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THE MECHANISM AND BIOCHEMISTRY IN OXIDATIVE STRESS A BRIEF OVERVIEW

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Abstract
This article provides a comprehensive overview of oxidative stress, emphasizing its pivotal role in human health and disease progression. Oxidative stress, characterized by an imbalance between reactive oxygen species (ROS) and antioxidative defense, leads to cellular damage and exacerbates chronic diseases. Initially perceived solely as harmful, recent studies reveal ROS's dual role in cellular signaling and metabolic regulation. This shift in perspective underlines the nuanced nature of redox biology, where ROS can be both beneficial and detrimental. The article highlights the importance of antioxidative strategies, including enzymes like SOD, catalase, glutathione peroxidase, and small molecular weight antioxidants such as vitamins E and C. Advancements in redox signaling and sensor understanding have redefined oxidative stress, focusing on the need for precise diagnostic and therapeutic strategies. The paper underscores the necessity for ongoing research to characterize redox biomarkers and establish robust clinical and epidemiological reference values.

Keywords: Antioxidative Defense, biomarkers, Oxidative Stress, Reactive Oxygen Species (ROS).

1. INTRODUCTION

Based on the premise that the study of oxidative stress (Gelpi et al., 2017) plays a key role in the initiation, progression (Sies, 1986), and exacerbation of many diseases, we have conducted a concise assessment of this concept, addressing free radicals of oxygen and nitrogen. We have examined the effects of antioxidants, both enzymatic and non-enzymatic, and the dynamics of the essential process of investigating the interaction between free radicals, the impact of their production, and how oxidative stress influences the development of pathologies and the connection with human health (Halliwell, 2007).

The subject is highly relevant today (Sadiq, 2023), as it expands and enhances recent discoveries in the field of oxidative stress, a concept that has rapidly evolved over the past decades in redox biology and medicine. The essential role lies in the use of oxidative stress at low levels for signaling and redox regulation, called eustress oxidative (Kannan and Jain, 2000), while high levels can cause oxidative deterioration of biomolecules.

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Various studies have highlighted a direct connection between free radicals produced by metabolism, their involvement in physiological and pathological states, and the effects of oxidative stress. The imbalance between antioxidants and oxidants leads to the accumulation of free radicals, which can damage essential macromolecules such as nucleic acids, proteins, and lipids, thus causing tissue damage and severely exacerbating the progression of diseases (Qian et al., 2023).

2. MATERIALS AND METHODS

As this paper is a brief review of the literature on the mechanism and biochemistry of oxidative stress, the method used was the analysis of the specialized literature. A comprehensive review was conducted to extract relevant data from the published literature. The total number of articles analyzed was 79. Databases such as Web of Science, PubMed, and Google Scholar were analyzed, searching for studies that focus on the addressed subject.

3. RESULTS AND DISCUSSIONS

3.1. A Brief History – Oxidative Stress

Oxidative stress is characterized by an abundant generation of reactive oxygen species (Metcalfe and Alonso-Alvarez, 2010) (ROS) that exceeds the body's defensive system's capacity (Bhattacharya, 2015). These highly reactive molecules are chemical compounds derived from oxygen. The balance between the formation of ROS and the body's ability to neutralize them defines the "redox condition" (Apel and Hirt, 2004). An excessive level of ROS (Bulkeley et al., 2023) can result from the toxicity of compounds foreign to the body. The combination of ROS with reactive nitrogen molecules can lead to the formation of peroxynitrite (Jomova et al., 2023), an even more harmful molecule (Andrés et al., 2023). While the body is equipped to cope with normal levels of oxidative stress, an excess (Aschbacher et al., 2013) can have harmful effects (Kruk et al., 2019). Furthermore, the growing role of ROS in the natural aging process and in the development of many chronic diseases (Sharma et al., 2015) such as cancer, atherosclerosis, cardiovascular diseases, diabetes (Cojocaru et al., 2023), neurodegenerative diseases, and liver and immune dysfunctions is recognized (Jomova et al., 2023).

