OXIDATIVE STRESS AND ANTIOXIDANT DEFENSE SYSTEMS

EDITOR

ASSIST. PROF. DR. SUZAN ONUR

SERÜVEN YAYINEVİ

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web: www.seruvenyayinevi.com
e-mail: seruvenyayinevi@gmail.com

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CONTENTS

Chapter 1

OXIDATIVE STRESS AND FORMATION MECHANISMS

Suzan ONUR[1]
Mustafa CENGİZ[2]
Adnan AYHANCI[3]

1 Dr. Öğr. Üyesi, Karabük Üniversitesi, Sağlık Bilimleri Fakültesi, Fizyoterapi ve Rehabilitasyon Bölümü, Nörolojik Rehabilitasyon Anabilim Dalı, Karabük suzanonur@karabuk.edu.tr
2 Doç. Dr., Eğitim Fakültesi, Matematik ve Fen Bilimleri Eğitimi Bölümü, Fen Eğitimi Anabilim Dalı, Siirt, m.cengiz@siirt.edu.tr
3 Prof. Dr., Eskişehir Osmangazi Üniversitesi, Fen Fakültesi, Biyoloji Bölümü, Genel Biyoloji Anabilim Dalı, Meşelik, Eskişehir, aayhanci@ogu.edu.tr

Oxidative stress (OS) signifies an unstable condition occurring at the cellular level; here, the ability of cells to cope with free radicals in order to perform their normal physiological functions becomes overwhelmed due to either excessive production of free radicals or inadequacy of antioxidant defense systems. OS has been associated with many pathological conditions in biological systems and plays a significant role in the pathogenesis of a range of diseases such as aging, cancer, cardiovascular diseases, and neurological disorders.

In this section, the molecular mechanisms of oxidative stress, its cellular effects, and potential impacts on health will be discussed. Understanding the complex processes underlying OS is critical not only for identifying new targets in disease prevention and treatment but also for serving as a valuable resource for researchers, clinical specialists, and students interested in comprehending the fundamental principles and clinical significance of OS.

INTRODUCTION

Humans with aerobic metabolism potentially generate many free radicals (FRs) throughout their lives, which can affect various systems and metabolism. These FRs can arise during normal metabolic processes as well as due to various external factors. FRs are defined as molecules with one or more unpaired electrons, high energy, instability, low molecular weight, and can cause oxidative damage in the cell by altering macromolecules such as DNA, proteins, lipids, leading to functional loss (1). Under normal physiological conditions, there is a balance between reactive oxygen species (ROS) constantly produced in cells and antioxidants that interact with them. The disruption of this balance in favor of ROS accumulation in the cell or inadequate endogenous defense systems is defined as oxidative stress (2, 3). Oxidative stress (OS) arises from an imbalance between the generation of reactive oxygen species and the body's antioxidant defense system, leading to an excess of free oxygen radicals (1) (Figure 1).

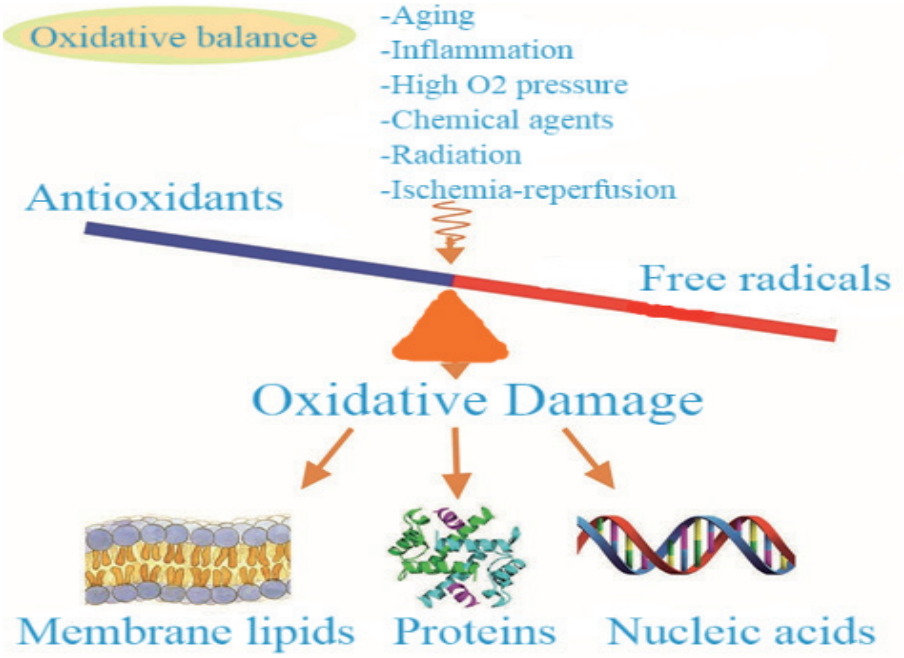

Figure 1. *Oxidative balance*

FREE RADICALS (FRs)

Biological Impact, Molecular Mechanism and Damage Mechanism of Free Radicals

Free radicals are reactive chemical species that play a significant role in biological systems (4). These are molecules that have lost one or more electrons and are therefore highly reactive chemically. They can arise from normal physiological processes as well as from environmental factors (5). FRs can damage biological molecules at the cellular level, such as lipids, proteins, and DNA (6). These damages are associated with a phenomenon called OS and play an important role in the pathophysiology of various diseases (7).

Substances with unpaired electrons in their outer orbitals are termed free radicals. In simpler terms, these are atoms or molecules that have an unpaired electron in their structure due to an open electron shell configuration (8, 9). While the terms radical and free radical are often used interchangeably, the term radical refers to the form of free radicals bound by water molecules (8). Molecules carrying unpaired electrons (e-) in their outer orbitals, defined as FRs (10), are reactive and short-lived. FRs are any atoms or molecules that are produced in many physiological or pathological conditions and contain one or more unpaired electrons. Also known as oxidant molecules or reactive

oxygen species, these molecules readily engage in electron exchange with other molecules (11). It is acknowledged that FRs, which can be generated as a continuation of normal metabolism or by many reactions necessary for energy production in the cell, are primarily formed by three basic mechanisms (12).

1.Covalent cleavage of a normal molecule with one of the shared electrons remaining in each fragment,

X:Y → X• + Y•

2.The loss of a single electron from a normal molecule or the heterolytic cleavage of a molecule, (where in heterolytic cleavage, both electrons forming the covalent bond remain with one of the atoms)

A - e- → A• + e

3. The addition of a single electron to a normal molecule,

A + e- → A•-

A compound can become a free radical through either losing one electron (reduction) or gaining an extra electron (oxidation). Free radicals may be present within larger molecules or exist as small, freely diffusible entities (13-16).

In biological systems, free radicals predominantly arise as a result of electron transfer (17). Radicals produced in the body should not always be considered as hazardous chemical species. Indeed, it is essential to convert oxygen into reactive forms for biochemical reactions. Controlled production of free radicals facilitates the occurrence of essential life cycles. The production of nitric oxide radicals, like oxygen radicals, is an indispensable biological event. Nitric oxide is one of the most common signaling molecules and participates in cellular activities and organ functions in the body (18). Factors determining whether these radicals are termed 'good' or 'bad' are related to where and how much they are produced. While reactive free radicals regulate important physiological reactions in the organism, such as killing microorganisms, regulating smooth muscle tone, and cytochrome P450 oxidations, excessive production can lead to harmful effects and OS in cells and tissues (19). OS is defined as the disruption of the pro-oxidant and antioxidant balance in the organism. Free radicals are extremely detrimental as they induce lasting damage to the DNA, protein, and lipid structures within our cells (16, 20, 21). Radicals exhibit a highly variable structure in terms of reactivity and are less stable compared to non-radical structures. Furthermore, once radicals are formed, they can react with other radicals as well as other molecules. The rate and selectivity of these reactions depend on the radical concentration and the presence of weak bonds in the molecule (21).

The beneficial effects of free radicals can only be considered when they are maintained at low levels. They are known to participate in cellular signaling processes, including the release of calcium from intracellular reservoirs, activation of tyrosine amino acids through phosphorylation, and initiation of growth factor signaling pathways. Additionally, they serve defensive roles such as combating infections, destroying cancer cells, and detoxifying xenobiotics (22).

Free radicals are constantly generated by mitochondria during the body's regular oxygen consumption. These radicals, formed during energy generation processes, have the potential to alter the structure of lipids, proteins, and nucleic acids. Free radicals originating from various internal and external sources can yield both advantageous and detrimental effects (22). FRs that can occur during oxidation and reduction reactions in the body, can also be produced by external (exogenous) factors (6, 16, 20). The most significant exogenous sources of free radicals are cigarette smoke, radiation, toxic gases, carcinogens, certain drugs, and pesticides. Excessive alcohol consumption, smoking, electromagnetic radiation, sunlight, chronic inflammations, excessive iron overload, excessive physical activity, aging, birth control pills, and pregnancy are also sources of FRs (16, 20). Endogenous and exogenous sources constantly producing free radicals in the cell and the environment are shown in Table 1.

Table 1. *Endogenous and Exogenous Sources of Free Radicals (22-27)*

Endogenous Sources
1.During oxidative phosphorylation in the mitochondria, oxygen radicals are produced as byproducts catalyzed by the electron transport system.
2.Inflammatory conditions release cytokines, leading to the production of free radicals by neutrophils and macrophages.
3.Free radicals can originate from different sources, including lipid peroxidation, xanthine oxidase, and mitochondrial cytochrome oxidase.
4.Smooth muscle cells, platelets, and arachidonic acid metabolism can generate free radicals.
5.Leakage of electrons in the endoplasmic reticulum through enzymes like xanthine oxidase (XO) and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase during autoxidation reactions and in the cytochrome P450 system.
6.Stress due to mental stress or physical fatigue can generate free radicals as toxic byproducts. Additionally, hormones like cortisol and catecholamines, which can induce stress reactions in the body, can themselves be converted into free radicals.
7.Immune system cells can produce reactive oxygen species (ROS) and oxy-radicals in response to pathogens.
Exogenous Sources
1.X-rays, UV rays, microwaves, and gamma rays,
2.Combustion of organic matter during cooking,
3.Volcanic activities and forest fires,
4.Air pollutants such as benzene, asbestos, formaldehyde, carbon monoxide, toluene, and ozone,
5.Chemicals found in adhesives, cleaning products, solvents, paints, insecticides, and perfumes,
6.Water pollutants such as chloroform and other trihalomethanes,
7.Smoking and exhaust fumes, alcohol, and tobacco use can induce free radical production.

Free radicals cause numerous effects at the molecular level in the organism. An increase in ROS which are toxic to cells, leads to damage to proteins, lipids, and nucleic acids within the cell, disrupting intracellular signaling pathways (28). Radicals can react with all cellular macromolecules, and three types of reactions are particularly important in the formation of cellular damage:

- Lipid peroxidation
- Oxidative modification of proteins
- DNA damage

The effects of oxidative stress on these pathological conditions occur through various mechanisms, including the disruption of cellular signaling pathways, increased inflammation, and increased cellular damage. The biological effects of free radicals and the pathophysiological role of OS are of great interest in modern medicine. Therefore, research on free radicals is considered an important area for developing new approaches for disease prevention and treatment. The symbols and characteristics of free radicals are shown in Table 2.

Table 2. *Symbols and Characteristics of Free Radicals (29)*

Free Radical	Symbol	Characteristic
Hydrogen	**H**	The simplest radical.
Superoxide	**$O_2 \cdot^-$**	The first intermediate of oxygen metabolism.
Hydroxyl	**$OH^\bullet$**	The most toxic (reactive) oxygen metabolite radical.
Hydrogen peroxide	**H_2O_2**	Low reactivity, weak ability to cause molecular damage.
Singlet oxygen	**O_2^-**	Short half-life, potent oxidative oxygen form.
Hydroperoxyl radical	**$HO_2^\cdot$**	Rapidly soluble in lipids, increasing lipid peroxidation.
Peroxide radical	**ROO^-**	Less effective than hydroxyl radical, localized in lipids.
Trichloromethyl	**CCl_3**	A radical produced in the liver as a metabolite of CCl_4 metabolism.
Thyl radical	**$RS^\bullet$**	General name for species containing sulfur and unpaired electrons.
Alkoxyl	**$RO^\bullet$**	An oxygen metabolite produced by the breakdown of organic peroxides.
Nitrogen oxide	**NO**	Produced in vivo from the amino acid L-arginine.
Nitrogen dioxide.	**NO_2**	Produced from the reaction of NO with oxygen.

The most important free radicals that occur are:

- Superoxide radical ($O_2 \cdot^-$),
- Hydrogen peroxide (H_2O_2),

- Hydroxyl radical ($OH^{\bullet}$),
- Singlet oxygen (O_2^{-}) (30, 31).

Superoxide Radicals ($O_2^{\bullet -}$)

Superoxide radical is a weak oxidant that alone may not lead to serious cellular damage. However, it can initiate a series of reactions that can lead to oxidative stress (OS) (16, 32, 33). One of the primary sources of superoxide production is Coenzyme Q, which is generated not only within the mitochondrial electron transport chain but also at other locations along this chain of electron transfer. This radical gives rise to other reactive oxygen species (ROS), which generally do not stray far from their origin (30, 34).

$$O_2 + é \rightarrow O_2^{\bullet -}$$

$$H_2O_2 + O_2 \rightarrow HO^- + OH^- + O_2$$

The OH- radicals generated are exceedingly reactive and can inflict considerable harm by interacting with structures such as DNA (16, 35, 36).

The half-lives of superoxide radicals, which generate H_2O_2 and oxygen via dismutation reaction, are relatively brief. The dismutation reaction happens spontaneously and is catalyzed by the Superoxide Dismutase (SOD) enzyme (16).

$$O_2^- + O_2^- + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

They are formed as a result of molecular oxygen (O_2) being reduced by accepting one electron in aerobic cells. They are especially produced endogenously in electron-rich environments such as the inner mitochondrial membrane and by flavoenzymes like xanthine oxidase (a).

$$HO_2 \rightarrow H^+ + O_2^{\bullet -} \text{ (a)}$$

Additionally, the autoxidation of reduced transition metals can generate superoxide radicals (b) (37).

$$Fe^{2+} + O_2 \rightarrow Fe^{3+} + O_2^{\bullet -} \text{ (b)}$$

Hydrogen Peroxide (H_2O_2)

Although not a free radical, hydrogen peroxide forms free radicals by reacting with a transition metal (e.g., Fe^{+2}) (34).

