Chapter 8

# Redoxomics and Oxidative Stress: From the Basic Research to the Clinical Practice

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Additional information is available at the end of the chapter

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**Abstract**

Potentially oxidant chemical species, which include not only free radicals but also other oxidizing chemical species such as reactive oxygen species (ROS), for example, hydroxyl radical and hydrogen peroxide, and nitrogen reactive species (RNS), for example, nitric oxide, play a relevant role in all biological processes and especially in cell defenses and molecular signaling. Their action is finely modulated by the antioxidant network that is composed either by endogenous or exogenous compounds (e.g., enzymes, peptides, lipids, and vitamins). An impaired modulation of oxidant species can lead to the so-called oxidative stress that is now considered an emerging health risk factor in almost all living organisms including plants, animals, and humans. Indeed, oxidative stress is related to a reduced lifespan and many diseases (e.g., cardiovascular diseases, neurodegenerative disorders, and metabolic diseases) both in humans and in animals. Unfortunately, oxidative stress does not show any clinical picture, but it can be detected only by means of specific laboratory tests. The recent recognition of a specific "redox code" and the definition of a redoxomics as a new "omics" are now enlarging the horizon of the traditional oxidative stress field leading to the definition of the so-called electrophilic stress. The aim of this chapter is to review the basic principles of redox reaction starting from the concept of free radicals and antioxidant in order to define the "electrophilic stress" as an emerging health risk factor for early aging and almost 1000 illness from infectious diseases to cancer. A paragraph is dedicated to the tests to measure oxidative stress in clinical practice either in humans or in animals in order to prevent, to treat and to monitor electrophilic-related diseases.

**Keywords:** ROS, RNS, antioxidants, redoxomics

## 1. Reactive species, free radicals, and oxidative processes

Free radicals play a fundamental role in the metabolic activity and function of different organs. Interactions between prooxidants (free radicals) and antioxidants lead to the maintenance of the intracellular homeostasis. A state of oxidative stress begins when there is an imbalance between the prooxidants and antioxidants, in favor of free radicals. Oxidative stress is a health risk factor involved in aging and in several diseases, in humans and/or in animals. In normal conditions, paired electrons create stable bonds in biomolecules; a free radical is defined as any independent species that contains one or more unpaired electrons in external orbital. Free radicals have a greater or lesser reactivity for the spontaneous tendency to exist as molecules with all electrons arranged in couples; this state is equivalent to the chemical stability.

The radicals do not show the same reactivity. Their increase of charge and the volumetric ratio is directly proportional to their reactivity. They will only reach their stability stripping electrons to other chemical species with which they are in contact and oxidize them [1].

Free radicals are classified according to the nature of atom that owns the orbital with unpaired electron. Reactive species include either radical or nonradical chemical species with oxidant potential. There are, therefore, free radicals centered on oxygen, carbon, nitrogen, or chlorine, and so on. Free radicals and other reactive species act as signaling molecules. Reactive species modulate transcription and epigenetics.

Free radicals/reactive species can be produced either by a "nonenzymatic" or an "enzymatic" way (**Figure 1**).

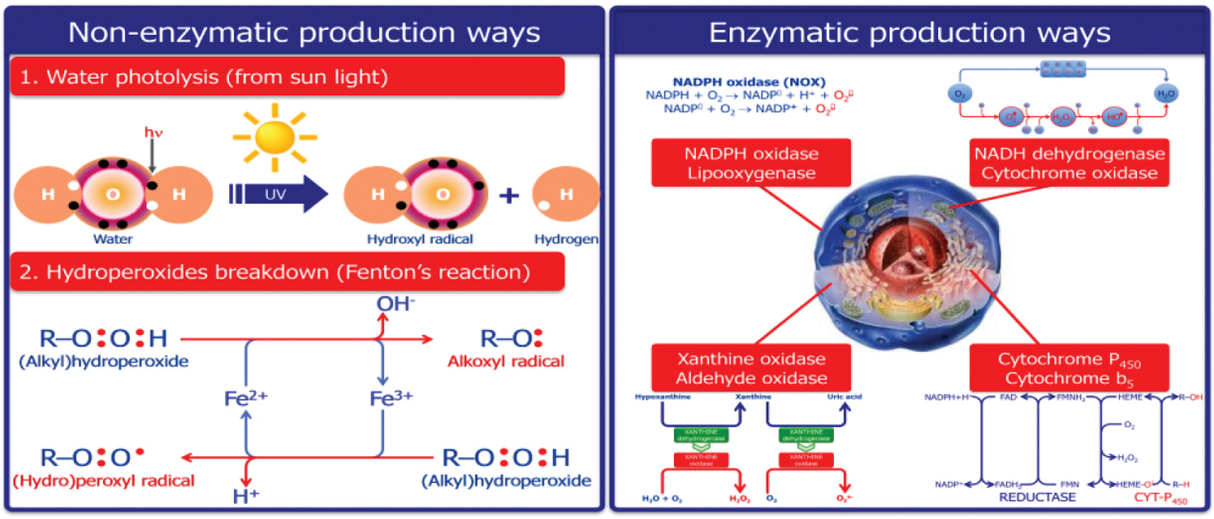


**Figure 1.** The "origin" of free radicals and other reactive species in living organisms.

The cell is the first target of oxidative damage. The destructive action of free radicals on cells is addressed mainly through the following reactions: membrane lipid peroxidation, oxidative modification of proteins and amino acids, nucleic acids' damage, and sugar oxidation (**Figure 2**).