Oxidative stress has a significant impact on cellular degradation, contributing to various pathologies (Radi, 2018). The interaction between oxidants and antioxidants and the resultant formation of ROS and/or RNS (reactive nitrogen species) (Di Meo et al., 2016) are involved in a wide range of conditions (Kurutas, 2015). It is assumed that therapies that enhance antioxidative function could reduce cellular damage (Fransen et al., 2012).

Decades ago, the concept of the oxidation of biomolecules through the action of free radicals was not considered viable (Gutteridge and Halliwell, 2000). The discovery of the enzyme superoxide dismutase in the 1960s paved the way (Bannister, 1988) for extensive studies that have highlighted how mitochondria produce free radicals (Foo et al., 2022) and other oxidants (Powers and Jackson, 2008). To counter the threats from an oxygenated environment, the body has developed antioxidant strategies to prevent and correct potential oxidative damage. Sies introduced the term oxidative stress, emphasizing the importance of the dynamic balance between oxidants and antioxidants.

More recently, it has been recognized that free radicals and oxidants are not only harmful factors at the cellular level but play a vital role in cellular signaling and redox metabolic regulation (Sadiq, 2023). Specific redox pathways have been identified that suggest evolved mechanisms for this signaling. This has led to a reevaluation of the concept of oxidative stress, focusing on specific and compartmentalized redox circuits at the cellular level (Ray et al., 2012). Thus, the current

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perspective on oxidative stress opens the way to the development of new therapeutic strategies, targeted towards specific targets and newly distinguished conditions.

In the fifth decade of the 20th century, Gerschman (2013) made the correlation between the harmful effects of oxygen and the generation of free radicals, while Harman postulated that the aging process is correlated with cumulative cellular damage caused by these reactive compounds (Harman, 1962; 1978; 1998). However, these hypotheses did not capture significant attention from the scientific community of biologists and biochemists, remaining marginalized for an extended period.

The transformation of perspective occurred in 1969 when McCord and Fridovich isolated an enzyme from bovine erythrocytes that catalyzes the conversion of the superoxide radical (O^{2-}) into diatomic oxygen and hydrogen peroxide (H_2O_2) (McCord and Fridovich, 1969a, 1969b). This catalyst was named superoxide dismutase (SOD). Although the superoxide radical had been identified in the 1930s by Linus Pauling, interest in it was limited to the chemical community. In the sixth decade, free radicals were considered too reactive to have a significant biological role. The discovery of SOD was pivotal in integrating the chemistry of free radicals into the fields of biology and medicine.

Subsequently, it was highlighted that the superoxide radical is an enzymatic product of xanthine oxidase and has been implicated in biological defensive mechanisms (Jomova et al., 2023). Moreover, it was recognized that, besides the complete reduction of molecular oxygen to water by cytochrome c oxidase in the mitochondrial respiratory chain, concomitant reactions can generate partially reduced oxygen species, including radicals. This class of reactive intermediaries, resulting from the incomplete reduction of oxygen, incorporates the superoxide radical (by adding one electron) and hydrogen peroxide (Joardar and Babu, 2020) (by adding two electrons). Hydrogen peroxide, having no unpaired electrons in its valence orbitals, is not classified as a free radical, but it can be converted into the hydroxyl radical (Pala and Tabakçıoğlu, 2007), an extremely powerful oxidant in a biological environment, by the cleavage of the O-O bond in the presence of ferrous iron - a reaction known as the Fenton reaction. In contrast to superoxide and hydrogen peroxide, which are relatively less reactive and therefore more selective in their attack (Yan et al., 2020), the hydroxyl radical attacks and oxidizes any biomolecule non-selectively and immediately upon formation (Reilly et al., 1991).

Reactive oxygen species (ROS) constitute a collective term that encompasses both free radicals, such as superoxide and hydroxyl radicals, and non-radical species, including hydrogen peroxide and singlet oxygen, the latter generated by photosensitization (Upadhyay, 2023), in the presence of a reactive photosensitizer with molecular oxygen in its ground state. Although the triplet state of molecular oxygen, characterized by low reactivity, falls into the category of free radicals due to the presence of two unpaired electrons in different anti-bonding π orbitals, spin restrictions impose barriers to its reactivity with biomolecules.

