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Review

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Oxidative stress and antioxidants in health and disease

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Abstract: The increase in the formation of reactive oxygen and reactive nitrogen species of endogenous or exogenous origin causes oxidative stress due to pro-oxidant and antioxidant imbalance that causes cellular damage in metabolism. This can increase inflammation of cells, apoptosis and necrosis, damage to DNA base damage, DNA and protein cross-links, lipid membrane peroxidation, and mitochondrial dysfunction. Antioxidants can be described as a system that protects biomolecules and the organism against the harmful effects of free radicals, reduces or repairs the damage done by reactive oxygen species (ROS) to the target molecule, and this is called antioxidant defense. It is known that the mechanisms caused by the increase in ROS resulting from oxidative stress are positively related to the pathology of many diseases such as cancer, metabolic syndrome, atherosclerosis, malaria, Alzheimer's disease, rheumatoid arthritis, neurodegenerative diseases and preeclampsia.

Keywords: antioxidants; diseases; free radicals; nitrogen oxygen species; oxidative stress; reactive oxygen species.

Introduction

Oxidative stress can be defined as an oxidative imbalance stemming from the inability to detoxify there reactive products, which are formed by the production of reactive oxygen species (ROS) during cellular metabolism [1]. The increase in free radicals formed as a result of biological oxidation causes disruption in the structure and functions of intracellular proteins, membrane damage due to polyunsaturated fatty acid peroxidation with lipids, nucleic acid base modifications and chromosome changes (DNA single-strand and double-strand breaks, DNA and protein cross-links) and cell death and oxidative damage to cellular structures and components such as polysaccharide depolymerization and carbohydrate degeneration [2].

Antioxidants are the system that protects the organism against the harmful effects of free radicals, reduces or repairs the damage. It is known that the mechanisms caused by the increase in ROS resulting from oxidative stress are positively related to the pathology of many diseases such as cancer, metabolic syndrome, atherosclerosis, malaria, Alzheimer's disease, rheumatoid arthritis, neurodegenerative diseases and preeclampsia [3–7]. This chapter aims to provide an overview of free radicals, their mechanisms, the role of oxidative stress in the formation of diseases, and the importance of antioxidants in a healthy life.

Free radicals

Free radicals, which are an integral part of many chemical and biological processes, are atoms, groups of atoms or molecules with unstable and reactive unpaired valence electrons in their atomic orbits [8]. Having one or more unpaired electrons gives them the freedom to exchange and receive electrons. Thus, their ability to react is much higher than non-radical compounds [9]. In addition to all these, when the free radical concentration is kept at low or moderate levels, it provides benefits for the organism by playing roles such as fighting pathogens by the host defense system, preventing smooth muscle cell proliferation, regulating systemic circulation, acting as regulatory mediators in signal transmission processes. In this case, aerobic cells faced with the oxygen paradox require homeostatic regulation in order to keep the free radical level in metabolism at physiological concentrations [1, 10, 11].

It is accepted that free radicals occur by three different mechanisms:

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(1) **Homolytic fragmentation:** It is the homolytic cleavage of a covalent bonded molecule by separation of the bond, leaving one of the common electrons in each fragment.

$$A:B \longrightarrow A\cdot + B\cdot \tag{1}$$

(2) **Heterolytic fragmentation:** It is an electron loss or heterolytic splitting of the molecule. Two different charged ions are formed.

$$A:B \longrightarrow A:^{-} + B^{+} \tag{2}$$

(3) **Electron transfer:** It occurs by adding or removing an electron from a non-radical molecule [8].

$$A + e^{-} \longrightarrow A^{-}\bullet\ (\text{radical anion}) \tag{3}$$

$$A - e^{-} \longrightarrow A^{+}\bullet\ (\text{radical cation}) \tag{4}$$

Free radicals consist of ROS and reactive nitrogen species (RNS). The most important radicals in biological systems are those derived from oxygen.

Reactive oxygen species

ROS describe molecules with unpaired electrons consisting of molecular oxygen. Oxygen in the ground state can be transformed into more reactive forms by energy or electron transfer. During the reduction of oxygen to water in aerobic metabolism, reactive molecules or particles are formed as a result of a series of reactions that occur with electron transfer. Among the ROS derived from oxygen, superoxide radical ($O_2^{-}\cdot$), hydroxyl radical (OH·) and H_2O_2 as prooxidant can be given as examples. $O_2^{-\cdot}$ and OH· are defined as “radicals”, and H_2O_2 as a “non-radical” species [2].

Singlet oxygen ($^1O_2$) is a non radical reactive oxygen molecule since it does not have unpaired electrons in its outer orbit. Since it is formed by the energetic excitation of oxygen, it has no spin restriction and has a very high reactivity. Singlet oxygen can transfer its available energy to other molecules and react with DNA, RNA, proteins, lipids and sterols. It can also generate the peroxy radical (ROO·) and initiate lipid peroxidation [8, 12]. Since the superoxide radical, which is formed by the reduction of triplet oxygen with an electron, is a strong reductant in the cell, the radicals it produces secondary at the molecular level are important. The superoxide radical is formed by the transfer of an electron to the O_2 atom while moving from complex I to III during mitochondrial respiration. While 2% of the oxygen used in the mitochondria turns into superoxide, the primary product formed by the reduction of oxygen in the mitochondria is water [13].