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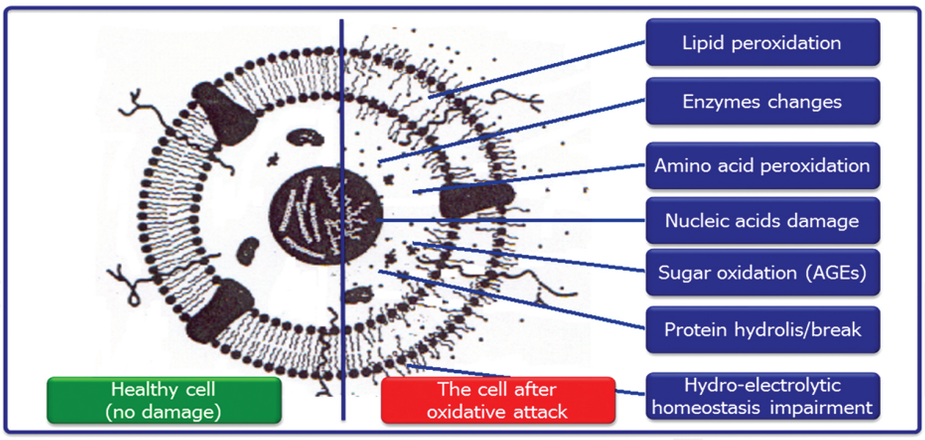


**Figure 2.** The cell: the first target of oxidative damage. Reactive species can hit not only lipids but also proteins and nucleic acids.

The damage, initially cellular, if prolonged through time, spreads to the tissues, organs and then it becomes a systemic damage. Oxidative stress is responsible, then, of cardiovascular diseases, dementia, Parkinson, early senescence, inflammation, cancers, and other diseases (**Figure 3**).

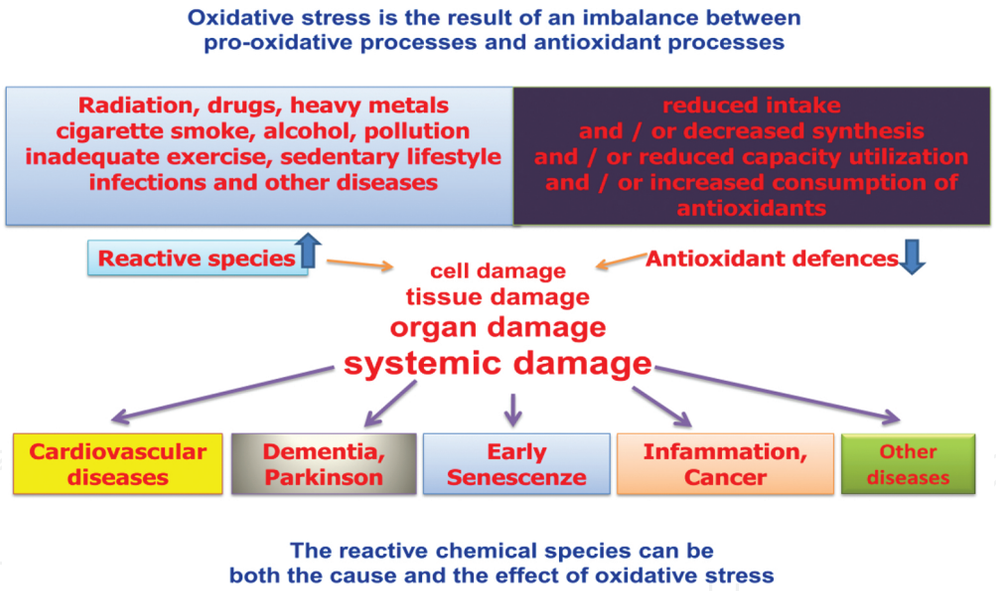


**Figure 3.** Consequences of the imbalance between pro-oxidants and antioxidants.

## 2. The antioxidants and biochemical classification

Antioxidants are substances that, when they are present at a low concentration compared to those of an oxidizable substrate, retard or prevent the oxidation of the same substrate. The keyword "oxidized substrate" includes every kind of molecule that is located *in vivo*.

In nature, there are no universal best antioxidants, but there are different antioxidants that are required to protect several molecules *in vivo* [2].

Antioxidants can be classified into enzymatic and nonenzymatic. The enzymatic antioxidants include glutathione reductase (GSH), superoxide dismutase (SOD), and catalase (CAT). Among the nonenzymatic antioxidants are vitamins (C, E, and B), carotenoids, carnitine, cysteine, some metals, taurine, and albumin [3]. Reductase and peroxidase glutathione are the main reducing endogenous agents and act as scavenger antioxidants especially in the epididymis and testes [4].

SOD is an enzyme that catalyzes the dismutation reactions of the superoxide anion ($O_2^-$). It can be found in intra- and extracellular forms. The intracellular forms are copper-zinc SOD present in the cytoplasm and contain copper and zinc in the active site (Cu, ZnSOD, SOD1); and manganese SOD localized mainly in the mitochondrial matrix and contains manganese in the active site (MnSOD, SOD2). Instead, SOD extracellular form (EC-SOD, SOD3), working into the extracellular space, is correlated to the polysaccharides of surface or in a free form [5].

CAT catalyzes the conversion of $H_2O_2$ to $O_2$ and $H_2O$ and presents a heme system with a central iron atom. It acts mainly in endoplasmic reticulum, peroxisomes, mitochondria, and the cytosol of many cell types [6].

Glutathione peroxidase (GPX) catalyzes the reduction of $H_2O_2$ and organic peroxides [5].GPX contains selenium in the form of selenocysteine in its active site. It is located in the sperm in the mitochondrial matrix.

The nonenzymatic exogen antioxidants are vitamins. Vitamin E encompasses a group of potent, lipid-soluble, chain-breaking antioxidants. Structural analyses have revealed that molecules having vitamin E antioxidant activity include four tocopherols and four tocotrienols. Vitamin E ($\alpha$-tocopherol) neutralizes $H_2O_2$ and quenches free radicals, therefore, stopping chain reactions that develop lipid peroxides and protecting the membrane from the oxidative damage.

Vitamin C (L-ascorbic acid or ascorbate), a pivotal nutrient for organisms, is present in the extracellular fluid. It is a principal chain-breaking antioxidant neutralizing superoxide, hydroxyl, and hydrogen peroxide radicals. Also, it has an important action to recycle vitamin E [7].

A class of natural pigments, carotenoids, is synthesized from plants and microorganisms, but not animals. They, present as microcomponents in fruits and vegetables, are responsible for their colors (yellow, orange, and red). Carotenoids are held liable for the beneficial effects of fruits and vegetables to prevent illnesses such as cardiovascular disease, cancer, and different chronic disease [7].