Initial studies confirmed that the electron transport chain in mitochondria can be the source of hydrogen peroxide and superoxide radical production (Miwa et al., 2003). The localization of superoxide dismutase in the mitochondrial matrix suggests the efficiency of dismutation of the superoxide radical into hydrogen peroxide, which corroborates with mitochondrial ROS production (Al-Nu'airat, 2018).

The production of ROS is not limited to the inner mitochondrial membrane; other structures, such as the outer mitochondrial membrane and various metabolic pathways in mammalian cells, also contribute to this process. Specific enzymes, such as monoamine oxidase in the outer mitochondrial

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membrane and amino acid oxidases in peroxisomes, generate hydrogen peroxide during their catalytic activity. At the same time, the microsomal electron transport chain, uncoupled nitric oxide synthase, and cyclooxygenase are other sources of ROS (Padovan et al., 2023). NADPH (nicotinamide adenine dinucleotide phosphate) oxidase and xanthine dehydrogenase produce the superoxide radical, the former playing a role (Cipriano et al., 2023) in immune defense mechanisms through phagocytosis, and the latter, once converted to xanthine oxidase, contributes to the increased production of superoxide radical under ischemia-reperfusion conditions.

The establishment of the production of superoxide radical and hydrogen peroxide by mitochondria laid the foundation for the theory of free radicals in biology and medicine, with significant contributions from Briton Chance (Boveris and Chance, 1973), Slater (1988), and others, culminating in exhaustive publications on this topic. Although the causal link between free radicals and pathologies remains complex, it is clear that free radicals contribute to the perpetuation of a cycle of damage that promotes cellular dysfunction and pathology. The generation of free radicals has been metaphorically named "the dark side of metabolism" because, in the absence of neutralization (Golubev et al., 2017), they can cause the oxidation of the double bonds of polyunsaturated lipids in membranes, can cause injuries to nuclear and mitochondrial DNA, and can accelerate the oxidation of proteins, leading to amplified degradation or loss of functionality.

In aerobic environments, organisms have developed complex antioxidant strategies to counteract (Artem et al., 2021) the harmful effects of reactive oxygen species (ROS), evolving enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase to neutralize reactive by-products (Landis and Tower, 2005). These efficient and specific enzymatic systems are complemented by low molecular weight antioxidant molecules, such as vitamins E and C and glutathione, which can themselves become radicals after electron transfer. The antioxidative strategy, described by Cadenas (2018), involves overlapping levels of prevention, interception, and repair, and adapts to cellular needs.

In redox biology, there has been a shift from characterizing antioxidant enzymes and compounds to understanding redox signaling and sensors (Forman et al., 2014). This progress has rewritten the definition of oxidative stress as a pro-oxidant imbalance that disturbs redox signaling and induces molecular damage, from beneficial eustress (Steinert and Amal, 2023) to toxic distress. This dichotomy reflects how low doses can serve redox signaling, while high levels can lead to disturbances and damage. In this context, the principles of redox regulation are synthesized in what is called the "Redox Code," an extension of the toxicological principle of Paracelsus that the dose defines the toxicity, emphasizing the importance of the dose in a spatio-temporal framework (Sies et al., 2017).

Oxidative stress, an imbalance between the production of oxidants and the capacity for antioxidative neutralization, influences redox signaling. Moderate exposure to oxidants promotes positive redox signaling (eustress), while high levels disturb this signaling and can damage biomolecules. Cellular adaptations may moderate these effects. Sies conceptualized oxidative stress as an imbalance favoring oxidants, leading to potential cellular damage (Sies et al., 2017). Oxidants such as hydrogen peroxide influence redox signaling and regulation via transcription factors, alongside other oxidants like the superoxide anion radical and singlet oxygen (Sies, 2019). Gagné (2014) highlighted oxidative stress as a common factor of toxicity, generated by various agents and involved in vital processes such as cellular respiration and metabolism. Enzymatic antioxidant systems and small mass molecules counteract the effects of reactive oxygen species. However, exposure to toxins can disrupt this delicate balance, leading to oxidative injuries and inflammation.

