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# Antioxidants in human disease: Potential therapeutic opportunities

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**Abstract:** It has been increasingly accepted that oxidative stress is implicated in the underlying mechanism of a variety of disease states. Free radicals and other reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced continuously in the cells; moreover, these molecules at low concentrations can behave as secondary messengers of cell signaling transduction pathways regulating physiologic processes for the maintenance of cell homeostasis. Conversely, increased ROS are associated to a broad spectrum of pathological effects ranging from functional impairment to cell death. Consequently, the resulting organ damage may account for the pathophysiological events leading to disease. In turn, cells are endowed with defense mechanisms including endogenous antioxidant systems able to exert protective effects against the cytotoxicity of ROS, which could be also reinforced by the administration of exogenous antioxidants. While the antioxidant defense system could be enhanced by low-to-moderate ROS levels, mainly through the up-regulation of the activity of antioxidant enzymes, this system could be overwhelmed by higher ROS levels, thereby giving rise to the development of oxidative stress and the related pathophysiological cascades. This paradigm constitutes the rationale central core leading to the understanding of many pathological disorders, such as those occurring in cardiovascular disease, atherosclerosis, cancer, metabolic disorders, and neurodegenerative diseases, among others. Therefore, the recognition of potential pharmacologic targets against oxidative stress related in these disorders should contribute to the design of interventions aimed to blunt or at least attenuate the harmful impact of increased ROS. However, the demonstration of a therapeutic role for antioxidants in diseases still remains controversial and needs further scientific bases. The present review presents an updated overview of the studies accounting for the protective role of antioxidants against oxidative stress-mediated cellular toxicity. In addition, a novel therapeutic approach is provided for the prevention and treatment of some prevalent human diseases that account for a major portion of deaths today.

## Introduction

The concept of a potential role for antioxidants in the prevention and treatment of human disease is supported by the evidence provided by some clinical and experimental studies performed during the last decades [1-6]. Nevertheless, the rational for these interventions arise from our knowledge about the biological effects of oxidative stress, a mechanism underlying the onset and development of many diseases. Reactive oxygen species (ROS), which are free radicals containing one or more unpaired electrons in the outer orbit and other related compounds, as well as reactive nitrogen species (RNS), derived from the endogenous metabolic processes in the human body, are potentially cytotoxic compounds. These reactive species at moderate concentrations play an important role as regulatory mediators in signaling processes [7, 8]. In contrast, at high concentrations they can damage cells and organs by either direct attack to biomolecules or starting chain reactions resulting in deleterious effects [9]. Acute or mild-to-moderate ROS exposure could be appropriately counteracted through the adaptive response of the antioxidant defense system. However, ROS production or long-term exposure could reach a level high enough to overwhelm the capacity of this system, thus giving rise to the occurrence of functional and/or structural organ damage and their clinical consequences. Accordingly, oxidative stress has been considered as a pathological mechanism participating in the initiation and progression of organs dysfunction [10]. Therefore, it seems reasonable to hypothesize that a reinforcement of the antioxidant system should result in a decreased risk to the development of many diseases mediated by a mechanism involving the contribution of oxidative stress. An example illustrating this view is postoperative atrial fibrillation, a clinical setting of ischemia-reperfusion associated with the occurrence of oxidative stress which may be counteracted by antioxidants [11, 12]. Although this paradigm has a major experimental support and has been successfully demonstrated [13], the results of several clinical trials designed to test the effectiveness of antioxidants in prevention of oxidative stress have been disappointing [13-17]. The reason for this discrepancy has not been elucidated yet, but it may be related with factors such as the subject enrollment criteria, genetic characteristics, diet differences, appropriateness of chosen biomarkers, dosage and type of antioxidant used in each trial, pharmacokinetic and pharmacodynamic properties of the antioxidants and their metabolites, among others [18]. More accurately designed clinical trials with updated basic scientific knowledge are still lacking to have a better understanding of the actual contribution of each individual antioxidant compound in the therapy of human diseases [19-23].The aim of this review was to present an updated survey of the studies accounting for a protective role of antioxidants against oxidative stress-mediated cellular toxicity, as a novel approach in the prevention and treatment of some prevalent human diseases.

