

# Oxidative stress, aging, and diseases

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**Abstract:** Reactive oxygen and nitrogen species (RONS) are produced by several endogenous and exogenous processes, and their negative effects are neutralized by antioxidant defenses. Oxidative stress occurs from the imbalance between RONS production and these antioxidant defenses. Aging is a process characterized by the progressive loss of tissue and organ function. The oxidative stress theory of aging is based on the hypothesis that age-associated functional losses are due to the accumulation of RONS-induced damages. At the same time, oxidative stress is involved in several age-related conditions (ie, cardiovascular diseases [CVDs], chronic obstructive pulmonary disease, chronic kidney disease, neurodegenerative diseases, and cancer), including sarcopenia and frailty. Different types of oxidative stress biomarkers have been identified and may provide important information about the efficacy of the treatment, guiding the selection of the most effective drugs/dose regimens for patients and, if particularly relevant from a pathophysiological point of view, acting on a specific therapeutic target. Given the important role of oxidative stress in the pathogenesis of many clinical conditions and aging, antioxidant therapy could positively affect the natural history of several diseases, but further investigation is needed to evaluate the real efficacy of these therapeutic interventions. The purpose of this paper is to provide a review of literature on this complex topic of ever increasing interest.

**Keywords:** elderly, reactive oxygen species, reactive nitrogen species, antioxidants

## Pathophysiology of oxidative stress

Free radicals are highly reactive atoms or molecules with one or more unpaired electron(s) in their external shell and can be formed when oxygen interacts with certain molecules.<sup>1</sup> These radicals can be produced in cells by losing or accepting a single electron, therefore, behaving as oxidants or reductants.<sup>2</sup> The terms reactive oxygen species (ROS) and reactive nitrogen species (RNS) refer to reactive radical and non-radical derivatives of oxygen and nitrogen, respectively.<sup>3</sup> Reactive oxygen and nitrogen species (RONS) are produced by all aerobic cells and play an important role in aging as well as in age-related diseases.<sup>4</sup> RONS generation is not only limited to determine deleterious effects but also involved in the extraction of energy from organic molecules, in immune defense, and in the signaling process.<sup>5</sup> There are endogenous and exogenous sources of RONS:

- Endogenous sources of RONS include nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, myeloperoxidase (MPO), lipoxygenase, and angiotensin II.<sup>6</sup> NADPH oxidase is the prevalent source of the radical superoxide anion ( $O_2^{\bullet -}$ ) which is formed by the one-electron reduction of molecular oxygen, with electrons supplied by NADPH, during cellular respiration. Most of the  $O_2^{\bullet -}$  is dismutated into the hydrogen peroxide ( $H_2O_2$ ) by superoxide dismutase (SOD).<sup>5</sup>  $H_2O_2$  is not a free radical because it has no unpaired electrons, but it is able to form the highly reactive ROS hydroxyl ion ( $OH^{\bullet}$ ) through the Fenton or Haber–Weiss

reaction. Hydroxyl radicals are extremely reactive and react especially with phospholipids in cell membranes and proteins. In neutrophils,  $H_2O_2$  in the presence of chloride and MPO can be converted to hypochlorous acid, an ROS particularly damaging cellular proteins.<sup>5</sup> Nitric oxide (NO) is produced from L-arginine by three main isoforms of nitric oxide synthase (NOS): epithelial NOS, related to vasodilation and vascular regulation, neuronal NOS, linked to intracellular signaling, and inducible NOS, activated in response to various endotoxin or cytokine signals.<sup>7</sup> Finally,  $O_2$  may react with NO to form another relatively reactive molecule, peroxynitrite ( $ONOO^-$ ).<sup>5,6</sup>

- Exogenous sources of RONS are air and water pollution, tobacco, alcohol, heavy or transition metals, drugs (eg, cyclosporine, tacrolimus, gentamycin, and bleomycin), industrial solvents, cooking (eg, smoked meat, waste oil, and fat), and radiation, which inside the body are metabolized into free radicals.<sup>8</sup>

RONS, whether they are endogenous or exogenous, cause oxidative modification of each of the major cellular macromolecules (carbohydrates, lipids, proteins, and DNA),<sup>6</sup> which can also be used as markers of oxidative stress.<sup>9</sup> Several oxidative changes in proteins have been described. Protein carbonyl (PC) is formed by Fenton reaction of oxidants with lysine, arginine, proline, and threonine residues of the protein side chains.<sup>10</sup> Carbonyl groups may also derive from the binding of aldehydic lipid oxidation products to lysine, cysteine, or histidine residues called Michael-addition reactions.<sup>9</sup> Reactions between RNS and tyrosine residues free or within polypeptide sequences induce the formation of nitrotyrosine (NT).<sup>9</sup> Low-density lipoproteins (LDLs) are the major carriers of cholesterol to body tissues. The oxidation of LDL is a complex process during which both the protein and the lipids undergo oxidative changes that can cause cholesterol accumulation.<sup>11</sup> Poly-unsaturated fatty acids (PUFAs), in particular linoleic and arachidonic acids, are other important targets of lipid peroxidation, mediated by hydroxyl and peroxy radicals. Depending on the type of PUFAs undergoing lipid oxidation, several different reactive aldehydes are produced, such as *trans*-4-hydroxy-2-nonenal (4-HNE), malondialdehyde (MDA), and isoprostanes ( $F_2$ -IsoPs).<sup>9</sup> The amino groups of lysine and arginine react with the carbonyl groups of carbohydrates in a process called glycoxidation, resulting in advanced glycation end products (AGEs). Major AGEs include hydroimidazolone, N $\epsilon$ -carboxymethyl-lysine, pentosidine, and glucosepane.<sup>12</sup> Oxidative damage to DNA results in several mutagenic

lesions including 2-hydroxy adenine, 8-oxoadenine, 5-hydroxycytosine, cytosine glycol, thymine, and glycol. The most mutagenic consequences of oxidative stress on DNA are 8-oxo-7,8-dihydro-guanine (8-oxoGuo) and 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) lesions, but, among these, the most recurring is 8-oxoGuo, which can result in G-to-T transversion events.<sup>13</sup>

Antioxidant defense protects biological systems from free radical toxicity and includes both endogenous and exogenous molecules.

- Endogenous antioxidants include enzymatic and non-enzymatic pathways.

The primary antioxidant enzymes are SOD, catalase (CAT), and glutathione peroxidase (GSH-Px). As mentioned above,  $O_2$  is converted by SOD to  $H_2O_2$ , which is decomposed to water and oxygen by CAT, preventing hydroxyl radicals production. Additionally, GSH-Px converts peroxides and hydroxyl radicals into nontoxic forms by the oxidation of reduced glutathione (GSH) into glutathione disulfide and then reduced to GSH by glutathione reductase. Other antioxidant enzymes are glutathione-S-transferase and glucose-6-phosphate dehydrogenase.<sup>14</sup>

The non-enzymatic antioxidants are molecules that interact with RONS and terminate the free radical chain reactions: bilirubin,  $\alpha$ -tocopherol (vitamin E), and  $\beta$ -carotene are present in blood while albumin and uric acid account for 85% of antioxidant capacity in plasma.<sup>15</sup>

