. At the cellular level, oxidative stress and inflammation are counteracted by transcription factor nuclear factor erythroid p45-related factor 2 (NRF2), and we describe the regulation of NRF2, highlighting its role in OA pathology. We also discuss the beneficial effect of some phytonutrients, including the therapeutic potential of NRF2 activation, in OA.] Marchev AS, Dimitrova PA, et al. *Anal. of the New York Academy of Sciences*, Vol 1401, Issue 1, Aug 2017. <https://nyaspubs.onlinelibrary.wiley.com/doi/abs/10.1111/nyas.13407>

Oxidative stress, redox signaling, and autophagy: cell death versus survival. [SIGNIFICANCE: The molecular machinery regulating autophagy has started becoming elucidated, and a number of studies have undertaken the task to determine the role of autophagy in cell fate determination within the context of human disease progression. Oxidative stress and redox signaling are also largely involved in the etiology of human diseases, where both survival and cell death signaling cascades have been reported to be modulated by reactive oxygen species (ROS) and reactive nitrogen species (RNS). RECENT ADVANCES: To date, there is a good understanding of the signaling events regulating autophagy, as well as the signaling processes by which alterations in redox homeostasis are transduced to the activation/regulation of signaling cascades. However, very little is known about the molecular events linking them to the regulation of autophagy. This lack of information has hampered the understanding of the role of oxidative stress and autophagy in human disease progression. CRITICAL ISSUES: In this review, we will focus on (i) the molecular mechanism by which ROS/RNS generation, redox signaling, and/or oxidative stress/damage alter autophagic flux rates; (ii) the role of autophagy as a cell death process or survival mechanism in response to oxidative stress; and (iii) alternative mechanisms by which autophagy-related signaling regulate mitochondrial function and antioxidant response. FUTURE DIRECTIONS: Our research efforts should now focus on understanding the molecular basis of events by which autophagy is fine tuned by oxidation/reduction events. This knowledge will enable us to understand the mechanisms by which oxidative stress and autophagy regulate human diseases such as cancer and neurodegenerative disorders.] Navarro-Yepes J, Burns M, et al. *Antioxid Redox Signal.* 2014 Jul 1;21(1):66-85. <https://www.ncbi.nlm.nih.gov/pubmed/24483238>

Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. [Reactive oxygen species (ROS) are generated during mitochondrial oxidative metabolism as well as in cellular response to xenobiotics, cytokines, and bacterial invasion. Oxidative stress refers to the imbalance due to excess ROS or oxidants over the capability of the cell to mount an effective antioxidant response. Oxidative stress results in macromolecular damage and is implicated in various disease states such as atherosclerosis, diabetes, cancer, neurodegeneration, and aging. Paradoxically, accumulating evidence indicates that ROS also serve as critical

signaling molecules in cell proliferation and survival. While there is a large body of research demonstrating the general effect of oxidative stress on signaling pathways, less is known about the initial and direct regulation of signaling molecules by ROS, or what we term the “oxidative interface.” Cellular ROS sensing and metabolism are tightly regulated by a variety of proteins involved in the redox (reduction/oxidation) mechanism. This review focuses on the molecular mechanisms through which ROS directly interact with critical signaling molecules to initiate signaling in a broad variety of cellular processes, such as proliferation and survival (MAP kinases, PI3 kinase, PTEN, and protein tyrosine phosphatases), ROS homeostasis and antioxidant gene regulation (thioredoxin, peroxiredoxin, Ref-1, and Nrf-2), mitochondrial oxidative stress, apoptosis, and aging (p66Shc), iron homeostasis through iron–sulfur cluster proteins (IRE–IRP), and ATM-regulated DNA damage response.] Ray, PD, Huang B, et al. *Cell Signal*. 2012 May; 24(5): 981–990. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3454471/>

Reactive oxygen species in cell signaling. [Reactive oxygen species (ROS) are generated as by-products of cellular metabolism, primarily in the mitochondria. When cellular production of ROS overwhelms its antioxidant capacity, damage to cellular macromolecules such as lipids, protein, and DNA may ensue. Such a state of “oxidative stress” is thought to contribute to the pathogenesis of a number of human diseases including those of the lung. Recent studies have also implicated ROS that are generated by specialized plasma membrane oxidases in normal physiological signaling by growth factors and cytokines. In this review, we examine the evidence for ligand-induced generation of ROS, its cellular sources, and the signaling pathways that are activated. Emerging concepts on the mechanisms of signal transduction by ROS that involve alterations in cellular redox state and oxidative modifications of proteins are also discussed.] Thannickal VJ, Fanburg BL. *Am J Physiol Lung Cell Mol Physiol*. 2000 Dec;279(6):L1005-28. <https://www.ncbi.nlm.nih.gov/pubmed/11076791>

Recent advances towards understanding redox mechanisms in the activation of nuclear factor kappaB. [The transcription factor, nuclear factor-kappaB (NF-kappaB) has been studied extensively due to its prominent role in the regulation of immune and inflammatory genes, apoptosis, and cell proliferation. It has been known for more than a decade that NF-kappaB is a redox-sensitive transcription factor. The contribution of redox regulation and the location of potential redox-sensitive sites within the NF-kappaB activation pathway are subject to intense debate due to many conflicting reports. Redox regulation of NF-kappaB has been extensively addressed in this journal and the reader is referred to two comprehensive reviews on the subject [1,2]. With the identification of signaling intermediates proximal to the degradation of the inhibitor, I-kappaB, the number of potential redox-sensitive sites is rapidly increasing. The purpose of this review is to address recent insights into the NF-kappaB signaling cascades that are triggered by proinflammatory cytokines such as TNF-alpha and IL-1beta. In addition, the role of nitrogen monoxide (.NO) in the regulation of NF-kappaB will be reviewed. Opportunities for redox regulation that occur upstream of I-kappaB-alpha degradation, as well as the potential for redox control of phosphorylation of NF-kappaB subunits, will be discussed. Redox-sensitive steps are likely to depend on the nature of the NF-kappaB activator, the type of reactive oxygen or nitrogen species involved, the selectivity of signaling pathways activated, as well as the cell type under investigation. Lastly, it is discussed how redox regulation of NF-kappaB activation is likely to involve multiple subcellular compartments.] Janssen-Heininger YM, et al. *Free Radic Biol Med*. 2000 May 1;28(9):1317-27. <https://www.ncbi.nlm.nih.gov/pubmed/10924851>

Redox Control of the Cell Cycle in Health and Disease. [The cellular oxidation and reduction (redox) environment is influenced by the production and removal of reactive oxygen species (ROS). In recent years, several reports support the hypothesis that cellular ROS levels could function as “second messengers” regulating numerous cellular processes, including proliferation. Periodic oscillations in the cellular redox environment, a redox cycle, regulate cell-cycle progression from quiescence (G0) to proliferation (G1, S, G2, and M) and back to quiescence. A loss in the redox control of the cell cycle could lead to aberrant proliferation, a hallmark of various human pathologies. This review discusses the literature that supports the concept of a redox cycle controlling the mammalian cell cycle, with an emphasis on how this control relates to proliferative disorders including cancer, wound healing, fibrosis, cardiovascular diseases, diabetes, and neurodegenerative diseases. We hypothesize that reestablishing the redox control of the cell cycle by manipulating the cellular redox environment could improve many aspects of the proliferative disorders.] Sarsour EH, Kumar MG, et al. *Antioxidants & Redox Signaling* Vol. 11, No. 12. <https://www.liebertpub.com/doi/abs/10.1089/ARS.2009.2513>

Redox control of transcription: sensors, response regulators, activators and repressors. [In a growing number of cases, transcription of specific genes is known to be governed by oxidation or reduction of electron carriers with which the gene products interact. The biological function of such control is to activate synthesis of appropriate redox proteins, and to repress synthesis of inappropriate ones, in response to altered availability of specific electron sources and sinks. In prokaryotic systems this control appears to operate by two general classes of mechanism: by two-component regulation involving protein phosphorylation on histidine and aspartate; and by direct oxidation-reduction of gene repressors or activators. For the first class, termed ‘two-component redox regulation’, the term ‘redox sensor’ is proposed for any electron carrier that becomes phosphorylated upon oxidation or reduction and thereby controls phosphorylation of specific response regulators, while the term ‘redox response regulator’ is proposed for the corresponding sequence-specific DNA-binding protein that controls transcription as a result of its phosphorylation by one or more redox sensors. For the second class of redox regulatory mechanism, the terms ‘redox activator protein’ and ‘redox repressor protein’ are proposed for single proteins containing both electron transfer and sequence-specific DNA-binding domains. The structure, function

and biological distribution of these components are discussed.] Allen JF. FEBS Lett. 1993 Oct 18;332(3):203-7. <https://www.ncbi.nlm.nih.gov/pubmed/8405457>

Redox mechanisms in age-related lung fibrosis. [Redox signaling and oxidative stress are associated with tissue fibrosis and aging. Aging is recognized as a major risk factor for fibrotic diseases involving multiple organ systems, including that of the lung. A number of oxidant generating enzymes are upregulated while antioxidant defenses are deficient with aging and cellular senescence, leading to redox imbalance and oxidative stress. However, the precise mechanisms by which redox signaling and oxidative stress contribute to the pathogenesis of lung fibrosis are not well understood. Tissue repair is a highly regulated process that involves the interactions of several cell types, including epithelial cells, fibroblasts and inflammatory cells. Fibrosis may develop when these interactions are dysregulated with the acquisition of pro-fibrotic cellular phenotypes. In this review, we explore the roles of redox mechanisms that promote and perpetuate fibrosis in the context of cellular senescence and aging.] Kurundkar A, Thannickal VJ. Redox Biol. 2016 Oct;9:67-76. doi: 10.1016/j.redox.2016.06.005. Epub 2016 Jun 25. <https://www.ncbi.nlm.nih.gov/pubmed/27394680>

Redox Proteomics: A Key Tool for New Insights into Protein Modification with Relevance to Disease. [Oxidatively modified proteins are characterized by elevations in protein-resident carbonyls or 3-nitrotyrosine, measures of protein oxidation, or protein bound reactive alkenals such as 4-hydroxy-2-nonenal, a measure of lipid peroxidation. Oxidatively modified proteins nearly always have altered structure and function. Redox proteomics is that branch of proteomics used to identify oxidized proteins and determine the extent and location of oxidative modifications in the proteomes of interest. This technique nearly always employs mass spectrometry as the major platform to achieve the goals of identifying the target proteins. Once identified, oxidatively modified proteins can be placed in specific molecular pathways to provide insights into protein oxidation and human disease. Both original research and review articles are included in this Forum on Redox Proteomics. The topics related to redox proteomics range from basic chemistry of sulfur radical-induced redox modifications in proteins, to the thiol secretome and inflammatory network, to reversible thiol oxidation in proteomes, to the role of glutamine synthetase in peripheral and central environments on inflammation and insulin resistance, to bioanalytical aspects of tyrosine nitrated proteins, to protein oxidation in human smokers and models thereof, and to Alzheimer disease, including articles on the brain ubiquitinylome and the “triangle of death” composed of oxidatively modified proteins involved in energy metabolism, mammalian target of rapamycin activation, and the proteostasis network. This Forum on Redox Proteomics is both timely and a critically important resource to highlight one of the key tools needed to better understand protein structure and function in oxidative environments in health and disease.] Butterfield DA, Perluigi M. Antioxid. Redox Signal. 26, 277–279. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5327050/>

Redox regulation in regenerative medicine and tissue engineering: The paradox of oxygen. [One of the biggest challenges in tissue engineering and regenerative medicine is to incorporate a functioning vasculature to overcome the consequences of a lack of oxygen and nutrients in the tissue construct. Otherwise, decreased oxygen tension leads to incomplete metabolism and the formation of the so-called reactive oxygen species (ROS). Cells have many endogenous antioxidant systems to ensure a balance between ROS and antioxidants, but if this balance is disrupted by factors such as high levels of ROS due to long-term hypoxia, there will be tissue damage and dysfunction. Current attempts to solve the oxygen problem in the field rarely take into account the importance of the redox balance and are instead centred on releasing or generating oxygen. The first problem with this approach is that although oxygen is necessary for life, it is paradoxically also a highly toxic molecule. Furthermore, although some oxygen-generating biomaterials produce oxygen, they also generate hydrogen peroxide, a ROS, as an intermediate product. In this review, we discuss why it would be a superior strategy to supplement oxygen delivery with molecules to safeguard the important redox balance. Redox sensor proteins that can stimulate the anaerobic metabolism, angiogenesis, and enhancement of endogenous antioxidant systems are discussed as promising targets. We propose that redox regulating biomaterials have the potential to tackle some of the challenges related to angiogenesis and that the knowledge in this review will help scientists in tissue engineering and regenerative medicine realize this aim.] Sthins MM, van Blitterswijk CA et al. Send to J Tissue Eng Regen Med. 2018 Oct;12(10):2013-2020. doi: 10.1002/term.2730. <https://www.ncbi.nlm.nih.gov/pubmed/30044552>

Redox regulation of genome stability by effects on gene expression, epigenetic pathways and DNA damage/repair. [Reactive oxygen and nitrogen species (e.g. H₂O₂, nitric oxide) confer redox regulation of essential cellular signaling pathways such as cell differentiation, proliferation, migration and apoptosis. In addition, classical regulation of gene expression or activity, including gene transcription to RNA followed by translation to the protein level, by transcription factors (e.g. NF-κB, HIF-1α) and mRNA binding proteins (e.g. GAPDH, HuR) is subject to redox regulation. This review will give an update of recent discoveries in this field, and specifically highlight the impact of reactive oxygen and nitrogen species on DNA repair systems that contribute to genomic stability. Emphasis will be placed on the emerging role of redox mechanisms regulating epigenetic pathways (e.g. miRNA, DNA methylation and histone modifications). By providing clinical correlations we discuss how oxidative stress can impact on gene regulation/activity and vice versa, how epigenetic processes, other gene regulatory mechanisms and DNA repair can influence the cellular redox state and contribute or prevent development or progression of disease.] Redox Biol. 2015 Aug; 5: 275–289. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4475862/>

Redox Regulation of NF- κ B Activation: Distinct Redox Regulation Between the Cytoplasm and the Nucleus.

[Reduction/oxidation (redox) regulation mediates numerous cellular responses and contributes to several physiological diseases. The transcription factor nuclear factor κ B (NF- κ B) is known to be a redox-sensitive factor. NF- κ B plays a central role in immune responses and inflammation, through regulation of the gene expression of a large number of cytokines and other immune response genes. NF- κ B is trapped in the cytoplasm in stimulated cells and translocates into the nucleus in response to several stimuli, including oxidative stress. Reactive oxygen species enhance the signal transduction pathways for NF- κ B activation in the cytoplasm and translocation into the nucleus. In contrast, the DNA binding activity of oxidized NF- κ B is significantly diminished, and that activity is restored by reducing enzymes, such as thioredoxin or redox factor 1. This review describes the signal transduction pathways for NF- κ B activation and redox regulation of NF- κ B activation in the cytoplasm and nucleus.] *Antioxid. Redox Signal.* 7, 395–403. <https://www.liebertpub.com/doi/10.1089/ars.2005.7.395>

Redox Regulation of T-Cell Function: From Molecular Mechanisms to Significance in Human Health and Disease. [Reactive oxygen species (ROS) are thought to have effects on T-cell function and proliferation. Low concentrations of ROS in T cells are a prerequisite for cell survival, and increased ROS accumulation can lead to apoptosis/necrosis. The cellular redox state of a T cell can also affect T-cell receptor signaling, skewing the immune response. Various T-cell subsets have different redox statuses, and this differential ROS susceptibility could modulate the outcome of an immune response in various disease states. Recent advances in T-cell redox signaling reveal that ROS modulate signaling cascades such as the mitogen-activated protein kinase, phosphoinositide 3-kinase (PI3K)/AKT, and JAK/STAT pathways. Also, tumor microenvironments, chronic T-cell stimulation leading to replicative senescence, gender, and age affect T-cell susceptibility to ROS, thereby contributing to diverse immune outcomes. Antioxidants such as glutathione, thioredoxin, superoxide dismutase, and catalase balance cellular oxidative stress. T-cell redox states are also regulated by expression of various vitamins and dietary compounds. Changes in T-cell redox regulation may affect the pathogenesis of various human diseases. Many strategies to control oxidative stress have been employed for various diseases, including the use of active antioxidants from dietary products and pharmacologic or genetic engineering of antioxidant genes in T cells. Here, we discuss the existence of a complex web of molecules/factors that exogenously or endogenously affect oxidants, and we relate these molecules to potential therapeutics.] Kesarwani P, Murali AK, et al. *Antioxid. Redox Signal.* 18, 1497–1534. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3603502/>

Redox Regulation of the Nuclear Factor Kappa B (NF- κ B) Signaling Pathway and Disease Control. [Nuclear factor kappa B (NF- κ B) is an inducible cellular transcription factor that activates various cellular and viral genes. NF- κ B usually exists as a molecular complex with an inhibitory molecule, I κ B, in the cytosol. On stimulation of the cells, such as by proinflammatory cytokines IL-1 and tumor necrosis factor (TNF), I κ B is dissociated and NF- κ B is translocated to the nucleus and activates the expression of the target genes. We found that a redox control mechanism is involved in the DNA-binding activity of NF- κ B and that a cellular-reducing catalyst thioredoxin (Trx), together with kinases, is primarily involved as an effector molecule in this signaling pathway. Trx was recently demonstrated to associate with the redox-sensitive cysteine within the DNA-binding loop of NF- κ B. Effects of antioxidants in blocking NF- κ B activation can be explained by the involvement of radical oxygen intermediates (ROI) in this pathway. These findings support the idea that redox regulation involving ROI and Trx plays a crucial role in the signal transduction pathway leading to NF- κ B activation, thus contributing substantially to understanding of the pathogenetic processes of various diseases including AIDS, hematogenic cancer cell metastasis, and rheumatoid arthritis (RA). Okamoto T, Sakurada S, et al. *Oxygen Homeostasis and Its Dynamics. Keio University Symposia for Life Science and Medicine*, vol 1. Springer, Tokyo. https://doi.org/10.1007/978-4-431-68476-3_55 https://link.springer.com/chapter/10.1007%2F978-4-431-68476-3_55

Redox regulation: a new challenge for pharmacology. [Redox signaling is evolving as a new field of biochemical and pharmacological research. Unlike oxidative stress which is characterized by a macroscopic shift in cellular redox potentials and usually accompanied by oxygen radical induced damage, redox regulation involves subtle and more chemically defined oxidations of short duration. Most important is the reductive component as a necessary part of a reversible regulatory process. Examples of redox regulation occur during early stages of the immune response, in hypoxia or in endothelial dysfunction. Persistent oxidative events together with a decline in the cellular reduction potential lead to oxidative stress as is seen in the pathophysiology of sepsis, reperfusion damage, atherosclerosis and diabetes. Oxidative signals involve superoxide and nitric oxide as the main players which form a system of oxidizing, nitrating or nitrosating species leading to posttranslational modifications of proteins. Modern techniques of immunohistochemistry and mass spectrometry allow a correlation of protein modification, e.g., disulfide, S-oxide, S-nitroso or nitrotyrosine formation, with enzyme activities and cellular responses. In this commentary, examples of the control of prostanoid synthesis by the NO/O₂- system are described. Redox regulation represents an interesting challenge for the development of drugs that modulate the oxidative trigger mechanisms or enforce the reductive pathways.] Frein D, Schildknecht S, et al. *Biochem Pharmacol.* 2005 Sep 15;70(6):811-23. <https://www.ncbi.nlm.nih.gov/pubmed/15899473>

Redox-Sensitive Signaling Factors and Antioxidants. [The redox state, like the pH or the osmotic pressure, represents chemical characteristics of the intracellular environment. The intracellular redox homeostasis can be disturbed by the installation of the “oxidative stress”, which manifests itself through the dysregulation of the balance between the systems that produce oxidant agents

and the antioxidant defense mechanisms (the redox balance). Essential cellular functions, such as gene expression, are influenced by the balance between pro- and antioxidant conditions. The mechanism by which the transcription of specific eukaryotic genes can be redox regulated, is complex, however, recent findings suggest that redox-sensitive transcription factors play an essential role in this process. This review is focused on the recent knowledge concerning some eukaryotic transcription factors, whose activation and DNA binding is controlled by the thiol redox status of the cell.[Mohora M, Greabu M, et al. *FARMACIA*, 2009, Vol. 57, 4 <http://www.revistafarmacia.ro/20094/issue42009art01.pdf>

Redox signaling: Potential arbitrator of autophagy and apoptosis in therapeutic response. [Redox signaling plays important roles in the regulation of cell death and survival in response to cancer therapy. Autophagy and apoptosis are discrete cellular processes mediated by distinct groups of regulatory and executioner molecules, and both are thought to be cellular responses to various stress conditions including oxidative stress, therefore controlling cell fate. Basic levels of reactive oxygen species (ROS) may function as signals to promote cell proliferation and survival, whereas increase of ROS can induce autophagy and apoptosis by damaging cellular components. Growing evidence in recent years argues for ROS that below detrimental levels acting as intracellular signal transducers that regulate autophagy and apoptosis. ROS-regulated autophagy and apoptosis can cross-talk with each other. However, how redox signaling determines different cell fates by regulating autophagy and apoptosis remains unclear. In this review, we will focus on understanding the delicate molecular mechanism by which autophagy and apoptosis are finely orchestrated by redox signaling and discuss how this understanding can be used to develop strategies for the treatment of cancer.] Zhang L, Wang K, et al. *Free Radical Biology and Medicine*. Volume 89, December 2015, Pages 452-465.

<https://www.sciencedirect.com/science/article/abs/pii/S0891584915006103>

Regulation of glutathione synthesis. [Glutathione (GSH) is a ubiquitous intracellular peptide with diverse functions that include detoxification, antioxidant defense, maintenance of thiol status, and modulation of cell proliferation. GSH is synthesized in the cytosol of all mammalian cells in a tightly regulated manner. The major determinants of GSH synthesis are the availability of cysteine, the sulfur amino acid precursor, and the activity of the rate-limiting enzyme, glutamate cysteine ligase (GCL). GCL is composed for a catalytic (GCLC) and modifier (GCLM) subunit and they are regulated at multiple levels and at times differentially. The second enzyme of GSH synthesis, GSH synthase (GS) is also regulated in a coordinated manner as GCL subunits and its up-regulation can further enhance the capacity of the cell to synthesize GSH. Oxidative stress is well known to induce the expression of GSH synthetic enzymes. Key transcription factors identified thus far include Nrf2/Nrf1 via the antioxidant response element (ARE), activator protein-1 (AP-1) and nuclear factor kappa B (NFkappaB). Dysregulation of GSH synthesis is increasingly being recognized as contributing to the pathogenesis of many pathological conditions. These include diabetes mellitus, pulmonary fibrosis, cholestatic liver injury, endotoxemia and drug-resistant tumor cells. Manipulation of the GSH synthetic capacity is an important target in the treatment of many of these disorders.] Lu SC. *Mol Aspects Med*. 2009 Feb-Apr;30(1-2):42-59. <https://www.ncbi.nlm.nih.gov/pubmed/18601945>

Regulation of inflammation and redox signaling by dietary polyphenols. [Reactive oxygen species (ROS) play a key role in enhancing the inflammation through the activation of NF-kappaB and AP-1 transcription factors, and nuclear histone acetylation and deacetylation in various inflammatory diseases. Such undesired effects of oxidative stress have been found to be controlled by the antioxidant and/or anti-inflammatory effects of dietary polyphenols such as curcumin (diferuloylmethane, a principal component of turmeric) and resveratrol (a flavonoid found in red wine). The phenolic compounds in fruits, vegetables, tea and wine are mostly derivatives, and/or isomers of flavones, isoflavones, flavonols, catechins, tocopherols, and phenolic acids. Polyphenols modulate important cellular signaling processes such as cellular growth, differentiation and host of other cellular features. In addition, they modulate NF-kappaB activation, chromatin structure, glutathione biosynthesis, nuclear redox factor (Nrf2) activation, scavenge effect of ROS directly or via glutathione peroxidase activity and as a consequence regulate inflammatory genes in macrophages and lung epithelial cells. However, recent data suggest that dietary polyphenols can work as modifiers of signal transduction pathways to elicit their beneficial effects. The effects of polyphenols however, have been reported to be more pronounced in vitro using high concentrations which are not physiological in vivo. This commentary discusses the recent data on dietary polyphenols in the control of signaling and inflammation particularly during oxidative stress, their metabolism and bioavailability.] *Biochem Pharmacol*. 2006 Nov 30;72(11):1439-52. Epub 2006 Aug 21. <https://www.ncbi.nlm.nih.gov/pubmed/16920072>

Regulation of oxidative stress by Nrf2 in the pathophysiology of infectious diseases. [The innate immune system, including phagocytic cells, is the first line of defense against pathogens. During infection by microorganisms such as viruses, bacteria, or parasites, phagocytic cells produce an excess of oxidants, a crucial process for the clearance of pathogens. This increase in oxidants creates an imbalance between oxidants and endogenous antioxidants. Left unchecked, this acute or chronic oxidative stress can lead to apoptotic cell-death and oxidative stress-induced diseases including neurodegenerative and cardiovascular disorders, premature aging, secondary infections, and cancer. The activation of nuclear factor E2-related factor 2 (Nrf2) is an efficient antioxidant defensive mechanism used by host cells to counteract oxidative stress. The transcription factor Nrf2 has been identified as the master regulator of several hundred of genes involved in the antioxidant defense response. The review objectives were to collect recent findings on the contribution of oxidative stress to complications of infection, and to highlight the beneficial impact of antioxidants in reducing inflammation and oxidant-related tissue damage. Furthermore, a direct relationship between infection and decline in Nrf2 activity has

been demonstrated. Thus, an interesting therapeutic approach in disease prevention and treatment of stress-related diseases may consist in optimizing antibiotic or antiviral therapy with a combination of Nrf2 inducer treatment.] Deramautd TB, Dill C, et al. *Med Mal Infect.* 2013 Mar;43(3):100-7. <https://www.ncbi.nlm.nih.gov/pubmed/23499316>

Simultaneous Activation of Nrf2 and Elevation of Dietary and Endogenous Antioxidant Chemicals for Cancer Prevention in Humans. [Despite extensive studies in cancer prevention, the incidence of cancer is increasing. We review studies that have identified several biochemical and genetic defects as well as potential carcinogens in the diet, environmental factors, and lifestyle-related habits. Two of the biochemical abnormalities increased oxidative stress and chronic inflammation, and chronic exposure to carcinogens and mutagens play a significant role in the initiation of multistage carcinogenesis. Therefore, attenuation of these biochemical defects may be useful in reducing the incidence of cancer. Activation of the transcriptional factor called nuclear factor (erythroid-derived 2)-like 2 (Nrf2), which enhances the levels of antioxidant enzymes and phase-2-detoxifying enzymes by complex mechanisms, may be one of the ways to reduce oxidative stress and chronic inflammation. Antioxidant enzymes destroy free radicals by catalysis, whereas phase-2-detoxifying enzymes remove potential carcinogens by converting them to harmless compounds for elimination from the body. However, increasing the levels of antioxidant enzymes by activating Nrf2 may not be sufficient to decrease oxidative stress and chronic inflammation optimally, because antioxidant chemicals, which are decreased in a high oxidative environment, must also be elevated. This review discusses the regulation of activation of Nrf2 and proposes a hypothesis that an elevation of the levels of antioxidant enzymes and dietary and endogenous antioxidant chemicals simultaneously may reduce the incidence of cancer by decreasing oxidative stress and chronic inflammation. The levels of antioxidant chemicals can be increased by supplementation, but increasing the levels of antioxidant enzymes requires activation of Nrf2 by reactive oxygen species (ROS)-dependent and-independent mechanisms. Several phytochemicals and antioxidant chemicals that activate Nrf2 have been identified. This review also describes clinical studies on antioxidants in cancer prevention that have produced inconsistent results. It discusses the possible reasons for the inconsistent results and proposes criteria that should be included in the experimental designs of future clinical studies to obtain consistent results. KEY TEACHING POINTS: • Reducing oxidative stress and chronic inflammation optimally requires an elevation of the levels of antioxidant enzymes and phase-2-detoxifying enzymes as well as dietary and endogenous antioxidant chemicals. • How the levels of antioxidant enzymes and phase-2-detoxifying enzymes are regulated by a nuclear transcriptional factor Nrf2. • How the activation and transcription of Nrf2 is regulated. • Identification of antioxidants that activate Nrf2 by ROS-dependent and-independent mechanisms, those that destroy free radicals by scavenging, and those that exhibit both functions. • Possible reasons for the inconsistent results produced by the previous clinical studies on antioxidants in cancer prevention. • The criteria that should be included in the experimental designs of future clinical studies on antioxidants in cancer prevention in high-risk populations to obtain consistent results.] Prasad Kn. *Am Coll Nutr.* 2016;35(2):175-84. <https://www.ncbi.nlm.nih.gov/pubmed/26151600>

Specificity in reactive oxidant signaling: think globally, act locally [Although reactive oxidants have long been stigmatized as unwanted metabolic byproducts, the expression of oxidases specifically functioning to produce these same molecules in a regulated fashion is surprisingly pervasive throughout metazoan and plant evolution. Although the involvement of oxidants in many signaling pathways is well documented, the cellular strategies for conferring pathway specificity to such reactive molecules have remained more recalcitrant. Recent studies now suggest that cells may spatially restrict oxidant production to allow microdomain-specific signaling.] Terada LS. *J Cell Biol.* 2006 Aug 28; 174(5): 615–623. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2064304/>

The Basics of Thiols and Cysteines in Redox Biology and Chemistry. [Cysteine is one of the least abundant amino acids, yet it is frequently found as a highly conserved residue within functional (regulatory, catalytic or binding) sites in proteins. It is the unique chemistry of the thiol or thiolate group of cysteine that imparts functional sites with their specialized properties (e.g., nucleophilicity, high affinity metal binding, and/or ability to form disulfide bonds). Highlighted in this review are some of the basic biophysical and biochemical properties of cysteine groups and the equations that apply to them, particularly with respect to pKa and redox potential. Also summarized are the types of low molecular weight thiols present in high concentrations in most cells, as well as the ways in which modifications of cysteinyl residues can impart or regulate molecular functions important to cellular processes including signal transduction.] Poole LB. *Free Radic Biol Med.* 2015 Mar; 0: 148–157. Published online 2014 Nov 27. doi: 10.1016/j.freeradbiomed.2014.11.013 <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4355186/>

The complexity of the Nrf2 pathway: beyond the antioxidant response. [The NF-E2-related factor 2 (Nrf2)-mediated signalling pathway provides living organisms an efficient and pivotal line of defensive to counteract environmental insults and endogenous stressors. Nrf2 coordinates the basal and inducible expression of antioxidant and Phase II detoxification enzymes to adapt to different stress conditions. The stability and cellular distribution of Nrf2 is tightly controlled by its inhibitory binding protein Kelch-like ECH-associated protein 1. Nrf2 signalling is also regulated by posttranslational, transcriptional, translational and epigenetic mechanisms, as well as by other protein partners, including p62, p21 and IQ motif-containing GTPase activating protein 1. Many studies have demonstrated that Nrf2 is a promising target for preventing carcinogenesis and other chronic diseases, including cardiovascular diseases, neurodegenerative diseases and pulmonary injury. However, constitutive activation of Nrf2 in advanced cancer cells may confer drug resistance. Here, we review the molecular mechanisms of Nrf2 signalling, the diverse classes of Nrf2 activators, including

bioactive nutrients and other chemicals, and the cellular functions and disease relevance of Nrf2 and discuss the dual role of Nrf2 in different contexts.] Huang Y, Li W, et al. *J Nutr Biochem*. 2015 Dec;26(12):1401-13.