A superoxide radical is formed by transferring an electron to oxygen.

$$O_2 + e^{-} \longrightarrow O_2^{-}\bullet \tag{5}$$

The superoxide radical reacts with nitric oxide and forms the peroxynitrite molecule (ONOO), which can turn into toxic products such as nitrogen dioxide (NO_2), hydroxyl radical, nitronium ion (NO_2^{+}) [14].

$$O_2^{-}\bullet + NO \longrightarrow ONOO^{-} \longrightarrow H^{+} \longrightarrow OH\bullet \tag{6}$$

The peroxide radical in aqueous solution acts as a reducing agent by donating an electron to cytochrome c.

$$\text{cyt c}\,(\text{Fe (III)}) + O_2^{-}\bullet \longrightarrow O_2 + \text{cyt c}\left(\text{Fe}^{2+}\right) \tag{7}$$

The reduction of oxygen with two electrons creates hydrogen peroxide (H_2O_2).

$$O_2 + 2e^{-} + 2H^{+} \longrightarrow H_2O_2 \tag{8}$$

The main production of hydrogen peroxide in biological systems occurs by superoxide dismutation and conversion of two superoxide molecules to hydrogen peroxide and molecular oxygen by taking two protons. Hydrogen peroxide is considered in the category of non-radical ROS that play a role in radical production [2, 8].

$$2O_2^{-}\bullet + 2H^{+} \longrightarrow H_2O_2 + O_2 \tag{9}$$

With the Haber–Weiss reaction, hydrogen peroxide interacts with the superoxide radical in the presence of Fe/Cu to form the highly reactive hydroxyl radical. Since hydrogen peroxide has the ability to pass through the cell membrane, it can reach membrane-protected structures [2, 15].

$$H_2O_2 + O_2^{-}\bullet \longrightarrow \bullet OH + OH^{-} + O_2\ (\text{Haber–Weiss Reaction}) \tag{10}$$

Reactions catalyzed by iron (or copper) can be considered both as a source of hydrogen peroxide and dependent on superoxide. Ferric iron (Fe^{3+}) is reduced to ferrous iron (Fe^{2+}) by superoxide. OH· and OH^{-} are produced from hydrogen peroxide through the Fenton reaction with ferro-iron. The hydroxyl radical has the capacity to cause damage in the biological system through the free radical chain reaction known as lipid peroxidation [2, 14].

$$O_2^{-}\bullet + Fe^{3+} \longrightarrow O_2 + Fe^{2+} \tag{11}$$

$$Fe^{2+}/Cu^{+} + H_2O_2 \longrightarrow Fe^{3+}/Cu^{2+} + OH^{-} + OH\bullet\ (\text{Fenton reaction}) \tag{12}$$

The hydroxyl radical, which is very reactive and has a short-lived oxidizing feature, can cause great damage due to its capacity to initiate a chain reaction with non-radical molecules. It can also contribute to addition reactions, electron transfer reactions, and hydrogen removal reactions with potential electron gain affinity [2, 15].

The hydroxyl radical causes damage by removing a proton from various molecules such as thiols and fatty acids, causing the formation of new radicals such as thiyl radicals (RS·), carbon-centered organic radicals (R·), organic peroxides (RCOO·). Antioxidants such as glutathione (GSH) and ascorbic acid act as prooxidants in the presence of metal ions [2, 16, 17].

$$\text{nGSH} \xrightarrow{Fe^{2+},Cu^{2+}} O_2^{-}\bullet,\ H_2O_2,\ \bullet OH,\ RS\bullet,\ RSOO\bullet,\ RSOOH,\ \text{carbonyls.} \quad (13)$$

Reactive nitrogen species

Nitric oxide (nitrogen monoxide, NO) is a colorless gas with moderate solubility in water and greater in organic solvents. Due to these properties, NO, which can easily diffuse between membranes and cells, is considered a free radical because it has unpaired electrons. NO is synthesized from L-arginine and oxygen by the enzyme nitric oxide synthase. For this reaction to occur, besides molecular oxygen, nicotinamide adenine dinucleotide (NADPH), flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN) and tetrahydrobiopterin (BH_4) are needed as cofactors. The entire synthesis reaction is a five-electron oxidation reaction with three electrons from NADPH and two electrons from O_2 (Figure 1) [8, 18, 19].

