Review

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Fundamentals of Reductive Stress

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Reductive stress is a cellular insult stemming from the overgeneration of reducing equivalents and heightened antioxidant potential within the body. Maintaining redox homeostasis necessitates a delicate balance between oxidant and antioxidant production. Key indicators of this balance include ratios such as Reduced glutathione (GSH) to Oxidized glutathione (GSSG), Nicotinamide adenine dinucleotide phosphate (NADP) to Reduced nicotinamide adenine dinucleotide phosphate (NADPH), and Nicotinamide adenine dinucleotide (NAD^+) to Reduced nicotinamide adenine dinucleotide (NADH). Glutathione, an endogenous antioxidant, can also be supplemented through natural food sources such as okra, spinach, broccoli, and sweet potatoes among others. These reducing equivalents primarily stem from cellular metabolic processes such as the Krebs cycle and glycolysis. When present in excess, they can modulate signaling pathways, disrupt transcriptional activity, and reduce cellular metabolism, paving the way for various diseases. Conditions associated with reductive stress include cancer, protein aggregation cardiomyopathy, muscular dystrophy, and Alzheimer's disease, among others. Moreover, prolonged use of antioxidant supplements like Vitamins and/or flavonoids may have pro-oxidant effects, disturbing cellular redox balance, inducing reductive stress, and potentially shortening life expectancy. Therefore, the consumption of antioxidant supplements should be moderate and appropriate, as excessive or haphazard intake can be detrimental to overall health.

Keywords: reductive stress; antioxidants; reducing equivalents; Kreb's cycle

Introduction

In vivo, simultaneous oxidation/reduction reactions are constantly occurring. The primary participants in these reactions are redox pairs such as Nicotinamide adenine dinucleotide (NAD^+) and Reduced nicotinamide adenine dinucleotide (NADH), Nicotinamide adenine dinucleotide phosphate (NADP) and Reduced nicotinamide adenine dinucleotide phosphate (NADPH), and Reduced glutathione (GSH) and Oxidized glutathione (GSSG). These redox pairs play crucial roles in the body's metabolism. For instance, NAD^+ serves as an electron sink, facilitating glycolysis. NADPH plays a pivotal role as an electron source for mitochondrial oxidative phosphorylation (OXPHOS) and in the reductive synthesis of nucleic acids and fatty acids [1].

Several pathological conditions are associated with an imbalance in the redox state of these molecules, as their reducing equivalents are critical for maintaining cellular energy metabolism and redox homeostasis. An excess of reducing equivalents can hinder cell proliferation, disrupt the formation of disulfide bonds in proteins, impair mitochondrial activity, and decrease cellular metabolism. Certain disorders, particularly those closely tied to inflammatory and oxidative stress situations, such as muscular dystrophy, hypertrophic cardiomyopathy, and protein aggregation cardiomyopathy, among others, may be influenced by this imbalance [2].

The metabolic pathways of the body's reactions act as sources of these reducing equivalents. For instance, NAD^+, a major cofactor, originates from tryptophan, serving as its precursor. It can be synthesized through the *de novo* pathway from nicotinic acid or through salvage pathways using nicotinamide as the precursor [3]. On the other hand, NADH is metabolized by enzymes involved in glycolysis, the pentose phosphate pathway, etc.

For the human body to maintain homeostasis, antioxidants and oxygen reactive species must be in equilibrium. Antioxidants can be either endogenous, such as catalase and superoxide dismutase, or exogenous, including compounds like resveratrol, and Vitamins E and C. Their role is to scavenge excess reactive oxygen species (ROS) in the body.

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While beneficial, an excess of antioxidants can lead to pro-oxidant effects, which can be harmful and counterproductive to their intended purpose. This review will comprehensively explore the defensive mechanisms of antioxidants, their potential as pro-oxidants, their metabolic origins, and the effects of these reducing equivalents on human health.

Reductive Stress in View

Reductive stress (RS) refers to a condition where the ratios of glutathione-to-glutathione disulfide and NADH to NAD^+ within the cell become elevated over time. It is increasingly recognized as a significant pathophysiological phenomenon akin to oxidative stress, playing a role in various disease states. Reductive stress has emerged as a noteworthy pathophysiological factor, particularly in conditions like cardiomyopathy and coronary artery disease, with its presence noted in numerous cardiomyopathies. The first discovery of reductive stress was made by Gores and colleagues in 1989 [4].

Chronic reductive stress can lead to oxidative stress (OS), which in turn activates reductive stress again through feedback regulation. For example, certain redox proteins may donate electrons to molecular oxygen (O_2).

Instead of the expected majority of electron acceptors during reductive stress, thereby enhancing the formation of reactive oxygen species (ROS) [5]. Reductive stress develops within cells due to the reduction of ROS resulting from an elevation in the ratios of NADP:NADPH, GSH:GSSG, and NAD^+:NADH, or due to excessive expression of antioxidant enzymes [6].

Cellular Homeostasis

Cellular homeostasis, particularly redox homeostasis, is the process through which balance is maintained between the production of reactive oxygen species (ROS), including superoxide anion radicals (O_2^-), hydrogen peroxide (H_2O_2), and antioxidants. To ensure proper transmission of intracellular signaling involved in typical physiological activities, cellular redox equilibrium is essential. However, the intracellular redox milieu may undergo changes due to a unilateral shift towards either an oxidative or a reductive state. Disruptions in redox status can occur upon exposure to infectious organisms (such as bacteria, viruses, parasites, etc.), radiation, toxins, and certain medical conditions.

Reactive oxygen species (ROS), often referred to as pro-oxidants, encompass reactive nitrogen species (RNS) such as nitrous oxide (NOO^-). Principal producers of ROS and RNS species include the mitochondrial electron transport chain, NAD (P) H oxidases (NOX), uncoupled nitric oxide synthases, and other enzymes [7]. To counteract the actions of these oxidants, the body has developed antioxidant defenses, which may be enzymatic or endogenous, such as catalase and superoxide dismutase, among others, capable of scavenging hydrogen peroxide radicals. Additionally, non-enzymatic defenses like ascorbate, α-tocopherol, and GSH play a role.

The failure of cells to maintain this homeostasis leads to redox stress, which can result from an imbalance between oxidative stress and reductive stress.

Redox Stress

Redox stress is a condition in which the normal balance between reactive oxygen species (ROS) and antioxidants in the body is disrupted, resulting in cellular insults such as reductive stress and oxidative stress. The concept of oxidative stress was originally established by Paniker and colleagues [8] in 1970 during their research on the GSH/GSSG pair in H_2O_2-driven human erythrocytes, particularly those with and without a deficiency in glutathione reductase (GR) [6]. The imbalance between pro-oxidants and antioxidants, with a favor towards pro-oxidants, is currently understood to be the underlying cause of oxidative stress, indicating an excess of the former. It is widely recognized that oxidative stress, induced by high levels of ROS and RNS, is detrimental to biological processes and contributes to the development of various pathological conditions, including cancer, aging, and diseases affecting the heart, nervous system, and skin [3,7,9].

Contrary to oxidative stress, reductive stress is not as well conceptualized. Gores and colleagues [4] made the initial discovery in 1989 through experiments where they induced anoxia by exposing rat hepatocytes to drugs that inhibited Adenosine Triphosphate (ATP) production and mitochondrial respiration, effectively simulating chemical hypoxia. According to the researchers, reductive stress, also referred to as reductive stress, occurs when electron carriers undergo reduction due to oxygen deprivation (hypoxia) and subsequently undergo re-oxidation upon re-oxygenation [4].

Redox buffers increase to a specific level referred to as compensatory Redox buffer capacity (ReBC) when cells encounter oxidative or reductive insults, aiming to counteract these redox stresses and maintain redox equilibrium. In this scenario, oxidants and reductants in cells are maintained within physiological levels [5]. However, oxidative or reductive stress occurs when this compensatory response reaches its maximum, surpassing the Redox buffer capacity (ReBC) (Fig. 1, Ref. [7]). It is noteworthy that reductive stress reduces cellular reactive oxygen species (ROS) levels below their physiological values, thereby disrupting signaling processes. Alternatively, it is proposed that reductive stress may indeed induce “oxidative stress, depending on the redox couples in which these ROS are engaged”, as it can also elevate ROS generation (for example, by partially reducing oxygen) [5,10].