The emerging role of Nrf2 in mitochondrial function. [The transcription factor NF-E2 p45-related factor 2 (Nrf2; gene name NFE2L2) allows adaptation and survival under conditions of stress by regulating the gene expression of diverse networks of cytoprotective proteins, including antioxidant, anti-inflammatory, and detoxification enzymes as well as proteins that assist in the repair or removal of damaged macromolecules. Nrf2 has a crucial role in the maintenance of cellular redox homeostasis by regulating the biosynthesis, utilization, and regeneration of glutathione, thioredoxin, and NADPH and by controlling the production of reactive oxygen species by mitochondria and NADPH oxidase. Under homeostatic conditions, Nrf2 affects the mitochondrial membrane potential, fatty acid oxidation, availability of substrates (NADH and FADH₂/succinate) for respiration, and ATP synthesis. Under conditions of stress or growth factor stimulation, activation of Nrf2 counteracts the increased reactive oxygen species production in mitochondria via transcriptional upregulation of uncoupling protein 3 and influences mitochondrial biogenesis by maintaining the levels of nuclear respiratory factor 1 and peroxisome proliferator-activated receptor γ coactivator 1 α , as well as by promoting purine nucleotide biosynthesis. Pharmacological Nrf2 activators, such as the naturally occurring isothiocyanate sulforaphane, inhibit oxidant-mediated opening of the mitochondrial permeability transition pore and mitochondrial swelling. Curiously, a synthetic 1,4-diphenyl-1,2,3-triazole compound, originally designed as an Nrf2 activator, was found to promote mitophagy, thereby contributing to the overall mitochondrial homeostasis. Thus, Nrf2 is a prominent player in supporting the structural and functional integrity of the mitochondria, and this role is particularly crucial under conditions of stress.] Dinkova-Kostova AT, Abramov AY. *Free Radical Biology and Medicine*, Volume 88, Part B, November 2015, Pages 179-188. <https://www.sciencedirect.com/science/article/pii/S0891584915002129>

The expanding network of redox signaling: new observations, complexities, and perspectives: [Over thirty years ago, the observations that eventually led to the discovery of the NADPH oxidase were made by Baehner, Karnovsky, and colleagues. These have served as a focal point of interest in placing reactive oxygen species (ROS) in the conceptual forefront of the biomedical community. Over the last decade, the examination of the roles of oxygen and redox tone in regulating cell function has turned inward to the intracellular environment. Because oxidative metabolism is central to the biology and health of all humans, how we respond to conditions of low and high oxygen stress has become a critical consideration in biology and medicine. Humans live in a world where we continually balance the use of oxygen as a source of energy, and as a source of cellular injury. The generation of oxygen radicals secondary to mitochondrial disruption, the activation of cellular NADPH oxidases, the metabolism of xenobiotics, or other forms of oxidative stress can lead to mutations in DNA, lipid peroxidation, and protein damage. We have therefore evolved a marvelously complex system of both defense mechanisms and sensing mechanisms for changes in cellular redox tone. These include the enzymes superoxide dismutase, catalase, and glutathione peroxidase that detoxify ROS. We have also developed signaling mechanisms that utilize ROS to initiate processes that allow cells to survive exposure to oxidative stress within certain tolerances, but also, when stress and damage become too great, to ensure cell death. How these pathways are initiated and controlled on a molecular basis by ROS and also molecular oxygen is at the heart of what is generally considered redox signaling and the response to oxidative stress. The molecular species that fall under the term ROS include superoxide, hydrogen peroxide (H₂O₂), hydroxyl radical, and singlet oxygen. Each of these can play a role in a variety of intracellular processes. Finally, we have adapted these molecular species, particularly H₂O₂ and (NO), as signaling molecules in multiple biological processes.] Soberman RJ. *J Clin Invest*. 2003 Mar 1; 111(5): 571–574. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC151913/>

The Plasma Membrane: A Platform for Intra- and Intercellular Redox Signaling. [Membranes are of utmost importance to allow for specific signal transduction due to their ability to localize, amplify, and direct signals. However, due to the double-edged nature of reactive oxygen species (ROS)-toxic at high concentrations but essential signal molecules-subcellular localization of ROS-producing systems to the plasma membrane has been traditionally regarded as a protective strategy to defend cells from unwanted side-effects. Nevertheless, specialized regions, such as lipid rafts and caveolae, house and regulate the activated/inhibited states of important ROS-producing systems and concentrate redox targets, demonstrating that plasma membrane functions may go beyond acting as a securing lipid barrier. This is nicely evinced by nicotinamide adenine dinucleotide phosphate (NADPH)-oxidases (NOX), enzymes whose primary function is to generate ROS and which have been shown to reside in specific lipid compartments. In addition, membrane-inserted bidirectional H₂O₂-transporters modulate their conductance precisely during the passage of the molecules through the lipid bilayer, ensuring time-scaled delivery of the signal. This review aims to summarize current evidence supporting the role of the plasma membrane as an organizing center that serves as a platform for redox signal transmission, particularly NOX-driven, providing specificity at the same time that limits undesirable oxidative damage in case of malfunction. As an example of malfunction, we explore several pathological situations in which an inflammatory component is present, such as inflammatory bowel disease and neurodegenerative disorders, to illustrate how dysregulation of plasma-membrane-localized redox signaling impacts normal cell physiology.] Nordzиеke DE, Medrano-Fernandez. *Antioxidants (Basel)*. 2018 Nov 20;7(11). pii: E168. doi: 10.3390/antiox7110168. <https://www.ncbi.nlm.nih.gov/pubmed/30463362>

The Redox Basis of Epigenetic Modifications: From Mechanisms to Functional Consequences. [Epigenetic modifications represent mechanisms by which cells may effectively translate multiple signaling inputs into phenotypic outputs. Recent research is revealing that redox metabolism is an increasingly important determinant of epigenetic control that may have significant ramifications in both human health and disease. Numerous characterized epigenetic marks, including histone methylation, acetylation, and ADP-ribosylation, as well as DNA methylation, have direct linkages to central metabolism through critical redox intermediates such as NAD⁺, S-adenosyl methionine, and 2-oxoglutarate. Fluctuations in these intermediates caused by both normal and pathologic stimuli may thus have direct effects on epigenetic signaling that lead to measurable changes in gene expression. In this comprehensive review, we present surveys of both metabolism-sensitive epigenetic enzymes and the metabolic processes that may play a role in their regulation. To close, we provide a series of clinically relevant illustrations of the communication between metabolism and epigenetics in the pathogenesis of cardiovascular disease, Alzheimer disease, cancer, and environmental toxicity. We anticipate that the regulatory mechanisms described herein will play an increasingly large role in our understanding of human health and disease as epigenetics research progresses.] Cyr AR, Domann FE. *Antioxid. Redox Signal.* 15, 551–589. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3118659/>

The Redox Code. [SIGNIFICANCE: The redox code is a set of principles that defines the positioning of the nicotinamide adenine dinucleotide (NAD, NADP) and thiol/disulfide and other redox systems as well as the thiol redox proteome in space and time in biological systems. The code is richly elaborated in an oxygen-dependent life, where activation/deactivation cycles involving O₂ and H₂O₂ contribute to spatiotemporal organization for differentiation, development, and adaptation to the environment. Disruption of this organizational structure during oxidative stress represents a fundamental mechanism in system failure and disease. RECENT ADVANCES: Methodology in assessing components of the redox code under physiological conditions has progressed, permitting insight into spatiotemporal organization and allowing for identification of redox partners in redox proteomics and redox metabolomics. CRITICAL ISSUES: Complexity of redox networks and redox regulation is being revealed step by step, yet much still needs to be learned. FUTURE DIRECTIONS: Detailed knowledge of the molecular patterns generated from the principles of the redox code under defined physiological or pathological conditions in cells and organs will contribute to understanding the redox component in health and disease. Ultimately, there will be a scientific basis to a modern redox medicine.] Jones DP, Sies H. *Antioxid Redox Signal.* 2015 Sep 20;23(9):734-46. <https://www.ncbi.nlm.nih.gov/pubmed/25891126> ; <https://www.liebertpub.com/doi/pdfplus/10.1089/ars.2015.6247?src=recsys>

White Paper on In-Vitro Bioactivity of ASEA™ Related to Toxicity, Glutathione Peroxidase, Superoxide Dismutase Efficacy and Related Transcription Factors. [This is a technical paper describing a variety of preliminary results from in vitro experiments, performed at national research institutions, investigating the bioactivity of a certain redox signaling compound, ASEA™, when placed in direct physical contact with living cells. Specific investigations include in vitro toxicity and antioxidant efficiencies of the master antioxidants glutathione peroxidase (GPx) and Superoxide Dismutase (SOD) inside living cells and the translocation of two well-studied transcription factors (NFκB, NRF2) known to regulate toxic response and antioxidant production in human cells. Some preliminary work on concentration dependence was also done as well as cell proliferation, counts associated with induced oxidative stress in human cells.] Samuelson G. <http://www.amazingmolecules.com/pdf/Antioxidant-Efficacy-White-Paper.pdf>

Oral Health

Antioxidants and its Role in Periodontitis – A Short Review. [Periodontitis results from the loss of balance between microbial virulence factors and a proportionate host response. Antioxidant is a substance that is present at low concentrations which significantly delays or prevents oxidation of that substrate. Diets low in antioxidant vitamin cannot increase the risk of any developing gum disease. For prevention and treatment of periodontitis daily nutrition should include sufficient antioxidants, vitamin D, and calcium. Inadequate antioxidant levels may be managed by higher intake of vegetables, berries, and fruits (e.g. kiwi fruit), or by phytonutrient supplementation. Nutritional counselling and supplementation may very well reduce inflammation and thereby enhance outcomes of conventional periodontal therapy. Hence this review discusses about the antioxidant nutrients which can play a major role in preventing and treating the periodontal diseases.] Pooja, A. J. *Pharm. Sci & Res.* Vol. 8(8), 2016, 759-763. <http://www.jpsr.pharmainfo.in/Documents/Volumes/vol8Issue08/jpsr08081614.pdf>

Analysis of superoxide dismutase activity levels in gingiva and gingival crevicular fluid in patients with chronic periodontitis and periodontally healthy controls. [OBJECTIVES: Superoxide dismutase (SOD) is an antioxidant enzyme that acts against superoxide, an oxygen radical, released in inflammatory pathways and causes connective tissue breakdown. In this study, SOD activities in gingiva and gingival crevicular fluid (GCF) from patients with chronic periodontitis (CP) and periodontally healthy controls were compared. MATERIAL AND METHODS: Twenty-six CP patients and 18 controls were studied. In patients, teeth with moderate-to-severe periodontal breakdown and > or =5 mm pockets that required full-thickness flap surgery in the right or left maxillary quadrant, and in controls, teeth scheduled for extraction for orthodontic reasons were studied. After the clinical measurements (probing depth, clinical attachment level, gingival index, gingival bleeding index, plaque index), GCF samples were

collected. Tissue samples were harvested from the same teeth, during flap operation in patients and immediately after tooth extraction in controls. SOD activities were spectrophotometrically assayed. The results were statistically analysed. RESULTS: Gingival SOD activity was significantly higher in the CP group than in controls ($p < 0.05$). No significant difference was found in GCF SOD activity between the groups ($p > 0.05$). Correlations between gingival and GCF SOD activities were not statistically significant in CP and control groups ($p > 0.05$). CONCLUSION: In CP, SOD activity seems to increase in gingiva, probably as a result of a higher need for SOD activity and protection in gingiva in CP than in periodontal health, while not significantly changing in GCF, suggesting a weak SOD activity in GCF in periodontal disease state. The weak correlation between gingival and GCF SOD activities suggests distinct actions of these SODs.] Akalin FA, Toklu E, et al. J Clin Periodontol. 2005 Mar;32(3):238-43. <https://www.ncbi.nlm.nih.gov/pubmed/15766365>.

Compromised GCF total antioxidant capacity in periodontitis: cause or effect? [BACKGROUND: Oxidative stress is implicated in the pathogenesis of periodontitis. The total antioxidant capacity (TAOC) of gingival crevicular fluid volume (GCF) and plasma appears compromised in periodontitis, but it is unclear whether this predisposes to, or results from the inflammatory process. AIM: To investigate longitudinal changes in GCF and plasma TAOC following reductions in periodontal inflammation with successful non-surgical therapy. MATERIALS AND METHODS: Two longitudinal studies were run in series on non-smokers with chronic periodontitis (CP). Study-1 ($n=17$) assessed index sites with mild disease; Study-2 ($n=18$) investigated deep sites. GCF sampling and clinical measures were performed at baseline and 3 months post-therapy. Plasma and GCF TAOC was determined by enhanced chemiluminescence and 32 age/sex-matched periodontally healthy controls were used. RESULTS: Therapy improved clinical outcomes consistent with the literature. There were no differences in plasma TAOC between periodontitis patients (507 ± 92 microMTeq) and controls (520 ± 100 microMTeq; $p=0.57$) at baseline, but GCF TAOC was lower ($p < 0.0001$) in CP patients (680 ± 371 microMTeq) than controls (1129 ± 722 microMTeq). Successful periodontal therapy did not alter plasma TAOC ($p=0.56$), but GCF TAOC increased (by 449 ± 722 microMTeq, $p < 0.001$) to control subject levels ($p=0.47$). CONCLUSIONS: Local total antioxidant capacity in CP appears to reflect increased oxygen radical activity during periodontal inflammation and can be restored to control subject levels by successful non-surgical therapy.] Chapple IL, Brock GR, et al. J Clin Periodontol. 2007 Feb;34(2):103-10. <https://www.ncbi.nlm.nih.gov/pubmed/17214737>.

Crevicular fluid glutathione levels in periodontitis and the effect of non-surgical therapy. [AIM: To quantify reduced and oxidized glutathione (GSH and GSSG) levels in gingival crevicular fluid (GCF) of periodontitis patients pre-therapy (versus periodontally healthy controls) and ascertain whether successful non-surgical therapy alters glutathione levels. MATERIALS AND METHODS: Thirty-second GCF samples (6/subject) were collected on Periopaper strips from starved, non-smokers ($n=20$; mean age 43.6 years) with chronic periodontitis, before and 3 months after non-surgical therapy, and periodontally healthy, age- and gender-matched controls ($n=20$). GSH and GSSG levels were determined using reversed-phase high-performance liquid chromatography with fluorescence detection. RESULTS: Lower concentrations of GSH ($p < 0.01$) and GSSG ($p < 0.05$) were detected in GCF from patients (pre- and post-therapy) than controls and treatment had no significant effect. Amounts per 30-second sample did not differ between patients and controls. However, the amount of GSSG per 30-second sample decreased in patients after therapy ($p < 0.05$). Consequently, therapy increased the GSH:GSSG ratio ($p < 0.05$) in patients compared with the controls ($p=0.8$). CONCLUSION: These data demonstrate high concentrations of GSH within GCF, which are compromised in chronic periodontitis. While therapy does not appear to fully restore GSH concentrations in GCF, it does restore the redox balance (GSH:GSSG ratio), suggesting that the abnormal redox balance arises secondary to oxidative stress resulting from periodontal inflammation.] Grant MM, Brock GR, et al. Clin Periodontol. 2010 Jan;37(1):17-23. doi: 10.1111/j.1600-051X.2009.01504.x. <https://www.ncbi.nlm.nih.gov/pubmed/19968740> .

Evaluation of glutathione level in gingival crevicular fluid in periodontal health, in chronic periodontitis and after nonsurgical periodontal therapy: A clinicobiochemical study. [CONTEXT: Periodontitis is predominantly due to exaggerated host response to pathogenic microorganisms and their products which causes an imbalance between the reactive oxygen species-antioxidant in gingival crevicular fluid (GCF). Glutathione is an important redox regulator in GCF and maintenance of stable reduced glutathione (GSH):oxidized glutathione (GSSG) ratio is essential for periodontal health. AIMS: The present study was undertaken to evaluate and compare the level of glutathione and redox balance (GSH: GSSG ratio) in GCF of chronic periodontitis patients, periodontally healthy controls and also to evaluate the effect of nonsurgical periodontal therapy on the level of glutathione and redox balance during 3 months postoperative visit. STUDY DESIGN: Baseline GCF samples were collected from 20 chronic periodontitis patients and 20 periodontally healthy subjects for GSH and GSSG levels estimation. Periodontitis patients were recalled 3 months postnonsurgical periodontal therapy to re-sample GCF. MATERIALS AND METHODS: GSH and GSSG levels were measured by high-performance liquid chromatography. The values were statistically analyzed by Paired t-test. RESULTS: The mean GSH and GSSG values in GCF were found to be significantly lower in periodontitis patients pre- and 3 months post-nonsurgical periodontal therapy, compared with those in the control group subjects. In addition, the successful nonsurgical therapy even though leading to a significant improvement in the GSH and GSSG levels, does not restore glutathione concentration to the levels seen in healthy subjects. CONCLUSION: Successful nonsurgical periodontal therapy leads to significant improvement in the redox balance (GSH: GSSG ratio) in chronic periodontitis patients.] Savita AM, Sarun E, et al. Contemp Clin Dent. 2015 Apr-Jun;6(2):206-10. doi: 10.4103/0976-237X.156047. <https://www.ncbi.nlm.nih.gov/pubmed/26097356> .

Evaluation of non-surgical therapy on glutathione levels in chronic periodontitis. [OBJECTIVE: To compare the levels of glutathione (GSH), both oxidized and reduced forms in patients with and without chronic periodontitis in gingival crevicular fluid (GCF). MATERIALS AND METHODS: Twenty GCF samples from maxillary quadrants were collected using capillary micropipettes from the chronic periodontitis patients (test group) at baseline before treatment, at 1-month, 3 months, and 6 months after scaling and root planing and samples from 20 patients without chronic periodontitis (control group) from maxillary quadrants were also collected. GSH, oxidized glutathione (GSSG) levels and GSH: GSSG ratios were determined using the spectrophotometric method. STATISTICAL ANALYSIS: Results were concluded for the test over control groups using paired Student's t-test. RESULTS: Lower concentrations of GSH ($P < 0.001$) and GSSG ($P < 0.001$) were detected in GCF in patients with chronic periodontitis (test group) than patients without chronic periodontitis (control group) at baseline. Treatment had a significant effect in improving the GSH and reducing GSSG levels postscaling and root planing at 1-month and 3 months but not significant effect at 6 months. Scaling and root planing increased the GSH: GSSG ratio ($P < 0.001$) in the test group as compared to the control group ($P < 0.001$). CONCLUSIONS: The concentrations of GSH within GCF are reduced in chronic periodontitis patients. Scaling and root planing (nonsurgical therapy) restores GSH concentration in GCF post 1-month and 3 months along with redox balance (GSH: GSSG ratio), but at 6 months the balance is not maintained. Adjunctive use of micronutritional supplements to boost antioxidant concentration in tissues by preserving GSH or by elevating its level at the inflamed sites is recommended, as nonsurgical periodontal therapy alone is not able to maintain redox balance for longer duration.] Palwankar P, Rana M. et al. Eur J Dent. 2015 Jul-Sep;9(3):415-22. doi: 10.4103/1305-7456.163226. <https://www.ncbi.nlm.nih.gov/pubmed/26430373>.

Glutathione in gingival crevicular fluid and its relation to local antioxidant capacity in periodontal health and disease. [AIMS: To determine possible changes in gingival crevicular fluid (GCF) antioxidant defence in chronic adult periodontal disease and to investigate the nature of the local radical scavenging mechanisms, with particular reference to glutathione. METHODS: GCF and plasma were collected from patients with chronic periodontitis and age and sex matched control subjects ($n = 10$). Polymorphonuclear leucocytes (PMNLs) were prepared and gingival epithelial cells (GECs) were collected by conventional methods from periodontally healthy subjects. PMNL were stimulated with F-Met-Leu-Phe after cytochalasin B treatment. Enhanced chemiluminescence was used to determine the total antioxidant capacity and to investigate the activity of cell fractions and reducing agents. GCF concentrations of reduced (GSH) and oxidised (GSSG) glutathione were determined by high performance liquid chromatography. RESULTS: Plasma and GCF from patients contained lower mean (SD) total antioxidant capacity (501.8 (123) micro M Teq/litre and 658.3 (392) micro M Teq/litre, respectively) compared with controls (577.9 (99.8) and 1351.5 (861) micro M Teq/litre, respectively). Antioxidant light recovery profiles for GCF demonstrated a stepped response, not seen in plasma, which was inhibited by N-ethylmaleimide. This response was also detected in the cytosolic fraction of GEC and anaerobically stimulated PMNL. Similar antioxidant profiles, inhibitable by N-ethylmaleimide, were obtained with cysteamine, cysteine, and GSH. Control GCF contained high mean (SD) concentrations of glutathione (GSH, 1899.8 (494.4) micro M; GSSG, 256.8 (152.4) micro M). GCF from patients with periodontitis contained significantly lower amounts of GSH (mean, 1183.1; SD, 580.3 micro M) and GSSG (mean, 150.1; SD, 44.9 micro M). CONCLUSIONS: GSH values and total antioxidant capacity are reduced in chronic periodontal disease. The high concentrations of GSH present in GCF in health are similar to those found extracellularly in the lung and may represent an important antioxidant and anti-inflammatory defence strategy common to exposed epithelial surfaces.] Chapple IL, Brock G, et al. Mol Pathol. 2002 Dec;55(6):367-73. <https://www.ncbi.nlm.nih.gov/pubmed/12456773>.

Glutathione levels in plasma, saliva and gingival crevicular fluid after periodontal therapy in obese and normal weight individuals. [BACKGROUND AND OBJECTIVE: The purpose of this study was to investigate the effects of obesity on reduced and oxidized glutathione (GSH and GSSG) levels in the gingival crevicular fluid, plasma and saliva of patients with chronic periodontitis and to evaluate the changes after nonsurgical periodontal therapy. MATERIAL AND METHODS: The study included 60 patients: 30 patients with chronic periodontitis (15 obese patients and 15 normal weight patients) and 30 healthy control subjects (15 obese patients and 15 normal weight patients). Gingival crevicular fluid, plasma and saliva samples were collected, and clinical periodontal measurements were recorded at baseline and at the first month after periodontal therapy from patients with chronic periodontitis. GSH and GSSG levels were analyzed with spectrophotometry. RESULTS: The GSH levels in the plasma, saliva and gingival crevicular fluid in obese individuals with chronic periodontitis were lower than in normal weight individuals at baseline ($p < 0.01$). There was a significant difference in the GSH/GSSG ratio in plasma and gingival crevicular fluid between the obese and normal weight groups at baseline ($p < 0.01$). The GSH levels in plasma, gingival crevicular fluid and saliva were significantly increased in both chronic periodontitis groups after nonsurgical periodontal therapy ($p < 0.01$). A significant positive correlation was found between GSH levels in saliva, plasma and gingival crevicular fluid in all groups ($p < 0.001$). CONCLUSIONS: The study revealed that obesity in patients with chronic periodontitis is associated with decreased GSH levels and the GSH/GSSG ratio. Moreover, nonsurgical periodontal therapy may be helpful for improvement in glutathione values in obese and normal weight individuals with chronic periodontitis.] Ongoz D, Bozkurt DS, et al. J Periodontal Res. 2016 Dec;51(6):726-734. doi: 10.1111/jre.12349. <https://www.ncbi.nlm.nih.gov/pubmed/26740476>.