Nitric oxide synthase (NOS), known as constitutive and inducible NOS according to their physicochemical and kinetic properties, are synthesized by three genes. Each of these genes creates NOS1, NOS2, and NOS3 isoforms. The isoforms of NOS enzyme exist in three forms as neuronal (nNOS), endothelial (eNOS) and inducible (iNOS) [20, 21].

nNOS and eNOS isoforms are called constitutive enzymes because they need Ca^{2+} to become active. Low concentration NO produced by these enzymes acts as an intracellular and intercellular messenger in the nervous system and smooth muscles. Thus, it activates cytoplasmic guanylate cyclase and increases the concentration of 3′,5′-cyclic guanosine monophosphate (cGMP) in cells. cGMP regulates the intracellular calcium concentration through various enzymes. The reason why nNOS and eNOS isoforms are synthesized in low amounts is that the enzyme becomes inactive with the decrease of intracellular ionized calcium concentration [22–24].

The iNOS isoform is secreted by stimulation of macrophages and other cell types in response to endotoxin and/or various cytokines, primarily phagocytic leukocytes. iNOS, whose activity is independent of calcium, is active as long as arginine is present. Thus, it has the ability to catalyze long-term and high concentration NO synthesis [24–26].

Nitric oxide has a role in regulating vascular tone, controlling blood pressure and kidney function. NO is also an important vasodilator formed in vascular endothelial cells. NO enters smooth muscle cells and stimulates soluble guanylate cyclase (sGC) to form cGMP. With an increase in cGMP concentration in the cell, cGMP activates one or more protein kinases, contributing to smooth muscle relaxation and vessel dilation [20].

Since the diffusion of NO radical between tissues is dependent on its reaction with oxyhemoglobin, NO radical can react locally within the tissue. The biological half-life of nitric oxide, which can dissolve in low molecular weight aqueous or hydrophobic environments, is 1–10 s, and its diffusion coefficient is determined as 3,300 $\mu m^2\ s^{-1}$ [21, 27].

Nitric oxide reacts with hydrogen peroxide and hypochlorous acid to produce dinitrogen thyroxide (N_2O^3), nitrite ($NO^-{}_2$) and nitrate ($NO^-{}_3$). The reaction between superoxide and nitric oxide radicals under physiological conditions is one of the most important reactions resulting in the formation of peroxynitrite. This reaction between the superoxide radical and nitric oxide is of great importance in terms

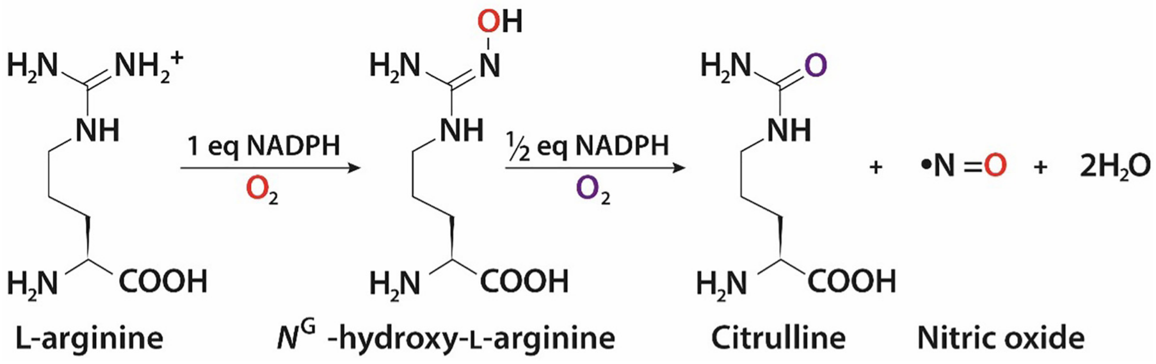

Figure 1: NO synthesis reaction catalyzed by NOS [18].

of maintaining the balance of ROS and the continuation of redox regulation [28, 29].

The superoxide radical can react with NO in aqueous solution to give the peroxynitrite anion.

$$O_2^- \bullet + \bullet NO \longrightarrow ONOO^- \qquad (14)$$

Peroxynitrite has been reported as a biologically cytotoxic intermediate. It is a potential source of toxicity with the formation of peroxynitrite-mediated hydroxyl radical or other radical species such as hydroxyl radical.

$$ONOO^- + H^+ \longrightarrow ONOOH \longrightarrow \bullet OH + NO_2 \qquad (15)$$

As a strong oxidant, peroxynitrite causes lipid peroxidation and protein oxidation in membranes, and the formation of toxic nitro-derivatives (nitrotyrosine) by nitrating phenolic amino acids such as tyrosine. In addition, it has high toxicity as it can oxidize and inactivate DNA, enzyme, protein, lipid and thiol groups [21].

$$ONOOH + RS^- \longrightarrow RSOH + NO_2^- \qquad (16)$$

Nitric oxide also reacts with lipophilic peroxyl radicals formed by the chain reaction of lipid peroxidation to form alkyl peroxynitrates (LOONO). If LOONO derivatives can be metabolized without releasing toxic free radicals, the interaction of NO with peroxyl radicals causes lipid peroxidation arrest [30].

Free radical sources

Endogenous sources

The substrates of $O_2^{-\cdot}$ and NO, which are among the two main pro-reactive species in biological systems, are O_2 and arginine, respectively. These radicals, which are the precursors of many reactions, are formed in compartments such as the membrane, mitochondria, endoplasmic reticulum or Golgi apparatus. In addition, stress and fatigue, immune system-induced free radical production may occur in response to pathogens [31, 32].