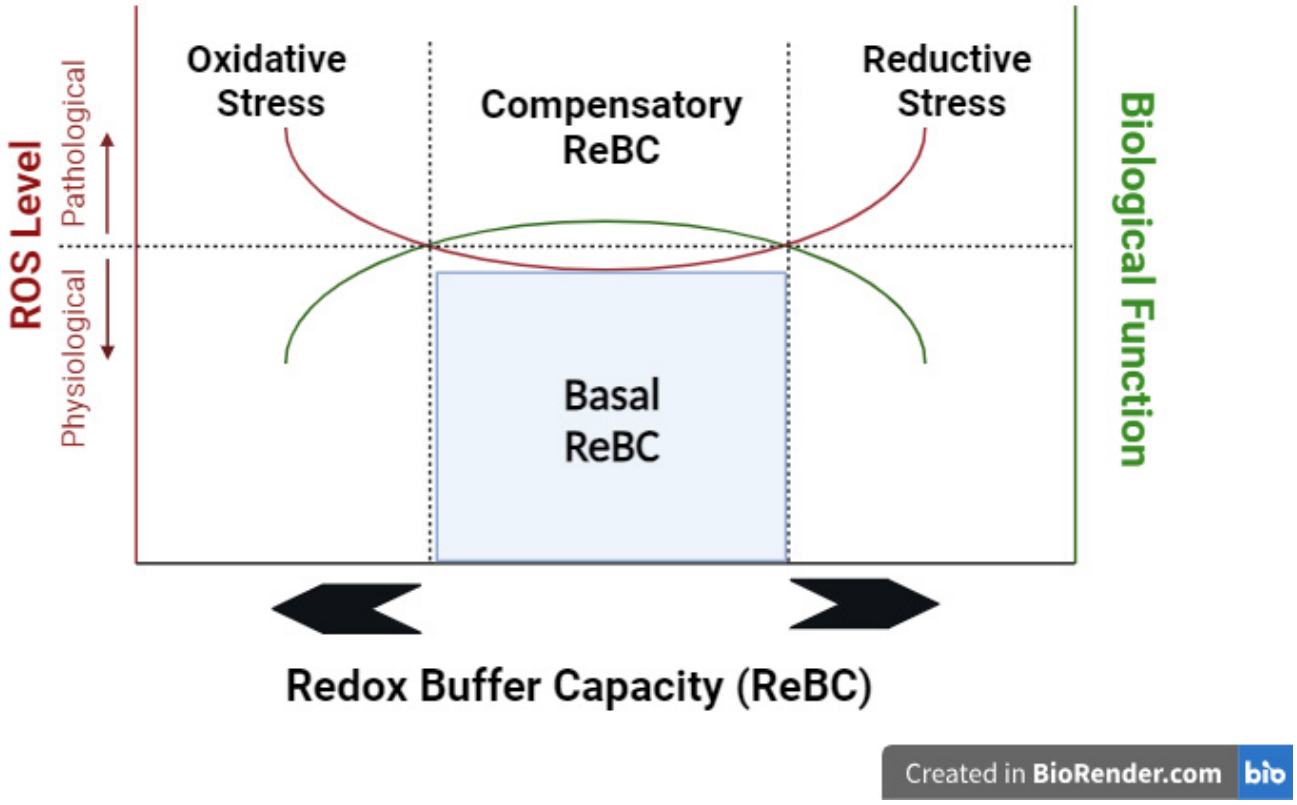

Fig. 1. Redox stress-NAD$^+$/NADH, NADP/NADPH, GSH/GSSG. The main cellular redox buffers in cells are redox couples. Under normal or unstressed conditions, these redox couples help in maintaining redox homeostasis. This maintenance capacity is termed basal redox capacity (green color box). On the other hand, when reactive oxygen species production rises, these redox couples act in response to these changes and also develop a regulatory process called compensatory basal capacity. This occurs by elevating the basal redox capacity above a certain level. When the compensatory response continues beyond the threshold level such that the maximal Redox buffer capacity (ReBC) is exceeded, reductive stress can occur. In contrast, when the ReBC decreases beyond normal, it leads to the generation of reactive oxygen species which consequently leads to oxidative stress. Redox Stress is the umbrella word for these two concepts: reductive stress and oxidative stress [7] reactive oxygen species (ROS). GSH, Reduced glutathione; GSSG, Oxidized glutathione; NADP, Nicotinamide adenine dinucleotide phosphate; NADPH, Reduced nicotinamide adenine dinucleotide phosphate; NAD$^+$, Nicotinamide adenine dinucleotide; NADH, Reduced nicotinamide adenine dinucleotide.

Antioxidants

The proposed definition of an antioxidant by Halliwell and Gutteridge states, "any chemical that, when present at low concentrations relative to those of an oxidizable substrate, considerably retards or prevents oxidation of that substrate" [11]. Others have described antioxidants as "any chemical that directly scavenges ROS or indirectly upregulates antioxidant defenses or decreases the generation of oxygen reactive species".

The body's intricate antioxidant defense system utilizes both endogenous enzymatic and non-enzymatic antioxidants (Fig. 2). Together, these compounds work to counteract free radical damage to vital biomolecules and ultimately protect bodily tissues. Within biological systems, antioxidant molecules function at various levels in defense against free radicals. These levels include radical abatement, radical scavenging, and damage repair caused by radicals. Depending on their role in the defense system, antioxidants can be categorized into four groups: first-line defense, second-line defense, third-line defense, and fourth-line defense antioxidants (Fig. 2).

Classification of Antioxidants

Various attempts have been made to classify antioxidants. Primarily, they are categorized as either exogenous (e.g., resveratrol, Vitamins, carotenoids, phytochemicals, polyphenols, flavonoids, and trace elements) or endogenous (enzymatic antioxidants such as catalase, superoxide dismutase, glutathione reductase, glucose-6-phosphate dehydrogenase, and glutathione peroxidase, among others). Additionally, some schools of thought include synthetic antioxidants (e.g., tert-butylhydroquinone (TBHQ), butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT)). Certain endogenous compounds like uric acid, conjugated bilirubin, melatonin, lipoic acid, and plasmalogens

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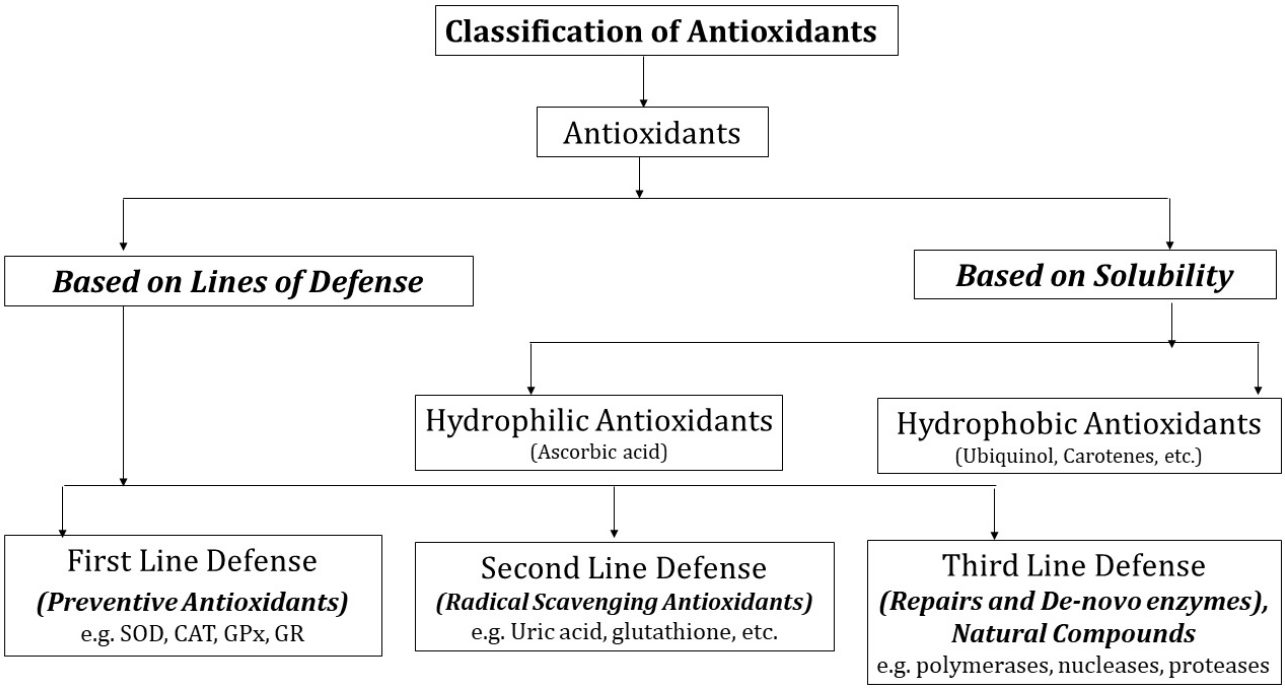

Fig. 2. Classes of antioxidants: a flowchart showing the different classes of antioxidants. These classes are based on their lines of defense and their solubility. The first line of defense consists of enzymatic or endogenous antioxidants such as superoxide dismutase, glutathione peroxidase, and catalase, which prevent the production of oxygen reactive species in the first place. The second line of defense helps to scavenge the free radicals after they are being produced while the third line of defense only becomes relevant until the free radical damage has taken place. SOD, superoxide dismutase; GPx, glutathione peroxidase; GR, glutathione reductase; CAT, catalase.

have also been classified as antioxidants. For the purposes of this review, however, the classification will be limited to categorization based on the line of defense and solubility.