Cysteines, intracellular GSH precursors, enhancement the quantity of GSH synthesized, which avoids oxidative damage to the cell membrane and DNA.

In addition, albumin, taurine/hypotaurine, inositol and any metal are other minor antioxidants which help to reduce oxidative stress.

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One of the plasma proteins, the albumin, reacts with peroxyl radicals and prevents the chain reactions that produce ROS (Reactive Oxygen Species) formation.

Taurine, nonenzymatic antioxidant, scavenges ROS, inositol enhances GSH activity.

Selenium is an important component in the regular development and maturation of cells and contributes to the protection of DNA and cell membranes, particularly when used as an adjunct to vitamin E.

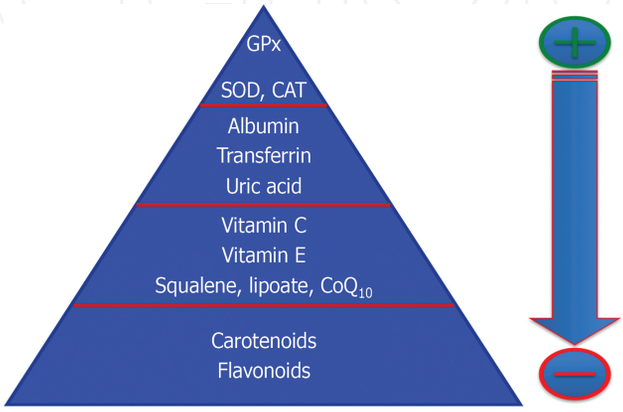


**Figure 4.** The antioxidant pyramid. Vertical stratification (power hierarchy).

Zinc acts as a chelator and binds ROS [8]. Chrome, another essential micronutrient, is a component of enzymes involved in carbohydrate metabolism. Its supplementation reduces fat deposition in rats, preventing obesity, initial phase of inflammation and oxidative stress [9].

**Figure 4** shows the power hierarchy of antioxidant pyramid. Endogenous enzyme antioxidants have a higher antioxidant capacity and are located at the top of the pyramid.

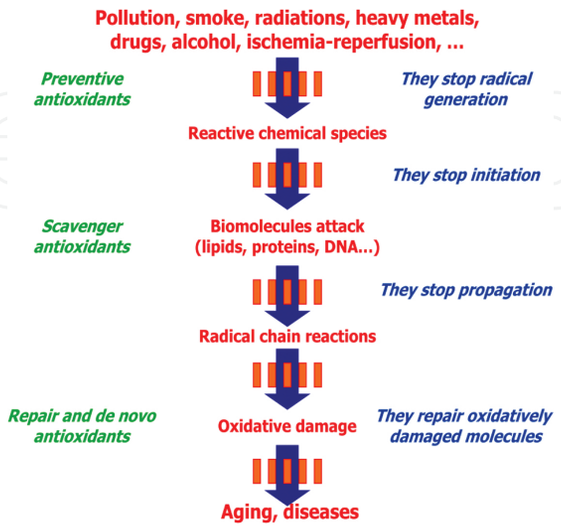


**Figure 5.** Antioxidant defense mechanisms.

The mechanisms of antioxidant action are shown in **Figure 5**. According to their function, they can be classified into preventive, scavenger, and repair antioxidants. Preventive antioxidants stop radical generation; scavenger antioxidants stop initiation and propagation; and repair and *de novo* antioxidants repair oxidated damaged molecules.

Polyphenols are abundant micronutrients in our diet, and there is evidence for their role in the prevention of degenerative diseases. Their bioavailability differs greatly among the polyphenol groups, depending on their composition, dietary sources, forms, and their containing so that the most abundant polyphenols in our diet are not necessarily those leading to the highest concentrations of active metabolites in target tissues. The plasma concentrations of total metabolites range from 0 to 4 mol/L with an intake of 50-mg aglycone equivalents. Among the polyphenols, isoflavones and gallic acid are the ones that are absorbed most by humans following, with different kinetics, flavonoids, catechins, and quercetin glycosides. Less absorbed polyphenols are proanthocyanidins, catechins galloylated tea, and anthocyanins. The data for other polyphenols remain poorly understood. Other studies would be necessary for the investigations on the health effects of polyphenols (**Figure 6**) [10].

| Classes of compounds | Active principles |
|---|---|
| Hydroxybenzoic acids | Gallic acid |
| Hydroxycinamic acids | Ferulic acid |
| Isoflavones | Daidzeidin, glycetin |
| Catechines | Epicatechin, epigallocatechin |
| Flavanones | Naringin, esperidin |
| Flavanols | Quercetin, rutin |
| Proanthocyanidines | Dimeric, trimeric |
| Anthocyanidines | Cyanidin, malvidin |

**Figure 6.** Antioxidant: polyphenol classes.

## 3. Oxidative stress: from the biochemistry to the clinics

Oxidative stress is a particular kind of chemical stress, which is induced—locally and/or systemically—by an excess of potentially oxidant reactive species, mostly centered on the oxygen (reactive oxygen species, ROS). It can be due to an increased production of reactive species and/or to a reduced efficiency of antioxidant defense system. The effects of oxidative stress can range from the impairment of cell signaling to the apoptosis or necrosis.

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The systematic assessment in biological samples of primary oxidizing chemical species, such as free radicals and their derivatives, such as hydroperoxides, as well as the dosage of antioxidant compounds and/or antioxidant activities (selenium and/or glutathione peroxidase), are not a "ring terminal" in the diagnostic chain of the information flows in biological systems (DNA > RNA > protein > metabolites > oxidants), but should make a "central place" compared to genomics, transcriptomics, proteomics, and metabolomics [11]. Precisely for this reason it has been newly introduced the new concept of "redoxomics" [12], a word previously used to detect only a few oxidized byproducts in the area of proteomics [13].

