Review Article

CMJ

www.cmj.ac.kr

Reactive Oxygen Species: Role in Pathophysiology, and Mechanism of Endogenous and Dietary Antioxidants during Oxidative Stress

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Redox imbalances, which result from excessive production of reactive oxygen species (ROS) or malfunctioning of the antioxidant system, are the source of oxidative stress. ROS affects all structural and functional components of cells, either directly or indirectly. In addition to causing genetic abnormalities, excessive ROS also oxidatively modifies proteins by protein oxidation and peroxidation and alters lipid structure via advanced lipoxidation, decreasing function and promoting damage or cell death. On the other hand, low levels of ROS constitute important redox-signaling molecules in various pathways that maintain cellular homeostasis and regulate key transcription factors. As a result, ROS can affect various cellular processes, such as apoptosis, migration, differentiation, and proliferation. ROS can act as signaling molecules, controlling various normal physiological activities at the cellular level. Furthermore, there is an increasing body of evidence indicating the role of ROS in various clinical conditions. In this review, we will summarize the role of ROS in physiological and pathological processes and antioxidant action during oxidative stress.

Key Words: *Oxidative Stress; Reactive Oxygen Species; Antioxidant; Homeostasis*

Article History:
Received September 11, 2024
Revised November 17, 2024
Accepted December 4, 2024

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INTRODUCTION

Reactive oxygen species (ROS) consist of radical and non-radical highly reactive oxygen species formed by the partial reduction of oxygen. Cellular ROS can be produced endogenously, or exogenously. Overproduction of ROS induces oxidative stress which is caused by imbalance in a biological system between oxidants and antioxidants.[1,2] While oxygen is vital for life, playing a crucial role in signal transmission, gene transcription, and various cellular functions, it also harms biomolecules in the form of free radicals or ROS. ROS-generated free radicals cause lipid peroxidation, DNA fragmentation, cell death, DNA damage, protein structure alteration, and membrane degradation.[3] This oxidative stress is involved not only in the toxicity of xenobiotics, but also in the pathophysiology of a variety of diseases such as cancer, ischemia-reperfusion injury, vascular endothelium, cardiovascular diseases, deep injuries, inflammation, sepsis, diabetic retinopathy, and organ dysfunction.[3]

ROS is produced during physiological cell functions such as aerobic respiration or inflammatory responses. They are primarily signaling molecules that promote gene expression, cell differentiation, anprocess process contributing to natural aging. They additionally play an important role in muscular contraction and vascular tone modulation and determine bactericidal and bacteriostatic activities.[1]

CATALOG OF ROS AND THEIR MECHANISMS OF ACTION

1. Superoxide anion ($O_2^{\bullet -}$)

The superoxide anion is a free radical, which means it possesses an unpaired electron making it highly reactive. Superoxide is classified as a radical as well as a one-charged anion.[4] It is a rapidly unstable molecule with a half-life of milliseconds, a moderately strong oxidant that is reduced

https://doi.org/10.4068/cmj.2025.61.1.32

to hydrogen peroxide and can also operate as a reductant and convert to oxygen (O_2). $O_2^{\bullet -}$ is a tiny anion that is extremely soluble in water, where it is solvated by four hydrogen-bonded water molecules and interacts with a proton or proton donor to generate hydroperoxyl radical.[5]

Superoxide is predominantly produced in cells by the addition of one electron to molecular oxygen (O_2) (Fig. 1). Many enzymes, including NADPH oxidase (NOX), xanthine oxidase (XO), lipoxygenase, cyclooxygenase, and cytochrome P450. CYP/cytochrome P450 reductase (POR), and electron transport chains found in the endoplasmic reticulum, peroxisomes, nuclear membrane, and cytoplasmic membrane, convert O_2 to superoxide. This can also be produced by a variety of non-enzymatic processes (glycation) or non-biochemical sources, including photolysis, chemical, and electrochemical pathways.[4]

Although superoxide is commonly perceived as a potentially harmful molecule due to its reactivity, it does play a functional role in some biological processes. For example, it is involved in signaling pathways and immunological responses. In some situations, Superoxide is purposely produced by cells as part of their defense systems against infections.[6] Excessive superoxide in conjunction with other ROS can result in oxidative stress, which has been linked to a variety of diseases, including neurological disorders, cardiovascular diseases, and cancer.[4] The damage inflicted by superoxide on cellular components, including DNA, proteins, and lipids has been associated with the etiology of various disorders. It causes damage to proteins with Fe-S centers, such as aconitase, succinate dehydrogenase, and NADH-ubiquinone oxidoreductase.

2. Radical hydroxyl ($^{\bullet}OH$)

The hydroxyl radical is a highly reactive and unstable molecule that is a member of the ROS family. Because of its high reactivity, the hydroxyl radical is especially strong and capable of causing harm to biological molecules within the cellular components.

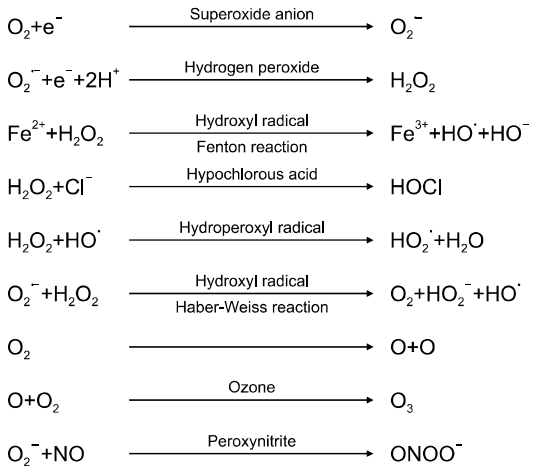

FIG. 1. Chemical reactions involved in forming Reactive Oxygen Species (ROS).

The hydroxyl radical is frequently produced in cells via the Fenton reaction (Fig. 1).[7] The interaction of hydrogen peroxide (H_2O_2) with transition metal ions, commonly iron (Fe^{2+}) or copper (Cu^{2+}), occurs in this process. The half-life of the hydroxyl radical is on a scale of microseconds. Because of its short lifespan, it has a limited diffusion range in biological systems and frequently reacts with molecules close to its creation site.

Due to its unpaired electron, the hydroxyl radical is very reactive. It can react with and demolish a variety of biomolecules, including DNA, proteins, and lipids.[8] The hydroxyl radical can harm cellular components by removing hydrogen atoms from DNA, resulting in DNA strand breakage and mutations. Cells have evolved numerous antioxidant defense systems, such as enzymes like catalase and peroxidases, which help break down hydrogen peroxide and prevent it from converting to the hydroxyl radical.

3. Hydroperoxyl radical ($HO_2^{\bullet}$)

The hydroperoxyl radical is highly reactive due to unpaired electrons and can participate in various chemical processes, contributing to oxidative stress. Hydroperoxyl radical can start chain reactions that lead to the oxidation of lipids, proteins, and DNA. The reactivity of hydroperoxyl radicals can potentially affect cellular signaling pathways. The hydroperoxyl radical can contribute to oxidative stress, which has been linked to a variety of diseases such as neurological disorders, cardiovascular diseases, and cancer.[9] Antioxidant defense systems exist in cells to counteract the damaging effects of ROS such as hydroperoxyl radicals. Enzymes such as superoxide dismutase (SOD) and catalase, as well as non-enzymatic antioxidants such as glutathione, play critical roles in maintaining cellular redox equilibrium.

4. Hydrogen peroxide (H_2O_2)

Hydrogen peroxide is an endogenous oxidant, the most stable ROS.[10] Because of cellular respiration, hydrogen peroxide is produced naturally in the body. H_2O_2 has also been shown to have antibacterial and antiviral action and has been used in clinical settings since the early twentieth century as a popular topical antiseptic for wound irrigation or disinfection of instruments/surfaces.[10] As part of their defense systems against invading pathogens, white blood cells produce hydrogen peroxide.[11] The oxidative environment formed by hydrogen peroxide aids in the destruction or neutralization of bacteria. H_2O_2 reduces phosphatase activity during insulin signaling to regulate tyrosine phosphorylation, activates mitogen-activated protein kinase (MAPK) in endothelial cells, and regulates ion channels and Ca^{2+} signaling in neurons.[12]

H_2O_2 can be produced in a variety of cellular compartments, including mitochondria, peroxisomes, and cytoplasm. Hydrogen peroxide is mostly produced via enzyme-catalyzed superoxide dismutation, though it can also be produced by the two-electron reduction of oxygen in reactions catalyzed by oxidases such as xanthine oxidase,

glucose oxidase, amino acid oxidase, urate oxidase, and other enzymatic processes.[13] H_2O_2 does not directly interact with pure biopolymers; the only exceptions may be thiol-based sensor proteins (like OxyR in bacteria), which react to the presence of micromolar levels of H_2O_2 by forming disulfide bonds that are used for signaling.[14] Hydrogen peroxide easily breaches biological membranes and enters neighboring blood vessels. H_2O_2 inhibits the activity of Krebs cycle enzymes such as aconitase, alpha-ketoglutarate dehydrogenase, and succinate dehydrogenase, which can collapse the mitochondrial proton gradient and affect the proton motive force required for pyruvate translocase to function in the inner mitochondrial membrane and transport pyruvate into the mitochondria. H_2O_2 is also implicated in β-cell dysfunction, which causes type 1 and type 2 diabetes Mellitus. Hydrogen peroxide is subsequently reduced enzymatically by glutathione peroxidases (GPxs), such as GPx-1, catalase, and peroxiredoxins (Prxs).[3] Peroxiredoxins are antioxidant enzymes that protect cells from oxidative stress by reducing hydrogen peroxide, lipid peroxide, and peroxynitrite.