First Line Defence Antioxidants (Radical Preventive). These antioxidants comprise a group that effectively slows down or halts the synthesis of reactive species within cells, including free radicals. They promptly neutralize any compound with the potential to generate free radicals or any other reactive species. Catalase, superoxide dismutase, and glutathione peroxidase are the primary enzymes in this category. The biological system's antioxidant defense mechanism against free radical attack relies on these antioxidant enzymes, as illustrated in Fig. 3 [12].

Superoxide dismutase (SOD) catalyzes the conversion of singlet oxygen radical ($^*O_2$) and superoxide anion radicals (O_2^-)—which form in tissues due to metabolic or cellular processes—into hydrogen peroxide (H_2O_2) and molecular oxygen (O_2). Accumulation of H_2O_2 can be toxic to body tissues or cells. Moreover, in the presence of Fe_2^+, it undergoes a Fenton reaction to generate harmful hydroxyl radicals (OH^-). Peroxisomes are a major source of the antioxidant catalase, which acts to prevent this occurrence by converting H_2O_2 into water and molecular oxygen, thereby reducing the damage caused by free radicals (Fig. 3) [12].

Lipid peroxides are reduced by glutathione peroxidase (GPx) to their corresponding alcohols, and hydrogen peroxide (H_2O_2) is converted to water. This process occurs because catalase is not present in mitochondria. These actions constitute the first-line antioxidant defense, as they form a group of protective antioxidants also known as primary defensive antioxidants. Therefore, the function and efficiency of the first line of antioxidants, primarily consisting of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), are crucial to the overall antioxidant defense strategy. This is particularly relevant in reference to the singlet oxygen radical ($^*O_2$), which is continuously generated in the body's regular metabolism through various mechanisms.

Second Line Defense Antioxidants (Radical Scavenging). This group of antioxidants is often referred to as "scavenging antioxidants" because they scavenge active radicals to halt chain initiation and interfere with chain propagation reactions. They achieve this by providing free radicals with an electron, causing them to transform into new, less harmful radicals. These "new radicals" can then be swiftly neutralized by other antioxidants in this class, rendering them completely harmless. The scavenging antioxidants include hydrophilic compounds such as Ascorbic Acid (Vitamin C), uric acid, and glutathione, as well as lipophilic compounds like α-tocopherol (Vitamin E), ubiquinol, and uric acid.

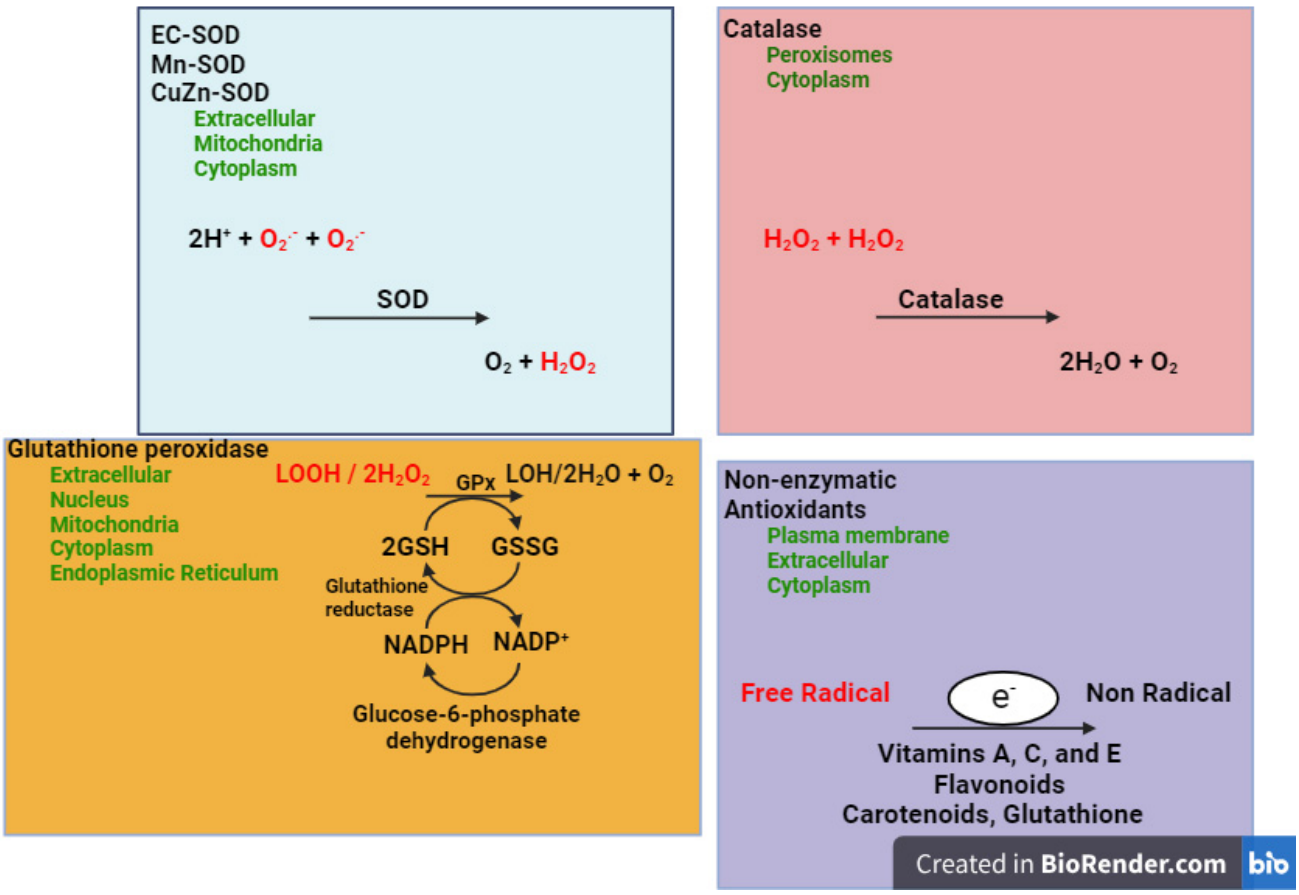

Fig. 3. Pathway showing mechanism of action of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). Since the superoxide dismutase catalyzes the dismutation of two molecules of superoxide anion ($O_2^{\cdot -}$) into hydrogen peroxide (H_2O_2) and molecular oxygen (O_2), the potentially dangerous superoxide anion is reduced in danger. SOD is usually bound with copper and zinc metals. It is usually called a metalloenzyme because it requires metal cofactors. The detoxification process started by SOD is finished by catalase, which catalyzes the breakdown or reduction of hydrogen peroxide (H_2O_2) to water and molecular oxygen using either iron or manganese as a cofactor. In the mitochondria, and occasionally in the cytoplasm, glutathione peroxidase converts hydrogen peroxides to water and lipid peroxides to the corresponding alcohols. EC, Extracellular; Mn, manganese; Cu, copper; Zn, zinc; LOOH, lipid peroxide; LOH, Lipid alcohol.

Third Line Defense Antioxidants (Radical-Induced Damage Repair). This group of antioxidants primarily comes into play after damage from free radicals has already occurred. These enzymes play a vital role in restoring the integrity of the cell membrane and repairing damage to biomolecules caused by free radicals. They are responsible for repairing lipid, protein, and DNA damage. Moreover, they undertake a crucial "cleanup" task, which involves identifying, breaking down, and eliminating oxidized or damaged proteins, DNA, and lipids to prevent their accumulation, which could be harmful to bodily organs. The DNA repair enzyme systems, including polymerases, glycosylases, and nucleases, as well as the proteolytic enzymes such as proteinases, proteases, and peptidases found in both the cytosol and mitochondria of mammalian cells, are typical examples of this group.