Local and systemic total antioxidant capacity in periodontitis and health. [BACKGROUND: The involvement of reactive oxygen species (ROS) in periodontal pathology is unclear but will be modulated by in vivo antioxidant defence systems. The aim of this cross-

sectional study was to determine both local (saliva and gingival crevicular fluid (GCF) and peripheral (plasma and serum) antioxidant capacity in periodontal health and disease. **MATERIALS AND METHODS:** Twenty non-smoking volunteers with chronic periodontitis were sampled together with twenty age- and sex-matched, non-smoking controls. After overnight fasting, saliva (whole unstimulated and stimulated) and blood were collected. Total antioxidant capacity (TAOC) was determined using a previously reported enhanced chemiluminescence method. **RESULTS:** GCF antioxidant concentration was significantly lower ($p<0.001$) in periodontitis subjects compared to healthy controls. Although mean levels of peripheral and salivary TAOC were also lower in periodontitis the difference was only significant for plasma ($p<0.05$). Healthy subjects' GCF antioxidant concentration was significantly greater than paired serum or plasma ($p<0.001$). Data stratified for gender did not alter the findings and a male bias was revealed in all clinical samples except GCF. **CONCLUSIONS:** These findings suggest that the antioxidant capacity of GCF is both qualitatively and quantitatively distinct from that of saliva, plasma and serum. Whether changes in the GCF compartment in periodontitis reflect predisposition to or the results of ROS-mediated damage remains unclear. Reduced plasma total antioxidant defence could result from low-grade systemic inflammation induced by the host response to periodontal bacteria, or may be an innate feature of periodontitis patients.] Brock GR, Butterworth CJ, et al. *J Clin Periodontol*. 2004 Jul;31(7):515-21. <https://www.ncbi.nlm.nih.gov/pubmed/15191586>.

Microbicidal activity of MDI-P against *Candida albicans*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Legionella pneumophila*. [Background: MDI-P (Medical Discoveries, Inc–Pharmaceutical, Layton, Utah) is a clear, colorless liquid generated by electrolysis of preservative-free and endotoxin-free, nonpyrogenic, sterile, injection saline (0.9% NaCl, wt/vol). It contains numerous highly reactive chlorine and oxygen species, including HOCl–1, OCl–, Cl–, Cl₂, O₂–, and O₃. This report presents data on the in vitro microbicidal activity of MDI-P against 4 clinically relevant microbial pathogens that are often difficult to eradicate. Methods: MDI-P was generated from injection saline by using a patented electrolysis instrument. It was then tested for microbicidal activity at concentrations ranging from 0.01% to 50% against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Legionella pneumophila*, and *Candida albicans* (10^5 to 10^9 colony-forming units/mL). The effect of serum (50% and 90%) and pH on MDI-P activity were also tested. The morphologic effects of MDI-P on microbial cells were studied by light microscopy of cells stained by Gram's method and by transmission electron microscopy. Morbidity, mortality, and the effect of MDI-P on tissues were studied by using a mouse model. Results: The microbicidal activity of MDI-P occurred within the first minute of exposure for all the organisms tested. When 50% MDI-P was tested against cell titers of 10^5 or 10^7 colony-forming units/mL, all test organisms were killed within 1 minute; at lower MDI-P concentrations, *C albicans* was the most sensitive organism, and *L pneumophila* was the most resistant. Even with beginning cell titers of 10^9 colony-forming units/mL, killing by 50% MDI-P was >99.9% for all test strains. Furthermore, at the same beginning cell titer, killing of *C albicans* by MDI-P diluted to 50% with normal human serum rather than injection saline was only slightly reduced. No acute morbidity, mortality, or tissue damage was detected in mice that were intravenously given 17 mL/kg of undiluted MDI-P. Conclusions: MDI-P is a very fast-acting, broad-spectrum microbicidal material. The lack of evidence for acute morbidity, mortality, or tissue injury, ease of preparation, and low cost suggest that it may be useful for various sterilization and disinfection applications.] Baltch AL, Smith RP, et al. *AJIC Am J Infect Control* 2000;28:251-7. <https://www.ncbi.nlm.nih.gov/pubmed/10840346>

Novel antioxidative nanotherapeutics in a rat periodontitis model: Reactive oxygen species scavenging by redox injectable gel suppresses alveolar bone resorption. [The excessive production of reactive oxygen species (ROS) has been implicated in a variety of disorders, but to date, ROS scavengers have not been widely used for local treatment of inflammation, because they are rapidly eliminated from the inflamed site. We have designed a novel redox injectable gel (RIG) that is formed at 37 °C after disintegration of nano-assembled flower micelles allowing nitroxide radicals to act locally as specific ROS scavengers for the treatment of periodontitis. In the present study, we have confirmed retention of the RIG in the periodontal region, along with its antioxidant-related anti-inflammatory effects, and we have subsequently evaluated the inhibitory effect of the RIG against *Porphyromonas gingivalis* (*P. gingivalis*)-induced alveolar bone loss attributed to ROS. Alveolar bone loss was estimated by morphometry, gingival blood flow was measured using laser Doppler flowmetry, and osteoclast differentiation was evaluated by tartrate-resistant acid phosphatase staining. The results show that the RIG can inhibit *P. gingivalis*-induced bone loss by antioxidant-related anti-inflammatory actions, and this suggests that the RIG is a promising novel therapeutic agent for the treatment of *P. gingivalis*-induced periodontitis.] Saita M, Kaneko J, et al. *Biomaterials*. Vol 76, Jan 2016, P 292-301. <https://doi.org/10.1016/j.biomaterials.2015.10.077>

Nuclear Factor Erythroid 2-Related Factor 2 Down-Regulation in Oral Neutrophils Is Associated with Periodontal Oxidative Damage and Severe Chronic Periodontitis. [The balance between reactive oxygen species and antioxidants plays an important role in periodontal health. We previously demonstrated that high reactive oxygen species production by oral polymorphonuclear neutrophils (oPMNs) in chronic periodontitis (CP) refractory to conventional therapy is associated with severe destruction of periodontium. Herein, we show that inhibition of antioxidant production through down-regulation of nuclear factor erythroid 2-related factor 2 (Nrf2) pathway in oPMN, despite enhanced recruitment in the oral cavity, is associated with severe CP. Twenty-four genes in the Nrf2-mediated oxidative stress response pathway were down-regulated in PMNs of diseased patients. Downstream of Nrf2, levels of oPMN superoxide dismutase 1 and catalase were decreased in severe CP, despite increased recruitment. Nrf2–/– mice had more severe loss of periodontium in response to periodontitis-inducing subgingival ligatures compared with wild-types. Levels of 8-hydroxy-deoxyguanosine were increased in periodontal lesions of Nrf2–/– mice, indicating high oxidative damage. We report, for the

first time, Nrf2 pathway down-regulation in oPMNs of patients with severe CP. PMNs of CP patients may be primed for low antioxidant response in the context of high recruitment in the oral cavity, resulting in increased oxidative tissue damage.] Sima C, Aboodi, GM, et al. *American Journal Of Pathology* 186(6) · April 201. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4901128/>

Oxidative Stress and Antioxidant System in Periodontitis. [Periodontitis is a common inflammatory disease, which is initiated by bacterial infection and subsequently progressed by aberrant host response. It can result in the destruction of teeth supporting tissues and have an influence on systemic health. When periodontitis occurs, reactive oxygen species, which are overproduced mostly by hyperactive neutrophils, could not be balanced by antioxidant defense system and cause tissues damage. This is characterized by increased metabolites of lipid peroxidation, DNA damage and protein damage. Local and systemic activities of antioxidants can also be influenced by periodontitis. Total antioxidant capacity, total oxidant status and oxidative stress index have been used to evaluate the oxidative stress associated with periodontitis. Studies have confirmed that inflammatory response in periodontitis is associated with an increased local and systemic oxidative stress and compromised antioxidant capacity. Our review focuses on increased oxidative stress in periodontal disease, specifically, on the relationship between the local and systemic biomarkers of oxidative stress and periodontitis and their association with the pathogenesis of periodontitis. Also, the relationship between periodontitis and systemic inflammation, and the effects of periodontal therapy on oxidative stress parameters will be discussed.] Wang Y, Andrukhov O, et al. *Front Physiol.* 2017 Nov 13;8:910. doi: 10.3389/fphys.2017.00910. <https://www.ncbi.nlm.nih.gov/pubmed/29180965>.

Oxidative Stress Induced Mechanisms in the Progression of Periodontal Diseases and Cancer: A Common Approach to Redox Homeostasis? [There is documented evidence of significant associations between cancer of the lung, kidney, pancreas, hematological and oral cancers and periodontal diseases of the supporting structures of the teeth. Enhanced lipid peroxidation, raised levels of TBARS and the oxidative stress marker malondealdehyde have been detected in breast cancer with reduced antioxidant capacity, also characteristic of periodontal diseases. Antioxidants could overcome this deficit and attenuate disease progression by down regulating glutathione detoxification/redox buffering system and inhibiting key transcription factors. Periodontal disease may be a critical marker of a susceptible immune system, or initiate cancer risk with a pro-oxidant inflammatory profile.] Soory M. *Cancers (Basel).* 2010 Jun; 2(2): 670–692. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3835098/>

Pathways that Regulate ROS Scavenging Enzymes, and Their Role in Defense Against Tissue Destruction in Periodontitis. [Periodontitis, an inflammatory disease that affects the tissues surrounding the teeth, is a common disease worldwide. It is caused by a dysregulation of the host inflammatory response to bacterial infection, which leads to soft and hard tissue destruction. In particular, it is the excessive inflammation in response to bacterial plaque that leads to the release of reactive oxygen species (ROS) from neutrophils, which, then play a critical role in the destruction of periodontal tissue. Generally, ROS produced from immune cells exhibit an anti-bacterial effect and play a role in host defense and immune regulation. Excessive ROS, however, can exert cytotoxic effects, cause oxidative damage to proteins, and DNA, can interfere with cell growth and cell cycle progression, and induce apoptosis of gingival fibroblasts. Collectively, these effects enable ROS to directly induce periodontal tissue damage. Some ROS also act as intracellular signaling molecules during osteoclastogenesis, and can thus also play an indirect role in bone destruction. Cells have several protective mechanisms to manage such oxidative stress, most of which involve production of cytoprotective enzymes that scavenge ROS. These enzymes are transcriptionally regulated via NRF2, Sirtuin, and FOXO. Some reports indicate an association between periodontitis and these cytoprotective enzymes' regulatory axes, with superoxide dismutase (SOD) the most extensively investigated. In this review article, we discuss the role of oxidative stress in the tissue destruction manifest in periodontitis, and the mechanisms that protect against this oxidative stress.] Kanzaki H, Wada S, et al. *Front Physiol.* 2017 May 30;8:351. doi: 10.3389/fphys.2017.00351. eCollection 2017. <https://www.ncbi.nlm.nih.gov/pubmed/28611683>.

Periodontitis and redox status: a review. Periodontal diseases are common inflammatory conditions of the supporting apparatus of the teeth which lead to early tooth loss. This review discusses the evidence for a role of reactive oxygen species in inducing periodontal tissue damage and focuses on recent evidence showing increased local and systemic alterations in the redox balance of periodontitis. An appraisal of the methods for analysis of oxidative stress in periodontal disease research is provided, showing an increase in oxidative stress measures and oxidative damage fingerprints detected in studies investigating periodontitis cases compared to healthy controls. Hypotheses on the relationships between oxidative stress and inflammatory responses and on the role of redox status in periodontal medicine are discussed. Finally, the review provides an overview of possible intervention pathways for the use of antioxidants as adjuncts to mechanical biofilm removal for the treatment of periodontitis.] Nibali, L, Donos N, *Current Pharmaceutical Design* 2013, 19 (15): 2687-97. <https://www.readbyqxdm.com/read/23092320/periodontitis-and-redox-status-a-review>.

Reactive Oxygen Species & Its Role in Periodontal Disease. [] Bhusari GM, Mahajan R, et al. *IOSR-JDMS*, Vol 13, Issue 8 Ver. 1, Aug 2014, p 52-59. <http://www.iosrjournals.org/iosr-jdms/papers/Vol13-issue8/Version-1/M013815259.pdf>

Reactive oxygen species in periodontitis. [Recent epidemiological studies reveal that more than two-third of the world's population suffers from one of the chronic forms of periodontal disease. The primary etiological agent of this inflammatory disease is a polymicrobial complex, predominantly Gram negative anaerobic or facultative bacteria within the sub-gingival biofilm. These

bacterial species initiate the production of various cytokines such as interleukin-8 and TNF- α , further causing an increase in number and activity of polymorphonucleocytes (PMN) along with these cytokines, PMNs also produce reactive oxygen species (ROS) superoxide via the respiratory burst mechanism as the part of the defence response to infection. ROS just like the interleukins have deleterious effects on tissue cells when produced in excess. To counter the harmful effects of ROS, human body has its own defence mechanisms to eliminate them as soon as they are formed. The aim of this review is to focus on the role of different free radicals, ROS, and antioxidants in the pathophysiology of periodontal tissue destruction.] *J Indian Soc Periodontol.* 2013 Jul-Aug; 17(4): 411–416. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3800399/>

Serum levels of antioxidants and superoxide dismutase in periodontitis patients with diabetes type 2. [AIMS AND

OBJECTIVES: The present study was designed to estimate and compare the superoxide dismutase and total antioxidant capacity levels in the serum of diabetes mellitus - Type 2 patients and healthy individuals with and without periodontal disease. MATERIALS AND METHODS: The study was designed as a case-control study comprising of 150 subjects, inclusive of both genders in the age group of 35-65 years. They were divided into three groups of 50 patients each. Patients were categorized into diabetic with chronic periodontitis (DM-CP) and systemically healthy groups with and without periodontitis. Serum samples were collected and sent for biochemical analysis to estimate the total antioxidant capacity (TAOC) and superoxide dismutase (SOD) levels. Results obtained were then statistically analysed using ANOVA test. RESULTS: The results showed a higher level of serum TAOC in the systemically healthy group without periodontitis (PH) compared to the other groups. The P value was found to be <0.05 . The SOD levels were found to be highest in the DM-CP group. CONCLUSION: The results of this study indicate that serum TAOC were found to be highest in the PH group and lowest in the systemically healthy with chronic periodontitis (CP). The serum SOD levels were found to be highest in the DM-CP group. The increased levels of SOD seen in DM patients may be a result of a protective and adaptive mechanism against the oxidative stress developing in the tissue.] Thomas B, Rao A, et al. *J Indian Soc Periodontol.* 2014 Jul;18(4):451-5. doi: 10.4103/0972-124X.138686. <https://www.ncbi.nlm.nih.gov/pubmed/2521025>.

The antioxidant master glutathione and periodontal health. [Glutathione, considered to be the master antioxidant (AO), is the most-important redox regulator that controls inflammatory processes, and thus damage to the periodontium. Periodontitis patients have reduced total AO capacity in whole saliva, and lower concentrations of reduced glutathione (GSH) in serum and gingival crevicular fluid, and periodontal therapy restores the redox balance. Therapeutic considerations for the adjunctive use of glutathione in management of periodontitis, in limiting the tissue damage associated with oxidative stress, and enhancing wound healing cannot be underestimated, but need to be evaluated further through multi-centered randomized controlled trials]. Bains, VK, Bains, R. *Dent Res J (Isfahan).* 2015 Sep-Oct; 12(5): 389–405. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4630702/>

The effect of periodontal therapy on oxidative stress biomarkers: A systematic review. [To systematically review the literature about the effect of periodontal treatment on oxidative stress (OxS) biomarkers. MATERIAL AND METHODS: Three databases (PubMed, EMBASE, and Scopus) were searched up to February 2018. Clinical trials with a follow-up of at least 6 weeks after mechanical periodontal treatment were included. Due to the high heterogeneity among the units and indices of measurements used in the studies, a meta-analysis was not performed. RESULTS: Overall, 3,199 studies were retrieved, of which 20 were included. Four studies were randomized clinical trials (RCT), and 16 studies were non-RCTs. The most common OxS biomarkers used were 8-hydroxydeoxyguanosine (8-OHdG), total oxidant status (TOS), and total antioxidant status (TAS). After treatment, most of the studies reported a decrease in 8-OHdG concentration in the gingival crevicular fluid (GCF) and saliva. In addition, the salivary concentration of this biomarker was similar to periodontally healthy patients. Periodontal therapy was effective in reducing TOS in GCF, saliva, and serum in most studies. TAS, however, responded inconsistently to the periodontal intervention. CONCLUSION: Periodontal therapy reduces the levels of OxS biomarkers, even to values similar to those found in periodontally healthy individuals. Additional RCTs are warranted, as the information is mainly based on nonrandomized studies.] da Silva JC, Muniz FWMG et al. *J Clin Periodontol.* 2018 Oct;45(10):1222-1237. doi: 10.1111/jcpe.12993. <https://www.ncbi.nlm.nih.gov/pubmed/30076616>.

The effects of non-surgical periodontal therapy on oxidant and anti-oxidant status in smokers with chronic periodontitis.

[AIM: The aim of this study was to determine the effect of non-surgical periodontal treatment on gingival crevicular fluid (GCF) and serum oxidant-antioxidant levels in smoking and non-smoking patients with chronic periodontitis. METHODS: Twenty-nine patients with chronic periodontitis (15 smokers (CP-S) and 14 non-smokers (CP-NS)) and 20 periodontally healthy subjects (10 smokers (H-S) and 10 non-smokers (H-NS)) totalling 49 subjects were included in this study. GCF was collected from at least two pre-selected sites (one moderate and one deep pocket) in patients with CP. In the healthy group, GCF samples were collected from one site. Probing pocket depth, clinical attachment level (CAL), gingival and plaque indices, and bleeding on probing were measured. To determine serum total oxidant status (TOS) and total antioxidant status (TAS), venous blood was drawn from each subject. The GCF, serum sampling, and clinical measurements were recorded at baseline and 6 weeks after periodontal treatment. RESULTS: The study showed statistically significant improvement of clinical parameters after periodontal treatment in both smokers and non-smokers. In the CP-S group, there were no significant differences in GCF TAS levels at both moderate and deep pocket sites between baseline and 6 weeks ($p>0.05$). GCF TAS levels in the CP-NS groups were significantly increased ($p<0.05$) at moderate and deep pocket sites between baseline and 6 weeks. GCF TOS levels in the CP-S groups were significantly decreased ($p<0.05$) at deep pocket sites between baseline

and 6 weeks. There was no significant difference in serum TAS levels of the all periodontitis patient groups between at baseline and 6 weeks ($p>0.05$). Serum TOS levels in the CP-S and CP-NS groups were significantly decreased ($p<0.05$) after periodontal treatments. CONCLUSIONS: The periodontal treatment improves the clinical parameters in both smokers and non-smokers. These results confirm that non-surgical periodontal therapy can reduce oxidative stress.] Arch Oral Biol. 2013 Jun;58(6):717-23. doi: 10.1016/j.archoralbio.2012.11.009. <https://www.ncbi.nlm.nih.gov/pubmed/23261252>.

The Redox Basis of Epigenetic Modifications: From Mechanisms to Functional Consequences. [Epigenetic modifications represent mechanisms by which cells may effectively translate multiple signaling inputs into phenotypic outputs. Recent research is revealing that redox metabolism is an increasingly important determinant of epigenetic control that may have significant ramifications in both human health and disease. Numerous characterized epigenetic marks, including histone methylation, acetylation, and ADP-ribosylation, as well as DNA methylation, have direct linkages to central metabolism through critical redox intermediates such as NAD⁺, S-adenosyl methionine, and 2-oxoglutarate. Fluctuations in these intermediates caused by both normal and pathologic stimuli may thus have direct effects on epigenetic signaling that lead to measurable changes in gene expression. In this comprehensive review, we present surveys of both metabolism-sensitive epigenetic enzymes and the metabolic processes that may play a role in their regulation. To close, we provide a series of clinically relevant illustrations of the communication between metabolism and epigenetics in the pathogenesis of cardiovascular disease, Alzheimer disease, cancer, and environmental toxicity. We anticipate that the regulatory mechanisms described herein will play an increasingly large role in our understanding of human health and disease as epigenetics research progresses.] Cyr AR, Domann FE. Antioxid. Redox Signal. 15, 551–589. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3118659/>.

The Role of Nrf2 in the Regulation of Periodontal Health and Disease. [Immune-related disease tolerance is an important defense strategy that facilitates the maintenance of health in organs and tissues that are commonly colonized by bacteria. Immune tolerance to dysbiotic, tooth-borne biofilms is a poorly understood yet clinically relevant concept in the immunopathological mechanisms that are involved in the pathogenesis of periodontitis, particularly those related to neutrophil and macrophage responses. In periodontal health, neutrophils and macrophages respond to the formation of pathogenic bacterial biofilms by the production of bactericidal reactive oxygen species (ROS). However, when released in excess, ROS cause tissue damage and exacerbate inflammation. To counter these destructive responses, many cell types, including neutrophils and macrophages, launch a dedicated antioxidant system that limits the cell and tissue-damaging effects of ROS. The expression of antioxidants is primarily regulated by genetic response elements in their promoters. Here we consider the roles of nuclear factor erythroid 2-related factor (Nrf2), a transcription factor, and other key regulators of antioxidants. The concept of disease tolerance, neutrophil and macrophage-generated oxidative stress, and their relationship to the pathogenesis of periodontitis is reviewed. We focus on the regulation of Nrf2 and recent evidence suggesting that Nrf2 plays a central role in host protection against tissue destruction in periodontitis.] Chiu AV, Saigh MA, et al. J Dent Res. 2017 Aug;96(9):975-983. doi: 10.1177/0022034517715007. <https://www.ncbi.nlm.nih.gov/pubmed/28617616>

The Role of Reactive Oxygen Species and Autophagy in Periodontitis and Their Potential Linkage. [Periodontitis is a chronic inflammatory disease that causes damage to periodontal tissues, which include the gingiva, periodontal ligament, and alveolar bone. The major cause of periodontal tissue destruction is an inappropriate host response to microorganisms and their products. Specifically, a homeostatic imbalance between reactive oxygen species (ROS) and antioxidant defense systems has been implicated in the pathogenesis of periodontitis. Elevated levels of ROS acting as intracellular signal transducers result in autophagy, which plays a dual role in periodontitis by promoting cell death or blocking apoptosis in infected cells. Autophagy can also regulate ROS generation and scavenging. Investigations are ongoing to elucidate the crosstalk mechanisms between ROS and autophagy. Here, we review the physiological and pathological roles of ROS and autophagy in periodontal tissues. The redox-sensitive pathways related to autophagy, such as mTORC1, Beclin 1, and the Atg12-Atg5 complex, are explored in depth to provide a comprehensive overview of the crosstalk between ROS and autophagy. Based on the current evidence, we suggest that a potential linkage between ROS and autophagy is involved in the pathogenesis of periodontitis.]. Liu C, Mo, L, et al. Front. Physiol., 23 June 2017. <https://doi.org/10.3389/fphys.2017.00439>

The role of reactive oxygen and antioxidant species in periodontal tissue destruction. [Oxidative stress lies at the heart of the periodontal tissue damage that results from host–microbial interactions, either as a direct result of excess ROS activity/antioxidant deficiency or indirectly as a result of the activation of redox-sensitive transcription factors and the creation of a pro-inflammatory state (Fig. 13). A body of literature supports peripheral blood neutrophil hyperreactivity in chronic and aggressive forms of periodontitis, with respect to total Fcc-receptor-mediated ROS generation. On balance currently available data suggest that this hyperreactivity has a constitutional element rather than being entirely the result of peripheral priming (e.g. by cytokines or lipopolysaccharide). Furthermore, it seems possible that baseline hyperactivity (i.e. low-level extracellular ROS release in the absence of exogenous stimulus) is also a constitutional property of peripheral neutrophils from periodontitis patients. This, together with the evidence for compromised plasma antioxidant capacity, independent of smoking, suggests an underlying environment of oxidative stress, within periodontitis patients. In addition to this albeit subtle systemic compromise, consider-able evidence has emerged over the last 2 years that oxidative stress and depressed antioxidant function are features of periodontal tissues and fluids in periodontitis

subjects. Several avenues of enquiry now exist for the development of novel antioxidant-based approaches to periodontal therapy. Moreover, specific pathways, such as the glycation and glyoxidation of proteins to produce advanced glycation end products (and increased receptor for advanced glycation end product expression), are dependent on oxidative mechanisms and are highly prevalent in type 2 diabetes and smokers the two major risk factors for periodontitis. Such oxidation products increase neutrophil adhesion, chemotaxis and priming and in hyper-active/reactive neutrophils, may augment the damaging effects of periodontal bacteria-mediated increases in ROS and oxidative stress, providing one explanation for the increased risk of periodontitis in type 2 diabetes and smokers. Besides the largely unexplored traditional routes of increasing the antioxidant capacity of periodontal tissues and extracellular fluids to manage or prevent the excess ROS-mediated tissue damage associated with periodontal hyper-inflammation (e.g. from the activities of neutrophils and fibroblasts), newer routes based upon the modulation of redox-sensitive transcription factors have recently emerged. Such transcription factors (nuclear factor- κ B and activating protein-1) are not only activated by receptor-ligand initiated pathways, but also by non-receptor-mediated shifts in intracellular redox state largely involving GSH depletion (oxidation/glutathionylation) and ROS (e.g. hydrogen peroxide) as metabolic triggers. This array of pathways provides opportunities to develop novel antioxidant therapies that target extracellular, membrane-bound or intra-cellular processes (Fig 12 and 13) and which function not only as antioxidants in the traditional sense but also as powerful anti-inflammatory agents. This review has attempted to bring together 40 years of periodontal research on reactive oxygen and antioxidant species and more recent work on redox biology, and to set this against the huge body of evidence within the biomedical literature, to high-light common mechanistic pathways and exciting new opportunities for the future development of host modulation therapies in periodontology.] Chapple LC, Matthews JB. *Periodontology* 2000, Vol.43, 2007, 160-232.

https://www.researchgate.net/publication/288231635_The_role_of_reactive_oxygen_and_antioxidant_species_in_periodontal_tissue_destruction

Total antioxidant capacity and superoxide dismutase activity levels in serum and gingival crevicular fluid in post-menopausal women with chronic periodontitis. [OBJECTIVES: Menopause has been linked with oxidative stress and decreased antioxidant (AO) defence. A connection has been established between menopause and certain periodontal conditions. The objective of this study is to compare serum and gingival crevicular fluid (GCF) total antioxidant capacity (TAOC) and superoxide dismutase (SOD) concentrations in post-menopausal patients with chronic periodontitis (PMCP) with those of pre-menopausal chronic periodontitis patients (CP). MATERIAL AND METHODS: Thirty-two PMCP patients, 31 CP patients, 25 post-menopausal periodontally healthy controls (PMPH) and 26 pre-menopausal controls (PH) were studied. After clinical measurements and samplings, serum and GCF TAOC and SOD concentrations were established in turn using an automated TAOC assay and spectrophotometric end point measurement. The results were analysed statistically. RESULTS: Serum and GCF TAOC and SOD concentrations were significantly lower in menopause and periodontitis ($p < 0.05$). The lowest values were in the PMCP group, whereas the highest values were in the PH group. While the effect of menopause was more evident in serum antioxidant analysis, the effect of periodontitis was observed to be more apparent in GCF. CONCLUSIONS: A decrease in systemic and local AO defence was observed owing to both menopause and periodontitis. The lowest AO values in the PMCP group suggest that menopause may be a risk factor for periodontitis.] Baltacioglu E, Akaliln FA, et al. *J Clin Periodontol.* 2006 Jun;33(6):385-92. <https://www.ncbi.nlm.nih.gov/pubmed/16677326>.