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Chronic oxidative stress may contribute to the accumulation of age-related pigments and physiopathological damage. In the scientific literature, the concept of oxidative stress has evolved towards a better understanding of biological responses and redox signaling (Sies et al., 2022).

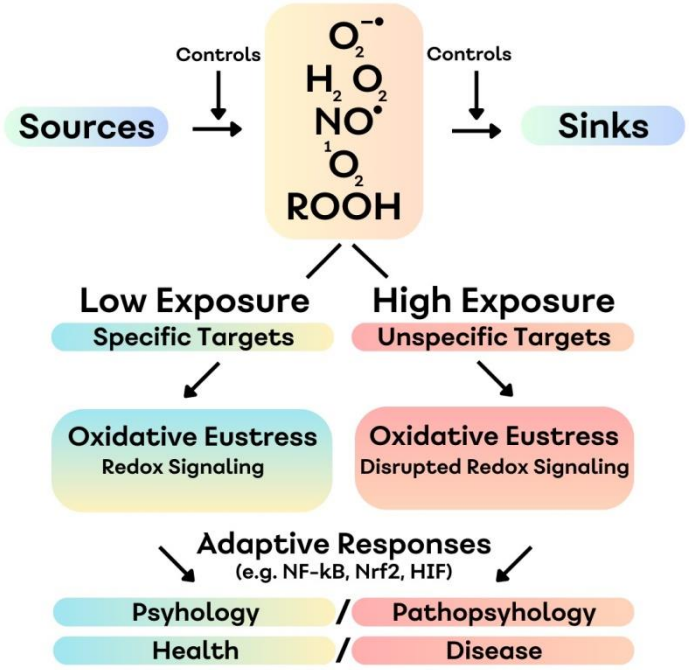

Figure 1. Oxidative stress and its relationship with redox signaling (processing after Sies, 2018 and Rajlic et al., 2023)

3.2. The Biochemistry of Oxidative Stress

Assessing oxidative stress in biology involves measuring redox biomarkers (Marrocco et al., 2017), which should be chemically unique, detectable, and fluctuate according to the level of oxidative stress. These markers need to be stable and specific, unaffected by other biological processes such as the cell cycle or metabolism.

Oxidative stress biomarkers are divided into four main categories: oxidants, antioxidants, oxidation products, and the pro-oxidant/antioxidant ratio. Confirming oxidative stress requires analyzing at least two categories of biomarkers due to the interconnection of chemical and biological systems (Frijhoff et al., 2015).

Oxidative stress is classified by severity, from benign eustress to toxic distress that compromises biomolecule integrity.

Measuring oxidative stress with sensitive, specific, reproducible, and reliable markers and controlling the significance of their fluctuations are important aspects in fundamental research, as well as in the clinic, because identifying increased oxidative stress allows the development of diagnostic, therapeutic, and preventive strategies, given its involvement in the onset and complications of many human diseases (Juan et al., 2021).

These markers should be easy to perform analytically, and their determination should be based on non-invasive methods, applicable to plasma or serum, to blood cellular elements, and to urine. In the absence of a "gold standard" test for free radical activity, a combination of two general

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approaches is used to measure the state of oxidative stress: measuring the products created by the attack of reactive species on the main macromolecular targets (lipids, proteins, and nucleic acids) and determining the endogenous content of antioxidants (enzymatic and non-enzymatic) (Vodičková Kepková and Vodička, 2023).