Antioxidants are also Classified Based on their Solubility in Water. Antioxidants typically react with oxidants in blood plasma and cell cytoplasm, but lipid-soluble antioxidants play a crucial role in protecting cell membranes against lipid peroxidation. Ascorbic acid, a primary hydrophilic antioxidant, is particularly effective as a lipid peroxidation inhibitor. Examples of hydrophobic antioxidants such as ubiquinol, carotenes, and α-tocopherol have also been demonstrated to shield cell membranes from lipid peroxidation. These antioxidants are obtained either through dietary sources or synthesized internally.

Mechanisms of Action of Named Antioxidants

Superoxide Dismutase. The first enzyme, involved in detoxification within the cell and possessing the highest potency as an antioxidant, is superoxide dismutase (SOD). It serves as a crucial endogenous antioxidant enzyme, func-

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tioning as the body's initial line of defense against reactive oxygen species (ROS). Its primary role is to catalyze the dismutation of two superoxide anion molecules (O_2*) into hydrogen peroxide (H_2O_2) and molecular oxygen (O_2), thus reducing the risk posed by superoxide anions (Fig. 3). As a metalloenzyme, SOD requires a metal cofactor to function properly. There are several variants of the enzyme, each based on the type of metal ion required as a cofactor [11,13]. SOD commonly binds to metal ions such as iron (Fe), zinc (Zn), copper (Cu), and manganese (Mn). Based on the metal ion cofactor, SODs are classified into three types.

These are:

(i) Fe-SOD, which is found in prokaryotes and in the chloroplasts of certain plants;

(ii) Mn-SOD, which is found in prokaryotes and eukaryotic mitochondria; and

(iii) Cu/Zn-SOD, which is more widely distributed in eukaryotes and is primarily located in the cytosol but is also present in the chloroplast [14].

It is common to have deficiencies in the SOD enzyme. Consequently, the enzyme is crucial for maintaining healthy cells by shielding them from free radicals, excessive oxygen radicals, and other harmful substances that accelerate cellular aging or death. As individuals age, SOD levels decline while free radical production increases. According to certain theories, proper daily supplementation of SOD can enhance the immune system, significantly reduce the risk of developing diseases, and ultimately decelerate the aging process. Krishnamurthy and Wadhwani [15] recommended cabbage, brussels sprouts, wheatgrass, barley grass, and broccoli as natural sources of SOD.

Catalase (CAT). Nearly all oxygen-utilizing living tissues possess the ubiquitous antioxidant enzyme catalase (CAT). This enzyme completes the detoxification process initiated by SOD by catalyzing the breakdown or reduction of hydrogen peroxide (H_2O_2) into molecular oxygen and water, utilizing either manganese or iron as a cofactor [12,13]. SOD is widely distributed within cells, constantly scavenging hydrogen peroxide molecules. CAT exhibits remarkable efficiency, capable of breaking down a million molecules of hydrogen peroxide in a single second. Although primarily located in peroxisomes, CAT is absent in mitochondria in mammalian cells, with the exception of mitochondria in the rat heart [13]. This suggests that a different enzyme, glutathione peroxidase, functions within the mitochondria of mammalian cells to break down hydrogen peroxide into oxygen and water.

Furthermore, certain alcohols such as ethanol, methanol, and phenols, among others, exhibit peroxidase activity and act as effective hydrogen donors that catalase (CAT) interacts with favorably. Catalase activity involves a two-step process: a hydrogen peroxide molecule converts heme into oxyferryl. Then, one oxidation equivalent from iron and one from the porphyrin ring are removed, resulting in the formation of a porphyrin cation radical. A second hydrogen peroxide molecule, acting as a reducing agent, restores the enzyme to its resting state, producing oxygen and water molecules [16].

While low concentrations of hydrogen peroxide regulate various physiological processes such as cell proliferation signaling, cell death, carbohydrate metabolism, mitochondrial function, platelet activation, and the maintenance of normal thiol redox balance [13,17], high concentrations have been reported to be extremely harmful to cells [13,18]. The ability of CAT to effectively reduce the levels of H_2O_2 in cells underscores its importance in the aforementioned physiological processes, as well as its role as a first-line antioxidant defense enzyme. Deficiencies or mutations in the enzyme have been linked to various disease states and abnormalities [19].

Glutathione Peroxidases (GPx). The crucial intracellular enzyme glutathione peroxidase (GPx) plays a pivotal role in breaking down lipid peroxides into their corresponding alcohols and hydrogen peroxide (H_2O_2) into water, primarily within the mitochondria and occasionally in the cytoplasm [20]. Its function often relies on selenium, a micronutrient cofactor, which is why GPx is commonly referred to as a selenocysteine peroxidase. The enzyme's primary role is to suppress the lipid peroxidation process, thereby shielding cells from oxidative stress [21].

Morón and Cortázar [22] assert that humans possess at least eight GPx enzymes, numbered from GPx1 to GPx8, with corresponding genes located on chromosomes 3, 14, 5, 19, 6, 6, 1, and 5, respectively. The most prevalent glutathione peroxidase, GPx1, is nearly ubiquitous in all cells and functions as a selenoperoxidase [13,23]. Several studies have underscored the clinical significance of GPx. According to Chabory *et al.* [24], individuals with reduced GPx activity are more susceptible to deficient antioxidant defenses, leading to oxidative damage to membrane fatty acids and functional proteins, thereby potentially causing neurotoxic harm [24]. In a previous hypothesis, Forgione and colleagues [25] proposed that GPx1 deficiency induces endothelial dysfunction by directly increasing vascular oxidative stress. Glutathione Peroxidases, especially GPx1, have been associated with a range of common and complex disorders, including cancer and cardiovascular disease [26,27].

Ascorbic Acid (Vtamin C). Lipid peroxidation (LPO) exemplifies a radical chain reaction, and Vitamin C plays a significant role in this process. Reactive oxygen species (ROS) which are generated by various enzymes, including those in the electron transport chain, xanthine oxidase, myeloperoxidase, and NADPH oxidase, initiate the radical reaction by abstracting hydrogen atoms from bisallylic C-H bonds, forming lipid radicals [28]. Lipids are often

the primary targets of oxygen radicals due to the presence of numerous ROS-producing enzymes within lipid bilayers and the relatively weak bisallylic C-H bonds in polyunsaturated fatty acids (PUFAs) compared to other C-H bonds. In the absence of membrane-bound α-tocopherol, carbon-centered lipid radicals react with molecular oxygen to generate peroxyl radicals, which can further participate in radical propagation reactions. Lipid hydroperoxides serve as precursors to various LPO products, including 2-alkenals, epoxides, and malondialdehyde, when they are not converted to hydroxy-fatty acids by glutathione-dependent reductases. Vitamin C can prevent LPO by scavenging ROS and utilizing the Vitamin E redox cycle to reduce lipid hydroperoxyl radicals by one electron [28].

Vitamin E (α-Tocopherol). As a potent peroxyl radical scavenger and antioxidant that interrupts antioxidant chains, Vitamin E halts the propagation of free radicals in membranes and plasma lipoproteins [29]. When peroxyl radicals (ROO·) are generated, they react with Vitamin E (Vit E-OH) approximately 1000 times faster than they do with polyunsaturated fatty acids (PUFAs) [30]. The combination of the hydroxyl group of tocopherol with the peroxyl radical leads to the formation of the tocopheryl radical (Vit E-O·) and the corresponding lipid hydroperoxide. When vitamin C (or other hydrogen donors, AH) interacts with the tocopheryl radical (Vit E-O·), the latter is oxidized, and vitamin E returns to its reduced state [31].

Vitamin E recycling refers to the continuous regeneration of the antioxidant function of oxidized Vitamin E through interactions with other antioxidants, particularly vitamin C. This process is influenced by the availability of aqueous antioxidants and cellular metabolism, constituting an "antioxidant network". It is worth noting that the interaction of free metals such as iron or copper with lipid hydroperoxides (ROOH) can initiate lipid peroxidation (LPO) by generating alkoxy radicals. Similarly, if other antioxidants are not present, tocopheryl radicals (Vit E-O·) can also trigger LPO [32].

In vivo, oxidized tocopherols are generally scarce because the tocopheroxyl radical can be converted back to tocopherol by ascorbate or other reducing agents. However, several biologically significant oxidation products are formed from α-tocopherol, including 4a,5-epoxy-, 7,8-epoxy-8a (hydroperoxy) tocopherones and their respective hydrolysis products, 2,3-epoxy-tocopherol quinone, and 5,6-epoxy-tocopherol quinone [33].