Redoxomics is a new field of "applied biochemistry" and "molecular diagnostic" with the following objectives: to examine the structure, the physiological role, and the deploying of oxidant and antioxidant systems into a living organism; to identify the mutual interactions of oxidant and antioxidant systems in a biological system (e.g., cell, tissue, organ, apparatus, and whole organism) in a defined phase of its development, under basic conditions as well as after stimulation potentially stressful; to assess the implications of these results from the point of view of epidemiology, pathophysiology, clinic, pharmacology, and so on [14].

The aspiring objective of redoxomics (as well as for another "-omics" in other areas) is "mapping" dynamically—through all the analytical and sophisticated techniques, from electron spin resonance to imaging—the whole oxidative-antioxidant repertoire [15].

This "integrated" approach allows us to track any qualitative/quantitative changes of oxidative balance and can support clinicians to give an optimum and "customized" solution for fixing any anomalies of redox status related to human diseases, in particular, in the area of aesthetic and antiaging medicine [16].

## 4. The breakdown of oxidant/antioxidant balance

The biological concept of stress: the word "stress", as it is currently in use, was first coined by Hans Selye (1907–1982) in 1936, he defined "stress" as "the nonspecific response of the body to any demand for change."

Selye had observed in many experiments that laboratory animals underwent sharp stimuli but different physical and emotional harmful (e.g., high beam, loud noise, extreme heat or cold, and constant frustration) all shown the identical pathologic changes of gastric ulcers narrowing of the lymphoid tissue and widening of the adrenal glands. Following they showed that persistent stress could be the cause of the development of different diseases in these animals similar to those observed in humans, like heart attacks, strokes, kidney diseases, and rheumatoid arthritis. The Selye's concept and dynamics of stress overlaps with that of oxidative stress (**Figure 7**) [17]. Most of oxidative stress-related diseases are related to life style [13].

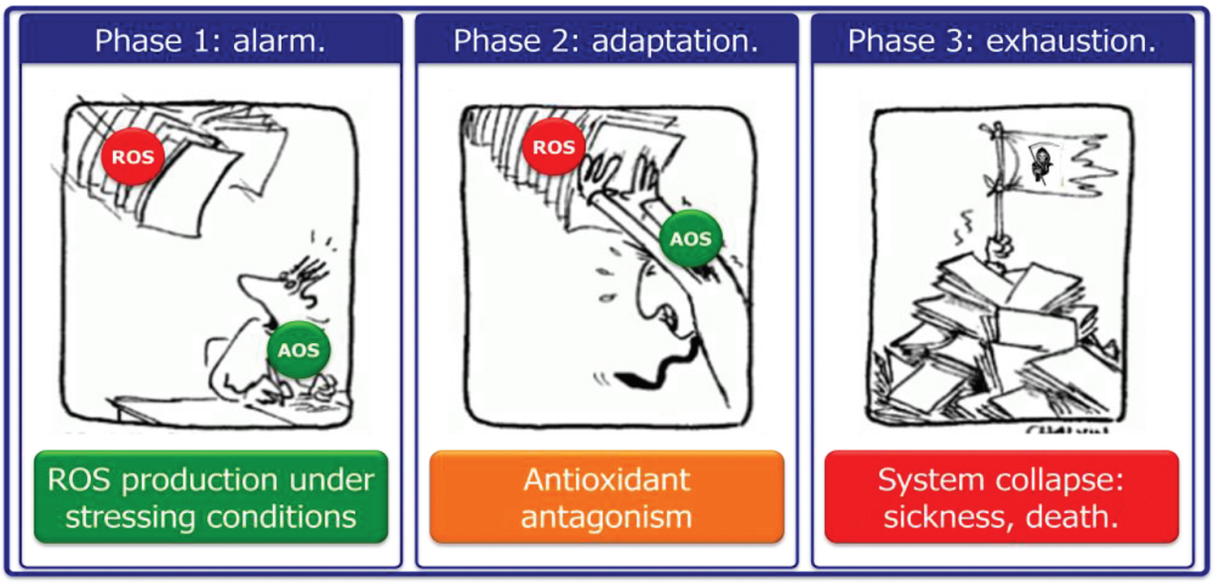


**Figure 7.** Classical stress and oxidative stress: two overlapping concepts.

Oxidative distress from oxidative eustress can happen for several reasons: formation of reactive species from stimulated polymorphonuclear leukocytes that can hit not only bacteria but also tissues; an overload of oxygen (e.g., by strong aerobic exercise) into the mitochondria and consequent increase ROS production; detoxification from acetaminophen (paracetamol) into the microsomes may increase ROS liver production; reperfusion after ischemia may lead to reactive oxygen species production due to xanthine oxidase activation.

In any case, an amount of oxidants is synthesized and by the old Greek language, *oxys* means *acids*, it is possible to establish a new paradigma: oxidative stress + acidosis = electrophilic stress.

## 5. Measurement of oxidative stress

The focus is now put on oxidative stress biomarkers that are objectively measured and assessed as markers of normal biological processes, pathogenic processes, or pharmacological responses to therapy performed. A biomarker, to be used as a predictor of illness, must first be endorsed. The validation criteria are intrinsic qualities like the specificity, sensitivity, the degree of inter- and intraindividual variability, and understanding of factors that can change.

In particular, features of the sample and analytical procedures are significant, therefore, noninvasiveness of the sampling, biomarker stability, sensitivity, specificity, velocity and simplicity of the analytical method are important. Below, the most commonly used biomarkers for the evaluation of oxidative/nitrosative damage are listed.

Oxidative stress depends on an imbalance that is created between the production of ROS (pro-oxidants) and the action of the antioxidants. Direct assays, which measure the oxidation of the cell membrane of many cell types, are available.

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The most widespread assay assesses the concentration of malondialdehyde (MDA), one of the end products of lipid peroxidation [18, 19]. The increased levels of MDA may be related, for example, to the decrease of sperm parameters. To quantify the damage of sperm DNA, another assay is also used; it measures the concentration of a specific product of oxidative DNA damage, 8-oxo-7,8, dihydro 2′deoxyguanosine (8-OHdG). This product is particularly employed as a specific marker of oxidative damage to sperm DNA [20].