- Exogenous antioxidants include ascorbic acid (vitamin C), which scavenges hydroxyl and superoxide radical anion,  $\alpha$ -tocopherol (vitamin E), which is involved against lipid peroxidation of cell membranes, and phenolic antioxidants, which include stilbene derivatives (resveratrol, phenolic acids, and flavonoids), oil lecithins, selenium, zinc, and drugs such as acetylcysteine.<sup>16</sup> Oxidative stress occurs when there is an imbalance between the formation and the removal of RONS because of an overproduction and/or an impaired ability to neutralize them or to repair the resulting damage.<sup>6</sup>

## Oxidative stress and theory of aging

Aging is the progressive loss of tissue and organ function over time.<sup>17</sup> The free radical theory of aging, later termed as oxidative stress theory of aging, is based on the structural damage-based hypothesis that age-associated functional losses are due to the accumulation of oxidative damage to macromolecules (lipids, DNA, and proteins) by RONS.<sup>18</sup> The exact mechanism of oxidative stress-induced aging is

still not clear, but probably increased RONS levels lead to cellular senescence, a physiological mechanism that stops cellular proliferation in response to damages that occur during replication. Senescent cells acquire an irreversible senescence-associated secretory phenotype (SASP) involving secretion of soluble factors (interleukins, chemokines, and growth factors), degradative enzymes like matrix metalloproteases (MMPs), and insoluble proteins/extracellular matrix (ECM) components.<sup>1,19</sup> RONS induce cellular senescence acting on various components of SASP:

- regulation of mammalian target of rapamycin complexes' functions;<sup>1</sup>
- production of IL-1 $\alpha$  leading to a proinflammatory state, which increases nuclear factor kappa-B (NF $\kappa$ B) activity and epithelial–mesenchymal transition and tumor metastatic progression;<sup>1</sup>
- induction of MMPs expression, which is associated with age-related and chronic diseases such as cancer, Alzheimer's, atherosclerosis, osteoarthritis, and lung emphysema;<sup>1</sup>
- inhibition of FOXO (Forkhead box) proteins activity, which is involved in insulin/insulin-like growth factor-1-mediated protection from oxidative stress;<sup>1</sup>
- reduction of sarco/endoplasmic reticulum Ca<sup>2+</sup>-ATPase activity leading to cardiac senescence;
- inhibition of sirtuins activity leading to an increased production of RONS by SOD inhibition, a proinflammatory state by preventing their inhibition of tumor necrosis factor alpha (TNF $\alpha$ ) and NF $\kappa$ B, and tumorigenic effect by preventing their inhibitory effect on c-Jun and c-Myc;<sup>20</sup>
- regulation of p16INK4a/pRB and p53/p21 pathways leading to senescence.<sup>1</sup>

## Oxidative stress and age-related diseases

Oxidative stress, cellular senescence, and consequently, SASP factors are involved in several acute and chronic pathological processes, such as CVDs, acute and chronic kidney disease (CKD), neurodegenerative diseases (NDs), macular degeneration (MD), biliary diseases, and cancer. Cardiovascular (CV) risk factors (ie, obesity, diabetes, hypertension, and atherosclerosis) are associated with the inflammatory pathway mediated by IL-1 $\alpha$ , IL-6, IL-8, and increased cellular senescence.<sup>1</sup> Moreover, vascular calcification is linked to an SASP-driven osteoblastic transdifferentiation of senescent smooth muscle cells. In many neurodegenerative

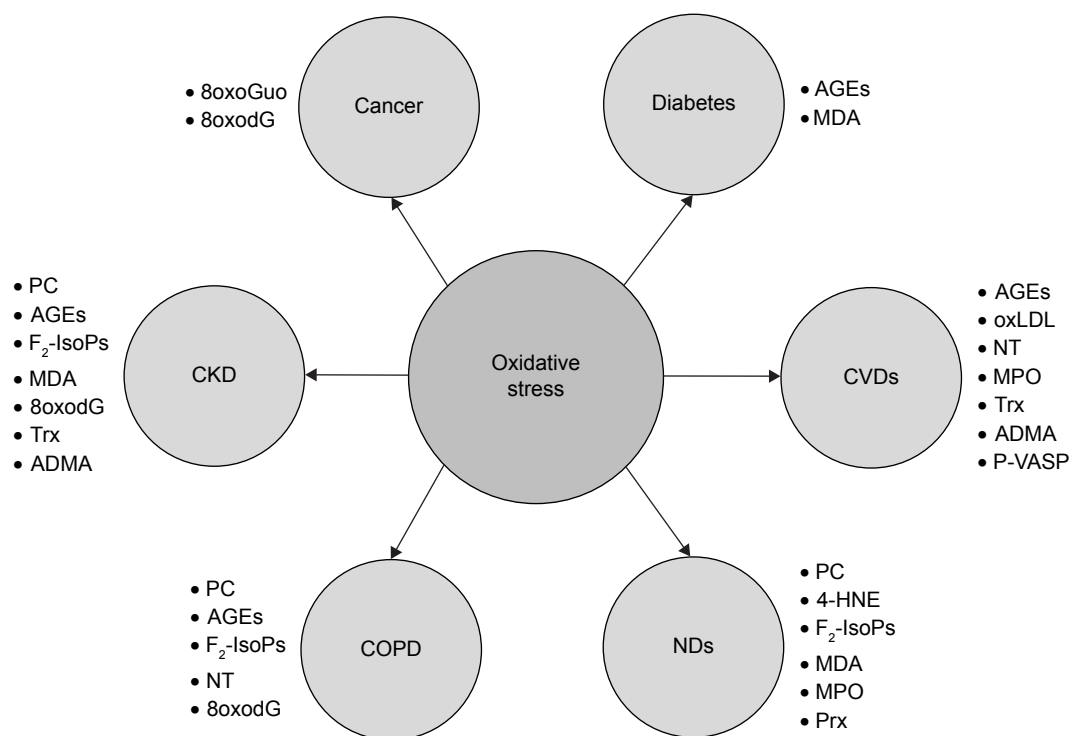
conditions, including Alzheimer's disease (AD), brain tissue biopsies show increased levels of p16, MMP, and IL-6.<sup>21</sup> Chronic obstructive pulmonary disease, biliary cirrhosis, cholangitis, and osteoarthritis share several damaging SASP profiles including IL-6, IL-8, and MMP.<sup>1</sup> The induction of epithelial to mesenchymal transition mediated by RONS promotes cancer metastasis.<sup>22</sup> In synthesis, given the close relationship between oxidative stress, inflammation, and aging, the oxidation-inflammatory theory of aging or oxi-inflamm-aging has been proposed: aging is a loss of homeostasis due to a chronic oxidative stress that affects especially the regulatory systems, such as nervous, endocrine, and immune systems. The consequent activation of the immune system induces an inflammatory state that creates a vicious circle in which chronic oxidative stress and inflammation feed each other, and consequently, increases the age-related morbidity and mortality.<sup>23</sup>

The connection between oxidative stress and the main age-related diseases is described in the following sections (Figure 1).