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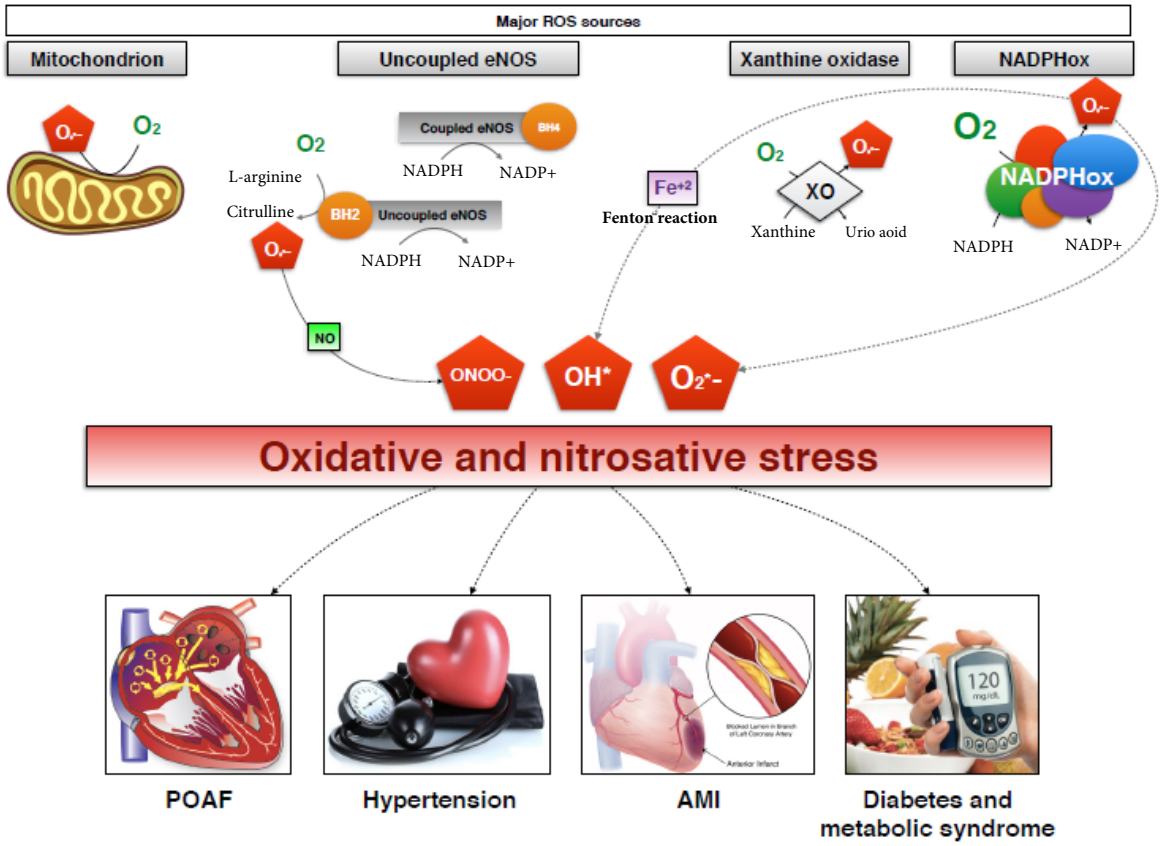
## Oxidative Stress and the Antioxidant Defense System

The mechanisms of human disease involve the increased generation of ROS (superoxide anion radical, hydrogen peroxide, hydroxyl radical and singlet oxygen) and reactive nitrogen species (RNS) (nitric oxide, peroxynitrite anion, and nitrogen dioxide radical), both types of which are highly reactive substances likely to attack biomolecules, such as proteins, lipids or DNA [9]. These species are endogenously generated, in part enzymatically from the reduction of oxygen to water, or directly through chemical reactions, such as Fenton and Haber-Weiss reactions. The progress of the latter occurs with the catalysis of transition metal ions leading to the production of hydroxyl radical, being iron overload one of the most highly deleterious effects for cell structures [24]. On the other hand, there is an antioxidant defense system to counteract the effects of increased ROS and RNS. The components of the antioxidant defense systems are enzymes that catalyze biochemical reactions which are able of removing free radicals and other reactive species from the cells and body fluids (mainly, catalase, superoxide dismutase and glutathione peroxidase), and antioxidant molecules reacting directly with ROS and RNS to form less reactive products, thus avoiding the development of oxidative stress and/or nitrosative stress, respectively. It is of interest to mention that a disease state will occur when the potential activity of the antioxidant system is overwhelmed by prooxidant effects that are enough active to maintain increased ROS and RNS concentrations in the metabolic steady state of the body, thereby resulting in structural and functional organ injuries. The regulation of the activity of antioxidant enzymes is dependent on the cell redox state, as their genomic expression is enhanced through the increased cellular ROS concentration. The mechanism of this regulation is triggered by the nuclear translocation of nuclear factor erythroid 2-related factor 2 (Nrf2) on the antioxidant response elements [25, 26], thus leading to an increased antioxidant response aimed to decrease ROS and RNS levels. This mechanism operates whenever reactive species concentrations are increased but being not high enough to activate the nuclear factor kappaB (NF-κB) pathway [27]. Otherwise the latter would result in deleterious effects for the cell, because it leads to a prooxidant state associated to increased productions of cytokines, chemokines and adhesion molecules, among others. It has been suggested from both basic research and human epidemiologic studies that a diet rich in antioxidant molecules could play a preventive role in the development of diseases caused by oxidative challenges [28]. Accordingly, studies in rats demonstrated that the antioxidant vitamin E may exert protection against the adverse effects of antibiotics such as vancomycin [29] or gentamicin [30]. In agreement with this point of view, the antioxidant hypothesis was postulated [31], linking the high content of diet antioxidants with their health benefits through a direct effect on the reduction of oxidative stress for preventing atherosclerosis. Although there are many other examples of protection against organ injury by antioxidants, the findings of clinical trials in humans up to date remain inconsistent. Indeed, the role of diet antioxidants is even more complex than expected, acting its components as signaling molecules in enzymatic pathways involved in the energetic balance, among other cell physiology processes [1]. Figure 1. summarizes the four main sources of reactive oxygen/nitrogen species in human disease. The contents of the following sections include studies of specific noncomunicable diseases known to be related with the occurrence of oxidative stress, providing the available evidence for a protective role of antioxidants.