5. Singlet oxygen ($^1O_2$)

ROS can be produced in two ways: electron transfer (type I) or energy transfer (type II).[15] Most oxygen radical anions, such as superoxide radical ($O_2^{\bullet -}$) and hydroxyl radical, are formed by electron transfer from an electron donor, whereas $^1O_2$ is formed by energy transfer from a photosensitizer's triplet state.[16] Peroxidase, peroxygenase, and catalase produce singlet oxygen by accepting H_2O_2 as a substrate.[16] Superoxide anions are produced by the membrane NADPH-oxidase and dismutase to hydrogen peroxide. H_2O_2 can generate nonradical $^1O_2$ by reacting with superoxide anions, HOCl, or chloramines. Since NADPH-oxidase is found in a wide range of cells, varied cells appear to produce the signal/messenger $^1O_2$ for inter or intracellular signaling.[17] NADPH-oxidase is present mostly in polymorphonuclear leukocytes and activated polymorphonuclear neutrophil granulocytes produce significant amounts of $^1O_2$. They produce hypochlorite (NaOCl) and chloramines (particularly N-chlorotaurine) by activating the respiratory burst (NADPH oxidase and myeloperoxidase). Chloramines are selective and stable producers of $^1O_2$. Singlet oxygen can also be formed through the dismutation of alkoxyl radicals. Singlet oxygen is produced by a variety of cell types, including neutrophils, and macrophages.[17] $^1O_2$ functions as both a signal and a therapeutic agent against a wide range of pathogens, including bacteria, viruses, cancer cells, and thrombi.[17] Being $^1O_2$ is a potent oxidant, it causes permanent photo-oxidative damage to surround biomolecules such as proteins, carotenoids, nucleic acids, and lipids, particularly polyunsaturated fatty acids (PUFA).[18]

6. Ozone (O_3)

Ozone is generated when oxygen molecules combine with ultraviolet (UV) radiation from the sun (Fig. 1). The breakdown of oxygen molecules into individual oxygen atoms can subsequently react with additional oxygen molecules to generate ozone. Antibodies and white blood cells produce ozone in the human body and employ it as a weapon against germs.[19]

Ozone treatment involves its infusion into the body for therapeutic objectives such as osteoarthrosis, wound healing, immune system stimulation, and decreased back pain. It is utilized in some medical procedures, although its use remains contentious, and its safety and efficacy are still being studied. Ozone therapy has been linked to several side effects, including cancer, infection, inflammation, and pain.[20] Ozone-induced oxidative stress induces respiratory tract cellular injury and altered cell signaling, as well as increased heart rate, diastolic pressure, vascular oxidative stress, inflammation, and decreased heart rate variability.[21]

7. Peroxynitrite ($ONOO^-$)

Nitric oxide and superoxide are both signaling chemicals produced by diverse cells, and their interaction makes peroxynitrite, a powerful oxidant involved in cellular signaling and oxidative stress. Peroxynitrite may directly react with electron-rich groups such as sulfhydryl, iron-sulfur centers, zinc-thiolates, and the active site sulfhydryl in tyrosine phosphatases.[22] Peroxynitrite can bind to proteins having heme prosthetic groups, such as hemoglobin, myoglobin, or cytochrome C. It reacts with lipids in membranes, liposomes, and lipoproteins by removing a hydrogen atom from polyunsaturated fatty acids (PUFA) and forming hydroperoxyl radicals.[22] Peroxynitrite can cause DNA damage and guanine is the most reactive with peroxynitrite of the four nucleobases due to its low reduction potential.

8. Alkoxyl radical ($RO^\bullet$)

An alkoxyl radical has the general form $RO^\bullet$, where R denotes an alkyl group.[23] Traditional sources of O-radicals include lead (IV) alkoxides, peroxides, sulfonates, hypohalites nitrite esters, *N*-alkoxylpyridine-2-thiones, and *N*-alkoxy phthalimides.[23] In biological systems, alkoxyl radicals are frequently generated by the interaction of organic hydroperoxides with transition metal ions such as iron or copper. They are extremely reactive and can cause oxidative stress and damage to biological systems. Alkoxyl radicals may trigger chain reactions by absorbing hydrogen atoms from neighboring molecules. This process yields more free radicals, which contributes to oxidative damage. Alkoxyl radicals, like other ROS, can cause damage to biological components such as proteins, lipids, and DNA.

9. Peroxyl radical ($ROO^\bullet$)

$ROO^\bullet$ are generated by the direct interaction of oxygen with alkyl radicals ($R^\bullet$).[24] Decomposition of alkyl peroxides (ROOH) produces $ROO^\bullet$ and $RO^\bullet$ radicals. When free PUFAs react with molecular oxygen, they form lipid hydroperoxide molecules (LOOHs). UV light or the presence of transition metal ions can trigger hemolysis of peroxides, re-

sulting in peroxyl and alkoxyl radicals. Peroxyl radicals can also be produced during the autoxidation of unsaturated lipids, which is a critical stage in lipid peroxidation. These radicals can damage almost all biomolecules, including lipids, carbohydrates, proteins, nucleic acids, and enzymes, affecting their biological processes.[25] Monounsaturated fatty acids and polyunsaturated fatty acids are the most vulnerable to peroxyl radical assault.

10. Hypochlorous acid (HOCl)

Hypochlorous acid is a strong oxidizing agent that is also an important component of the human immune system. Neutrophils, eosinophils, and monocytes release HOCl during reaction to injury.[26] Myeloperoxidase is the sole peroxidase that catalyzes the formation of HOCl from H_2O_2 and chloride (Fig. 1). Hypochlorous acid's antibacterial activity is pH-dependent. The stomach's acidic environment offers an extra line of defense against swallowed germs since it is most efficient at acidic pH values.[27] The United States Environmental Protection Association has authorized stabilized HOCl as a disinfectant against SARS-CoV-2 in liquid form on non-porous surfaces.[28]

HOCl is engaged in bacterial cell death, entering cell walls with water-like ease, owing to its low molecular weight and electroneutrality.[29] Once within the cell, it can oxidize a wide range of biological components, including DNA, RNA, thiols, heme-protein, amino groups, carbohydrates, and lipids. Hypochlorous acid reacts with unsaturated bonds in lipids but not with saturated bonds. HOCl can kill a wide variety of cells, including endothelial cells, epithelial cells, fibroblast cells, T-cells, and tumor cells.[30] Glutathione (GSH) is a classic example of a scavenging antioxidant. The chlorination of GSH by HOCl results in glutathione sulphonamide, a particular marker of HOCl oxidation in biological systems.

REACTIVE OXYGEN SPECIES PRODUCTION

ROS can be produced by the induction of various cell organelles such as mitochondria, microsomes, peroxisomes, endoplasmic reticulum, and other cytosolic enzymes due to the involvement of NADPH and NADH in the respiratory chain and cellular metabolisms.[31] Toll-like receptor 1 (TLR1), TLR2, and TLR4 can increase ROS production by recruiting mitochondria to macrophage phagosomes and translocating tumor necrosis factor receptor-associated factor 6 (TRAF6) to mitochondria to engage in an evolutionarily conserved signaling intermediate in Toll pathways (ECSIT) (Fig. 2).[32] However, respiratory chain complexes are the primary sources of ROS generation.

1. Mitochondrial electron transport chain

In mammalian cells, mitochondria are the major producers of $O_2^{\bullet-}$ and H_2O_2. The mitochondrial electron transport chain, which is essential for cellular respiration, is a primary generator of ROS. Electrons leaking from the respiratory chain can combine with molecular oxygen to generate superoxide anion, a major ROS. Complexes I and III are known superoxide-generating sites inside the mitochondria.[33] Reverse electron transfer (RET) causes complex I to produce a considerable amount of $O_2^{\bullet-}$. It was discovered that isolated mitochondria respiring on succinate produce substantial amounts of $O_2^{\bullet-}$ via RET.[33]

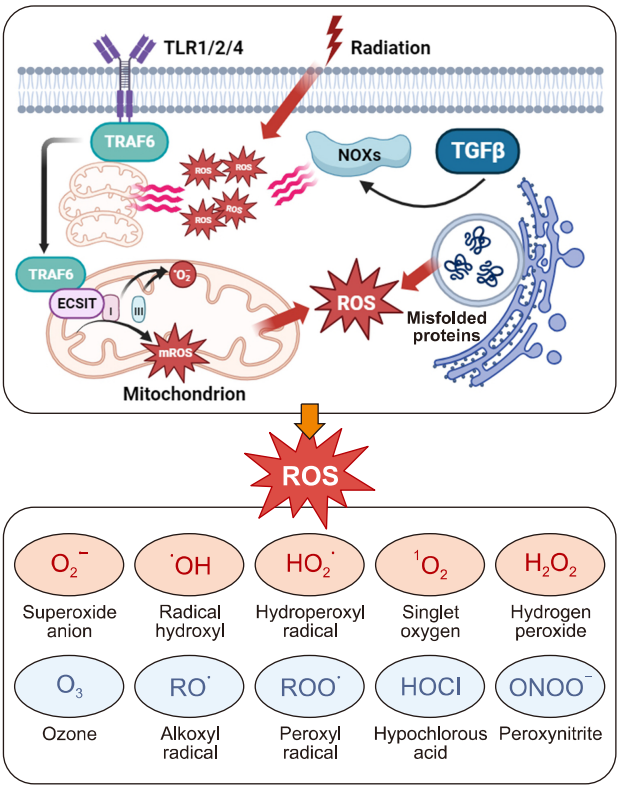

FIG. 2. Production of Reactive Oxygen Species (ROS). ROS can be generated by various cell organelles such as mitochondria, peroxisomes, endoplasmic reticulum, and other cytosolic enzymes. Toll-like receptor 1 (TLR1), TLR2, and TLR4 can increase ROS production by recruiting mitochondria and translocating tumor necrosis factor receptor-associated factor 6 (TRAF6) to mitochondria to engage in an evolutionarily conserved signaling intermediate in Toll pathways (ECSIT). During immune response, TGF-β secreted by Tregs activates the NOXs to produce ROS.

