



THE JANUS FACE OF OXIDATIVE STRESS IN HEALTH AND DISEASE: THE CAUSE OR THE CURE?

Silvia Francati¹, Marco Fiore², and Giampiero Ferraguti^{1*}

¹ *Department of Experimental Medicine, Sapienza University of Rome, Rome, Italy*

² *Institute of Biochemistry and Cell Biology (IBBC-CNR), c/o Department of Sensory Organs, Sapienza University of Rome, Rome, Italy*

*Reactive oxygen species (ROS), reactive nitrogen species (RNS) and diffusible reactive species (DRS), under certain concentrations, play an important role at the physiological level. The role of antioxidant molecules, endogenous and exogenous, is to maintain adequate amounts of ROS and RNS. The increase in ROS and RNS due to an overproduction of these species or a decrease in antioxidant molecules leads to the phenomenon called oxidative stress. Oxidative stress is involved in the physiological process of aging but is implicated in pathologies such as some types of tumors, neurodegenerative and autoimmune disorders, male infertility, cardiovascular disorders such as atrial fibrillation, and lung disorders such as chronic obstructive pulmonary disorders and pulmonary hypertension. This involvement has aroused widespread interest, especially because many studies try to exploit it at a therapeutic level. The purpose of this review is to discuss pathologies in which oxidative stress has an important role and for this reason, it can be targeted for a therapeutic intervention to improve or cure the pathology; sometimes modulation of oxidative stress can be used to improve the effect of the therapy. **Biomed Rev 2023; 34: 13-26***

Keywords: Antioxidant compounds, reactive oxygen species (ROS), reactive nitrogen species (RNS), diffusible reactive species (DRS)

ABBREVIATIONS USED

ROS: reactive oxygen species
 RNS: reactive nitrogen species
 DRS: diffusible reactive species
 8-OHdG: 8-oxo-2'-deoxyguanosine
 AD: Alzheimer's disease
 PD: Parkinson's disease
 SOD: superoxide dismutase
 NOX: NADPH oxidase
 Nrf2: NF-E2-related factor 2
 Keap1: Kelch-like ECH-associated protein 1
 HIF-1a: hypoxia-inducible transcription factor
 AF: atrial fibrillation

OXIDATIVE STRESS AND NITROSATIVE STRESS

The terms oxidative stress and nitrosative stress are used to describe the condition of imbalance between the production and accumulation of reactive oxygen species (ROS), reactive nitrogen species (RNS) and diffusible reactive species (DRS) and their detoxification in cells and tissues (1,2). The cause of this imbalance in favor of oxidants is due to an increased production of ROS or a decrease in their antioxidant-mediated detoxification (Fig. 1) (3). ROS and RNS are free radicals or molecules capable of generating free radicals. Free radicals are unstable and reactive molecules due to the presence of an unpaired electron in their outer shell (4).

A book entitled "Oxidative Stress" was published in 1985 (revision 1997), in which this imbalance phenomenon was described for the first time (5). Since then this topic has aroused the interest of many scientists from biochemists to biologists and doctors for the role it played both in physiological and pathological conditions of the cell and because of its involvement in various pathologies (6–9).

Thanks to many studies focused on this topic, it was soon discovered that ROS and RNS are essential at the physiological level in the cell for redox signals involved in several biological processes including proliferation, gene expression, migration, and differentiation (10). This phenomenon was called "eustress" (11). For example, H_2O_2 is a low molecular weight molecule important in signaling for the regulation of protein functions (12).

When levels of free radicals, ROS, and RNS exceed the ability of antioxidants to regulate them, lipids, proteins, and nucleic acids are subjected to significant damage (13,14). The oxidation of proteins can affect amino acid residues, methionine, arginine, cysteine, and histidine are the most

involved (15). Free radicals can then modify proteins, making them unstable and susceptible to enzymatic proteolysis. Oxidized protein products can contribute to cell damage, and affect signal transduction machinery, enzyme activity, and membrane transport (13). The effect that oxidation has on proteins depends on the oxidizing agent and its concentration and the structural and biochemical characteristics of the proteins involved. Lipid peroxidation is the process of adding a hydroperoxyl group to a lipid (16). The most affected lipids in case of oxidative stress are the polyunsaturated fatty acids (PUFA) present in glycerolipids, phospholipids, and cholesterol (17). Numerous compounds (alkanes, malondialdehyde, and isoprostanes) are generated with lipid peroxidation and act as markers in lipid peroxidation tests. Their presence has been noted in many diseases such as neurodegenerative diseases, ischemic reperfusion injury, and diabetes (13).

More than 100 oxidative modifications of DNA have been identified. The most frequent is the formation of 8-oxo-2'-deoxyguanosine (8-OHdG), an important DNA lesion that can cause mutagenesis and epigenetic changes (1,14,18–20). In the case of oxidative DNA damage, the role played by base excision repair (BER) is important. It has been seen that the alteration of this mechanism leads to the development of Alzheimer's (AD) and Parkinson's (PD) disease (21). More recently, nucleotide excision repair (NER) proteins have also been shown to be involved (22).

The effects that ROS and RNS have on cellular components cause tissue degeneration and premature aging, but also cell death by apoptosis, cell transformation, and cancer (23).

OXIDATIVE AGENTS

ROS are highly reactive chemicals, a variety of molecules and free radicals (chemical species with one unpaired electron), formed from diatomic oxygen (O_2) (24). Reduction of molecular oxygen produces superoxide anion ($\bullet O_2^-$), a precursor to many other reactive oxygen species (24). Hydrogen peroxide (H_2O_2) is formed from the dismutation, spontaneous or catalyzed by superoxide dismutase (SOD), of superoxide (24,25). Hydrogen peroxide can be partially reduced forming hydroxide ions and hydroxyl radicals ($\bullet OH$) (Table 1).

H_2O_2 has a low overall reactivity, it can cross the cell membrane by passive diffusion and shows the greatest stability compared to other ROS (26). Direct generation of H_2O_2 can form from enzymes such as NADPH oxidase (NOX) 4, monoamine oxidase, and xanthine oxidase (26).

Xanthine oxidase, NOX, the mitochondrial electron

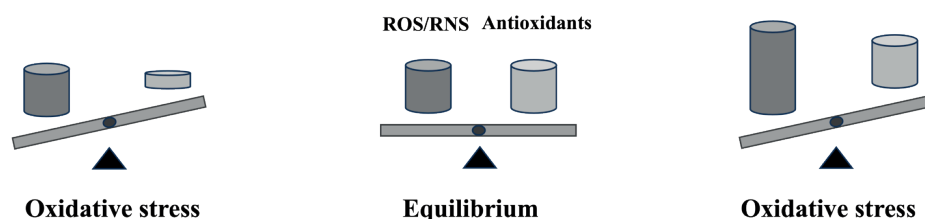


Figure 1. Graphical representation of the maintenance of homeostasis through the balance between ROS and antioxidants. The balance between ROS and RNS is lost in case of an increase in the species themselves or a decrease in antioxidant compounds. In both cases, the result is the occurrence of oxidative stress.

Table 1. Classification of ROS

ROS			
Radicals		Non-radicals	
Superoxide	$O_2^{\cdot -}$	Ozone	O_3
Hydroxyl	$OH^{\cdot}$	Singlet oxygen	O_2
Peroxyl	$RO_2^{\cdot}$	Hydrogen peroxide	H_2O_2
Alkoxy	$RO^{\cdot}$	Hypochlorous acid	$HOCl$
Hydroperoxyl	$HO_2^{\cdot}$		

transport chain (ETC), uncoupled endothelial nitric oxide synthase, xanthine oxidase, cytochrome P-450 oxygenase, and cyclooxygenase are the major sources of ROS (11,27). In fact, under physiological conditions, the major production of ROS occurs during oxidative respiration in the mitochondria, from complexes I, III, and IV of the respiratory chain. In addition to the cytochrome chain, ROS are also produced by mitochondrial outer membrane enzymes, including monoamine oxidase (MAO) and cytochrome b5 reductase (Cb5R), and by other inner membrane enzymes, such as glycerol-3-phosphate dehydrogenase (GPDH) and various cytochrome P450 enzymes. In the mitochondrial matrix the complexes of aconitase, pyruvate dehydrogenase (PDH) and -ketoglutarate dehydrogenase (αKGDH) produce superoxide (28). In addition to the mitochondrion, ROS production can occur in other organelles such as peroxisomes and endoplasmic reticulum. Furthermore, the production of ROS varies according to the cell type (29). The hormonal environment also influences the production of ROS, as they control the metabolic activities that deplete oxygen (30).