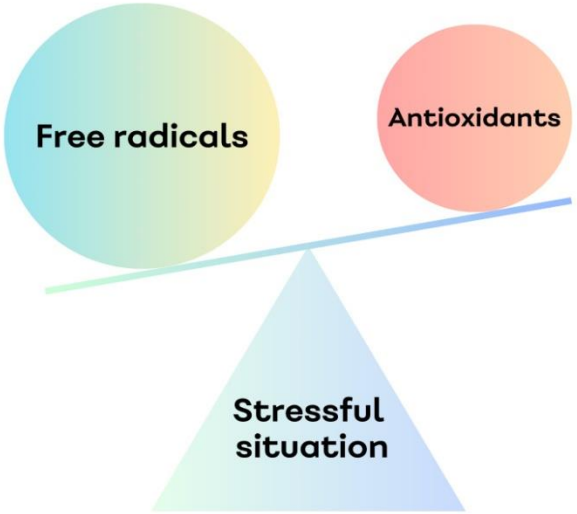

Figure 2. Oxidative stress balance (processing after Hamann, 2015)

An excessive accumulation of free radicals damages essential biological molecules such as DNA, proteins, lipids, and carbohydrates, causing indirect harm through the resulting cytotoxic and mutagenic metabolites, especially during lipid oxidation (Griffiths et al., 2002). Specific biomarkers include products of the oxidation of lipids, proteins, and nucleic acids.

According to the World Health Organization, a biomarker is a measurable substance or process in the body that can indicate the health status or prognosis of a disease. Oxidative stress biomarkers are frequently measurable and suggest an influence on disease (Więdłocha et al., 2023). However, for clinical relevance, a biomarker must indicate disease specificity, predict prognosis, and reflect disease stability, thus contributing to the assessment of therapeutic efficacy. Clinically, it is essential for biomarkers to be stable, detectable in accessible tissues, and allow for cost-effective and reproducible measurements.

Lipid Oxidation Markers - The oxidation of lipids, especially polyunsaturated fatty acids as well as cholesterol by ROS, is called "lipid peroxidation" (Chinko and Umeh, 2023). These non-enzymatic reactions are the basis for the production of many markers of oxidative stress, indicating the accumulation of damage in lipids. The most commonly used compounds in exploring oxidative stress are:

• Hydroperoxides

Hydroperoxides are early products of lipid peroxidation; due to their instability and the great variability of plasma concentrations in "healthy" individuals, they are relatively little used in the assessment of a redox imbalance in humans (Fu et al., 1995). Their determination is carried out by

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high-performance liquid chromatography (HPLC) coupled with electrochemical or fluorometric detection or by mass spectrometry.

• Aldehydes

Malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) are detectable products of lipid peroxidation in plasma or urine by advanced techniques such as high-performance liquid chromatography (HPLC) or gas chromatography (GC) associated with mass spectrometry (Pignoli et al., 2009).

MDA, resulting from the peroxidation of polyunsaturated fatty acids, can react with proteins and contribute to atherogenesis by forming cross-links between lysine residues, which may influence macrophage behavior towards oxidized low-density lipoproteins (OxLDL) and thus promote atherosclerosis.

In practice, MDA is often quantified from plasma samples using colorimetric tests based on the reaction with thiobarbituric acid (TBA). However, TBA-based TBARS tests can also react with other aldehydes, reducing their specificity for MDA. Alternatives such as ELISA kits for MDA are available and offer improved specificity, often validated by comparison with HPLC (De Leon and Borges, 2020).

• Oxysterols

Oxysterols are oxidation products of cholesterol formed by radical attack. The main circulating oxysterols are 7-ketocholesterol and 7-β-hydroxycholesterol (Samadi et al., 2019). Oxysterols are useful for monitoring type 2 diabetic patients, particularly those with cardiovascular risk factors. Oxysterols can be measured by GC-MS or LC-MS/MS.