2. Phagocytosis

Activated phagocytes (neutrophils, eosinophils, monocytes, and lymphocytes) can generate high levels of ROS via NOXs during respiratory bursts.[34] Direct interaction with phagocytes or cytokines can also cause activated T-cells to produce respiratory bursts. During immune response, transforming growth factor-β (TGF-β) activates Treg NOXs, causing ROS generation. During phagocytosis, immune cells such as neutrophils and monocytes produce ROS as part of their antimicrobial activity to fight pathogens that are too big to be phagocytosed. This involves the activation of NOXs and the subsequent production of superoxide and other ROS. Neutrophils have a particular FcR signaling pathway that is involved in the production of ROS against malaria.[35]

3. Endoplasmic reticulum stress

The Endoplasmic Reticulum (ER) redox environment determines the fate of incoming proteins, and the quantity of redox signaling mediators affects the level of ROS. The interaction of ER stress and ROS with redox signaling mediators such as protein disulfide isomerase (PDI) endoplasmic reticulum oxidoreductin (ERO)-1, glutathione/glutathione disulfide (GSSG), NADPH oxidase 4 (Nox4), NADPH-P450 reductase (NPR), and calcium.[36] In the case of the ER, oxidative stress can cause a buildup of misfolded proteins, resulting in the development of ER stress and misfolded proteins also induce ROS production. To fight ER stress, cells activate a highly conserved stress response known as the unfolded protein response (UPR).

4. Peroxisome

Peroxisomes are highly dynamic and metabolically active organelles that are a major source of ROS and reactive nitrogen species (RNS) such as H_2O_2, $O_2^{\bullet -}$, $^{\bullet}OH$, nitric oxide ($NO^{\bullet}$), and $ONOO^-$, which are primarily produced in various metabolic pathways such as fatty acid oxidation, photorespiration, nucleic acid and polyamine catabolism, ureide metabolism, and others. Peroxisomes can rapidly produce and scavenge H_2O_2 and $O_2^{\bullet -}$, allowing them to control dynamic fluctuations in ROS levels. Peroxisomal ROS are involved in more sophisticated signaling networks in cells that involve calcium, hormones, and redox homeostasis.[37]

5. Enzymatic reactions

ROS are produced within cells by various enzymatic processes. NADPH Oxidases (NOXs), xanthine oxidase, lipoxygenases, cyclooxygenases, Cytochrome P450 Enzymes, Uncoupled Nitric Oxide Synthase, Monoamine Oxidases, and cyclo-oxygenase are some examples of reactions that can generate superoxide and other ROS. NOXs are a group of enzymes that produce reactive ROS, mainly superoxide, as part of the immunological response. These enzymes produce superoxide by transferring electrons from NADPH to molecular oxygen. NOXs can be present in intracellular organelle membranes such as those of mitochondria, endoplasmic reticulum, perinuclear regions, and even the nuclear membrane, where they can emit ROS.

6. Metal-catalyzed reactions and radiation

Transition metal ions, such as iron and copper, can participate in ROS-producing processes. For example, the Fenton reaction includes the reaction of hydrogen peroxide with iron ions to form hydroxyl radicals. UV and ionizing radiation exposure can cause the development of ROS.[38] These types of radiation can directly interact with biological components, resulting in the production of free radicals. UV-A and UV-B wavelengths penetrate the skin, interacting with molecules like melanin to produce ROS that damage DNA, proteins, and lipids, accelerating skin aging and increasing cancer risk. Pollutants like ozone, particulate matter, heavy metals, and polycyclic aromatic hydrocarbons (PAHs) also generate ROS through cellular interactions, weakening antioxidant defenses and causing inflammation, respiratory and cardiovascular diseases, and skin aging. Together, UV radiation and pollutants significantly increase ROS levels, stressing the importance of antioxidant protection to reduce aging and disease risks.

ROLE OF ROS IN PATHOPHYSIOLOGY

ROS act as signaling molecules in a variety of intracellular signaling pathways, triggering specific target proteins such as transcription factors, kinases, and phosphatases to further orchestrate cellular processes and thus regulate cell cycle progression (proliferation or arrest), differentiation, quiescence, senescence, or apoptosis (Figs. 3 and 4). ROS are significant signal modifiers and mediators in response to growth factors, cytokines, hypoxia, shear stress, and cyclic strain.[39] Many key pathways are activated in response, including G protein-coupled receptors (GPCRs), Notch, Wnt-catenin, mitogen-activated protein kinase (MAPK), JAK-STAT, Nuclear factor kappa B (NF-κB), and PI3K/AKT. Here is an overview of the role of ROS in different pathophysiologies.

1. Cancer

ROS are implicated in the initiation and progression of cancer. Elevated ROS levels can cause DNA damage, leading to mutations and genomic instability. ROS can directly induce oxidative DNA damage. Such damage consists of DNA double-stranded breaks and the producing mutagenic 8-oxo-7-hydro-2-deoxyguanosine (8-oxodG).[40,41] 8-oxodG is a major cause of spontaneous mutagenesis because it in-

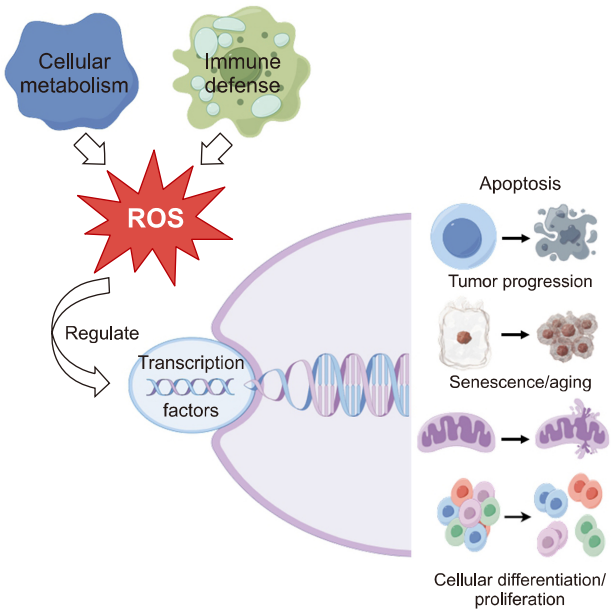

FIG. 3. Pathophysiology of Reactive Oxygen Species (ROS). ROS are produced by cellular metabolism and the immunological defense system. Excess ROS regulate numerous transcription factors inducing apoptosis, tumor growth, senescence, and aging-related diseases.

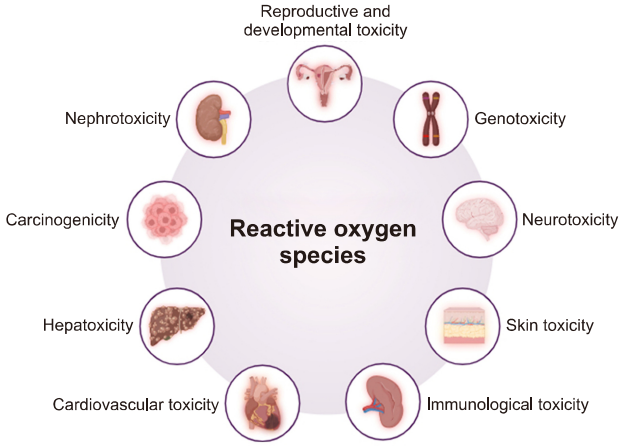

FIG. 4. Pathophysiological disorders due to excessive production of ROS.

duces guanine to thymine transversion through its capacity to pair with both cytosine and adenine. Therefore, the buildup of 8-oxodG in cellular genomes promotes carcinogenesis. At low to moderate levels, ROS operate as signal transducers, activating cell proliferation, migration, invasion, and angiogenesis. High quantities of ROS, on the other hand, cause damage to proteins, nucleic acids, lipids, membranes, and organelles, ultimately leading to cell death.