Total antioxidant capacity and superoxide dismutase activity levels in serum and gingival crevicular fluid in pregnant women with chronic periodontitis. [BACKGROUND: There is evidence of reduced antioxidant (AO) defense in periodontitis and pregnancy and adverse interactions between periodontitis and pregnancy. METHODS: In this study, serum and gingival crevicular fluid (GCF) total AO capacity (TAOC) and superoxide dismutase (SOD) enzyme concentrations in pregnant patients with chronic periodontitis (CP) were compared to those in non-pregnant patients. Periodontal examinations were performed and GCF/serum samples were obtained from 33 pregnant patients with CP (PCP), 18 pregnant patients with gingivitis (PG), and 21 periodontally healthy pregnant controls (P-controls), monitored in the first and third trimesters; 27 non-pregnant women with CP; and 25 non-pregnant control women. The concentrations of TAOC (automated measurement method) and SOD (spectrophotometric method) were determined. RESULTS: Periodontal parameters were higher in pregnant patients versus non-pregnant patients and in the CP group compared to controls, whereas TAOC and SOD concentrations were lower ($P < 0.05$). All parameters, except plaque index, increased in pregnant subjects in the third trimester compared to the first trimester, whereas TAOC and SOD levels decreased ($P < 0.05$). Periodontal parameters were highest and TAOC and SOD levels were lowest in the PCP group in the third trimester ($P < 0.05$). CONCLUSIONS: Systemic and local GCF AO levels decreased in pregnancy and periodontitis, and AO defense reached the lowest levels in the last phase of pregnancy, whereas periodontal status deteriorated. These results suggest that reduced AO capacity may be associated with adverse periodontitis-pregnancy interactions, and each situation can be a provocative risk factor for the other.] Akalin FA, Baltacioglu E, et al. *J Periodontol.* 2009 Mar;80(3):457-67. doi: 10.1902/jop.2009.080218. <https://www.ncbi.nlm.nih.gov/pubmed/19254130>.

Aging

Oxidative stress and protein aggregation during biological aging. [Biological aging is a fundamental process that represents the major risk factor with respect to the development of cancer, neurodegenerative, and cardiovascular diseases in vertebrates. It is,

therefore, evident that the molecular mechanisms of aging are fundamental to understand many disease processes. In this regard, the oxidation and nitration of intracellular proteins and the formation of protein aggregates have been suggested to underlie the loss of cellular function and the reduced ability of senescent animals to withstand physiological stresses. Since oxidatively modified proteins are thermodynamically unstable and assume partially unfolded tertiary structures that readily form aggregates, it is likely that oxidized proteins are intermediates in the formation of amyloid fibrils. It is, therefore, of interest to identify oxidatively sensitive protein targets that may play a protective role through their ability to down-regulate energy metabolism and the consequent generation of reactive oxygen species (ROS). In this respect, the maintenance of cellular calcium gradients represents a major energetic expense, which links alterations in intracellular calcium levels to ATP utilization and the associated generation of ROS through respiratory control mechanisms. The selective oxidation or nitration of the calcium regulatory proteins calmodulin and Ca-ATPase that occurs in vivo during aging and under conditions of oxidative stress may represent an adaptive response to oxidative stress that functions to down-regulate energy metabolism and the associated generation of ROS. Since these calcium regulatory proteins are also preferentially oxidized or nitrated under in vitro conditions, these results suggest an enhanced sensitivity of these critical calcium regulatory proteins, which modulate signal transduction processes and intracellular energy metabolism, to conditions of oxidative stress. Thus, the selective oxidation of critical signal transduction proteins probably represents a regulatory mechanism that functions to minimize the generation of ROS through respiratory control mechanisms. The reduction of the rate of ROS generation, in turn, will promote cellular survival under conditions of oxidative stress, when reactive oxygen and nitrogen species overwhelm cellular antioxidant defense systems, by minimizing the non-selective oxidation of a range of biomolecules. Since protein aggregation occurs if protein repair and degradative systems are unable to act upon oxidized proteins and restore cellular function, the reduction of the oxidative load on the cell by the down-regulation of the electron transport chain functions to minimize protein aggregation. Thus, ROS function as signaling molecules that fine-tune cellular metabolism through the selective oxidation or nitration of calcium regulatory proteins in order to minimize wide-spread oxidative damage and protein aggregation. Oxidative damage to cellular proteins, the loss of calcium homeostasis and protein aggregation contribute to the formation of amyloid deposits that accumulate during biological aging. Critical to understand the relationship between these processes and biological aging is the identification of oxidatively sensitive proteins that modulate energy utilization and the associated generation of ROS. In this latter respect, oxidative modifications to the calcium regulatory proteins calmodulin (CaM) and the sarco/endoplasmic reticulum Ca-ATPase (SERCA) function to down-regulate ATP utilization and the associated generation of ROS associated with replenishing intracellular ATP through oxidative phosphorylation. Reductions in the rate of ROS generation, in turn, will minimize protein oxidation and facilitate intracellular repair and degradative systems that function to eliminate damaged and partially unfolded proteins. Since the rates of protein repair or degradation compete with the rate of protein aggregation, the modulation of intracellular calcium concentrations and energy metabolism through the selective oxidation or nitration of critical signal transduction proteins (i.e. CaM or SERCA) is thought to maintain cellular function by minimizing protein aggregation and amyloid formation. Age-dependent increases in the rate of ROS generation or declines in cellular repair or degradation mechanisms will increase the oxidative load on the cell, resulting in corresponding increases in the concentrations of oxidized proteins and the associated formation of amyloid.] Suier TC. *Exp Gerontol.* 2001 Sep;36(9):1539-50. <https://www.ncbi.nlm.nih.gov/pubmed/11525876>

Redox theory of aging: implications for health and disease. [Genetics ultimately defines an individual, yet the phenotype of an adult is extensively determined by the sequence of lifelong exposures, termed the exposome. The redox theory of aging recognizes that animals evolved within an oxygen-rich environment, which created a critical redox interface between an organism and its environment. Advances in redox biology show that redox elements are present throughout metabolic and structural systems and operate as functional networks to support the genome in adaptation to environmental resources and challenges during lifespan. These principles emphasize that physical and functional phenotypes of an adult are determined by gene–environment interactions from early life onward. The principles highlight the critical nature of cumulative exposure memories in defining changes in resilience progressively during life. Both plasma glutathione and cysteine systems become oxidized with aging, and the recent finding that cystine to glutathione ratio in human plasma predicts death in coronary artery disease (CAD) patients suggests this could provide a way to measure resilience of redox networks in aging and disease. The emerging concepts of cumulative gene–environment interactions warrant focused efforts to elucidate central mechanisms by which exposure memory governs health and etiology, onset and progression of disease.] Young-Mi G, Jones DP. *Clin Sci (Lond).* 2017 Jul 15; 131(14): 1669–1688. Published online 2017 Jun 30. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5773128/>

Role of reactive oxygen species in the defective regeneration seen in aging muscle. [The ability of muscles to regenerate successfully following damage diminishes with age and this appears to be a major contributor to the development of muscle weakness and physical frailty. Successful muscle regeneration is dependent on appropriate reinnervation of regenerating muscle. Age-related changes in the interactions between nerve and muscle are poorly understood but may play a major role in the defective regeneration. During aging there is defective redox homeostasis and an accumulation of oxidative damage in nerve and muscle that may contribute to defective regeneration. The aim of this review is to summarise the evidence that abnormal reactive oxygen species (ROS) generation in nerve and/or muscle may be responsible for the defective regeneration that contributes to the degeneration of skeletal muscle observed during aging. Identifying the importance of ROS generation in skeletal muscle during aging could have fundamental

implications for interventions to prevent muscle degeneration and treatments to reverse the age-related decline in muscle mass and function.] Vasilaki A, Jackson MJ. *Free Radic Biol Med.* 2013 Dec;65:317-323 <https://www.ncbi.nlm.nih.gov/pubmed/23851030/>

Roles of sedentary aging and lifelong physical activity in exchange of glutathione across exercising human skeletal muscle.

[Reactive oxygen species (ROS) are important signaling molecules with regulatory functions, and in young and adult organisms, the formation of ROS is increased during skeletal muscle contractions. However, ROS can be deleterious to cells when not sufficiently counterbalanced by the antioxidant system. Aging is associated with accumulation of oxidative damage to lipids, DNA, and proteins. Given the pro-oxidant effect of skeletal muscle contractions, this effect of age could be a result of excessive ROS formation. We evaluated the effect of acute exercise on changes in blood redox state across the leg of young (23 ± 1 years) and older (66 ± 2 years) sedentary humans by measuring the whole blood concentration of the reduced (GSH) and oxidized (GSSG) forms of the antioxidant glutathione. To assess the role of physical activity, lifelong physically active older subjects (62 ± 2 years) were included. Exercise increased the venous concentration of GSSG in an intensity-dependent manner in young sedentary subjects, suggesting an exercise-induced increase in ROS formation. In contrast, venous GSSG levels remained unaltered during exercise in the older sedentary and active groups despite a higher skeletal muscle expression of the superoxide-generating enzyme NADPH oxidase. Arterial concentration of GSH and expression of antioxidant enzymes in skeletal muscle of older active subjects were increased. The potential impairment in exercise-induced ROS formation may be an important mechanism underlying skeletal muscle and vascular dysfunction with sedentary aging. Lifelong physical activity upregulates antioxidant systems, which may be one of the mechanisms underlying the lack of exercise-induced increase in GSSG.] Nyberg M, Mortensen SP, et al. *Free Radic Biol Med.* 2014 Aug;73:166-73. <https://www.ncbi.nlm.nih.gov/pubmed/24858720>

Asthma

Glutathione Redox Control of Asthma: From Molecular Mechanisms to Therapeutic Opportunities. [Asthma is a chronic inflammatory disorder of the airways associated with airway hyper-responsiveness and airflow limitation in response to specific triggers. Whereas inflammation is important for tissue regeneration and wound healing, the profound and sustained inflammatory response associated with asthma may result in airway remodeling that involves smooth muscle hypertrophy, epithelial goblet-cell hyperplasia, and permanent deposition of airway extracellular matrix proteins. Although the specific mechanisms responsible for asthma are still being unraveled, free radicals such as reactive oxygen species and reactive nitrogen species are important mediators of airway tissue damage that are increased in subjects with asthma. There is also a growing body of literature implicating disturbances in oxidation/reduction (redox) reactions and impaired antioxidant defenses as a risk factor for asthma development and asthma severity. Ultimately, these redox-related perturbations result in a vicious cycle of airway inflammation and injury that is not always amenable to current asthma therapy, particularly in cases of severe asthma. This review will discuss disruptions of redox signaling and control in asthma with a focus on the thiol, glutathione, and reduced (thiol) form (GSH). First, GSH synthesis, GSH distribution, and GSH function and homeostasis are discussed. We then review the literature related to GSH redox balance in health and asthma, with an emphasis on human studies. Finally, therapeutic opportunities to restore the GSH redox balance in subjects with asthma are discussed.] Fitzpatrick AM, Jones DP, et al. *Antioxid. Redox Signal.* 17, 375–408. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3353819/>

Autism

Potential Role of Selenoenzymes and Antioxidant Metabolism in relation to Autism Etiology and Pathology. [Autism and autism spectrum disorders (ASDs) are behaviorally defined, but the biochemical pathogenesis of the underlying disease process remains uncharacterized. Studies indicate that antioxidant status is diminished in autistic subjects, suggesting its pathology is associated with augmented production of oxidative species and/or compromised antioxidant metabolism. This suggests ASD may result from defects in the metabolism of cellular antioxidants which maintain intracellular redox status by quenching reactive oxygen species (ROS). Selenium-dependent enzymes (selenoenzymes) are important in maintaining intercellular reducing conditions, particularly in the brain. Selenoenzymes are a family of ~25 genetically unique proteins, several of which have roles in preventing and reversing oxidative damage in brain and endocrine tissues. Since the brain's high rate of oxygen consumption is accompanied by high ROS production, selenoenzyme activities are particularly important in this tissue. Because selenoenzymes can be irreversibly inhibited by many electrophiles, exposure to these organic and inorganic agents can diminish selenoenzyme-dependent antioxidant functions. This can impair brain development, particularly via the adverse influence of oxidative stress on epigenetic regulation. Here we review the physiological roles of selenoproteins in relation to potential biochemical mechanisms of ASD etiology and pathology.] Raymond LJ, Deth RC, et al. *Autism Res Treat.* 2014; 2014: 164938. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3966422/?fbclid=IwAR2AvJDjGfgyBWZZX0GHDQVowvHxhuymA3--44CJtdqxe1AXm1iyFW5EV0g>

Bowel & Colon Health

Inflammatory bowel disease: mechanisms, redox considerations, and therapeutic targets. [Oxidative stress is thought to play a key role in the development of intestinal damage in inflammatory bowel disease (IBD), because of its primary involvement in intestinal cells' aberrant immune and inflammatory responses to dietary antigens and to the commensal bacteria. During the active disease phase, activated leukocytes generate not only a wide spectrum of pro-inflammatory cytokines, but also excess oxidative reactions, which markedly alter the redox equilibrium within the gut mucosa, and maintain inflammation by inducing redox-sensitive signaling pathways and transcription factors. Moreover, several inflammatory molecules generate further oxidation products, leading to a self-sustaining and auto-amplifying vicious circle, which eventually impairs the gut barrier. The current treatment of IBD consists of long-term conventional anti-inflammatory therapy and often leads to drug refractoriness or intolerance, limiting patients' quality of life. Immune modulators or anti-tumor necrosis factor α antibodies have recently been used, but all carry the risk of significant side effects and a poor treatment response. Recent developments in molecular medicine point to the possibility of treating the oxidative stress associated with IBD, by designing a proper supplementation of specific lipids to induce local production of anti-inflammatory derivatives, as well as by developing biological therapies that target selective molecules (i.e., nuclear factor- κ B, NADPH oxidase, prohibitins, or inflammasomes) involved in redox signaling. The clinical significance of oxidative stress in IBD is now becoming clear, and may soon lead to important new therapeutic options to lessen intestinal damage in this disease.] Biasi F, Leonarduzzi G, et al. *Antioxid Redox Signal*. 2013 Nov 10;19(14):1711-47. doi: 10.1089/ars.2012.4530. <https://www.ncbi.nlm.nih.gov/pubmed/?term=inflammatory+bowel+disease%3A+mechanisms%2C+redox+considerations>

Cancer

Diagnosing and exploiting cancer's addiction to blocks in apoptosis. [Cancer cells survive despite violating rules of normal cellular behaviour that ordinarily provoke apoptosis. The blocks in apoptosis that keep cancer cells alive are therefore attractive candidates for targeted therapies. Recent studies have significantly increased our understanding of how interactions among proteins in the BCL2 family determine cell survival or death. It is now possible to systematically determine how individual cancers escape apoptosis. Such a determination can help predict not only whether cells are likely to be killed by antagonism of BCL2, but also whether they are likely to be sensitive to chemotherapy that kills by the intrinsic apoptotic pathway.] Letai AG. *Nat Rev Cancer*. 2008 Feb;8(2):121-32.. <https://www.ncbi.nlm.nih.gov/pubmed/18202696>

GPx3-mediated redox signaling arrests the cell cycle and acts as a tumor suppressor in lung cancer cell lines. [Glutathione peroxidase 3 (GPx3), a major scavenger of reactive oxygen species (ROS) in plasma, acts as a redox signal modulator. However, the mechanism underlying GPx3-mediated suppression of cancer cell growth is unclear. The aim of this study was to identify these mechanisms with respect to lung cancer. To enhance the redox modulating properties of GPx3, lung cancer cells were subjected to serum starvation for 12 h, resulting in ROS generation in the absence of oxidant treatment. We then investigated whether suppression of tumorigenesis under conditions of oxidative stress was dependent on GPx3. The results showed that GPx3 effectively suppressed proliferation, migration, and invasion of lung cancer cells under oxidative stress. In addition, GPx3 expression led to a significant reduction in ROS production by cancer cells and induced G2/M phase arrest. We also found that inactivation of cyclin B1 significantly suppressed by nuclear factor- κ B(NF- κ B) inactivation in lung cancer cells was dependent on GPx3 expression. To further elucidate the mechanism(s) underlying GPx3-mediated suppression of tumor proliferation, we next examined the effect of GPx3-mediated redox signaling on the ROS-MKP3-extracellular signal-regulated kinase (Erk)-NF- κ B-cyclin B1 pathway and found that GPx3 strongly suppressed activation of the Erk-NF- κ B-cyclin B1 signaling cascade by protecting MKP3 (an Erk-specific phosphatase) from the effects of ROS. Thus, this study demonstrates for the first time that the GPx3 suppresses proliferation of lung cancer cells by modulating redox-mediated signals.] Byung CA, Choi YD, et al. *PLOS One*, Sept 27, 2018. <https://doi.org/10.1371/journal.pone.0204170>

Hallmarks of cancer: the next generation. [The hallmarks of cancer comprise six biological capabilities acquired during the multistep development of human tumors. The hallmarks constitute an organizing principle for rationalizing the complexities of neoplastic disease. They include sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis. Underlying these hallmarks are genome instability, which generates the genetic diversity that expedites their acquisition, and inflammation, which fosters multiple hallmark functions. Conceptual progress in the last decade has added two emerging hallmarks of potential generality to this list-reprogramming of energy metabolism and evading immune destruction. In addition to cancer cells, tumors exhibit another dimension of complexity: they contain a repertoire of recruited, ostensibly normal cells that contribute to the acquisition of hallmark traits by creating the "tumor microenvironment." Recognition of the widespread applicability of these concepts will increasingly affect the development of new means to treat human cancer.] Hanahan D, Weinberg RA. *Cell*. 2011 Mar 4;144(5):646-74. <https://www.ncbi.nlm.nih.gov/pubmed/21376230>

Hydrogen peroxide – production, fate and role in redox signaling of tumor cells. [Hydrogen peroxide (H₂O₂) is involved in various signal transduction pathways and cell fate decisions. The mechanism of the so called “redox signaling” includes the H₂O₂-mediated reversible oxidation of redox sensitive cysteine residues in enzymes and transcription factors thereby altering their activities. Depending on its intracellular concentration and localization, H₂O₂ exhibits either pro- or anti-apoptotic activities. In comparison to normal cells, cancer cells are characterized by an increased H₂O₂ production rate and an impaired redox balance thereby affecting the microenvironment as well as the anti-tumoral immune response. This article reviews the current knowledge about the intracellular production of H₂O₂ along with redox signaling pathways mediating either the growth or apoptosis of tumor cells. In addition it will be discussed how the targeting of H₂O₂-linked sources and/or signaling components involved in tumor progression and survival might lead to novel therapeutic targets.] Lennicke C, Rahn J, et al. *Cell Communication and Signaling* 2015;13:39. <https://biosignaling.biomedcentral.com/articles/10.1186/s12964-015-0118-6>

Redox Control of Apoptosis: An Update. [The redox environment of the cell is currently thought to be extremely important to control cell growth, differentiation, and apoptosis as many redox-sensitive proteins characterize these networks. A recent, widely accepted theory is that free radicals are not only dangerous species but, at low concentration, they have been designed by evolution to participate in the maintenance of cellular redox (reduction/oxidation) homeostasis. This notion derives from the evidence that cells constantly generate free radicals both as waste products of aerobic metabolism and in response to a large variety of stimuli. Free radicals, once produced, provoked cellular responses (redox regulation) against oxidative stress transducing the signals to maintain the cellular redox balance. Growing evidence suggests that in many instances the production of radical species is tightly regulated and their downstream targets are very specific, indicating that reactive oxygen species and reactive nitrogen species actively participate in several cell-signalling pathways as physiological "second messengers." In this review, we provide a general overview and novel insights into the redox-dependent pathways involved in programmed cell death.] Filomeni G, Ciriolo MR. *Antioxidants and Redox Signaling* 8(11-12):2187-92 · November 2006. https://www.researchgate.net/publication/6761655_Redox_Control_of_Apoptosis_An_Update

Redox control of cancer cell destruction. [Redox regulation has been proposed to control various aspects of carcinogenesis, cancer cell growth, metabolism, migration, invasion, metastasis and cancer vascularization. As cancer has many faces, the role of redox control in different cancers and in the numerous cancer-related processes often point in different directions. In this review, we focus on the redox control mechanisms of tumor cell destruction. The review covers the tumor-intrinsic role of oxidants derived from the reduction of oxygen and nitrogen in the control of tumor cell proliferation as well as the roles of oxidants and antioxidant systems in cancer cell death caused by traditional anticancer weapons (chemotherapeutic agents, radiotherapy, photodynamic therapy). Emphasis is also put on the role of oxidants and redox status in the outcome following interactions between cancer cells, cytotoxic lymphocytes and tumor infiltrating macrophages.] Hagedus C, Kovacs K, et al. *Redox Biol.* 2018 Jun;16:59-74 <https://www.ncbi.nlm.nih.gov/pubmed/29477046>

Redox Homeostasis and Cellular Antioxidant Systems: Crucial Players in Cancer Growth and Therapy. [Reactive oxygen species (ROS) and their products are components of cell signaling pathways and play important roles in cellular physiology and pathophysiology. Under physiological conditions, cells control ROS levels by the use of scavenging systems such as superoxide dismutases, peroxiredoxins, and glutathione that balance ROS generation and elimination. Under oxidative stress conditions, excessive ROS can damage cellular proteins, lipids, and DNA, leading to cell damage that may contribute to carcinogenesis. Several studies have shown that cancer cells display an adaptive response to oxidative stress by increasing expression of antioxidant enzymes and molecules. As a double-edged sword, ROS influence signaling pathways determining beneficial or detrimental outcomes in cancer therapy. In this review, we address the role of redox homeostasis in cancer growth and therapy and examine the current literature regarding the redox regulatory systems that become upregulated in cancer and their role in promoting tumor progression and resistance to chemotherapy.] Marengo B, Nitti M., et al. *Oxidative Medicine and Cellular Longevity*. Volume 2016, Article ID 6235641, 16 pages. <http://dx.doi.org/10.1155/2016/6235641>. <https://www.hindawi.com/journals/omcl/2016/6235641/>

Redox regulation in cancer: a double-edged sword with therapeutic potential. [Oxidative stress, implicated in the etiology of cancer, results from an imbalance in the production of reactive oxygen species (ROS) and cell's own antioxidant defenses. ROS deregulate the redox homeostasis and promote tumor formation by initiating an aberrant induction of signaling networks that cause tumorigenesis. Ultraviolet (UV) exposures, gamma-radiation and other environmental carcinogens generate ROS in the cells, which can exert apoptosis in the tumors, thereby killing the malignant cells or induce the progression of the cancer growth by blocking cellular defense system. Cancer stem cells take the advantage of the aberrant redox system and spontaneously proliferate. Oxidative stress and gene-environment interactions play a significant role in the development of breast, prostate, pancreatic and colon cancer. Prolonged lifetime exposure to estrogen is associated with several kinds of DNA damage. Oxidative stress and estrogen receptor-associated proliferative changes are suggested to play important roles in estrogen-induced breast carcinogenesis. BRCA1, a tumor suppressor against hormone responsive cancers such as breast and prostate cancer, plays a significant role in inhibiting ROS and estrogen mediated DNA damage; thereby regulate the redox homeostasis of the cells. Several transcription factors and tumor suppressors are involved during stress response such as Nrf2, NF-kappaB and BRCA1. A promising strategy for targeting redox status

of the cells is to use readily available natural substances from vegetables, fruits, herbs and spices. Many of the phytochemicals have already been identified to have chemopreventive potential, capable of intervening in carcinogenesis.] Acharya A, Das I, et al. *Oxid Med Cell Longev*. 2010 Jan-Feb;3(1):23-34. doi: 10.4161/oxim.3.1.10095. <https://www.ncbi.nlm.nih.gov/pubmed/20716925>

Redox sensing: orthogonal control in cell cycle and apoptosis signaling. [Living systems have three major types of cell signalling systems that are dependent upon high-energy chemicals, redox environment and transmembranal ion-gating mechanisms. Development of integrated systems biology descriptions of cell signalling require conceptual models incorporating all three. Recent advances in redox biology show that thiol–disulphide redox systems are regulated under dynamic, nonequilibrium conditions, progressively oxidized with the life cycle of cells and distinct in terms of redox potentials amongst subcellular compartments. This article uses these observations as a basis to distinguish ‘redox-sensing’ mechanisms, which are more global biologic redox control mechanisms, from ‘redox signalling’, which involves conveyance of discrete activating or inactivating signals. Both redox sensing and redox signalling use sulphur switches, especially cysteine (Cys) residues in proteins which are sensitive to reversible oxidation, nitrosylation, glutathionylation, acylation, sulfhydration or metal binding. Unlike specific signalling mechanisms, the redox-sensing mechanisms provide means to globally affect the rates and activities of the high-energy, ion-gating and redox-signalling systems by controlling sensitivity, distribution, macromolecular interactions and mobility of signalling proteins. Effects mediated through Cys residues not directly involved in signalling means redox-sensing control can be orthogonal to the signalling mechanisms. This provides a capability to integrate signals according to cell cycle and physiologic state without fundamentally altering the signalling mechanisms. Recent findings that thiol–disulphide pools in humans are oxidized with age, environmental exposures and disease risk suggest that redox-sensing thiols could provide a central mechanistic link in disease development and progression.] Jones DP. *J Intern Med* 2010; 268: 432–448. <https://onlinelibrary.wiley.com/doi/full/10.1111/j.1365-2796.2010.02268.x>

Regulation of redox balance in cancer and T cells. [Reactive oxygen species (ROS) mediate redox signaling necessary for numerous cellular functions. Yet, high levels of ROS in cells and tissues can cause damage and cell death. Therefore, regulation of redox homeostasis is essential for ROS-dependent signaling that does not incur cellular damage. Cells achieve this optimal balance by coordinating ROS production and elimination. In this Minireview, we discuss the mechanisms by which proliferating cancer and T cells maintain a carefully controlled redox balance. Greater insight into such redox biology may enable precisely targeted manipulation of ROS for effective medical therapies against cancer or immunological disorders.] Kong H, Chandel NS. *J Biol Chem*. 2018 May 18;293(20):7499-7507. <https://www.ncbi.nlm.nih.gov/pubmed/29282291>

ROS homeostasis and metabolism: a dangerous liason in cancer cells. [Tumor cells harbor genetic alterations that promote a continuous and elevated production of reactive oxygen species. Whereas such oxidative stress conditions would be harmful to normal cells, they facilitate tumor growth in multiple ways by causing DNA damage and genomic instability, and ultimately, by reprogramming cancer cell metabolism. This review outlines the metabolic-dependent mechanisms that tumors engage in when faced with oxidative stress conditions that are critical for cancer progression by producing redox cofactors. In particular, we describe how the mitochondria has a key role in regulating the interplay between redox homeostasis and metabolism within tumor cells. Last, we will discuss the potential therapeutic use of agents that directly or indirectly block metabolism.] Panieri E, Santoro MM. *Cell Death & Disease* volume 7, page e2253 (2016). <https://www.nature.com/articles/cddis2016105>

The Role of Cellular Glutathione Peroxidase Redox Regulation in the Suppression of Tumor Cell Growth by Manganese Superoxide Dismutase. [Manganese-containing superoxide dismutase (MnSOD) is an essential primary antioxidant enzyme that converts superoxide radical to hydrogen peroxide and molecular oxygen within the mitochondrial matrix. Cytosolic glutathione peroxidase (GPX) converts hydrogen peroxide into water. MnSOD is reduced in a variety of tumor types and has been proposed to be a new kind of tumor suppressor gene, but the mechanism(s) by which MnSOD suppresses malignancy is unclear. According to the enzymatic reactions catalyzed by MnSOD and cytosolic GPX, change in the cellular redox status, especially change attributable to accumulation of hydrogen peroxide or other hydroperoxides, is a possible reason to explain the suppression of tumor growth observed in MnSOD-overexpressing cells. To test this possible mechanism, we transfected human cytosolic GPX cDNA into human glioma cells overexpressing MnSOD. The results showed that GPX overexpression not only reversed the tumor cell growth inhibition caused by MnSOD overexpression but also altered the cellular contents of total glutathione, reduced glutathione, oxidized glutathione, and intracellular reactive oxygen species. Overexpression of GPX also inhibited degradation of the inhibitory subunit α of nuclear factor- κ B. These results suggest that hydrogen peroxide or other hydroperoxides appear to be key reactants in the tumor suppression by MnSOD overexpression, and growth inhibition correlates with the intracellular redox status. This work suggests that manipulations that inhibit peroxide removal should enhance the tumor suppressive effect of MnSOD overexpression.] Shijun Li, Tao Y, et al. *Cancer Research*, July 2000, Vol 60, Issue 14. <http://cancerres.aacrjournals.org/content/60/14/3927.short>