The production of free radicals and other reactive oxygen species also occurs in inflammation, strenuous exercise, ischemia, and reperfusion injury (13). There are also sources of ROS external to the body such as cigarette smoke, environmental pollution, radiation, ozone, chemical and industrial solvents, drugs, and pesticides (Fig. 2) (13).

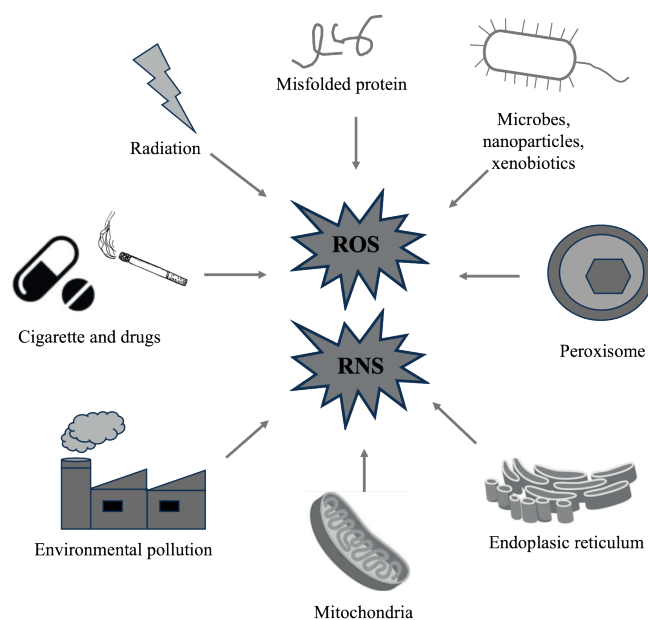


Figure 2. Source of ROS and RNS. Reactive oxygen and nitrogen species can be generated by both exogenous and endogenous agents.

NITROSATIVE AGENTS

RNS are formed from the oxidation of nitric oxide (NO) and their presence is due to its overproduction by inducible nitric oxide synthase or by uncoupled endothelial nitric oxide synthase (31). The NO radical ($NO^{\cdot}$) is produced by the oxidation of one of the terminal guanidino nitrogen atoms of L-arginine. RNS include nitroxide anion (NO^-), nitrosonium cation (NO^+), peroxynitrite ($ONOO^-$), peroxynitrous acid (HNO_3), nitrogen dioxide ($\cdot NO_2$), nitric oxide ($NO^{\cdot}$), nitrite (NO_2^-), nitrate (NO_3^-), nitroxyl (HNO), dinitrogen trioxide (N_2O_3), peroxynitric acid (O_2NOOH) and peroxynitrate (O_2NOO^-) (Table 2) (32,33).

Table 2. Classification of RNS

RNS			
Radicals		Non-radicals	
Nitric oxide	NO [•]	Peroxynitrite	ONOO ⁻
Nitrogen dioxide	NO ₂ [•]	Dinitrogen trioxide	N ₂ O ₃
Nitrate radical	NO ₃ [•]	Dinitrogen tetroxide	N ₂ O ₄
		Peroxynitric acid	HNO ₄
		Peroxynitrous acid	HNO ₃
		Nitrous acid	HNO ₂
		Nitroxyl	HNO
		Nitrosonium cation	NO ⁺
		Nitroxide anion	NO ⁻
		Nitrite	NO ₂ ⁻
		Nitrate	NO ₃ ⁻
		Peroxynitrate	NO ₄ ⁻
		Alkyl peroxynitrite	ROONO
		Nitronium anion	NO ₂ ⁺
		Nitryl chloride	NO ₂ Cl

ANTIOXIDANT AGENTS

Halliwell and Gutteridge define antioxidants as substances capable, at relatively low concentrations, of competing with other oxidizable substrates, delaying or inhibiting their oxidation (34). These molecules can neutralize free radicals by donating them an electron (13). Antioxidant molecules can be produced by the body following certain metabolic pathways (for example glutathione, ubiquinol, and uric acid) and are defined as endogenous. Among the endogenous antioxidants, the enzymatic ones, such as SOD, catalase (CAT), and glutathione peroxidase, are distinguished from the non-enzymatic ones: lipoic acid, glutathione, L-arginine, and coenzyme Q10. Normally •O₂⁻ levels are kept low by their spontaneous and/or enzyme-mediated dismutation catalyzed by SOD1-3 into H₂O₂. Mn-dependent SOD2 acts at the mitochondrial matrix level while CuZn-dependent SOD1 and SOD3 act in the cytosol and extracellularly (26).

Exogenous antioxidants, on the other hand, are of animal or vegetable origin and are introduced into the body through the diet: flavonoids, vitamins A, E, and C, curcumin, tempol, date palm pollen extract, N-acetyl cysteine, resveratrol, and α-lipoic acid (1,13,30). Antioxidants perform their function through different mechanisms: by donating an electron, by chelating metal ions, or by regulating gene expression (35). The action of antioxidants occurs at different levels (13).

Preventive antioxidants, work by blocking the formation of free radicals, for example by reducing hydroperoxides and hydrogen peroxide to alcohols and water. These glutathione-s-transferase, phospholipid hydroperoxide, glutathione peroxidase and peroxidase (13). Others work by scavenging active radicals. These include vitamin E, vitamin C, uric acid, bilirubin, albumin, and ubiquinol (13). A group of antioxidant molecules instead acts to repair damages: the enzymes that have the task of recognizing and eliminating the proteins that have undergone oxidation mutations or the DNA repair systems (13). Another action of antioxidants is referred to as adaptive where the antioxidants are directed to the right site when free radicals are produced (13).

To maintain redox homeostasis within the cell, NF-E2-related factor 2 (Nrf2) plays a key role. It is considered the master regulator for the activation of the expression of genes involved in anti-oxidation and the synthesis of antioxidants (36). NRF2 is a transcription factor that controls the expression of over 250 genes through the binding to the enhancer antioxidant response elements(37). In fact under normal conditions NRF2 is linked to Kelch-like ECH- associated protein 1 (Keap1) and this complex is degraded once it is bound to the Cullin-3 E3 ligase by the proteasome (36,38). In case of an increase of oxidizing agents, cysteine residues of Keap1 act as sensors undergoing oxidation; through this complex mechanism, Nrf2 accumulates within the cell is not degraded, and translocated to the nucleus where is complexed with transcription factor avian musculoaponeurotic fibrosarcoma (Maf), activating transcription factor 4 and Fos-related antigen proteins forming a heterodimer. This complex induces transcription of all genes that have antioxidant response elements in the regulatory region, thus activating the antioxidant response (36,39).

ROS AND RNS IN PHYSIOLOGICAL CONDITIONS

For many decades it was thought that ROS only had a toxic role in the cell by damaging cellular components. However, now it is known that controlled levels of oxidizing agents play fundamental roles in many cellular processes (12,26). Some specific ROS have been shown to modify the function, localization, and activity of proteins through oxidative post-translational modifications (PTMs) (26). ROS and RNS can modify proteins reversibly at cysteine and methionine residues and at those residues that contain sulfur. Proteins containing oxidized reactive cysteines are subject to changes in localization, and physical interactions, and undergo further post-translational modifications (40).