**Figure 1.** Major ROS and RNS sources in the human organism.




The figure summarizes the four main sources of reactive oxygen/nitrogen species, including NADPH oxidase, xanthine oxidase, uncoupled eNOS and the mitochondrion, all of which plays a key role in oxidative stress-mediated cardiovascular and metabolic diseases.

## Cardiovascular Diseases

### Acute Myocardial Infarction

Coronary-heart disease is the most common cause of morbidity and mortality at the global level and its prevalence is steadily increasing [32]. During the last two decades, the advances of interventional cardiology and thrombolytic pharmacology have significantly reduced acute myocardial infarction (AMI)-derived mortality burden. Paradoxically, oxygen flow restoration of the ischemic area triggers a rapid and massive myocardial necrosis along with less intense but yet more prolonged apoptosis and autophagy processes. These events lead to the configuration of a phenomenon known as myocardial reperfusion injury (MRI), whose damage may account for up to 50% of the final size of the AMI [33]. Myocardial damage caused by ischemia-reperfusion events is mainly associated to four clinical conditions: lethal reperfusion (increasing myocardial final infarct size), myocardial stunning (reversible mechanical dysfunction), no-reflow phenomenon and post-reperfusion arrhythmias.

There is a large amount of evidence proposing oxidative stress as a central mediator in MRI. The level of tissue oxygenation increases following blood flow restoration, which is followed by an early burst of ROS generation [34], mainly superoxide anion, being these ROS major initiators of myocardial damage during ischemia-reperfusion [35]. Endothelial cells, vascular smooth muscle and cardiomyocytes are the primary source of ROS during reperfusion [36]. Increased ROS production is mainly due to the activation of xanthine oxidase in endothelial cells, mitochondrial electron transport chain reactions in cardiomyocytes, uncoupled endothelial eNOS and NADPH oxidase in inflammatory cells [37]. It is of interest to remark that ROS also stimulates the production of inflammatory cytokines and, in turn, inflammatory cytokines stimulate ROS formation.

In the last years, it has been postulated that the iron homeostasis could have an important role in the development of MRI in the cardiomyocytes [38]. Following reperfusion, both iron and copper are released to the coronary circulation [39], which can contribute to ROS generation. This catalytic free iron can generate ROS through the Fenton reaction catalyzing the production of hydroxyl radical [40]. It has also been reported that susceptibility to injury from H2O2 in rat hearts is associated with the magnitude of the intracellular low molecular weight iron pool [41].

### Antioxidants in AMI

The consistent success of antioxidant strategies to ameliorate lethal reperfusion in animal models, which also correlated with a diminution of oxidative stress markers, stimulated the interest to perform clinical trials. However, the results have not been as expected, mainly due to methodological issues [35].

Ascorbate is an essential antioxidant that performs its roles in different cell locations by acting in water-soluble components [42, 43]. The most studied mechanism in which vitamin C acts is partly based on its ability to directly reduce ROS [44, 45]. Besides its ROS scavenger actions, vitamin C exerts a complex modulation of numerous enzymes involved in ROS production, endothelial dysfunction, platelet aggregation and smooth muscle cell tone regutation [46-48]. NADPH oxidase, the most important superoxide source in the cardiovascular system, can be directly down-regulated by vitamin C [46-48]. Vitamin C has also shown to increase eNOS activity, by pre-preventing the oxidation of tetrahydrobiopterin and by inhibiting the p47phox subunit expression [49]. Therefore, ascorbate increases NO synthesis, reduces ROS formation and contributes to the vascular tone regulation [50-52]. In relation to antioxidant enzymes up-regulation, some studies have demonstrated a positive correlation between antioxidant vitamins concentration and antioxidant enzyme activity, particularly SOD [46].