Cardiovascular Health

Insights into the Redox Control of Blood Coagulation: Role of Vascular NADPH Oxidase-Derived Reactive Oxygen Species in the Thrombogenic Cycle. [Various cardiovascular diseases including thrombosis, atherosclerosis, (pulmonary) hypertension and diabetes, are associated with disturbed coagulation. Alterations in the vessel wall common to many cardiovascular disorders have been shown to initiate the activity of the coagulation system, but also to be the result of an abnormal coagulation system. The primary link between the coagulation and the vascular system appears to be tissue factor (TF), which is induced on the surface of vascular cells and initiates the extrinsic pathway of the blood coagulation cascade, leading to the formation of thrombin. Thrombin can also interact with the vascular wall via specific receptors and can increase vascular TF expression. Such a "thrombogenic cycle" may be essentially involved in the pathogenesis of cardiovascular disorders associated with an abnormal coagulation. Therefore, the identification of the signaling pathways regulating this cycle and each of its relevant connecting links is of fundamental importance for the understanding of these disorders and their putative therapeutic potential. Reactive oxygen species (ROS) and the ROS-generating NADPH oxidases have been shown to play important roles as signaling molecules in the vasculature. In this review, we summarize the data supporting a substantial role of ROS in promoting a thrombogenic cycle in the vascular system.] *Antioxidants and Redox Signaling* 6(4):765-76 · September 2004. <https://www.liebertpub.com/doi/abs/10.1089/1523086041361695>

Modulation of Protein Kinase Activity and Gene Expression by Reactive Oxygen Species and Their Role in Vascular Physiology and Pathophysiology. [Abstract—Emerging evidence indicates that reactive oxygen species, especially superoxide and hydrogen peroxide, are important signaling molecules in cardiovascular cells. Their production is regulated by hormone-sensitive enzymes such as the vascular NAD(P)H oxidases, and their metabolism is coordinated by antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase. Both of these reactive oxygen species serve as second messengers to activate multiple intracellular proteins and enzymes, including the epidermal growth factor receptor, c-Src, p38 mitogen-activated protein kinase, Ras, and Akt/protein kinase B. Activation of these signaling cascades and redox-sensitive transcription factors leads to induction of many genes with important functional roles in the physiology and pathophysiology of vascular cells. Thus, reactive oxygen species participate in vascular smooth muscle cell growth and migration; modulation of endothelial function, including endothelium-dependent relaxation and expression of a proinflammatory phenotype; and modification of the extracellular matrix. All of these events play important roles in vascular diseases such as hypertension and atherosclerosis, suggesting that the sources of reactive oxygen species and the signaling pathways that they modify may represent important therapeutic targets.] *Griendling KK, Sorescu D, et al. Arteriosclerosis, Thrombosis, and Vascular Biology, Vol 20, No 10.* <https://www.ahajournals.org/doi/full/10.1161/01.ATV.20.10.2175>

Nuclear factor E2-related factor 2-dependent myocardial cytoprotection against oxidative and electrophilic stress. [Nuclear factor E2-related factor 2 (Nrf2) is a critical regulator of cytoprotective gene expression. However, the role of this transcription factor in myocardial cytoprotection against oxidative and electrophilic stress remains unknown. This study was undertaken to investigate if Nrf2 signaling could control the constitutive and inducible expression of antioxidants and phase 2 enzymes in primary cardiomyocytes as well as the susceptibility of these cells to oxidative and electrophilic injury. The basal expression of a series of antioxidants and phase 2 enzymes was significantly lower in cardiomyocytes from Nrf2(-/-) mice than those from wild-type littermates. Incubation of wild-type cardiomyocytes with 3H-1,2-dithiole-3-thione (D3T) led to significant induction of various antioxidants and phase 2 enzymes, including catalase, glutathione, glutathione peroxidase (GPx), glutathione reductase, glutathione S-transferase, NAD(P)H:quinone oxidoreductase 1, and heme oxygenase-1. The inducibility of the above cellular defenses except GPx by D3T was abolished in Nrf2(-/-) cardiomyocytes. As compared to wild-type cells, Nrf2(-/-) cardiomyocytes were much more susceptible to cell injury induced by H(2)O(2), peroxynitrite, and 4-hydroxy-2-nonenal. Treatment of wild-type cardiomyocytes with D3T, which upregulated the cellular defenses, resulted in increased resistance to the above oxidant- and electrophile-induced cell injury, whereas D3T treatment of Nrf2(-/-) cardiomyocytes provided no cytoprotection. This study demonstrates that Nrf2 is an important factor in controlling both constitutive and inducible expression of a wide spectrum of antioxidants and phase 2 enzymes in cardiomyocytes and is responsible for protecting these cells against oxidative and electrophilic stress. These findings also implicate Nrf2 as an important signaling molecule for myocardial cytoprotection.] *Zhu H, Jia Z, et al. Cardiovasc Toxicol. 2008 Summer;8(2):71-85.* <https://www.ncbi.nlm.nih.gov/pubmed/18463988>

Nrf2-dependent upregulation of antioxidative enzymes: a novel pathway for proteasome inhibitor-mediated cardioprotection. [AIMS: We have shown previously that non-toxic inhibition of the ubiquitin-proteasome system upregulates antioxidative defence mechanisms and protects endothelial cells from oxidative stress. Here, we have addressed the question whether the induction of antioxidative enzymes contributes to cardioprotection by non-toxic proteasome inhibition. METHODS AND RESULTS: Treatment with 0.5 micromol/L MG132 for 48 h proved to be non-toxic and protected neonatal rat cardiac myocytes against H(2)O(2)-mediated oxidative stress in lactate dehydrogenase assays. This correlated with reduced levels of intracellular reactive oxygen species as determined by loading myocytes with dichlorofluorescein. Immunoblots showed significant upregulation of superoxide dismutase 1 (SOD1), haem oxygenase 1, and catalase upon proteasome inhibition. Luciferase assays using a reporter driven by the SOD1 promoter

revealed proteasome inhibitor-mediated induction of luciferase activity. Deletion and mutation analyses identified an antioxidant response element (ARE) in the SOD1 promoter to be not only essential but also sufficient for transcriptional upregulation by proteasome inhibition. An essential role for the antioxidative transcription factor NF-E2-related factor 2 (Nrf2)-which was stabilized by proteasome inhibition-in ARE-mediated transcriptional activation was revealed in cardiac myocytes from Nrf2 wild-type and knockout mice: proteasome inhibition upregulated antioxidative enzymes and conferred protection against H₂O₂-mediated oxidative stress in Nrf2 wild-type cells. In contrast, the induction of antioxidative enzymes and cytoprotection were completely abolished in cardiac myocytes from Nrf2 knockout mice. CONCLUSION: Non-toxic proteasome inhibition upregulates antioxidative enzymes via an Nrf2-dependent transcriptional activation of AREs and confers cardioprotection.] Dreger H, Westphal K, et al. *Cardiovasc Res.* 2009 Jul 15;83(2):354-61. <https://www.ncbi.nlm.nih.gov/pubmed/19351736>

Oxidative Stress, Vascular Remodeling, and Vascular Inflammation in Hypertension. [the observed elevation in inflammatory parameters in subjects who subsequently go on to develop hypertension is particularly relevant and creates options for potential primary prevention strategies. A number of therapeutic agents have been identified which are able to influence this inflammatory process and positively influence cardiovascular outcomes.] Renna, NF. *Int J Hypertens.* 2013; 2013: 710136. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3818852/>

Reactive Oxygen Species, Vascular Oxidative Stress, and Redox Signaling in Hypertension. [Metabolism of oxygen by cells generates potentially deleterious reactive oxygen species (ROS). Under normal conditions the rate and magnitude of oxidant formation is balanced by the rate of oxidant elimination. However, an imbalance between prooxidants and antioxidants results in oxidative stress, which is the pathogenic outcome of oxidant overproduction that overwhelms the cellular antioxidant capacity. The kidney and vasculature are rich sources of NADPH oxidase-derived ROS, which under pathological conditions play an important role in renal dysfunction and vascular damage. Strong experimental evidence indicates that increased oxidative stress and associated oxidative damage are mediators of renovascular injury in cardiovascular pathologies. Increased production of superoxide anion and hydrogen peroxide reduced nitric oxide synthesis, and decreased bioavailability of antioxidants have been demonstrated in experimental and human hypertension. These findings have evoked considerable interest because of the possibilities that therapies targeted against free radicals by decreasing ROS generation or by increasing nitric oxide availability and antioxidants may be useful in minimizing vascular injury and renal dysfunction and thereby prevent or regress hypertensive end-organ damage. This article highlights current developments in the field of ROS and hypertension, focusing specifically on the role of oxidative stress in hypertension-associated vascular damage. In addition, recent clinical trials investigating cardiovascular benefits of antioxidants are discussed, and some explanations for the rather disappointing results from these studies are addressed. Finally, important avenues for future research in the field of ROS, oxidative stress, and redox signaling in hypertension are considered.] Touyz RM. *Hypertension*, September 2004, Vol 44, Issue 3. P 248-252. <https://www.ahajournals.org/doi/abs/10.1161/01.HYP.0000138070.47616.9d>

Redox Control of Endothelial Function and Dysfunction: Molecular Mechanisms and Therapeutic Opportunities. [The endothelium is essential for the maintenance of vascular homeostasis. Central to this role is the production of endothelium-derived nitric oxide (EDNO), synthesized by the endothelial isoform of nitric oxide synthase (eNOS). Endothelial dysfunction, manifested as impaired EDNO bioactivity, is an important early event in the development of various vascular diseases, including hypertension, diabetes, and atherosclerosis. The degree of impairment of EDNO bioactivity is a determinant of future vascular complications. Accordingly, growing interest exists in defining the pathologic mechanisms involved. Considerable evidence supports a causal role for the enhanced production of reactive oxygen species (ROS) by vascular cells. ROS directly inactivate EDNO, act as cell-signaling molecules, and promote protein dysfunction, events that contribute to the initiation and progression of endothelial dysfunction. Increasing data indicate that strategies designed to limit vascular ROS production can restore endothelial function in humans with vascular complications. The purpose of this review is to outline the various ways in which ROS can influence endothelial function and dysfunction, describe the redox mechanisms involved, and discuss approaches for preventing endothelial dysfunction that may highlight future therapeutic opportunities in the treatment of cardiovascular disease.] Thomas, SR, Sitting PK, et al. *Antioxidants & Redox Signaling*, Vol. 10, No. 10. Published Online:15 Aug 2008<https://doi.org/10.1089/ars.2008.2027> . <https://www.liebertpub.com/doi/abs/10.1089/ars.2008.2027>.

Redox signaling in cardiac physiology and pathology. [Redox signaling refers to the specific and usually reversible oxidation/reduction modification of molecules involved in cellular signaling pathways. In the heart, redox signaling regulates several physiological processes (eg, excitation-contraction coupling) and is involved in a wide variety of pathophysiological and homeostatic or stress response pathways. Reactive oxygen species involved in cardiac redox signaling may derive from many sources, but NADPH oxidases, as dedicated sources of signaling reactive oxygen species, seem to be especially important. An increasing number of specific posttranslational oxidative modifications involved in cardiac redox signaling are being defined, along with the reactive oxygen species sources that are involved. Here, we review current knowledge on the molecular targets of signaling reactive oxygen species in cardiac cells and their involvement in cardiac physiopathology. Advances in this field may allow the development of targeted therapeutic strategies for conditions such as heart failure as opposed to the general antioxidant approaches that have failed to date.] Burgoyne JR, Mongue-Din H, et al. *Circ Res.* 2012 Sep 28;111(8):1091-106. <https://www.ncbi.nlm.nih.gov/pubmed/23023511>

Redox signaling in cardiovascular health and disease. [Spatiotemporal regulation of the activity of a vast array of intracellular proteins and signaling pathways by reactive oxygen species (ROS) governs normal cardiovascular function. However, data from experimental and animal studies strongly support that dysregulated redox signaling, resulting from hyper-activation of various cellular oxidases or mitochondrial dysfunction, is integral to the pathogenesis and progression of cardiovascular disease (CVD). In this review, we address how redox signaling modulates the protein function, the various sources of increased oxidative stress in CVD, and the labyrinth of redox-sensitive molecular mechanisms involved in the development of atherosclerosis, hypertension, cardiac hypertrophy and heart failure, and ischemia–reperfusion injury. Advances in redox biology and pharmacology for inhibiting ROS production in specific cell types and subcellular organelles combined with the development of nanotechnology-based new in vivo imaging systems and targeted drug delivery mechanisms may enable fine-tuning of redox signaling for the treatment and prevention of CVD.] Madamanchi NR, Runge MS. *Free Radic Biol Med.* 2013 Aug; 0: 473–501. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3883979/>

ROS signaling and redox biology in endothelial cells. [The purpose of this review is to provide an overview of redox mechanisms, sources and antioxidants that control signaling events in ECs. In particular, we describe which molecules are involved in redox signaling and how they influence the relationship between ECs and other vascular component with regard to angiogenesis. Recent and new tools to investigate physiological ROS signaling will be also discussed. Such findings are providing an overview of the ROS biology relevant for endothelial cells in the context of normal and pathological angiogenic conditions.] Panieri E, Santoro MM. *Mol. Life Sci.* (2015) 72: 3281. <https://doi.org/10.1007/s00018-015-1928-9>. <https://link.springer.com/article/10.1007/s00018-015-1928-9>.

The Role of Redox Signaling in Epigenetics and Cardiovascular Disease. [The term epigenetics refers to the changes in the phenotype and gene expression that occur without alterations in the DNA sequence. There is a rapidly growing body of evidence that epigenetic modifications are involved in the pathological mechanisms of many cardiovascular diseases (CVDs), which intersect with many of the pathways involved in oxidative stress. Recent Advances: Most studies relating epigenetics and human pathologies have focused on cancer. There has been a limited study of epigenetic mechanisms in CVDs. Although CVDs have multiple established genetic and environmental risk factors, these explain only a portion of the total CVD risk. The epigenetic perspective is beginning to shed new light on how the environment influences gene expression and disease susceptibility in CVDs. Known epigenetic changes contributing to CVD include hypomethylation in proliferating vascular smooth muscle cells in atherosclerosis, changes in estrogen receptor- α (ER- α) and ER- β methylation in vascular disease, decreased superoxide dismutase 2 expression in pulmonary hypertension (PH), as well as trimethylation of histones H3K4 and H3K9 in congestive heart failure. Critical Issues: In this review, we discuss the epigenetic modifications in CVDs, including atherosclerosis, congestive heart failure, hypertension, and PH, with a focus on altered redox signaling. Future Directions: As advances in both the methodology and technology accelerate the study of epigenetic modifications, the critical role they play in CVD is beginning to emerge. A fundamental question in the field of epigenetics is to understand the biochemical mechanisms underlying reactive oxygen species-dependent regulation of epigenetic modification.] Kim GH, Ryan JJ, et al. *Antioxid. Redox Signal.* 18, 1920–1936. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3624767/>

VEGF receptor signaling – in control of vascular function. [Vascular endothelial growth-factor receptors (VEGFRs) regulate the cardiovascular system. VEGFR1 is required for the recruitment of haematopoietic precursors and migration of monocytes and macrophages, whereas VEGFR2 and VEGFR3 are essential for the functions of vascular endothelial and lymphendothelial cells, respectively. Recent insights have shed light onto VEGFR signal transduction and the interplay between different VEGFRs and VEGF co-receptors in development, adult physiology and disease.] Olsson, AK, Dimberg, A, et al. *Nature Reviews – Molecular Cell Biology*, Vol 7, May 2006, p 359. <http://web.mit.edu/hst527/www/readings/Lecture%206/VEGF.pdf>

Cellular Healing and Wound Repair

Dermal wound healing is subject to redox control. [Previously we have reported in vitro evidence suggesting that that H₂O₂ may support wound healing by inducing VEGF expression in human keratinocytes (JBC 277: 33284–90). Here, we test the significance of H₂O₂ in regulating wound healing in vivo. Using the Hunt-Schilling cylinder approach we present first evidence that the wound site contains micromolar concentration of H₂O₂. At the wound site, low concentrations of H₂O₂ supported the healing process especially in p47phox and MCP-1 deficient mice where endogenous H₂O₂ generation is impaired. Higher doses of H₂O₂ adversely influenced healing. At low concentrations, H₂O₂ facilitated wound angiogenesis in vivo. H₂O₂ induced FAK phosphorylation both in wound-edge tissue in vivo as well as in human dermal microvascular endothelial cells (HMEC). H₂O₂ induced site-specific (Tyr-925 & Tyr-861) phosphorylation of FAK. Other sites, including the Tyr-397 autophosphorylation site, were insensitive to H₂O₂. Adenoviral gene delivery of catalase impaired wound angiogenesis and closure. Catalase over-expression slowed tissue remodeling as evident by a more incomplete narrowing of the hyperproliferative epithelium region and incomplete eschar formation. Taken together, this work presents the first in vivo evidence indicating that strategies to influence the redox environment of the wound site may have a bearing on healing outcomes.] RoyS, Khanna S, et al. *Mol Ther.* 2006 Jan; 13(1): 211–220. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1389791/>

Generating and Reversing Chronic Wounds in Diabetic Mice by Manipulating Wound Redox Parameters. [By 2025, more than 500 M people worldwide will suffer from diabetes; 125 M will develop foot ulcer(s) and 20 M will undergo an amputation, creating a major health problem. Understanding how these wounds become chronic will provide insights to reverse chronicity. We hypothesized that oxidative stress (OS) in wounds is a critical component for generation of chronicity. We used the db/db mouse model of impaired healing and inhibited, at time of injury, two major antioxidant enzymes, catalase and glutathione peroxidase, creating high OS in the wounds. This was necessary and sufficient to trigger wounds to become chronic. The wounds initially contained a polymicrobial community that with time selected for specific biofilm-forming bacteria. To reverse chronicity we treated the wounds with the antioxidants α -tocopherol and N-acetylcysteine and found that OS was highly reduced, biofilms had increased sensitivity to antibiotics, and granulation tissue was formed with proper collagen deposition and remodeling. We show for the first time generation of chronic wounds in which biofilm develops spontaneously, illustrating importance of early and continued redox imbalance coupled with the presence of biofilm in development of wound chronicity. This model will help decipher additional mechanisms and potentially better diagnosis of chronicity and treatment of human chronic wounds.] Dhall S, Do DC, et al. *Journal of Diabetes Research*. Volume 2014, Article ID 562625, 18 pages, <http://dx.doi.org/10.1155/2014/562625>. <https://www.hindawi.com/journals/jdr/2014/562625/abs/>

Oxidative stress in normal and impaired wound repair. [A large percentage of the population suffers from wound healing abnormalities, in particular aged individuals, patients with diabetes, and those treated with immunosuppressive drugs, chemo- or radiotherapy. The mechanisms underlying the impaired healing response are still poorly understood. Recent studies provided strong evidence for a role of oxidative stress in the pathogenesis of non-healing ulcers. Therefore, it is of major importance to identify and functionally characterize the factors involved in the generation and detoxification of reactive oxygen species (ROS). This will provide the basis for the development of new strategies for therapeutic intervention. In this review we summarize the current information about the roles of low molecular weight antioxidants and ROS-detoxifying enzymes in normal and impaired wound repair, and we report on the consequences of their modulation at the wound site.] Matthias S, Sabine W. *Pharmacological Research* Volume 58, Issue 2, August 2008, Pages 165-171. <https://doi.org/10.1016/j.phrs.2008.06.004>

Redox Signaling in Diabetic Wound Healing Regulates Extracellular Matrix Deposition. [Significance: Impaired wound healing is a major complication of diabetes, and can lead to development of chronic foot ulcers in a significant number of patients. Despite the danger posed by poor healing, very few specific therapies exist, leaving patients at risk of hospitalization, amputation, and further decline in overall health. Recent Advances: Redox signaling is a key regulator of wound healing, especially through its influence on the extracellular matrix (ECM). Normal redox signaling is disrupted in diabetes leading to several pathological mechanisms that alter the balance between reactive oxygen species (ROS) generation and scavenging. Importantly, pathological oxidative stress can alter ECM structure and function. Critical Issues: There is limited understanding of the specific role of altered redox signaling in the diabetic wound, although there is evidence that ROS are involved in the underlying pathology. Future Directions: Preclinical studies of antioxidant-based therapies for diabetic wound healing have yielded promising results. Redox-based therapeutics constitute a novel approach for the treatment of wounds in diabetes patients that deserve further investigation.] Kunkemoeller B, Kyriakides TR. *Antioxid. Redox Signal.* 27, 823–838. <https://www.liebertpub.com/doi/abs/10.1089/ars.2017.7263>

Redox signals in wound healing. [Physical trauma represents one of the most primitive challenges that threatened survival. Healing a problem wound requires a multi-faceted comprehensive approach. First and foremost, the wound environment will have to be made receptive to therapies. Second, the appropriate therapeutic regimen needs to be identified and provided while managing systemic limitations that could secondarily limit the healing response. Unfortunately, most current solutions seem to aim at designing therapeutic regimen with little or no consideration of the specific details of the wound environment and systemic limitations. One factor that is centrally important in making the wound environment receptive is correction of wound hypoxia. Recent work have identified that oxygen is not only required to disinfect wounds and fuel healing but that oxygen-dependent redox-sensitive signaling processes represent an integral component of the healing cascade. Over a decade ago, it was proposed that in biological systems oxidants are not necessarily always the triggers for oxidative damage and that oxidants such as H₂O₂ could actually serve as signaling messengers and drive several aspects of cellular signaling. Today, that concept is much more developed and mature. Evidence supporting the role of oxidants such as H₂O₂ as signaling messenger is compelling. A complete understanding of the continuum between the classical and emergent roles of oxygen requires a thorough consideration of current concepts in redox biology. The objective of this review is to describe our current understanding of how redox-sensitive processes may drive dermal tissue repair.] *Biochimica et Biophysica Acta (BBA) - General Subjects*. Volume 1780, Issue 11, November 2008, Pages 1348-1361. <https://www.sciencedirect.com/science/article/pii/S030441650800007X>

Roles of Antioxidative Enzymes in Wound Healing. [Since skin is the first barrier separating the body from the external environment, impaired wound healing can be life threatening to living organisms. Delayed healing processes are observed in animals under certain circumstances, such as advanced age, diabetes, and immunosuppression, but the underlying mechanisms of the abnormality remain elusive. Redox homeostasis is defined as the balance between the levels of reactive oxygen species (ROS) and antioxidants in which antioxidative enzymes play central roles in scavenging ROS. In addition to deleterious effects, ROS also exert

beneficial functions on some cellular processes such as transducing phosphorylation signaling, but excessive antioxidants may impede the healing process. Hence, strict control over the amounts of antioxidants is desirable when applied for therapeutic purposes. Here we overview recent findings regarding the relationships between antioxidative enzymes and wound healing. Unveiling the role of antioxidative enzymes is expected to contribute to our understanding of the wound healing processes.] Kurahashi T, Fujii J. . J. Dev. Biol. 2015, 3(2), 57-70; <https://doi.org/10.3390/jdb3020057> www.mdpi.com/2221-3759/3/2/57 .