The role played by oxidation on STAT1 and STAT3 proteins has been studied over the years (41–44). These are redox-sensitive proteins, but the effect of oxidation depends on the biological system examined and on the type of oxidative insult (45). H_2O_2 and electrophiles derived from lipid peroxidation or metabolism play important roles in major signaling pathways and the regulation of transcription factors (46). In some studies, it has been shown that H_2O_2 activates NF- κ B by activating protein kinase C (PKC) (47). Among the pathways with redox regulation, those for cell adhesion have been identified. ROS levels can signal changes in oxygen concentration, in fact, in a 2022 study, it was seen that under hypoxic conditions the release of mitochondrial ROS increased, and this phenomenon caused the stabilization of the hypoxia-inducible transcription factor (HIF-1 α) (48). Some studies have highlighted the role of NO radicals and superoxide in the regulation of vascular function (49,50). In immunological defense, ROS play an important role (51). Lymphocytes, granulocytes, and phagocytes produce, for defense against microbes, high levels of ROS (52). The increase of inflammatory cytokine TNF expression that occurs during systemic infection causes an increase in the production of ROS and RNS (51,53). This crosstalk leads to increased macrophage-killing activity and necroptosis by activating the ERK1/2 and NF- κ B pathways (51). It has been shown that ROS also induce dendritic cells to differentiate and to perform their function of presenting antigen to T cells (54,55).

Further evidence of the importance of ROS in human homeostasis is the fact that hydrogen peroxide acts as a cofactor for thyroid peroxidase, the enzyme involved in the synthesis of thyroid hormones (56).

Neutrophils, once activated by inflammatory mediators, are capable of engulfing microorganisms and inactivating them through the phagosome, a cellular compartment in which a complex of ROS and enzymes are enclosed (29). These cells are large producers of ROS which they exploit as a response to infections. The NOX enzyme was first described in neutrophils (57).

Furthermore, ROS and RNS are considered “alarm molecules” because in case of poor availability of extracellular nutrients they send intracellular signals to the autophagic mechanism which, when activated, restore homeostasis (40).

ROS AND RNS ROLE IN AGING

The term “hormesis” describes those substances whose effect depends on their dose (58). The Swiss physician Paracelsus and

father of toxicology said, “Only the dose makes the poison” (59). In 2006 Tapia described the concept of mitohormesis as the theory in which sub-lethal mitochondrial stress induces a hormetic response that promotes health and vitality, where low levels of ROS and RNS are required for a range of events leading to benefits ranging from the transient cytoprotective longevity to that of organismal level (60).

Aging is a natural and physiological process characterized however by the shortening of telomeres and more frequent DNA damage. These damages cause the activation of the DNA damage response (DDR) which generates an increase in ROS levels. This condition causes cell cycle arrest or senescence. Senescence results in mitochondrial dysfunction due to a decrease in the GRSF1 factor and a further increase in ROS levels (61).

Among the techniques proposed to delay aging and related diseases is that of targeting ROS-producing senescent cells (52). According to Denham Harman’s 1956 free radical theory of aging (FRTA), the loss of function that occurs in old age results from the cellular accumulation of free radicals (62). In addition to having confirmed this theory, more recent studies have highlighted that oxidative stress can also play a role in pathologies whose frequency increases with age, such as cancer, neurodegeneration, arthritis, atherosclerosis, and metabolic pathologies such as diabetes and obesity (63–65).

ROS AND RNS IN PATHOLOGICAL CONDITIONS: CAUSE AND CURE

The uncontrolled increase in ROS causes oxidative stress which harms all cellular components its involvement in many pathologies is known (Table 3) (50,66–68). Such pathologies include neoplastic transformations in the lung, liver, kidney, breast, and colon, but also neurodegenerative diseases, atherosclerosis, male infertility, vitiligo, obstructive sleep apnea syndrome, and neuronal disorders such as depression (69,70). The role they play in disease may vary from onset to progression, but they can also influence the prognosis and the effect of therapy (56).

Currently, the involvement of ROS and RNS in the development and progression of tumors has been widely demonstrated and has also been shown to influence their heterogeneity (71). For example, it has been shown that the increase in ROS causes a rapid expression of activating protein-1 (AP-1), important in cell transformation and tumor progression, and a stabilization of HIF-1 α (72). In renal cell carcinoma, for example, stabilization of HIF-1 α by ROS promotes tumor growth, invasion,

and angiogenesis (56). In patients with chronic hepatitis C, oxidative stress has been shown to alter the state of chromatin leading to abnormal methylation of tumor suppressor genes, thus promoting hepatocellular carcinoma (20). Colorectal cancer is promoted by the formation of ROS and electrophilic species, such as acetaldehyde and lipid peroxidation products, which are formed following the processing of some substances, including ethanol (73–78). The increase of ROS acts by increasing the inflammatory state which favors the process of tumorigenesis, inducing the expression of CYP2E1 involved in the metabolic activation of some gastric carcinogens and inhibiting the expression of antioxidant molecules (79). In some tumors, increased ROS production has been demonstrated even in those non-tumor cells that play a crucial role in the tumor such as cancer-associated fibroblasts (CAF), cells of the immune system, pericytes, adipocytes, and endothelial (71). It has also been shown that the progression and suppression of cancer depend on the levels of intracellular ROS (69). In their breast cancer study, Wang et al. demonstrated that the curcumin analog molecule WZ35 had a greater effect than curcumin in increasing ROS production. The increase of ROS activates the YAP and JNK pathways which leads tumor cells to apoptosis with beneficial effects on tumor progression (80). However, tumor cells often develop resistance to apoptosis; this causes an increase in chemoresistance. Nrf2 is implicated in this process and is therefore a possible target to overcome chemoresistance due to ROS (81). Genetic mutations in the Keap1/NRF2 pathway are involved in many tumors and sometimes in chemotherapy resistance (82–86). Despite the implication of ROS in different tumor aspects, the majority of studies using ROS-based therapies are mostly in phase I or II (81).

Oxidative and nitrosative stress can lead to heart failure. Furthermore, ROS at high levels in cardiomyocytes can lead to damage at the level of proteins and channels involved in contraction-decontraction (87). Moreover, ROS have a profibrotic action which leads to remodeling of the extracellular matrix (87).

Oxidative stress plays an important role in cardiac arrhythmias, including atrial fibrillation (AF) (88,89). AF is the most common arrhythmia and the one most correlated with a high mortality rate (90). Studies conducted, mainly on animal models, demonstrate that ROS influence miRNAs which, in turn, are directly or indirectly responsible for arrhythmogenesis (90). Since oxidative stress is implicated in AF and its risk factors, it is increasingly being evaluated as a possible upstream target of anti-AF therapies (89,91).

ROS also play a role in the pathogenesis and worsening of sickle cell anemia. Indeed an increase in oxidative stress is correlated to greater severity of the pathology (92).

A 2022 study conducted on patients with chronic obstructive pulmonary disease showed that serum 8-OHdG levels are related to the severity and adverse prognosis of the disease because they increase with the loss of lung function. Despite this evidence, how 8-OHdG is involved in the pathology is still unknown (93).

Many studies confirm that oxidative imbalances contribute to the pathogenesis of pulmonary hypertension. For this reason, therapeutic strategies that aim to directly or indirectly reduce ROS and RNS in PH have been studied with promising results (94).

The central nervous system is among the compartments most at risk for the effects of oxidative stress. The brain requires a large amount of oxygen and is also a compartment very rich in lipids (95). It is known that oxidative stress has a key role in the development of neurodegenerative diseases such as AD, PD, Huntington's disease, and amyotrophic lateral sclerosis (95).

AD is a severe incurable neurodegenerative disease. In most cases it is sporadic and the risk increases with age (96). It is characterized by increasingly marked memory loss, a progressive loss of cognitive abilities, and an inability to do the simplest things (97). Accumulation of amyloid beta has been observed to be the initiating cause of the development of AD (98). Oxidative stress and mitochondrial dysfunction contribute to the development of the disease and it is known that ROS increase the levels of beta amyloids (99,100). To date, antioxidants are used to limit the damage of oxidative stress at the mitochondrial level, among these are MitoTEMPO, MitoQ, MitoVitE, 4,5-dihydroxy-benzene-1,3-disulfonate (Tiron), astaxanthin. Furthermore, to inhibit the effect of inflammatory processes, N-acetyl-5-methoxytryptamine, or melatonin, is used to limit the release of NO (101).