ROS production in the mitochondrial electron transport system

For the production of ATP, the electrons in NADH and $FADH_2$ in the mitochondria pass through the electron transport chain, and the oxygen is reduced to water by gaining four electrons. In the tetravalent reduction to H_2O, approximately 95% of O_2 is used without the formation of a free radical intermediate. While the remaining 5% O_2 is reduced, ROS such as superoxide and hydrogen peroxide are formed. Production of mitochondrial ROS is increased in mature cells with mitochondrial dysfunction and damaged membrane integrity [33, 34].

In the electron transport chain complex 1 and complex 3, during the transfer of electrons, as a result of the leakage of a few electrons, O_2 is reduced and $O_2^{-\cdot}$ radical formation occurs. The resulting superoxide radical is converted to H_2O_2 by the enzymes superoxide dismutase 1 (SOD1) and superoxide dismutase 2 (SOD2). H_2O_2 is also reduced to water by glutathione peroxidase [34, 35].

ROS production in the endoplasmic reticulum

The endoplasmic reticulum (ER) is the intracellular organelle responsible for post-translational modifications such as protein folding, disulfide bond formation, and protein folding. Protein disulfide isomerase (PDI) enzyme, which has a chaperone effect and prevents protein aggregation together with thiol oxidoreductase, plays a role in disulfide bond formation. ROS are produced during electron transfer from protein thiol to O_2 via oxidoreductin-1 (ERO-1) protein and protein disulfide isomerase (PDI), which oxidizes PDI [36, 37].

Production of ROS and RNS in peroxisomes

Peroxisomes, which play an important role in metabolic pathways such as pentose phosphate oxidative pathway, amino acid catabolism and fatty acid oxidation, are cellular hydrogen peroxide production sites. Peroxisomes containing ROS-producing enzymes such as palimtoyl-CoA oxidase, pipecolic acid oxidase, nitric oxide synthase, xanthine oxidase, D-aspartate oxidase, pristanoyl-CoA oxidase, hydrogen peroxide, superoxide, hydroxyl radical, nitric oxide, free radicals such as peroxynitrite contribute to the production [38, 39].

Production of ROS mediated by plasma membrane enzymes

As a result of the reactions catalyzed by lipooxygenase and cyclooxygenase enzymes, unstable peroxides can be formed from prostaglandin, thromboxane, and locotrienes, and ROS such as HO· and RO· may be formed from these peroxides [40].

Exogenous sources

Exogenous sources such as narcotic drugs and anesthetic gases, environmental agents, heavy metals, exposure to gamma radiation, exposure to non-ionizing radiation, exhaust gas, exposure to cigarette smoke, industrial pollutants, and xenobiotics induce the formation of ROS [41].

ROS-induced oxidative damage

Since free radicals are reactive molecules, they react with other molecules in biological systems and form oxidative stress. Here, the most affected cellular components can be listed as lipid, nucleic acids, proteins and enzymes. These effects are summarized in Figure 2.

Effect on lipids

As it is known, lipid peroxidation occurs by three types of mechanisms: non-enzymatic non-radical oxidation and free radical-mediated oxidation, enzymatic oxidation. Polyunsaturated fatty acids (PUFA), such as linoleic acid, linolenic acid, arachidonic acid, and eicosapentaenoic acid, are highly susceptible to free radical-mediated oxidation because they have multiple bonds. Lipid peroxidation begins when free radicals such as hydroxyl radical, alkoxyl radical (RO·) and peroxyl radical (ROO·) cleave the hydrogen atom of the methylene ($–CH_2–$) group of PUFA in the membrane structure [43–45].

$$RH + \bullet OH \longrightarrow H_20 + R\bullet \tag{17}$$

The lipid peroxyl radical is formed by adding oxygen to the alkyl radical formed.

$$R \bullet + O_2 \longrightarrow ROO\bullet \tag{18}$$

The lipid peroxyl radical cleaves a hydrogen atom from another polyunsaturated fatty acid, forming lipid hydroperoxide and a new lipid radical. These chain reactions are repeated over and over to form lipid hydroperoxides.

$$ROO \bullet + RH \longrightarrow R \bullet + ROOH \tag{19}$$

RO·, ROO· with perhydroxyl radical, superoxide anion, transition metal ions (Fe^{2+} and Cu^{+}) or metal complexes that are stable under physiological conditions form radicals such as [46].

$$ROOH + Fe^{2+} \longrightarrow Fe^{3+} + \bullet OH + RO \bullet \bullet \tag{20}$$

$$ROOH + Fe^{3+} \longrightarrow Fe^{2+} + H^{+} + ROO \bullet \bullet \tag{21}$$

$$ROOH + O_2 \longrightarrow O_2 + \bullet OH + RO\bullet \tag{22}$$

As a result of lipid peroxidation, reactive aldehydes and hydroperoxides such as malondialdehyde (MDA), 4-hydroxy-