The assay of the indirect chemiluminescence is one of the most popular methods for the determination of ROS in the spermatic semen. Luminol (5-amino-2,3, dihydro 1,4, phthalazinedione) and lucigen are substances used to determine the redox activity in the cells [20]. Lucigen quantizes only extracellular superoxide radicals, while the luminol is able to determine the extracellular and intracellular levels of ROS.

In order to use the nitrobluetetrazolium assay an optical microscope is needed: it allows us to determine the differentiation of ROS in different cell types. This reagent, nitrobluetetrazolium, reacts with superoxide radicals, for example, in spermatozoa and in leukocytes, changing to diformazan, a blue pigment. The concentration of diformazan is directly proportional to the intracellular concentration of ROS [21].

To assess serum total oxidant and antioxidant levels, commercially available d-ROMs and anti-ROM (Reactive Oxygen Metabolites) tests (Diacron International, Grosseto, Italy) are utilized. These tests are performed using Free Carpe Diem, a dedicated spectrophotometer (Diacron International, Grosseto, Italy).

Oxidative status is evaluated by measuring hydroperoxides in the serum using d-ROMs' test. The d-ROMs test measures the oxidant ability of a serum sample toward a particular substance (modified aromatic amine) used as an indicator (chromogen, N,N-diethyl-para-phenylendiamine) (DEPPD). The phenomenon is associated with the progressive and gradual color change to pink reaction mixture (serum + chromogen), which was initially colorless. In the d-ROMs test, the metabolites of reactive oxygen species (ROMs), particularly hydroperoxides (ROOH), of a biological sample, in the presence of iron, issued by the serum proteins by an acid buffer, can produce alkoxyl and peroxyl radicals, in accordance with Fenton's reaction.

Such radicals are able to oxidize an alkyl-substituted aromatic amine that is solubilized in a chromogenic mixture, thus producing a pink-colored derivative which is photometrically quantified at 505 nm [22]. The intensity of developed color is directly proportional to the concentration of ROMs, according to the Lambert–Beer's law and is expressed as Carratelli units (1 CARR U = 0.08 mg hydrogen peroxide/dl). The method is linear up to 1000 CARR U.

The measurement of antioxidant capacity in serum samples can be performed by the anti-ROM test. The anti-ROM test measures the antioxidant capacity of serum in terms of iron-reducing capacity; in fact, it is based on the ability of serum antioxidants to reduce ferric iron to ferrous iron, which reacting with $\alpha\alpha$-dipyridyl, gives rise to a reddish purple. The intensity of color increases in proportion to the amount of iron reduced by antioxidants present in the sample.

In the BAP (Biological Antioxidant Potential) test, adding a sample of plasma to a dye solution, obtained as a mixture of ferric chloride with a derived thiocyanate solution, a discoloration is caused. The intensity of this discoloration is determined photometrically by using a wavelength of 505 nm and it results proportional to the ability of plasma to reduce ferric ions [23]. The results obtained are evaluated as μmol/L or reduced ferric ions.

The antioxidant levels can be evaluated in the semen too, both with a chemiluminescence and through a colorimetric assay. The antioxidant amounts are determined by the addition of a well-known concentration of ROS to semen, developing the chemiluminescent signal or the color changes. The antioxidants present in the sperm behave toward ROS as a scavenger, so it is possible to measure the residual levels of ROS. Then, the intensity of the developed signal is inversely proportional to the total antioxidant activity of the sample [24].

The total oxidant capacity/potential of a blood plasma/serum sample can be evaluated by exploiting the ability of N,N-diethyl-paraphenylendiamine to give electrons (oxidation) after the reaction with a biological sample. The newly generated radical cation can be detected—thus providing a suitable measure of oxidant capacity—either by evaluating the absorbance change at 505 nm (the solution becomes pink to red, depending on the concentration) or the specific spin resonance signal [22].

## 6. The oxidative stress evaluation in clinical practice

Oxidative stress does not show any clinical picture; thus, the study of basic mechanisms of oxidative stress can lead to the identification of suitable biomarkers. Searching for the "ideal" biomarker of oxidative stress is not an easy matter; it should be validated by means of the golden standard technique (e.g., electron spin resonance); acceptably high levels of sensitivity, specificity, and precision; chemically stable over the time; able to measure suitably oxidative stress level; able to provide reliable information even in an early stage of the disease; able to anticipate the progression of disease during a systemic monitoring; modifiable with adequate sensitivity after medical/surgery/antioxidant treatments; minimally invasive, highly compliant, fast; and optimal cost/benefit ratio. Unfortunately, an accomplished biomarker of oxidative stress with all these features is not yet available.

The blood plasma/serum of apparently healthy people (and that one of many animal species) is able to oxidize the DEPPD (as described above) in a precise range of absorbance change as a function of either genetic or environment factors (age, gender, race, physiological conditions like pregnancy, and so on) and according to a Gauss-like curve of distribution (**Figure 8**) [25].

| Primary mechanism | Primary cell site involved | Main biochemical mechanisms | Main reactive species involved | Causes and clinical correlations |
|---|---|---|---|---|
| Reactive changes of cell surface | Plasmamembrane | Activation of NADPH oxidase | Superoxide anion | Inflammatory diseases |
| | | Activation of arachidonic acid metabolism | Hydroperoxides Superoxide anion | Infectious diseases |
| Impaired cell respiration | Mitochondria | Metabolic activation | Superoxide anion Hydrogen peroxide Hydroxyl radical | Caloric excess Strong erobic activity Thyroid hyperactivity |
| | | Mithocondrial dysfunction | Superoxide anion Hydrogen peroxide | Nervous system and muscle genetic disorders |
| Pharmaco-metabolic induction | Endoplasmic reticulum/ microsomes | Activation of cytochrome $P_{450}$ and $b_5$ | Variable | Alcohol abuse Drugs, xenobiotics |
| Abnormal changes of intracellular $pO_2$ | Citosol | Activation of xanthine oxidase | Superoxide anion Hydrogen peroxide Hydroxyl radical | Ischemia/reperfusion diseases (e. g. infarction) |
| Multiple | Variable | Variable | Variable | Cigarette smoke,toxicities, radiations, metabolism |

**Figure 8.** Causes, mechanisms, and clinical pictures of oxidative stress.

The blood plasma/serum of apparently healthy people, which are exposed to factors that are classically able to induce a condition of oxidative stress, shows a total oxidant capacity constantly and significantly higher than that found in apparently healthy nonexposed peoples, and patients suffering from diseases classically related to oxidative stress show significantly higher levels of blood plasma/serum total oxidant capacity compared to those found in apparently healthy controls (**Figure 9**).