The lack of positive results using ascorbate to prevent MRI is probably due to pharmacological concerns [35]. Kinetic data indicate that superoxide reacts with NO at a rate $10^5$-fold greater than the rate at which superoxide reacts with ascorbate [53]. As a consequence, high plasma levels are needed to support its competition with superoxide, which cannot be achieved by oral doses [35]. Therefore, new clinical trials are needed to elucidate the role of ascorbate in preventing MRI.

Another antioxidant used in MRI is N-acetylcysteine (NAC). Although it has been shown that NAC interacts with numerous biochemical pathways, its main mechanism involves serving as a precursor of cysteine and replenishment of cellular GSH levels [54]. Given its properties, NAC has been widely used in different experimental and clinical settings to counteract oxidative stress. An interesting study published in 2006, shows that administration of NAC in combination with streptokinase significantly diminishes oxidative stress and improves left ventricular function in patients with AMI [55]. However, it has also been reported that although a high-dose of NAC prior to percutaneous coronary angioplasty (PCA) reduces oxidative stress, it does not provide an additional advantage in the prevention of MRI [56]. Finally, a recent study using a rat model of MRI demonstrated that treatment with NAC continuous infusion before occlusion produces a significant limitation of the infarct and recovers the decreased total glutathione when compared to control [57]. In summary, due to the known antioxidant and cardioprotective effect, and its role as GSH-donor, it is plausible to suggest that NAC might have a role in preventing MRI, but further studies are still lacking.

Given the known role of iron in the lethal reperfusion, iron chelators have been tested to ameliorate this injury. Deferoxamine (DFO) has been one of the most used drugs for this purpose. In animal models of AMI, DFO has exhibited positive results. Some studies performed in dogs reported a decrease in the infarct size when they used DFO during the reperfusion, suggesting that iron-catalyzed ROS production contributes to cardiomyocyte necrosis in the setting of MRI [56, 57]. Furthermore, it has been described improved recovery of myocardial function after ischemia with iron chelation [39, 58]. In the setting of clinical trials, Paraskevaidis et al. suggested that DFO infusion was able to reduce myocardial stunning after elective coronary artery bypass grafting and to improve long-term ejection fraction [59]. In a recent clinical study, Chan et al. randomized 60 patients with STEMI to intravenous DFO before PCA and then for 12 hours versus placebo [60]. The serum iron levels and lipid peroxidation biomarkers were reduced in the DFO group without differences in the infarct size. Iron chelators could be used combined with other antioxidants in order to prevent MRI, but limited data is available [61].

### Postoperative Atrial Fibrillation

Atrial fibrillation (AF) represents the most common arrhythmia in clinical practice and is associated with poor clinical outcome. In the general population, it affects approximately 2.3 million people in

the USA and increasing in fivefold the risk for stroke [62]. The efficacy of currently available treatments is sub-optimal. In turn, AF is the most common complication associated with coronary artery bypass graft surgery and other surgical procedures performed with extracorporeal circulation (postoperative atrial fibrillation, POAF). Frequently, It occurs within the first few days in 10% to 65% of patients after major cardiothoracic surgery and results in increased morbidity and length of hospital stay, having enormous cost implications in these patients. Its appearance increases with age and with the presence of known risk factors as arterial hypertension, coronary heart disease, diabetes mellitus and valve disease, among others. Management of POAF is often frustrating, and strategies vary widely from one institution to another. Despite all the efforts put into preventing POAF, including the use of β-blockers and amiodarone, a considerable percentage of the patients still presents the arrhythmia [63].

POAF genesis and pathophysiology have been heavily studied in the last years, however the exact mechanisms behind its appearance and perpetuation, have not been clearly described so far. The involvement of oxidative stress in the mechanism of POAF is supported by an increasing body of evidence indicating that ROS formation following extracorporeal circulation are involved in the structural and functional myocardial impairment derived from the ischemia–reperfusion event [64-66]. Following cardiac surgery, and especially with extracorporeal circulation, ischemic phenomena and posterior reperfusion are mandatory. This leads to high ROS synthesis, which could impair the normal operation of several physiological processes in the organism [67].