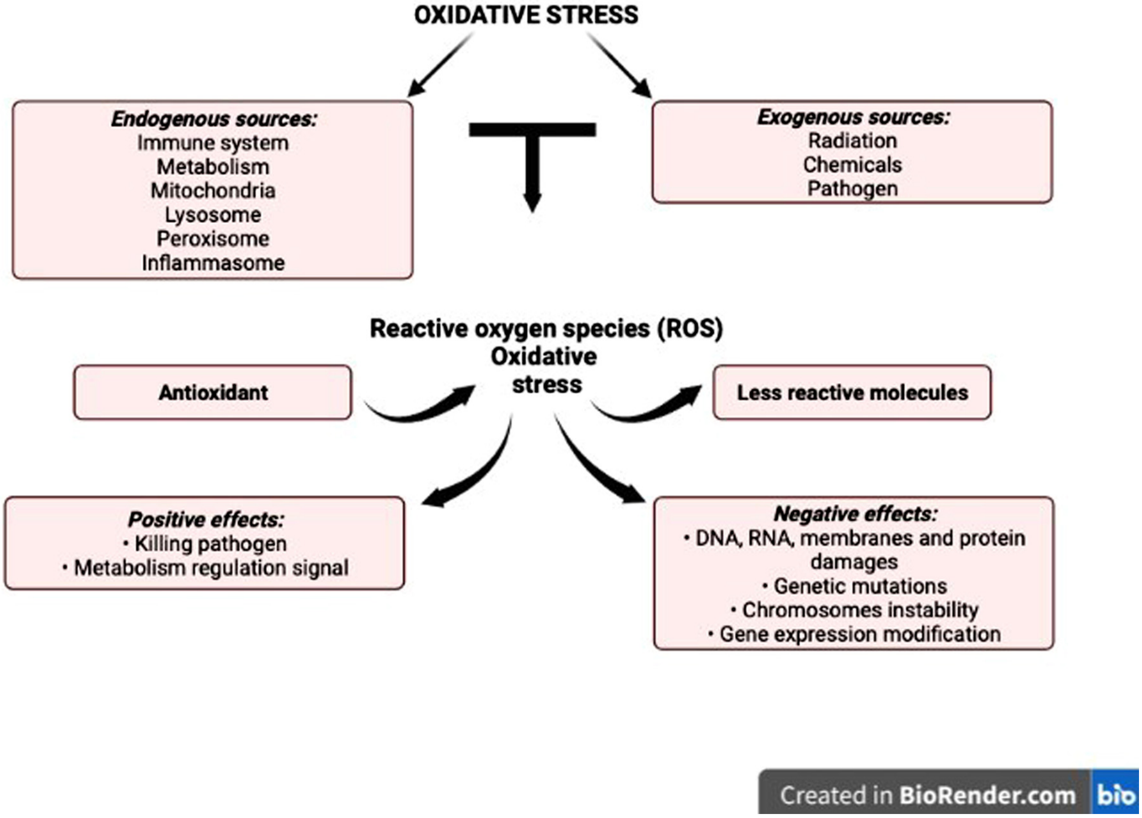

Figure 2: Sources, positive and negative effects of oxidative stress [42].

2-nonenal (HNE) and acrolein, prostanes and hydroxyoctadecanoic acid (HODE) are produced as end products. Lipid peroxidation associated with changes in a variety of biochemical pathways and it can damage the cell membrane by disrupting its fluidity, permeability and biophysical properties [44, 45, 47].

Effect on proteins

Proteins contain various functional groups such as simple hydrocarbons, thiol groups, alcohols and amines in their amino acid side chains. Proteins containing unsaturated bonds and sulfur can undergo oxidation by reacting with reactive oxygen and reactive nitrogen species. As a result of the interaction of free radicals and proteins, the function and activation of structural proteins can be inhibited. Thus, they can cause protein damage, as well as the formation of stable and highly reactive products such as protein hydroperoxides, and the formation of electrophilic molecules that react with proteins with nucleophilic side chains [48].

For example, under oxidative stress, cysteine may encounter oxidants $O_2^{-}\cdot$, HO·, ·NO, H_2O_2. Sulfenic acids, formed by the oxidation of the thiol in the side chain of cysteine, exhibit both electrophilic and nucleophilic characteristics and are highly reactive. The sulfenic and sulfonic acids formed cannot be reduced by thioredoxin or glutathione and undergo irreversible modification [49].

Effect on DNA

ROS such as hydroxyl radical and singlet oxygen acting on DNA cause disruption of cellular function through harmful modification of proteins and other molecules. They cause DNA base changes, single and double strand breaks, DNA-protein crosslinking and damage to the repair system [50].

Oxidative stress and diseases

The increase in the formation of reactive oxygen and reactive nitrogen species of endogenous or exogenous origin causes oxidative stress due to pro-oxidant and antioxidant imbalance that causes cellular damage in metabolism [1]. This can increase inflammation of cells, apoptosis and necrosis, damage to DNA base damage, DNA and chromosomal aberration, DNA single-stranded and double-stranded breaks, DNA and protein cross-links, lipid membrane, collagen structure and mitochondrial function. It is known that oxidative stress plays a role in many diseases such as cardiovascular diseases, chronic obstructive pulmonary disease, chronic kidney disease, neurodegenerative diseases and cancer.