PD is the second most common cause of neurodegeneration. It is characterized by the degeneration of dopaminergic neurons located within the substantia nigra (98). Many therapeutic approaches targeting mitochondrial abnormalities and oxidative stress are still being studied to counteract AD and PD (98,102). Antioxidants are a very powerful tool in combating oxidative stress and improving the effects of age on the brain (103). Although oxidative stress is an early molecular process involved in the development of these diseases, the Food and Drug Administration (FDA) has not yet approved

any antioxidant therapy (96,104,105).

Vitiligo is an autoimmune disease characterized by the loss of skin pigmentation due to the attack of melanocytes by immune cells (106). It has been shown that oxidative stress and free radicals are important factors in the development of the pathology. Oxidative stress induced by hydrogen peroxide leads to melanocyte stress resulting in the production of damage-associated molecular patterns and the release of melanosomal antigens which increase the immune response (107). In addition to phototherapy and phenol treatment, many alternative treatments that target oxidative stress are emerging. There are many studies using antioxidant substances that target the Nrf2 pathway and the Hsp70 chaperone (108–111). Despite promising results, their therapeutic use has not been approved (108).

Physiological levels of ROS are essential to guarantee the processes that the spermatozoon must carry out, such as capacitation, hyperactivation, and the acrosome reaction. Excessive levels of ROS are the main cause of most male infertility (112). The lack of essential cytoplasmic enzymes makes spermatozoa very susceptible to damage caused by oxidative stress. The cytoplasmic membrane of the spermatozoon is strongly subject to lipid peroxidation as it is rich in unsaturated fatty acids. High levels of ROS are related to an increase in immature sperm, because of the effect they have on the production of male hormones (113). In the case of oxidative stress, cortisol is released by the adrenal gland. This hormone negatively regulates the hypothalamic-pituitary-gonadal axis, decreasing testosterone levels and consequently sperm maturation (112). In unfavorable oxidative conditions, sperm count, motility, and morphology are compromised. The condition of oligospermia is due to the damage induced by ROS on sperm cells causing their apoptosis. Furthermore, oxidative stress also causes a reduction in sperm motility, thus decreasing the chances of reaching the oocyte (114,115). Correct sperm morphology is important for successful fertilization and a healthy pregnancy (116). Furthermore, the sperm genome is very sensitive to damage caused by stress due to its limited levels of repair enzymes. DNA fragmentation caused by ROS is related to a greater risk of embryonic anomalies such as genetic mutations or chromosomal rearrangements (114). There are no clinically stable treatments for idiopathic (without an identified cause) male infertility. First of all, a healthy lifestyle, without smoking or alcohol, and with a healthy diet, is recommended to have a greater chance of conceiving (117–122). Empirical medical treatment for idiopathic infertility may be hormonal

treatment or antioxidant supplementation (123). Many studies with antioxidant therapy have demonstrated positive results on the viability, morphology, motility, and integrity of the sperm genome, and on fertility in general (124–127). Among the molecules tested for supplemental antioxidant treatment, are vitamins C and E, folic acid, zinc, L-carnitine, and coenzyme Q10 (122,128).

THE MURBURN CONCEPT AND THE DIFFUSIBLE REACTIVE SPECIES (DRS)

The term Murburn first used by Kelath Murali Manoj describes the mechanism of some enzymes with redox activity. Literally this term derives from “closed burning”, an oxidative process. DRS are involved in this process. The enzymes that carry out the Murburn system are defined as Murzymes while the term Murzone indicates the region where DRS interact with the terminal substrate. The reaction can occur under both aerobic and anaerobic conditions (129). Among the murzymes, there are free ions, radical species, or molecules with cofactors that have an extended pi- electronic system or metal centers with d- electron or proteins that have many charged residues. These include chloroperoxidase, hemoglobin, plastocyanin, cytochrome c, and lactate dehydrogenase (130). The reaction that occurs, according to the Murburn scheme, does not follow standard models of kinetics; in fact, it is subject to multiple equilibria, and the product is formed if the reactants are present at discrete concentrations. DRS are important for the performance of routine metabolic and physiological functions including cellular respiration, homeostasis, thermogenesis, and drug metabolism (131,132).

CONCLUSION

The balance between oxidizing and antioxidant substances is essential for maintaining the body's homeostasis. The increase in levels of oxidizing substances to the detriment of antioxidants causes oxidative stress. This latter is implicated in the development and progression of many pathologies. Over the years, this phenomenon has aroused much interest in scientific research for the development of therapies based on those pathways implicated in maintaining ROS and RNS adequate levels. Various therapeutic approaches targeting different molecules are currently being studied, but despite the promising results none have yet been officially approved. However, antioxidant molecules are often used as adjuvants in therapy for various pathologies.

Table 3. Diagram of the role of ROS and RNS in pathologies and possible therapeutic approaches that target this involvement (80,100,108).

Pathology	Role of ROS	ROS as a therapeutic approach
Renal cell carcinoma	ROS increase the stability of the HIF-1 α factor.	N.E.
Hepatocellular carcinoma	ROS cause chromatin instability in chronic hepatitis C.	N.E.
Colorectal cancer	ROS increase inflammation and CYP2E1 expression and inhibit the expression of antioxidant molecules.	N.E.
Breast cancer	ROS activate YAP and JNK pathways to induce apoptosis.	WZ35 induces ROS generation in tumoral cells
Heart failure	ROS damage channel proteins involved in cardiomyocyte contraction and have a profibrotic action towards the extracellular matrix.	N.E.
Atrial fibrillation	ROS regulate FA-related miRNAs.	ROS as targets of anti-FA therapies under study.
Sickle cell anemia	ROS implicated in pathogenesis.	N.E.
Chronic Obstructive Pulmonary Disease	Increased serum 8-OHdG levels correlate with poor prognosis.	N.E.
Pulmonary hypertension	ROS implicated in pathogenesis.	Therapeutic approaches to target ROS with promising results.
Alzheimer's Disease	ROS increase the levels of beta-amyloids	Melatonin and other antioxidants are used to limit the effects of ROS and RNS.
Parkinson's Disease	Oxidative stress affects nigral neurons which are highly susceptible to mitochondrial dysfunction.	N.E.
Vitiligo	Stressed melanocytes undergo functional impairment and death.	Therapeutic approaches targeting the Nrf2 pathway under study.
Male infertility	Oxidative stress causes damage to the sperm membrane, increases cellular immaturity, decreases sperm motility, number, and morphology, and fragments their DNA.	Antioxidant supplementation therapy in empirical medical treatment.

N.E.= no evidence

CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

ACKNOWLEDGMENTS

The authors thank Sapienza Università di Roma, Italy, and IBBC-CNR, Rome, Italy.

REFERENCES

- Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, *et al.* Oxidative Stress: Harms and Benefits for Human Health. *Oxid Med Cell Longev* 2017;2017. doi:10.1155/2017/8416763.
- Uys JD, Xiong Y, Townsend DM. Nitrosative stress-induced S-glutathionylation of protein disulfide isomerase. *Methods Enzymol* 2011;490:321–32. doi:10.1016/B978-0-12-385114-7.00018-0.
- Schafer FQ, Buettner GR. Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. *Free Radic Biol Med* 2001;30:1191–212. doi:10.1016/S0891-5849(01)00480-4.
- Pham-Huy LA, He H, Pham-Huy C. Free radicals, antioxidants in disease and health. *Int J Biomed Sci* 2008;4:89–96.
- Sies H. Oxidative stress: oxidants and antioxidants. *Exp Physiol* 1997;82:291–5.
- Herrmann JM, Dick TP. Redox Biology on the rise. *Biol Chem* 2012;393:999–1004. doi:10.1515/hsz-2012-0111.
- Sies H. Biochemistry of Oxidative Stress. *Angew Chemie Int Ed English* 1986;25:1058–71. doi:10.1002/anie.198610581.