Hydroxyl Radicals ($OH^{\bullet}$)

The most potent free radical found in biological systems is the hydroxyl radical. In irradiated tissues, a portion of the energy is absorbed by intracellular

water, leading to the formation of a covalent bond between oxygen and hydrogen by radiation, resulting in the production of hydrogen (H) and hydroxyl radicals.

$$H - O - H \rightarrow H + OH^{\cdot}$$

Hydroxyl radicals can participate in radical formation and engage in a series of reactions. They incorporate into the structure of bases found in DNA and RNA, causing strand breaks in DNA by inducing damage to the bases and sugars of DNA. If the damage is extensive, it may not be repairable by cellular protective systems, leading to mutations and cell death (30, 35, 38).

Singlet oxygen (O_2^-)

Singlet oxygen is the name given to the excited state of oxygen, and it is a non-radical reactive oxygen species with very high reactivity. It reacts directly with unsaturated fatty acids to form peroxyl radicals, initiating lipid peroxidation as potent as hydroxyl radicals (29).

REACTIVE OXYGEN SPECIES

Biological Effect, Formation Mechanism, and Function of Reactive Oxygen Species

Reactive oxygen species (ROS) are oxygen derivatives that occur naturally in biological systems or due to various internal and external factors (39). They are produced as a normal part of cellular metabolism, but imbalances can trigger OS (4). ROS can damage various biological molecules, from cell membranes to DNA, and play a significant role in the pathogenesis of various pathological conditions (5).

Though oxygen is vital for human existence, certain reactive oxygen species (ROS) generated during regular metabolism possess the capacity to inflict substantial damage on the body. In contrast to typical oxygen molecules, ROS, predominantly comprising free radicals, manifest as oxygen species with heightened chemical reactivity (40).

The biological effects of ROS occur through various molecular mechanisms at the cellular level (7). For example, ROS can trigger lipid peroxidation and disrupt the structure and function of cell membranes (41). Additionally, ROS can cause oxidation of proteins, leading to changes in protein functions and disruption of cellular homeostasis (42).

The oxygen present in the atmosphere is called molecular oxygen or dioxygen. A small portion of normal oxygen is converted to ROS during metabolism in cellular compartments, mainly mitochondria. The main reactive oxygen species are superoxide radical, hydroxyl radical and hydrogen peroxide.

The first two are free radicals, while hydrogen peroxide is a prooxidant (43). The endogenous and exogenous sources of reactive oxygen species are shown in Figure 2.

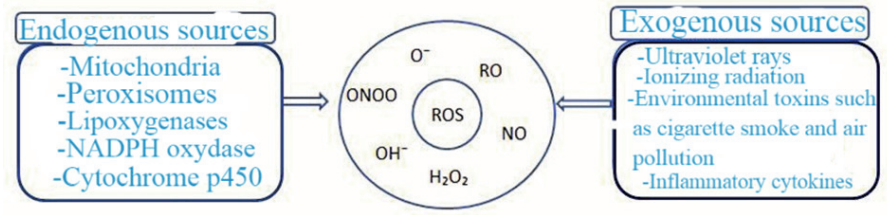

Figure 2. *Endogenous and Exogenous Sources of Reactive Oxygen Species*

Hydrogen Peroxide (H_2O_2)

Hydrogen peroxide, although not a free radical, falls within the scope of ROS and plays a significant role in the formation of free radicals. Numerous enzymes located within cellular compartments, including urate oxidase, glucose oxidase, and D-amino acid oxidase, directly generate hydrogen peroxide by transferring two electrons to oxygen. In the presence of Fe^{2+} or other transition metals (c) and superoxide radicals (d), it generates the most potent radical, hydroxyl radical (44, 45).

$$H_2O_2 + Fe^{2+}\ Fe^{3+} + OH^- + OH^{\bullet}$$ (c) Fenton reaction

$$O_2^{\cdot -} + H_2O_2\ O_2 + H_2O + OH^{\bullet}$$ (d) Haber-Weiss reaction

Hydrogen peroxide, unlike the superoxide radical, is soluble in fat, so it can cause damage to cellular membranes containing Fe^{2+} even at a distance from where it is formed.

Hydroxyl radicals ($OH^{\bullet}$)

Despite having a very short half-life of about 10-9 seconds, hydroxyl radicals are the most powerful ROS due to their extreme reactivity (46). Hydroxyl radicals are formed from hydrogen peroxide through the Fenton and Haber-Weiss reactions in the presence of transition metals (c, d). They can abstract a proton from various molecules such as thiols and fatty acids at their site of formation, leading to the generation of new radicals and causing damage to the cell (47).

OS caused by ROS plays a critical role in the pathogenesis of many diseases (48). Various pathological conditions such as cancer, cardiovascular diseases, neurodegenerative diseases, and inflammatory diseases are affected by the

biological effects of ROS (6). The effects of OS on these pathological conditions occur through various mechanisms, including disruption of cellular signaling pathways, increased inflammation, and increased cell damage. The biological effects of ROS and the pathophysiological role of OS are a major focus in modern medicine (39). Therefore, research on ROS is considered an important area for developing new approaches for the prevention and treatment of diseases.

When the body's defense systems against OS are inadequate, oxidative damage occurs in cells. As a result of this damage, significant disruptions occur in body functions. It is crucial to prevent and treat these disruptions with naturally occurring and externally obtained antioxidants. It is known that antioxidants have protective effects on lipids, proteins, nucleic acids, and other target macromolecules by preventing the oxidation of unsaturated fatty acids in cell membranes. Therefore, maintaining the balance between oxidants and antioxidants in the human body is essential for maintaining a healthy life (50).

CONCLUSION

In this section, we have discussed how oxidative stress occurs at the cellular and molecular levels, the important characteristics of free radicals, and their effects on biological systems. Oxidative stress is defined as an imbalance at the cellular and molecular levels, while free radicals are chemical species with unpaired electrons.

It has been observed that oxidative stress is associated with pathophysiological conditions and plays a role in the development of many diseases such as aging, cancer, and diabetes. Furthermore, it has been emphasized that a better understanding of the cellular and molecular mechanisms is needed for the prevention and intervention of oxidative stress.

In conclusion, understanding the complex effects of oxidative stress and free radicals on biological systems continues to be an important area of research and clinical application in the field of health. Advances in this area could potentially have significant impacts on the prevention and treatment of future diseases. This conclusion summarizes the topics covered in the book chapter and provides some recommendations for future research directions.

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Chapter 2

THE EFFECTS OF OXIDATIVE STRESS ON CELLULAR STRUCTURES: LIPID PEROXIDATION

Ilknur KULCANAY ŞAHIN[1]

Canan VEJSELOVA SEZER[2]

Adnan AYHANCI[3]

1 Dr. Öğr. Üyesi İlknur KULCANAY ŞAHİN, Kırıkkale Üniversitesi, Sağlık Hizmetleri MYO, Tıbbi Teknikler ve Hizmetler Bölümü, Kırıkkale, ilknurkulcanay@hotmail.com

2 Dr. Öğr. Üyesi, Eskişehir Teknik Üniversitesi, Fen Fakültesi, Biyoloji Bölümü, Eskişehir, cananveyselova@gmail.com

3 Prof. Dr. Adnan AYHANCİ, Eskişehir Osmangazi Üniversitesi, Fen Fakültesi, Biyoloji Bölümü, Genel Biyoloji Anabilim Dalı, Meşelik, Eskişehir, aayhanci@ogu.edu.tr

Lipid peroxidation occurs when free radicals (in example OH·, O_2^-) and H_2O_2 attack the phospholipids or polyunsaturated fatty acids of cellular and/or organelle membranes. This process, known as "autoxidation," produces various aldehydes, ketones, alkanes, carboxylic acids, and polymerization products, which are highly reactive with other cellular components and act as biological indicators of lipid peroxidation. When free radicals attack polyunsaturated fatty acids of the cell membrane, they initiate an lipid peroxidation chain reaction, forming a carbon-centered radical (R*). The carbon-centered radical reacts with O_2, creating a lipid hydroperoxide (ROOH), which propagates the chain reaction by generating lipid peroxyl radicals (ROO). One of the particularly hazardous aldehyde end product of lipid peroxidation is malondialdehyde. It induces structural changes in DNA and proteins, like fragmentation, modification, and aggregation. The detrimental effects of oxidative damage can trigger pathways leading to either necrotic or apoptotic cell death. Lipid peroxidation and macromolecular oxidation disrupt membrane permeability and electrolyte balance through the excessive binding of reactive aldehydes to cellular proteins. Lipid hydroperoxides impair membrane function by allowing uncontrolled ion passage and increasing rigidity. Protein degradation caused by oxidative stress leads to the progressive breakdown of biological systems. These cascading effects can overwhelm cellular repair mechanisms, ultimately triggering uncontrolled cell death, and contribute to the development and progression of various pathological conditions, underscoring the importance of mitigating free radical-mediated damage for maintaining cellular and organismal health.

INTRODUCTION

In living organisms, highly reactive chemical species are generated as unavoidable byproducts of essential metabolic and energetic processes. The primary source of highly reactive chemical species within cells is the leakage of electrons to oxygen during the oxidative reactions occurring in the mitochondria and endoplasmic reticulum. This process produces the superoxide anion (O_2•–), which is then converted into hydrogen peroxide (H_2O_2) that is another reactive oxygen compound. When H_2O_2 interacts with certain transition metal ions, such as ferrous iron (Fe^{2+}) or cuprous copper (Cu^+), it can undergo the Fenton reaction, forming the highly reactive hydroxyl radical (OH•). This peroxyl radical is considered the most reactive radical species present in the body, with an extremely short half-life and a propensity to indiscriminately interact with a wide range of biological macromolecules. Due to their extreme reactivity, these free radical species can modify the structure and function of crucial biological macromolecules, including lipids, proteins, and nucleic acids, leading to damage at the cellular, tissue, and organ levels. One of the most significant consequences of free radical-induced damage is lipid peroxidation, which occurs when the polyunsaturated fatty acids within

cell membrane lipids are exposed to and interact with these reactive species. In case of disrupt of peroxyl radical neutralization generated during lipid peroxidation by the cell's antioxidant defense mechanisms, the radicals can trigger a self-sustaining chain reaction that continues to generate intense lipid peroxide compounds. This process is especially hazardous, as the degradation of lipid peroxides can result in the production of various aldehydes, many of which have potent biological activity. These reactive aldehydes, such as malondialdehyde and 4-hydroxynonenal, can either be metabolized within the cell or diffuse from their site of origin to damage other cellular components. The presence of these aldehyde byproducts of lipid peroxidation is often used as a biomarker of oxidative stress, as they can contribute to the disruption of cellular signaling pathways, the impairment of protein function, and the induction of further oxidative damage. The delicate balance between the generation of these reactive oxygen species and the antioxidant defense mechanisms of the cell are crucial for maintaining cellular homeostasis and overall organismal health. Disruption of this equilibrium can lead to the accumulation of oxidative damage and contribute to the development and/or progression of a variety of pathological conditions, including cardiovascular diseases, neurodegenerative disorders, cancer, and accelerated aging. Ongoing research in the field of free radical biology and oxidative stress continues to elucidate the complex interplay between different reactive oxygen and nitrogen species, their specific cellular targets, and the intricate mechanisms by which they can influence cellular function and organismal wellbeing. This knowledge is essential for the design of targeted interventions and the development of effective therapeutic strategies aimed at mitigating the detrimental effects of oxidative stress on human health.

1.Free Radicals as Reactive Compounds

According to the quantum chemistry principles, a bond can accommodate a maximum of two electrons in a highly stable configuration. In biological systems, electrons predominantly exist in paired states. When a bond is disrupted, the two electrons can either remain together within the same atom, forming an ion, or they can separate, resulting in the formation of a free radical. Free radicals are defined as atoms or molecules possessing an unpaired electron in their outer orbital, rendering them incomplete and unstable. Reactive Oxygen Species (ROS) are characterized by the presence of these unpaired, high-energy electrons in their outer orbitals. This allows them to readily interact with surrounding molecules, exchanging electrons in an attempt to stabilize themselves. This interaction can trigger a chain reaction of damage, as the molecules that react with free radicals also become free radicals themselves. The term "Reactive Oxygen Species" is often used interchangeably with "free oxygen radicals," as it encompasses both radical and non-radical oxygen-containing molecules that can initiate the formation of oxygen radicals

through their reactions. While oxygen is essential for sustaining life, it can also cause cellular damage under certain conditions due to the increased production of ROS. Under normal physiological conditions, the level of ROS generated does not exceed the antioxidant defense mechanisms of the body. However, when the balance is disrupted, these highly reactive species can interact with and damage a wide range of organic and inorganic molecules, including proteins, lipids, and carbohydrates. Within cells, these radicals can interact with nucleic acids and membrane molecules, causing structural breakdown and compromising cellular function. Free radicals are generated under various physiological and pathological conditions and are often referred to as oxidant molecules or reactive oxygen particles due to their ability to readily exchange electrons with other molecules. Mitochondria routinely produce free radicals as a byproduct of normal oxygen consumption and energy production. These free radicals can alter the structure of lipids, proteins, and nucleic acids, leading to cellular damage. Free radicals originate from numerous endogenous and exogenous sources, including mitochondria, and cause a range of damage while also providing certain benefits. At low concentrations, free radicals play a role in activating cellular signals such as calcium release from intracellular stores, tyrosine phosphorylation, growth factor signaling, and defense mechanisms against infections, cancer cells, and xenobiotic detoxification. However, free radicals are beneficial only at low concentrations. While oxygen is essential for sustaining life, it can also cause cellular damage under certain conditions due to the increased production of ROS. Under normal physiological conditions, the level of ROS generated does not exceed the antioxidant defense mechanisms ot the body (11, 13, 15, 26, 36, 37, 40).