Nuclear factor-erythroid 2 related factor 2 (NRF2) is an important regulator of antioxidant responses in cancer cells. NRF2 is activated and overexpressed in cancer to enhance cancer cell survival. NRF2 and its related genes regulate the cellular antioxidant system. Under normal settings, kelch-like ECH-associated protein 1 (KEAP1) closely regulates NRF2 expression and function. Under oxidative conditions, NRF2 dissociates from KEAP1 and translocates to the nucleus, where it binds to and activates the antioxidant response element (ARE) in a variety of target genes. ARE regulates antioxidant enzymes such as NADPH quinone dehydrogenase 1, heme oxygenase 1, thioredoxin reductase 1, superoxide dismutase, glutathione peroxidase, and catalase. As a result, cancer cells protect themselves from excessive ROS.

2. Neurodegenerative disorders

Increased ROS levels, as well as reduced antioxidant defenses, are typical characteristics of neurodegenerative disorders. Oxidative stress is a common cause of neuronal cell loss in neurodegenerative disorders such as Alzheimer's disease (AD), Parkinson's disease (PD), Amyotrophic Lateral Sclerosis (ALS), and Huntington's disease (HD), as well as brain and spinal cord damage after stroke and traumatic brain injury.[42,43] ROS buildup has also been linked to mitochondrial malfunction, which results in decreased energy production, alterations in metal homeostasis, and the deposition of toxic protein aggregates inducing many neurodegenerative disorders. These cause cell death mechanisms such as apoptosis, necrosis, ferroptosis, or autophagy to be activated. Oxidative stress is involved in a variety of mechanisms, including proteins, nucleic acids, and lipid oxidation which induce the formation of more advanced glycation final products, mitochondrial dysfunction, glial cell stimulation, apoptosis, developing defects in the ubiquitin-proteasome system, oligomerization of proteins such as alpha-synuclein (α-Syn) or beta-amyloid (Aβ), cytokine production and inflammatory response, and proteasome dysfunction.[44] NOX activation in microglia or astrocytes is responsible for ROS production and ultimately neuronal death.[45] High levels of ROS are strongly associated to the manifestation of neuronal death in a variety of neurological diseases. These include chronic diseases (Parkinson's or Alzheimer's disease), acute brain injuries (brain trauma and cerebral ischemia), and psychiatric disorders (autism, attention deficit hyperactivity disorder, depression, and schizophrenia).

3. Cardiovascular diseases

Overproduction of oxidative stress-related factors such as ROS can lead to a heart attack, atherosclerosis, hypertension, hypercholesterolemia, and heart failure.[46] Cardiomyocytes and vascular layers including endothelium, smooth muscle, and adventitia produce ROS.[47] The endothelium regulates vascular tone, modulates inflammation, and promotes or inhibits vascular development, platelet aggregation, and coagulation.[48]

ROS are important in angiotensin II (Ang-II)-induced cardiac hypertrophy/remodeling.[49] Ang-II promotes the generation of ROS after binding to the receptor, which leads to the activation of different signaling kinases. The activation of the ERK kinase signaling cascade by ROS promotes the production of the hypertrophic factor beta-myosin heavy chain in myocytes triggered by Ang-II. Furthermore, Ang-II could stimulate NADPH oxidase to produce ROS, leading to p38MAP kinase activation and subsequent AP-1 activation, while Rac1 activation appears to be a key stage in the development of hypertrophy.[50]

Cardiovascular risk factors such as hypercholesterolemia, hypertension, diabetes mellitus, and smoking all enhance ROS generation while decreasing endothelial NO production. The major ROS produced by NOXs in cardiovascular cells are H_2O_2 and $O_2^{\bullet -}$. Substantial evidence supports the function of ROS in intimal thickening and the progression of atherosclerosis.

ROS also drive endothelial dysfunction, atherosclerosis, and hypertension by promoting oxidative stress and inflammation in the vascular system. ROS reduce NO availability, forming peroxynitrite, which impairs vasodilation and promotes vessel inflammation. This dysfunction facilitates atherosclerosis, as oxidized LDL (oxLDL) attracts immune cells, leading to foam cell and plaque formation that narrows arteries and raises heart attack or stroke risks. ROS also contribute to hypertension by constricting blood vessels and activating pathways that increase vascular resistance. These effects elevate blood pressure, leading to vascular remodeling and increasing cardiovascular.

4. Inflammatory diseases

ROS can also activate the MAPK pathway leading to the increased expression of vascular endothelial growth factor (VEGF). Cancer cells can generate and secrete VEGF to maintain their proliferation and migration. ROS-regulated factors (transcription factors, cytokines, and chemokines) are involved in various aspects of cellular and regulate inflammation, immune cell migration, cellular proliferation, survival, and apoptosis.[51] ROS-induced activation of signaling molecules such as Src, Ras, PI3K, EGFR, MAPK, (p38MAPK), JNK, and ERK leads to the activation of transcription factors, which in turn trigger the generation of pro-inflammatory cytokines and chemokines. Besides NF-κB, other transcription factors, such as nuclear factor-like 2 (Nrf-2), the hypoxia-inducible factor-1α (HIF-1α), β-catenin/Wnt, peroxisome proliferator activator receptor gamma (PPAR-γ), and activator protein-1 (AP-1), have also been shown to be regulated by ROS.[52] The production of cytokines and chemokines is triggered by ROS. These cytokines and chemokines bind to their respective receptors such as platelet-derived growth factor receptor, VEGF receptor, and epidermal growth factor receptor (EGFR) regulating various cellular functions such as cell adhesion, phagocytosis, cytokine secretion, cell activation, cell proliferation, cell survival and cell death, apoptosis, angiogenesis, and proliferation.[53]

H_2O_2 is the main secondary messenger in wound-healing responses inducing angiogenesis, various immunocyte recruitments, and keratinocyte proliferation and migration.[54,55]

5. Coronavirus disease 2019 (COVID-19)

The SARS-CoV-2 virus can cause the generation of ROS, which contributes to oxidative stress. The immunological response to the virus involves the activation of inflammatory pathways, and immune cells such as neutrophils and macrophages produce ROS as part of their defense against the virus. COVID-19 might increase oxidative stress by affecting Reactive Oxygen Species Modulator 1 (ROMO1) through activating NF-κB pathways.[55] ROMO1 is a mitochondrial protein that interacts to certain proteins in the mitochondrial inner membrane and was initially discovered in several malignant tissues that produce medication resistance. Furthermore, ROMO1, a ROS-producing protein, has been identified in a variety of malignancies and has been linked to cancer cell invasion and progression. SARS-CoV-2 may affect mitochondrial activity in infected cells. Disruption of mitochondrial function can result in increased ROS production, contributing to oxidative stress by activating NADPH oxidases in infected cells, resulting in increased superoxide production. SARS-CoV-2 infections can cause endoplasmic reticulum stress, a physiological reaction to unfolded or misfolded proteins that cause the formation of ROS and activation of the NF-κB, JAK-STAT, and NLRP3 inflammatory pathways.[56]

6. Diabetes

High glucose levels in diabetes stimulate the generation of ROS and activate apoptosis in cells.[57] Insulin resistance, β-cell malfunction, and diabetic complications are all exacerbated by oxidative stress.

7. Respiratory diseases

Airway inflammation is caused by oxidative stress during respiratory disorders such as chronic obstructive pulmonary disease (COPD) and asthma.[54] It contributes to the remodeling of airways and the development of lung dysfunction. Particulate matter <2.5 μm ($PM_{2.5}$) is a leading cause of death and disability worldwide. The research to date suggests that reactive oxygen species (ROS) generation in response to $PM_{2.5}$ may include disturbance of cellular redox signaling and/or rising of endogenous ROS production, resulting in excessive responses.

8. Age-related diseases

Oxidative stress is thought to play a role in age-related disorders such as cardiovascular diseases, neurological disorders, and cancer.[36] The mitochondria produce ROS that cause aging, and an organism's lifespan is thus determined by the rate of oxygen consumption by the mitochondrial respiratory chain. Aging and the Free Radical Theory support some observations:

(1) increasing levels of ROS formation and oxidative stress with aging

(2) a steady increase in mitochondrial dysfunction with aging

(3) an increase in ROS generation following inhibition of electron transport chain components

(4) a decrease in peroxisomal function with aging

According to available data, cellular senescence of mesenchymal stem cells (MSC) occurs with aging due to excessive ROS produced by complex I functional deficiency combined with depressed Ndufs6.[58] Overproduction of ROS with aging limits bone formation and promotes bone breakdown by disrupting the balance of oxidative and antioxidant defense mechanisms.[59]

ROLE OF ANTIOXIDANTS IN THE REDUCTION OF ROS

Antioxidants play a crucial role in reducing reactive oxygen species (ROS) by directly neutralizing them through electron donation, effectively acting as "scavengers" that prevent oxidative damage to cellular components like proteins, DNA, and lipids, thus mitigating oxidative stress and protecting against various diseases associated with excessive ROS production; essentially, they help maintain a balanced redox state within the body by counteracting the harmful effects of free radicals.

1. Donation of electrons

Antioxidants stabilize free radicals by giving electrons, preventing them from causing damage to biological com-

ponents. Natural and synthesized phenolic compounds with electron-donating groups (EDG) such as methoxy in the ortho or para position have more antioxidant activity than those with meta-EDG.[60]

2. Free radical scavenging

Scavengers are antioxidants that seek for and neutralize free radicals. This prevents free radicals from damaging cellular components by interacting with them. Various enzyme systems in the body scavenge free radicals, while micronutrient (vitamins) antioxidants such as vitamin E (tocopherol), vitamin C (ascorbic acid), and ß-carotene play an important role in the ROS scavenging system (Table 1).[61-79]

3. Metal chelation

Certain antioxidants, such as enzymes and chemicals, can chelate or bind to metal ions. Antioxidants assist in inhibiting the production of free radicals by chelating these metals. Curcumin, an antioxidant, can bind to metal ions such as Mn^{2+}, Fe^{2+}, and Zn^{2+}, producing metal chelates and inhibiting free radical production.