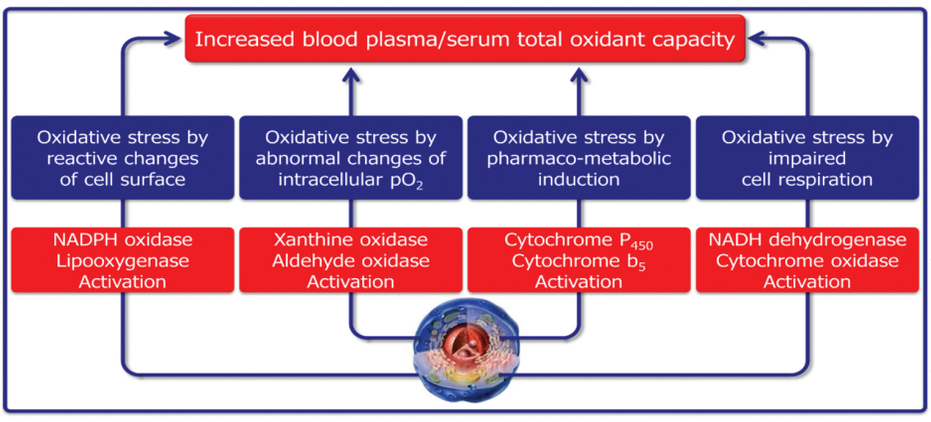


**Figure 9.** Oxidative stress-related diseases. Pathophysiological and clinical pictures.

Some studies have shown that between the work done on standing and chronic venous insufficiency of the lower limbs, there is a statistically significant correlation. This correlation has been associated with an oxidative stress in an advanced stage that, according to several studies, would represent a risk factor for cardiovascular systemic disorders; in fact, standing workers exhibit significantly higher mean levels of ROS after work [26].

It is known that elevated levels of blood reactive oxygen species are correlated with the severity of periodontitis. An improvement of clinical parameters in chronic periodontitis patients, through a nonsurgical periodontal treatment, is able to determine a decrease in blood reactive oxygen species [27].

Moreover, free radicals' cigarette smoke is a complex chemical system and there are many potential pathways for these species to interact with one another and with biopolymers in a smoker's lung. There is ample evidence that the free radical system plays a significant role in cigarette smoke toxicology. It is becoming increasingly strong and necessary to try to place some possibilities in perspective [28, 29].

Chronic alcohol abuse appears to be linked to increased serum levels of reactive oxygen species, such as hydroperoxides, with a normal antioxidant capacity. The study performed by Trotti et al. [30] suggested that both alterations in the redox balance and a thrombophilic condition can be observed in heavy drinkers without severe liver diseases, such as cirrhosis, hepatitis, and hepatitis C virus (HCV).

Oxidative stress by impaired cell respiration increased oxidant capacity in strenuous exercise. Physical activity increases the free radicals in several ways, such as oxidative phosphorylation enhances in answer to exercise; therefore, there is a simultaneous production of free radicals. Catecholamines, issued when exercising, can produce free radicals. The free radicals that enhance with exercise are produced by the metabolism of prostanoids, by xanthine oxidase, NADPH (Nicotinamide Adenine dinucleotide Phosphate-Oxidase) oxidase, and other secondary sources, for example, by macrophages hired to fix the injured tissue that can produce free radicals [31].

Antioxidant supplements are sold and used by athletes to counteroxidative stress due to strenuous exercise. It is not yet clear if strenuous exercise increases the need for supplementary antioxidants in the diet. When the rise of free radicals exceeds the capacity of antioxidants to neutralize them, the radicals have the cellular components as targets in particular lipids. The lipid attack starts a chain reaction known as lipid peroxidation, which results in formation of a high number of radicals and reactive species that can damage other cellular components. The organism is able to bear a limited increase of free radicals; indeed, the evidence suggests that an ROS enhancement is needed for a muscle adjustment [32, 33].

In a study of Chen and Kotani [34], oxidative stress in premenopausal women with oral contraceptive therapy, which is now commonly used in millions of women worldwide, was investigated. This treatment is associated with an increased risk of deep vein thrombosis, venous thromboembolism, and stroke, so it is critically important to evaluate risks and benefits of therapies. The results of the study show that use of oral contraceptive therapy may increase oxidative stress levels, independently on traditional cardiovascular risk factors, in premenopausal women, providing new perspectives to the prevention of vascular complications in these subjects.

The cells normally generate reactive oxygen species (ROS) during energy metabolism for the respiratory chain. ROS at low or moderate concentrations have important physiological roles. However, an excessive enhancement of ROS in conditions of oxidative stress may be very

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harmful. The central nervous system (CNS) is helpless to oxidative stress for its high oxygen consume, for the weak antioxidant systems and terminal differentiation of neurons. Therefore, oxidative stress causes several neurodegenerative illnesses. Also, chemotherapy can cause serious side effects on the CNS and peripheral nervous system (PNS) in treated cancer patients, and then several studies show the involvement of ROS in the neurotoxicity induced by drugs. For this reason, the growth of neuroprotective drugs such as antioxidants can be considered a beneficial strategy for clinical treatment [35].

The worldwide incidence of diabetes mellitus (DM) has recently increased rapidly due to lifestyle changes, with DM projected to affect over 300 million people in the last years. DM is related to several complications and poor quality of life of the affected patients; this also leads to an increase in health spending. DM can lead not only to microangiopathy (related to major complications of diabetes, such as diabetic retinopathy, nephropathy, and neuropathy) but can also be considered a major risk factor for macroangiopathy, like coronary heart and cerebrovascular diseases. Moreover, oxidative stress can be an important factor for the development and progression of diabetic complications, related to insulin resistance and reduced insulin secretion with consequent development of DM. Therefore, the oxidative stress in DM is an important factor involved in the development of diabetic complications and in that of the same DM [36].