• Isoprostanes (IsoPs)

Isoprostanes are oxidation products of arachidonic acid. 8-isoprostaglandin F2α (8-iso-PGF2α) is the most studied isoprostane. The relevance of isoprostanes in the assessment of redox imbalance has been demonstrated in many pathological situations, including cardiovascular and neurodegenerative diseases. Their determination in plasma and urine is based on mass spectrometry coupled to GC or HPLC and on immunoassay tests (ELISA). Isoprostanes (IsoPs) are generated through the process of oxidation of arachidonic acid (AA) induced by free radicals. This oxidation of AA occurs regardless of its esterification in phospholipids, triacylglycerides, or cholesterol esters (Czerska et al., 2015). Variations in the types and proportions of IsoP formed are determined by oxygen levels and glutathione concentration in the system.

• Oxidized Low-Density Lipoproteins (LDL) and Corresponding Antibodies

Oxidized LDL and the antibodies they generate are proposed as markers for exploring oxidative injuries in cardiovascular diseases, related to the atherogenic properties of oxidized forms of LDL. These tests are performed on plasma (or isolated LDL) and are based on immunological tests (Trpkovic et al., 2015).

The determination of oxLDL in plasma or purified LDL is a common marker of oxidative stress, linked to the theory of oxidative atherosclerosis. OxLDL is measured by immunological methods using specific monoclonal antibodies, such as 4E6, which detect aldehyde-modified lysine on LDL, and DLH3 and E06, which identify oxidized phosphatidylcholine and phosphorylcholine variants (Afonso and Spickett, 2019).

Protein Oxidation Markers

• Carbonylated Proteins

Carbonylated proteins are widespread products resulting from the oxidation of certain amino acids such as lysine, arginine, proline, and threonine, detectable by ELISA (Alamdari et al., 2005)

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techniques and spectrometry (Baraibar et al., 2013). High concentrations of these carbonylated proteins are often associated with conditions like neurodegenerative diseases and diabetes.

Advanced lipoxidation end products form when lipid aldehydes bind to the amino groups of lysine, cysteine, and histidine through Michael addition. Similarly, advanced glycation end products (AGEs) arise from the interaction between lysine and arginine residues and carbohydrates through glyoxidation. AGEs, a diverse set of compounds, are the result of non-enzymatic reactions between reducing sugars and the amino groups in long-lived lipids, DNA, and proteins, a process amplified in states of hyperglycemia, hyperlipidemia, and oxidative stress.

• Nitrotyrosine (3-nitrotyrosine)

3-nitrotyrosine results from the oxidation of tyrosine by the degradation products of peroxynitrites. It is determined by HPLC coupled with mass spectrometry. High concentrations of 3-nitrotyrosine are found in diabetes, cardiovascular diseases, and some neurodegenerative diseases (Teixeira et al., 2016). Nitrotyrosine (Tyr-NO_2) is recognized as a stable biomarker of oxidative/nitrative stress, particularly in the context of inflammatory conditions. Tyrosine nitration involves the substitution of a hydrogen atom from the aromatic ring of tyrosine with a nitro group. This chemical alteration can occur both at the level of tyrosine incorporated into polypeptides and at free tyrosine. The nitration process, which involves reactive nitrogen species (RNS), typically proceeds in two stages: initially, tyrosine is oxidized to a tyrosine radical, followed by a reaction between this radical and the nitrogen dioxide radical (-NO_2).

• *Advanced Glycation End Products*

Proteins can undergo glycation by an ose to give rise to advanced glycation end products (AGEs), some of which are obtained through oxidation. The concentration of AGEs can be determined by immunoassay, spectrofluorimetry, or chromatography. Among the AGEs used are pentosidine and carboxymethyllysine. The latter could constitute an interesting marker of glycoxidation, which would be correlated with the development of microvascular lesions in diabetics (Kalousova et al., 2002).

Nucleic Acid Oxidation Markers - Purine and pyrimidine bases are sensitive to ROS attack. Guanine is the most sensitive base to oxidation and is the most studied biomarker of oxidative DNA damage, especially in urine (Zabel et al., 2018). Its oxidation product, 8-hydroxy-2'-deoxyguanosine (8-OH-dG), is determined by HPLC (coupled with electrochemistry or mass spectrometry) and by immunoassay. Recently, the test for 8-oxo-guanosine (8-oxoG) has been proposed to evaluate RNA oxidation.