Before being determined the specific molecular pathways through which ROS exert their actions, the first evidences accounting for this hypothesis were the high levels of serum myocardial oxidation biomarkers (peroxide, derivatives of reactive oxidative metabolites of oxygen and/or nitrogen) in AF presenting patients in relation to healthy individuals [44]. There is also evidence for oxidative injury in atrial tissues from AF patients [67]. On this line, it was found that patients developing POAF had increased levels and expression of NOX subunit NOX2 and in NOX-derived superoxide generation [68]. Together with NOX, it has been found that other pro-oxidative enzymes are in higher activity in the context of POAF; this is the case of xanthine oxidase and uncoupled eNOS [68].

Electric remodeling is one of the most important mechanisms whereby ROS disturbs the normal electric conduction of the heart. Fibrillating atria is characterized by a diminished action potential and effective refractory period, due to changes in several currents that normally maintain the cardiomyocyte electric potential [69]. Between those currents, the L-type voltage-gated $Ca^2+$ current has been found to be the principal target of ROS action. This current has been found to be diminished in cells extracted of fibrillating atria, as a result of cardiomyocyte calcium overloading [69]. Experiments using canine sarcoplasmic reticule vesicles demonstrated the existence of a superoxide activated calcium releases from Ryanodine receptor (RyRC) [70]. Hence, ischemia-reperfusion dependent ROS could activate the RyRC and produce calcium overloading. As a consequence, L-type current is reduced, thus leading to the electrophysiological changes, like shortening of the refractory period, involved in the initiation and perpetuation of POAF.

Finally, it has to be mentioned the effect of ROS in the disruption of cardiomyocyte connexins. Connexins are a set of proteins assembled between two adjacent cardiac cells, forming the structure known as gap junction. Under conditions of oxidative stress, following an ischemia/reperfusion cycle, increased ROS directly interact with the connexins, particularly with connexin 43, thereby disrupting its organization, leading to electrical remodeling and therefore to propensity to present AF [71, 72].The exact molecular mechanism by which ROS disrupt normal connexin distribution has not been completely identified. However, the ROS-mediated activation of protein kinase C gamma, unique isoform present only in neural and optic tissue, leads to the phosphorylation and posterior disassembly of connexin 43 [73].

## Antioxidants in POAF

Based on the numerous evidence supporting the hypothesis that oxidative stress is a cornerstone in the pathogenesis underlying POAF it could be noted that the use of antioxidants as therapeutic tools appears to be a rational line of study. Substances with antioxidant properties such as statins, NAC, and specially vitamins C and E have proved to be efficient not only in decreasing the serum oxidative levels in patients undergoing cardiac surgery, but also in diminishing the occurrence of POAF [74,75]. Furthermore it has been hypothesized that one of the mechanisms whereby classic anti-AF drugs act is related with the ability to scavenge ROS and protect against membrane lipid peroxidation [76].

NAC was used in a prospective, randomized, placebo-controlled trial to study its potential anti-arrhythmic effect [74]. In this study of 115 patients undergoing coronary artery bypass and/or valve surgery, 58 patients received pre-operative NAC and 57 patients received placebo (both groups received also standard medical therapy, including β-blockers). The results showed that POAF incidence was 5.2% in the NAC group and 21.1% in the placebo group. These data demonstrated that an antioxidant agent, such as NAC, used in combination with classic anti-arrhythmic drugs, could contribute to prevent the appearance of arrhythmias like POAF. More studies using NAC are still lacking to assess the actual potential of this agent.

Antioxidant vitamins and AF related clinical trials have not been heavily studied. One of the most paradigmatic studies involving ascorbate anti-arrhythmic properties was conducted to test not only the effects of vitamin C supplementation in POAF incidence, but also to assess the biochemical changes in oxidative and electric status after canine atrial pacing. In the first part, 43 patients subjected to coronary artery bypass were given 2 g of vitamin C the day before the surgery, followed by 500 mg until the fifth post-operative day. The POAF incidence in the ascorbate treated group was 16% v/s 35% in the control group. In the other part of the study, eleven dogs were subjected to rapid atrial pacing, which led to shortening of the effective refractory period (ERP), associated with accumulation of 3-nitrotyrosine residues, a peroxynitrite oxidative biomarker, and decreased levels of ascorbate compared with non-paced controls. Ascorbate treatment attenuated the ERP shortening and diminished the 3-nitrotyrosine residues concentration found after atrial pacing [77]. This study showed, on the one hand that antioxidant vitamins could decrease the incidence of POAF, and on the other hand showed that this effect could be a reflection of a stabilization of the electrophysiological properties of the heart, that are impaired in individuals presenting this arrhythmia.

It was also shown that oral vitamin C in association with β-blockers was more effective in preventing POAF than β-blockers alone.