Pro-Oxidant Effects of Antioxidants

While antioxidants offer significant benefits to the body, maintaining them at an optimal level is crucial. Excess antioxidants can pose a threat to the body by inadvertently generating reactive oxygen species (ROS), which they are originally meant to scavenge. To mitigate this risk, it is advisable to supplement antioxidants only when oxidative stress has been identified. Prooxidants, on the other hand, are reduced antioxidants that can react with molecular oxygen. In aerobic environments, they can produce superoxide radicals, which subsequently dismutate to form H_2O_2. This H_2O_2 can then interact with reduced metal ions and superoxide to generate hazardous ROS. For example, flavonoids may act as prooxidants in the presence of reduced metals, while tocopherols may exhibit similar behavior in the presence of transition metals like Cu (I), depending on the specific matrix environment in which they are found.

Prooxidants are substances that induce oxidative stress either by generating reactive oxygen species (ROS) or by inhibiting the antioxidant system. Several factors, including the presence of metal ions, the concentration of antioxidants in matrix settings, and their redox potential, can influence an antioxidant's function and potentially transform it into a prooxidant [34,35]. Despite its potent antioxidant properties, Vitamin C can also exhibit prooxidant activity when interacting with iron and copper. This interaction leads to the reduction of Fe^{3+} to Fe^{2+} (or Cu^{3+} to Cu^{2+}), subsequently converting hydrogen peroxide into hydroxyl radicals [36]. Thus, while Vitamin C is a robust antioxidant, its effect can vary depending on dosage. Low doses (30–100 mg/kg body weight) may have antioxidant effects, while high doses (1000 mg/kg body weight) may exhibit prooxidant effects [36].

While α-tocopherol is renowned for its potent antioxidant properties, excessive doses can lead to pro-oxidant activity. If there is inadequate ascorbic acid available for regenerating Vitamin E, it may persist in a highly reactive state and contribute to the autoxidation of linoleic acid. Under such circumstances, Vitamin E can engage with free radicals, eventually becoming a radical itself [36,37]. Additionally, there is speculation regarding the pro-oxidant effects of carotenoids, particularly through autoxidation in the presence of significant concentrations of oxygen, resulting in the formation of hydroxyl radicals. However, conclusive evidence supporting this hypothesis is lacking [36].

There have been reports regarding the pro-oxidant potential of resveratrol. Resveratrol, a natural polyphenol found in grapes, is often associated with cardiovascular protection due to its antioxidant effects on the endothelium. However, studies have shown that while resveratrol exhibits beneficial effects at low to moderate doses, higher doses can lead to an increase in intracellular oxidative stress in endothelial cells. This oxidative state can subsequently cause mitochondrial membrane depolarization and eventual endothelial cell death. Consequently, resveratrol has been implicated in inducing oxidative stress and impairing mitochondrial function, ultimately resulting in endothelial cell death [38].

Furthermore, another study reported that both resveratrol and coumaric acid demonstrate antioxidant effects at moderately high doses. However, at higher doses, they ex-

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hibit a dose-dependent pro-oxidant effect, which is associated with apoptosis, cell damage, and downregulation of phospho-Akt [39].

Furthermore, an additional study highlighted that at low doses, resveratrol exhibited cytoprotective effects and bolstered the antioxidant system. This was characterized by scavenging of reactive oxygen species (ROS), augmentation of antioxidant defense system activity (such as Nuclear factor erythroid 2-related factor 2 (Nrf2), CAT, SOD, GSH, etc.), inhibition of lipid peroxidation, enhancement of immune function, protection against DNA damage, prevention of mitochondrial respiratory dysfunction, and elevation of sirtuin 1 level/activity. However, at high doses, resveratrol led to increased ROS production and diminished levels of antioxidant defense system activity (including Nrf2, CAT, SOD, GSH, etc.), resulting in reduced cell proliferation, impaired immune function, induction of heat shock response, promotion of DNA damage, especially in the presence of Cu^{2+}, induction of senescence, and inhibition of epithelial-to-mesenchymal transition (EMT) [40].

Essential Markers for Reductive Stress (NAD$^+$:NADH and GSH:GSSG Ratio)

NAD$^+$:NADH Ratio as a Marker of Reductive Stress

NAD$^+$/NADH constitutes a crucial redox pair serving as a cofactor in numerous metabolic reactions. Synthesized from tryptophan or Niacin (Vitamin B3), NAD$^+$ represents the oxidized form, whereas NADH is its reduced counterpart. These molecules play integral roles in Adenosine Triphosphate (ATP) formation via glycolysis, the tricarboxylic acid (TCA) cycle, mitochondrial oxidative phosphorylation, and various other pathways. Their levels are meticulously regulated in a homeostatic manner, ensuring that the oxidation of one is balanced by the reduction of the other. While NADH functions as an antioxidant, excessive accumulation can induce reductive stress and the generation of reactive oxygen species (ROS).

Furthermore, an excess of NADH or a deficiency in NAD$^+$ can lead to an accumulation of NADH [10]. Mitochondrial complex I (NADH dehydrogenase) responds to the electron pressure resulting from excessive NADH synthesis by oxidizing more NADH to NAD$^+$. This process elevates electron leakage, leading to the reduction of oxygen to produce superoxide anion radicals (O_2^-). Consequently, these free radicals exacerbate oxidative stress. The accumulation of reducing equivalents, such as NADH, results in an oxidative state [41]. Dysfunction of mitochondrial complex I, responsible for recycling NADH, can lead to reductive stress and NADH buildup. This accumulation may impede β-cells from releasing insulin [42].

In a hyperglycemic state, increased glucose levels lead to heightened flux through the glycolytic pathway, resulting in elevated production of pyruvate and acetyl-CoA, and subsequently, NADH [43]. The surplus of NADH exerts pressure on the mitochondrial electron transport chain, given its role as an electron carrier [43]. This phenomenon is particularly pronounced in hepatocytes and pancreatic beta-cells due to the uninhibited activity of glucokinase (hexokinase D) by glucose-6-phosphate (G6P) [44]. Consequently, as glucose levels escalate, there is a corresponding increase in G6P generation, which undergoes breakdown via glycolysis and the Krebs cycle, thereby augmenting NADH production and, ultimately, contributing to reductive stress.

Metabolic Sources of NAD$^+$/NADH in Reductive Stress

The balance of NAD$^+$/NADH is critical for cellular metabolism, particularly in energy production and biosynthetic pathways. NAD$^+$/NADH serves as a key player in ATP generation through oxidative phosphorylation, acting as the primary energy source. Additionally, it contributes to energy production during glycolysis, although to a lesser extent compared to mitochondrial oxidative phosphorylation. An imbalance in the NAD$^+$:NADH ratio has been implicated in mitochondrial dysfunction, highlighting its importance in cellular homeostasis. Furthermore, NADH plays a crucial role in generating NADPH via the pentose phosphate pathway (PPP), which is essential for reductive biosynthesis processes such as nucleotide, amino acid, and lipid synthesis [45]. Glutathione is intricately linked to cellular redox state and amino acid metabolism, further emphasizing the interconnectedness of these metabolic pathways.

One key regulator of mitochondrial NADPH levels is nicotinamide nucleotide transhydrogenase (NNT). Overexpression of NNT has been associated with reductive stress, characterized by elevated NADP:NADPH ratios, particularly observed in melanoma cells [46]. This reductive stress induces glutaminolysis, leading to the production of α-ketoglutarate, which can be further metabolized to succinyl CoA or citrate in the TCA cycle. Conversely, silencing of NNT results in a decrease in glutamine metabolism and an increase in glucose oxidation and energy production via the TCA cycle [46]. These findings underscore the metabolic flexibility of melanoma cells in response to changes in redox balance, highlighting the intricate interplay between reductive stress, energy metabolism, and cell survival.