The general case for redox control of wound repair. [The orthodox view has been that reactive oxygen species are primarily damaging to cells. There is general agreement that while high (3%) doses of H₂O₂ may serve as a clinical disinfectant, its overall effect on healing is not positive. Current work shows that at very low concentrations, reactive oxygen species may regulate cellular signaling pathways by redox-dependent mechanisms. Recent discoveries show that almost all cells of the wound microenvironment contain specialized enzymes that utilize O₂ to generate reactive oxygen species. Numerous aspects of wound healing are subject to redox control. An understanding of how endogenous reactive oxygen species are generated in wound-related cells may influence the healing process and could result in new redox-based therapeutic strategies. Current results with growth factor therapy of wounds have not met clinical expectations. Many of these growth factors, such as platelet-derived growth factor, rely on reactive oxygen species for functioning. Redox-based strategies may serve as effective adjuncts to jump-start healing of chronic wounds. The understanding of wound-site redox biology is also likely to provide novel insights into the fundamental mechanisms that would help to optimize conditions for oxygen therapy. While a window of therapeutic opportunity seems to exist under conditions of low concentrations of reactive oxygen species, high levels may complicate regeneration and remodeling of nascent tissue.] Sen CK. The International Journal of Tissue Repair and Regeneration. Nov 12, 2003 <https://doi.org/10.1046/j.1524-475X.2003.11607.x> <https://onlinelibrary.wiley.com/doi/abs/10.1046/j.1524-475X.2003.11607.x>

The role of redox mechanisms in hepatic chronic wound healing and fibrogenesis. [Under physiological conditions, intracellular and tissue levels of reactive oxygen species (ROS) are carefully controlled and employed as fine modulators of signal transduction, gene expression and cell functional responses (redox signaling). A significant derangement in redox homeostasis, resulting in sustained levels of oxidative stress and related mediators, plays a role in the pathogenesis of human diseases characterized by chronic inflammation, chronic activation of wound healing and tissue fibrogenesis, including chronic liver diseases. In this chapter major concepts and mechanisms in redox signaling will be briefly recalled to introduce a number of selected examples of redox-related mechanisms that can actively contribute to critical events in the natural history of a chronic liver diseases, including induction of cell death, perpetuation of chronic inflammatory responses and fibrogenesis. A major focus will be on redox-dependent mechanisms involved in the modulation of phenotypic responses of activated, myofibroblast-like, hepatic stellate cells (HSC/MFs), still considered as the most relevant pro-fibrogenic cells operating in chronic liver diseases.] Novo, E, Parola M. Fibrogenesis Tissue Repair. 2012; 5(Suppl 1): S4. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3368756/>

Cystic Fibrosis

Redox balance in Cystic Fibrosis. [The homeostatic balance between oxidants and antioxidants in biological systems is known as redox balance, and is regulated by complex processes. Redox balance regulates many of the known cellular pathways and disease processes. The dysregulation of redox balance can lead to acute or long-term oxidative or reductive stresses that are associated with many of the abnormalities observed in cystic fibrosis (CF). Over the past 5 decades researchers have examined contributors to redox dysregulation, their molecular products, and their impact on ion transport, cell proliferation, inflammation, bacterial killing, and the metabolism of nucleic acids, proteins, and lipids in CF. CF patients exhibit elevated markers of oxidative stress when compared to non-CF healthy controls; however, whether the reported redox imbalance is sufficient to produce pathology has been controversial. In addition, comparisons between CF and non-CF disease controls have been lacking. To better understand the mechanisms which mediate the generation of oxidants and antioxidants in CF and the importance of their balance in effecting oxidative or reductive stress, we will review the determinants of redox balance in the blood, lumen, and cellular compartments. From the perspective of methodological application, we will focus on the approaches most often used to study oxidant and antioxidants in CF, including biochemical, proteomic, metabolomic, and lipidomic studies, with a discussion of the few transcriptomic analyses that predict changes in the expression of regulators of redox. Finally, we will discuss the utility of oxidants and antioxidants as biomarkers of disease and the use of antioxidant therapy in CF.] Ziady AG, Hansen J. Int J Biochem Cell Biol. 2014 Jul; 0: 113–123. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4035434/>

Diabetes

Hyperglycemia-induced oxidative stress and its role in diabetes mellitus related cardiovascular diseases. [Diabetes mellitus is associated to an increased risk of cardiovascular diseases. Hyperglycemia is an important factor in cardiovascular damage, working through different mechanisms such as activation of protein kinase C, polyol and hexosamine pathways, advanced glycation end

products production. All of these pathways, in association to hyperglycemia-induced mitochondrial dysfunction and endoplasmic reticulum stress, promote reactive oxygen species (ROS) accumulation that, in turn, promote cellular damage and contribute to the diabetic complications development and progression. ROS can directly damage lipids, proteins or DNA and modulate intracellular signaling pathways, such as mitogen activated protein kinases and redox sensitive transcription factors causing changes in protein expression and, therefore, irreversible oxidative modifications. Hyperglycemia-induced oxidative stress induces endothelial dysfunction that plays a central role in the pathogenesis of micro- and macro-vascular diseases. It may also increase pro-inflammatory and pro-coagulant factors expression, induce apoptosis and impair nitric oxide release. Oxidative stress induces several phenotypic alterations also in vascular smooth-muscle cell (VSMC). ROS is one of the factors that can promote both VSMC proliferation/migration in atherosclerotic lesions and VSMC apoptosis, which is potentially involved in atherosclerotic plaque instability and rupture. Currently, there are contrasting clinical evidences on the benefits of antioxidant therapies in the prevention/treatment of diabetic cardiovascular complications. Appropriate glycemic control, in which both hypoglycemic and hyperglycemic episodes are reduced, in association to the treatment of dyslipidemia, hypertension, kidney dysfunction and obesity, conditions which are also associated to ROS overproduction, can counteract oxidative stress and, therefore, both microvascular and macrovascular complications of diabetes mellitus.] Fiorentino TV, Prioletta A, et al. *Curr Pharm Des.* 2013;19(32):5695-703. <https://www.ncbi.nlm.nih.gov/pubmed/23448484>

Mitochondrial Dysfunction in Diabetes: From Molecular Mechanisms to Functional Significance and Therapeutic Opportunities. [Given their essential function in aerobic metabolism, mitochondria are intuitively of interest in regard to the pathophysiology of diabetes. Qualitative, quantitative, and functional perturbations in mitochondria have been identified and affect the cause and complications of diabetes. Moreover, as a consequence of fuel oxidation, mitochondria generate considerable reactive oxygen species (ROS). Evidence is accumulating that these radicals per se are important in the pathophysiology of diabetes and its complications. In this review, we first present basic concepts underlying mitochondrial physiology. We then address mitochondrial function and ROS as related to diabetes. We consider different forms of diabetes and address both insulin secretion and insulin sensitivity. We also address the role of mitochondrial uncoupling and coenzyme Q. Finally, we address the potential for targeting mitochondria in the therapy of diabetes.] Sivitz WI, Yorek MA. *Antioxid Redox Signal.* 2010 Feb 15; 12(4): 537–577. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2824521/>

Oxidative stress and diabetic complications. [Oxidative stress plays a pivotal role in the development of diabetes complications, both microvascular and cardiovascular. The metabolic abnormalities of diabetes cause mitochondrial superoxide overproduction in endothelial cells of both large and small vessels, and also in the myocardium. This increased superoxide production causes the activation of five major pathways involved in the pathogenesis of complications: polyol pathway flux, increased formation of advanced glycation end-products (AGEs), increased expression of the receptor for AGEs and its activating ligands, activation of protein kinase C (PKC) isoforms, and overactivity of the hexosamine pathway. It also directly inactivates two critical antiatherosclerotic enzymes, eNOS and prostacyclin synthase. Through these pathways, increased intracellular ROS cause defective angiogenesis in response to ischemia, activate a number of pro-inflammatory pathways, and cause long-lasting epigenetic changes which drive persistent expression of proinflammatory genes after glycemia is normalized ('hyperglycemic memory'). Atherosclerosis and cardiomyopathy in type 2 diabetes are caused in part by pathway-selective insulin resistance, which increases mitochondrial ROS production from free fatty acids and by inactivation of anti-atherosclerosis enzymes by ROS. Overexpression of superoxide dismutase in transgenic diabetic mice prevents diabetic retinopathy, nephropathy, and cardiomyopathy. The aim of this review is to highlight advances in understanding the role of metabolite-generated ROS in the development of diabetic complications.] Ferdinando G, Brownlee M. *Circ Res.* 2010 Oct 29; 107(9): 1058–1070. doi: 10.1161/CIRCRESAHA.110.223545. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2996922/>

Redox Signaling in Diabetic Nephropathy: Hypertrophy versus Death Choices in Mesangial Cells and Podocytes. [This review emphasizes the role of oxidative stress in diabetic nephropathy, acting as trigger, modulator, and linker within the complex network of pathologic events. It highlights key molecular pathways and new hypothesis in diabetic nephropathy, related to the interferences of metabolic, oxidative, and inflammatory stresses. Main topics this review is addressing are biomarkers of oxidative stress in diabetic nephropathy, the sources of reactive oxygen species (mitochondria, NADPH-oxidases, hyperglycemia, and inflammation), and the redox-sensitive signaling networks (protein kinases, transcription factors, and epigenetic regulators). Molecular switches deciding on the renal cells fate in diabetic nephropathy are presented, such as hypertrophy versus death choices in mesangial cells and podocytes. Finally, the antioxidant response of renal cells in diabetic nephropathy is tackled, with emphasis on targeted therapy. An integrative approach is needed for identifying key molecular networks which control cellular responses triggered by the array of stressors in diabetic nephropathy. This will foster the discovery of reliable biomarkers for early diagnosis and prognosis, and will guide the discovery of new therapeutic approaches for personalized medicine in diabetic nephropathy.] Manda G, et al. <https://www.hindawi.com/journals/mi/2015/604208/>

The molecular biology of chronic wounds and delayed healing in diabetes. [Apoptosis of various cell types, including epithelial cells and fibroblasts; Disruption of the redox balance and alterations to the level or activity of reducing enzymes that help to

maintain the normal redox state could also contribute to poor healing... Wound healing is a complicated and integrated process. Although there is some tolerance in terms of redundancy and interrelated control mechanisms, pushing beyond such limits may contribute to delayed wound healing, and in extreme cases lead to chronic wounds/ulcers and thus potentially to lower extremity amputation. Diabetes is associated with such disruption in wound healing. Research in humans and in animal models has identified a large number of changes associated with diabetes at the molecular level in delayed wound healing and to a lesser extent in chronic diabetic ulcers. Better overall understanding of these changes and how they are interrelated would allow for specifically targeted treatment, thus ensuring improved quality of life for patients and providing savings to the high costs that are associated with all aspects of chronic diabetic ulcers. This review examines the work done at the molecular level on chronic diabetic ulcers, as well as considering changes seen in diabetes in general, both in humans and animal models, that may in turn contribute to ulcer formation.] Blakytyn, R, Jude E. *Diabetic Medicine*, 13 February 2006 <https://doi.org/10.1111/j.1464-5491.2006.01773.x>

Type 2 diabetes as a redox disease. [Physical exercise has long been widely regarded as essential to human health. 1 Yet, we do not know how exercise-stressed skeletal muscle cells that generate reactive oxygen species such as hydrogen peroxide (H_2O_2) delay—if not prevent—the occurrence and severity of diseases such as type 2 diabetes (as well as dementias, cardiovascular disease, and some cancers). Also unexplained is the recent finding that metformin—the most commonly used drug to treat type 2 diabetes 2, 3, 4 —and physical exercise seem to be beneficial for several of the same diseases, including cancer, Alzheimer's disease, and cardiovascular disease. 5, 6 New evidence 7 shows that combinations of short-term metformin treatment with single acute bouts of exercise do not, as generally expected, enhance insulin sensitivity. In fact, metformin alone can attenuate much of the oxidative effect of exercise. 7 The reason why exercise and metformin have opposing physiological consequences (oxidative vs reducing) has been shown by studies 8 that suggest that giving mice metformin increases synthesis of the transcription factor Nrf2, which controls the downstream synthesis of RNA molecules coding for major cellular antioxidant enzymes.] Watson JD *Lancet*. 2014 Mar 1;383(9919):841-3 <https://www.ncbi.nlm.nih.gov/pubmed/24581668>

Endocrine

Overcoming Thyroid Disorders. [Understanding the etiology and treatment of failing thyroid glands lie in the Redox molecule pathways of Iodine metabolism and the manufacturing of thyroxin by the thyroid gland.] Brownstein D, (2012), *WebMd Article ID: 302537*. <http://www.theredoxdoc.com/wp-content/uploads/NewsLetterMar17.pdf>

Selenium and endocrine systems. [The trace element selenium (Se) is capable of exerting multiple actions on endocrine systems by modifying the expression of at least 30 selenoproteins, many of which have clearly defined functions. Well-characterized selenoenzymes are the families of glutathione peroxidases (GPXs), thioredoxin reductases (TRs) and iodothyronine deiodinases (Ds). These selenoenzymes are capable of modifying cell function by acting as antioxidants and modifying redox status and thyroid hormone metabolism. Se is also involved in cell growth, apoptosis and modifying the action of cell signalling systems and transcription factors. During thyroid hormone synthesis GPX1, GPX3 and TR1 are up-regulated, providing the thyrocytes with considerable protection from peroxidative damage. Thyroidal D1 in rats and both D1 and D2 in humans are also up-regulated to increase the production of bioactive 3,5,3'-tri-iodothyronine (T3). In the basal state, GPX3 is secreted into the follicular lumen where it may down-regulate thyroid hormone synthesis by decreasing hydrogen peroxide concentrations. The deiodinases are present in most tissues and provide a mechanism whereby individual tissues may control their exposure to T3. Se is also able to modify the immune response in patients with autoimmune thyroiditis. Low sperm production and poor sperm quality are consistent features of Se-deficient animals. The pivotal link between Se, sperm quality and male fertility is GPX4 since the enzyme is essential to allow the production of the correct architecture of the midpiece of spermatozoa. Se also has insulin-mimetic properties, an effect that is probably brought about by stimulating the tyrosine kinases involved in the insulin signaling cascade. Furthermore, in the diabetic rat, Se not only restores glycaemic control but it also prevents or alleviates the adverse effects that diabetes has on cardiac, renal and platelet function.] Beckett GJ, Arthur JR. *J Endocrinol*. 2005 Mar;184(3):455-65. <https://www.ncbi.nlm.nih.gov/pubmed/15749805>

Testosterone and oxidative stress: the oxidation handicap hypothesis. [Secondary sexual traits (SST) are usually thought to have evolved as honest signals of individual quality during mate choice. Honesty of SST is guaranteed by the cost of producing/maintaining them. In males, the expression of many SST is testosterone-dependent. The immunocompetence handicap hypothesis has been proposed as a possible mechanism ensuring honesty of SST on the basis that testosterone, in addition to its effect on sexual signals, also has an immunosuppressive effect. The immunocompetence handicap hypothesis has received mixed support. However, the cost of testosterone-based signalling is not limited to immunosuppression and might involve other physiological functions such as the antioxidant machinery. Here, we tested the hypothesis that testosterone depresses resistance to oxidative stress in a species with a testosterone-dependent sexual signal, the zebra finch. Male zebra finches received subcutaneous implants filled with flutamide (an anti-androgen) or testosterone, or kept empty (control). In agreement with the prediction, we found that red blood cell resistance to a free radical attack was the highest in males implanted with flutamide and the lowest in males implanted with testosterone. We also found that cell-mediated immune response was depressed in testosterone-treated birds, supporting the immunocompetence handicap

hypothesis. The recent finding that red blood cell resistance to free radicals is negatively associated with mortality in this species suggests that benefits of sexual signaling might trade against the costs derived from oxidation.] Alonso-Alvarez C, Bertrand S, et al. Proc Biol Sci. 2007 Mar 22;274(1611):819-25. <https://www.ncbi.nlm.nih.gov/pubmed/17251089>

Eye Health

The role of oxidative stress in the pathogenesis of age-related macular degeneration. [Age-related macular degeneration (AMD) is the leading cause of blind registration in the developed world, and yet its pathogenesis remains poorly understood. Oxidative stress, which refers to cellular damage caused by reactive oxygen intermediates (ROI), has been implicated in many disease processes, especially age-related disorders. ROIs include free radicals, hydrogen peroxide, and singlet oxygen, and they are often the byproducts of oxygen metabolism. The retina is particularly susceptible to oxidative stress because of its high consumption of oxygen, its high proportion of polyunsaturated fatty acids, and its exposure to visible light. In vitro studies have consistently shown that photochemical retinal injury is attributable to oxidative stress and that the antioxidant vitamins A, C, and E protect against this type of injury. Furthermore, there is strong evidence suggesting that lipofuscin is derived, at least in part, from oxidatively damaged photoreceptor outer segments and that it is itself a photoreactive substance. However, the relationships between dietary and serum levels of the antioxidant vitamins and age-related macular disease are less clear, although a protective effect of high plasma concentrations of alpha-tocopherol has been convincingly demonstrated. Macular pigment is also believed to limit retinal oxidative damage by absorbing incoming blue light and/or quenching ROIs. Many putative risk-factors for AMD have been linked to a lack of macular pigment, including female gender, lens density, tobacco use, light iris color, and reduced visual sensitivity. Moreover, the Eye Disease Case-Control Study found that high plasma levels of lutein and zeaxanthin were associated with reduced risk of neovascular AMD. The concept that AMD can be attributed to cumulative oxidative stress is enticing, but remains unproven. With a view to reducing oxidative damage, the effect of nutritional antioxidant supplements on the onset and natural course of age-related macular disease is currently being evaluated.] Beatty S, Koh H, et al. Surv Ophthalmol. 2000 Sep-Oct;45(2):115-34. <https://www.ncbi.nlm.nih.gov/pubmed/11033038>

Exercise

Antioxidant and redox regulation of cellular signaling: introduction. [Oxidation-reduction (redox) based regulation of gene expression appears to be a fundamental regulatory mechanism in cell biology. This basic information has been exploited to develop novel strategies in clinical therapeutics. In contrast to the conventional idea that reactive species mostly serve as a trigger for oxidative damage of biological structures, we now know that low physiologically relevant concentration of reactive oxygen species can regulate a variety of key molecular mechanisms. Physical exercise causes redox changes in various cells and tissues. The molecular implications of such change are yet uncharacterized. The five component articles of this symposium discuss skeletal muscle contraction, cell adhesion, heat shock proteins, programmed cell death, and carbohydrate metabolism as they relate to physical exercise.] Sen CK. Medicine and Science in Sports and Exercise, 01 Mar 2001, 33(3):368-370. <https://europepmc.org/abstract/med/11252060>

Effects of ASEA beverage intake on endurance performance in mice. [Previous research with ASEA (a saline beverage with stable superoxide complexes) found increased fatty acid mobilization in cyclists after 7 d ingestion. We hypothesized that run time to exhaustion would be favorably influenced by ASEA intake due to enhanced fatty acid oxidation and muscle glycogen sparing. Sixty mice were randomized to 1 of 4 treatment groups (n=15 each): placebo sedentary (PS), ASEA sedentary (AS), placebo run (PR), and ASEA run (AR). Mice were gavaged daily with ASEA or placebo (0.3 ml/d) for 7 d. PR and AR groups were run to exhaustion (24 m/min) at the end of the 7-d gavage period, with AR running significantly longer than PR (68.0±9.2 vs. 52.8±7.4 min, respectively) (p<0.001). At the point of exhaustion, liver glycogen was undetectable for both AR and PR. When adjusted to run time, the estimated rate of muscle glycogen depletion was different between AR and PR (0.036±0.014 and 0.052±0.018 ug/mg protein per min, respectively, p=0.017). Skeletal muscle phosphorylated acetyl-CoA carboxylase (p-ACC) was significantly increased in AR compared to AS (p=0.020) and PR (p=0.045). Fatty acyl CoA transport (CPT1), and beta-oxidation (beta-HAD) were not different between AR and PR. ASEA increased run time to exhaustion by 29% in mice, potentially through less inhibition of fatty acid oxidation via increased P-ACC, and muscle glycogen sparing percent (30%).] Knab AM, Nieman DC, et al. The FASEB Journal, Vol 27, No. 1_Supplement; April 2013. https://www.fasebj.org/doi/abs/10.1096/fasebj.27.1_supplement.713.1

Influence of a redox-signaling supplement on biomarkers of physiological stress in athletes: a metabolomics approach. [We determined if drinking ASEA™, a redox-signaling molecule beverage, would improve cycling performance, counter inflammation, oxidative stress, and immune dysfunction, and alter the metabolite profile. Cyclists (n = 20) participated in a randomized, double blinded, placebo controlled, crossover study. Subjects completed two 75km time trials (TT) after 1 wk ingestion of 118 mL/d ASEA or placebo (PL). Subjects ingested 473 mL ASEA or PL during the TT. Blood samples were taken pre, post, 1h post-exercise (Ex).

ASEA did not improve TT performance ($p = .95$). The Ex-induced pattern of change in inflammation, oxidative stress, and immunity did not differ ($p > .05$) between ASEA vs. PL. The metabolomic profiles (GC/MS) of ASEA and PL samples were clearly separated by partial least square discriminant analysis when using pre-Ex data ($Q^2Y = .71$), and when using the ratio of 1h post-Ex to pre-Ex ($Q^2Y = .68$). Linear modeling found that ASEA supplementation caused a significant shift in 43 metabolites pre-Ex, especially free fatty acids, suggesting an enhanced fat oxidation and amino acid sparing, with an increase in ascorbic acid, during Ex. Within the context of this study, 1-wk ASEA supplementation caused extensive fatty acid mobilization before and during Ex, with no apparent influence on TT performance and traditional biomarkers of inflammation, oxidative stress, and immunity.] Shanely RA, Nieman DC, et al. The FASEB Journal, Vol 26, No 1_Supplement, April 2012. https://www.fasebj.org/doi/abs/10.1096/fasebj.26.1_supplement.lb713

Redox Mechanism of Reactive Oxygen Species in Exercise. [It is well known that regular exercise can benefit health by enhancing antioxidant defenses in the body. However, unaccustomed and/or exhaustive exercise can generate excessive reactive oxygen species (ROS), leading to oxidative stress-related tissue damages and impaired muscle contractility. ROS are produced in both aerobic and anaerobic exercise. Mitochondria, NADPH oxidases and xanthine oxidases have all been identified as potential contributors to ROS production, yet the exact redox mechanisms underlying exercise-induced oxidative stress remain elusive. Interestingly, moderate exposure to ROS is necessary to induce body's adaptive responses such as the activation of antioxidant defense mechanisms. Dietary antioxidant manipulation can also reduce ROS levels and muscle fatigue, as well as enhance exercise recovery. To elucidate the complex role of ROS in exercise, this review updates on new findings of ROS origins within skeletal muscles associated with various types of exercises such as endurance, sprint and mountain climbing. In addition, we will examine the corresponding antioxidant defense systems as well as dietary manipulation against damages caused by ROS.] He F, Li J, et al. *Front Physiol.* 2016; 7: 486. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5097959/>

Urinary F2-Isoprostanes and Metabolic Markers of Fat Oxidation. [Metabolomic studies of increased fat oxidation showed increase in circulating acylcarnitines C2, C8, C10, and C12 and decrease in C3, C4, and C5. We hypothesize that urinary F2-isoprostanes reflect intensity of fatty acid oxidation and are associated with circulating C2, C8, C10, and C12 directly and with C3, C4, and C5 inversely. Four urinary F2-isoprostane isomers and serum acylcarnitines are quantified using LC-MS/MS within the Insulin Resistance Atherosclerosis Study nondiabetic cohort ($n = 682$). Cross-sectional associations between fasting urinary F2-isoprostanes (summarized as a composite index) and the selected acylcarnitines are examined using generalized linear models. F2-isoprostane index is associated with C2 and C12 directly and with C5 inversely: the adjusted beta coefficients are 0.109, 0.072, and -0.094 , respectively ($P < 0.05$). For these acylcarnitines and for F2-isoprostanes, the adjusted odds ratios (ORs) of incident diabetes are calculated from logistic regression models: the ORs (95% CI) are 0.77 (0.60–0.97), 0.79 (0.62–1.01), 1.18 (0.92–1.53), and 0.51 (0.35–0.76) for C2, C12, C5, and F2-isoprostanes, respectively. The direction of the associations between urinary F2-isoprostanes and three acylcarnitines (C2, C5, and C12) supports our hypothesis. The inverse associations of C2 and C12 and with incident diabetes are consistent with the suggested protective role of efficient fat oxidation.] Li'yasova D, Wagenknecht LE, et al. *Oxid Med Cell Longev.* 2015; 2015: 729191. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4352765/>

Hair

Oxidative Damage Control in a Human (Mini-) Organ: Nrf2 Activation Protects against Oxidative Stress-Induced Hair Growth Inhibition. [The in situ control of redox insult in human organs is of major clinical relevance, yet remains incompletely understood. Activation of nuclear factor (erythroid-derived 2)-like 2 (Nrf2), the "master regulator" of genes controlling cellular redox homeostasis, is advocated as a therapeutic strategy for diseases with severely impaired redox balance. It remains to be shown whether this strategy is effective in human organs, rather than only in isolated human cell types. We have therefore explored the role of Nrf2 in a uniquely accessible human (mini-) organ: scalp hair follicles. Microarray and qRT-PCR analysis of human hair follicles after Nrf2 activation using sulforaphane identified the modulation of phase II metabolism, reactive oxygen species clearance, the pentose phosphate pathway, and glutathione homeostasis. Nrf2 knockdown (small interfering RNA) in cultured human hair follicles confirmed the regulation of key Nrf2 target genes (i.e., heme oxygenase-1, NAD(P)H dehydrogenase, quinone 1, glutathione reductase, glutamate-cysteine ligase catalytic subunit, ABCC1, peroxiredoxin 1). Importantly, Nrf2 activation significantly reduced reactive oxygen species levels and associated lipid peroxidation. Nrf2 preactivation reduced premature catagen and hair growth inhibition induced by oxidative stress (H_2O_2 or menadione), significantly ameliorated the H_2O_2 -dependent increase in matrix keratinocyte apoptosis and reversed the reactive oxygen species-induced reduction in hair matrix proliferation. This study thus provides direct evidence for the crucial role of Nrf2 in protecting human organ function (i.e., scalp hair follicles) against redox insult.] Haslam IS, Jadkauskaite L, et al. *J Invest Dermatol.* 2017 Feb;137(2):295-304. <https://www.ncbi.nlm.nih.gov/pubmed/27702566>

Fibromyalgia

Total antioxidant capacity and the severity of the pain in patients with fibromyalgia. [The purpose of the study was to determine the oxidative and antioxidative status of plasma in patients with fibromyalgia. Total antioxidant capacity (TAC) of plasma was significantly lower in patients with fibromyalgia (n = 20) than in healthy controls (n = 20) [1.5 (SD 0.3) and 1.9 (SD 0.3) mmol Trolox equiv./l, P = 0.001]. In contrast, the total peroxide level of plasma was significantly higher in patients than in healthy controls [37.4 (SD 6.7) and 33.0 (SD 2.7) micromol H₂O₂/l; P = 0.01]. The oxidative stress index (OSI) level was significantly higher in patients with fibromyalgia than in healthy controls [2.5 (SD 1.0) and 1.8 (SD 0.4); P = 0.007]. A significant negative correlation between visual analogue scale (VAS) and TAC level was determined (r = -0.79, P < 0.001). The present results indicate that patients with fibromyalgia are exposed to oxidative stress and this increased oxidative stress may play a role in the etiopathogenesis of the disease. Supplementation of antioxidant vitamins such as vitamins C and E to the therapy may be indicated.] Altındag O, Celik H. Redox Rep. 2006;11(3):131-5.

<https://www.ncbi.nlm.nih.gov/pubmed/?term=Total+antioxidant+capacity+and+the+severity+of+the+pain+in+patients+with+fibromyalgia>

Free radicals and antioxidants in primary fibromyalgia: an oxidative stress disorder? [The role of free radicals in fibromyalgia is controversial. In this study, 85 female patients with primary fibromyalgia and 80 age-, height-, and weight-matched healthy women were evaluated for oxidant/antioxidant balance. Malondialdehyde is a toxic metabolite of lipid peroxidation used as a marker of free radical damage. Superoxide dismutase is an intracellular antioxidant enzyme and shows antioxidant capacity. Pain was assessed by visual analog scale. Tender points were assessed by palpation. Age, smoking, body mass index (BMI), and duration of disease were also recorded. Malondialdehyde levels were significantly higher and superoxide dismutase levels significantly lower in fibromyalgic patients than controls. Age, BMI, smoking, and duration of disease did not affect these parameters. We found no correlation between pain and number of tender points. In conclusion, oxidant/antioxidant balances were changed in fibromyalgia. Increased free radical levels may be responsible for the development of fibromyalgia. These findings may support the hypothesis of fibromyalgia as an oxidative disorder.] Bagis S, Tamer L, et al. Rheumatol Int. 2005 Apr;25(3):188-90.

<https://www.ncbi.nlm.nih.gov/pubmed/14689230>

Immune System

Are Reactive Oxygen Species Always Detrimental to Pathogens? [Reactive oxygen species (ROS) are deadly weapons used by phagocytes and other cell types, such as lung epithelial cells, against pathogens. ROS can kill pathogens directly by causing oxidative damage to biocompounds or indirectly by stimulating pathogen elimination by various nonoxidative mechanisms, including pattern recognition receptors signaling, autophagy, neutrophil extracellular trap formation, and T-lymphocyte responses. Thus, one should expect that the inhibition of ROS production promote infection. Increasing evidences support that in certain particular infections, antioxidants decrease and prooxidants increase pathogen burden. In this study, we review the classic infections that are controlled by ROS and the cases in which ROS appear as promoters of infection, challenging the paradigm. We discuss the possible mechanisms by which ROS could promote particular infections. These mechanisms are still not completely clear but include the metabolic effects of ROS on pathogen physiology, ROS-induced damage to the immune system, and ROS-induced activation of immune defense mechanisms that are subsequently hijacked by particular pathogens to act against more effective microbicidal mechanisms of the immune system. The effective use of antioxidants as therapeutic agents against certain infections is a realistic possibility that is beginning to be applied against viruses.] Paiva, CN, Bozza MT. Antioxid. Redox Signal. 20, 1000–1037..