This study consisted in 100 patients undergoing coronary artery bypass grafting, separated in a β-blockers group and a β-blockers/ ascorbate group, which received 2 g of ascorbic acid on the night before the surgery and 2 g daily for 5 days after surgery. The POAF incidence was 4% in β-blockers/ ascorbate group and 26% in the β-blockers group [78]. Consequently, antioxidant vitamins not only have shown favor-able anti-arrhythmogenic results compared with non-vitamin patie-nts, but also with patients receiving classical anti-AF drug treatment. This concept has major relevance, as the study for alternative thera-peutic tools was originated because of the lack of effective and risk-free treatment for AF.

The future task is to continue testing antioxidant therapies under different protocols and contexts, to assess their real potential in preventing and/or treating AF and POAF.

## Hypertension

Hypertension is a major risk factor for cardiovascular disease [79]. Recently, a growing body of evidence has involved oxidative stress in the mechanism of development of hypertension. Indeed, ROS contribute to regulating the biological processes occurring in the vascular wall, both in normal physiological conditions, as well as in the occurrence of hypertension [80, 81]. Available evidence of the contribution of oxidative stress in the pathogenesis of human hypertension includes enhancement of ROS production, together with decreased bioavailability of both nitric oxide (NO) and antioxidants. The first formed ROS is superoxide anion radical, which is produced from NOX, an enzyme subjected to regulation by hormones such as angiotensin II (AT-II), endothelin-1 (ET-1), and urotensin II (UT-II), among others. Furthermore, mechanical stimuli known to occur in blood pressure elevation further contribute to increased ROS production. It is of interest to mention that increased intracellular calcium concentration may result from ROS-induced vasoconstriction, thus enhancing the development of hypertension [82]. The regulation of vasomotor tone depends upon a delicate balance between vasoconstrictor and vasodilator forces, the latter being likely to be modulated by oxidative stress. This view has stimulated the interest for searching novel antihypertensive therapies aimed to decrease ROS generation and/or increase NO bioavailability.

The occurrence of oxidative stress is due to an imbalance between ROS generation and the antioxidant potential in the body, the latter being overwhelmed by the increased ROS concentration in the steady state. It should be noted that although ROS are mediators of normal biological effects related to vascular function at the cell level, the increased levels of these species can give rise to pathological changes, as those observed in cardiovascular disease. ROS behave as redox-sensitive blood pressure modulators [83-85]. Accordingly, increased ROS concentration has been demonstrated both in patients with essential hypertension and in various animal models of hypertension [86-90]. In addition, this derangement is accompanied by a decreased antioxidant potential [91]. Therefore, these data provide evidence of the involvement of vascular oxidative stress in the mechanism of development of essential hypertension. Furthermore, a strong association between blood pressure and oxidative stress-related parameters has been found, such as plasma 8-isoprostane levels [92]. Interestingly, studies performed in mice models having genetic deficiency in ROS-generating enzymes showed that these animals had lower blood pressure than control with wild-type mice [93]. Moreover, at the cellular level, it has been reported that ROS production is enhanced in cultured vascular smooth muscle cells (VSMC) isolated from both hypertensive rats and isolated arteries of hypertensive human patients; these findings are associated with amplified, redox-dependent signaling and reduced antioxidant bioactivity [94] . These reports could support the view that the modulation of oxidative stress could be expressed in blood pressure lowering in the case of known antihypertensive agents, such as β-adrenergic blockers, angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor antagonists, and calcium channel blockers [95, 96].

There are various ROS sources formed in blood vessels, from both enzymatic and nonenzymatic origin. Together with the mitochondrion, the major enzymatic sources comprise NOX, xanthine oxidase (XO), and uncoupled NO synthase [97, 98].

## Antioxidants in Hypertension

Recent advances in understanding the complexity of redox signaling in the vascular system points to a central role of oxidative stress in the pathogenesis of vascular dysfunction. This is how hypertension is associated with impaired endothelium-dependent vasodilation with inactivation of endothelium-derived nitric oxide by oxygen free radicals. In this regard, it has arisen a growing interest concerning the therapeutic possibilities to target ROS in the management of essential hypertension.

Antihypertensive effects of vitamin C were hypothesized as early as 1946, and it has been proven that vitamin C enhances endothelial function through effects on nitric oxide production [51]. Most studies have demonstrated an inverse relation between vitamin C plasma levels and blood pressure, in normotensive and hypertensive populations [99]. However, evidence for blood pressure-lowering effects of vitamin C in clinical trials is still inconsistent.

Despite the evidence points to the use of vitamin C as an adjunct in the treatment on essential hypertension, there is still lack of long-term studies that support its use. Up to date there are few trials that have used chronic supplementation [100, 101].The strongest evidence of the possible role of vitamin C on hypertension treatment was handed by a recent meta-analysis that included twenty-nine trials, concluding that in short-term trials, vitamin C supplementation reduces systolic and diastolic blood pressure. But it also highlights that long-term trials on the effects of vitamin C on blood pressure and clinical events are still needed to elucidate its true benefit [102].