- 8 Sies H, Jones D. Oxidative stress. 2nd 2007.
- 9 Go YM, Jones DP. The redox proteome. *J Biol Chem* 2013;288:26512–20. doi:10.1074/jbc.R113.464131.
- 10 Alp NJ, Channon KM. Regulation of Endothelial Nitric Oxide Synthase by Tetrahydrobiopterin in Vascular Disease. *Arterioscler Thromb Vasc Biol* 2004;24:413–20. doi:10.1161/01.ATV.0000110785.96039.f6.
- 11 Fukai T, Ushio-Fukai M. Cross-Talk between NADPH Oxidase and Mitochondria: Role in ROS Signaling and Angiogenesis. *Cells* 2020;9. doi:10.3390/cells9081849.
- 12 Sies H. Oxidativer Stress, Eustress und Distress: H₂O₂ als Signalmolekül. *BioSpektrum* 2022;28:685–90. doi:10.1007/s12268-022-1862-y.
- 13 Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacogn Rev* 2010;4:118–26. doi:10.4103/0973-7847.70902.
- 14 Slimen IB, Najar T, Ghram A, Dabbebi H, Ben Mrad M, Abdrabbah M. Reactive oxygen species, heat stress and oxidative-induced mitochondrial damage. A review. *Int J Hyperth* 2014;30:513–23. doi:10.3109/02656736.2014.971446.
- 15 Freeman BA, Crapo JD. Biology of disease. Free radicals and tissue injury. *Lab Investig* 1982;47:412–26.
- 16 Lermant A, Murdoch CE. Cysteine glutathionylation acts as a redox switch in endothelial cells. *Antioxidants* 2019;8. doi:10.3390/antiox8080315.
- 17 Anthonymuthu TS, Kenny EM, Bayir H. Therapies targeting lipid peroxidation in traumatic brain injury. *Brain Res* 2016;1640:57–76. doi:10.1016/j.brainres.2016.02.006.
- 18 Gunes AE, Yilmaz O, Erbas C, Dagli SN, Celik H. High serum 8-hydroxy-2'-deoxyguanosine levels predict DNA damage and aging in professional divers. *Rev Assoc Med Bras* 2021;67:1701–5. doi:10.1590/1806-9282.20210748.
- 19 Watanabe S, Li YS, Kawasaki Y, Ootsuyama Y, Kawai K. Health examination results and work environment factors affecting urinary 8-hydroxy-2'-deoxyguanosine levels. *J Occup Health* 2021;63:e12210. doi:10.1002/1348-9585.12210.
- 20 Nishida N, Arizumi T, Takita M, Kitai S, Yada N, Hagiwara S, et al. Reactive oxygen species induce epigenetic instability through the formation of 8-hydroxydeoxyguanosine in human hepatocarcinogenesis. *Dig Dis* 2013;31:459–66. doi:10.1159/000355245.
- 21 Rao KS. Free radical induced oxidative damage to DNA: Relation to brain aging and neurological disorders. *Indian J Biochem Biophys*. 2007;46:9–15.
- 22 D'Errico M, Parlanti E, Dogliotti E. Mechanism of oxidative DNA damage repair and relevance to human pathology. *Mutat Res - Rev Mutat Res* 2008;659:4–14. doi:10.1016/j.mrrev.2007.10.003.
- 23 Kaspar JW, Niture SK, Jaiswal AK. Nrf2:INrf2 (Keap1) signaling in oxidative stress. *Free Radic Biol Med* 2009;47:1304–9. doi:10.1016/j.freeradbiomed.2009.07.035.
- 24 Turrens JF. Mitochondrial formation of reactive oxygen species. *J Physiol* 2003;552:335–44. doi:10.1113/jphysiol.2003.049478.
- 25 Zorov DB, Juhaszova M, Sollott SJ. Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. *Physiol Rev* 2014;94:909–50. doi:10.1152/physrev.00026.2013.
- 26 Lennicke C, Cochemé HM. Redox metabolism: ROS as specific molecular regulators of cell signaling and function. *Mol Cell* 2021;81:3691–707. doi:10.1016/j.molcel.2021.08.018.
- 27 Chattopadhyay M, Khemka VK, Chatterjee G, Ganguly A, Mukhopadhyay S, Chakrabarti S. Enhanced ROS production and oxidative damage in subcutaneous white adipose tissue mitochondria in obese and type 2 diabetes subjects. *Mol Cell Biochem* 2015;399:95–103. doi:10.1007/s11010-014-2236-7.
- 28 Finkel T. Signal transduction by reactive oxygen species. *J Cell Biol* 2011;194:7–15. doi:10.1083/jcb.201102095.
- 29 Holmström KM, Finkel T. Cellular mechanisms and physiological consequences of redox-dependent signaling. *Nat Rev Mol Cell Biol* 2014;15:411–21. doi:10.1038/nrm3801.
- 30 Sahoo DK, Chainy GBN. Hormone-linked redox status and its modulation by antioxidants. In: Litwack GBT-V and H, editor. *Vitam. Horm.*, vol. 121, Academic Press; 2023, p. 197–246. doi:10.1016/bs.vh.2022.10.007.
- 31 Pérez-Torres I, Guarner-Lans V, Rubio-Ruiz ME. Reductive stress in inflammation-associated diseases and the pro-oxidant effect of antioxidant agents. *Int J Mol Sci* 2017;18. doi:10.3390/ijms18102098.
- 32 Dröge W. Free radicals in the physiological control of cell function. *Physiol Rev* 2002;82:47–95. doi:10.1152/physrev.00018.2001.
- 33 Pérez-Torres I, Manzano-Pech L, Rubio-Ruiz ME, Soto ME, Guarner-Lans V. Nitrosative stress and its association with cardiometabolic disorders. *Molecules* 2020;25. doi:10.3390/molecules25112555.