It has been stated that hydrogen peroxide plays a role in the initiation and progression of vitiligo [51]. It has been reported that ROS has a role in the development of two forms of dermatitis, such as irritant contact dermatitis (ICD) and allergic contact dermatitis (ACD) [52].

ROS accumulation leads to adipose oxidative stress and is associated with clinical outcomes such as signaling, fat cell metabolism, type 2 diabetes, obesity, and insulin resistance [53]. Production of ROS as well as lipid peroxidation products and malondialdehyde increases in obese patients [54]. Nutritional status, hyperglycemia, hyperlipidemia and chronic inflammation increase oxidative stress in obesity [55, 56]. It has been determined that there is more ROS activity in the mitochondria of the airway smooth muscle and a decrease in the antioxidant level in asthma [57]. In the pathology of atherosclerosis, hypertension, myocardial infarction and ischemia, the imbalance between ROS secretion and endogenous antioxidant level is highly correlated [58].

The relationship between metabolic syndrome and obesity, diabetes, dyslipidemia, hypertension and impaired glucose tolerance, cardiovascular diseases is oxidative stress and redox imbalance caused by the continuation of chronic inflammation [59].

It has been observed that modified bases increase in the brain in Parkinson's disease, and protein carbonylation and nitration in Alzheimer's disease. Protein damage, lipid peroxidation, and DNA and RNA oxidation are present in patients with amyotrophic lateral sclerosis, whereas neuronal degeneration mediated by oxidative stress and mitochondrial dysfunction is present in Huntington's disease [60, 61]. An imbalance in intracellular and extracellular glutathione levels and a decrease in antioxidant levels were detected in children with autism diagnosis where oxidative stress markers increased [62]. An increase in ROS levels and a decrease in antioxidant levels in patients with rheumatoid arthritis indicate the presence of oxidative stress [63].

Recent studies indicate that the overproduction of ROS or RNS may cause damage to the male and female reproductive systems leading to infertility [64]. High levels of ROS cause seminal oxidative stress. This impair sperm quality and function by decreasing motility and progression, hyperactivation, capacitation, acrosome reaction and zona-pellucida penetration prior to fertilization [65, 66]. The increase in serum MDA, xanthine oxidase (XO) and SOD levels and the decrease in GSH levels of polycystic ovary syndrome (PCOS) patients reflect the effectiveness of oxidative stress [67]. In chronic kidney disease, oxidative stress-induced damage may occur as a result of high oxidation reactions in the mitochondria, decreased antioxidant levels, and increased ROS production [68, 69].

In osteoarthritis disease, ROS production induces the expression of matrix-degrading proteases. As a result, cartilage extracellular matrix synthesis decreases and may cause joint dysfunction [70]. Moderate ROS contribute to mutation by promoting stress signaling, while oxidative modifications in proteins and lipids favor tumor initiation and progression [71].

Finally, in Figure 3, oxidative stress-induced diseases in humans are summarized.

Defense systems against free radicals

Substances that can prevent or delay the oxidation of substances such as proteins, lipids, carbohydrates and DNA in biological systems are called antioxidants. Antioxidants can be described as a system that protects biomolecules and the organism against the harmful effects of free radicals, reduces or repairs the damage done by ROS to the target molecule, and this is called antioxidant defense [11, 73].

Antioxidants generally act by converting oxidants into weaker molecules, inactivating them by adding H^+, breaking chain reactions or repairing damaged biomolecules. In addition, they show their effects by preventing peroxidation chain reactions in lipid oxidation and/or collecting reactive oxygen species, inhibiting lipid peroxidation, and playing an active role in the conversion of peroxides to nonradical products such as alcohol. The level and composition of antioxidant defense systems differ from tissue to tissue and cell to cell. Antioxidant defense is not 100% effective in the organism, as it is constantly exposed to radical production in an O_2 environment [1, 11, 74].

Intracellular antioxidants

Superoxide dismutase (SOD)

SODs are metalloproteins that can catalyze the conversion of superoxide anion ($\cdot O_2^-$) to hydrogen peroxide (H_2O_2). This