4. Enzyme activity

Antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase play essential roles in ROS neutralization (Table 2). These enzymes catalyze and transform ROS into less reactive or non-toxic molecules.

5. Quenching singlet oxygen

Singlet oxygen is a highly reactive form of oxygen. Certain antioxidants, such as carotenoids, can quench singlet oxygen by removing energy from it, preventing it from causing damage to the biological system.[80]

6. Antioxidant enzyme activity increment

Some antioxidants can increase enzymatic activity, which improves the cell's ability to neutralize ROS. Essential trace minerals, such as iron (Fe) as part of catalase; copper (Cu), manganese (Mn), and zinc (Zn) that activate SOD, and selenium (Se) that can activate glutathione peroxidase to neutralize ROS.

7. Metallic nanozymes (MNZs)

Metallic nanozymes are promising possibilities for treating ROS-related inflammatory diseases because they mimic the endogenous enzymatic mechanism.[81] These are extremely stable in a variety of physiological circumstances, including low pH and high temperature. MNZs function as synthetic antioxidants, capturing and scavenging surplus ROS during inflammation and protecting the tissues from damage.

ANTIOXIDANT ENZYMES

1. Superoxide dismutase (SOD)

The superoxide anion is converted into hydrogen peroxide and molecular oxygen through the action of three types of SODs, each characterized by distinct protein folds and catalytic metal ions. These include Cu,ZnSODs (SOD1), MnSOD/FeSODs (SOD2), and NiSODs (SOD3). SOD1 mutations in humans are associated with the fatal neurological disease amyotrophic lateral sclerosis (ALS, widely known as Lou Gehrig's disease).[82] The functional divergence between FeSOD and MnSOD is very important, preventing the two metals from substituting for each other in most Mn/FeSODs. Notably, NiSOD, a more recently discovered variant, has only been identified in bacteria.

2. Catalase

Catalase uses an iron or manganese cofactor to catalyze the conversion of hydrogen peroxide to water and oxygen. It is located mostly in peroxisomes, where it serves an important role in detoxifying peroxides produced during fatty acid metabolism. Catalase is made up of four protein subunits, each of which contains a heme group and a NADPH molecule.[83]

3. Glutathione peroxidase (GPx)

Glutathione Peroxidase lowers hydrogen peroxide and lipid hydroperoxides by using reduced glutathione as a substrate. With eight members, GPx is the most well-known protein family.[82] However, each member of the GPx family has a unique mode of action and site of action in maintaining redox balance. GPx1-4 and GPx6 use selenocysteine as the active center to catalyze the reduction of H_2O_2 or organic hydroperoxides to water or corresponding alcohols, lowering their toxicity, and preserving redox equilibrium. GPx4 can reduce complex lipid molecules in addition to H_2O_2. It is the only GPx enzyme that directly lowers and eliminates lipid hydroperoxides. The active sites of GPx5 and GPx7-GPx8 do not contain selenium cysteine (Secys), but instead contain cysteine residues (Cys). GPx5 is mostly expressed in epididymal tissue and protects sperm from oxidative damage. Expression of selenium-independent glutathione peroxidase 5 (GPx5) in the epididymis of Small Tail Han sheep.[84] Both GPx7 and GPx8 are endoplasmic reticulum enzymes involved in the oxidative folding of endoplasmic reticulum proteins, and GPx8 also plays an important role in Ca^{2+} control in the endoplasmic reticulum.

4. Peroxiredoxins (Prx)

Peroxiredoxins are a widely distributed family of cysteine-dependent peroxidase enzymes capable of rapidly clearing peroxides and serving as thiol-dependent peroxidases.[85] Prxs are classified into six evolutionary subfamilies (Prx1, Prx5, Prx6, Tpx, PrxQ, and AhpE), which differ in their oligomeric states and interfaces, as well as the placement of the resolving Cys.

5. Thioredoxin reductase (TrxR)

Thioredoxin Reductase is a selenium (selenocysteine)-containing protein that exists in three forms: TrxR1, TrxR2, and TrxR3. Thioredoxin Reductase (TrxR) enzymes

TABLE 1. List of antioxidants with mechanism and potential food sources

Antioxidants	Antioxidant mechanism	Food sources	References
α-carotene	Scavenging singlet oxygen and peroxyl radicals	Apricot, carrot, sweet potato, pumpkin, and beans	61,62
Astaxanthin	Donating electrons, bonding with the free radical	Algae, trout, krill, yeast, salmon, shrimp, and crayfish	63
Anthocyanins	Hydrogen atom transfer and single-electron transfer	Red and purple berries, plums, apples, grapes, and cabbage	64
β-carotene	Scavenging singlet oxygen and peroxyl radicals	Yellow and green leafy fruits and vegetables	62
Capsaicin	Scavenging $^{\bullet}$OH radicals in water, and DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals in ethanol	Bell peppers, cayenne peppers, jalapeño peppers, and other chili peppers	65
Catechin	Free-radical scavenging and metal chelating activities	Fresh tea leaves, black grapes, apricots, broad beans, and strawberries	62
Canthaxanthin	Scavenging singlet oxygen and peroxyl radicals	Mushrooms, fish, crustaceans, and eggs	62,66
Curcumin	Scavenge various ROS including superoxide anions, hydrogen peroxide, and nitrite radicals	Turmeric root	67
Cryptoxanthin	Scavenging singlet oxygen and peroxyl radicals	Oranges, peaches, papaya, and tangerines	62
Coenzyme Q10	Increase the production of key antioxidant enzymes such as superoxide dismutase	Oily fish (such as salmon and tuna), organ meats (such as liver), and whole grains	68,69
Glutathione	Scavenging reactive hydroxyl free radicals, other oxygen-centered free radicals, and cofactor of glutathione peroxidase	Avocado, oranges, cabbage, watermelon, asparagus, grapefruit, strawberries, broccoli, cucumber, green bean, and green tea	70
Lycopene	Scavenging singlet oxygen and peroxyl radicals	Red fruits and vegetables	62
Lutein	Scavenging singlet oxygen and peroxyl radicals	Green leafy vegetables (e.g., kale, broccoli, spinach, peas, and lettuce) and egg yolks	62
Lipoic acid	Scavenging superoxide anion and reactive nitrogen species	Organ meats (such as liver, heart, and kidney, muscle meats), fruits, and vegetables	71
Manganese	Component or activator of the antioxidant enzyme superoxide dismutase	Shellfish, especially clams, chickpeas, oysters, nuts, beans, bananas, spinach, and pineapples	72
Melatonin	Scavenging hydroxyl radical and the peroxynitrite anion, singlet oxygen, superoxide anion radical, hydrogen peroxide, nitric oxide, and hypochlorous acid	Milk, rice, cherries, nuts, fish, goji berries, and oats	73
Quercetin	Stimulate the activity of antioxidant enzymes SOD and catalase	Citrus fruits, onions, apples, sage, parsley, and tea	74
Resveratrol	Scavenging free radicals include superoxide radicals, hydroxyl radical, hydrogen peroxide, nitric oxide, and nitrogen dioxide Improving endogenous antioxidant enzyme activity (e.g., SOD, catalase, and GSH)	Grapes, blueberries, raspberries, plums, and peanuts	75
Selenium	Involved in synthesizing and regulating the activity of endogenous antioxidant enzymes glutathione peroxidase	Brazil nuts, seafood, and organ meats	76
Vitamin A	Quenching singlet oxygen, neutralizing thiyl radicals, and stabilizing peroxyl radicals	Liver, eggs, fish, dairy products, leafy green vegetables, orange, yellow vegetables, tomato products, fruits, and vegetable oils	77
Vitamin C	Quenching singlet oxygen and stabilizing peroxyl radicals	Citrus fruits	78
Vitamin E	Quenching singlet oxygen, and stabilizing peroxyl radicals	Nuts, seeds, and vegetable oils	78
Zeaxanthin	Scavenging singlet oxygen and peroxyl radicals	Green leafy vegetables (e.g., kale, broccoli, spinach, green peas, and lettuce) and egg yolks	62
Citric acid	Scavenging hydrogen peroxide, hydroxyl, alkoxy, and peroxide radicals and superoxide anions	Lemons, grapefruits, limes, oranges, tangerines, and pomelos	79

are found primarily in the cytoplasm and mitochondria. TrxR and Thioredoxin (Trx) are two key antioxidant systems that regulate redox homeostasis in cells. The thioredoxin system, which consists of TrxR, Trx, and NADPH, operates by a disulfide-dithiol exchange process.[86] TrxR is required for the regeneration of decreased thioredoxin,

TABLE 2. Antioxidant enzymes, their functions, Subcellular locations, and associated health implications

Antioxidant enzymes	Functions	Subcellular locations	Implications on diseases
Superoxide dismutase	Neutralizes superoxide anion radical	Widely distributed	Neurodegenerative diseases, aging, cancer, cardiovascular
Catalase	The conversion of hydrogen peroxide to water and oxygen	Peroxisome	Diabetes mellitus, vitiligo, cardiovascular diseases, hypertension, anemia, and neurodegenerative diseases
Glutathione peroxidase	Lowers hydrogen peroxide and lipid hydroperoxides	Cytosol	Cancer, cardiovascular
Peroxiredoxins	Rapidly clearing peroxides	Cytosol, mitochondria, and nucleus	Inflammatory diseases, neurodegenerative diseases, cancer, cardiovascular
Thioredoxin reductase	Reducing thioredoxin	Cytosol and mitochondria	Cancer, neurodegenerative diseases, and cardiovascular diseases
Glutathione reductase	Maintains cellular GSH levels	Cytosol and mitochondria	Aging processes, and cell death
Paraoxonase	Functions as a lactonase and an arylesterase	Bloodstream, mitochondria	Cardiovascular diseases, neurological disorders, diabetes mellitus, atherosclerosis, obesity, NAFLD, and inflammatory diseases

which is required for some peroxiredoxins' peroxidase activity.