2. Generation of Free Radicals in the Cell

Oxygen, an essential element for sustaining life, can also become a source of cellular damage in case of presence in excessive or imbalanced quantities. This paradox arises from the metabolic processes, particularly respiration, that involve reduction-oxidation (redox) reactions. Within the mitochondria, the final addition of four electrons to molecular oxygen culminates in the formation of water, yet this process also generates deleterious intermediate products such as superoxide (O_2^-), H_2O_2, and the highly reactive hydroxyl radicals (OH^-). Intracellular enzymes, including xanthine oxidase, can directly generate superoxide radicals as unintended byproducts of their normal functions. These superoxide species can then participate in the Fenton reaction, a metal-catalyzed process that facilitates the production of additional free radicals. Inıtial step of the Fenton reaction is reduce of intracellular ferric iron (Fe^{3+}) to its ferrous state (Fe^{2+}), with a process exacerbated by the presence of superoxide. Furthermore, external sources of energy, such as ultraviolet light (UV) and X-rays, have the capacity to convert water into equally reactive hydroxyl (OH^-) and hydrogen (H^+) free radicals. Even the

metabolic breakdown of certain exogenous substances or drugs, like carbon tetrachloride (CCl_4), can lead to the formation of carbon-based free radicals (CCl_3). Nitric oxide (NO), an important chemical mediator in various cell types, interacts with oxygen to produce non-radical peroxynitrite, as well as the radical species nitrogen dioxide (NO_2) and nitrogen trioxide (NO_3). These nitrogen-based reactive species can collectively slow down mitochondrial respiration, potentially impacting cellular energy production and metabolism. The superoxide anion radical ($O_2–$) is generated through the single-electron reduction of oxygen and serves as an intermediary in numerous metabolic activities within the body. While it is a relatively weak oxidant and cannot independently cause significant cellular damage, superoxide can initiate processes that ultimately culminate in oxidative stress. Coenzyme Q, a crucial component of the electron transport chain within the mitochondria, is a primary source of superoxide generation. In contrast, hydroxyl radicals (OH•) are exceptionally reactive and can inflict severe damage by interacting with a wide range of cellular structures and biomolecules. Unlike the relatively short-lived superoxide radicals, the hydroxyl radical is considered one of the most potent oxidizing species in biological systems. This is due to its extremely high reactivity and lack of selectivity, enabling it to indiscriminately target and disrupt a variety of cellular components, including proteins, lipids, and nucleic acids. The diverse array of reactive oxygen species, each with its unique properties and reactivity, highlights the complex and multifaceted nature of oxidative stress within the biological context. While some ROS, like superoxide, may serve as intermediates in normal metabolic processes, the overproduction or imbalance of these species can lead to cascading cellular damage. The hydroxyl radical, in particular, stands out as a particularly destructive entity, capable of wreaking havoc on the structural and functional integrity of critical cellular constituents, ultimately contributing to the pathogenesis of various disease states. The hydroxyl radical can indiscriminately attack and disrupt the structural integrity of various cellular components, including DNA, proteins, and lipids. When the hydroxyl radical comes into contact with DNA, it can cause direct oxidative damage to the nitrogenous bases and the deoxyribose sugar backbone, leading to the formation of single-strand and double-strand breaks, as well as the generation of mutagenic DNA lesions. These DNA alterations can result in genetic instability, impaired cellular function, and the potential for the development of various pathological conditions, such as cancer. In contrast to the highly reactive and destructive nature of the hydroxyl radical, superoxide radicals ($O_2•–$) have a relatively brief half-life, as they readily undergo a dismutation reaction, either spontaneously or catalyzed by the enzyme superoxide dismutase, to form hydrogen peroxide and molecular oxygen (O_2). Within muscle tissues, superoxide radicals can be generated through various mechanisms, such as the leakage of electrons from components of the mitochondrial electron transport chain, the

autoxidation of heme proteins, and the enzymatic activity of xanthine oxidase. Additionally, the activation of leukocytes in the muscle tissue vasculature, in response to bacterial invasion or other inflammatory stimuli, can contribute to the generation of superoxide radicals, which serve as a primary bactericidal mechanism. The relatively short-lived nature of superoxide radicals and their ability to be rapidly dismutated into less reactive species, such as hydrogen peroxide, suggest that they may play a more nuanced role in cellular signaling and the regulation of various physiological processes, compared to the indiscriminate and destructive nature of the hydroxyl radical. Nevertheless, the presence of superoxide radicals and their potential to be converted into other reactive oxygen species, like the hydroxyl radical, highlights the importance of maintaining a delicate balance between free radical generation and the cell's antioxidant defense mechanisms. Disruption of this equilibrium can lead to the accumulation of oxidative damage and contribute to the development and/or progression of pathological conditions like neurodegenerative disorders, cardiovascular diseases, and skeletal muscle dysfunction. Ongoing research continues to elucidate the complex interplay between different reactive oxygen species, their specific cellular targets, and the intricate mechanisms by which they can influence cellular homeostasis and overall organismal health. This knowledge is crucial for the design of targeted interventions and the development of effective therapeutic strategies aimed at mitigating the detrimental effects of oxidative stress on human wellbeing (9, 26, 35, 41, 43).

2.1. Hydrogen Peroxide as a Reactive Oxygen Compound

The ubiquitous presence of hydrogen peroxide (H_2O_2) within aerobic cells, albeit at low concentrations, is a testament to its intricate involvement in various cellular compartments, including mitochondria, microsomes, peroxisomes, and phagocytic cells. This formation of H_2O_2 is inextricably linked to the production of molecular oxygen (O_2), facilitated by both nonenzymatic pathways and the catalytic action of the enzyme superoxide dismutase. The types of enzymes which directly generate H_2O_2 is diverse, encompassing the oxidase enzymes of xanthine, aldehyde, urate, glucose, glycolate, and D-amino acid. Although H_2O_2 itself does not possess the hallmarks of a free radical, its ability to interact with transition metals, such as Fe^{2+}, endows it with the capacity to increase these highly reactive species. The critical role of H_2O_2 in the process of lipid peroxidation is underscored by its generation during the oxidation of oxymyoglobin. Quantitative studies have revealed the production of H_2O_2 at a rate of approximately 3.9 × 10^-9 M/hg in hemoglobin, with a steady-state concentration of about 2 × 10^-10 M in red blood cells. Intriguingly, the storage of turkey muscle tissues at 37°C for 30 minutes has been shown to result in the formation of around 14.0 nmol of H_2O_2 per gram of fresh weight, with a notable increase observed during storage at 4°C. Despite the lack of unpaired electrons that would classify H_2O_2 as a radical species, its capacity

to induce significant damage cannot be overlooked. This potential for harm lies in its ability to generate more reactive species, such as hydroxyl radicals, through the catalysis of Fe (II). Moreover, the concentration-dependent effects of H_2O_2 extend beyond the generation of free radicals, as it can also disrupt the integrity of heme proteins, leading to the release of iron and heme groups or the conversion of these proteins into ferryl or perferryl radicals (7, 19, 22, 23, 27, 30).

2.2. Reactive Hydroxyl Radicals

The profound reactivity of the hydroxyl radical (OH-) within biological systems has earned it the distinction of being the most reactive oxygen radical. In tissues exposed to radiation, a significant portion of the absorbed energy is channeled into the formation of covalent bonds between oxygen and hydrogen, culminating in the generation of hydrogen (H-) and hydroxyl (OH-) radicals. The crucial role of OH- radicals in the cascade of radical formation is undeniable, as they engage in a series of reactions that can result in DNA strand breaks by attacking the bases and sugars. The severity of the damage inflicted by OH- radicals may surpass the repair capabilities of cellular protective systems, potentially leading to mutations and cell death. Remarkably, the *in vivo* steady-state concentration of hydroxyl (OH•) radicals is virtually negligible, a testament to their exceptionally rapid reactivity with a vast array of molecules within living cells. These highly reactive species can indiscriminately target and interact with DNA, proteins, phospholipids, amino acids, and sugars, typically near their site of formation. This exceptional reactivity is attributed to the unique combination of properties possessed by hydroxyl radicals, such as their high electrophilicity, thermochemical reactivity, and the ability to form close to target molecules. The formation of hydroxyl radicals has been demonstrated in living erythrocytes under the influence of adriamycin, a process that has been observed through the use of the spin trap electron paramagnetic resonance technique. However, the majority of in vivo or in situ hydroxyl radicals are generated from the decomposition of hydrogen peroxide (H_2O_2), a process catalyzed by the presence of ferrous iron (Fe(II)). This Fenton-like reaction represents a critical pathway for the production of these highly reactive species within biological systems. The rapid and indiscriminate nature of hydroxyl radical interactions underscores their potential to inflict widespread cellular damage. Their ability to react with a diverse range of biomolecules, including those essential for cellular structure, function, and genetic integrity, highlights the importance of tightly regulating their formation and mitigating their deleterious effects. Maintaining a delicate balance between hydroxyl radical generation and the antioxidant defense mechanisms of the body is crucial for preserving cellular homeostasis and preventing the onset of oxidative stress-related pathologies. Furthermore, OH- radicals can be produced from diverse sources, such as sunlight, ultraviolet radiation, ionizing irradiation,

the reaction of hypochlorous acid with superoxide anions, and the sonolysis of water. Inhibitors of OH- radicals, known as scavengers, include methanol, ethanol, 1-butanol, mannitol, formate, thiourea, dimethyl thiourea, glucose, tris-buffer, and sorbitol. However, the effectiveness of these scavengers is not always guaranteed, as their ability to prevent OH- from reacting with other molecules, including lipids, can be influenced by various factors. The highly reactive hydroxyl radical (OH•) is capable of interacting with various molecular scavengers, leading to the formation of scavenger-derived radical species. These newly formed scavenger radicals may then go on to react with additional molecules within the biological system, propagating a cascade of oxidative reactions. Increasing attention has been directed towards the potential involvement of a metal-mediated mechanism in the generation of hydroxyl radicals. In this process, the hydroxyl radicals are produced from the reaction of hydrogen peroxide with metal ions that are bound to macromolecules within the cellular environment. This localized generation of OH• radicals can have significant consequences, as they can directly interact with and damage nearby biomolecules. It has been well documented that the interaction of ferrous iron (Fe(II)) ions with hydrogen peroxide results in the formation of hydroxyl radicals through the Fenton reaction. This immediate production of OH• radicals can lead to direct damage to nearby DNA molecules, as the Fe(II)-deoxyribose complex can readily oxidize the sugar moiety, causing strand breaks and other deleterious modifications. Furthermore, it has been observed that ferric iron (Fe(III)) ions can bind to cellular membranes and generate free radicals at the binding site. Iron is proposed to primarily associate with the sulfone groups and carboxyl groups of sialic acids on the membrane, as well as the sulfate groups of glycolipids and the phosphate head groups of glycoproteins and phospholipids. Conversely, the presence of hydroxyl radical scavengers has been shown to effectively inhibit the formation of OH• radicals in the presence of the chelating agent EDTA, which facilitates the removal of Fe(II) ions from these membrane-bound binding sites. This highlights the critical role that the localization and availability of metal ion catalysts play in promoting the generation of highly reactive hydroxyl radicals within the cellular environment. In summary, the toxicity associated with molecular oxygen (O_2) and hydrogen peroxide may be largely attributed to the presence and distribution of metal ion catalysts that can facilitate the formation of hydroxyl radicals, leading to widespread oxidative damage to cellular macromolecules and disruption of normal physiological processes (3, 4, 9, 20, 24, 25, 27, 29, 31, 32, 34)

2.3. Formation and Functions of Singlet Oxygen ($O_2\uparrow\downarrow$)

The highly reactive singlet oxygen species, which is a non-radical excited state of oxygen, can directly interact with the unsaturated fatty acids that are essential components of cellular membranes. This interaction results in the

generation of the peroxyl radical, a potent oxidizing agent that can initiate the process of lipid peroxidation. The formation of the peroxyl radical through this singlet oxygen-mediated pathway is comparable in its destructive potential to the damaging effects induced by the highly reactive hydroxyl radical (OH•). Singlet oxygen ($1O_2$) is a unique form of oxygen that is characterized by the excitation of one of its unpaired electrons to a higher energy level. This excited state of oxygen is considerably more reactive than the ground state triplet oxygen ($3O_2$) that is typically present in the environment. Singlet oxygen can be generated through a variety of photochemical and enzymatic processes, both within the cell and in the surrounding environment. When singlet oxygen encounters the unsaturated fatty acids that are abundant in cellular membranes, it can directly abstract a hydrogen atom from the carbon-carbon double bonds, initiating the process of lipid peroxidation. This reaction leads to the formation of the peroxyl radical (ROO•), a highly reactive species that can propagate the chain reaction of lipid peroxidation, resulting in the oxidative degradation of polyunsaturated fatty acids within the cell membrane. The damaging effects of the peroxyl radical generated by singlet oxygen are comparable in strength to the deleterious impacts induced by the hydroxyl radical (OH•), which is considered one of the most potent oxidizing species in biological systems. Both the peroxyl radical and the hydroxyl radical can induce significant structural and functional alterations to cellular components, including proteins, nucleic acids, and lipids. The susceptibility of unsaturated fatty acids to singlet oxygen-mediated lipid peroxidation highlights the critical importance of maintaining an appropriate balance between the generation of these reactive oxygen species and the cell's antioxidant defense mechanisms. Disruption of this equilibrium can have far-reaching consequences, contributing to the development and progression of various pathological conditions, such as cardiovascular diseases, neurodegenerative disorders, and certain types of cancer. Understanding the mechanisms by which singlet oxygen and other reactive oxygen species can initiate and propagate the process of lipid peroxidation is crucial for the design of targeted interventions and therapeutic strategies aimed at mitigating the detrimental effects of oxidative stress on human health and well-being. Ongoing research in this field continues to elucidate the complex interplay between free radical generation, lipid peroxidation, and cellular integrity, paving the way for the development of more effective approaches to the prevention and management of oxidative stress-related diseases (2, 6, 8, 10, 14, 16, 18, 21).