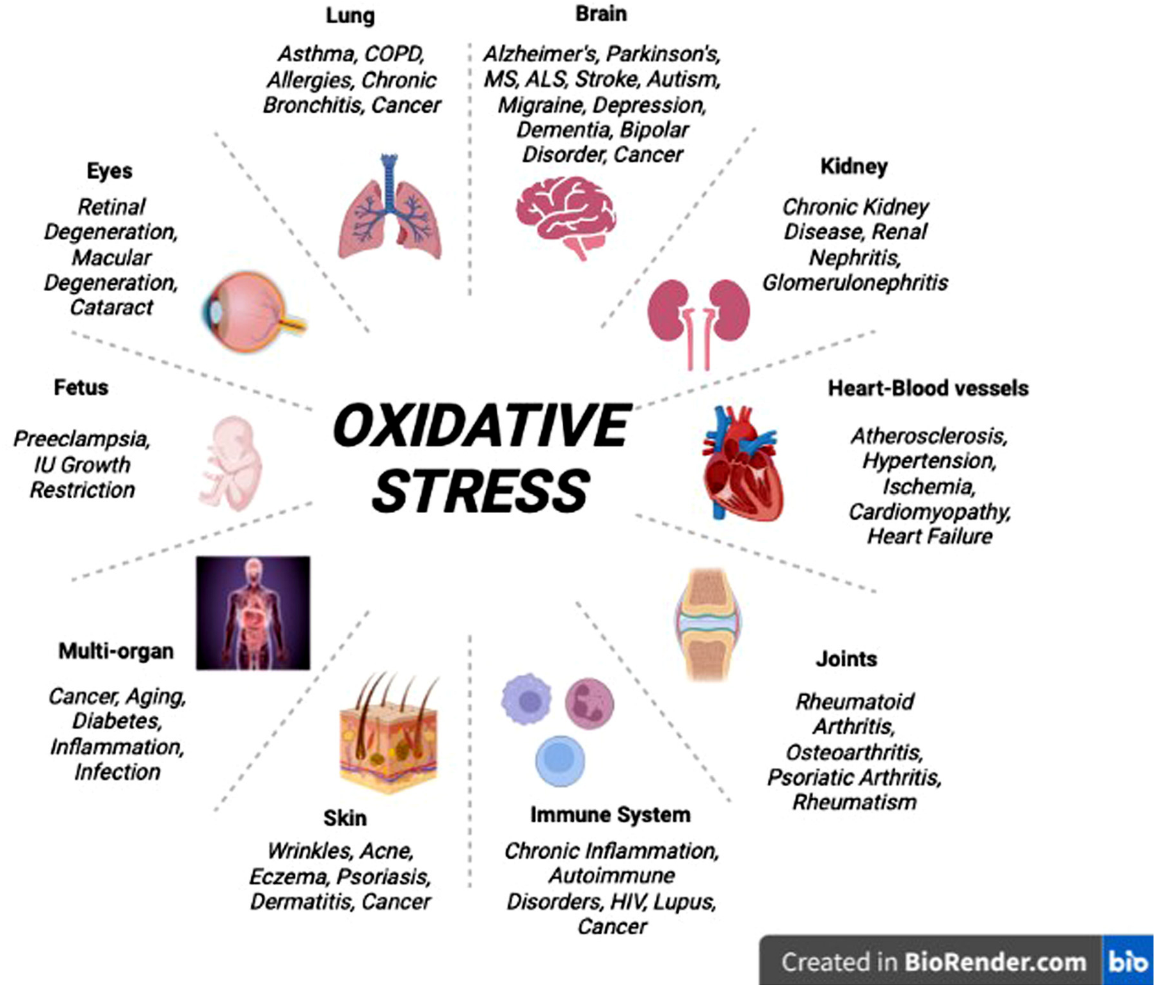

Figure 3: Oxidative stress-induced diseases in humans [72].

reaction is the cellular source of H_2O_2. SODs detoxify superoxide anions, preventing their reaction with NO and preventing the formation of peroxynite. In mammals, there are three isoforms of superoxide dismutase found in the cytosol: copper, zinc superoxide dismutase (Cu, ZnSOD or SOD1), manganese superoxide dismutase (MnSOD or SOD2) found in the mitochondrial matrix, and superoxide dismutase (SOD3) found in the extracellular space [75].

$$2O_2^{-}\bullet + 2H \xrightarrow{\text{SOD}} H_2O_2 + O_2 \tag{23}$$

Catalase (CAT)

The enzyme catalase, which is a hemoprotein, can detoxify hydrogen peroxide into water under high concentration hydrogen peroxide conditions.

$$2H_2O_2 \xrightarrow{\text{CAT}} 2H_2O + O_2 \tag{24}$$

In mammals, catalases can also catalyze peroxidase-type reactions, provided that substrates have limited access to heme. It is mostly found in peroxisomes. Its activity is high in the liver, kidney, myocardium, striated muscle and erythrocytes [76, 77].

Glutathione peroxidase (GPx)

Glutathione peroxidase is the multiple isozymes responsible for the reduction of hydrogen peroxides. There are six GPx isozymes in mammalian tissues, expressed as GPx1, 2, 3, 4, 5, and 6. GPx1, 2, 3 and 4 isozymes are selenoprotein. The interlocking enzyme system GPx and glutathione reductase (GR) catalyze the reduction of H_2O_2 by consuming glutathione. GPx enzymes catalyze the GSH-dependent reduction of fatty acid hydroperoxides other than H_2O_2 and various synthetic hydroperoxides such as cumene and *t*-butyl hydroperoxides [78, 79].