## Oxidative stress and CVDs

CVDs are a leading cause of morbidity and mortality in the elderly, and atherosclerosis plays a crucial role as main causal event.<sup>24</sup> Several studies have proven that heart tolerance to oxidative stress decreases with age because of a reduction in the concentrations of the antioxidant enzymes (ie, GSH-Px and SOD), contributing to the development of CV alterations.<sup>25</sup>

The evidence currently available links atherosclerosis with oxidized LDL-cholesterol (oxLDL) as the compound mainly responsible for its production, also in elderly.<sup>26</sup> In fact, different studies showed a significant association between oxLDL and higher arterial stiffness, independent of other traditional CVD risk factors.<sup>27</sup> A study conducted in 2,944 healthy women (aged 30–79 years) underlined increases in oxLDL levels in plasma after 50 years.<sup>28</sup> The increase of oxLDL with aging may amplify LDL atherogenicity because of the prooxidant and proinflammatory environments that characterize elderly subjects.<sup>26</sup> Conversely, data for the InCHIANTI dataset, a 9-year follow-up population-based study, showed no association between higher oxLDLs levels (measured with antibody 4E6) and CVD/cardiac mortality, suggesting that in advanced age, the prognostic information added by oxLDLs might not be significant.<sup>29</sup> Moreover, the development of oxidative stress contributes to vascular endothelial dysfunction with aging. In healthy adults varying in age, brachial artery flow-mediated dilation is inversely



**Figure 1** Oxidative stress, age-related diseases, and relative biomarkers.

**Abbreviations:** 4-HNE, *trans*-4-hydroxy-2-nonenal; 8oxodG, 7,8-dihydro-8-oxo-2'-deoxyguanosine; 8oxoGuo, 7,8-dihydro-8-oxoguanosine; ADMA, asymmetric dimethyl L-arginine; AGEs, advanced glycation end products; CKD, chronic kidney disease; CVDs, cardiovascular diseases; F<sub>2</sub>-IsoPs, F<sub>2</sub>-isoprostanes; MDA, malondialdehyde; MPO, myeloperoxidase; NDs, neurodegenerative diseases; NT, nitrotyrosine; oxLDL, oxidized low-density lipoprotein; PC, protein carbonyl; Prx, peroxiredoxins; P-VASP, phosphorylated vasodilator-stimulated phosphoprotein; Trx, thioredoxin.

related to NT in vascular endothelial cells.<sup>30</sup> Furthermore, the expression of endothelin-1, the most effective vasoconstrictor molecule produced by the vascular endothelium, is increased in vascular endothelial cells of older compared with younger adults and inversely related to endothelium-dependent dilation and positively related to NT.<sup>31</sup> Endothelial dysfunction and vascular remodeling are recognized as early determinants in the development of hypertension and atherosclerosis.<sup>32</sup>

## Oxidative stress and diabetes

Diabetes mellitus (type 1 and 2) is a metabolic disease associated with increased formation of free radicals and decreased antioxidant potential, leading to macro- and microvascular complications.<sup>33</sup> The precise mechanism by which oxidative stress may accelerate the development of complications in diabetes is only partly known. Oxidant stress in type 2 diabetes (T2D) promotes prothrombotic reactions, leading to CV complications.<sup>34</sup> Diabetes damages can be considered tissue-oxidative-damaging effects of chronic hyperglycemia. Increased intracellular glucose leads to an increased RONS production, which exceeds the antioxidant capability of the cell to neutralize them.<sup>35</sup> RONS induce, in this way, the

activation of four important molecular pathways involved in hyperglycemia-induced oxidative tissue: activation of protein kinase C (PKC), increased hexosamine pathway flux, increased AGEs, and increased polyol pathway flux.<sup>36</sup> In particular, the activation of the AGEs pathway can damage cells modifying the regulation of gene transcription, the signaling between the matrix and other cells and blood proteins (eg, albumin), causing them to bind to AGEs receptors (RAGEs) on macrophages/mesangial cells and increase the production of growth factors and proinflammatory cytokines.<sup>37</sup> Regarding the geriatric population, a study conducted on 61 elderly subjects has shown increased levels of antioxidants (ie, CAT and GSH-Px) in subjects with impaired glucose tolerance (IGT), compared with healthy controls, and increased levels of oxidative stress biomarkers (ie, MDA) in T2D, but not in IGT group. These results suggest that there is an increased oxidative stress in the elderly T2D patients, which can be partly balanced by increased antioxidant defense system in subjects with IGT.<sup>38</sup>

## Oxidative stress and COPD

COPD is a major cause of morbidity and mortality worldwide<sup>39</sup> and its prevalence increases with age.<sup>40</sup> Several

mechanisms related to aging are potentially involved in its pathogenesis, such as shortened telomere length, cellular and immune senescence, inflammation and also oxidative stress.<sup>41</sup> RONS in COPD result from both cellular and environmental sources. Environmental oxidants from cigarette smoke activate macrophage and epithelial cells triggering proinflammatory cytokine and chemokine generation, which induce immune response. Released proteases break down connective tissues in the lung, potentially resulting in chronic bronchitis or emphysema. Moreover, excessive RONS released via oxidative bursts of polymorphonuclear leukocytes (PMNs) and alveolar macrophages have been shown to inhibit antiprotease processes and accelerate the degradation of lung tissue. RONS also delay the resolution of inflammation via compromising the phagocytic ability of alveolar macrophages, leading to necrosis and emphysema. Likewise, long-term cigarette smoke exposure impairs PMNs and alveolar macrophage phagocytosis and antigen-presentation functions, which could predispose patients to bacterial/viral infection.<sup>42</sup> Several studies have shown increased levels of biomarkers of oxidative stress in COPD patients, such as 8-oxodG, NT, F2-IsoPs, AGEs, and PC, with a strong correlation with the severity of airflow limitation in COPD elderly patients.<sup>41</sup> In particular, some authors have shown that several age-related diseases, including COPD, are associated with a skeletal muscle dysfunction. Oxidative stress is a major player in the etiology of this condition in the elderly, and specifically, protein carbonylation was shown to modify the function of key enzymes and structural proteins involved in muscle contractile performance.<sup>10</sup> Furthermore, a decline in some isoforms of the receptor for AGEs has been detected in COPD elderly patients, and a study has shown that its levels have a significant and independent association with FEV<sub>1</sub>, FEV<sub>1</sub>/FVC, and diffusing capacity of the lung for carbon monoxide in COPD patients, suggesting that it may be a marker of disease severity and consequently a marker of accelerated aging in this cohort.<sup>41,43</sup>

## Oxidative stress and CKD

Oxidative stress plays a pivotal role in the progression of CKD, through glomerular damage and renal ischemia and, indirectly, with inflammation, hypertension, and endothelial dysfunction.<sup>44</sup> CKD patients are in a chronic inflammation state characterized by the activation of PMNs and monocytes. These inflammatory cells increase the secretion of NADPH oxidase and MPO that enhance the formation of ROS.<sup>45</sup> Leukocytes of CKD patients produce superoxide anions, which inactivate NO, reducing the ability of blood vessels

dilatation that contributes to hypertension. NO levels are also reduced because of the lack of its precursor L-arginine, which is formed from L-citrulline in the kidney. Superoxide anion is also capable of reacting with NO itself forming peroxynitrite, which can oxidize NOS making it unstable. This unstable NOS will further augment superoxide production.<sup>46</sup> NOS activity is also inhibited by NOS inhibitors, such as the asymmetric dimethyl-arginine (ADMA), which accumulates in CKD and contributes to hypertension through vasoconstriction.<sup>47</sup> CKD patients also have high levels of homocysteine, which raises ROS production, and ADMA, reduces NO synthesis, and increases the risk of CV events.<sup>45</sup> Endothelial dysfunction, induced by oxidative stress, changes vascular permeability and leads to the entry of LDL cholesterol into intimal layer and increased oxLDL levels, which are involved in the atherosclerosis process, as mentioned earlier.<sup>48</sup> ADMA-induced endothelial dysfunction leads to proteinuria, a sign of glomerular damage associated, in turn, with proinflammatory cytokines secretion, which increases the production of ROS, worsening renal damage.<sup>45</sup> Finally, in a state of oxidative stress, the oxidation of red blood cells lipid membranes decreases their elasticity shortening their lifespan and increasing the hemolysis probability. This might explain the cause of anemia in patients with CKD in addition to the reduction of erythropoietin synthesis.<sup>49</sup> In particular, the global prevalence of CKD is rising, particularly among the elderly population, and CKD accelerates normal aging and leads to worsening frailty in elderly patients through several mechanisms, in particular, oxidative stress.<sup>50</sup>