- 34 Land ET. Free radicals in biology and medicine. *Int J Radiat Biol* 1990;58:725–725. doi:10.1080/09553009014552071.
- 35 Krinsky NI. Mechanism of Action of Biological Antioxidants (43429). *Proc Soc Exp Biol Med* 1992;200:248–54. doi:10.3181/00379727-200-43429.
- 36 Kasai S, Shimizu S, Tatara Y, Mimura J, Itoh K. Regulation of Nrf2 by mitochondrial reactive oxygen species in physiology and pathology. *Biomolecules* 2020;10. doi:10.3390/biom10020320.
- 37 Casas AI, Nogales C, Mucke HAM, Petrain A, Cuadrado A, Rojo AI, *et al.* On the clinical pharmacology of reactive oxygen species. *Pharmacol Rev* 2020;72:801–28. doi:10.1124/pr.120.019422.
- 38 Bhakkiyalakshmi E, Sireesh D, Ramkumar KM. Redox sensitive transcription via Nrf2-keap1 in suppression of inflammation. In: Chatterjee S, Jungraithmayr W, Bagchi D, editors. *Immun. Inflamm. Heal. Dis. Emerg. Roles Nutraceuticals Funct. Foods Immune Support*, Academic Press; 2017, p. 149–61. doi:10.1016/B978-0-12-805417-8.00012-3.
- 39 Chen F, Liu Y, Wong NK, Xiao J, So KF. Oxidative Stress in Stem Cell Aging. *Cell Transplant* 2017;26:1483–95. doi:10.1177/0963689717735407.
- 40 Filomeni G, De Zio D, Cecconi F. Oxidative stress and autophagy: The clash between damage and metabolic needs. *Cell Death Differ* 2015;22:377–88. doi:10.1038/cdd.2014.150.
- 41 Gorina R, Sanfeliu C, Galitó A, Messeguer À, Planas AM. Exposure of glia to pro-oxidant agents revealed selective Stat1 activation by H₂O₂ and Jak2-independent antioxidant features of the Jak2 inhibitor AG490. *Glia* 2007;55:1313–24. doi:10.1002/glia.20542.
- 42 Gorina R, Petegnief V, Chamorro Á, Planas AM. AG490 prevents cell death after exposure of rat astrocytes to hydrogen peroxide or proinflammatory cytokines: Involvement of the Jak2/STAT pathway. *J Neurochem* 2005;92:505–18. doi:10.1111/j.1471-4159.2004.02878.x.
- 43 Madamanchi NR, Li S, Patterson C, Runge MS. Reactive oxygen species regulate heat-shock protein 70 via the JAK/STAT pathway. *Arterioscler Thromb Vasc Biol* 2001;21:321–6. doi:10.1161/01.ATV.21.3.321.
- 44 Carballo M, Conde M, El Bekay R, Martín-Nieto J, Camacho MJ, Monteseirín J, *et al.* Oxidative stress triggers STAT3 tyrosine phosphorylation and nuclear translocation in human lymphocytes. *J Biol Chem* 1999;274:17580–6. doi:10.1074/jbc.274.25.17580.
- 45 Butturini E, de Prati AC, Mariotto S. Redox regulation of STAT1 and STAT3 signaling. *Int J Mol Sci* 2020;21:1–18. doi:10.3390/ijms21197034.
- 46 Forman HJ. Redox signaling: An evolution from free radicals to aging. *Free Radic Biol Med* 2016;97:398–407. doi:10.1016/j.freeradbiomed.2016.07.003.
- 47 Kaul N, Gopalakrishna R, Gundimeda U, Choi J, Forman HJ. Role of protein kinase C in basal and hydrogen peroxide-stimulated NF- κ B activation in the murine macrophage J774A.1 cell line. *Arch Biochem Biophys* 1998;350:79–86. doi:10.1006/abbi.1997.0487.
- 48 Willson JA, Arienti S, Sadiku P, Reyes L, Coelho P, Morrison T, *et al.* Neutrophil HIF-1 α stabilization is augmented by mitochondrial ROS produced via the glycerol 3-phosphate shuttle. *Blood* 2022;139:281–6. doi:10.1182/blood.2021011010.
- 49 Faraci FM. Reactive oxygen species: Influence on cerebral vascular tone. *J Appl Physiol* 2006;100:739–43. doi:10.1152/jappphysiol.01044.2005.
- 50 Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol* 2007;39:44–84. doi:10.1016/j.biocel.2006.07.001.
- 51 Yang S, Lian G. ROS and diseases: role in metabolism and energy supply. *Mol Cell Biochem* 2020;467:1–12. doi:10.1007/s11010-019-03667-9.
- 52 Davalli P, Mitic T, Caporali A, Lauriola A, D’Arca D. ROS, Cell Senescence, and Novel Molecular Mechanisms in Aging and Age-Related Diseases. *Oxid Med Cell Longev* 2016;2016:3565127. doi:10.1155/2016/3565127.
- 53 Blaser H, Dostert C, Mak TW, Brenner D. TNF and ROS Crosstalk in Inflammation. *Trends Cell Biol* 2016;26:249–61. doi:10.1016/j.tcb.2015.12.002.
- 54 Sun L, Wang X, Saredy J, Yuan Z, Yang X, Wang H. Innate-adaptive immunity interplay and redox regulation in immune response. *Redox Biol* 2020;37:101759. doi:10.1016/j.redox.2020.101759.
- 55 Del Prete A, Zaccagnino P, Di Paola M, Saltarella M, Oliveros Celis C, Nico B, *et al.* Role of mitochondria and reactive oxygen species in dendritic cell differentiation and functions. *Free Radic Biol Med* 2008;44:1443–51. doi:10.1016/j.freeradbiomed.2007.12.037.
- 56 Brieger K, Schiavone S, Miller FJ, Krause KH. Reactive oxygen species: From health to disease. *Swiss Med Wkly* 2012;142:w13659. doi:10.4414/smw.2012.13659.
- 57 Hampton MB, Kettle AJ, Winterbourn CC. Inside the neutrophil phagosome: Oxidants, myeloperoxidase, and bacterial killing. *Blood* 1998;92:3007–17. doi:10.1182/blood.v92.9.3007.421k47_3007_3017.

- 58 Bárcena C, Mayoral P, Quirós PM. Mitohormesis, an Antiaging Paradigm. *Int Rev Cell Mol Biol* 2018;340:35–77. doi:10.1016/bs.ircmb.2018.05.002.
- 59 Deichmann WB, Henschler D, Holmstedt B, Keil G. What is there that is not poison? A study of the Third Defense by Paracelsus. *Arch Toxicol* 1986;58:207–13. doi:10.1007/BF00297107.
- 60 Tapia PC. Sublethal mitochondrial stress with an attendant stoichiometric augmentation of reactive oxygen species may precipitate many of the beneficial alterations in cellular physiology produced by caloric restriction, intermittent fasting, exercise and dietary phytonutrients: “Mitohormesis” for health and vitality. *Med Hypotheses* 2006;66:832–43. doi:10.1016/j.mehy.2005.09.009.
- 61 Lesmana R, Parameswari C, Mandagi GF, Wahyudi JF, Permana NJ, Radhiyanti PT, *et al.* The Role of Exercise-Induced Reactive Oxygen Species (ROS) Hormesis in Aging: Friend or Foe. *Cell Physiol Biochem* 2022;56:692–706. doi:10.33594/000000594.
- 62 Hemagirri M, Sasidharan S. Biology of aging: Oxidative stress and RNA oxidation. *Mol Biol Rep* 2022;49:5089–105. doi:10.1007/s11033-022-07219-1.
- 63 Ferrucci L, Fabbri E. Inflammageing: chronic inflammation in ageing, cardiovascular disease, and frailty. *Nat Rev Cardiol* 2018;15:505–22. doi:10.1038/s41569-018-0064-2.
- 64 Liu Z, Zhou T, Ziegler AC, Dimitrion P, Zuo L. Oxidative Stress in Neurodegenerative Diseases: From Molecular Mechanisms to Clinical Applications. *Oxid Med Cell Longev* 2017;2017. doi:10.1155/2017/2525967.
- 65 Terracina S, Ferraguti G, Petrella C, Bruno SM, Blaconà G, Di Certo MG, *et al.* Characteristic Hallmarks of Aging and the Impact on Carcinogenesis. *Curr Cancer Drug Targets* 2022;23:87–102. doi:10.2174/1568009622666220816120353.
- 66 Juránek I, Nikitovic D, Kouretas D, Hayes AW, Tsatsakis AM. Biological importance of reactive oxygen species in relation to difficulties of treating pathologies involving oxidative stress by exogenous antioxidants. *Food Chem Toxicol* 2013;61:240–7. doi:10.1016/j.fct.2013.08.074.
- 67 Meliante PG, Zoccali F, Cascone F, Di Stefano V, Greco A, de Vincentiis M, *et al.* Molecular Pathology, Oxidative Stress, and Biomarkers in Obstructive Sleep Apnea. *Int J Mol Sci* 2023;24:5478. doi:10.3390/ijms24065478.
- 68 Micangeli G, Menghi M, Profeta G, Tarani F, Mariani A, Petrella C, *et al.* The Impact of Oxidative Stress on Pediatrics Syndromes. *Antioxidants* 2022;11:1983. doi:10.3390/antiox11101983.
- 69 Ismail T, Kim Y, Lee H, Lee DS, Lee HS. Interplay between mitochondrial peroxiredoxins and ROS in cancer development and progression. *Int J Mol Sci* 2019;20. doi:10.3390/ijms20184407.
- 70 Mastino P, Rosati D, de Soccio G, Romeo M, Pentangelo D, Venarubea S, *et al.* Oxidative Stress in Obstructive Sleep Apnea Syndrome: Putative Pathways to Hearing System Impairment. *Antioxidants* 2023;12. doi:10.3390/antiox12071430.
- 71 De Sá Junior PL, Câmara DAD, Porcacchia AS, Fonseca PMM, Jorge SD, Araldi RP, *et al.* The Roles of ROS in Cancer Heterogeneity and Therapy. *Oxid Med Cell Longev* 2017;2017:2467940. doi:10.1155/2017/2467940.
- 72 Prasad S, Gupta SC, Tyagi AK. Reactive oxygen species (ROS) and cancer: Role of antioxidative nutraceuticals. *Cancer Lett* 2017;387:95–105. doi:10.1016/j.canlet.2016.03.042.
- 73 Carito V, Ceccanti M, Cestari V, Natella F, Bello C, Coccorello R, *et al.* Olive polyphenol effects in a mouse model of chronic ethanol addiction. *Nutrition* 2017;33:65–9. doi:10.1016/j.nut.2016.08.014.
- 74 Fiore M, Minni A, Cavalcanti L, Raponi G, Puggioni G, Mattia A, *et al.* The Impact of Alcohol Consumption and Oral Microbiota on Upper Aerodigestive Tract Carcinomas: A Pilot Study. *Antioxidants* 2023;12. doi:10.3390/antiox12061233.
- 75 Ceci FM, Francati S, Ferraguti G, Coriale G, Ciccarelli R, Minni A, *et al.* Behavioral dysregulations by chronic alcohol abuse. Motivational enhancement therapy and cognitive behavioral therapy outcomes. *Riv Psichiatr* 2022;57:1–9. doi:10.1708/3749.37321.
- 76 Ferraguti G, Terracina S, Petrella C, Greco A, Minni A, Lucarelli M, *et al.* Alcohol and Head and Neck Cancer: Updates on the Role of Oxidative Stress, Genetic, Epigenetics, Oral Microbiota, Antioxidants, and Alkylating Agents. *Antioxidants* 2022;11. doi:10.3390/antiox11010145.
- 77 Petrella C, Carito V, Carere C, Ferraguti G, Ciafrè S, Natella F, *et al.* Oxidative stress inhibition by resveratrol in alcohol-dependent mice. *Nutrition* 2020;79–80:110783. doi:10.1016/j.nut.2020.110783.
- 78 Derme M, Piccioni MG, Brunelli R, Crognale A, Denotti M, Ciolli P, *et al.* Oxidative Stress in a Mother Consuming Alcohol during Pregnancy and in Her Newborn: A Case Report. *Antioxidants* 2023;12:1216. doi:10.3390/antiox12061216.