6. Glutathione reductase (GR)

Glutathione reductase is responsible for maintaining the supply of reduced GSH, which is one of the most prevalent reducing thiols in most cells. GSH, in its reduced form, performs critical roles in the cellular regulation of ROS.[87] Reduces oxidized glutathione to its reduced form GSH, hence preserving the cellular GSH pool. Because of its characteristics and reactivity, GSH is a crucial antioxidant molecule that serves as a marker of the redox state in numerous diseases, aging processes, and cell death.

7. Paraoxonase (PON)

Paraoxonase enzymes regulate inflammation and serve as antioxidants in human tissues. PON1, PON2, and PON3 have been identified as PON family subgroups (Table 2).[88] PON1 is produced in the liver and is found in the bloodstream in association with high-density lipoprotein (HDL). PON1 enzyme contains calcium ion sites, which are necessary for enzyme stability and catalytic activity. PON1 is the most studied enzyme in the PON family, and its significance has been demonstrated in cardiovascular diseases, diabetes mellitus, atherosclerosis, obesity, non-alcoholic fatty liver disease (NAFLD), and inflammatory diseases.

DIETARY FOODS AND DRUGS INDUCING ROS PRODUCTION

Dietary macronutrients promote ROS generation through increased metabolic rate and β-oxidation in mitochondria and peroxisomes.[89-91] Acetyl CoA, produced by glycolysis and β-oxidation of fatty acids, is metabolized in mitochondria via the tricarboxylic acid cycle, resulting in NADH and FADH2. NADH and FADH2 supply electrons to complex I and II of the mitochondrial electron transport chain, respectively. The leakage of these electrons to oxygen is the primary cause of $O_2^{-\bullet}$ production. Similarly, quickly following consuming a meal, ROS concentrations in peripheral cells are significantly elevated, which promotes additional ROS formation.[90,92]

Consuming glucose leads to an increase in ROS production by polymorphonuclear leukocytes (PMNs) and mononuclear cells (MNCs), as well as a drop in plasma-tocopherol concentrations. Glucose and meal challenges also enhance lipid peroxidation and deplete antioxidant reserves. These findings raise the question of whether macronutrients other than carbs can boost ROS production and oxidative stress. Protein intake boosts $O_2^{-\bullet}$ production in polymorphonuclear leukocytes and mononuclear cells. The data indicating protein-dependent postprandial ROS generation are less extensive when compared to glucose ingestion, and one study found no significant increase in oxidative stress following protein intake by healthy males. In contrast to glucose and proteins, lipids are a primary source of ROS production and accumulation in the plasma and ER membranes.[93,94] Longer chain polyunsaturated fatty acids (PUFAs) have a higher β-oxidation rate compared to monounsaturated and saturated fatty acids. As a result, PUFAs generate more reactive ROS compared to monounsaturated and saturated fatty acids. Consuming a high-fat diet raises aROS levels, which can lead to proinflammatory signaling, activation of the NF-κB transcription factor, and deleterious effects on organs including the liver and kidneys.[95-97]

Some pharmaceutical preparations, such as Diclofenac, Paracetamol, and Cisplatin, are also responsible for the formation of ROS leading to oxidative stress because of overconsumption.[98] Traditional medicine, such as Ayurvedic medicine, contains heavy metals like mercury, arsenic, and lead, which reduce the activity of antioxidant enzymes, causing an imbalance of the redox system and oxidative

stress, which can also severely affect the liver and other tissues.[99-101]

RECENT ADVANCES IN THE STUDY OF REACTIVE OXYGEN SPECIES (ROS)

1. Precise ROS measurement

Researchers are actively developing advanced fluorescent probes designed to detect specific reactive oxygen species (ROS) with exceptional sensitivity and spatial resolution.[102,103] This innovative technology allows for improved monitoring of redox changes within cells, enhancing our understanding of the complex processes that occur within our bodies. Such advancements in the scientific community hold great promise for significant breakthroughs in cellular health and disease management, underscoring the importance of these efforts in contributing to society's greater good.

2. ROS-responsive drug delivery

The field of targeted cancer therapies has advanced by developing refined drug delivery systems.[104] These systems are designed to release therapeutic agents selectively in areas with elevated reactive oxygen species (ROS) levels, such as tumors. This targeted approach enhances treatment efficacy while reducing systemic side effects, ultimately improving patient outcomes. By leveraging tumors' unique biochemical environments, researchers are pioneering a transformative approach to cancer treatment that could redefine oncology.

3. Wound healing applications

Understanding the role of ROS in different stages of wound healing has led to the development of ROS-scavenging agents that promote skin regeneration.

4. Immune system modulation

Researchers are exploring ways to manipulate ROS levels in immune cells to enhance their anti-tumor activity and control immune responses in various diseases.

CONCLUSIONS

ROS are generated by various endogenous cellular activities. Mitochondria are the primary source of endogenous cellular ROS, which, in considerable quantities, can damage macromolecules such as DNA, proteins, and lipids. Such damage has been linked to the development of a variety of pathological conditions, including cancer, inflammatory, neurodegenerative, respiratory, cardiovascular, and age-related diseases. The body contains built-in antioxidant defenses against free radicals. Antioxidants in the diet are beneficial in the prevention of disease. However, many questions regarding antioxidant supplements and disease prevention mechanisms remain unsolved. The paired character of ROS with their advantageous and detrimental qualities reveals the complexity of their specialized roles in a biological compartment, as well as the difficulty in developing relevant techniques to treat ROS-related disorders. Future advances in genomes, metabolomics, and proteomics will be helpful in gaining a precise understanding of biochemical networks involved in cellular responses to oxidative stress.

CONFLICT OF INTEREST STATEMENT

None declared.

REFERENCES

1. Jakubczyk K, Dec K, Kałduńska J, Kawczuga D, Kochman J, Janda K. Reactive oxygen species - sources, functions, oxidative damage. Pol Merkur Lekarski 2020;48:124-7.
2. Singh A, Kukreti R, Saso L, Kukreti S. Oxidative stress: a key modulator in neurodegenerative diseases. Molecules 2019;24: 1583.
3. Chaudhary P, Janmeda P, Docea AO, Yeskaliyeva B, Abdull Razis AF, Modu B, et al. Oxidative stress, free radicals and antioxidants: potential crosstalk in the pathophysiology of human diseases. Front Chem 2023;11:1158198.
4. Andrés CMC, Pérez de la Lastra JM, Andrés Juan C, Plou FJ, Pérez-Lebeña E. Superoxide anion chemistry-its role at the core of the innate immunity. Int J Mol Sci 2023;24:1841.
5. Hyodo K, Suzuki N, Mise K, Okuno T. Roles of superoxide anion and hydrogen peroxide during replication of two unrelated plant RNA viruses in Nicotiana benthamiana. Plant Signal Behav 2017;12:e1338223.
6. Fujii J, Homma T, Osaki T. Superoxide radicals in the execution of cell death. Antioxidants (Basel) 2022;11:501.
7. Lipinski B. Hydroxyl radical and its scavengers in health and disease. Oxid Med Cell Longev 2011;2011:809696.
8. Charrier JG, Anastasio C. Impacts of antioxidants on hydroxyl radical production from individual and mixed transition metals in a surrogate lung fluid. Atmos Environ (1994) 2011;45:7555-62.
9. Baschieri A, Jin Z, Amorati R. Hydroperoxyl radical (HOO•) as a reducing agent: unexpected synergy with antioxidants. A review. Free Radic Res 2023;57:115-29.
10. Francis R. The effects of acute hydrogen peroxide exposure on respiratory cilia motility and viability. PeerJ 2023;11:e14899.
11. Kutzsche S, Ilves P, Kirkeby OJ, Saugstad OD. Hydrogen peroxide production in leukocytes during cerebral hypoxia and reoxygenation with 100% or 21% oxygen in newborn piglets. Pediatr Res 2001;49:834-42.
12. Biswas K, Alexander K, Francis MM. Reactive oxygen species: angels and demons in the life of a neuron. NeuroSci 2022;3: 130-45.
13. Andrés CMC, Pérez de la Lastra JM, Juan CA, Plou FJ, Pérez-Lebeña E. Chemistry of hydrogen peroxide formation and elimination in mammalian cells, and its role in various pathologies. Stresses 2022;2:256-74.
14. Mahaseth T, Kuzminov A. Potentiation of hydrogen peroxide toxicity: from catalase inhibition to stable DNA-iron complexes. Mutat Res Rev Mutat Res 2017;773:274-81.
15. Nam JS, Hong Y, Lee CG, Kim TI, Lee C, Roh DH, et al. Singlet