3. Main Sources of Reactive Free Species

Reactive free species, commonly referred to as oxidative radicals, are continually generated within cells and the surrounding environment. These highly reactive compounds originate from a diverse array of both internal and external factors. Internally, the respiratory process within the mitochondria,

inflammatory reactions, the breakdown of lipids, the activity of certain enzymes, and even psychological strain can all trigger the formation of these oxidative radicals. Externally, exposure to ionizing radiation, the byproducts of combustion, environmental pollutants, and the consumption of tobacco and alcohol can also induce the generation of these reactive species. This wide-ranging assortment of endogenous and exogenous sources contributes to the ubiquitous presence of these highly reactive compounds, which can ultimately result in significant physiological harm to the cell and the organism as a whole. Due to the inherently destructive impact of free radicals on cellular components, the plasma membrane and organelle membranes of the cell undergo disruption, allowing the uncontrolled influx of sodium and calcium ions, as well as water. This alteration in membrane permeability is morphologically characterized by the presence of a pale, granular cytoplasm and the swelling of the cell. As these structural abnormalities progress, the cell experiences a cascade of irreversible changes, ultimately culminating in cell death. The damaging effects of oxidative radicals are not limited to the direct disruption of cellular membranes. These reactive species can also target and disrupt the structural integrity of other critical cellular components, such as proteins and nucleic acids. The oxidation of proteins can lead to the denaturation and loss of their essential functions, while the oxidation of DNA can result in genetic mutations and the impairment of crucial cellular processes, including transcription and replication. Furthermore, the presence of transition metal ions, such as iron (Fe) and copper (Cu), can exacerbate the detrimental effects of free radicals. These transition metals can participate in redox reactions, leading to the generation of even more potent oxidative species, such as the highly reactive hydroxyl radical (OH•). The introduction of these metal ions can significantly accelerate the rate of lipid peroxidation, a self-propagating chain reaction that results in the oxidative degradation of polyunsaturated fatty acids within the cell membrane. The ubiquitous nature of oxidative radicals and their multifaceted impact on cellular integrity highlight the critical importance of maintaining a delicate balance between free radical production and the body's antioxidant defense mechanisms. Disruption of this equilibrium can have far-reaching consequences in contributing pathological conditions as cardiovascular diseases, neurodegenerative disorders, and certain types of cancer. Understanding the intricacies of free radical-mediated cellular damage is crucial for the development of targeted interventions and therapeutic strategies aimed at mitigating the detrimental effects of oxidative stress on human health (5, 28, 39, 41, 42).

3.1. Lipids as targets for free radicals

The unsaturated bonds present in the fatty acids and cholesterol that comprise the cell membrane exhibit a high vulnerability to the damaging effects of free radicals. This susceptibility of the cell's lipid components is

a key factor in the initiation of lipid peroxidation, a self-propagating chain reaction that results in the oxidative degradation of polyunsaturated fatty acids. This process is highly detrimental to the overall integrity and function of the cell. The presence of transition metals, such as iron (Fe) and copper (Cu), within the cellular environment plays a crucial role in exacerbating the effects of lipid peroxidation. In the presence of these transition metals, the lipid peroxides that are essential components of the cell membrane undergo further transformations, leading to the formation of additional radical species, including RS- and ROO-. The introduction of iron and copper salts, therefore, significantly accelerates the rate of lipid peroxidation, causing a cascade of deleterious effects on the cell membrane. As the lipid peroxidation process progresses, the cell membrane experiences a reduction in fluidity and permeability, ultimately culminating in the disruption of its integrity. This disruption can have far-reaching consequences for the cell, as the cell membrane is responsible for regulating the movement of various molecules, maintaining the appropriate electrochemical gradients, and facilitating essential cellular processes. The high susceptibility of unsaturated lipids to free radical-mediated damage, coupled with the catalytic role of transition metals, underscores the importance of understanding and managing the complex interplay between oxidative stress and lipid peroxidation within the cellular environment. Disruption of this delicate balance can lead to cardiovascular diseases, neurodegenerative disorders and cancer development. Ongoing research in this field aims to elucidate the underlying mechanisms and develop targeted interventions to mitigate the detrimental effects of lipid peroxidation, with the ultimate goal of improving human health and well-being (1, 26, 41).

3.2. Lipid peroxidation as a chain reaction

Lipid peroxidation represents a complex and multifaceted chain reaction that is initiated by the introduction of free radicals. This process consists of three primary stages: initiation, propagation, and termination. During the initiation stage, highly reactive radical species, such as the hydroxyl radical (OH•), target the polyunsaturated fatty acids present within cell membranes. These reactive radicals extract a hydrogen atom from the methylene (—CH_2—) group of the fatty acid, thereby commencing the lipid peroxidation process. Polyunsaturated fatty acids are particularly susceptible to this peroxidation, as the increasing number of double bonds in the fatty acid side chain facilitates the cleavage of hydrogen atoms. The resulting conjugated dienes formed in this initial stage may then undergo structural changes, transitioning from the *cis* to *trans* configuration. This structural alteration can potentially contribute to the development of more rigid domains within the oxidized lipid bilayer, significantly impacting the fluidity and permeability of the cell membrane. The propagation stage follows, with the carbon-centered radical reacting with molecular oxygen to produce the lipid peroxyl radical (LOO•). Although less

reactive than the hydroxyl radical, the peroxyl radical can still travel to distant regions within the cell and perpetuate the lipid peroxidation chain reaction by abstracting hydrogen atoms from neighboring fatty acid chains. This perpetuation of the chain reaction can lead to a cascade of deleterious effects, including decreased membrane fluidity, altered membrane potential, increased permeability to ions, and ultimately the disruption of organelle or cellular integrity. The final stage is the termination of lipid peroxidation, during which the LOO radicals either undergo a chain-breaking reaction or self-destruct, forming non-radical products. While the resulting lipid hydroperoxides can remain relatively stable at physiological temperatures, they may still break down when exposed to high temperatures or the presence of transitional metal ions, such as iron (Fe) and copper (Cu). These free radicals and electrophilic products (e.g., 4-hydroxynonenal) generated during this stage can then react with nearby membrane proteins or diffuse to interact with distant molecules such as DNA, perpetuating the cycle of cellular damage. The complex and multifaceted nature of lipid peroxidation underscores the importance of maintaining a delicate balance between free radical production and the body's antioxidant defense mechanisms. Disruption of this balance can have far-reaching consequences, contributing to the development and progression of various pathological conditions, including cardiovascular diseases, neurodegenerative disorders, and certain types of cancer. Understanding the intricacies of the lipid peroxidation process is crucial for the development of targeted interventions and therapeutic strategies aimed at mitigating the detrimental effects of free radical-mediated damage (12, 17, 27, 38).

CONCLUSION

In conclusion, lipid peroxidation is a complex chain reaction initiated by free radicals that attack polyunsaturated fatty acids in cellular membranes, leading to the formation of highly reactive compounds such as malondialdehyde. These reactive species induce structural changes in DNA and proteins, disrupting cellular function and integrity. The consequences extend to membrane permeability, electrolyte balance, and protein stability, ultimately overwhelming cellular repair mechanisms and potentially triggering necrotic or apoptotic cell death pathways. Mitigating free radical-mediated damage is crucial for preserving cellular and organismal health, as oxidative stress and lipid peroxidation contribute significantly to the development and progression of various pathological conditions. Therefore, strategies aimed at reducing oxidative stress are essential for maintaining cellular homeostasis and preventing disease progression.

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Chapter 3

ANTIOXIDANT SYSTEMS AND MECHANISMS OF ACTION

Rıfat ERTEKİN[1]

Suzan ONUR[2]

Adnan AYHANCI[3]

1 Dr., Eskişehir Osmangazi Üniversitesi, Tıp Fakültesi, Fizik Tedavi ve Rehabilitasyon Bölümü, Meşelik, Eskişehir, rertekin@ogu.edu.tr

2 Dr. Öğr. Üyesi, Karabük Üniversitesi, Sağlık Bilimleri Fakültesi, Fizyoterapi ve Rehabilitasyon Bölümü, Nörolojik Rehabilitasyon Anabilim Dalı, Karabük, suzanonur@karabuk.edu.tr

3 Prof. Dr., Eskişehir Osmangazi Üniversitesi, Fen Fakültesi, Biyoloji Bölümü, Genel Biyoloji Anabilim Dalı, Meşelik, Eskişehir, aayhanci@ogu.edu.tr

With the pace of modern life and the widespread consumption of industrial food products, preserving human health and preventing diseases are becoming increasingly crucial. At this point, antioxidants play a critical role. Antioxidants are compounds that help protect our bodies from oxidative stress, thereby preventing cellular damage. This chapter deals with the significance of antioxidants and their modes of operation.

Antioxidants have a positive influence on health by neutralising the harmful effects of oxidative stress caused by harmful molecules known as free radicals. Free radicals tend to be generated during the body's regular metabolic activities but can induce harm to cells if their balance is disrupted. Antioxidants work by rendering these free radicals harmless, thereby preventing cellular damage.

Key topics covered in this chapter include the types of antioxidants, their natural sources, their effects on health, and their mechanisms of action. Antioxidants can be obtained from various sources such as vitamins, minerals, flavonoids, and many other plant compounds. Each of these acts through different biochemical pathways and assumes different roles in various parts of the body.

This chapter addresses the fundamental mechanisms to understand the health benefits of antioxidants and examines various antioxidant examples. Additionally, information is provided on the use of antioxidant supplements and their potential risks.

In conclusion, antioxidants have a significant impact on one's overall well-being. The objective of this chapter is to offer a thorough comprehension of how antioxidants might be utilised to mitigate the impacts of oxidative stress and maintain a state of good health.

INTRODUCTION

Free radicals, or FR, are naturally occurring byproducts of physiological activity in organisms. Oxidative stress (OS) arises when the concentration of free radicals escalates within the organism, resulting in cellular harm and consequential health complications. To prevent oxidative stress in organisms, it is crucial to maintain a balance between the levels of free radicals and antioxidants. The physiological processes that impede the formation of reactive oxygen species (ROS), shield against the detrimental effects of these substances, and assist in the elimination of toxins are referred to as "antioxidant defence systems" or simply "antioxidants." Antioxidants (AO) rapidly react with FR, thereby preventing the progression of autooxidation and peroxidation (1, 2).

Antioxidants function by neutralising an excess of radicals, protecting cells from the detrimental effects of free radicals, and assisting in the prevention of diseases (3). The consequences of AO may be briefly outlined in four key elements.

1. Scavenging effect: The process of capturing or converting FR into a less active structure is called scavenging effect. Bilirubin, tracheobronchial mucus, antioxidant enzymes, and some small AO molecules exhibit scavenging effects (4, 5).

2. Quenching effect: The process of neutralizing or reducing the activities of ROS by transferring hydrogen to them is called quenching effect. Vitamins exhibit quenching effects (4).

3. Repair effect: The process of renewing or repairing biomolecules damaged by oxidation is called repair effect. Enzymes that repair oxidatively damaged DNA are examples of repair effects (4, 6).

4. Chain-breaking effect: The process of breaking the chemical chain structures of free radicals to eliminate their oxidizing properties is called the chain-breaking effect. Bilirubin, ceruloplasmin, haemoglobin, and minerals can be given as examples of this effect (4, 7).

Antioxidants are categorised into two groups: endogenous and exogenous AOs (Table 1). Endogenous and exogenous antioxidants counteract free radicals (FR) and safeguard the organism against their harmful effects. Endogenous antioxidants can be categorised into two groups: enzymatic and non-enzymatic ones. Several enzymes perform the role of enzymatic antioxidants (AOs), including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and glutathione reductase (GSH-R). Glutathione, ferritin, ceruloplasmin, transferrin, ascorbic acid, α-tocopherol, uric acid, albumin, and bilirubin are non-enzymatic antioxidants. Exogenous antioxidants (AOs) encompass a range of substances such as vitamins A, C, E, non-steroidal anti-inflammatory medications, folic acid, carotene, allopurinol, adenosine, mannitol, acetylcysteine, and iron chelators (8).

Table 1. *Classification of Antioxidants*

ENDOGENOUS ANTIOXIDANTS		
ENZYMATIC ANTIOXIDANTS	**NONENZYMATIC ANTIOXIDANTS**	
Superoxide dismutase (SOD)	Glutathione	Coenzyme Q10
Catalase (CAT)	Melatonin	Selenium
Glutathione peroxidase (GSH-Px)	Uric acid	α-lipoic acid
Glutathione reductase (GR)	Bilirubin	Transferrin
	Albumin	Ceruloplasmin
	Lycopene	Flavinoids
EXOGENOUS ANTIOXIDANTS		
ANTIOXIDANT VITAMINS	**ANTIOXIDANT DRUGS**	
α-Tocopherol (Vitamin E)	Xanthine oxidase inhibitors (allopurinol, oxypurinol, pterin aldehyde, tungsten)	
β-carotene (Vitamin A)	NADPH oxidase inhibitors (adenosine, local anesthetics, calcium channel blockers, nonsteroidal anti-inflammatory drugs)	
Ascorbic acid (Vitamin C)	Recombinant superoxide dismutase	
Folic acid (Vitamin B9)	Trolox-C (vitamin E analogue)	
	Those enhancing the endogenous antioxidant activity (ebselen and acetylcysteine, hence increasing GPx activity)	
	Nonenzymatic free radical scavengers (mannitol, albumin)	
	Iron redox cycle inhibitors (desferrioxamine)	
	Neutrophil adhesion inhibitors	
	Cytokines (TNF and IL-1)	
	Barbiturates	
	Iron chelators	

A) ENZYMATIC ENDOGENOUS ANTIOXIDANTS

The enzymatic antioxidants (AOs) consist of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH_Px), and glutathione reductase (GR) (3, 8, 9).