Another crucial source of NAD$^+$/NADH is the malate-aspartate shuttle (Fig. 4b). It achieves this by maintaining high NAD$^+$ levels in the cytosol and high NADH levels in the mitochondria via a critical compartmental exchange of NADH and NAD$^+$ (Fig. 5). If this exchange pathway is disrupted, cellular redox homeostasis and energy metabolism may suffer, potentially affecting NAD$^+$/NADH levels in both compartments. GOT2, mitochondrial glutamate oxaloacetate (OAA) transaminase, is a key enzyme in the malate-aspartate shuttle (Fig. 4a). Consequently, cytosolic NADH levels rise while mito-

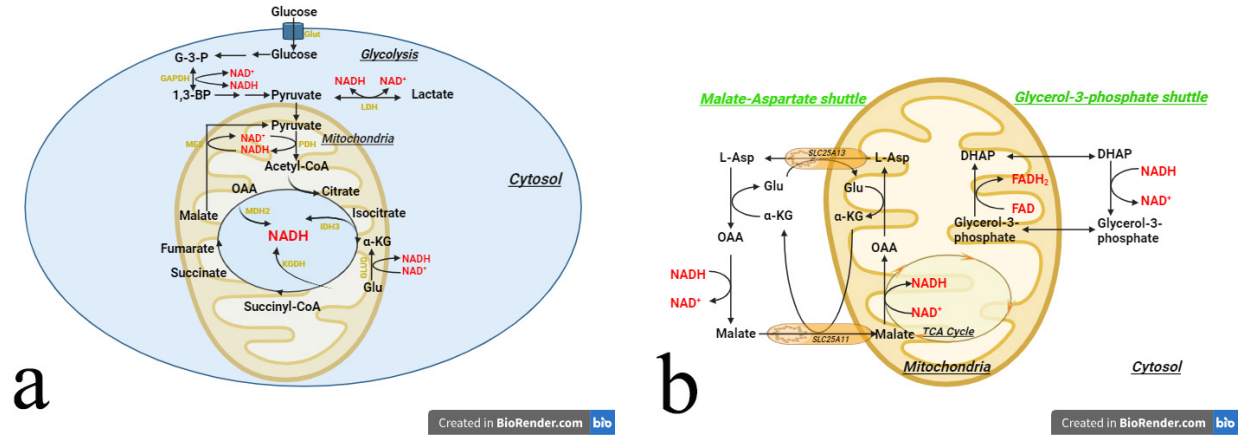

Fig. 4. Metabolic sources of NAD^+/NADH via glycolysis (a) malate -aspartate shuttle, and glycerol-3-phosphate shuttle (b). OAA, oxaloacetate; Glu, glutamate; TCA, tricarboxylic acid; FAD, flavin adenine dinucleotide; $FADH_2$, Reduced flavin adenine dinucleotide; α-KG, Alpha-ketoglutarate; L-Asp, L-Aspartate; DHAP, Dihydroxyacetone phosphate.

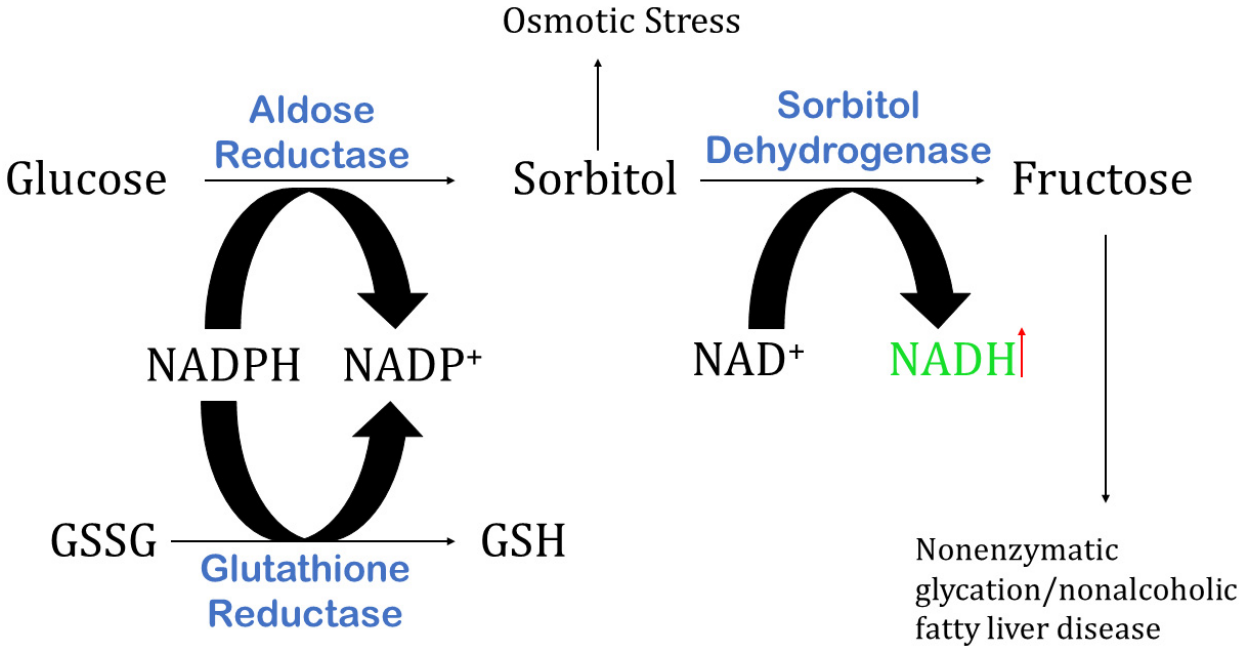

Fig. 5. Polyol pathway. The two processes that are respectively conducted by sorbitol dehydrogenase and aldose reductase are shown above. From glucose, the routes produce sorbitol, which is then converted to NADH. The buildup of this NADH may result in reductive stress, which then progresses to oxidative stress.

chondrial NADH levels fall, resulting in a decrease in NADPH/$NADP^+$ and an increase in cellular ROS production.

NADH Production via Polyol Pathway. Aldose reductase and sorbitol dehydrogenase catalyze two sequential events in the polyol pathway, depicted in Fig. 5. Under euglycemic conditions, this route is typically rather inactive [47], but it can become a highly active glucose disposal pathway under diabetic hyperglycemia. The generation of NADH, sorbitol, and fructose is a key characteristic of this pathway. Each of these by-products or intermediates contributes to the development of diabetes and its consequences. For instance, fructose can lead to nonenzymatic protein glycation or nitration and contribute to the pathophysiology of nonalcoholic fatty liver disease, while sorbitol can accumulate in retinal and renal tissues, causing osmotic stress and cell death [48]. More crucially, a large amount of NADH produced by this pathway is known to disturb the redox balance between NADH and NAD^+, and consuming NADPH can impede glutathione reductase activity, leading to a buildup of oxidized glutathione and aggravating the redox imbalance [10,49].

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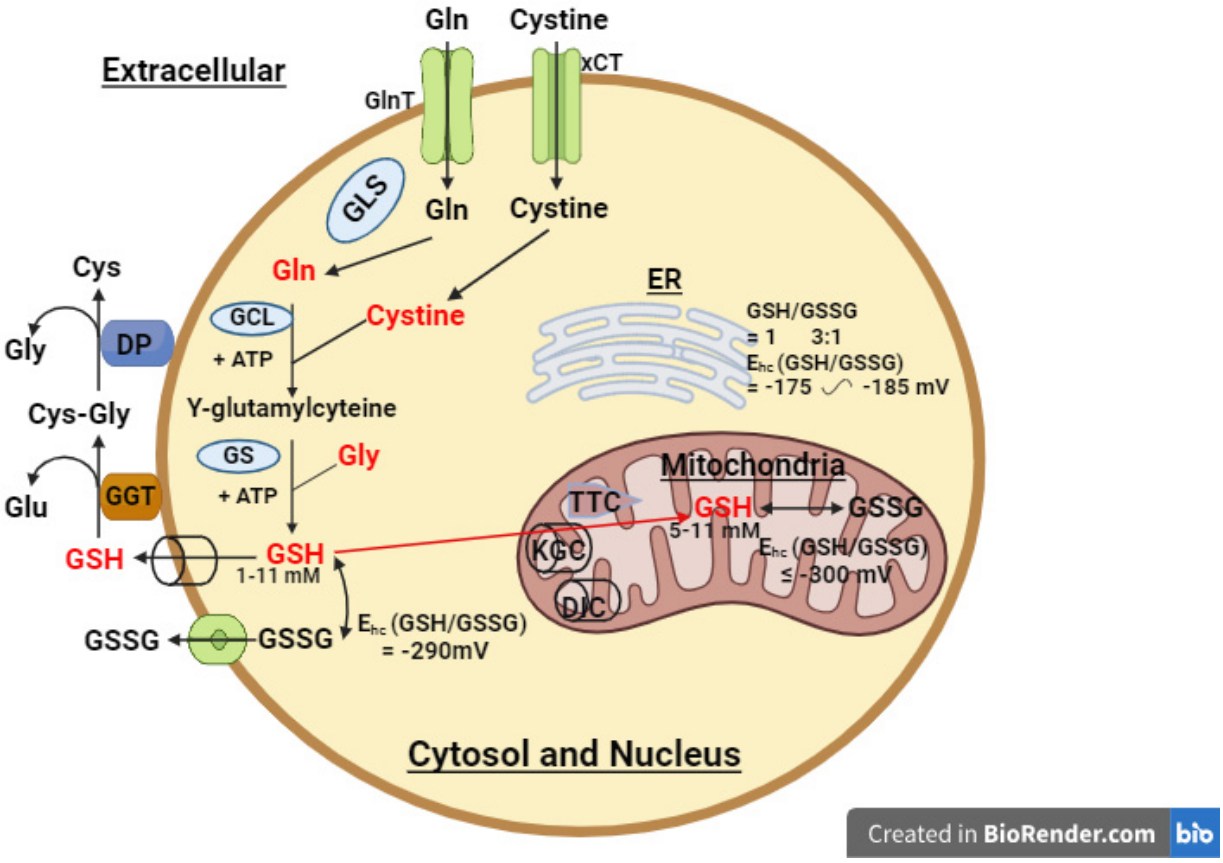