Because supplementation made only with vitamin C has achieved inconsistent clinical outcomes, the scientific rational approach has led to the suggestion that the combined intake of antioxidants could achieve better clinical results. Following that consideration, a randomized double-blind placebo-controlled clinical trial was conducted to test the hypothesis that oral administration of vitamin C and E together causes decrease in blood pressure in patients with mild-to-moderate essential hypertension, 110 men with recent diagnosis of grade 1 essential hypertension were randomly assigned to receive either vitamin C (1g) plus vitamin E (400IU) daily or placebo for 8 weeks. The results of this study, showed for the first time a specific association between oxidative-stress related parameters and blood pressure. Following administration of vitamins C plus E, patients with essential hypertension had significantly lower systolic, diastolic and mean arterial blood pressure [103].

According to the theoretical possibility of the role of antioxidants, further trials have been performed using different compounds with antioxidant activity. This is how Barrios et al. in 2002 conducted a pati-

ent crossover study with the aim to investigate the potential effect of N-acetylcysteine (NAC) added to the ACE inhibitors antihypertensive action. A significant decrease in systolic and diastolic blood pressure was achieved with the combination of ACE inhibitor and NAC compared to the ACE inhibitor-only period [104].

Although there is objective compelling evidence supporting the use of antioxidants in the management of hypertensive patients, there are also several studies that have not shown beneficial effects. As an example: Vitamin E [105] [Palumbo et al., 2000], Coenzyme Q10 [106] and NAC [107] have failed to obtain beneficial effects on clinical settings.

## Metabolic Diseases

### Type 2 Diabetes

Diabetes mellitus is a major cause of morbidity in the western world, and of the most common severe chronic illnesses, affecting over 230 million people worldwide with an estimated global prevalence of 5.1% [108]. The associated complications possess enormous public health and economic burdens.

This illness is characterized by a chronic metabolic disorder caused by defects in both insulin secretion and action. An elevated rate of basal hepatic glucose production in the presence of hyperinsulinemia is the primary cause of fasting hyperglycemia. The reason for the injury related to hyperglycemia is the formation of advanced glycation end products (AGE) such as glycated proteins, glucose oxidation-derived metabolites, and increased free fatty acids [109]. These effects result in oxidative stress in the mitochondria, as well as in the activation of oxidative and inflammatory signaling pathways.

The latter is continued with damage to the insulin-producing cells, resulting in various complications of diabetes, including retinopathy, nephropathy, atherosclerosis and subsequent coronary artery disease, cerebral vascular disease, and peripheral artery disease, which are related to microangiopathy or endothelial injury [110].

Under hyperglycemic conditions, ROS are produced in various tissues such as nerve cells and vascular cells, which are involved in the development of diabetic complications [111]. Recently, pancreatic β-cells emerged as a target of oxidative stress–mediated tissue damage [112]. These cells present a relatively low expression of antioxidant enzymes, such as catalase and glutathione peroxidase [113], providing an increased susceptibility to oxidative damage. There are several sources of ROS production in cells: the nonenzymatic glycosylation reaction [114], the electron transport chain in mitochondria [115], and the hexosamine pathway [116]. In that regard, elevated oxidative stress biomarkers have been reported in patients with diabetes [117, 118].

### Antioxidants in Diabetes

In the case of the antioxidant supplement, various studies support the preventive effects on metabolic and clinical complications of diabetes, mainly in type 1 diabetes [119]. However, the potential role of these agents in the treatment of type 2 diabetes is not well characterized. This can be mainly due to methodological issues, including exclusion criteria.

The supplement of vitamin C showed an attenuation of oxidative damage in diabetes mellitus patients [120]. It is known that vitamin C at pharmacologic doses decreases sorbitol accumulation, which contributes to the progression of chronic diabetic complications. One trial used vitamin C supplements intake of 100 or 600 mg daily for 58 days in young adults with insulin-dependent diabetes mellitus and nondiabetic adults [121]. This intervention diminished significantly the sorbitol accumulation in the erythrocytes of diabetics, displaying low toxicity. Other study, a placebo-controlled trial, tested the hypothesis that oral prophylaxis with vitamin C attenuates rest and exercise induced free radical-mediated lipid peroxidation in type 1 diabetes mellitus [122]. Venous blood samples were obtained at rest, after a maximal exercise challenge and before and 2h after oral ingestion of 1g ascorbate or placebo. Vitamin C supplementation increased plasma vitamin C concentration to a similar degree in both groups and attenuated the exercise-induced oxidative stress response compared with healthy individuals.