<https://www.liebertpub.com/doi/10.1089/ars.2013.5447>

Killing activity of neutrophils is mediated through activation of proteases by K⁺ flux. [According to the hitherto accepted view, neutrophils kill ingested microorganisms by subjecting them to high concentrations of highly toxic reactive oxygen species (ROS) and bringing about myeloperoxidase-catalysed halogenation. We show here that this simple scheme, which for many years has served as a satisfactory working hypothesis, is inadequate. We find that mice made deficient in neutrophil-granule proteases but normal in respect of superoxide production and iodinating capacity, are unable to resist staphylococcal and candidal infections. We also show that activation provokes the influx of an enormous concentration of ROS into the endocytic vacuole. The resulting accumulation of anionic charge is compensated for by a surge of K⁺ ions that cross the membrane in a pH-dependent manner. The consequent rise in ionic strength engenders the release of cationic granule proteins, including elastase and cathepsin G, from the anionic sulphated proteoglycan matrix. We show that it is the proteases, thus activated, that are primarily responsible for the destruction of the bacteria.] Reeves EP, Lu H, et al. Nature. 2002 Mar 21;416(6878):291-7. <https://www.ncbi.nlm.nih.gov/pubmed/11907569/>

Reactive oxygen species in the immune system. [Reactive oxygen species (ROS) are a group of highly reactive chemicals containing oxygen produced either exogenously or endogenously. ROS are related to a wide variety of human disorders, such as chronic

inflammation, age-related diseases and cancers. Besides, ROS are also essential for various biological functions, including cell survival, cell growth, proliferation and differentiation, and immune response. At present there are a number of excellent publications including some reviews about functions of these molecules either in normal cell biology or in pathophysiology. In this work, we reviewed available information and recent advances about ROS in the main immune cell types and gave summary about functions of these highly reactive molecules both in innate immunity as conservative defense mechanisms and in essential immune cells involved in adaptive immunity, and particularly in immune suppression.] Yang Y, Bazhin AV, et al. *Int Rev Immunol*. 2013 Jun;32(3):249-70. doi: 10.3109/08830185.2012.755176. <https://www.ncbi.nlm.nih.gov/pubmed/23617726>

Redox regulation of the immune response. [Reactive oxygen and nitrogen species (ROS–RNS) and other redox active molecules fulfill key functions in immunity. Beside the initiation of cytotoxic reactions within the pathogen defense strategy, redox reactions trigger and shape the immune response and are further involved in termination and initialization of cellular restorative processes. Regulatory mechanisms provided by redox-activated signaling events guarantee the correct spatial and temporal proceeding of immunological processes, and continued imbalances in redox homeostasis lead to crucial failures of control mechanisms, thus promoting the development of pathological conditions. Interferon-gamma is the most potent inducer of ROS–RNS formation in target cells like macrophages. Immune-regulatory pathways such as tryptophan breakdown via indoleamine 2,3-dioxygenase and neopterin production by GTP-cyclohydrolase-I are initiated during T helper cell type 1 (Th1-type) immune response concomitant to the production of ROS–RNS by immunocompetent cells. Therefore, increased neopterin production and tryptophan breakdown is representative of an activated cellular immune system and can be used for the in vivo and in vitro monitoring of oxidative stress. In parallel, the activation of the redox-sensitive transcription factor nuclear factor-kappa B is a central element in immunity leading to cell type and stimulus-specific expression of responsive genes. Furthermore, T cell activation and proliferation are strongly dependent on the redox potential of the extracellular microenvironment. T cell commitment to Th1, Th2, regulatory T cell, and other phenotypes appears to crucially depend on the activation of redox-sensitive signaling cascades, where oxidative conditions support Th1 development while ‘antioxidative’ stress leads to a shift to allergic Th2-type immune responses.] Gostner, JM, Becker K, et al. *Redox Report, Communications in Free Radical Research*, Volume 18, 2013 - Issue 3. <https://www.tandfonline.com/doi/abs/10.1179/1351000213Y.0000000044>

Redox Regulation of T-Cell Function: From Molecular Mechanisms to Significance in Human Health and Disease. [Reactive oxygen species (ROS) are thought to have effects on T-cell function and proliferation. Low concentrations of ROS in T cells are a prerequisite for cell survival, and increased ROS accumulation can lead to apoptosis/necrosis. The cellular redox state of a T cell can also affect T-cell receptor signaling, skewing the immune response. Various T-cell subsets have different redox statuses, and this differential ROS susceptibility could modulate the outcome of an immune response in various disease states. Recent advances in T-cell redox signaling reveal that ROS modulate signaling cascades such as the mitogen-activated protein kinase, phosphoinositide 3-kinase (PI3K)/AKT, and JAK/STAT pathways. Also, tumor microenvironments, chronic T-cell stimulation leading to replicative senescence, gender, and age affect T-cell susceptibility to ROS, thereby contributing to diverse immune outcomes. Antioxidants such as glutathione, thioredoxin, superoxide dismutase, and catalase balance cellular oxidative stress. T-cell redox states are also regulated by expression of various vitamins and dietary compounds. Changes in T-cell redox regulation may affect the pathogenesis of various human diseases. Many strategies to control oxidative stress have been employed for various diseases, including the use of active antioxidants from dietary products and pharmacologic or genetic engineering of antioxidant genes in T cells. Here, we discuss the existence of a complex web of molecules/factors that exogenously or endogenously affect oxidants, and we relate these molecules to potential therapeutics.] Kesarwani P, Murali AK, et al. *Antioxid. Redox Signal*. 18, 1497–1534. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3603502/>

Mental Health

3-Nitrotyrosine and glutathione antioxidant system in patients in the early and late stages of bipolar disorder. [Background There has been an increasing interest in the role of oxidative stress in the pathophysiology of bipolar disorder. To explore this further, we evaluated the activity of glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione S-transferase (GST), as well as 3-nitrotyrosine levels and carbonyl content in patients in the early (within 3 years of illness onset) and late (a minimum of 10 years of illness) stages of bipolar disorder. Methods We matched 30 patients in the early stage and 30 patients in the late stage of bipolar disorder, diagnosed according to DSM-IV criteria, with 60 healthy controls (30 matched for each group of patients). We measured symptomatic status using the Hamilton Rating Scale for Depression and the Young Mania Rating Scale. Results We found a significant increase in 3-nitrotyrosine levels among patients in the early ($p < 0.010$) and late ($p < 0.010$) stages of bipolar disorder. The activity of GR and GST was increased only among patients in the late stage of illness. Glutathione peroxidase activity and carbonyl content did not differ among the groups. Limitations Limitations of our study include its cross-sectional design, which did not allow us to examine direct causative mechanisms or the effects of progression of illness, and the potential environmental bias introduced by comparing patient groups recruited from different regions of the world. Conclusion Our data indicate a possible tyrosine nitration-induced damage in patients with bipolar disorder that is present from the early stage of illness. Our data also indicate that patients in

the late stage of illness demonstrate enhanced activity of GR and GST, which could suggest the involvement of a compensatory system in bipolar disorder.] Andrezza AC, Kapczinski F, et al. *J Psychiatry Neurosci.* 2009 Jul; 34(4): 263–271. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2702443/>

Oxidative stress and anxiety: relationship and cellular pathways. [High O₂ consumption, modest antioxidant defenses and a lipid-rich constitution make the brain highly vulnerable to redox imbalances. Oxidative damage in the brain causes nervous system impairment. Recently, oxidative stress has also been implicated in depression, anxiety disorders and high anxiety levels. The findings which establish a link between oxidative stress and pathological anxiety have inspired a number of other recent studies focusing on the link between oxidative status and normal anxiety and also on a possible causal relationship between cellular oxidative stress and emotional stress. This review examines the recent discoveries made on the link between oxidative status and normal anxiety levels and the putative role of oxidative stress in genesis of anxiety. We discuss the different opinions and questions that exist in the field and review the methodological approaches that are being used to determine a causal relationship between oxidative and emotional stress.] Bouayed J, Rammal H, et al. *Oxid Med Cell Longev.* 2009 Apr-Jun;2(2):63-7. <https://www.ncbi.nlm.nih.gov/pubmed/20357926>

Mitochondria

Aging is associated with decreased mitochondrial enzymes and altered morphology in human muscles. [Aging is the accumulation of molecular and cellular defects leading to dysfunction of tissues, organs, and systems whose obvious feature is decline of muscle mass and strength. The majority of energy provided to skeletal muscle is produced and cycled in form of ATP which stems mainly from oxidative phosphorylation occurring at the electron transport chain(ETC) of the mitochondria. Over the years, aging theory has been extended to the “Mitochondrial theory of aging” and substantial evidence has emerged from bioenergetic, biochemical and genetic studies to lend support to this theory. Although several lines of evidence support the role for substantial declines in enzyme activity in the etiology of aging, it remains unclear if the expression of polypeptide components of the ETC complexes alter with aging. Here, we determined to observe the morphology change and quantify the expression of several enzymes in ETC and cytoplasm of mitochondrial. Taking these findings together, we suggest that the altered morphology and decreased expression of enzymes in mitochondrial may contribute to aging in human muscles.] Qian W, Jiang P, et al. *Biomedical Research* (2014) Volume 25, Issue 3. <http://www.alliedacademies.org/articles/aging-is-associated-with-decreased-mitochondrial-enzymes-and-alteredmorphology-in-human-muscles.html>

Decline in skeletal muscle mitochondrial function with aging in humans. [Cumulative mtDNA damage occurs in aging animals, and mtDNA mutations are reported to accelerate aging in mice. We determined whether aging results in increased DNA oxidative damage and reduced mtDNA abundance and mitochondrial function in skeletal muscle of human subjects. Studies performed in 146 healthy men and women aged 18 – 89 yr demonstrated that mtDNA and mRNA abundance and mitochondrial ATP production all declined with advancing age. Abundance of mtDNA was positively related to mitochondrial ATP production rate, which in turn, was closely associated with aerobic capacity and glucose tolerance. The content of several mitochondrial proteins was reduced in older muscles, whereas the level of the oxidative DNA lesion, 8-oxo-deoxyguanosine, was increased, supporting the oxidative damage theory of aging. These results demonstrate that age-related muscle mitochondrial dysfunction is related to reduced mtDNA and muscle functional changes that are common in the elderly. In summary, the current study demonstrates that age-related reduction in muscle mtDNA and increased DNA oxidation is associated with reduced levels of mitochondrial gene transcripts and proteins. These changes are closely related to declining capacity for mitochondrial ATP production in skeletal muscle and collectively may contribute to lower physical function and higher insulin resistance that are common in older people.] Short KR, Bigelow ML, et al. *PNAS*, April 12, 2005, Vol 102, No. 15. <https://www.pnas.org/content/pnas/102/15/5618.full.pdf>

Mitochondrial dysfunction and redox imbalance as a diagnostic marker of free radical diseases. [The intracellular redox balance (redox status) is a dynamic system that may change via many factors. Mitochondria are one of the most important among them. These organelles are the main intracellular source of energy. They are essential for maintaining cellular homeostasis due to regulation of many biochemical processes. The mitochondrial dynamics change during cellular activities and in some cases, can cause an overproduction of reactive oxygen species (ROS), which encourages the induction of oxidative DNA damage and up- or down-regulation of phosphatases, proliferative/anti-proliferative factors, apoptotic/anti-apoptotic factors, etc. Moreover, mitochondrial dysfunction and redox imbalance can continuously support and contribute to a wide range of pathologies, termed as "free radical diseases" (e.g., cancer, neurodegeneration, atherosclerosis, inflammation, etc.). This review article is focused on the mitochondrial dysfunction and cellular redox status as a hallmark of cell homeostasis and diagnostic marker of cancer. It is intended to broad readership - from students to specialists in the field.] Georgieva Em Ivanova D. et al. *Anticancer Res.* 2017 Oct;37(10):5373-5381. <https://www.ncbi.nlm.nih.gov/pubmed/28982845>

Mitochondrial metabolism of reactive oxygen species. [Abstract: For a long time mitochondria have mainly been considered for their role in the aerobic energy production in eukaryotic cells, being the sites of the oxidative phosphorylation, which couples the

electron transfer from respiratory substrates to oxygen with the ATP synthesis. Subsequently, it was showed that electron transfer along mitochondrial respiratory chain also leads to the formation of radicals and other reactive oxygen species, commonly indicated as ROS. The finding that such species are able to damage cellular components, suggested mitochondrial involvement in degenerative processes underlying several diseases and aging. More recently, a new role for mitochondria, as a system able to supply protection against cellular oxidative damage, is emerging. Experimental evidence indicates that the systems, evolved to protect mitochondria against endogenously produced ROS, can also scavenge ROS produced by other cellular sources. It is possible that this action, particularly relevant in physio-pathological conditions leading to increased cellular ROS production, is more effective in tissues provided with abundant mitochondrial population. Moreover, the mitochondrial dysfunction, resulting from ROS-induced inactivation of important mitochondrial components, can be attenuated by the cell purification from old ROS-overproducing mitochondria, which are characterized by high susceptibility to oxidative damage. Such an elimination is likely due to two sequential processes, named mitoptosis and mitophagy, which are usually believed to be induced by enhanced mitochondrial ROS generation. However, they could also be elicited by great amounts of ROS produced by other cellular sources and diffusing into mitochondria, leading to the elimination of the old dysfunctional mitochondrial subpopulation.] Venditti P, Di Stefano L, et al. *Mitochondrion*, Vol 13, Issue 2, March 2013, p 71-82. <https://www.sciencedirect.com/science/article/pii/S1567724913000093>

Mitochondrial metabolism of reactive oxygen species. [Oxidative stress is considered a major contributor to etiology of both “normal” senescence and severe pathologies with serious public health implications. Mitochondria generate reactive oxygen species (ROS) that are thought to augment intracellular oxidative stress. Mitochondria possess at least nine known sites that are capable of generating superoxide anion, a progenitor ROS. Mitochondria also possess numerous ROS defense systems that are much less studied. Studies of the last three decades shed light on many important mechanistic details of mitochondrial ROS production, but the bigger picture remains obscure. This review summarizes the current knowledge about major components involved in mitochondrial ROS metabolism and factors that regulate ROS generation and removal. An integrative, systemic approach is applied to analysis of mitochondrial ROS metabolism, which is now dissected into mitochondrial ROS production, mitochondrial ROS removal, and mitochondrial ROS emission. It is suggested that mitochondria augment intracellular oxidative stress due primarily to failure of their ROS removal systems, whereas the role of mitochondrial ROS emission is yet to be determined and a net increase in mitochondrial ROS production in situ remains to be demonstrated.] Andreyev, A.Y., Kushnareva, Y.E. et. Al. *Biochemistry (Moscow)* (2005) 70: 200. <https://doi.org/10.1007/s10541-005-0102-7>. <https://link.springer.com/article/10.1007/s10541-005-0102-7>

Skeletal muscle aging and the mitochondrion. [Decline in human muscle mass and strength (sarcopenia) is a hallmark of the aging process. A growing body of research in the areas of bioenergetics and protein turnover has placed the mitochondria at the center of this process. It is now clear that, unless an active lifestyle is rigorously followed, skeletal muscle mitochondrial decline occurs as humans age. Increasing research on mitochondrial biology has elucidated the regulatory pathways involved in mitochondrial biogenesis, many of which are potential therapeutic targets, and highlight the beneficial effects of vigorous physical activity on skeletal muscle health for an aging population.] Johnson M, Robinson M, et al. *Trends in Endocrinology & Metabolism*, Vol 24, Issue 5, May 2013. P 247-25. <https://www.sciencedirect.com/science/article/pii/S1043276012002238>

The role of mitochondria in aging. [Over the last decade, accumulating evidence has suggested a causative link between mitochondrial dysfunction and major phenotypes associated with aging. Somatic mitochondrial DNA (mtDNA) mutations and respiratory chain dysfunction accompany normal aging, but the first direct experimental evidence that increased mtDNA mutation levels contribute to progeroid phenotypes came from the mtDNA mutator mouse. Recent evidence suggests that increases in aging-associated mtDNA mutations are not caused by damage accumulation, but rather are due to clonal expansion of mtDNA replication errors that occur during development. Here we discuss the caveats of the traditional mitochondrial free radical theory of aging and highlight other possible mechanisms, including insulin/IGF-1 signaling (IIS) and the target of rapamycin pathways, that underlie the central role of mitochondria in the aging process.] Bratic A, Larsson N. *Journal of Clinical Investigation*, 10.1172/JCI64125 <https://www.jci.org/articles/view/64125>

Neurology / Nervous System

A novel small molecule, N-(4-(2-pyridyl)(1,3-thiazol-2-yl))-2-(2,4,6-trimethylphenoxy) acetamide, selectively protects against oxidative stress-induced cell death by activating the Nrf2-ARE pathway: therapeutic implications for ALS. [Antioxidant defense is crucial in restoring cellular redox homeostasis. Recent findings have suggested that oxidative stress plays pivotal roles in the pathogenesis of many neurodegenerative diseases. Thus, an anti-oxidative stress remedy might be a promising means for the treatment of such disorders. In this study, we employed a novel ligand-based virtual screening system and identified a novel small molecule, N-(4-(2-pyridyl)(1,3-thiazol-2-yl))-2-(2,4,6-trimethylphenoxy) acetamide (CPN-9), which selectively suppressed oxidative stress-induced cell death in a cell-type-independent manner. CPN-9 upregulates NF-E2-related factor 2 (Nrf2), a key transcriptional regulator of the expression of phase II detoxification enzymes and antioxidant proteins, and Nrf2-regulated factors such as heme oxygenase-1 (HO-1), NAD(P)H quinone oxidoreductase 1 (NQO1), and glutamate-cysteine ligase modifier subunit (GCLM). The

CPN-9-mediated upregulation of HO-1, NQO1, and GCLM was abolished by Nrf2 knockdown. Moreover, the antioxidant N-acetylcysteine reduced the protective effect of CPN-9 against oxidative stress-induced cell death with concomitant diminishing of Nrf2 nuclear translocation. These results indicate that CPN-9 exerts its activity via the reactive oxygen species-dependent activation of the Nrf2 signaling pathway in cultured cells. It is noteworthy that the postonset systemic administration of CPN-9 to a transgenic ALS mouse model carrying the H46R mutation in the human Cu/Zn superoxide dismutase (SOD1) gene sustained motor functions and delayed disease progression after onset. Collectively, CPN-9 is a novel Nrf2 activator and a neuroprotective candidate for the treatment of neurodegenerative diseases, including ALS.] Kanno T, Tanaka K, et al. *Free Radic Biol Med*. 2012 Dec 1;53(11):2028-42. <https://www.ncbi.nlm.nih.gov/pubmed/23000247> .

Cerebrovascular and Neurological Disorders: Protective Role of NRF2. [Cellular defense mechanisms, intracellular signaling, and physiological functions are regulated by electrophiles and reactive oxygen species (ROS). Recent works strongly considered imbalanced ROS and electrophile overabundance as the leading cause of cellular and tissue damage, whereas oxidative stress (OS) plays a crucial role for the onset and progression of major cerebrovascular and neurodegenerative pathologies. These include Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), Huntington's disease (HD), stroke, and aging. Nuclear factor erythroid 2-related factor (NRF2) is the major modulator of the xenobiotic-activated receptor (XAR) and is accountable for activating the antioxidative response elements (ARE)-pathway modulating the detoxification and antioxidative responses of the cells. NRF2 activity, however, is also implicated in carcinogenesis protection, stem cells regulation, anti-inflammation, anti-aging, and so forth. Herein, we briefly describe the NRF2-ARE pathway and provide a review analysis of its functioning and system integration as well as its role in major CNS disorders. We also discuss NRF2-based therapeutic approaches for the treatment of neurodegenerative and cerebrovascular disorders.] Sivandzade F, Bhalerao A, et al. *Int J Mol Sci*. 2019 Jul 12;20(14). <https://www.ncbi.nlm.nih.gov/pubmed/31336872>

NRF2 and NF- κ B interplay in cerebrovascular and neurodegenerative disorders: Molecular mechanisms and possible therapeutic approaches. [Electrophiles and reactive oxygen species (ROS) play a major role in modulating cellular defense mechanisms as well as physiological functions, and intracellular signaling. However, excessive ROS generation (endogenous and exogenous) can create a state of redox imbalance leading to cellular and tissue damage (Ma and He, 2012) [1]. A growing body of research data strongly suggests that imbalanced ROS and electrophile overproduction are among the major prodromal factors in the onset and progression of several cerebrovascular and neurodegenerative disorders such as amyotrophic lateral sclerosis (ALS), stroke, Alzheimer's disease (AD), Parkinson's disease (PD), and aging (Ma and He, 2012; Ramsey et al., 2017; Salminen et al., 2012; Sandberg et al., 2014; Sarlette et al., 2008; Tanji et al., 2013) [1-6]. Cells offset oxidative stress by the action of housekeeping antioxidative enzymes (such as superoxide dismutase, catalase, glutathione peroxidase) as well direct and indirect antioxidants (Dinkova-Kostova and Talalay, 2010) [7]. The DNA sequence responsible for modulating the antioxidative and cytoprotective responses of the cells has been identified as the antioxidant response element (ARE), while the nuclear factor erythroid 2-related factor (NRF2) is the major regulator of the xenobiotic-activated receptor (XAR) responsible for activating the ARE-pathway, thus defined as the NRF2-ARE system (Ma and He, 2012) [1]. In addition, the interplay between the NRF2-ARE system and nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B, a protein complex that controls cytokine production and cell survival), has been further investigated in relation to neurodegenerative and neuroinflammatory disorders. On these premises, we provide a review analysis of current understanding of the NRF2-NF- κ B interplay, their specific role in major CNS disorders, and consequent therapeutic implication for the treatment of neurodegenerative and cerebrovascular diseases.] Send to *Redox Biol*. 2018 Nov 28;21:101059. doi: 10.1016/j.redox.2018.11.017. <https://www.ncbi.nlm.nih.gov/pubmed/30576920>

Oxidative stress and nerve damage: role in chemotherapy induced peripheral neuropathy. [Peripheral neuropathy is a severe dose limiting toxicity associated with cancer chemotherapy. Ever since it was identified, the clear pathological mechanisms underlying chemotherapy induced peripheral neuropathy (CIPN) remain sparse and considerable involvement of oxidative stress and neuroinflammation has been realized recently. Despite the empirical use of antioxidants in the therapy of CIPN, the oxidative stress mediated neuronal damage in peripheral neuropathy is still debatable. The current review focuses on nerve damage due to oxidative stress and mitochondrial dysfunction as key pathogenic mechanisms involved in CIPN. Oxidative stress as a central mediator of apoptosis, neuroinflammation, metabolic disturbances and bioenergetic failure in neurons has been highlighted in this review along with a summary of research on dietary antioxidants and other nutraceuticals which have undergone prospective controlled clinical trials in patients undergoing chemotherapy.] Areti A, Yerra VG, et al. *Redox Biol*. 2014 Jan 18;2:289-95. doi: 10.1016/j.redox.2014.01.006. <https://www.ncbi.nlm.nih.gov/pubmed/24494204>

Oxidative stress in the brain: Novel cellular targets that govern survival during neurodegenerative disease. [Despite our present knowledge of some of the cellular pathways that modulate central nervous system injury, complete therapeutic prevention or reversal of acute or chronic neuronal injury has not been achieved. The cellular mechanisms that precipitate these diseases are more involved than initially believed. As a result, identification of novel therapeutic targets for the treatment of cellular injury would be extremely beneficial to reduce or eliminate disability from nervous system disorders. Current studies have begun to focus on pathways of oxidative stress that involve a variety of cellular pathways. Here we discuss novel pathways that involve the generation of reactive

oxygen species and oxidative stress, apoptotic injury that leads to nuclear degradation in both neuronal and vascular populations, and the early loss of cellular membrane asymmetry that mitigates inflammation and vascular occlusion. Current work has identified exciting pathways, such as the Wnt pathway and the serine–threonine kinase Akt, as central modulators that oversee cellular apoptosis and their downstream substrates that include Forkhead transcription factors, glycogen synthase kinase-3 β , mitochondrial dysfunction, Bad, and Bcl-xL. Other closely integrated pathways control microglial activation, release of inflammatory cytokines, and caspase and calpain activation. New therapeutic avenues that are just open to exploration, such as with brain temperature regulation, nicotinamide adenine dinucleotide modulation, metabotropic glutamate system modulation, and erythropoietin targeted expression, may provide both attractive and viable alternatives to treat a variety of disorders that include stroke, Alzheimer's disease, and traumatic brain injury.] Chong ZZ, Li F, et al. *Progress in Neurobiology*, Volume 75, Issue 3, February 2005, Pages 207-246. <https://www.sciencedirect.com/science/article/pii/S0301008205000134>

Redox Regulation of Cellular Stress Response in Aging and Neurodegenerative Disorders: Role of Vitagenes. [Reduced expression and/or activity of antioxidant proteins lead to oxidative stress, accelerated aging and neurodegeneration. However, while excess reactive oxygen species (ROS) are toxic, regulated ROS play an important role in cell signaling. Perturbation of redox status, mutations favoring protein misfolding, altered glycosylation, overloading of the product of polyunsaturated fatty acid peroxidation (hydroxynonenals, HNE) or cholesterol oxidation, can disrupt redox homeostasis. Collectively or individually these effects may impose stress and lead to accumulation of unfolded or misfolded proteins in brain cells. Alzheimer's (AD), Parkinson's and Huntington's disease, amyotrophic lateral sclerosis and Friedreich's ataxia are major neurological disorders associated with production of abnormally aggregated proteins and, as such, belong to the so-called "protein conformational diseases". The pathogenic aggregation of proteins in non-native conformation is generally associated with metabolic derangements and excessive production of ROS. The "unfolded protein response" has evolved to prevent accumulation of unfolded or misfolded proteins. Recent discoveries of the mechanisms of cellular stress signaling have led to new insights into the diverse processes that are regulated by cellular stress responses. The brain detects and overcomes oxidative stress by a complex network of "longevity assurance processes" integrated to the expression of genes termed vitagenes. Heat-shock proteins are highly conserved and facilitate correct protein folding. Heme oxygenase-1, an inducible and redox-regulated enzyme, has having an important role in cellular antioxidant defense. An emerging concept is neuroprotection afforded by heme oxygenase by its heme degrading activity and tissue-specific antioxidant effects, due to its products carbon monoxide and biliverdin, which is then reduced by biliverdin reductase in bilirubin. There is increasing interest in dietary compounds that can inhibit, retard or reverse the steps leading to neurodegeneration in AD. Specifically any dietary components that inhibit inappropriate inflammation, A β P oligomerization and consequent increased apoptosis are of particular interest, with respect to a chronic inflammatory response, brain injury and β -amyloid associated pathology. Curcumin and ferulic acid, the first from the curry spice turmeric and the second a major constituent of fruit and vegetables, are candidates in this regard. Not only do these compounds serve as antioxidants but, in addition, they are strong inducers of the heat-shock response. Food supplementation with curcumin and ferulic acid are therefore being considered as a novel nutritional approach to reduce oxidative damage and amyloid pathology in AD. We review here some of the emerging concepts of pathways to neurodegeneration and how these may be overcome by a nutritional approach.] Calabrese V, Guagliano E, et al., *Neurochem Res* (2007) 32: 757. <https://doi.org/10.1007/s11064-006-9203-y>. <https://link.springer.com/article/10.1007%2Fs11064-006-9203-y#citeas>

PEMF, Photobiomodulation, Low-level Light Therapy

Coupling of pulsed electromagnetic fields (PEMF) therapy to molecular grounds of the cell. [In this review we compile results cited in reliable journals that show a ratio for the use of pulsed electromagnetic fields (PEMF) in therapy, indeed. This is true especially for chronically inflamed joints. Furthermore, we try to link this therapeutic approach to the molecular background of chronic inflammation and arthritis. At first we start with the clinical outcome of PEMF therapy. Then, we look for possible triggers and an electromagnetic counterpart that is endogenously inherent in cell biology and in the tissues of interest. Finally, we want to investigate causal molecular and cellular mechanisms of possible PEMF actions. It shows that there are endogenous mechanisms, indeed, which can act as triggers for PEMF like the resting membrane potential as well as resonance mechanisms in charged moieties like membrane transporters. Especially voltage-gated calcium channels can be triggered. These may lead into specific signaling pathways and also may elicit nitric oxide as well as moderate radical reactions, which can ultimately lead to e.g. NF κ B-like reactions. Concerted in the right way, these reactions can cause a kind of cell protection and ultimately lead to a dampening of inflammatory signals like interleukins.] Funk R. *m J Transl Res*. 2018; 10(5): 1260–1272. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5992548/>