oxygen generation from polyaminoglycerol by spin-flip-based electron transfer. JACS Au 2022;2:933-42.
16. Ingenbosch KN, Quint S, Dyllick-Brenzinger M, Wunschik DS, Kiebist J, Süss P, et al. Singlet-oxygen generation by peroxidases and peroxygenases for chemoenzymatic synthesis. Chembiochem 2021;22:398-407.
17. Stief TW. The physiology and pharmacology of singlet oxygen. Med Hypotheses 2003;60:567-72.
18. Dogra V, Kim C. Singlet oxygen metabolism: from genesis to signaling. Front Plant Sci 2020;10:1640.
19. Onyango AN. Endogenous generation of singlet oxygen and ozone in human and animal tissues: mechanisms, biological significance, and influence of dietary components. Oxid Med Cell Longev 2016;2016:2398573.
20. Serra MEG, Baeza-Noci J, Mendes Abdala CV, Luvisotto MM, Bertol CD, Anzolin AP. The role of ozone treatment as integrative medicine. An evidence and gap map. Front Public Health 2023; 10:1112296.
21. Nuvolone D, Petri D, Voller F. The effects of ozone on human health. Environ Sci Pollut Res Int 2018;25:8074-88.
22. Pacher P, Beckman JS, Liaudet L. Nitric oxide and peroxynitrite in health and disease. Physiol Rev 2007;87:315-424.
23. Khlestkin VK, Mazhukin DG. Recent advances in the application of N,O-dialkylhydroxylamines in organic chemistry. Curr Org Chem 2003;7:967-93.
24. Spiteller G, Afzal M. The action of peroxyl radicals, powerful deleterious reagents, explains why neither cholesterol nor saturated fatty acids cause atherogenesis and age-related diseases. Chemistry 2014;20:14928-45.
25. Chamulitrat W, Takahashi N, Mason RP. Peroxyl, alkoxyl, and carbon-centered radical formation from organic hydroperoxides by chloroperoxidase. J Biol Chem 1989;264:7889-99.
26. Winterbourn CC, Hampton MB, Livesey JH, Kettle AJ. Modeling the reactions of superoxide and myeloperoxidase in the neutrophil phagosome: implications for microbial killing. J Biol Chem 2006;281:39860-9.
27. Wang L, Bassiri M, Najafi R, Najafi K, Yang J, Khosrovi B, et al. Hypochlorous acid as a potential wound care agent: part I. Stabilized hypochlorous acid: a component of the inorganic armamentarium of innate immunity. J Burns Wounds 2007;6:e5.
28. Nguyen K, Bui D, Hashemi M, Hocking DM, Mendis P, Strugnell RA, et al. The potential use of hypochlorous acid and a smart prefabricated sanitising chamber to reduce occupation-related COVID-19 exposure. Risk Manag Healthc Policy 2021;14: 247-52.
29. Andrés CMC, Pérez de la Lastra JM, Juan CA, Plou FJ, Pérez-Lebeña E. Hypochlorous acid chemistry in mammalian cells-influence on infection and role in various pathologies. Int J Mol Sci 2022;23:10735.
30. Pullar JM, Vissers MC, Winterbourn CC. Living with a killer: the effects of hypochlorous acid on mammalian cells. IUBMB Life 2000;50:259-66.
31. Zhang Y, Wong HS. Are mitochondria the main contributor of reactive oxygen species in cells? J Exp Biol 2021;224:jeb221606.
32. Silwal P, Kim JK, Kim YJ, Jo EK. Mitochondrial reactive oxygen species: double-edged weapon in host defense and pathological inflammation during infection. Front Immunol 2020;11:1649.
33. Murphy MP. How mitochondria produce reactive oxygen species. Biochem J 2009;417:1-13.
34. Chen X, Song M, Zhang B, Zhang Y. Reactive oxygen species regulate T cell immune response in the tumor microenvironment. Oxid Med Cell Longev 2016;2016:1580967.
35. Ofori EA, Garcia-Senosiain A, Naghizadeh M, Kana IH, Dziegiel MH, Adu B, et al. Human blood neutrophils generate ROS through FcγR-signaling to mediate protection against febrile P. falciparum malaria. Commun Biol 2023;6:743.
36. Chaudhary MR, Chaudhary S, Sharma Y, Singh TA, Mishra AK, Sharma S, et al. Aging, oxidative stress and degenerative diseases: mechanisms, complications and emerging therapeutic strategies. Biogerontology 2023;24:609-62.
37. Del Río LA, López-Huertas E. ROS generation in peroxisomes and its role in cell signaling. Plant Cell Physiol 2016;57:1364-76.
38. Milkovic L, Cipak Gasparovic A, Cindric M, Mouthuy PA, Zarkovic N. Short overview of ROS as cell function regulators and their implications in therapy concepts. Cells 2019;8:793.
39. Burtenshaw D, Kitching M, Redmond EM, Megson IL, Cahill PA. Reactive oxygen species (ROS), intimal thickening, and subclinical atherosclerotic disease. Front Cardiovasc Med 2019; 6:89.
40. Oka S, Nakabeppu Y. DNA glycosylase encoded by MUTYH functions as a molecular switch for programmed cell death under oxidative stress to suppress tumorigenesis. Cancer Sci 2011;102: 677-82.
41. Kasai S, Kokubu D, Mizukami H, Itoh K. Mitochondrial reactive oxygen species, insulin resistance, and Nrf2-mediated oxidative stress response-toward an actionable strategy for anti-aging. Biomolecules 2023;13:1544.
42. Houldsworth A. Role of oxidative stress in neurodegenerative disorders: a review of reactive oxygen species and prevention by antioxidants. Brain Commun 2024;6:fcad356.
43. Valero T. Mitochondrial biogenesis: pharmacological approaches. Curr Pharm Des 2014;20:5507-9.
44. Sienes Bailo P, Llorente Martín E, Calmarza P, Montolio Breva S, Bravo Gómez A, Pozo Giráldez A, et al. The role of oxidative stress in neurodegenerative diseases and potential antioxidant therapies. Adv Lab Med 2022;3:342-60.
45. Chay KO, Nam Koong KY, Hwang S, Kim JK, Bae CS. NADPH oxidase mediates β-amyloid peptide-induced neuronal death in mouse cortical cultures. Chonnam Med J 2017;53:196-202.
46. Ray PD, Huang BW, Tsuji Y. Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. Cell Signal 2012;24:981-90.
47. Bardaweel SK, Gul M, Alzweiri M, Ishaqat A, ALSalamat HA, Bashatwah RM. Reactive oxygen species: the dual role in physiological and pathological conditions of the human body. Eurasian J Med 2018;50:193-201.
48. Kim H, Yun J, Kwon SM. Therapeutic strategies for oxidative stress-related cardiovascular diseases: removal of excess reactive oxygen species in adult stem cells. Oxid Med Cell Longev 2016;2016:2483163.
49. Matsuda S, Umemoto S, Yoshimura K, Itoh S, Murata T, Fukai T, et al. Angiotensin Ⅱ activates MCP-1 and induces cardiac hypertrophy and dysfunction via toll-like receptor 4. J Atheroscler Thromb 2015;22:833-44.