1. Superoxide Dismutases

Superoxide dismutases (SODs) are metalloenzymes functioning as the primary defensive mechanism against reactive oxygen species (ROS). Superoxide dismutase (SOD) facilitates the conversion of the superoxide radical (O_2-) into molecular oxygen and hydrogen peroxide (H_2O_2), hence decreasing the levels of O_2-. High concentrations of O_2- can be detrimental to cells. CAT or GSH-Px is used to subsequently eliminate hydrogen peroxide. The process involves the simultaneous oxidation and reduction of metal ions found in the catalytic sites of SODs. SODs may be categorised into four main families based on the metal cofactors found in their catalytic sites: Copper-

Zinc SOD (Cu, Zn-SOD), Iron SOD (Fe-SOD), Manganese SOD (Mn-SOD), and Nickel SOD (10, 11).

$$2O_2^- + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

Superoxide dismutases (SODs) are crucial in safeguarding the organism from oxidative stress (OS) by functioning as potent antioxidants (AOs). The enzyme has anti-inflammatory properties and further inhibits the development of precancerous cellular changes. As individuals get older, the levels of SOD in their body naturally decline, which increases their vulnerability to illnesses associated with oxidative stress. Superoxide Dismutase (SOD) is employed in cosmetics and personal care products as an anti-ageing agent and antioxidant. It effectively reduces skin damage caused by free radicals, hence avoiding the development of wrinkles and age spots. Furthermore, it facilitates the process of wound healing, shields against UV radiation, and diminishes other manifestations of ageing (12, 13).

SOD exhibits therapeutic potential by exerting physiological effects in the treatment of illnesses resulting from OS. It has been shown to have remarkable effects on several health problems such as red blood cell-related disorders, steroid-dependent nephrotic syndrome, cystic fibrosis (CF), malignant breast disease, post-cholecystectomy pain syndrome, neuronal apoptosis, AIDS, amyotrophic lateral sclerosis and cancer. Several studies indicate a robust correlation between superoxide dismutase (SOD) activity and Alzheimer's disease. Moreover, it has been documented that SOD therapy can assist in the recovery process of mustard gas burns. SOD enzymes have shown promising results in animal models of cerebral ischemia-reperfusion injury, inflammation, cardiac ischemia-reperfusion injury, and other disorders. (10, 13, 14).

2. Catalase

Catalase is an important antioxidant enzyme composed of four protein components. Every component consists of a heme group and a NADPH molecule. The NADPH molecule is often situated in close proximity to the surface and exhibits strong binding in several catalases. Catalase is mostly located in intracellular organelles, specifically peroxisomes, and to a lesser degree in mitochondria and the endoplasmic reticulum (7). It has been extracted from both bacterial and eukaryotic organisms and is a cofactor required in aerobic species. Two molecules of hydrogen peroxide may be converted into one molecule of oxygen and two molecules of water by the catalytic enzyme catalase (15).

$$2H_2O_2 \xrightarrow{Catalase} 2H_2O + O_2$$

The reaction occurs in two stages: the initial stage includes the reduction of the H_2O_2 molecule, resulting in the creation of an oxyferryl species (FeIVO). In the second stage, the FeIVO complex undergoes reduction by a second H_2O_2 molecule, leading to the formation of O_2 and H_2O molecules. This process results in the release of the free enzyme (15, 16).

$$\mathbf{Catalase}(Fe^{(III)}\mathbf{(prophyrin)} + H_2O_2 \rightarrow \mathbf{Catalase}(Fe^{(IV)} - \mathbf{O(prophyrin)} + H_2O$$

$$\mathbf{Catalase}(Fe^{(IV)} - \mathbf{O(prophyrin)} + H_2O_2 \rightarrow \mathbf{Catalase}(Fe^{(III)}\mathbf{(prophyrin)} + H_2O + O_2$$

Catalase is crucial in situations related to oxidative stress (OS), such as inflammation, mutagenesis, and inhibition of apoptosis (17). Catalase deficiency is linked to a wide range of metabolic illnesses, including diabetes types I and II, insulin resistance, hypertension, asthma, anaemia, cancer, and a number of neurological disorders, including schizophrenia, bipolar disorder, Parkinson's, Alzheimer's, and Parkinson's. Catalase has a significant role in the management of disorders associated with oxidative stress (18).

3. Glutathione Peroxidase

Glutathione peroxidase is a collection of enzymes that depend on selenium and consists of cytosolic, plasma, phospholipid hydroperoxide, and gastrointestinal GSH-Px. GSH-Px is an enzyme that demonstrates peroxidase activity and has a selenocysteine residue in its catalytic site. GSH-Px transforms lipid hydroperoxides into alcohols and hydrogen peroxide into water. The GSH-Px family has many isoenzymes, with glutathione peroxidase-1 (GPH-Px-1) being the predominant one, and its substrate is hydrogen peroxide. GSHPx-1 functions within the cell and regulates the appropriate levels of hydrogen peroxide, which is essential for the proper functioning of mitochondria, maintaining a balanced thiol redox homeostasis and facilitating signal transduction (19-21).

$$ROOH + 2\mathbf{GSH} \xrightarrow{\mathbf{GPx}} ROH + GSSG + \mathbf{H_2}O$$

Various forms of reactive oxygen species (ROS) and reactive nitrogen species (RNS), such as those produced by inducible nitric oxide synthase (iNOS), including O_2-, peroxynitrite (ONOO-), and nitric oxide (NO), can deactivate GPx-1. Nevertheless, the extent of deactivation is contingent upon the intracellular redox state, primarily influenced by the levels of reduced glutathione (GSH) and the ratio of GSH to oxidised glutathione (GSSG). GPx-1 functions to safeguard DNA against genetic alterations and inhibit the development of cancer-causing properties. However, an extreme drop in oxidative stress level can lead to reductive stress, which is marked by a decline in physiological activities such as mitochondrial activity, cell proliferation, cardiomyopathies, and other disorders (22).

4. Glutathione Reductase

Glutathione reductase (GR) is an enzyme that belongs to the class of flavoproteins. It includes flavin adenine dinucleotide (FAD) and acts as an oxidoreductase, relying on NADPH for its activity, which is present in both prokaryotic and eukaryotic cells. GR facilitates the conversion of oxidised glutathione (GSSG) back to reduced glutathione (GSH) by transferring an electron from NADPH to the disulfide bonds of GSSG. Therefore, NADPH has a vital function in averting oxidative harm induced by free radicals (23, 24).

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

Glutathione reductase is made up of two identical subunits that form dimers. Each subunit possesses an active site that includes a flavin adenine dinucleotide (FAD). During the reaction, the enzyme facilitates the transport of electrons from NADPH to a disulphide bridge located in the active site. The two sulfhydryl (-SH) groups subsequently engage with GSSG, resulting in its reduction to GSH and reconfiguring the disulfide bond inside the protein (25). The NADPH needed for the functioning of the GR enzyme is provided in many ways. The pentose phosphate pathway serves as the primary source. An additional source of NADPH is the NADP+-dependent isocitrate dehydrogenase enzyme, mostly located in the mitochondria and to a lesser degree in the cytoplasm. This enzyme can also be susceptible to damage caused by free radicals. Nicotinamide nucleotide transhydrogenase is a crucial enzyme for providing NADPH in the mitochondria. Another source of the reaction is the enzyme that facilitates the conversion of malate into CO_2 and pyruvate by reducing NAD^+ or $NADP^+$ (25).

B) NONENZYMATIC ENDOGENOUS ANTIOXIDANTS

Glutathione, uric acid, albümin, selenium, melatonin, bilirubin, coenzyme Q10, ceruloplasmin, α-lipoic acid and transferrin are examples of non-enzymatic antioxidants (3, 8, 9).

1. Glutathione

Glutathione (GSH) is mostly found in the cytosol, endoplasmic reticulum, vacuoles, and mitochondria. The cytoplasm contains a minimum of 85% of GSH. Once GSH is produced in the cytoplasm, it has the ability to migrate to the mitochondria, nucleus, peroxisomes, and endoplasmic reticulum. GSH aids in the detoxification of xenobiotics because of its sulphur content. GSH functions as an antioxidant and is responsible for maintaining the cellular redox state. It also plays a central role in the function of the detoxification system, the formation of eicosanoids, the control of cell signalling pathways, gene expression and apoptosis. The synthesis of GSH occurs through a two-

step process. Initially, the enzyme glutamine cysteine ligase (GCL) combines glutamine and cysteine to produce ɣ-glutamylcysteine. Additionally, the enzyme glutathione synthetase (GSS) combines glycine with ɣ-glutamylcysteine to produce ɣ-glutamylcysteinylglycine (GSH). The glutamine cysteine ligase is composed of catalytic (GCLC) and regulatory (GCLM) subunits (26-28).

$$\textbf{Glutamin} + \textbf{Sistein} \xrightarrow{\textbf{GCL}} \gamma - \textbf{glutamilsistein}$$

$$\gamma - \textbf{glutamilsistein} + \textbf{Glisin} \xrightarrow{\textbf{GSS}} \textbf{Glutatyon}$$

At the same time as it scavenges singlet oxygen (O-) and hydroxyl radicals (OH-), glutathione is responsible for the detoxification of lipid peroxides and H_2O_2 through the catalytic activity of GSH-Px. GSH also enhances the transportation of amino acids across the cell membrane and replenishes crucial antioxidants. GSH regulates the levels of vitamin E and vitamin C. GSH has the ability to directly decrease the tocopherol radical of vitamin E and indirectly decrease ascorbate to semi-dehydroascorbate (9, 27).

$$\textit{ROOH} + 2\textbf{GSH} \xrightarrow{\textbf{GPx}} \textit{ROH} + \textit{GSSG} + \mathbf{H_2}\textit{O}$$

$$\textit{ROOH} + 2\textbf{GSH} \xrightarrow{\textbf{GST}} \textit{ROH} + \textit{GSSG} + \mathbf{H_2}\textit{O}$$

2. Melatonin

When exposed to darkness, the pineal gland is responsible for the production of melatonin, also known as N-acetyl-5-methoxytryptamine, which is then released into the circulation. Melatonin is present in several organs, including the pancreas, liver, brain, lens, and thyroid gland. In the hours of darkness, it is produced by synthesising tryptophan from the amino acid. Melatonin, which protects against damage, is produced by the pineal gland at a rate of around 0.5 mg per day, as determined by factors such as age, lifestyle, physical activity, and medication consumption. Melatonin, in conjunction with proteins and lipids, can shield both nuclear DNA and mitochondrial DNA from the damaging effects of oxidative stress, which tend to diminish with increasing age (29). Through its ability to mitigate the deleterious effects of free radicals (FR) on intracellular macromolecules, melatonin offers full protection. Melatonin is able to efficiently neutralise a wide range of reactive species because it acts as both a direct scavenger of free radicals and an indirect antioxidant. These reactive species include OH-, H_2O_2, O-, NO, ONOO-, and peroxynitric acid, in addition to a number of other types of FR (30).

To either boost the immune system or reduce the action of prooxidants, melatonin increases antioxidant enzymes such as SOD, CAT and GSH-Px.

Melatonin has been demonstrated to stimulate ɣ-glutamylcysteine synthetase, which in turn leads to an increase in the amounts of glutathione (GSH) found inside the cells. Melatonin also inhibits pro-oxidative enzymes including lipoxygenase and nitric oxide synthase, which are both components of the catabolic pathway. As a result of its ability to stabilise the cellular membranes, it assists the cell membranes in their ability to resist oxidative damage. In conclusion, melatonin improves the efficiency of the electron transport system by significantly lowering the formation of free radicals (FR) and the leakage of electrons. (31).

Melatonin has been proven to be an effective FR scavenger thanks to a multitude of research conducted both in vitro and in vivo. This is particularly true in the context of cancer, neurodegenerative illnesses, and psychiatric conditions. In addition, melatonin is quite beneficial in the treatment of inflammatory diseases. Melatonin molecules have a strong ability to concurrently scavenge numerous radicals that are produced from oxygen or nitrogen, and they can interact with one another through processes that are either receptor-dependent or receptor-independent with one another. Melatonin inhibits the inflammatory processes that are believed to be responsible for inflammation by interacting with cyclooxygenase (COX2), a key regulator of inflammation and a promoter of cell death in cancerous cells (32, 33).

3. Uric Acid

Uric acid is the end product of purine metabolism in humans. It is important to note that the concentration of uric acid in the serum varies greatly depending on the species. With regard to the human body, the kidneys are responsible for excreting two-thirds of uric acid, which is classified as a waste product, while the gastrointestinal system is responsible for excreting one-third. The creation of monosodium urate (MSU) crystals in and around the joints can be accelerated by having serum urate levels rise over the solubility threshold. This can occur when there is an increase in the generation of urate or when there is an insufficient excretion of uric acid. The mobilisation of MSU crystals has the potential to set off an inflammatory response that is commonly referred to as a gout attack (34).

On average, approximately fifty per cent of the total antioxidant capacity of the blood is accounted for by uric acid. Chelating transition metals and neutralising OH-, O-, O_2-, ONOO, and peroxynitric acid are some of the effects it has. Inhibiting lipid peroxidation is the mechanism by which it exerts its protective effect. For the same reason that it is an effective radical scavenger, uric acid also functions as a chelating agent for metal ions like Fe and Cu. Furthermore, the enzyme xanthine oxidoreductase is responsible for controlling the final metabolic step, which is the conversion of hypoxanthine

into uric acid. Also, ROS are produced as a result of this activity. There is evidence that the endothelium and myocardium are responsible for the creation of xanthine oxidoreductase on a local level, despite the fact that the liver and the small intestine are the primary suppliers of this enzyme (35, 36).