## Oxidative stress, cognitive impairment, and dementia

Cognitive impairment and dementia affect life quality and life span in the elderly. Not only NDs, which include AD, Parkinson's disease (PD), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS), but also vascular dementia have a massive impact especially in aging populations – progressive loss of memory, impairments in the movement, or progressive inability to move.<sup>51,52</sup> Oxidative stress has been shown to play a pivotal role in the pathophysiology of dementia.<sup>53</sup> In fact, several studies have evaluated the relationship between the levels of some oxidative stress biomarkers and cognitive function, evaluated with Mini-Mental State Examination (MMSE). A recent study showed that increased oxidative stress biomarkers (ie, MDA, GSH-Px, and PC) correlated with raised levels of inflammatory cytokines and both were associated with low cognitive performance in institutionalized elderly people.<sup>54</sup>



Another study showed that, despite age being the principle cause of cognitive decline, cognitive impairment is slower in patients with high GSH-Px activity, but high GSH levels seem to accelerate cognitive decline in the elderly. This seems to be a paradoxical finding because high intracellular GSH should have a protective function against cell damage caused by free radicals. A possible hypothesis is that, as GSH is a substrate of GSH-Px, high GSH levels could be a consequence of the increase in oxidative stress induced by a reduction of GSH-Px activity.<sup>55</sup> Other theories have been suggested to explain the involvement of oxidative stress in the pathophysiology of NDs. For example, oxidative stress is one of the conditions that induces stress granules (SGs) formation. Nuclear SGs contain heat-shock transcription factor 1/2 and pre-mRNA processing factors while cytoplasmic SGs are composed of proteins and non-translating mRNAs.<sup>53</sup> Normal cytoplasmic SGs assemble in response to various stressful conditions and are degraded once the stress is absent. SGs can also form super-stable aggregates under pathological conditions – more severe stress, mutations that promote SG assembly or amyloid formation, or mutations that reduce their clearance.<sup>56</sup> SGs produced in response to acute stress are protective and antiapoptotic. In cognitive impairment, the stress is a chronic condition that cannot be resolved; hence, SGs may interfere with neuronal function by silencing transcripts and by sequestering important proteins such as ribonucleoproteins.<sup>53</sup> Another hypothesis suggests the role of ROS and redox metals in the pathogenesis of AD. Abnormal homeostasis of bioactive metals could be involved in oxidative stress influencing AD – zinc directly affects amyloid-protein precursor,<sup>57</sup> and aluminum, zinc, iron, and copper directly bind to amyloid promoting its aggregation.<sup>58–60</sup> Similarly, the redox metals could promote Tau phosphorylation. ROS, amyloid, and Tau protein affect the activity of N-methyl-D-aspartate (NMDA) receptors, triggering an NMDA-mediated excessive influx of  $\text{Ca}^{2+}$  in post-synaptic neurons leading to a cascade of events that increase ROS production, oxidative stress, Tau phosphorylation, and lipid peroxidation, ultimately leading to synaptic dysfunction responsible for AD.<sup>61</sup>

## Oxidative stress and cancer

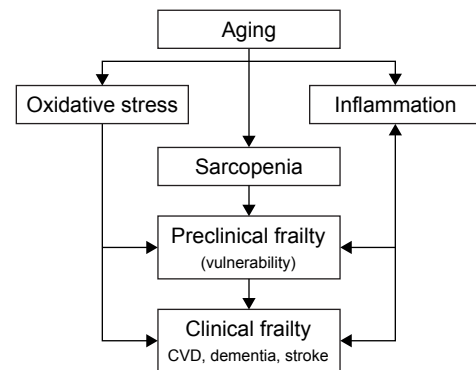
Many studies have demonstrated the direct relationship between chronic inflammation and carcinogenesis.<sup>62</sup> The main chemical effectors of these effects of the inflammatory response are free radical species derived from oxidative stress induced by inflammation. RONS may directly or indirectly damage other chemical or structural components in target

cells or recruit other inflammatory cells leading to additional RONS production that amplifies the damage.<sup>63</sup> In particular, RONS and inflammatory cytokines, such as  $\text{TNF}\alpha$ , activate the transcription factor  $\text{NF}\kappa\text{B}$ , which induces the expression of genes involved in cell proliferation, apoptosis, and carcinogenesis.<sup>64</sup> Chronic inflammation is also associated with angiogenesis, another characteristic of cancer, because RONS can also increase the expression of transcriptional factors (ie, c-fos and c-jun) involved in neoplastic transformation and enhancement of cancer angiogenesis.<sup>63</sup> Moreover, macrophages, platelets, fibroblasts, and cancer cells are a major source of angiogenic factors (ie, fibroblast growth factor, vascular endothelial growth factor, and prostaglandins-E1 and E2), which increase the production of RONS and, subsequently, increase the risk of cancer.<sup>62</sup> The mutagenic/carcinogenic potential of RONS is due to their capability of reacting with DNA and chemically modifying it. In particular, ROS-induced DNA damages can result in transcriptional arrest or induction/replication errors, or genomic instability, which are all associated with carcinogenesis.<sup>65</sup> Among oxidized-DNA products, 8-oxodG and 8-nitroguanine are considered biomarkers for inflammation-induced carcinogenesis.<sup>62</sup> There is a dramatic age-dependent escalation in cancer risk, and increased oxidative stress and oxidative stress and its damages over a lifetime may be responsible for this phenomenon.<sup>66</sup> In fact, there is an accumulation of RONS-induced DNA damages with age, which is confirmed by the progressive and statistically significant increase in the levels of 7,8-dihydro-8-oxo-2'-deoxyguanosine (8oxodG) observed with aging.<sup>62</sup> On the basis of these considerations, chronic inflammation and oxidative stress should be considered high risk factors for cancer, especially in elderly people.<sup>66</sup> Based on these analogies, it should be recommended that elderly people should consume higher antioxidant compounds, but the efficacy of antioxidant therapy in the prevention of carcinogenesis is currently under investigation.