50. Satoh M, Ogita H, Takeshita K, Mukai Y, Kwiatkowski DJ, Liao JK. Requirement of Rac1 in the development of cardiac hypertrophy. Proc Natl Acad Sci U S A 2006;103:7432-7.
51. Uchmanowicz I. Oxidative stress, frailty and cardiovascular diseases: current evidence. Adv Exp Med Biol 2020;1216:65-77.
52. Espinosa-Diez C, Miguel V, Mennerich D, Kietzmann T, Sánchez-Pérez P, Cadenas S, et al. Antioxidant responses and cellular adjustments to oxidative stress. Redox Biol 2015;6: 183-97.
53. Ramesh G, MacLean AG, Philipp MT. Cytokines and chemokines at the crossroads of neuroinflammation, neurodegeneration, and neuropathic pain. Mediators Inflamm 2013;2013: 480739.
54. Steiling H, Munz B, Werner S, Brauchle M. Different types of ROS-scavenging enzymes are expressed during cutaneous wound repair. Exp Cell Res 1999;247:484-94.
55. Amini MA, Karimi J, Talebi SS, Piri H. The association of COVID-19 and reactive oxygen species modulator 1 (ROMO1) with oxidative stress. Chonnam Med J 2022;58:1-5.
56. Yiang GT, Wu CC, Lu CL, Hu WC, Tsai YJ, Huang YM, et al. Endoplasmic reticulum stress in elderly patients with COVID-19: potential of melatonin treatment. Viruses 2023;15:156.
57. Kohnert KD, Freyse EJ, Salzsieder E. Glycaemic variability and pancreatic β-cell dysfunction. Curr Diabetes Rev 2012;8:345-54.
58. Zhang Y, Guo L, Han S, Chen L, Li C, Zhang Z, et al. Adult mesenchymal stem cell ageing interplays with depressed mitochondrial Ndufs6. Cell Death Dis 2020;11:1075.
59. An Y, Zhang H, Wang C, Jiao F, Xu H, Wang X, et al. Activation of ROS/MAPKs/NF-κB/NLRP3 and inhibition of efferocytosis in osteoclast-mediated diabetic osteoporosis. FASEB J 2019;33: 12515-27.
60. Lee CY, Nanah CN, Held RA, Clark AR, Huynh UG, Maraskine MC, et al. Effect of electron donating groups on polyphenol-based antioxidant dendrimers. Biochimie 2015;111:125-34.
61. Burri BJ, Chang JS, Neidlinger TR. β-cryptoxanthin- and α-carotene-rich foods have greater apparent bioavailability than β-carotene-rich foods in Western diets. Br J Nutr 2011;105: 212-9.
62. Stahl W, Sies H. Antioxidant activity of carotenoids. Mol Aspects Med 2003;24:345-51.
63. Ambati RR, Phang SM, Ravi S, Aswathanarayana RG. Astaxanthin: sources, extraction, stability, biological activities and its commercial applications--a review. Mar Drugs 2014;12:128-52.
64. Khoo HE, Azlan A, Tang ST, Lim SM. Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits. Food Nutr Res 2017;61: 1361779.
65. Kogure K, Goto S, Nishimura M, Yasumoto M, Abe K, Ohiwa C, et al. Mechanism of potent antiperoxidative effect of capsaicin. Biochim Biophys Acta 2002;1573:84-92.
66. Gaur V, Bera S. Microbial canthaxanthin: an orange-red keto carotenoid with potential pharmaceutical applications. BioTechnologia (Pozn) 2023;104:315-28.
67. Joe B, Vijaykumar M, Lokesh BR. Biological properties of curcumin-cellular and molecular mechanisms of action. Crit Rev Food Sci Nutr 2004;44:97-111.
68. Pravst I, Zmitek K, Zmitek J. Coenzyme Q10 contents in foods and fortification strategies. Crit Rev Food Sci Nutr 2010;50: 269-80.
69. Zhang Y, Huang X, Liu N, Liu M, Sun C, Qi B, et al. Discovering the potential value of coenzyme Q10 in oxidative stress: enlightenment from a synthesis of clinical evidence based on various population. Front Pharmacol 2022;13:936233.
70. Minich DM, Brown BI. A review of dietary (phyto)nutrients for glutathione support. Nutrients 2019;11:2073.
71. Anthony RM, MacLeay JM, Gross KL. Alpha-lipoic acid as a nutritive supplement for humans and animals: an overview of its use in dog food. Animals (Basel) 2021;11:1454.
72. Erikson KM, Aschner M. Manganese: its role in disease and health. Met Ions Life Sci 2019;19:253-66.
73. Kennaway DJ. Melatonin rich foods in our diet: food for thought or wishful thinking? Food Funct 2020;11:9359-69.
74. Dabeek WM, Marra MV. Dietary quercetin and kaempferol: bioavailability and potential cardiovascular-related bioactivity in humans. Nutrients 2019;11:2288.
75. Meng X, Zhou J, Zhao CN, Gan RY, Li HB. Health benefits and molecular mechanisms of resveratrol: a narrative review. Foods 2020;9:340.
76. Hu W, Zhao C, Hu H, Yin S. Food sources of selenium and its relationship with chronic diseases. Nutrients 2021;13:1739.
77. Palace VP, Khaper N, Qin Q, Singal PK. Antioxidant potentials of vitamin A and carotenoids and their relevance to heart disease. Free Radic Biol Med 1999;26:746-61.
78. Sies H, Stahl W, Sundquist AR. Antioxidant functions of vitamins. Vitamins E and C, beta-carotene, and other carotenoids. Ann N Y Acad Sci 1992;669:7-20.
79. Abdel-Salam OM, Youness ER, Mohammed NA, Morsy SM, Omara EA, Sleem AA. Citric acid effects on brain and liver oxidative stress in lipopolysaccharide-treated mice. J Med Food 2014; 17:588-98.
80. Ramel F, Birtic S, Cuiné S, Triantaphylidès C, Ravanat JL, Havaux M. Chemical quenching of singlet oxygen by carotenoids in plants. Plant Physiol 2012;158:1267-78.
81. Mohapatra A, Park IK. Recent advances in ROS-scavenging metallic nanozymes for anti-inflammatory diseases: a review. Chonnam Med J 2023;59:13-23.
82. Perry JJ, Shin DS, Getzoff ED, Tainer JA. The structural biochemistry of the superoxide dismutases. Biochim Biophys Acta 2010;1804:245-62.
83. Goyal MM, Basak A. Human catalase: looking for complete identity. Protein Cell 2010;1:888-97.
84. Li R, Fan X, Zhang T, Song H, Bian X, Nai R, et al. Expression of selenium-independent glutathione peroxidase 5 (GPx5) in the epididymis of Small Tail Han sheep. Asian-Australas J Anim Sci 2018;31:1591-7.
85. Perkins A, Nelson KJ, Parsonage D, Poole LB, Karplus PA. Peroxiredoxins: guardians against oxidative stress and modulators of peroxide signaling. Trends Biochem Sci 2015;40:435-45.
86. Bjørklund G, Zou L, Wang J, Chasapis CT, Peana M. Thioredoxin reductase as a pharmacological target. Pharmacol Res 2021;174: 105854.
87. Couto N, Wood J, Barber J. The role of glutathione reductase and related enzymes on cellular redox homoeostasis network. Free Radic Biol Med 2016;95:27-42.

88. Arab ZN, Khayatan D, Razavi SM, Zare K, Kheradkhah E, Momtaz S, et al. Phytochemicals as modulators of paraoxonase-1 in health and diseases. Antioxidants (Basel) 2022;11:1273.
89. Sikder MM, Uyama T, Sasaki S, Kawai K, Araki N, Ueda N. PLAAT1 expression triggers fragmentation of mitochondria in an enzyme activity-dependent manner. J Biochem 2023;175: 101-13.
90. Samoylenko A, Hossain JA, Mennerich D, Kellokumpu S, Hiltunen JK, Kietzmann T. Nutritional countermeasures targeting reactive oxygen species in cancer: from mechanisms to biomarkers and clinical evidence. Antioxid Redox Signal 2013;19: 2157-96.
91. Lismont C, Nordgren M, Van Veldhoven PP, Fransen M. Redox interplay between mitochondria and peroxisomes. Front Cell Dev Biol 2015;3:35.
92. Mohanty P, Ghanim H, Hamouda W, Aljada A, Garg R, Dandona P. Both lipid and protein intakes stimulate increased generation of reactive oxygen species by polymorphonuclear leukocytes and mononuclear cells. Am J Clin Nutr 2002;75:767-72.
93. Fisher-Wellman K, Bloomer RJ. Macronutrient specific postprandial oxidative stress: relevance to the development of insulin resistance. Curr Diabetes Rev 2009;5:228-38.
94. Tan BL, Norhaizan ME. Effect of high-fat diets on oxidative stress, cellular inflammatory response and cognitive function. Nutrients 2019;11:2579.
95. Dalvi PS, Chalmers JA, Luo V, Han DY, Wellhauser L, Liu Y, et al. High fat induces acute and chronic inflammation in the hypothalamus: effect of high-fat diet, palmitate and TNF-α on appetite-regulating NPY neurons. Int J Obes (Lond) 2017;41:149-58.
96. Ma Y, Lee G, Heo SY, Roh YS. Oxidative stress is a key modulator in the development of nonalcoholic fatty liver disease. Antioxidants (Basel) 2021;11:91.
97. Rahman SMK, Sasaki S, Uyama T, Hussain Z, Sikder MM, Saiga H, et al. PLAAT1 deficiency alleviates high-fat diet-induced hepatic lipid accumulation in mice. FASEB J 2023;37:e23032.
98. Deavall DG, Martin EA, Horner JM, Roberts R. Drug-induced oxidative stress and toxicity. J Toxicol 2012;2012:645460.
99. Mamun Sikder M, Rakib Hasan M, Sarkar Lopa S, Abedin N, Jashim Uddin M, Moklesur Rahman Sarker M, et al. Toxicological studies of an ayurvedic medicine “Karpur Ras” used in diarrhoea. Int J Pharm 2015;5:472-7.
100. Sikder MM, Zulfiquar TN, Chowdhury IH, Akter K, Asha UH, Haque E, et al. Preclinical lipid pro¬file studies of a classical Ayurvedic preparation, Siddha Makardhwaja (SMD), after chronic administration to male Sprague-Dawley rats. Biol Med (Aligarh) 2018;10:439.
101. Sikder MM. Ayurvedic medicine: a traditional medical system and its heavy metal poisoning. Chonnam Med J 2024;60:97-104.
102. Murphy MP, Bayir H, Belousov V, Chang CJ, Davies KJA, Davies MJ, et al. Guidelines for measuring reactive oxygen species and oxidative damage in cells and in vivo. Nat Metab 2022;4:651-62.
103. Wu L, Sedgwick AC, Sun X, Bull SD, He XP, James TD. Reaction-based fluorescent probes for the detection and imaging of reactive oxygen, nitrogen, and sulfur species. Acc Chem Res 2019;52:2582-97.
104. Liu J, Jia B, Li Z, Li W. Reactive oxygen species-responsive polymer drug delivery systems. Front Bioeng Biotechnol 2023;11: 1115603.