Assessment of Antioxidant Defense Systems

There are many markers that can be used to assess the body's antioxidant state. These include both enzymatic and non-enzymatic antioxidants (Kusano and Ferrari, 2008). Regarding enzymatic antioxidant markers, enzymatic activities of SOD, GPx, and catalase can be measured. Their determination is applied to whole blood. The activity of myeloperoxidase (MPO) is also measured in neutrophils, primarily by immunochemical methods such as ELISA (Cao et al., 2023). An increase in serum MPO has been described during myocardial infarction and unstable angina. Non-enzymatic antioxidant markers include vitamins E (α-tocopherol) and C, carotenoids (β-carotene), ubiquinol, and antioxidant trace elements (Zn, Se, Mn, Cu). All these compounds can be measured in plasma, but also in erythrocytes and platelets. Lipophilic antioxidants (vitamin E, carotenoids, and ubiquinol) can also be determined in different classes of lipoproteins (VLDL, LDL, HDL). Another approach to determining antioxidant status is the estimation of the total radical-trapping parameter (TRAP) in plasma (Silvestrini et al., 2023). This test is widely used as an index of the

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antioxidative capacity of non-enzymatic plasma defenses. The current lack of standardization of most oxidative stress markers and the establishment of reference values, as well as their validation in epidemiological studies, represent a limitation of their use.

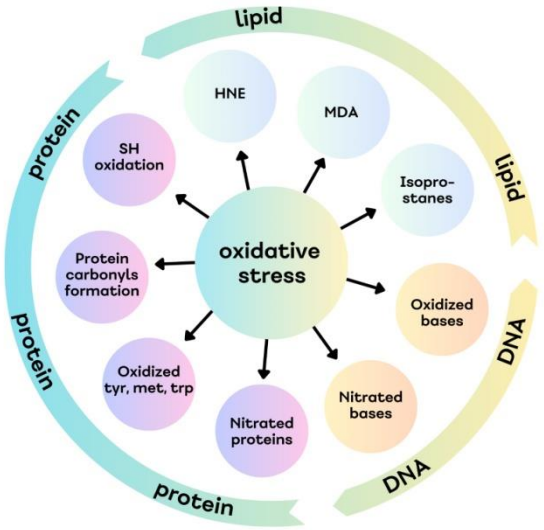

Figure 3. Diagram of markers oxidative stress (processing after Giustarini et al., 2009 and Masenga et al., 2023)

4. CONCLUSIONS

The article has synthesized the concept of oxidative stress, a biological phenomenon with significant implications for human health and disease pathology. Studies highlight that the balance between reactive oxygen species (ROS) and the body's antioxidative defense system is crucial for maintaining cellular homeostasis. An imbalance, where ROS generation exceeds antioxidative capacity, leads to oxidative stress, which can cause cellular damage and contribute to the progression of chronic diseases and the ageing process.

The history of research has revealed that, although initially free radicals and oxidants were considered solely harmful, more recent discoveries underline their dual role, including significant functions in cellular signaling and metabolic regulation. This aspect has shifted the paradigm in redox biology, from a negative perspective to one where reactive species play both beneficial and detrimental roles depending on the context.

The body's antioxidative strategies, such as enzymes (SOD, catalase, glutathione peroxidase) and small molecular weight antioxidants (vitamins E and C), work synergistically to limit oxidative damage. Advances in understanding redox signaling and sensors have led to a redefinition of oxidative stress as a pro-oxidant imbalance that disturbs redox signaling and induces molecular damage. This perspective has generated interest in developing redox biomarkers for diagnosing and monitoring oxidative stress in various pathologies.

In conclusion, the article emphasises the importance of oxidative stress in biology and medicine, as well as the need for a deeper assessment and understanding of the interaction between antioxidative systems and ROS production for the development of effective therapeutic strategies. It also encourages continued research for better characterization of redox biomarkers and the establishment of robust reference values in a clinical and epidemiological context.

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