4. Bilirubin

There is a significant amount of antioxidant (AO) activity in bilirubin (BIL), which is a breakdown product of haemoglobin (37). The haem molecule, also known as Fe-protoporphyrin IX, is broken down by the enzyme known as haem oxygenase (HO), which results in the formation of BIL. The bulk of haem is obtained from the breakdown of haemoglobin, which is released daily during the clearance of senescent erythrocytes in the liver and spleen. While haem is present in many intracellular enzymes, most of it is obtained via the degradation of haemoglobin. Haemoxygenase is responsible for the cleavage of the haem molecule at the α-meso-carbon bridge, resulting in the production of biliverdin, carbon monoxide, and iron in equal measurements. Biliverdin reductase enzyme catalyses the reduction of the biliverdin molecule, which is typically non-toxic and water-soluble, into bilirubin IXα. Bilirubin IXα has reduced solubility and increased toxicity, potentially serving as an evolutionary adaptation to produce this essential cytoprotectant (38). BIL is a tetrapyrrole molecule consisting of two rigid dipyrroles connected by a methylene bridge at carbon 10. Among the three isomers (IIIα, IXα, and XIIIα), IXα is the inherent form. The BIL molecule has carboxymethyl side chains with -COOH groups that have pKa values of 8.1 and 8.4. This indicates that at a neutral pH, BIL mostly occurs as a protonated diacid, as seen in Figure 2. The comparatively poor water solubility of BIL, at around 70 nM, is due to the internal bonding of polar groups at neutral pH. When the pH is increased above 9.5 using NaOH in a laboratory setting, the solubility of BIL is considerably enhanced to a concentration of above 60 mM. Within plasma, the solubility of BIL is increased at a pH of 7.4 due to a distinct mechanism.

More than 99% of BIL molecules attach to various binding sites on the albumin molecule. Hepatocytes take up a small amount of free and unconjugated bilirubin from the plasma by passive diffusion through the cell membrane. Afterwards, it attaches to proteins inside the cell and forms a bond with glucuronic acid with the help of the enzyme UDPGT. Bilirubin, when it becomes soluble in water, is eliminated from the body through the bile and is mostly expelled in the faeces (39, 40).

BIL primarily protects the kidneys, heart, and brain, which are the organs most susceptible to damage from ischemia/reperfusion caused by severe hypotension, stroke, cardiac bypass, and myocardial infarction. This protection is achieved by reducing oxidative stress (41-44). BIL exhibits a comparable impact in inhibiting nitrosative damage caused by ONOO-, a potent oxidant

generated from the merging of NO and O2 during renal ischemia/reperfusion injury (45). Individuals with Gilbert's syndrome who have elevated bilirubin (BIL) levels have been found to hinder the oxidation of proteins and lipids when exposed to hypochlorous acid, which suggests a potential method for protecting against atherosclerosis (46).

5. Albumin

Albumin (ALB) consists of 585 amino acids and has a molecular weight of 66 kilodaltons (kDa). This highly soluble protein is found in human plasma at typical levels of between 35 and 50 grammes per litre. ALB plays a decisive role in several physiological and pharmacological processes. It is responsible for the transport of metals, fatty acids, cholesterol, bile pigments and drugs. It has a vital function in controlling osmotic pressure and the allocation of fluids among various compartments. ALB typically has a half-life of around 20 days under normal circumstances. The concentration of ALB in the blood reflects the equilibrium between its production and breakdown in the liver, as well as its movement across blood vessel walls (47). ALB often denotes the main and prevailing antioxidant in plasma, which is a component of the body that is constantly subjected to oxidative damage. A substantial proportion of the overall antioxidant properties in the serum can be ascribed to ALB, whose concentrations in cerebral fluid, tears, synovial fluid, and lung bronchoalveolar fluids are often lower than those in plasma. Activated neutrophils emit molecules that primarily enhance vascular permeability, leading to inflammation. The observable harm leads to a positive outcome: ALB levels rise in regions of inflammation, showcasing the protein's many antioxidant qualities (48).

ALB is regarded as the primary constituent of the extracellular antioxidant defence system due to the possession of one sulfhydryl group per molecule, which largely neutralises free radicals (FR). ALB functions as a sacrificial molecule, exhibiting a great affinity for Cu and a lesser affinity for Fe. As a result, the metals are retained on the surface of the protein to which ALB is linked. These metals are capable of engaging in Haber-Weiss reactions; however, the hydroxyl group that is produced as a result is quickly neutralised by ALB. The damage to ALB is minimal since it is present at large levels in the plasma and is counteracted by other antioxidant compounds neutralising FR. Ceruloplasmin and transferrin, among other plasma proteins, also have antioxidant properties (49).

6. Coenzyme Q10

Coenzyme Q10 (CoQ10), commonly referred to as ubiquinone, is a lipid-soluble molecule with properties similar to those of vitamins. It is produced naturally in the human body from the amino acid tyrosine. The compound consists of a quinone group and a side chain consisting of 10 isoprenoid units. Ubiquinol, which is the completely reduced version of CoQ10, is an effective

lipophilic antioxidant that may counteract free radicals and restore the reduced state of vitamin E (50, 51).

CoQ10 has the ability to hinder the process of lipid peroxidation in biological membranes and safeguard mitochondrial proteins and DNA against oxidative harm. Cells have the ability to synthesise and renew the reduced form of this antioxidant, which is the only one that is lipophilic. CoQ10 is crucial in the production of cellular energy in the form of ATP because it is strongly linked to the inner mitochondrial membrane and is involved in the electron transport chain and oxidative phosphorylation. CoQ10 improves energy levels, stimulates the immune system and acts as an antioxidant. As a result, it is present in greater quantities in tissues with high metabolic activity, including the liver, heart, muscles and kidneys (52, 53).

CoQ10 acts as an antioxidant by eliminating free radicals and inhibiting the oxidation of lipids and proteins. Ubiquinol ($CoQH_2$), in its reduced state, functions as a lipophilic antioxidant and plays a role in the movement of electrons and protons within the electron transport system. Ubiquinol transfers electrons to counteract the effects of oxidants and has highly potent antioxidant activity. Therefore, CoQ10 offers an efficient defence against harmful reactive oxygen species (ROS) such as H_2O_2 and $O_2^{\cdot}$-. CoQ10 exhibits a similar ability to vitamin E in preventing lipid peroxidation. CoQ10 and α-tocopherol have a synergistic effect, where CoQ10 helps regenerate the active forms of α-tocopherol and both substances operate in similar ways to vitamin C (54).

7.Alpha-Lipoic Acid

Alpha-lipoic acid (LA) and its reduced derivative, dihydrolipoic acid (DHLA), have strong antioxidant properties. Lipoic acid (LA), a medicinal antioxidant, may be readily absorbed when taken with meals and is efficiently transformed into a bioavailable form by the cells. LA possesses many AO characteristics, which involve interactions with other AO molecules in both the membrane and aqueous phase, and has low toxicity. DHLA possesses these characteristics, which contribute to its status as one of the most potent naturally existing antioxidants. As an antioxidant (AO), LA has the ability to directly remove harmful ROS from the body. It can also help replenish natural antioxidants in the body, such as glutathione (GSH), vitamins E and C. Additionally, LA may bind to metals and remove them from the body through a process called metal chelation (55). The redox couple formed by LA and DHLA has a high standard reduction potential of 0.32 V, whereas the redox potential of the GSH/GSSG couple is 0.24 V. Hence, LA/DHLA is commonly known as a universal antioxidant. It has been noted that the redox of LA/DHLA can restore other antioxidants including vitamin C and E. While DHLA eliminates FR, it does not completely obliterate it; instead, FR may be restored

from LA. DHLA has demonstrated its significance as a cofactor for essential mitochondrial bioenergetic enzymes, including as pyruvate dehydrogenase and alpha-ketoglutarate dehydrogenase. The degradation of LA mostly occurs through mitochondrial oxidation (56).

LA has several positive effects, such as decreasing the production of ROS by NADPH oxidase and endothelial cells, restoring the levels of reduced and oxidised glutathione (GSH/GSSG), and enhancing the expression of important antioxidant enzymes, such as glutathione reductase, in mitochondria. Furthermore, the beneficial impact of LA on LPS-induced oxidative stress has been shown (57, 58). LA is a distinctive substance that originates from both internal and external sources. It acts as a direct scavenger of ROS in both its oxidised and reduced states. It demonstrates both hydrophilic and lipophilic properties, allowing it to exert antioxidant effects in many cellular compartments such as the cytosol, plasma membrane, serum, and lipoproteins. It can also remove OH radicals, hypochlorous acid, and O radicals by scavenging. DHLA acts as an antioxidant by removing O_2 and peroxyl radicals, hence inhibiting the peroxidation of proteins caused by free radicals (59).

8. Selenium

Selenium is a vital element that is naturally present in a variety of foods and supplements. It possesses both antioxidative and immune system regulating effects. It is a crucial constituent of several proteins referred to as selenoproteins. These are involved in the optimal functioning of the reproductive system and the thyroid gland. They provide cellular protection against damage and infections induced by ROS. They provide protection to cell membranes against damage caused by ROS and maintain blood platelets in a condition of good health. Se plays a crucial role in catalysing the peroxidation of peroxides by boosting the activity of GSH-Px. Additionally, Se inhibits the generation of ROS through its antioxidant properties (60).

9. Ceruloplasmin

Ceruloplasmin (Cp) is a glycoprotein that belongs to the alpha-2 group and has many sites where copper may bind. The process of converting Fe^{2+} to Fe^{3+} requires the transfer of electrons while oxygen is reduced to form water. The process of converting ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}) enables iron to participate in the systemic iron cycle and attach to the iron chaperone transferrin for transportation between different tissues.

The electrons of Fe^{2+} are transported to areas where molecular oxygen undergoes reduction to form water. Oxygen, an indispensable element for the life of cells, can have harmful consequences due to its role as a precursor for important nutrients and by-products. Cp is a protein that possesses distinct domains capable of enhancing cellular energy generation and inhibiting

the creation of oxygen radicals. The protein's capacity to perform these two functions is attributed to its intricate shape and structure, which incorporates strategically positioned copper ions capable of accepting and releasing electrons from various substrates, such as iron, oxygen, and iron-binding proteins. Copper plays a crucial role in many processes inside the SER that maximise iron metabolism. A flaw or mutation in the SER gene that hinders the attachment of copper inside SER hampers iron metabolism. The excessive accumulation of iron leads to the occurrence of substantial clinical symptoms in the resultant disease (61, 62).

Transferrin is a prevalent plasma protein present in the bloodstream of animals. The primary role of this substance is to attach to iron and carry it from the site of absorption to any cell that requires it. Iron is a crucial ingredient for the proper functioning of several metabolic processes, and its balance must be meticulously regulated. Transferrin that is saturated with iron transports the iron to cells that have transferrin receptors. Once the transferrin binds to the receptor, the whole transferrin-transferrin receptor complex is internalised within the cell within endocytic vesicles. The iron that is bound is then freed from the complex in order to carry out its further functions. Transferrin is transported back to the outer surface of the cell and is subsequently released (63, 64).

While free iron holds significance, it possesses the potential to be highly hazardous. Iron in its ferric form (Fe^{3+}) is not soluble, but in its ferrous form (Fe^{2+}), it produces free radicals.

$$Fe^{2+} + O_2 \rightarrow Fe^{3+} + O_2^{-\cdot}$$

$$2O_2^{-} + 2H^{+} \rightarrow H_2O_2 + O_2$$

$$Fe^{2+} + H_2O_2 \rightarrow OH^{\cdot} + OH^{-} + Fe^{3+}$$

These reactions generate very reactive and detrimental oxidation byproducts, including O_2-, H_2O_2, and OH-. To address this issue, iron is bound to transferrin, therefore enhancing its solubility and isolation while inhibiting the generation of free radicals (63, 64).

10. Lycopene

The biological functions of carotenoids are contingent upon their provitamin A activities. Nevertheless, lycopene does not possess provitamin activity because it lacks a β-ionone ring structure. Lycopene possesses a significant biological characteristic as it functions as a potent antioxidant and effectively eliminates free radicals. Of all carotenoids, lycopene is the most efficient in scavenging O_2- free radicals. (65, 66).

Lycopene is being increasingly recognised as a micronutrient that offers substantial health advantages. Numerous clinical investigations have shown that lycopene plays a crucial role in disorders related to oxidative stress (OS) and cancer. This substance is a potent antioxidant that may effectively eliminate oxygen radicals, decrease oxidative stress, and inhibit the production of ROS. It has the ability to safeguard against the oxidation of lipids, proteins, and DNA within a living organism. Tomatoes are the primary food source of lycopene, and lycopene is more easily absorbed by the body when tomatoes are cooked, made into tomato juice, or turned into tomato sauce compared to when they are consumed raw (67).

11. Flavonoids

Flavonoids are a class of polyphenolic chemicals that are widely distributed in the plant kingdom. More than 4000 flavonoids have been discovered based on their chemical structure. Antioxidants typically function by impeding, averting, or eradicating oxidative harm to certain molecules. Flavonoids have a wide range of actions, including the removal of harmful free radicals, binding to metals, inhibiting enzymes that produce free radicals, and promoting the activity of natural antioxidant enzymes in the body. Flavonoids are mostly known for their capacity to directly eliminate ROS, making them effective antioxidants. Flavonoids can immediately chelate free radicals by donating a hydrogen atom or transferring a single electron (68).

Flavonoids may also act by chelating transition metal elements. Flavonoids possess chelating abilities that allow them to form complexes with metal ions in the human body, therefore inhibiting their oxidative activity. Certain flavonoids possess the ability to form complexes with trace metal ions like Fe^{2+} and Cu^{+}, which are crucial for oxygen metabolism and the generation of free radicals (69).

Flavonoids have can function as intracellular antioxidants by directly eliminating free radicals and blocking enzymes generating free radicals, such as cyclooxygenase, xanthine oxidase, microsomal monooxygenase, lipoxygenase, protein kinase C, mitochondrial succin oxidase, and NADPH oxidase. Flavonoids may exert their antioxidant action via stimulating the activity of natural antioxidant enzymes. Phase II metabolising enzymes, among them sulphotransferases, N-acetyltransferases, UDP-glucuronosyltransferases, glutathione S-transferases, and methyltransferases, play a crucial role in protecting cells from harmful toxins and foreign substances (68).

C) EXOGENOUS ANTIOXIDANTS- VITAMINS

Exogenous antioxidants can be categorised into two distinct groups: exogenous antioxidants, both in the form of vitamins and as medicinal substances. Exogenous vitamin antioxidants, such as α-tocopherol (vitamin

E), β-carotene (vitamin A), ascorbic acid (vitamin C), and folic acid (vitamin B9), are generated from vitamins and are consumed externally (1, 2, 8).