## Oxidative stress, sarcopenia, and frailty

With improved life conditions and the availability of innovative treatments, life expectancy and, consequently, the number of elderly in the population have increased.<sup>67</sup> Aging is associated with strength deficits that are related to frailty, loss of independence, and physical disability.<sup>68</sup> Reduction in strength is due to both loss of muscle quantity, referred to as sarcopenia, and loss of muscle strength, termed as dynapenia.<sup>69</sup> Skeletal muscles consume large quantities of oxygen and can generate a great amount of RONS, whose

accumulation is thought to be a common determinant in the loss of both muscle quantity and quality, through several mechanisms.<sup>70</sup> First of all, with advancing age, the antioxidants defenses are compromised, and RONS accumulation induces posttranscriptional modifications, which can be used as biomarkers of oxidative stress, such as the nitration and nitrosylation (ie, NT), carbonylation (ie, PC, 4-HNE), and glycation (ie, AGEs).<sup>69</sup> Second, RONS contribute to sarcopenia also by increasing proteolysis and decreasing muscle protein synthesis, leading to a reduction in muscle mass quantity.<sup>3</sup> On the other hand, several mechanisms, acting on the neuromuscular junction, are involved in RONS-mediated reduction of muscle quality and strength. First, RONS reduce acetylcholine release at the synaptic cleft, which could lead to a failure in the generation of an action potential by the sarcolemma. Moreover, persistent oxidative stress may change the morphology of the neuromuscular junction causing a reduction in the innervation and fibers number. At the same time, RONS damage impair excitation–contraction (EC) coupling, leading to a lower release of calcium from the sarcoplasmic reticulum. Finally, RONS induce modification in actin and myosin structures significantly reducing the cross-bridge cycling within the myofibrillar apparatus.<sup>69</sup> Sarcopenia is an important alteration occurring in the elderly and predicts frailty, poor quality of life, and mortality.<sup>70</sup> Thus, sarcopenia can be assessed objectively and could be used as a predictor of these adverse health outcomes and a therapeutic target for muscle-building interventions in the elderly.<sup>71</sup> In particular, sarcopenia is said to be the “biological substrate of physical frailty”,<sup>72</sup> and the sarcopenia phenotype should be the central focus of frailty assessment and intervention.<sup>71</sup> A recent systematic review on oxidative stress and frailty showed that preclinical frailty is associated with higher peripheral levels of RONS biomarkers and lower antioxidant parameters. However, due to the cross-sectional design of the study, it was not possible to establish the directionality of the relationships between RONS and frailty, since it could be bidirectional (Figure 2).<sup>73</sup> Moreover, the Framingham Offspring Study showed increased levels of proinflammatory and oxidative stress biomarkers (ie, C reactive protein and F<sub>2</sub>-IsoPs) both in pre-frail and frail elderly subjects.<sup>74</sup> The same results were described in the elderly German population from the ESTHER Cohort Study.<sup>75</sup> All these studies defined frailty according to Fried et al, who describe a physical frailty phenotype consisting of the presence of three or more of the following characteristics: unintentional weight loss, weakness, exhaustion, low physical activity, and slowness in motor



**Figure 2** Relationship among oxi-inflamm-aging, preclinical and clinical frailty.  
**Abbreviation:** CVD, cardiovascular disease.

performance.<sup>76</sup> Since Fried’s phenotype of frailty focuses on physical frailty, the mechanisms proposed to explain the relationship between oxidative stress and frailty in the elderly are largely overlapped with those described above to explain how oxidative stress causes musculoskeletal system damages.<sup>74</sup> In other words, frailty in these studies has been defined with criteria of Fried’s frailty phenotype, ie, “primary” or “preclinical” frailty, which is not associated with a specific disease. In contrast, “secondary” or “clinical” frailty which is associated with known disability and/or comorbidity (ie, CVDs, NDs, CKD, T2D)<sup>77</sup> could significantly increase the generation of RONS products.<sup>73</sup>

## Biomarkers of oxidative stress

The World Health Organization defined a biomarker as any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease.<sup>78</sup> A clinically useful biomarker should have specificity for a certain disease (diagnostic), have a prognostic value, and correlate with disease activity.<sup>9</sup> The most clinically relevant oxidative stress biomarkers are described in Table 1<sup>10,12,26,27,30,31,38,41,43,54,62,66,79–101</sup> and Figure 1 and can be divided into RONS-induced modifications, markers of RONS generation, markers of antioxidant defense, and downstream functional markers of RONS-induced damage.<sup>9</sup>

RONS-induced modifications biomarkers include

- PC, whose increased levels are associated with low cognitive performance,<sup>54</sup> CKD,<sup>79</sup> COPD,<sup>10</sup> and sarcopenia;<sup>80</sup>
- AGEs, as mentioned above, derived from glycoxidation reactions. AGEs levels are increased in T2D<sup>12</sup> and are an independent risk factor for both all-cause and CV mortality,<sup>81</sup> also among elderly patients with heart failure.<sup>82</sup> There is an age-dependent increase of serum

**Table 1** Overview of the most important oxidative stress biomarkers

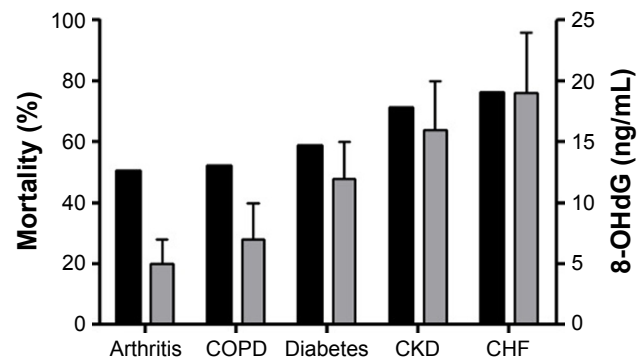
| Biomarkers  | Sample    | Disease   | References                           |
|---|-----------|---|--------------------------------------|
| <b>RONS-induced modifications</b>                           |           |   |                                      |
| PC  | Plasma    | High levels are associated with raised levels of cytokines and both are associated with low cognitive performance in institutionalized elderly subjects.<br>Higher in CKD.<br>Associated with skeletal muscle dysfunction in elderly COPD patients.<br>Increased levels are associated with sarcopenia in elderly subjects.   | 54<br>79<br>10<br>80                 |
| AGEs  | Serum     | Higher in T2D.<br>Independent risk factor for both all-cause and cardiovascular disease mortality in elderly subjects with or without CHF.<br>Correlate with lipid profiles and atherosclerotic characteristics in the elderly.<br>Higher in elderly subjects with CKD.<br>Higher in elderly subjects with osteoporosis.<br>Decreased levels of their receptors are markers of severity and accelerated aging in COPD elderly patients. | 12<br>81, 82<br>83<br>81<br>85<br>43 |
|   | Skin      | Increased levels are associated with sarcopenia in elderly subjects.<br>Correlation with risk of exhaustion and low energy expenditure, but not with prevalent or incident frailty in elderly subjects.   | 86<br>87                             |
| oxLDL   | Plasma    | High levels are associated with arterial stiffness and atherogenesis in the elderly.<br>Not predictive of cardiac mortality in elderly subject.   | 26<br>27                             |
| 4-HNE   | Plasma    | Increased in elderly subjects with AD, PD, HD, and ALS pathologies.<br>Increased levels are associated with sarcopenia in elderly subjects.   | 88<br>89                             |
| F <sub>2</sub> -IsoPs                                       | Urine     | High levels are associated with depressed mood in community-dwelling elderly subjects.<br>High levels in AMD.<br>High levels in AD and HD.<br>High levels in CKD and correlate with disease progression.  | 90<br>91<br>92<br>79                 |
| MDA   | Plasma    | High levels in COPD elderly patients.<br>Increased levels correlate with raised levels of inflammatory cytokines and both are associated with low cognitive performance in institutionalized elderly subjects.<br>Increased in elderly subject with T2D but not with IGT.<br>Elevated in CKD, inversely related to GFR, and positively correlated with uremic toxins and severity of glomerulosclerosis.                                | 41<br>54<br>38<br>79                 |
| NT  | Plasma    | Nitrotyrosine levels are associated with vascular endothelial dysfunction with aging.<br>High levels in COPD elderly patients.  | 30, 31<br>41                         |
|   | Tissue    | Increased levels are associated with sarcopenia in elderly subjects.  | 93                                   |
| 8oxodG  | Urine     | Markers of inflammation-induced carcinogenesis increasing with aging.<br>High levels in COPD elderly patients.<br>High levels in CKD.   | 62<br>41<br>79                       |
| 8oxoGuo   | Urine     | Markers of carcinogenesis in the elderly.   | 66                                   |
| <b>Markers of RONS generation</b>                           |           |   |                                      |
| MPO   | Plasma    | MPO predicts endothelial dysfunction and the development of CHF in the elderly.<br>MPO predicts all-cause mortality in frail and very old community-dwelling people.<br>MPO levels are elevated in elderly with AD.   | 94<br>95<br>96                       |
| <b>Markers of antioxidant defense</b>                       |           |   |                                      |
| Trx   | Plasma    | Significantly increased in CHF compared with healthy elderly subjects.<br>Inverse relationship with GFR, suggesting a protective mechanism.   | 97<br>79                             |
| Prx   | Plasma    | Potential biomarker of AD in elderly subjects.<br>Potential useful risk stratification tool to predict mortality in elderly patients with nonspecific complaints presenting to the emergency department.  | 98<br>99                             |
| <b>Downstream functional markers of RONS-induced damage</b> |           |   |                                      |
| ADMA  | Plasma    | Strong prognostic value for mortality and future CV events in the elderly.<br>Elevated in CKD and is an independent predictor of superoxide generation.   | 100<br>79                            |
| P-VASP  | Platelets | Establish the efficacy of antiplatelet drugs in the adults and the elderly.   | 101                                  |