- 79 Na HK, Lee JY. Molecular basis of alcohol-related gastric and colon cancer. *Int J Mol Sci* 2017;18. doi:10.3390/ijms18061116.
- 80 Wang L, Wang C, Tao Z, Zhao L, Zhu Z, Wu W, *et al.* Curcumin derivative WZ35 inhibits tumor cell growth via ROS-YAP-JNK signaling pathway in breast cancer. *J Exp Clin Cancer Res* 2019;38:460. doi:10.1186/s13046-019-1424-4.
- 81 Zhou X, An B, Lin Y, Ni Y, Zhao X, Liang X. Molecular mechanisms of ROS-modulated cancer chemoresistance and therapeutic strategies. *Biomed Pharmacother* 2023;165:115036. doi:10.1016/j.biopha.2023.115036.
- 82 Ma-on C, Sanpavat A, Whongsiri P, Suwannasin S, Hirankarn N, Tangkijvanich P, *et al.* Oxidative stress indicated by elevated expression of Nrf2 and 8-OHdG promotes hepatocellular carcinoma progression. *Med Oncol* 2017;34:1–12. doi:10.1007/s12032-017-0914-5.
- 83 Pirpour Tazehkand A, Akbarzadeh M, Velaie K, Sadeghi MR, Samadi N. The role of Her2-Nrf2 axis in induction of oxaliplatin resistance in colon cancer cells. *Biomed Pharmacother* 2018;103:755–66. doi:10.1016/j.biopha.2018.04.105.
- 84 Lister A, Nedjadi T, Kitteringham NR, Campbell F, Costello E, Lloyd B, *et al.* Nrf2 is overexpressed in pancreatic cancer: Implications for cell proliferation and therapy. *Mol Cancer* 2011;10:1–13. doi:10.1186/1476-4598-10-37.
- 85 Hayden A, Douglas J, Sommerlad M, Andrews L, Gould K, Hussain S, *et al.* The Nrf2 transcription factor contributes to resistance to cisplatin in bladder cancer. *Urol Oncol Semin Orig Investig* vol. 32, Elsevier; 2014, p. 806–14. doi:10.1016/j.urolonc.2014.02.006.
- 86 Djavaheri-Mergny M, Javelaud D, Wietzerbin J, Besançon F. NF- κ B activation prevents apoptotic oxidative stress via an increase of both thioredoxin and MnSOD levels in TNF α -treated Ewing sarcoma cells. *FEBS Lett* 2004;578:111–5. doi:10.1016/j.febslet.2004.10.082.
- 87 van der Pol A, van Gilst WH, Voors AA, van der Meer P. Treating oxidative stress in heart failure: past, present and future. *Eur J Heart Fail* 2019;21:425–35. doi:10.1002/ejhf.1320.
- 88 Xie W, Santulli G, Reiken SR, Yuan Q, Osborne BW, Chen BX, *et al.* Mitochondrial oxidative stress promotes atrial fibrillation. *Sci Rep* 2015;5:11427. doi:10.1038/srep11427.
- 89 Mason FE, Pronto JRD, Alhussini K, Maack C, Voigt N. Cellular and mitochondrial mechanisms of atrial fibrillation. *Basic Res Cardiol* 2020;115:72. doi:10.1007/s00395-020-00827-7.
- 90 Lee S, Choi E, Cha MJ, Hwang KC. Looking into a conceptual framework of ROS–miRNA–Atrial fibrillation. *Int J Mol Sci* 2014;15:21754–76. doi:10.3390/ijms151221754.
- 91 Yang KC, Dudley SC. Oxidative stress and atrial fibrillation: Finding a missing piece to the puzzle. *Circulation* 2013;128:1724–6. doi:10.1161/CIRCULATIONAHA.113.005837.
- 92 Antwi-Boasiako C, Dankwah GB, Aryee R, Hayfron-Benjamin C, Donkor ES, Campbell AD. Oxidative Profile of Patients with Sickle Cell Disease. *Med Sci (Basel, Switzerland)* 2019;7. doi:10.3390/medsci7020017.
- 93 Cao P, Zhang C, Hua DX, Li MD, Lv BB, Fu L, *et al.* Serum 8-Hydroxy-2'-deoxyguanosine Predicts Severity and Prognosis of Patients with Acute Exacerbation of Chronic Obstructive Pulmonary Disease. *Lung* 2022;200:31–9. doi:10.1007/s00408-021-00507-w.
- 94 Tabima DM, Frizzell S, Gladwin MT. Reactive oxygen and nitrogen species in pulmonary hypertension. *Free Radic Biol Med* 2012;52:1970–86. doi:10.1016/j.freeradbiomed.2012.02.041.
- 95 Singh A, Kukreti R, Saso L, Kukreti S. Oxidative stress: A key modulator in neurodegenerative diseases. *Molecules* 2019;24. doi:10.3390/molecules24081583.
- 96 Tönnies E, Trushina E. Oxidative Stress, Synaptic Dysfunction, and Alzheimer's Disease. *J Alzheimer's Dis* 2017;57:1105–21. doi:10.3233/JAD-161088.
- 97 Gaugler J, James B, Johnson T, Scholz K, Weuve J. 2016 Alzheimer's disease facts and figures. *Alzheimer's Dement* 2016;12:459–509. doi:10.1016/j.jalz.2016.03.001.
- 98 Macdonald R, Barnes K, Hastings C, Mortiboys H. Mitochondrial abnormalities in Parkinson's disease and Alzheimer's disease: Can mitochondria be targeted therapeutically? *Biochem Soc Trans* 2018;46:891–909. doi:10.1042/BST20170501.
- 99 Leuner K, Schütt T, Kurz C, Eckert SH, Schiller C, Ochipinti A, *et al.* Mitochondrion-derived reactive oxygen species lead to enhanced amyloid beta formation. *Antioxidants Redox Signal* 2012;16:1421–33. doi:10.1089/ars.2011.4173.
- 100 Beata BK, Wojciech J, Johannes K, Piotr L, Barbara M. Alzheimer's Disease—Biochemical and Psychological Background for Diagnosis and Treatment. *Int J Mol Sci* 2023;24. doi:10.3390/ijms24021059.