1. Vitamin E

Vitamin E is a lipid-soluble vitamin that possesses significant antioxidant capabilities. Vitamin E consists of eight stereoisomers, which are α, β, γ, δ-tocopherol and α, β, γ, δ-tocotrienol. Out of these isomers, α-tocopherol is the most physiologically potent form, protecting cell membranes from free radical harm. The primary role of α-tocopherol as an antioxidant is to safeguard against lipid peroxidation. Vitamin E exhibits a defensive impact against prostate, colon, and breast cancer, specific cardiovascular ailments, cataracts, ischaemia, arthritis, and some neurological problems. Vitamin E disrupts the process of peroxidation by stabilising free radicals, primarily by reducing O_2, particularly to OH or O_2-. Vitamin E carries out its antioxidant role by several processes, such as scavenging free radicals, disrupting the chain reaction, inhibiting, rebuilding damaged structures, and enhancing the body's defensive systems. Vitamin E's antioxidant impact on cell membranes is often facilitated by GSH-Px. Glutathione peroxidase and α-tocopherol have synergistic antioxidant actions. While α-tocopherol prevents the formation of peroxides, GSH-Px eliminates formed peroxides (70). Dietary sources of vitamin E include vegetable oils, wheat germ oil, whole grains, nuts, cereals, fruits, eggs, poultry, and meats. The natural α-tocopherol contained in food can be degraded by the process of heating and preserving food (71).

2. Vitamin C

Vitamin C, also known as ascorbic acid, is a kind of vitamin that dissolves in water and is necessary for the production of collagen, carnitine, and neurotransmitters. Vitamin C possesses antioxidant, anti-atherogenic, anti-carcinogenic, and immunomodulatory properties. Additionally, it has proven efficacy in preventing lung cancer, colorectal cancer, and stomach cancer. Vitamin C and vitamin E collaborate to eliminate harmful free radicals and concurrently restore the depleted form of vitamin E. Nevertheless, the use of large amounts of vitamin C (2000 mg or higher per day) is a subject of debate because of its possible pro-oxidant or carcinogenic characteristics. Natural sources of vitamin C include green vegetables, tomatoes, and acidic fruits. Ascorbic acid is chemically unstable and can degrade or be depleted during the heating process (72, 73).

Vitamin C may be found in a wide variety of plant cells, organelles, and apoplasts. This substance is referred to as the principal AOS scavenger because of its capacity to transfer electrons in a wide variety of enzymatic and non-enzymatic processes that take place in the aqueous phase. On the other hand, the leaves of plants and the stroma of chloroplasts are the primary locations where vitamin C may be found in its reduced form as ascorbate. This vitamin

serves as a cofactor for the enzyme violaxanthin deoxidase, which effectively eliminates surplus excitation energy in chloroplasts. It directly removes superoxides, hydroxyl radicals, and singlet oxygen. Additionally, it catalyses the reduction of hydrogen peroxide (H_2O_2) to water via the ascorbate peroxidase process. Additionally, it aids in safeguarding the membrane by replenishing tocopherol from tocopheroxyl radicals (74).

Vitamin C efficiently neutralises ROS and reactive nitrogen species (RNS) such as superoxide, hydroperoxyl, singlet oxygen, ozone, peroxynitrite, nitrogen dioxide and hypochlorous acid. Thanks to this ability, it is able to effectively protect against oxidative damage. Vitamin C can act as a co-antioxidant by forming α-tocopherol from α-tocopheroxyl radicals produced when lipid-soluble radicals are scavenged (75). Furthermore, vitamin C acts not only as an antioxidant but also as an oxidising agent by converting Fe^{+3} to Fe^{+2}, thus promoting lipid peroxidation (2).

3. Beta-Carotene

Beta-carotene is a kind of carotenoid that dissolves in fat. Carotenoids are classified as provitamins because they may be transformed into active Vitamin A. Retinol, which is crucial for eyesight, is formed through the conversion of beta-carotene. It possesses strong antioxidant properties and effectively scavenges singlet oxygen. Beta-carotene possesses antioxidant capabilities that involve the removal of singlet oxygen, elimination of free radicals, and safeguarding cell membrane lipids against oxidative degradation. Beta-carotene is present in a variety of fruits, cereals, oils, and vegetables, including spinach, carrots, squash, and green plants (76).

4. Folic acid

Folic acid, also known as pteroylglutamic acid, Vitamin B9, or Vitamin M, is a kind of water-soluble vitamin that belongs to the Vitamin B family. While plants and microorganisms have the ability to produce folate, humans and other animals lack the capacity to synthesise folic acid, which makes it a necessary nutrition for them. Folic acid deficiency is linked to several illnesses. It is essential for the process of DNA synthesis and the generation of red blood cells. It is essential for maintaining normal fertility in both females and males. Moreover, it has a significant function throughout periods of growth such as pregnancy and infancy, as well as during the process of cell division. Folic acid is a potent antioxidant that eliminates reactive oxygen species (ROS). Folic acid demonstrates efficacy in decreasing plasma homocysteine levels, both as a standalone treatment and when used in combination with other B vitamins. In addition, antioxidant vitamins such as vitamin C and vitamin E may have a beneficial effect in reducing oxidative vascular damage caused by homocysteine (77-79).

D) DRUG EXOGENOUS ANTIOXIDANTS

For the most part, antioxidant drugs can be classified into the following categories: Trolox-C, endogenous antioxidant activity enhancers, Xanthine oxidase inhibitors, recombinant superoxide dismutase, cytokines, neutrophil adhesion inhibitors, barbiturates, NADPH oxidase inhibitors, non-enzymatic free radical scavengers, iron redox cycle inhibitors, and iron chelators (8).

Generally speaking, antioxidants exert their effects through two distinct routes, which are referred to as primary and secondary antioxidants. There are three different types of primary antioxidants: radical scavengers, oxygen scavengers, and chelating agents. Primary antioxidants are responsible for preventing the generation of free radicals. However, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butylhydroquinone (TBHQ), and gallates are also examples of radical scavengers. Sulphites glucose oxidase, and ascorbyl palmitate are all examples of oxygen scavengers that are of significant importance. Chelating agents, on the other hand, consist of heavy metals or substances like iron and copper. The hydroperoxides that are produced as a result of lipid oxidation are converted into stable end products by secondary antioxidants such thiodipropionic acid and dilaurylthiodipropionate. These antioxidants are called secondary antioxidants. Fruits and vegetables that have been processed, soft drinks, margarine, and canned shellfish are all examples of products that contain these antioxidants.

Added to that, plants contain a wide variety of synthetic antioxidants, including gallates that have been created synthetically. Molecular, cellular, and organ levels are all affected by synthetic phenolic antioxidants in a manner that is comparable to other typical biological effects. Synthetic antioxidants that are widely employed, such as BHA, BHT, ethoxyquin, and propyl gallate, have the potential to induce positive interactions. These interactions include antimutagenic activity, radioprotection, protection against acute chemical toxicity, and anticancer effects. Antioxidants, on the other hand, have the ability to induce good interactions with both physical and chemical noxae. These interactions include radiosensitization, enhanced toxicity of other chemicals, higher mutagenesis activity, and greater tumour production from chemical carcinogens. However, these interactions are in direct opposition to interactions that result in detrimental consequences (80, 81).

Several synthetic compounds that possess antioxidant properties are now employed in the treatment of different diseases. For instance, 5-aminosalicylic acid, which is classified as a synthetic antioxidant, is considered one of the most potent medications for managing chronic inflammatory bowel conditions. Recent study indicates that Mexidol, an antioxidant, may have therapeutic benefits in treating acute pancreatitis. It has the potential to slow down the damaging processes occurring in the pancreas, hence lowering the severity of

complications and fatality rates associated with this condition. Administering potent antioxidants like carvedilol and melatonin by intraperitoneal injection has been shown to have positive effects in treating chronic fatigue syndrome. Xanthine oxidase inhibitors, including statins (such as atorvastatin, simvastatin, pravastatin, rosuvastatin) and allopurinol, possess antioxidant properties and are utilised to treat hyperlipoproteinemia and gout, respectively. Several synthetic antidiabetic medications, including repaglinide, metformin, and glibenclamide, and exhibit antioxidant properties (82-85).

CONCLUSION

This section provides a comprehensive analysis of the significance and operational processes of antioxidants. Antioxidants exert a beneficial impact on health by safeguarding the body from the deleterious consequences of oxidative stress. Oxidative stress is a fundamental contributor to several illnesses, and consistent intake of antioxidants can diminish the likelihood of developing these diseases. Antioxidants can be derived from many natural sources and fulfil distinct functions in various bodily systems. Vitamins, minerals, flavonoids, and other plant chemicals possess antioxidant capabilities and are crucial for sustaining a healthy lifestyle. Antioxidants thwart cellular harm by counteracting the impact of free radicals and hinder the onset of several diseases.

Antioxidants have intricate modes of action that contribute to several metabolic processes. These benefits encompass the capture of free radicals, the provision of anti-inflammatory actions, and the support of cellular repair processes. Gaining knowledge of these pathways enhances our comprehension of the impacts of antioxidants on health and enables us to optimise their use.

To summarise, antioxidants have a decisive influence on our well-being and it is important to include foods with a sufficient antioxidant content in our regular diet. However, it is important to remember that antioxidant supplements should not be used as a substitute for a balanced diet. The material presented in this section is intended to serve as an initial reference to emphasise the importance of antioxidants and promote understanding of their role in maintaining a healthy lifestyle.

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Chapter 4

ANTIOXIDANT DEFENSE SYSTEM AGAINST OXIDATIVE STRESS

Suzan ONUR[1]

Adnan AYHANCI[2]

Rıfat ERTEKİN[3]

1 Dr. Öğr. Üyesi, Karabük Üniversitesi, Sağlık Bilimleri Fakültesi, Fizyoterapi ve Rehabilitasyon Bölümü, Nörolojik Rehabilitasyon Anabilim Dalı, Karabük, suzanonur@karabuk.edu.tr

2 Prof. Dr., Eskişehir Osmangazi Üniversitesi, Fen Fakültesi, Biyoloji Bölümü, Genel Biyoloji Anabilim Dalı, Meşelik, Eskişehir, aayhanci@ogu.edu.tr

3 Dr., Eskişehir Osmangazi Üniversitesi, Tıp Fakültesi, Fizik Tedavi ve Rehabilitasyon Bölümü, Meşelik, Eskişehir, rertekin@ogu.edu.tr

The pace of modern life and changes in lifestyle bring about a number of factors affecting human health. one of these is oxidative stress. Reactive molecules formed during the normal metabolism of our cells are called free radicals (SR), and while they can cause oxidative damage to our cells, they can also lead to various health problems in the long term. Reactive oxygen species (ROS) are naturally produced by living organisms due to various factors including normal cellular metabolism and exposure to environmental pollutants. ROS are highly reactive molecules capable of damaging cellular structures such as carbohydrates, nucleic acids, lipids, and proteins, thereby disrupting their normal functions. When the balance between oxidants and antioxidants tilts in favor of oxidants, it leads to oxidative stress. However, the body possesses its own defense mechanisms that effectively counteract oxidative stress, primarily through antioxidant defense systems. Aerobic organisms typically have robust antioxidant systems comprising both enzymatic and non-enzymatic antioxidants, which play crucial roles in neutralizing the harmful effects of ROS. Nevertheless, under certain pathological conditions, these antioxidant systems may become overwhelmed. Oxidative stress is implicated in the development of various pathological conditions and diseases, including cancer, neurological disorders, atherosclerosis, hypertension, ischemia/reperfusion injury, diabetes, acute respiratory distress syndrome, idiopathic pulmonary fibrosis, chronic obstructive pulmonary disease, and asthma.

This section focuses on how conditions caused by oxidative stress are defended against by antioxidant defense systems. It explains how our bodies produce antioxidants and how these antioxidants function. Additionally, it aims to be a comprehensive resource for researchers, students, and healthcare professionals interested in understanding the complexity of oxidative stress and antioxidant defense systems. By deepening the existing knowledge in this field, it aims to contribute to the development of new approaches to reduce the effects of oxidative stress and promote a healthy lifestyle.

INTRODUCTION

In aerobic organisms, antioxidant (AO) defense mechanisms have evolved in response to oxygen-derived radicals that begin to form with oxygenated life. There exists a delicate balance between free radicals (SR) and antioxidants, and when this delicate balance is disrupted, numerous pathological changes leading to cellular damage can occur. Antioxidants are defense systems developed in the body to prevent damage resulting from the formation of reactive oxygen species (ROS) (1).

Substances capable of preventing or delaying the oxidation of materials such as lipids, proteins, and DNA by free radicals within the cell are called antioxidants, and these mechanisms are referred to as antioxidant defense systems (2). Antioxidants inhibit oxidative damage at the cellular level by

preventing the formation of active oxygen or by scavenging the active oxygen formed, thereby halting the formation of degenerative diseases (3).

Antioxidants prevent cellular damage by transferring electrons to free radicals. Antioxidants demonstrate their effects through four different mechanisms:

1. Cleansing effect: Involves capturing free oxygen radicals or neutralizing oxidants by converting them into weaker molecules. This is accomplished by antioxidant enzymes and micromolecules.

2. Suppression effect: Involves reducing the effectiveness or slowing down the reaction rates of free oxygen radicals by transferring hydrogen to them upon interaction. Vitamins and flavonoids demonstrate their effects through this mechanism.

3. Chain-breaking effect: Involves binding to free oxygen radicals, breaking their chains, and disrupting their functions, thereby stopping the chemical reactions that produce SR.

4. Repair effect: Facilitates the repair of biological molecular damage caused by free radicals in structures such as lipids, proteins, and DNA (2-4).

While the body produces some antioxidants at the cellular level (enzymatically), it acquires others through diet (non-enzymatically) (5). Endogenous and exogenous antioxidants are shown in Table 1.