Numerous studies have shown that the transition metals could be affected in the pathogenesis of various neurodegenerative diseases for their ability to produce oxidative stress. Alzheimer's disease is the most common cause of dementia in older people. The metals, such as iron and copper, which can catalyze the Fenton's reaction by reactive oxygen species, are highly concentrated within the neuritic plaques that represent the features of the Alzheimer's disease brain.

A large body of experimental and *postmortem* findings indicates that Alzheimer's disease is associated with increased oxidative stress levels in the brain. Despite the current limitations of oxidative stress assessment in living subjects, recent data suggest that oxidative challenge might increase early both in the central nervous system and peripheral fluids [37].

## 7. Antioxidants and disease prevention

Numerous studies show that diets high in fruits and vegetables are protective against cardiovascular diseases (CVD), several kinds of cancer, and other chronic diseases. However, although a broad consensus, it is still unclear what their mechanism(s) of action enable the protection against certain diseases. The antioxidant hypothesis connects the high content of antioxidant molecules found in plant foods and their health benefits by a direct impact on the decrease of oxidative stress.

Some clinical studies have shown contrasting results: some showing protective effects, while others do not. Antioxidants do not act just in isolation or in synergistic interactions; it should be taken into account that in part they are involved in the antioxidant regeneration. The data

emerged from these studies are that dietary and endogenous antioxidants, with various activities and features, act synergistically contributing to the overall effect of protective plant foods.

The efficacy of the nonenzymatic antioxidant barrier can be evaluated by determining the total antioxidant capacity (TAC), termed as moles of oxidizing neutralized by 1 L of the sample tested. TAC treats the cumulative action of all antioxidants in the matrix, thus providing an integrated parameter instead of the simple sum for measurable antioxidants, giving a view balance among antioxidant molecules. Some experiments have shown that plasma TAC of patients with various chronic diseases such as diabetes, AIDS, ulcerative colitis, Crohn's disease, meningitis, cardiovascular diseases, colorectal, lung and breast cancer, is much lower than in healthy controls, suggesting impairment of antioxidant barrier in the development of these pathologies.

In order to optimize the intake of dietary antioxidants, particular attention should be paid to the possibility that the interaction between foods consumed in a meal might affect the *in vivo* efficiency of dietary antioxidants [38].

Lycopenes, which represent more than 80% of the total tomato carotenoids, can reduce the risk of cardiovascular disease by inhibiting cholesterol synthesis, reducing the expression of cell surface adhesion molecules and the binding of monocytes to endothelial cells, and modulating LDL (low-density lipoprotein) susceptibility to oxidation. *In vitro* studies demonstrated that the highest beneficial effects as a cancer preventive of lycopene in the diet occur when it is associated with other compounds. A recent study suggests that $\alpha$-tocopherol or whole tomato lipophilic extracts (containing more than 80% lycopene along with other compounds) potentiate the effects of lycopene during oxidative stress [39].

Neurons are particularly prone to oxidative stress. Particularly, ROS were shown implicated in the pathology of a number of neurological disorders. The brain, mostly neuronal plasmamembranes, houses large concentrations of polyunsaturated fatty acids, which may undergo lipid peroxidation in such an oxygen-rich environment. Brain catecholamines easily undergo auto-oxidation phenomena, thus generating reactive oxygen species. Furthermore, brain contains conspicuous amount of iron (a powerful catalyst of free radical generation), although in an inactive form (chelated). Physiologically, brain exhibits low antioxidant defenses (vitamin C, vitamin E, glutathione, and superoxide dismutase); moreover, reduced levels of antioxidants such as vitamins E and C have been reported in many neurological conditions. It was demonstrated that vitamin E supplementation in deficient individuals is able to either prevent or at least halt the progression of many neurological features. However, supplementation of vitamin E in patients suffering from Parkinson's disease had no apparent benefits [40].

The study has compared the activity and bioavailability of some antioxidants, which have been used in doses very close to those of an average daily meal. Three different formulations (F1, F2, and F3) were tried. Each one was prepared both in fluid and dry formulations and given to the same group of subjects for 1 week. The antioxidants provided in combination with a dosage near to one RDA (Recommended Daily Allowance) decreased oxidative stress, and the fluid formulation was found to be more active and bioavailable than the dry one. The antiox-

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idants present in F1 are those with affinity for membranes (vitamin A, vitamin E, and carotene), minerals (selenium and zinc), components of antioxidant enzymes, and L-cysteine, which is needed for the synthesis of glutathione peroxidase. In F2, the antioxidants comprise circulating substances (vitamin C, bioflavonoids, and vitamin B-6) and a cytosol antioxidant (coenzyme Q10). In this study, F1 was significantly more active than F2, and F3 enhances the F1 activity without a true synergism. Nevertheless, especially in healthy subjects, the existence of a "roof" effect of antioxidants cannot be ruled. The antioxidant activity can be much more evident in subjects with a chronic oxidative stress. A group of antioxidants in low doses decreased oxidative stress as highlighted by the values expressed as U CARR. Since oxidative stress is important for the prevention and/or treatment of an illness, the dROMs' test appears to be a suitable instrument with which to identify the type and dose of antioxidants [41].