- 101 Twarowski B, Herbet M. Inflammatory Processes in Alzheimer's Disease—Pathomechanism, Diagnosis and Treatment: A Review. *Int J Mol Sci* 2023;24. doi:10.3390/ijms24076518.
- 102 Degirmenci Y, Angelopoulou E, Georgakopoulou VE, Bougea A. Cognitive Impairment in Parkinson's Disease: An Updated Overview Focusing on Emerging Pharmaceutical Treatment Approaches. *Medicina (Kaunas)* 2023;59. doi:10.3390/medicina59101756.
- 103 Ionescu-Tucker A, Cotman CW. Emerging roles of oxidative stress in brain aging and Alzheimer's disease. *Neurobiol Aging* 2021;107:86–95. doi:10.1016/j.neurobiolaging.2021.07.014.
- 104 Padurariu M, Ciobica A, Lefter R, Serban IL, Stefanescu C, Chirita R. The oxidative stress hypothesis in Alzheimer's disease. *Psychiatr Danub* 2013;25:401–9.
- 105 Collins AE, Saleh TM, Kalisch BE. Naturally Occurring Antioxidant Therapy in Alzheimer's Disease. *Antioxidants (Basel)* 2022;11. doi:10.3390/antiox11020213.
- 106 Frisoli ML, Essien K, Harris JE. Vitiligo: Mechanisms of Pathogenesis and Treatment. *Annu Rev Immunol* 2020;38:621–48. doi:10.1146/annurev-immunol-100919-023531.
- 107 Wang Y, Li S, Li C. Perspectives of new advances in the pathogenesis of vitiligo: From oxidative stress to autoimmunity. *Med Sci Monit* 2019;25:1017–23. doi:10.12659/MSM.914898.
- 108 Chang WL, Ko CH. The Role of Oxidative Stress in Vitiligo: An Update on Its Pathogenesis and Therapeutic Implications. *Cells* 2023;12. doi:10.3390/cells12060936.
- 109 Ma J, Li S, Zhu L, Guo S, Yi X, Cui T, *et al.* Baicalein protects human vitiligo melanocytes from oxidative stress through activation of NF-E2-related factor2 (Nrf2) signaling pathway. *Free Radic Biol Med* 2018;129:492–503. doi:10.1016/j.freeradbiomed.2018.10.421.
- 110 Mou K, Pan W, Han D, Wen X, Cao F, Miao Y, *et al.* Glycyrrhizin protects human melanocytes from H₂O₂-induced oxidative damage via the Nrf2-dependent induction of HO-1. *Int J Mol Med* 2019;44:253–61. doi:10.3892/ijmm.2019.4200.
- 111 Yang L, Yang F, Teng L, Katayama I. 6-Shogaol Protects Human Melanocytes Against Oxidative Stress Through Activation of the Nrf2-Antioxidant Response Element Signaling Pathway. *Int J Mol Sci* 2020;21:3537. doi:10.3390/ijms21103537.
- 112 Barati E, Nikzad H, Karimian M. Oxidative stress and male infertility: current knowledge of pathophysiology and role of antioxidant therapy in disease management. *Cell Mol Life Sci* 2020;77:93–113. doi:10.1007/s00018-019-03253-8.
- 113 Aitken RJ, Baker MA, Sawyer D. Oxidative stress in the male germ line and its role in the aetiology of male infertility and genetic disease. *Reprod Biomed Online* 2003;7:65–70. doi:10.1016/S1472-6483(10)61730-0.
- 114 Walke G, Gaurkar SS, Prasad R, Lohakare T, Wanjari M. The Impact of Oxidative Stress on Male Reproductive Function: Exploring the Role of Antioxidant Supplementation. *Cureus* 2023;15:e42583. doi:10.7759/cureus.42583.
- 115 Aitken RJ, Drevet JR, Moazamian A, Gharagozloo P. Male Infertility and Oxidative Stress: A Focus on the Underlying Mechanisms. *Antioxidants* 2022;11. doi:10.3390/antiox11020306.
- 116 Jakubik-Uljasz J, Gill K, Rosiak-Gill A, Piasecka M. Relationship between sperm morphology and sperm DNA dispersion. *Transl Androl Urol* 2020;9:405–15. doi:10.21037/tau.2020.01.31.
- 117 Fiore M, Messina MP, Petrella C, D'Angelo A, Greco A, Ralli M, *et al.* Antioxidant properties of plant polyphenols in the counteraction of alcohol-abuse induced damage: Impact on the Mediterranean diet. *J Funct Foods* 2020;71:104012. doi:10.1016/j.jff.2020.104012.
- 118 Ciafrè S, Carito V, Ferraguti G, Greco A, Chaldakov GN, Fiore M, *et al.* How alcohol drinking affects our genes: An epigenetic point of view. *Biochem Cell Biol* 2019;97:345–56. doi:10.1139/bcb-2018-0248.
- 119 Ciafrè S, Ferraguti G, Greco A, Polimeni A, Ralli M, Ceci FM, *et al.* Alcohol as an early life stressor: epigenetics, metabolic, neuroendocrine and neurobehavioral implications. *Neurosci Biobehav Rev* 2020;118:654–68. doi:10.1016/j.neubiorev.2020.08.018.
- 120 Terracina S, Ferraguti G, Tarani L, Messina MP, Lucarelli M, Vitali M, *et al.* Transgenerational Abnormalities Induced by Paternal Preconceptual Alcohol Drinking. Findings from Humans and Animal Models. *Curr Neuroparmacol* 2021;19:1158–1173. doi:10.2174/1570159x1966621101111430.
- 121 Carito V, Ceccanti M, Ferraguti G, Coccurello R, Ciafrè S, Tirassa P, *et al.* NGF and BDNF Alterations by Prenatal Alcohol Exposure. *Curr Neuroparmacol* 2019;17:308–17. doi:10.2174/1570159x15666170825101308.

- 122 Khaw SC, Wong ZZ, Anderson RA, da Silva SM. L-Carnitine and L-Acetylcarnitine Supplementation for Idiopathic Male Infertility. *Reprod Fertil* 2020;1:67–81. doi:10.1530/RAF-20-0037.
- 123 Jung JH, Seo JT. Empirical medical therapy in idiopathic male infertility: Promise or panacea? *Clin Exp Reprod Med* 2014;41:108–14. doi:10.5653/cerm.2014.41.3.108.
- 124 Busetto GM, Agarwal A, Virmani A, Antonini G, Ragonesi G, Del Giudice F, *et al.* Effect of metabolic and antioxidant supplementation on sperm parameters in oligo-astheno-teratozoospermia, with and without varicocele: A double-blind placebo-controlled study. *Andrologia* 2018;50. doi:10.1111/and.12927.
- 125 Martin-Hidalgo D, Bragado MJ, Batista AR, Oliveira PF, Alves MG. Antioxidants and male fertility: From molecular studies to clinical evidence. *Antioxidants* 2019;8. doi:10.3390/antiox8040089.
- 126 Agarwal A, Leisegang K, Majzoub A, Henkel R, Finelli R, Selvam MKP, *et al.* Utility of antioxidants in the treatment of male infertility: Clinical guidelines based on a systematic review and analysis of evidence. *World J Mens Health* 2021;39:1–58. doi:10.5534/WJMH.200196.
- 127 Menghi M, Micangeli G, Tarani F, Putotto C, Pirro F, Mariani A, *et al.* Neuroinflammation and Oxidative Stress in Individuals Affected by DiGeorge Syndrome. *Int J Mol Sci* 2023;24(4):4242. doi: 10.3390/ijms24044242.
- 128 Alahmar AT, Sengupta P. Impact of Coenzyme Q10 and Selenium on Seminal Fluid Parameters and Antioxidant Status in Men with Idiopathic Infertility. *Biol Trace Elem Res* 2021;199:1246–52. doi:10.1007/s12011-020-02251-3.
- 129 Manoj KM. Debunking chemiosmosis and proposing murburn concept as the operative principle for cellular respiration. *Biomed Rev* 2017;28:31–48. doi:10.14748/bmr.v28.4450.
- 130 Manoj KM. Murburn concept and murzymes in 2023: Celebrating 25th year of pursuit. *Biomed Rev* 2022;33:1–15.
- 131 Manoj KM, Gideon DA, Jacob VD. Murburn scheme for mitochondrial thermogenesis. *Biomed Rev* 2018;29:73. doi:10.14748/bmr.v29.5852.
- 132 Venkatachalam A, Parashar A, Manoj KM. Functioning of drug-metabolizing microsomal cytochrome P450s: In silico probing of proteins suggests that the distal heme ‘active site’ pocket plays a relatively ‘passive role’ in some enzyme-substrate interactions. *Silico Pharmacol* 2016;4:1–38. doi:10.1186/s40203-016-0016-7.