**Abbreviations:** 4-HNE, *trans*-4-hydroxy-2-nonenal; 8oxodG, 7,8-dihydro-8-oxo-2'-deoxyguanosine; 8oxoGuo, 7,8-dihydro-8-oxoguanosine; AD, Alzheimer's disease; ADMA, asymmetric dimethyl L-arginine; AGEs, advanced glycation end products; ALS, amyotrophic lateral sclerosis; AMD, age-related macular degeneration; CHF, chronic heart failure; CKD, chronic kidney disease; CV, cardiovascular; F<sub>2</sub>-IsoPs, F<sub>2</sub>-isoprostanes; GFR, glomerular filtration rate; HD, Huntington's disease; IGT, impaired glucose tolerance; MDA, malondialdehyde; MPO, myeloperoxidase; NT, nitrotyrosine; oxLDL, oxidized low-density lipoprotein; PC, protein carbonyl; PD, Parkinson's disease; Prx, peroxiredoxins; P-VASP, phosphorylated vasodilator-stimulated phosphoprotein; RONS, reactive oxygen and nitrogen species; T2D, type 2 diabetes; Trx, thioredoxin.



AGEs levels, and in the elderly, AGEs levels positively correlate with lipid profiles and atherosclerotic characteristics, suggesting that AGEs could be used as a marker to predict atherosclerotic lesions.<sup>83</sup> Increased AGEs levels are also associated with chronic kidney disease, predicting a greater decline of renal function.<sup>84</sup> Accumulation of AGEs in the collagen matrix of bone increases its stiffness and fragility; in fact, AGEs levels are significantly higher in patients with osteoporosis.<sup>85</sup> Moreover, a recent interesting French study analyzed the relationships between the accumulation of AGEs, assessed by skin autofluorescence (AF), and frailty in 423 older community-dwellers aged  $\geq 75$  years, showing that the accumulation of AGEs was not associated with prevalent or incident frailty but with the 4-year risk of exhaustion and low energy expenditure.<sup>87</sup> Nevertheless, increased levels of AGEs are associated with sarcopenia in elderly subjects,<sup>86</sup> while decreased levels of RAGEs are markers of severity and accelerated aging in COPD patients;<sup>43</sup>

- oxLDL levels, as described in the section “Oxidative stress and CVDs”, correlate with arterial stiffness and atherogenesis in the elderly, not predicting mortality;<sup>26,27</sup>
- 4-HNE levels are elevated in elderly subjects with NDs (ie, AD, PD, HD, and ALS)<sup>88</sup> and sarcopenia;<sup>89</sup>
- $F_2$ -isoprostanes urinary levels are increased in several diseases frequently diagnosed in elderly patients, such as depression,<sup>90</sup> age-related macular degeneration (AMD),<sup>91</sup> AD and HD,<sup>92</sup> COPD,<sup>41</sup> and CKD, where it correlates with disease progression;<sup>79</sup>
- MDA increased plasma levels correlate with low cognitive performance in institutionalized elderly subjects;<sup>54</sup> its levels increase in elderly subjects with T2D but not with IGT<sup>38</sup> and in CKD, where its levels are inversely related to glomerular filtration rate (GFR) and positively correlated with uremic toxins and severity of glomerulosclerosis;<sup>79</sup>
- NT levels are used as biomarkers of COPD<sup>41</sup> and sarcopenia<sup>93</sup> in elderly subjects, and high levels are associated with vascular endothelial dysfunction with aging;<sup>30,31</sup>
- DNA modifications, 8oxodG and 7,8-dihydro-8-oxoguanosine (8oxoGuo), are excreted into the urine and measured with enzyme-linked immunosorbent assay. Both are markers of inflammation and carcinogenesis in the elderly.<sup>62,66</sup> Furthermore, 8oxodG is also a marker of COPD<sup>41</sup> and CKD in elderly subjects.<sup>79</sup> In fact, data from the “Osservatorio Geriatrico Campano” showed that high levels of oxidative stress, quantified with 8oxodH, are associated with high mortality in age-related chronic diseases



**Figure 3** Chronic diseases, oxidative stress, and long-term mortality in community-dwelling elderly subjects. Data from a previous study.<sup>102</sup>

**Abbreviations:** CHF, chronic heart failure; CKD, chronic kidney disease; 8-OHdG, 8-hydroxy-2'-deoxyguanosine.

(ie, COPD, CKD, chronic heart failure [CHF], and T2D) in community-dwelling elderly subjects (Figure 3).<sup>102</sup>

The most important marker of RONS generation is MPO. MPO is a leukocyte-derived enzyme released in inflammatory conditions, which catalyzes the formation of several ROS and is involved in LDL oxidation and is considered a major contributor in the formation and rupture of atherosclerotic plaque.<sup>103</sup> Moreover, MPO directly catalyzes the consumption of NO resulting in endothelial dysfunction.<sup>94</sup> Therefore, MPO levels independently predict endothelial dysfunction and mortality for CVDs and CHF. Data from the Aging and Longevity Study in the Sirente Geographic Area (iSIRENTE Study), a prospective cohort study that collected data on all individuals aged  $\geq 80$  years living in a mountain community ( $n=363$ ), showed that high plasma levels of MPO are positively associated with all-cause death in frail and very old persons living in the community, independent of age, gender, and other clinical and functional variables. In particular, after adjusting for several clinical correlates of mortality risk, baseline MPO plasma concentration emerged as an independent predictor of mortality.<sup>95</sup> A study conducted on 3,733 elderly subjects measuring MPO levels showed an independent association between increased MPO levels and the development of CHF, particularly beyond myocardial infarction and traditional cardiac risk factors.<sup>94</sup> Moreover, among age-related diseases, MPO levels are also elevated in elderly subjects with AD, showing that MPO might be an important molecular link between atherosclerosis and AD and probably a promising biomarker for the detection and risk stratification of AD patients.<sup>96</sup>

Markers of antioxidant defense include the following:

- Thioredoxin, NADPH, and thioredoxin reductase (TrxR) are important defense systems against oxidative stress,

providing the electrons to thiol-dependent peroxidases (peroxiredoxins) to remove RONS.<sup>104</sup> Trx levels are significantly increased in CHF compared with healthy elderly subjects<sup>97</sup> and its levels show an inverse relationship with GFR, suggesting a protective mechanism.<sup>79</sup>

- Peroxiredoxins are thiol-specific proteins that react as scavenger of H<sub>2</sub>O<sub>2</sub>, peroxynitrite, and a wide range of hydroperoxides.<sup>105</sup> Peroxiredoxins are potential biomarkers of AD in elderly subjects<sup>98</sup> and, interestingly, they could be a useful risk stratification tool to predict mortality in elderly patients with nonspecific complaints presenting to the emergency department.<sup>99</sup>

There are also two markers that reflect oxidative stress downstream of the RONS-induced damage: ADMA and phosphorylated vasodilator-stimulated phosphoprotein (P-VASP).<sup>9</sup>

ADMA is a powerful inhibitor of NOS and also competes with L-arginine for the binding site of this enzyme. Reduced production of endogenous NO coincides with high ADMA levels, engenders endothelial dysfunction, and this dysfunction is reversed by L-arginine.<sup>106</sup> In particular, in the randomly selected, community-based sample of 1155 elderly, aged 65–102 years, of the “Invecchiare in Chianti Study” (InCHIANTI; aging in the Chianti area), higher ADMA levels independently predicted all-cause and CV mortality. Thus, ADMA could be used as a prognostic value for mortality and future CV events.<sup>100</sup>

P-VASP is probably the best-established marker for physiological cyclic guanosine monophosphate (cGMP) signaling, because it is phosphorylated mainly by cGMP-dependent protein kinases, and lowered P-VASP levels are indicative of pathological signaling.<sup>9</sup> However, in human blood samples, P-VASP levels are used to establish the efficacy of (or detect nonresponders to) antiplatelet drugs, also in the elderly, as shown in the GENERATIONS trial.<sup>101,107</sup>

Oxidative stress biomarkers may provide important information about the efficacy of a treatment, thus, providing guidance for the selection of the most effective drugs/dose regimens for patients. In addition, if a biomarker of oxidative stress is particularly relevant from a pathophysiological point of view, it may also be useful in research as a therapeutic target to identify novel treatments with antioxidant properties. However, further investigations are needed to confirm and explore their potential clinical applications.<sup>9</sup>

## Therapeutic approach

Since it has been established that oxidative stress plays an important role in the pathogenesis of many clinical conditions

and aging, several studies have been conducted to investigate the therapeutic effects of antioxidant therapy.

## Vitamins

The most extensively studied antioxidants are vitamin A, and its precursor  $\beta$ -carotene, vitamin C, and vitamin E.<sup>108</sup> Several large observational studies were conducted on the effect of intake of different vitamins and on the risk of CVDs, suggesting that higher intake of these vitamins significantly lowered the risk of these pathologic conditions.<sup>109</sup> Systematic reviews and meta-analysis conducted by Cochrane group investigators to study the effect of vitamins on all-cause mortality showed conflicting and, many times, disappointing results. In some trials, vitamins did not seem to significantly affect mortality, but in several other trials, they were administered alone or in combination showing a significant increase of all-cause mortality.<sup>110</sup> These conflicting findings can lead to the conclusion that vitamins cannot be used as effective antioxidant therapeutic agents.

## Coenzyme Q10

Coenzyme Q10 (CoQ10), ubiquinone (oxidized form) or ubiquinol (reduced form), is an endogenous lipid that takes part in the mitochondrial respiratory chain reactions.<sup>111</sup> Numerous pathological processes are associated with primary and secondary CoQ10 deficits, including mitochondrial diseases, fibromyalgia, CVDs, NDs, cancer, T2D, male infertility, and periodontal disease.<sup>112</sup> CoQ10 treatment is safe in humans, and new formulations that increase CoQ10 absorption and tissue distribution have been developed. Oral administration of CoQ10 is a frequent antioxidant strategy in many of the abovementioned diseases providing a significant to mild symptomatic benefit.<sup>112</sup>

## Selenium

Many of the physiological roles of the element selenium (Se) are directly attributed to its presence within at least 25 proteins, named selenoproteins, collectively essential for life and also involved in oxidative stress control.<sup>113</sup> Several selenoproteins have been characterized as antioxidant enzymes, such as GSH-Px, TrxR, and iodothyronine deiodinases.<sup>114</sup> Several clinical trials have provided convincing evidence of the central role of this element in the prevention and treatment of multiple diseases, such as CVDs, atherosclerosis, diabetes mellitus, stroke, NDs, depression, hypothyroidism, and cancer.<sup>115</sup> However, there are some controversies over whether Se administration increases the risk of some neuronal diseases (ie, ALS) because of its

neurotoxic effects – inhibition of prostaglandin D synthase in the brain, inhibition of squalene mono-oxygenase, increase in dopamine and its metabolites, inhibition of succinic dehydrogenase, acetylcholine esterase and Na<sup>+</sup>/K<sup>+</sup> ATPase, and induction of seizures.<sup>116</sup> Additional experimental evidence is needed to determine the optimal level of Se daily intake that can maximize health benefits, avoiding potential toxic effects.<sup>115</sup>

## Polyphenols

Polyphenols are secondary metabolites of plants, and they are found largely in fruits, vegetables, cereals, and beverages. Typically, a glass of red wine contains about 100 mg polyphenols.<sup>117</sup> The phenolic compounds of wine can be divided into flavonoids and nonflavonoids. Flavonoids account for >85% of the phenolic components in red wine and include different molecules, especially quercetin, and nonflavonoids include mainly resveratrol.<sup>118</sup> Quercetin is an antioxidant agent present in wine that has been shown to prevent or delay the initiation of CVDs or cancer through different biological effects. Its mechanisms of action are not completely understood, but it has shown an antiinflammatory effect through a decrease of TNF $\alpha$  and IL-1 $\beta$  and suppression of NF $\kappa$ B.<sup>119</sup> Resveratrol is an antioxidant agent derivative of stilbene, which passed unnoticed until it was related to the French paradox.<sup>118</sup> The “French paradox” is a term generated in 1992 based on epidemiological data from French people who had a low incidence of CVDs despite the high consumption of saturated fat. This paradox was attributed to their high wine consumption (20–30 g/day), which was suggested to decrease platelet aggregation.<sup>120</sup> Since 1992, the French paradox has considerably evolved, and its explanation has been attributed to resveratrol beneficial effects because it

- reduces vascular remodeling and inflammation, reducing vascular smooth muscle cells proliferation, vascular calcification, and interfering with the expression of proinflammatory enzymes;<sup>118</sup>
- prevents endothelial dysfunction increasing the expression of NOS and vascular dilation, contributing also to the prevention of hypertension;<sup>121</sup>
- reduces platelet recruitment and aggregation;<sup>122</sup>
- reduces LDL oxidation and LDL and triglycerides levels;<sup>118</sup>
- improves glucose metabolism by increasing insulin sensitivity and glucose absorption mediated by glucose transporter-4 and protecting pancreatic  $\beta$ -cells function.<sup>123</sup>

These effects are positively related to the reduction of CVDs. However, wine and/or resveratrol can also reduce the

incidence of other pathologies, such as neurodegenerative conditions, cancer, and osteoporosis.<sup>118</sup>