Finally, flavonoids are described to exert several biological activities, which are mostly ascribed to their radical-scavenging, metal chelating and enzyme modulation ability. Various authors have found that flavonoid consumption has a positive effect on insulin resistance and cardiovascular outcome measures [123]. Indeed, antioxidant effects have been described on serum and macrophages, which could contribute to attenuation of atherosclerosis development in non-insulin dependent diabetes mellitus patients [124].

### Metabolic Syndrome

One of the most important CVD risk factor is the Metabolic Syndrome (MS), which is a multifactorial cluster of metabolic abnormalities. The analysis of a representative sample of United States of America (USA) adult population showed that MS affects approximately 24% of the individuals, being highly prevalent [125]. A more recent study found that approximately 34% of adults met the National Cholesterol Education Program (NCEP)-ATP III criteria for MS, showing an important increase of its prevalence [126].

Several definitions of MS have been proposed. According to the ATP III of the NCEP, the diagnosis of MS requires the presence of at least three of the following risk factors: a) Fasting plasma glucose (FPG) ≥ 100mg/dL (5.6mmol/L); (b) blood pressure ≥ 130/85 mmHg; (c) triglyceride ≥ 150 mg/dL (1.7 mmol/L); (d) HDL cholesterol: men < 40mg/dL (1.03 mmol/L); women <50mg/dL (1.29 mmol/L); (e) men with WC > 102 cm. and women with WC >88 cm. [127]. Other MS criteria are those from the International Diabetes Federation (IDF). It says that a patient has MS if he/she has a WC ≥ 94 cm. in men and ≥ 80 cm. in women plus any two of these risk factors: (a) fasting plasma glucose (FPG) ≥ 100 mg/dL (5.6 mmol/L) or previously diagnosed impaired fasting glucose (b) blood pressure ≥ 130/85mmHg or treatment for hypertension; (c) Triglyceride ≥ 150 mg/dL (1.7 mmol/L); (d) HDL Cholesterol: men < 40 mg/dL (1.03 mmol/L); women < 50 mg/dL (1.29 mmol/L) or treatment for low HDL [128]. Despite the differences, all definitions of MS consider metabolic variables that largely depend on lifestyle. For example, the weight loss, mainly because of lifestyle changing instead of pharmacology treatment, has an important impact on reducing the prevalence of MS [129].

There is a large amount of studies that provide evidence about oxidative stress and its central role in the pathophysiology of MS. From a clinical point of view, reports from the Third National Health and Nutrition Examination Survey indicate diminished concentrations of the antioxidants vitamins C and E and several carotenoids in participants with MS [130], indicating a possibly disrupted antioxidant defense system in MS. Animal models also reinforce the idea described above. Studies in a diet-induced rat model of MS found increased oxidative stress and endothelial dysfunction, mainly by an increased NOX activity and a downregulation of superoxide dismutase [131]. An inter-

interesting fact is that obese mice treated with apocynin, a NOX inhibitor, presented a reduction in ROS levels and an improvement in glucose and lipid metabolism, independent of body weight [132].

Considering the insulin resistance as a central mechanism underlying MS, it is important to mention that, despite it develops mainly secondary to obesity, increased ROS have also been shown to have a causal role in insulin resistance [133]. In this regard, it can be mentioned that high doses of hydrogen peroxide and reagents that produce ROS [134] can induce insulin resistance in adipocytes. It has been also demonstrated that the up-regulation of genes responsible for ROS production occurs in adipose tissue before the onset of insulin resistance and obesity in mice fed a high fat-diet [135], suggesting that oxidative stress could be the triggering factor that leads to an insulin resistance state in MS.

Despite the evidence mentioned above, there are few clinical trials using antioxidants in MS, and clinical attempts are conducted to manage each component of the syndrome separately. The main objective of further trials should be to diminish the insulinresistance. In that regard, ascorbate has gained attention. Vitamin C is a water soluble compound and it prevents protein and lipid oxidation in the extracellular environment. In vivo studies confirmed that vitamin C administration improves arterial vasodilatation by increasing NO bioavailability [136]. In addition, there is direct evidence that vitamin C has a beneficial effect on insulin sensitivity and some components of the antioxidant defense system in an animal model of insulin resistance [137].

## Concluding Remarks and Future Perspectives

The available evidence regarding the potential use of antioxidant supplements in the prevention and treatment of human diseases suggests that these compounds may be effective in decreasing oxidative stress in vivo. Therefore, antioxidant therapy could be cautiously used to ameliorate the damage observed in diseases mediated by ROS. The limitations for the clinical use of antioxidant supplements are related to the actual scarce knowledge of their properties, bioavailability, adverse events, therapeutic margin, interaction and potential synergistic effect with other drugs, among others.

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