Ascorbic acid (ascorbate or vitamin C) has a controversial history in cancer treatment. Pharmacologic concentrations of ascorbate, only achievable by intravenous (i.v.) administration, produce $H_2O_2$, causing cancer and cell death *in vitro* [42]. Parenteral administration of ascorbate in pharmacologic doses produces millimolar concentrations in blood and extracellular fluid, with preferential generation of Asc•, the product of a loss of one electron from ascorbate, and $H_2O_2$ in extracellular fluid but not blood. When ascorbate is given parenterally, Asc• is detected preferentially in extracellular fluid compared with blood. Asc• generation in extracellular fluid depended on the ascorbate dose and the resulting concentrations. These findings are all consistent with the hypothesis that pharmacologic ascorbate concentrations *in vivo* serve as a prodrug for selective delivery of $H_2O_2$ to the extracellular space. In humans, these experiments are based on principles of tight control of ascorbate. After oral ingestion, control of intracellular and extracellular ascorbate concentrations is mediated by three mechanisms: intestinal absorption, tissue transport, and renal reabsorption. These three mechanisms work in coordination with each other, ensuring that ascorbate is tightly controlled. Parenteral administration bypasses tight control, which is restored as kidneys excrete ascorbate. The results demonstrate an explanation on why tight control happens. If the tight control is exceeded, $H_2O_2$ is formed into the extracellular space. When tight control is reset, the production of $H_2O_2$ stops. If there was no tight control, the formation of $H_2O_2$ and exposure to it could be steady, with disagreeable impact on division and cell growth. Tight control prevents continued exposure of tissues to high concentrations of $H_2O_2$. Bypassing provisionally the tight control with the ascorbate parenteral administration, $H_2O_2$ is able to form for only a fair period of time, decreasing the damage, and gives a drug for therapeutic of i.v. use of ascorbate [43].

Also, endothelium performs an important role in the regulation of vascular tone, platelet activity, leukocyte adhesion, and thrombosis, and is also implicated in the development of atherosclerosis. In patients with determined coronary heart disease or coronary risk factor, endothelial dysfunction was observed. Treatment with lipid lowering drugs, ACE (Angiotensin Converting Enzyme inhibitor) inhibitors, physical activity, and antioxidant agents has shown an improvement in endothelial function in the coronary and peripheral vessels. Vitamin C is a very efficient antioxidant, and is a scavenger of several reactive oxygen species, such as superoxide anion and peroxynitrite. Several researches have demonstrated that the beneficial effect of vitamin C (24 mg/min) on endothelial dysfunction in subjects with risk factors or

coronary artery disorders is specific, because it was observed neither in healthy control subjects nor on the endothelium-independent vasodilation induced by nitroglycerin or SNP (Sodium Nitroprusside) [44].

## 8. Conclusions

An unhealthy diet, alcohol abuse, chronic intake of drugs, cigarette smoking, inadequate exercise, and environmental pollution are just some causes of a particular form of "stress" which experts have called "oxidative stress" [45]. It is very different and definitely more dangerous than the more common "emotional distress" that affects every day much of the population in Western countries with high economic level [46]. Oxidative stress is a form of "chemical stress" induced by the presence, in our organism, of high quantities of harmful substances acting as oxidants, whose members are the most dangerous oxygen free radicals [47].

Oxidative stress is considered responsible for premature and many diseases ranging from hypertension to atherosclerosis, infarction to stroke, from Parkinson's to Alzheimer's disease, from colitis to pancreatitis, from obesity to diabetes, from chronic bronchitis to rheumatoid arthritis, from AIDS to several forms of cancer [48, 49]. Oxidative stress is much more sneaky, because it gives rise to characteristic symptoms, or to a particular clinical picture, because its causes are to be found in "invisible" entities, such as free radicals [50]. Therefore, the clinician could not suspect the existence; oxidative stress does not provide any evidence to suggest a more detailed diagnosis, when performing simple laboratory tests allow to understand immediately the problem, avoiding the patient a series of consequences such compromise the duration and/or the quality of life in the short or medium term. It is not currently provided for the execution of any preliminary laboratory tests, although available for clinical routine, to show—by means of the quantification in the blood of suitable biochemical markers—the objective need for such formulations. While it is now known that a cholesterol-lowering drug is taken only after a test that has documented a high blood level of cholesterol, it is now an increasingly widespread tendency to assume antioxidants even without the documentation in the blood, an increase in the level of free radicals and/or a reduction of its "physiological" antioxidant defenses. It is not yet a good practice to perform a preliminary evaluation of the laboratory of oxidative stress.

However, the scientific evidence supports that only adequate assessment biochemistry may allow the identification and the definition of a state of oxidative stress and make monitoring of a possible antioxidant therapy. Because of these specific tests for the evaluation and determination of free radicals and antioxidant defenses, it makes the initial diagnosis of oxidative stress extremely accurate and reliable, whether the two opposite components, either pro- or antioxidant, are measured separately [51]. It is possible to determine in real time whether oxidative stress is due to an increased production and/or a reduced ability to eliminate free radicals. It would be appropriate to undergo the oxidative stress evaluation, even in good health and, more so, when exposed to pro-oxidant

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factors (e.g., incorrect lifestyles, excessive aerobic exercise, and pollutants in the workplace) or when affected from chronic degenerative diseases (e.g., diabetes, atherosclerosis, cancer, dementia, and rheumatoid arthritis) or, eventually, when performed specific treatments (e.g., dialysis, by-pass, organ transplant, radiotherapy, and chemotherapy) [52, 53]. For this evaluation it will be possible to use specific therapies and to monitor the real efficacy of antioxidants, too often assumed without a preliminary test able to demonstrate its necessity. The same "prescription" of supplements, finally, will lean—in this sensitive field—on a more solid and leave the empirical phase in which it often finds itself.

The evaluation of a real state of oxidative stress may be covered in the predictive medicine area. Predictive medicine is the emerging field of medicine that entails predicting the probability of disease and taking proactive steps to either prevent the disease altogether or significantly decrease its impact upon the patient (such as by preventing mortality or limiting morbidity). The aim of predictive medicine is to predict the likelihood of disease so that healthcare providers and the patients will have an active role in changing the way of life and increase the medical surveillance, such as complete biannual skin examinations by a dermatologist or internist if their patient is found to have an increased risk of melanoma; an ECG and cardiac examination by a cardiologist if a patient has an increased risk of cardiac arrhythmia or alternating magnetic resonance imaging (MRI); or mammograms if a patient has an increased risk of breast cancer. Predictive medicine is useful for both healthy individuals (predictive health) and those with illnesses (predictive medicine); its aim is to provide information on the possibility of having a disease and to predict the progression and treatment for a specific disease. Aside from genetic testing, predictive medicine utilizes a wide variety of tools to predict health and disease, including assessments of exercise, nutrition, spirituality, quality of life, and oxidative stress.

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