Fig. 6. Metabolic sources of GSH/GSSG. The three amino acids that make up GSH as a precursor are glutamate (Glu), cysteine (Cys), and glycine. Glutamylcysteine ligase (GCL), the rate-limiting enzyme, and GS are two ATP-dependent enzymes required for the two sequential processes necessary to produce GSH (GSH synthetase). Through gls-mediated glutaminolysis, both endogenous and exogenous Gln can replace Glu. By reducing both exogenous and endogenous cystine, cys can be produced. GS, GSH synthetase; ER, endoplasmic reticulum; GLS, glutamate synthetase; Gln, glutamine; GlnT, glutamine transporter; ATP, Adenosine Triphosphate; GGT, gamma-glutamyl transpeptidase; Glu, glutamate; Cys, cysteine.

The GSH:GSSG Ratio

The tripeptide glutathione, composed of glutamate, cysteine, and glycine, and possessing a low molecular weight, has been associated with several diseases and is frequently used as an indicator of cellular redox state. According to Lu (2013) [50], it is synthesized by the enzymes glutathione reductase (GR), gamma-glutamyl-cysteine synthetase (GCL), and GSH synthetase (Fig. 6). GSH serves as a widely distributed, nonprotein thiol antioxidant crucial for defending against intracellular free radicals and eliminating electrophilic toxicants. Furthermore, GSH facilitates the conversion of Vitamins E and C into their active forms and neutralizes O_2 and OH radicals [51].

The presence of oxidative stress (OS) is often indicated by decreased levels of plasma and cellular GSH [52], as GSH is oxidized to GSSG in the presence of reactive oxygen species (ROS). Moreover, GSSG can accumulate within cells and interact with sulfhydryl groups on proteins to form GSH-disulfide proteins, which have longer half-lives and reduce the amount of improperly folded proteins [53]. Glucose-6-phosphate dehydrogenase, GSH synthase, gamma-glutamyl-cysteine synthetase (GCL), and glutathione reductase (GR) are enzymes involved in the biosynthesis and production of GSH. GCL, by regulating the conversion of glutamine and cysteine into gamma-glutamyl-cysteine, catalyzes the rate-limiting step in GSH synthesis. Its increased expression elevates GSH levels, which can be utilized to counteract ROS during OS conditions. The ability of GSH to confer resistance to anticancer medications like cisplatin has been demonstrated, as evidenced by increased GSH expression in non-small cell lung cancer (NSCLC) leading to resistance to cisplatin therapy [54].

The redox potential of the GSH:GSSG ratio varies in different subcellular compartments, with different ratios recorded in the cytoplasm, endoplasmic reticulum, mitochondria [55]. GSH is transported into mitochondria via the 2-oxoglutarate and dicarboxylate carriers, thereby increasing the availability of GSH for mitochondrial peroxidases and modulating H_2O_2 levels.

Table 1. Some of the diseases associated with reductive stress.

S/N	Disease	Indications from reductive stress (RS)	Ref(s)
1	Pulmonary Hypertension	Increased mitochondrial NADH	[58,59]
2	Stent Stenosis	Alteration of GSH:GSSG and NADPH:$NADP^+$ ratios	[2,60,61]
3	Parkinson's Disease	Increase in NAD^+/NADH	[62]
4	Insulin Resistance/Metabolic Syndrome	Increase in NADH	[63–65]
5	Rheumatoid Arthritis	Increase and accumulation of NADPH	[66]
6	Renal Disease	Increase in GSH	[67]
7	Infectious Disease	Increase in thiol levels	[68]
8	Alzheimer's Disease	Increased glucose 6-phosphate dehydrogenase (G6PD) and GSH	[69]

Metabolic Sources of GSH/GSSG in Reductive Stress

Glutamate serves as a precursor for the synthesis of glutathione (Fig. 6). Through a process called glutaminolysis, glutamine undergoes hydrolysis into glutamate catalyzed by glutamate synthetase (cytosolic GLS1 and mitochondrial GLS2), which is then converted into α-ketoglutarate by glutamate dehydrogenases. Enhancing glutaminolysis could alter cellular redox status, energy metabolism, and GSH levels. For example, in three human cancer cell lines, the overexpression of GLS2 led to a significant increase in the levels of glutamate, GSH, and NADH, as well as the GSH:GSSG ratio. This increase was associated with a reduction in basal levels of reactive oxygen species (ROS) in the cells, indicating the presence of reductive stress in these cells [56].

Deletion of prolyl hydroxylase (PHD2) resulted in increased glutamine uptake and upregulation of GLS1 expression, leading to enhanced glutaminolysis and elevated cellular GSH levels and GSH:GSSG ratio. These changes were correlated with increased expression of antioxidant genes (*SOD1*, *SOD2*, *Catalase*, GP*x1*, and *GR*) and reduced production of cellular and mitochondrial ROS, indicative of reductive stress [57]. Interestingly, this reductive stress was associated with heightened glycolytic activity and increased glycogen storage in these cells, alongside reduced oxygen consumption and palmitate β-oxidation [57]. This suggests a suppression of mitochondrial function concurrent with an enhancement of glycolysis and glycogenesis.

Diseases Associated with Reductive Stress

An excess of reducing equivalents can impair cellular proliferation, disrupt disulfide bond formation in proteins, compromise mitochondrial function, and reduce cellular metabolism. Several diseases closely associated with inflammatory conditions, such as cancer, Alzheimer's disease, metabolic syndrome, muscular dystrophy, pulmonary hypertension, and rheumatoid arthritis, may be influenced by this imbalance. Additionally, diseases like protein aggregation cardiomyopathy, hypertrophic cardiomyopathy, muscular dystrophy, and pulmonary hypertension may also be impacted. Specific examples of these diseases are highlighted in Table 1 (Ref. [2,58–69]).

Relationship between Reductive Stress and Pulmonary Hypertension

Vasoconstriction, vascular remodeling, and small thrombotic events are hallmark features of pulmonary hypertension, a complex, multifactorial, progressive condition. Inflammation characterizes this condition, attributed to the accumulation of perivascular inflammatory cells (such as macrophages, dendritic cells, T and B lymphocytes, and mast cells) and elevated levels of pro-inflammatory cytokines in the bloodstream [58]. The presence of reductive stress (RS) associated with hypoxia has been observed in pulmonary vascular cells, potentially contributing to the pathophysiology of pulmonary hypertension. Intracellular 2-oxoglutarate (2OG) and its reduced metabolite 2-hydroxyglutarate (2HG) both increase twofold in hypoxia. There are two enantiomers of 2HG: D and L. These enantiomers have been associated with neurological abnormalities in children due to rare inborn metabolic defects leading to increased urinary excretion of 2HG [59].

Mitochondrial reductive stress resulting from dysfunction in the tricarboxylic acid cycle of the respiratory chain is facilitated by the inhibition of 2OG-dependent dioxygenases by both enantiomers. Oldham and colleagues reported that these changes increase mitochondrial NADH levels, providing a substrate for the synthesis and accumulation of L-2-hydroxyglutarate (L2HG), thereby contributing to the elevation of reductive stress [59].

Stent Stenosis and Reductive Stress

Reduced levels of the enzyme GPx1 are associated with risk factors for vascular remodeling, including hypertension, endothelial dysfunction, diabetes, atherosclerotic plaque development, inflammation, and vascular damage post-stent placement [2]. Conversely, increases in this enzyme lead to reductive stress by improving the GSH:GSSG and NADPH:$NADP^+$ ratios. Reductive stress subsequently enhances the S-glutathionylation of critical proteins, promoting the proliferation, migration, and survival of vascular smooth muscle cells, ultimately leading to stent stenosis [60]. Dietary selenium levels are among the various factors influencing GPx1 expression and function [61].