Low-intensity light therapy: exploring the role of redox mechanisms. [Low-intensity light therapy (LILT) appears to be working through newly recognized photoacceptor systems. The mitochondrial electron transport chain has been shown to be photosensitive to red and near-infrared (NIR) light. Although the underlying mechanisms have not yet been clearly elucidated, mitochondrial photostimulation has been shown to increase ATP production and cause transient increases in reactive oxygen species (ROS). In some cells, this process appears to participate in reduction/oxidation (redox) signaling. Redox mechanisms are known to be involved in cellular homeostasis and proliferative control. In plants, photostimulation of the analogous photosynthetic electron transport chain

leads to redox signaling known to be integral to cellular function. In gene therapy research, ultraviolet lasers are being used to photostimulate cells through a process that also appears to involve redox signaling. It seems that visible and near visible low-intensity light can be used to modulate cellular physiology in some nonphotosynthetic cells, acting through existing redox mechanisms of cellular physiology. In this manner, LILT may act to promote proliferation and/or cellular homeostasis. Understanding the role of redox state and signaling in LILT may be useful in guiding future therapies, particularly in conditions associated with pro-oxidant conditions.] Tafur J, Mills PJ. *Photomed Laser Surg.* 2008 Aug;26(4):323-8. <http://www.medicinabiomolecular.com.br/biblioteca/pdfs/Biomolecular/luz-de-baixa-intensidade-mecanismos-no-sistema.pdf>

Mechanisms and Mitochondrial Redox Signaling in Photobiomodulation. [Photobiomodulation (PBM) involves the use of red or near-infrared light at low power densities to produce a beneficial effect on cells or tissues. PBM therapy is used to reduce pain, inflammation, edema, and to regenerate damaged tissues such as wounds, bones and tendons. The primary site of light absorption in mammalian cells has been identified as the mitochondria, and more specifically, cytochrome c oxidase (CCO). It is hypothesized that inhibitory nitric oxide can be dissociated from CCO thus restoring electron transport and increasing mitochondrial membrane potential. Another mechanism involves activation of light or heat-gated ion channels. This review will cover the redox signaling that occurs in PBM and examine the difference between healthy and stressed cells, where PBM can have apparently opposite effects. PBM has a marked effect on stem cells, and this is proposed to operate via mitochondrial redox signaling. PBM can act as a pre-conditioning regimen, and can interact with exercise on muscles.] Hamblin, MR. *Photochem Photobiol.* 2018 Mar; 94(2): 199–212. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5844808/>

Magnetic Fields and Reactive Oxygen Species. [Reactive oxygen species (ROS) ubiquitously exist in mammalian cells to participate in various cellular signaling pathways. The intracellular ROS levels are dependent on the dynamic balance between ROS generation and elimination. In this review, we summarize reported studies about the influences of magnetic fields (MFs) on ROS levels. Although in most cases, MFs increased ROS levels in human, mouse, rat cells, and tissues, there are also studies showing that ROS levels were decreased or not affected by MFs. Multiple factors could cause these discrepancies, including but not limited to MF type/intensity/frequency, exposure time and assay time-point, as well as different biological samples examined. It will be necessary to investigate the influences of different MFs on ROS in various biological samples systematically and mechanistically, which will be helpful for people to get a more complete understanding about MF-induced biological effects. In addition, reviewing the roles of MFs in ROS modulation may open up new scenarios of MF application, which could be further and more widely adopted into clinical applications, particularly in diseases that ROS have documented pathophysiological roles.] Wang H, Zang X et al *Int J Mol Sci.* 2017 Oct; 18(10): 2175. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5666856/>

Modulation of Redox Signaling in Chronic Diseases and Regenerative Medicine' [Over the last decades, major advances in therapeutic strategies for chronic diseases have significantly reduced death rates. Redox signaling is being implicated in the pathophysiology of such diseases, affecting most organs. Proliferation of cancer cells, damage to cardiomyocytes and vascular cells, and exacerbation of inflammation are just a few examples of events importantly controlled by reacting oxygen species (ROS). Physiologic levels of ROS act as signaling molecules and modulate healthy functions, while high levels of ROS can derange the homeostasis of most organs and systems in our body. Hence, fine-tuning the complexity of redox signaling is a very up-to-date field of research. Light-based technologies are emerging as powerful tools in several experimental and clinical arenas. Two manuscripts in this issue focus on the use of light to detect and interfere with ROS signaling. In their review, M. R. Antognazza et al. describe the state-of-the-art and recent advances in the field of photostimulation of oxidative stress (from photobiomodulation mediated by naturally expressed light-sensitive proteins to the latest optogenetic approaches) and highlight novel concepts based on optically driven ROS regulations mediated by polymeric materials. The possibility to modulate oxidative stress in disease conditions also paves the way to innovative therapeutic intervention.] Zacchigna S, Sartiani L, et al. *Oxid Med Cell Longev.* 2019 Apr 23;2019:6091587. <https://www.hindawi.com/journals/omcl/2019/6091587/>

Photobiomodulation of human adipose-derived stem cells using 810nm and 980nm lasers operates via different mechanisms of action. [Photobiomodulation (PBM) using red or near-infrared (NIR) light has been used to stimulate the proliferation and differentiation of adipose-derived stem cells. The use of NIR wavelengths such as 810nm is reasonably well accepted to stimulate mitochondrial activity and ATP production via absorption of photons by cytochrome c oxidase. However, the mechanism of action of 980nm is less well understood. Here we study the effects of both wavelengths (810nm and 980nm) on adipose-derived stem cells in vitro. Both wavelengths showed a biphasic dose response, but 810nm had a peak dose response at 3J/cm² for stimulation of proliferation at 24h, while the peak dose for 980nm was 10-100 times lower at 0.03 or 0.3J/cm². Moreover, 980nm (but not 810nm) increased cytosolic calcium while decreasing mitochondrial calcium. The effects of 980nm could be blocked by calcium channel blockers (capsazepine for TRPV1 and SKF96365 for TRPC channels), which had no effect on 810nm. To test the hypothesis that the chromophore for 980nm was intracellular water, which could possibly form a microscopic temperature gradient upon laser irradiation, we added cold medium (4°C) during the light exposure, or pre-incubated the cells at 42°C, both of which abrogated the effect of 980nm but not 810nm. We conclude that 980nm affects temperature-gated calcium ion channels, while 810nm largely affects

mitochondrial cytochrome c oxidase.] Wang Y, Huang YY, et al. *Biochim Biophys Acta Gen Subj.* 2017 Feb;1861(2):441-449. <https://www.ncbi.nlm.nih.gov/pubmed/27751953>

Photobiomodulation (660 nm) therapy reduces oxidative stress and induces BDNF expression in the hippocampus.

[Photobiomodulation therapy (PBMT) effects an important role in neural regeneration and function enhancement, such as expression of nerve growth factor and nerve regeneration, in neuronal tissues, and inhibition of cell death by amyloid beta in neurons is inhibited by PBMT. However, there no studies evaluated the effects of PBMT on oxidative stress in the hippocampus. The aim of this study is to evaluate the effects of PBMT on oxidative stress in the hippocampus. This study assessed the anti-oxidative effect, the expression of BDNF and antioxidant enzymes, as well as the activation of cAMP response element binding (CREB) and extracellular signal-regulated kinase (ERK) signal transduction pathways assess using a hippocampal cell line (HT-22) and mouse organotypic hippocampal tissues by PBMT (LED, 660 nm, 20 mW/cm²). PBMT inhibited HT-22 cell death by oxidative stress and increased BDNF expression via ERK and CREB signaling pathway activation. In addition, PBMT increased BDNF expression in hippocampal organotypic slices and the levels of phosphorylated ERK and CREB, which were reduced by oxidative stress, as well as the expression of the antioxidant enzyme superoxide dismutase. These data demonstrate that PBMT inhibits hippocampal damage induced by oxidative stress and increases the expression of BDNF, which can be used as an alternative to treat a variety of related disorders that lead to nerve damage. Activation and redox homeostasis in neuronal cells may be a notable mechanism of the 660-nm PBMT-mediated photobioreactivity.] Heo J, Park J, et al. *Scientific Reports* volume 9, Article number: 10114 (2019) <https://www.nature.com/articles/s41598-019-46490-4>

Pulsed electromagnetic field (PEMF) prevents pro-oxidant effects of H₂O₂ in SK-N-BE(2) human neuroblastoma cells.

[Purpose The redox milieu, together with reactive oxygen species (ROS) accumulation, may play a role in mediating some biological effects of extremely-low-frequency electromagnetic fields (ELF-EMF). Some of us have recently reported that a pulsed EMF (PEMF) improves the antioxidant response of a drug-sensitive human neuroblastoma SH-SY5Y cell line to pro-oxidants. Since drug resistance may affect cell sensitivity to redox-based treatments, we wanted to verify whether drug-resistant human neuroblastoma SK-N-BE(2) cells respond to a PEMF in a similar fashion. Materials and methods SK-N-BE(2) cells were exposed to repeated 2 mT, 75 Hz PEMF (15 min each, repeated 3 times over 5 days), and ROS production, Mn-dependent superoxide dismutase (MnSOD)-based antioxidant protection and viability were assessed after 10 min or 30 min 1 mM hydrogen peroxide. Sham controls were kept at the same time in identical cell culture incubators. Results The PEMF increased the MnSOD-based antioxidant protection and reduced the ROS production in response to a pro-oxidant challenge. Conclusions Our work might lay foundation for the development of non-invasive PEMF-based approaches aimed at elevating endogenous antioxidant properties in cellular or tissue models.] Falone S, Marchesi N, et al. *International Journal of Radiation Biology.* Volume 92, 2016 - Issue 5. <https://www.tandfonline.com/doi/abs/10.3109/09553002.2016.1150619?src=recsys&journalCode=irab20>

Platelets, Platelet-rich Fibrin (PRF) and Redox Signaling

Age associated non-linear regulation of redox homeostasis in the anucleate platelet: Implications for CVD risk patients.

[BACKGROUND: Aging is a complex physiological phenomenon, intricately associated with cardiovascular pathologies, where platelets play a central pathophysiological role. Although antiplatelets are commonly employed to prevent and treat major adverse cardiovascular events, aging associated intraplatelet changes remain largely unexplored. METHODS: Platelets were studied in high cardiovascular risk patients (aged 40-100 years) comparing them to younger healthy subjects. This was followed by cross sectional and longitudinal mice studies. Flow cytometry, biochemical and molecular assays were used to study platelets comprehensively. FINDINGS: CVD Patients were categorized in the age groups 40-59, 60-79, and 80-100 years. Progressive decline in platelet health was observed in the 40-79 years age cohort, marked by increase in oxidative stress, hyperactivation and apoptotic markers. Paradoxically, this was reversed in patients aged above 79 years and the improved platelet phenotype was associated with lower oxidative damage. The platelets from the very old (80-100 year) group were found to be preloaded with increased antioxidants, which also contributed to higher resistance against induced redox insults. Cross sectional mouse studies excluded the effect of comorbidities and medications. Longitudinal mouse studies implicate an adaptive increase in antioxidant levels as the mechanism. INTERPRETATION: We report a novel age associated, non-linear redox regulation in platelets in both humans and mice. In advanced age, there occurs an adaptive increase in platelet antioxidants, reducing the intracellular ROS and leading to a healthier platelet phenotype. Clinically, our results advocate the use of less aggressive antiplatelet therapies for CVD in the elderly population.] Jain K, Tyagi T, et al. *EBioMedicine.* 2019 Jun;44:28-40. [https://www.thelancet.com/article/S2352-3964\(19\)30323-8/pdf](https://www.thelancet.com/article/S2352-3964(19)30323-8/pdf)

Antimicrobial action of autologous platelet-rich plasma on MRSA-infected skin wounds in dogs. [Effective antimicrobial preparations, other than antibiotics, are important for the treatment of potentially fatal drug-resistant infections. Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the leading causes of hospital-acquired and post-operative infections. Fortunately, the antimicrobial properties of platelet-rich plasma (PRP) against various microorganisms enable its potential use as an alternative to conventional antibiotics. The present work was designed to evaluate the hypothesized antimicrobial activity of PRP against MRSA

infected skin wounds. Six adult male dogs were divided equally into control and PRP groups. Unilateral circular full-thickness skin wounds were created then a MRSA suspension was injected locally. Treatment started at 1st week post infection with subcutaneous infiltration of autologous activated PRP every week in the PRP group and with topical application of clindamycin cream twice daily in the control group. PRP decreased wound size and significantly increased wound contractility and re-epithelization, as confirmed by histopathological and immunohistochemical findings. Also PRP treated group showed significant decrease in ROS and redox imbalance with over expression of the TNF- α and VEGFA genes that indicate angiogenesis and maximum antibacterial activity after three weeks. In conclusion, CaCl₂-activated PRP exhibited antimicrobial activity against MRSA infection, which improved the infected wound healing re-epithelization and granulation tissue formation.] Farghali HA, AbdElKader, NA, et al. Scientific Reports volume 9, Article number: 12722 (2019) <https://www.nature.com/articles/s41598-019-48657-5>

Platelet receptor redox regulation. [Several recent findings point to an important role for redox regulation of platelet responses to collagen involving the receptor, glycoprotein (GP)VI. First, the antioxidant dietary compound, quercetin, was shown to inhibit GPVI-dependent platelet activation and signaling responses to collagen. Second, collagen increased platelet production of the oxygen radical, superoxide anion (O₂⁻), mediated by the multi-subunit enzyme nicotinamide adenine dinucleotide (phosphate) (NAD(P)H) oxidase. In that case, O₂⁻ was implicated in regulating not initial aggregation, but collagen-induced thrombus stabilization involving release of ADP. Third, our laboratory showed that an unpaired thiol in the GPVI cytoplasmic tail undergoes rapid oxidation to form GPVI homodimers following ligand binding, preceding GPVI signaling and ectodomain metalloproteolysis, and indicating formation of an oxidative submembranous environment in activated platelets. This review examines receptor/redox regulation in other cells, and relevance to the pathophysiological function of GPVI and other platelet receptors initiating thrombus formation in haemostasis or thrombotic diseases such as heart attack and stroke.] Arthur JF, Gardiner EE, et al. Platelets. 2008 Feb;19(1):1-8. <https://www.ncbi.nlm.nih.gov/pubmed/18231933>

Reactive Oxygen Species Play a Critical Role in Collagen-Induced Platelet Activation via SHP-2 Oxidation. [The collagen-stimulated generation of reactive oxygen species (ROS) regulates signal transduction in platelets, although the mechanism is unclear. The major targets of ROS include protein tyrosine phosphatases (PTPs). ROS-mediated oxidation of the active cysteine site in PTPs abrogates the PTP catalytic activity. The aim of this study was to elucidate whether collagen-induced ROS generation leads to PTP oxidation, which promotes platelet stimulation. Results: SH2 domain-containing PTP-2 (SHP-2) is oxidized in platelets by ROS produced upon collagen stimulation. The oxidative inactivation of SHP-2 leads to the enhanced tyrosine phosphorylation of spleen tyrosine kinase (Syk), Vav1, and Bruton's tyrosine kinase (Btk) in the linker for the activation of T cells signaling complex, which promotes the tyrosine phosphorylation-mediated activation of phospholipase C γ 2 (PLC γ 2). Moreover, we found that, relative to wild-type platelets, platelets derived from glutathione peroxidase 1 (GPx1)/catalase double-deficient mice showed enhanced cellular ROS levels, oxidative inactivation of SHP-2, and tyrosine phosphorylation of Syk, Vav1, Btk, and PLC γ 2 in response to collagen, which subsequently led to increased intracellular calcium levels, degranulation, and integrin α IIb β 3 activation. Consistent with these findings, GPx1/catalase double-deficiency accelerated the thrombotic response in FeCl₃-injured carotid arteries. Innovation: The present study is the first to demonstrate that SHP-2 is targeted by ROS produced in collagen-stimulated platelets and suggests that a novel mechanism for the regulation of platelet activation by ROS is due to oxidative inactivation of SHP-2. Conclusion: We conclude that collagen-induced ROS production leads to SHP-2 oxidation, which promotes platelet activation by upregulating tyrosine phosphorylation-based signal transduction.] Jang JY, Min JH, et al. Antioxid. Redox Signal. 20, 2528–2540. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4025609/>

Regulation of platelet activation and thrombus formation by reactive oxygen species/ [Reactive oxygen species (ROS) are generated within activated platelets and play an important role in regulating platelet responses to collagen and collagen-mediated thrombus formation. As a major collagen receptor, platelet-specific glycoprotein (GP)VI is a member of the immunoglobulin (Ig) superfamily, with two extracellular Ig domains, a mucin domain, a transmembrane domain and a cytoplasmic tail. GPVI forms a functional complex with the Fc receptor γ -chain (FcR γ) that, following receptor dimerization, signals via an intracellular immunoreceptor tyrosine-based activation motif (ITAM), leading to rapid activation of Src family kinase signaling pathways. Our previous studies demonstrated that an unpaired thiol in the cytoplasmic tail of GPVI undergoes rapid oxidation to form GPVI homodimers in response to ligand binding, indicating an oxidative submembranous environment in platelets after GPVI stimulation. Using a redox-sensitive fluorescent dye (H₂DCE-DA) in a flow cytometric assay to measure changes in intracellular ROS, we showed generation of ROS downstream of GPVI consists of two distinct phases: an initial Syk-independent burst followed by additional Syk-dependent generation. In this review, we will discuss recent findings on the regulation of platelet function by ROS, focusing on GPVI-dependent platelet activation and thrombus formation.] Qiao, J, Arthur, JF, et al. Redox Biol. 2018 Apr; 14: 126–130. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5596263/>

Skin

Biochemical markers of oxidative and nitrosative stress in acne vulgaris: correlation with disease activity. [BACKGROUND: Acne vulgaris is a multifactorial skin disorder of unknown etiology. Free radical-mediated reactions have been implicated but their role in eliciting this response and contributing to disease progress remains unexplored. This study was undertaken to investigate the status and contribution of oxidative/nitrosative stress in patients with acne vulgaris. METHODS: Sera from 50 acne vulgaris with varying levels of disease activity (mild, moderate, and severe) according to the Global Acne Grading System (GAGS) and 40 age- and sex-matched controls were evaluated for serum levels of oxidative/nitrosative stress markers, including protein oxidation, lipid peroxidation and nitric oxide (NO), superoxide dismutase (SOD), and glutathione (GSH). RESULTS: Serum analysis showed significantly higher levels of carbonyl contents, malondialdehyde (MDA) and NO, in acne patients compared with healthy controls ($P < 0.05$). Interestingly, not only there were an increased number of subjects positive for carbonyl contents, but also the levels of these oxidants were significantly increased with the increase of the disease activity ($P < 0.05$). In addition, a significant correlation was observed between the levels of carbonyl contents and the GAGS scores ($r = 0.341$, $r = 0.355$, and $r = 0.299$, respectively). Furthermore, sera from acne patients had lower levels of SOD and GSH compared with healthy control sera. CONCLUSION: These findings support an association between oxidative/nitrosative stress and acne. The stronger response observed in serum samples from patients with higher GAGS scores suggests that markers of oxidative/nitrosative stress may be useful in evaluating the progression of acne and in elucidating the mechanisms of disease pathogenesis.] Al-Shobali HA, Alzolibani AA, et al. *J Clin Lab Anal.* 2013 Jan;27(1):45-52. doi: 10.1002/jcla.21560.
<https://www.ncbi.nlm.nih.gov/pubmed/?term=Biochemical+Markers+of+Oxidative+and+Nitrosative+Stress+in+Acne+Vulgaris%3A+Correlation+With+Disease+Activity>

Dermal Wound Healing Is Subject to Redox Control. [Previously we have reported in vitro evidence suggesting that that H₂O₂ may support wound healing by inducing VEGF expression in human keratinocytes (C. K. Sen et al., 2002, *J. Biol. Chem.* 277, 33284–33290). Here, we test the significance of H₂O₂ in regulating wound healing in vivo. Using the Hunt–Schilling cylinder approach we present the first evidence that the wound site contains micromolar concentrations of H₂O₂. At the wound site, low concentrations of H₂O₂ supported the healing process, especially in p47phox- and MCP-1-deficient mice in which endogenous H₂O₂ generation is impaired. Higher doses of H₂O₂ adversely influenced healing. At low concentrations, H₂O₂ facilitated wound angiogenesis in vivo. H₂O₂ induced FAK phosphorylation both in wound-edge tissue in vivo and in human dermal microvascular endothelial cells. H₂O₂ induced site-specific (Tyr-925 and Tyr-861) phosphorylation of FAK. Other sites, including the Tyr-397 autophosphorylation site, were insensitive to H₂O₂. Adenoviral gene delivery of catalase impaired wound angiogenesis and closure. Catalase overexpression slowed tissue remodeling as evidenced by a more incomplete narrowing of the hyperproliferative epithelium region and incomplete eschar formation. Taken together, this work presents the first in vivo evidence indicating that strategies to influence the redox environment of the wound site may have a bearing on healing outcomes.] Roy S, Khanna S, et al. *Molecular Therapy*, Volume 13, Issue 1, January 2006, Pages 211-220. <https://www.sciencedirect.com/science/article/pii/S1525001605014085>

Redox Imbalance in T Cell-Mediated Skin Diseases [The skin is permanently exposed to physical, chemical, and biological aggression by the environment. In addition, acute and chronic inflammatory events taking place in the skin are accompanied by abnormal release of pro-oxidative mediators. In this paper, we will briefly overview the homeostatic systems active in the skin to maintain the redox balance and also to counteract abnormal oxidative stress. We will concentrate on the evidence that a local and/or systemic redox dysregulation accompanies the chronic inflammatory disorder events associated to psoriasis, contact dermatitis, and atopic dermatitis. We will also discuss the fact that several well-established treatments for the therapy of chronic inflammatory skin disorders are based on the application of strong physical or chemical oxidants onto the skin, indicating that, in selected conditions, a further increase of the oxidative imbalance may lead to a beneficial outcome.] Pastore S, Korkina L. *Mediators of Inflammation*. Volume 2010, Article ID 861949, 9 pages. <http://dx.doi.org/10.1155/2010/861949>
<https://www.hindawi.com/journals/mi/2010/861949/abs/>

Targeting the Redox Balance in Inflammatory Skin Conditions [Reactive oxygen species (ROS) can be both beneficial and deleterious. Under normal physiological conditions, ROS production is tightly regulated, and ROS participate in both pathogen defense and cellular signaling. However, insufficient ROS detoxification or ROS overproduction generates oxidative stress, resulting in cellular damage. Oxidative stress has been linked to various inflammatory diseases. Inflammation is an essential response in the protection against injurious insults and thus important at the onset of wound healing. However, hampered resolution of inflammation can result in a chronic, exaggerated response with additional tissue damage. In the pathogenesis of several inflammatory skin conditions, e.g., sunburn and psoriasis, inflammatory-mediated tissue damage is central. The prolonged release of excess ROS in the skin can aggravate inflammatory injury and promote chronic inflammation. The cellular redox balance is therefore tightly regulated by several (enzymatic) antioxidants and pro-oxidants; however, in case of chronic inflammation, the antioxidant system may be depleted, and prolonged oxidative stress occurs. Due to the central role of ROS in inflammatory pathologies, restoring the redox balance forms an innovative therapeutic target in the development of new strategies for treating inflammatory skin conditions. Nevertheless, the

clinical use of antioxidant-related therapies is still in its infancy] Wagener F, Carels C, et al. *Int. J. Mol. Sci.* 2013, 14(5), 9126-9167; <https://doi.org/10.3390/ijms14059126> <https://www.mdpi.com/1422-0067/14/5/9126> .

Vitamin D status is independently associated with plasma glutathione and cysteine thiol/disulphide redox status in adults.

[OBJECTIVE: Redox status and inflammation are important in the pathophysiology of numerous chronic diseases. Epidemiological studies have linked vitamin D status to a number of chronic diseases. We aimed to examine the relationships between serum 25-hydroxyvitamin D [25(OH)D] and circulating thiol/disulphide redox status and biomarkers of inflammation. DESIGN: This was a cross-sectional study of N = 693 adults (449 females, 244 males) in an apparently healthy, working cohort in Atlanta, GA. Plasma glutathione (GSH), cysteine (Cys) and their associated disulphides were determined with high-performance liquid chromatography, and their redox potentials (Eh GSSG and Eh CySS) were calculated using the Nernst equation. Serum inflammatory markers included interleukin-6 (IL-6), interleukin-8 (IL-8) and tumour necrosis factor- α , assayed on a multiplex platform, and C-reactive protein (CRP), assayed commercially. Relationships were assessed with multiple linear regression analyses. RESULTS: Serum 25(OH)D was positively associated with plasma GSH ($\beta \pm SE$: 0.002 ± 0.0004) and negatively associated with plasma Eh GSSG ($\beta \pm SE$: -0.06 ± 0.01) and Cys ($\beta \pm SE$: -0.01 ± 0.003) ($P < 0.001$ for all); statistical significance remained after adjusting for age, gender, race, percentage body fat and traditional cardiovascular risk factors ($P = 0.01-0.02$). The inverse relationship between serum 25(OH)D and CRP was confounded by percentage body fat, and full adjustment for covariates attenuated serum 25(OH)D relationships with other inflammatory markers to nonstatistical significance. CONCLUSIONS: Serum 25(OH)D concentrations were independently associated with major plasma thiol/disulphide redox systems, suggesting that vitamin D status may be involved in redox-mediated pathophysiology.] Alvarez JA, Chowdhury R, et al. *Clin Endocrinol (Oxf)*. 2014 Sep;81(3):458-66. doi: 10.1111/cen.12449. <https://www.ncbi.nlm.nih.gov/pubmed/?term=vitamin+d+status+is+independently+associated+with+plasma+glutathione+and+cysteine>

Wound Healing

An Essential Role of NRF2 in Diabetic Wound Healing. [The high mortality and disability of diabetic nonhealing skin ulcers create an urgent need for the development of more efficacious strategies targeting diabetic wound healing. In the current study, using human clinical specimens, we show that perilesional skin tissues from patients with diabetes are under more severe oxidative stress and display higher activation of the nuclear factor-E2-related factor 2 (NRF2)-mediated antioxidant response than perilesional skin tissues from normoglycemic patients. In a streptozotocin-induced diabetes mouse model, Nrf2(-/-) mice have delayed wound closure rates compared with Nrf2(+/+) mice, which is, at least partially, due to greater oxidative DNA damage, low transforming growth factor- β 1 (TGF- β 1) and high matrix metalloproteinase 9 (MMP9) expression, and increased apoptosis. More importantly, pharmacological activation of the NRF2 pathway significantly improves diabetic wound healing. In vitro experiments in human immortalized keratinocyte cells confirm that NRF2 contributes to wound healing by alleviating oxidative stress, increasing proliferation and migration, decreasing apoptosis, and increasing the expression of TGF- β 1 and lowering MMP9 under high-glucose conditions. This study indicates an essential role for NRF2 in diabetic wound healing and the therapeutic benefits of activating NRF2 in this disease, laying the foundation for future clinical trials using NRF2 activators in treating diabetic skin ulcers.] Long M, Rojo de la vega M, et al. *Diabetes*. 2016 Mar;65(3):780-93. <https://www.ncbi.nlm.nih.gov/pubmed/26718502>

Regulation of Wound Healing by the NRF2 Transcription Factor-More Than Cytoprotection. [The nuclear factor-erythroid 2-related factor 2 (NRF2) transcription factor plays a central role in mediating the cellular stress response. Due to their antioxidant properties, compounds activating NRF2 have received much attention as potential medications for disease prevention, or even for therapy. Accumulating evidence suggests that activation of the NRF2 pathway also has a major impact on wound healing and may be beneficial in the treatment of chronic wounds, which remain a considerable health and economic burden. While NRF2 activation indeed shows promise, important considerations need to be made in light of corresponding evidence that also points towards pro-tumorigenic effects of NRF2. In this review, we discuss the evidence to date, highlighting recent advances using gain- and loss-of-function animal models and how these data fit with observations in humans.] Hiebert P, Werner S. *Int J Mol Sci*. 2019 Aug 8;20(16). <https://www.ncbi.nlm.nih.gov/pubmed/31398789>