Table 1. *Endogenous and Exogenous Sources of Antioxidants*

ANTIOXIDANTS	
1-Endogenous Antioxidants	
Enzymatic Ones	**Non-enzymatic Ones**
• Superoxide dismutase (SOD)	• Vitamins A, C, E
• Glutathione peroxidase (GSH-Px)	• Hemoglobin, myoglobin
• Catalase (CAT)	• Transferrin, cysteine, albumin
• Glutathione S-transferase (GST)	•Uric acid, ceruloplasmin, lactoferrin
•Phospholipid hydroperoxide glutathione peroxidase	• Paraoxonase
	• Glutathione
	•Cytokines, bilirubin, hydroperoxide glutathione peroxidase
2-Exogenous Antioxidants	
• Enzyme inhibitors	
- Xanthine oxidase inhibitors (tungsten, allopurinol, pterin aldehyde)	
• Recombinant superoxide dismutase	
• Analogs of vitamins C and E	
• NADPH oxidase inhibitors (local anesthetics, calcium channel blockers, NSAIDs, iodonium, setile, adenosine, diphenylene)	

The primary line of defense that the body produces against free radicals includes:

- Superoxide dismutase (SOD)

- Glutathione peroxidase (GSH-Px)

- Catalase (CAT) (5).

Intracellular Environment of Antioxidant Defense

SOD, a widely distributed metalloproteinase enzyme in the body, catalyzes the conversion of superoxide radicals into hydrogen peroxide and molecular oxygen (6). It plays crucial roles in preventing lipid peroxidation and protecting against oxidative damage. Its functions also include facilitating the destruction of bacteria during phagocytic activity and protecting cells and DNA against the ionizing effects of radiation (7-10).

Superoxide dismutase (SOD) is the most important antioxidant defense system against ROS and superoxide anion radicals (11). SOD's role is to maintain low levels of superoxide in cells that metabolize oxygen and inhibit lipid peroxidation (12).

SOD catalyzes the conversion of a superoxide radical into an O_2 molecule and reduces another superoxide radical to the less reactive molecule hydrogen peroxide (H_2O_2) (11).

SOD

$$\mathbf{O_2\text{-}^{\cdot} + O_2\text{-}^{\cdot} + 2H^+ \rightarrow O_2 + H_2O_2}$$

SOD, which contains copper and zinc ions especially in the cytosol of human cells and manganese ions in mitochondrial SOD, has two isoenzymes (6).

SOD deficiency is common. decreased SOD levels increase the formation of free radicals. SOD levels decrease with aging. Daily SOD supplementation reduces the chance of contracting diseases and the aging process by protecting the immune system. Sources of SOD include cabbage, Brussels sprouts, wheatgrass, barley grass, and broccoli (5).

Superoxide radicals are detoxified by dismutation or directly converted to water by the GSH-Px and CAT enzymes. Under normal conditions, the detoxification of hydrogen peroxide in the cell primarily relies on the function of a selenoenzyme called GSH-Px (6). GSH-Px, a selenium-containing enzyme, has functions such as protecting membrane lipids and hemoglobin against oxidative damage in the cytosol, reducing hydrogen peroxide generated by SOD, and reducing fatty acid hydroperoxides. GSH-Px working at low concentrations of hydrogen peroxide prevents peroxidation of the membrane when vitamin E is deficient. It is considered the most effective antioxidant mechanism against

oxidative stress in erythrocytes. It prevents damage to phagocytic cells during respiratory burst reactions caused by SR peroxidation. Studies have shown that a decrease in GSH-Px activity is associated with increased hydrogen peroxide levels and cellular damage (13-16). GSH-Px is an important antioxidant that hydrolyzes hydrogen peroxide to water in mitochondria and sometimes in the cytosol (17). Thus, GSH-Px, which prevents the initiation and progression of lipid peroxidation, is an enzyme that detoxifies H_2O_2 present in cells under normal conditions (18). Its activity is often selenium-dependent. Therefore, it can be divided into selenium-dependent -GSH-Px and selenium-independent -GSH-Px. The main sites of expression are selenium-dependent GSH-Px1, which protects the cell against almost every oxidative stress (19).

According to research, low levels of GSH-Px lead to disruption of the antioxidant system. Consequently, the development of oxidative damage in membrane fatty acids and functional proteins, along with neurotoxic damage and neurodegeneration, resulting in permanent damage, indicate the impairment of the antioxidant defense system (20).

Another antioxidant enzyme, CAT, is considered to have significant activity in situations where hydrogen peroxide formation increases (6). While CAT is predominantly located in peroxisomes, it is absent in the mitochondria of most mammalian cells, with the exception of mitochondria found in the rat heart. During the process of reducing oxygen to water in mitochondria, approximately 1-2% of total oxygen consumption has the potential to generate cytotoxic species like superoxide and hydrogen peroxide. Increased levels of superoxide radicals can inflict damage on mitochondria. To counteract this, antioxidant defense systems are mobilized. Initially, superoxide radicals generated within mitochondria are neutralized by manganese superoxide dismutase (Mn-SOD or SOD2) and glutathione peroxidase (GSH-Px). However, a considerable amount of hydrogen peroxide (H_2O_2) exits the mitochondria and enters the cytoplasm. The detoxification of H_2O_2 transitioning from mitochondria to the cytoplasm is facilitated by the catalase enzyme, which is synthesized within peroxisomes. Catalase synthesized within peroxisomes efficiently reduces H_2O_2 levels to a more stable concentration compared to glutathione peroxidase. While GSH-Px breaks down H_2O_2 at lower concentrations, CAT can break down millions of H_2O_2 molecules per second (5).

$$\mathbf{H_2O_2 \xrightarrow{CAT} 2H_2O + O_2}$$

Hydrogen peroxide plays a role in regulating some physiological processes such as cell proliferation signaling, cell death, carbohydrate metabolism, mitochondrial function, thrombocyte activation, and maintaining normal thiol redox balance in low amounts (22). However, it is stated to be highly harmful to cells at high concentrations (5). Therefore, the ability of CAT to effectively limit H_2O_2 concentration in cells is important for its role as a primary

antioxidant defense enzyme in physiological processes. CAT deficiency or mutation is associated with various disease conditions and abnormalities. Therefore, CAT is one of the most studied enzymes and forms the basis of antioxidant studies in many different organisms (23).

In addition to SOD, GSH-Px, and CAT enzymes, vitamins E and C also exhibit intracellular antioxidant properties by breaking the lipid peroxidation chain reactions in cell membranes (6).

Extracellular Antioxidant Defense

Unlike the intracellular environment, the activity of the enzymatic antioxidant system in extracellular fluids is quite limited. Therefore, in extracellular environments, enzymes play a minor role in antioxidant defense, while vitamins E and C, transferrin, haptoglobin, ceruloplasmin, albumin, bilirubin, β-carotene, uric acid, glucose, cysteine, tracheobronchial mucus, and α-1 antitrypsin are responsible for major antioxidant activities (6).

Vitamin E, known as the main lipid-soluble and chain-breaking antioxidant in cells, primarily found in plasma, has the highest antioxidant activity. Its most important function is to prevent damage to fatty acids in membrane lipids against attacks by oxygen free radicals (24).

Vitamin C (ascorbic acid), which plays a crucial role in strengthening the immune system, is also protective against heart and cancer diseases. Due to its water-soluble nature, it is distinguished from other antioxidant vitamins. It is known that in healthy individuals, protein constitutes 49% of the Total Antioxidant Level (TAS), bilirubin constitutes 1.7%, and vitamin C constitutes 5% (25). Being a chain-breaking antioxidant and playing a significant role in protecting leukocytes against oxidative damage, especially during detoxification metabolism, it ensures the neutralization of SODs and ROSs (24).

However, vitamin C can also exhibit oxidizing properties when reacting with iron or copper ions in the presence of hydrogen peroxide. Normally, superoxide radicals and hydrogen peroxide are generated extracellularly by endothelial cells, lymphocytes, platelets, fibroblasts, and other cells. Superoxide radicals and hydrogen peroxide can transform into more dangerous radicals such as hydroxyl groups, especially in the presence of free iron and copper ions. Therefore, the extracellular antioxidant defense mechanism of the organism should involve the sequestration of iron and copper ions. Transferrin serves as an example of this (26). Transferrin binds iron and prevents or slows down lipid peroxidation and iron-catalyzed Haber-Weiss reactions (27). Transferrin, a transport protein for iron, is loaded with about 20-30% iron in healthy individuals. Thus, the activity of free ionic iron in the plasma decreases to zero. Iron bound to transferrin cannot initiate lipid peroxidation. However, in iron storage diseases, low molecular weight iron ion complexes stimulate lipid

peroxidation and hydroxyl radical processes, leading to multi-organ damage.

Hem-containing proteins such as hemoglobin and myoglobin can stimulate lipid peroxidation in the presence of hydrogen peroxide through two mechanisms:

-Protein and hydrogen peroxide reaction form OXO-heme radicals (especially tyrosine peroxyl radicals), which stimulate lipid peroxidation.

-Excessive hydrogen peroxide acts on myoglobin and hemoglobin, causing the release of free iron ions. Free iron ions also stimulate lipid peroxidation (26).

Seruloplasmin, which carries copper in the plasma, also plays a role in iron metabolism and has antioxidant properties (28). Although less significant, it inhibits Fe and Cu-dependent lipid peroxidation by reacting with O_2- (27).

Uric acid, a byproduct of purine metabolism, accumulates in the body since the human body does not contain urate oxidase enzyme. Uric acid not only effectively eliminates singlet oxygen, peroxyl, and hydroxyl radicals but also prevents radical formation by binding iron and copper (29).

Albumin, in addition to many functions in the body, also has the ability to bind copper ions, thereby inhibiting copper ion-dependent lipid peroxidation and hydroxyl radical formation (28). While albumin strongly binds copper, it binds iron weakly and clears OH- radicals and HOCl, a derivative of myeloperoxidase, on its surface (29).

Albumin also carries fatty acids in the blood, and bilirubin binds to albumin as well (28). Bilirubin, a toxic compound at high serum levels, is as effective as vitamin E in inhibiting chain reactions in lipid peroxidation. Recent studies have shown that bilirubin contains conjugate double bonds, making it a potent antioxidant both in vivo and in vitro. Bilirubin has a physiological protective role in rapid and prolonged oxidant-induced cell damage (30). In vivo, bilirubin acts as an antioxidant in lipid peroxidation. In vivo, bilirubin probably prevents the peroxidation of fatty acids bound to albumin (28).

Among non-enzymatic antioxidants, vitamin A (ß-carotene) is a fat-soluble antioxidant that can directly capture free radicals before they affect biological targets and inhibit the formation of peroxide radicals with its chain-breaking property (29). Diseases resulting from vitamin A deficiency, coronary heart diseases, and especially cancer can be prevented or delayed due to their antioxidant properties (31).

Phenols containing the OH- group attached to an aromatic ring are another type of antioxidant. Phenolic compounds, along with their various types, are notably effective in inhibiting autooxidation. Furthermore, they exhibit a wide range of beneficial properties including anti-allergic, anti-

inflammatory, anti-diabetic, antimicrobial, antipathogenic, antiviral, and antithrombotic activities. These compounds also offer protective effects against cardiovascular diseases, cancer, osteoporosis, diabetes mellitus, and neurodegenerative diseases (32).

As understood from all this information, the primary antioxidant activity in the extracellular environment is achieved by preventing metal ion-mediated SR reactions. This is accomplished not with antioxidant enzymes, but with vitamin E and C, transferrin, seruloplasmin, albumin, etc. However, the concentrations of antioxidants in all extracellular fluids are different. The increase in oxidant molecules, which can increase up to a certain level in the body, is neutralized by natural antioxidant molecules always present at a certain level. Thus, a balance is maintained between the level of oxidants and the power of antioxidants to balance this level in a healthy organism. However, if oxidants exceed a certain level or antioxidants are insufficient, a shift may occur, and in this case, oxidant molecules can cause harmful effects by damaging the building blocks of the organism, such as proteins, lipids, carbohydrates, nucleic acids, and beneficial enzymes (33).

In conclusion, the harmful effects of increased ROS can be summarized as follows: they disrupt the structure of cell organelles and membranes, render intracellular beneficial enzymes ineffective, lead to DNA damage and disruption of aerobic respiration in mitochondria, and activate lytic enzymes such as elastase, protease, phospholipase, lipoxygenase, cyclooxygenase, xanthine oxidase, indoleamine dioxygenase, tryptophan dioxygenase, and galactose oxidase. They increase potassium loss from cells and support platelet aggregation. Additionally, they facilitate the accumulation of phagocytic cells in tissues and break down collagen tissue components, defense enzymes, and neurotransmitters outside the cell (34).

CONCLUSION

Free radicals play a role in the pathophysiology of cardiovascular, inflammatory, cancer, and neurodegenerative diseases. Antioxidants prevent or reduce tissue damage by inhibiting free radical formation. Enzyme activities of SOD, CAT, and GSH-Px constitute the basic defense systems in cells and play a key role against diseases caused by oxidative damage.

ROS and antioxidant defense systems have become increasingly important topics of interest and research in the field of health. This section has focused on an in-depth examination of antioxidant defense mechanisms. It has extensively discussed how our body responds to oxidative stress with antioxidant defense systems and how these systems function. The mechanisms by which antioxidants neutralize free radicals and their roles in this process have been examined in detail.

In other sections, the effects of oxidative stress on health have been emphasized, and how oxidative stress is associated with many health problems such as cardiovascular diseases, cancer, neurological disorders, and aging has been explained. Understanding the role of oxidative stress in the pathophysiology of these diseases is a critical step in their prevention and treatment.

Finally, the benefits of antioxidants for health and strategies that can be used to combat oxidative stress have been discussed. Various approaches, such as nutrition, lifestyle changes, and antioxidant supplements, have been discussed on how they can effectively reduce the effects of oxidative stress. This section aims to provide a comprehensive resource for researchers, students, and healthcare professionals who seek to understand the complexity of oxidative stress and antioxidant defense systems. It aims to contribute to the development of new approaches to reduce the effects of oxidative stress and promote a healthy lifestyle. Further research and accumulation of knowledge in this area will be an important step in preserving and improving human health.

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