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Reductive Stress and Parkinson's Disease

Neuronal death in different regions of the nervous system, followed by degeneration in those affected areas, is the hallmark of neurodegenerative diseases. Inflammation is a common feature, often accompanied by increased protein aggregate formation and alterations in neurotransmitter concentrations. Despite this, the mechanism initiating and driving the chronic processes in these diseases remains unknown [70]. Parkinson's disease, for instance, is believed to be associated with an imbalance in NAD^+/NADH levels, which interferes with the ability of ubiquinone to transfer electrons from iron-sulfur centers to the respiratory electron transport chain (RETC) complex I [62].

Implication of Reductive Stress in Insulin Resistance Accompanying with Metabolic Syndrome (MS)

Persistent hyperglycemia, resulting from chronic overeating and excessive sucrose intake, can contribute to metabolic syndrome (MS). Insulin Resistance (IR), hyperinsulinemia [2], dyslipidemia, obesity, hypertension, and impaired insulin secretion are all hallmark features of MS. Elevated blood glucose levels lead to increased generation of pyruvate and acetyl-CoA, essential for NADH synthesis. Furthermore, excess glucose can activate glyceraldehyde-3-phosphate (G-3-P) dehydrogenase, stimulating both the Krebs cycle and glycolysis, thereby increasing NADH levels. Additionally, during hyperglycemic conditions, the polyol pathway consumes more than 30% of the body's glucose, exacerbating reductive stress symptoms [63].

Additionally, NADPH serves as a cofactor for inducible nitric oxide synthase (iNOS), contributing to hypertension in metabolic syndrome (MS). Therefore, in the presence of persistent hyperglycemia, reductive stress (RS) followed by oxidative stress (OS) may significantly contribute to glucotoxicity. RS, a condition associated with pancreatic β-cells, can hinder insulin release [10]. Previous study has linked obesity, type II diabetes, lipid accumulation in skeletal muscle, and reduced activity of the respiratory electron transport chain (RETC) complex I [64]. Furthermore, in a model of male mice overexpressing GPx1, Insulin Resistance (IR), hyperglycemia, hyperinsulinemia, increased fat deposition and plasma leptin, and decreased insulin sensitivity were observed. Overexpression of GPx-1 may impair insulin function by excessively scavenging intracellular reactive oxygen species (ROS) required for insulin sensitization [65].

In response to insulin stimulation, hepatoma and adipose cells release a significant amount of H_2O_2, leading to a reversible oxidative suppression of protein tyrosine phosphatase activity. The maintenance of protein tyrosine phosphatase activity and insulin sensitivity thus relies on the regulation of reversible tyrosine phosphorylation in the insulin signaling cascade. Normal or low levels of intracellular ROS or H_2O_2 are necessary for Akt to phosphorylate Ser473 and activate insulin signaling [65,71]. Overexpression of GPx-1 results in increased protein tyrosine phosphatase activity and decreased insulin receptor phosphorylation, accelerating the elimination of the intracellular H_2O_2 burst following insulin stimulation [64].

Reductive Stress and Rheumatoid Arthritis

In rheumatoid arthritis (RA) patients, CD4 T-cells play a role in promoting synovitis, autoantibody formation, osteoclast differentiation, endothelial dysfunction, and pro-inflammatory effector functions. These T-cells, akin to malignant cells, rely on oxidative glucose metabolism along with mitochondrial oxidative phosphorylation to efficiently generate ATP [72]. However, for replication from a single cell to thousands of copies, they also require a carbon source and the reducing power of NADH, in addition to ATP [66]. Naive rheumatoid arthritis (RA) T-cells exhibit a defect in glycolytic flux due to upregulated glucose-6-phosphate dehydrogenase (G6PD). Excessive G6PD activity diverts glucose into the pentose phosphate pathway (PPP), resulting in increased NADPH levels and accumulation, leading to the consumption of reactive oxygen species (ROS) and consequent reductive stress (RS). Inadequate oxidative signaling prevents ATM activation, allowing RA T-cells to bypass the G2/M cell cycle checkpoint, resulting in an inflammatory T-cell pool [66]. Various metabolic interventions, including drug therapies, can redirect glucose utilization away from the PPP and toward glycolytic breakdown, thereby alleviating RS and preventing the hyperproliferation and improper differentiation of RA T-cells.

Reductive Stress and Renal Diseases

Grp94 and *Grp78*, products of the prototypical glucose-regulated (*Grp*) genes, play critical chaperone roles during protein folding and processing in the endoplasmic reticulum (ER) [73], and have also been implicated in inflammatory conditions such as kidney disease. These genes are part of the gene battery that responds to reductive stress (RS), while heat shock protein (*HSP*) genes respond to oxidative stress (OS) [74,75]. Thiol reductions can also be cytotoxic and increase the expression of *Grp* genes. Agents such as thiols, which activate *Grp78* transcription, interfere with ER protein folding [76]. Treatment with dithiothreitol (DTT) increases *Grp78* gene expression and *gadd153* gene transcription in LLC-PK1 renal epithelial cells. Additionally, treatment with N-acetyl-L-cysteine results in a 3- to 4-fold increase in glutathione levels in human embryonic kidney cells, leading to increased mitochondrial oxidation and RS, which may subsequently result in oxidative stress [67]. The accumulation of L2HG enantiomers in pediatric renal cell carcinoma is attributed to RS associated with hypoxia. Mutant enzyme cytosolic isocitrate dehydrogenase-1 has been identified as the source of cell lines with defects in the respiratory electron transport chain (RETC) and elevated levels of D2HG [59].

Reductive Stress in Infectious Diseases

Disease-causing pathogens have also been associated with reductive stress (RS). Exposure to dithiothreitol (DTT) induces Mycobacterium tuberculosis (Mtb) to generate thiol RS, leading to the formation of an adherent biofilm in Mtb cultures. These biofilms harbor metabolically active bacteria that exhibit drug tolerance [68]. In response to thiol RS, bacteria develop an envelope where periplasmic proteins unfold. The presence of this envelope triggers a specific transcriptional response [77].

Reductive Stress in Alzheimer's Diseases

RS occurs at a young age and precedes disease onset in an Alzheimer's disease (AD) model, such as the APP/PS1 transgenic mice [78]. In this model, RS is marked by increased glucose-6-phosphate dehydrogenase (G6PD) activity and glutathione levels, contributing to damage of mitochondrial membrane sulfhydryl groups, which become susceptible due to H_2O_2 deficiency [69]. Young, healthy individuals at risk of Alzheimer's disease also exhibit RS, characterized by overexpression of antioxidant enzymes before disease onset. Consequently, it is perplexing why this hyper-response of antioxidant defenses leads to RS in subjects, which eventually collapses during disease progression, resulting in oxidative stress (OS) that contributes to dementia development [79]. Flavonoids, when consumed at normal levels, have been reported to protect the rat brain hippocampus against Alzheimer's disease [80,81], but high doses of flavonoids may elevate the risk of RS.

Conclusions

The balance of reducing equivalents, such as NAD^+/NADH and GSH/GSSG, is crucial in numerous metabolic pathways where they serve as cofactors and ATP generators, among other roles. Disruption of this balance can lead to reductive stress or oxidative stress, which can have detrimental effects on cellular homeostasis. To maintain homeostasis, exogenous and endogenous antioxidants play a crucial role These antioxidants have pro-oxidant effects when present in excess quantities. This pro-oxidant activity tends to produce reactive oxygen species which are the causal agents of oxidative stress. The NAD^+:NADH and GSH:GSSG ratios are influenced by various pathways, including glycolysis and the tricarboxylic acid (TCA) cycle. Abnormalities in these ratios are associated with inflammatory-related diseases such as cancer, stent stenosis, aging, and cardiovascular issues, among others. This area of research holds promise for further understanding the molecular mechanisms underlying inflammatory and pathological conditions, and we hope this review provides insights to facilitate deeper exploration of this topic.

Recommendations

Reductive stress remains a less explored concept compared to oxidative stress, and to some extent, certain fundamental aspects remain elusive. Consequently, further research is warranted to elucidate these uncertainties. For instance, understanding how reductive stress influences the body's metabolic reactions and how the body adapts to emerging changes are among the numerous ambiguous aspects that necessitate investigation.

Availability of Data and Materials

All data associated with this review are available on request from the corresponding author.

Author Contributions

JAF: conceived, designed, and wrote the sub-topics for the review, and proofread the manuscript. TBO: wrote the first draft of the manuscript. BOA, ORA, TFF and MI: technical support, review, and editing. All authors made significant contributions to the idea or design of the study. All authors contributed equally to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest. Marcello IRITI is serving as one of the Editorial Board of this journal. We declare that Marcello IRITI had no involvement in the peer review of this article and has no access to information regarding its peer review.

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