$$H_2O_2 + 2GSH \xrightarrow{\text{GPx}} GSSG + 2H_2O \tag{25}$$

$$LOOH + 2GSH \xrightarrow{\text{GPx}} LOH + GSSG + H_2O \tag{26}$$

Glutathione (GSH)

Glutathione (γ-Glutamyl Cysteinyl Glycine) is an intracellular antioxidant and a tripeptide found in low concentrations in the extracellular distance. In mammalian cells and tissues, GSH is involved in reactions with reactive oxygen species, electrophiles, non-enzymatic antioxidants, and protein degluthionylase. It protects the cell against oxidative damage by creating an environment with a high redox potential inside the cell. It protects cells against oxidative damage against glutathione, hydrogen peroxide, hydroxyl radical, superoxide anion and alkoxyl radicals and prevents inactivation of proteins and enzymes by keeping the sulfhydryl groups of proteins in a reduced state [80–82].

Glutathione S transferase (GSTs)

A family of enzymes that catalyze the detoxification of low concentration hydrogen peroxide and the conjugation of GSH to a wide variety of xenobiotics. In mammalian tissues, cytosolic, mitochondrial and microsomal GSTs are membrane-associated proteins in eicosanoid and glutathione metabolism. Some GSTs show GPx-like activity with organic hydroperoxides, previously called non-selenium glutathione peroxidase activity. They catalyze the reaction of organic peroxides with GSH to form GSSG and alcohols [83–85].

$$\text{Xenobiotic} + GSH \xrightarrow{\text{GST}} \text{Xenobiotic} - GS \tag{27}$$

$$LOOH + GSH \xrightarrow{\text{GST}} LOH + GSSG + H_2O \tag{28}$$

Glutathione reductase (GR)

Glutathione (GSSG), which is oxidized by reactions with reactive oxygen species, is converted back into reduced form by using NADPH as a cofactor by the glutathione reductase enzyme. This reaction is important in regulating cellular redox homeostasis and detoxification reactions of ROS [86].

$$GSSG + NADPH + H \xrightarrow{\text{GR}} 2GSH + NADP^{+} \tag{29}$$

In addition, thioredoxin reductase (TRX), thioredoxin peroxidase (PRX), which is characterized in human cells, is important in the detoxification of hydrogen peroxide. There are studies showing that PRX acts as peroxynitrite reductase and may have functionality as a protective molecule in ROS-mediated lung injury [87, 88].

Dietary antioxidants

The human diet contains a number of different compounds with antioxidant capacity [89]. Ascorbic acid, a water-soluble vitamin, neutralizes hydrogen peroxide, superoxide, and hydroxyl radicals. The monoanion form ascorbate, which is dominant at physiological pH, scavenges thiyl, nitroxide and oxysulfide radicals. Protects lipids against oxidation by neutralizing radicals that initiate lipid peroxidation. It reduces the tocopheroxyl radical, which is responsible for the regeneration of vitamin E, to α-tocopherol. It prevents LDL oxidation with vitamin E [90, 91].

α-Tocopherol, a fat-soluble vitamin, reacts with lipid peroxyl radicals. Tocopherols and tocotrienols inhibit lipid peroxidation. It also prevents the initiation of lipid peroxidation by scavenging $HO_2\cdot/O_2^{-}\cdot$ and hydroxyl radicals of α-tocopherol, protecting the membranes from oxidative damage. Tocopherols can exert pro-oxidant effects *in vitro* by reducing Fe^{3+} to Fe^{2+} and Cu^{2+} to Cu^{+} [92, 93].

Carotenoids such as β-carotene and lycopene act as antioxidants by scavenging singlet oxygen and inhibiting lipid peroxidation, preventing the formation of β-carotene peroxide radicals, protecting the cell from oxidative stress [94].

Polyphenols prevent the formation of ROS by chelating the free Fe and Cu involved in the Habern–Weiss and Fenton reactions. There is increasing evidence that polyphenols protect cells against oxidative damage, limiting the risk of various degenerative diseases associated with oxidative stress [95]. For example, Thymoquinone is the main component of the essential oil of *Nigella sativa*, has been reported to have many properties such as antioxidant, anti-inflammatory, antineoplastic, and antiviral [96].

Conclusions

The increase in the formation of reactive oxygen and reactive nitrogen species of endogenous or exogenous origin causes oxidative stress due to pro-oxidant and antioxidant imbalance that causes cellular damage in metabolism. The resulting unstable, reactive and unpaired valence-electron radicals have a high ability to react with biomolecules. Free radicals cause deterioration in the structure and functions of proteins, damage to the cell membrane structure by lipid peroxidation, nucleic acid base modifications and chromosomal changes, causing oxidative damage to cellular structure and components. Oxidative stress causes many diseases such as cancer, metabolic syndrome, Type 2 Diabetes, atherosclerosis, malaria, Alzheimer's and rheumatoid arthritis and neurodegenerative diseases. Antioxidants, on the other hand, are defense systems that protect biomolecules and the organism against oxidative damage and reduce the damage done by reactive oxygen species. In order to be protected from oxidative damage caused by free radicals, seasonal vegetables and fruits should be consumed regularly.

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