## Other antioxidant agents

Many studies have been conducted to identify new natural antioxidants.<sup>2</sup> In particular, even the fermented papaya preparation (FPP), produced by fermentation of *Carica papaya* Linn by using yeast, is considered a food supplement that exhibits antiinflammatory, antioxidant, and immunostimulatory functions that could be helpful against age-related and disease-related increase in oxidative stress. In fact, because of the important role of oxidative stress in the pathophysiology of chronic NDs, a study has been conducted to explore the effects of FPP in AD patients, by measuring urinary 8oxodG, showing that, after a supplementation with FPP 4.5 g/day for 6 months, urinary levels of 8oxodG were significantly decreased ( $14.1 \pm 1.7$  ng/mL to  $8.45 \pm 1.1$  ng/mL,  $p < 0.01$ ), with no significant changes in controls, showing a potential beneficial effect of FPP in patients with neurodegenerative conditions, such as AD.<sup>124</sup> Given the reported inverse relationship between the dietary intake of antioxidant-rich food and the incidence of human diseases, there has been a global trend toward increasing the intake of natural antioxidants, especially in the geriatric population.<sup>2,125</sup> Many antioxidant vegetables and fruits have been identified such as potato, spinach, tomatoes, and legumes or berries, cherries, citrus, prunes, and olives.<sup>2,126</sup> Interestingly, melatonin is a powerful cardioprotective agent and has shown beneficial effects on cardiac aging and many other age-related disorders.<sup>125</sup> Melatonin (N-acetyl-5-methoxytryptamine) is an endogenously produced indoleamine synthesized from the essential amino acid tryptophan and secreted by the pineal gland in a circadian manner, but also by extrapineal organs (ie, gastrointestinal tract, immune system cells, retina, spleen, liver, kidney, and heart) not in a circadian manner.<sup>127,128</sup> Melatonin has also been identified in a very large number of plant species, in plant food and medical herbs. In particular, among the plant products in which melatonin has been identified, the most important are diet products including wine, olive oil, tomato, and beer.<sup>125</sup> Melatonin acts through several mechanisms: directly scavenging free radicals and indirectly upregulating antioxidant enzymes and downregulating prooxidant enzymes.<sup>129</sup> Aging is associated with a significant reduction in endogenous melatonin secretion, resulting in an augmentation of oxidative stress and other metabolic changes.<sup>130</sup> Melatonin receptors have been identified in the human CV system, mainly localized in the ventricular wall, coronary arteries, aorta, and peripheral arteries, where they exert a

protective effect from serious CV events, such as ischemic heart disease, acute myocardial infarction, and also cardiac syndrome X, an angina-like chest discomfort.<sup>125</sup> In particular, some authors have observed an antihypertensive effect of the melatonin, administered orally at 1.5 mg each day, on elderly hypertensive volunteers of both genders (63–91 years old), hypothesizing as a possible explanation that melatonin binds to peripheral receptors in the arteries inducing vasodilatation followed by a decrease in arterial blood pressure.<sup>131</sup> Melatonin also shows a protective effect from cardiac ischemia-reperfusion (RI)-induced infarction.<sup>132</sup> Furthermore, a recent study showed that the oral administration of melatonin in patients who were undergoing elective coronary artery bypass grafting could ameliorate, in a dose-dependent way (10 mg capsule vs 20 mg capsule once daily), myocardial RI injury by interfering with the oxidative stress, inflammation, and apoptotic markers.<sup>133</sup> Melatonin may show a protective effect also from myocardial infarction but, at the present time, only two clinical trials, the MARIA and the IMPACT, are currently investigating the effects of melatonin in patients at high risk of acute myocardial infarction.<sup>125</sup> In the MARIA trial, patients undergoing revascularization for ST-elevation myocardial infarction (STEMI) received intravenous (51.7  $\mu$ mol) and intracoronary (8.6  $\mu$ mol) melatonin immediately before and during percutaneous coronary intervention (PCI);<sup>134</sup> whereas, in the IMPACT trial, acute myocardial infarction patients will receive an intracoronary (1 mg) and intravenous (49 mg) dose of melatonin.<sup>135</sup> In particular, the MARIA trial has showed that, when administered via intravenous and intracoronary during primary PCI for STEMI, melatonin had an acceptable safety and tolerability profile, but it did not appear to exert a significant effect on myocardial infarct size measured by magnetic resonance imaging (MRI) and it may have a detrimental effect after STEMI, mainly because it might facilitate left ventricular remodeling.<sup>134</sup> Given its cardioprotective effects and safety, increased blood melatonin levels could be obtained by moderate and chronic consumption of wine, beer, walnuts, and other food or beverages that contain melatonin, but further investigations are needed to expand its range of applications and also to better understand its specific mechanism(s) to improve CV physiology.<sup>125</sup>

## Physical exercise

Inactivity and aging are known to increase basal RONS concentrations in skeletal muscle, leading to sarcopenia.<sup>136</sup> In contrast, regular physical activity is an important determinant in maintaining an optimal state of health, reducing oxidative stress and preventing chronic diseases, but these beneficial effects are related to the features of physical exercise,

especially intensity.<sup>137</sup> The relationship between exercise intensity and oxidative stress adaptation in older adults shows that both inactivity and high-intensity physical exercise are related to increased oxidative stress, whereas moderate intensity exercise is related to a reduction in oxidative stress levels.<sup>136</sup> In fact, physical exercise training can be divided into acute, endurance, and resistance. In particular, acute exercise induces increased production of free radicals in elderly subjects, together with an increase in antioxidant defenses which, given its age-related decline, seems to be ineffective in neutralizing all of the free radicals produced during exercise, setting up a status of oxidative stress.<sup>138–140</sup> On the other hand, endurance training induces both a decrease in the production of free radicals and an increase in antioxidant defenses in elderly people. According to the findings in the literature, optimal aerobic training for oxidative/antioxidant balance effects can be achieved with intensities between 50% and 80% of  $\text{VO}_2\text{max}$  (the maximum rate of oxygen consumption measured during incremental exercise) and with a frequency of two to three sessions per week.<sup>136</sup> At the same time, resistance training improves antioxidant defenses in elderly population.<sup>141</sup> This effect can be achieved by training protocols provided of sufficient volume for each muscle group (3–5 sets, 10 repetitions) and intensities between 50% and 80 % of one-repetition maximum, the maximum amount of force that can be generated in one maximal contraction.<sup>136</sup> The role of oxidative stress in age-related sarcopenia suggests the importance of physical activity in limiting this process in elderly subjects and its association with increased RONS generation, but it can also induce an increase in antioxidants defense, positively affecting oxidative/antioxidant balance, if a moderate intensity protocol of endurance or resistance training is chosen, avoiding acute one.<sup>142</sup>

## Conclusion

RONS are produced by several endogenous and exogenous processes. Oxidative stress results from the imbalance between RONS production and antioxidants defense and is primarily involved in “aging theory”, in particular in the “oxi-inflamm-aging hypothesis”. Oxidative stress is also related to several chronic diseases and, together with chronic inflammation, to sarcopenia and frailty in elderly population. Biomarkers of oxidative stress may be useful as diagnostic tool or therapeutic target. Antioxidant therapy, such as resveratrol and other nutritional compounds, together with moderate aerobic exercise, may positively affect the clinical damage induced by oxidative stress. Further investigations are needed to evaluate the real efficacy of these therapeutic interventions.



## Disclosure

The authors report no conflicts of interest in this work.

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