

## REVIEW

# The role of oxidative stress in the pathophysiology of hypertension

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Hypertension is considered to be the most important risk factor in the development of cardiovascular disease. An increasing body of evidence suggests that oxidative stress, which results in an excessive generation of reactive oxygen species (ROS), has a key role in the pathogenesis of hypertension. The modulation of the vasomotor system involves ROS as mediators of vasoconstriction induced by angiotensin II, endothelin-1 and urotensin-II, among others. The bioavailability of nitric oxide (NO), which is a major vasodilator, is highly dependent on the redox status. Under physiological conditions, low concentrations of intracellular ROS have an important role in the normal redox signaling maintaining vascular function and integrity. However, under pathophysiological conditions, increased levels of ROS contribute to vascular dysfunction and remodeling through oxidative damage. In human hypertension, an increase in the production of superoxide anions and hydrogen peroxide, a decrease in NO synthesis and a reduction in antioxidant bioavailability have been observed. In turn, antioxidants are reducing agents that can neutralize these oxidative and otherwise damaging biomolecules. The use of antioxidant vitamins, such as vitamins C and E, has gained considerable interest as protecting agents against vascular endothelial damage. Available data support the role of these vitamins as effective antioxidants that can counteract ROS effects. This review discusses the mechanisms involved in ROS generation, the role of oxidative stress in the pathogenesis of vascular damage in hypertension, and the possible therapeutic strategies that could prevent or treat this disorder.

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## INTRODUCTION

Hypertension is considered the most important risk factor for the occurrence of cardiovascular disease.<sup>1</sup> Oxidative stress has gained attention as one of the fundamental mechanisms responsible for the development of hypertension. Reactive oxygen species (ROS) have an important role in the homeostasis of the vascular wall; hence, they could be part of the mechanism that leads to hypertension.<sup>2–4</sup> Thus, increased ROS production, reduced nitric oxide (NO) levels and reduced antioxidant bioavailability were demonstrated in experimental and human hypertension. Vascular superoxide is primarily derived from nicotinamide adenine dinucleotide phosphate (NADPH) oxidase when stimulated by hormones, such as angiotensin II (AT-II), endothelin-1 (ET-1) and urotensin II. In addition, increased ROS production may be generated by mechanical stimuli on the vascular wall, which increase with hypertension. ROS-induced vasoconstriction results from increased intracellular calcium concentration, thereby contributing to the pathogenesis of hypertension.<sup>2</sup> Vasomotor tone is dependent on a delicate balance between vasoconstrictor and vasodilator forces that result from the interaction between the components of the vascular wall and the blood, all of which can be altered by oxidative stress. These findings have stimulated interest in

antihypertensive therapies targeted toward decreasing ROS generation and/or increasing NO bioavailability. This review examines the available studies that highlight the role of oxidative stress in the underlying mechanism of high blood pressure and discusses those that consider the use of antioxidants in the prevention or treatment of this disorder.

## PATHOPHYSIOLOGY OF HYPERTENSION

### Endothelial dysfunction

Endothelial dysfunction has been implicated in the pathophysiology of different forms of cardiovascular disease, including hypertension. Endothelial dysfunction may be defined as impairment that is characterized by a shift in the actions of the endothelium toward reduced vasodilation, a proinflammatory state, and prothrombotic settings. These events lead to a state of vascular inflammation, which may be partially mediated by ROS formed by activated mononuclear cells.

### Vascular oxidative stress and hypertension

Oxidative stress constitutes a unifying mechanism of injury in many types of disease processes, and it occurs when there is an imbalance between the generation of ROS and the antioxidant defense systems in

the body. The ROS family comprises many molecules that have divergent effects on cellular function. Importantly, many of these actions are associated with pathological changes observed in cardiovascular disease. The effects of ROS are mediated through redox-sensitive regulation of multiple signaling molecules and second messengers.<sup>5–7</sup> Several studies have demonstrated excessive amounts of ROS in essential hypertensive patients and various animal models of hypertension;<sup>8–12</sup> these patients and experimental animals have a diminished antioxidant status,<sup>13</sup> which contributes to the accumulating evidence that increased vascular oxidative stress could be involved in the pathogenesis of essential hypertension.<sup>2,3,14</sup> Recently, a strong association between blood pressure and some oxidative stress-related parameters was demonstrated.<sup>15</sup> Other studies showed that mouse models that involve genetic deficiencies in ROS-generating enzymes have lower blood pressure compared with their wild-type counterparts.<sup>16,17</sup> In addition, in cultured vascular smooth muscle cells (VSMCs) and isolated arteries from hypertensive rats and humans, ROS production is enhanced, redox-dependent signaling is amplified, and antioxidant bioactivity is reduced.<sup>18</sup> The beneficial effects of classical antihypertensive agents, such as  $\beta$ -adrenergic blockers, angiotensin I converting enzyme (ACE) inhibitors, AT-II receptor antagonists, and calcium channel blockers, may be mediated, in part, by decreasing vascular oxidative stress.<sup>19,20</sup>

**Sources of ROS in the vascular wall.** A variety of enzymatic and non-enzymatic sources of ROS exist in blood vessels. The best-characterized source of ROS is NADPH oxidase. In addition to NADPH oxidase, several other enzymes may contribute to ROS generation, including xanthine oxidase, NO synthase (NOS) and mitochondrial enzymes.

**NADPH oxidase:** NADPH oxidase is the primary biochemical source of ROS, particularly superoxide, in the vasculature. The kidney and vasculature are rich sources of NADPH oxidase-derived ROS, which have an important role in renal dysfunction and vascular damage under pathological conditions.<sup>12,21</sup> This system catalyses the reduction of molecular oxygen by NADPH, which functions as an electron donor, thus generating superoxide. NADPH oxidase is upregulated in hypertension by humoral and mechanical signals. AT-II is the most studied stimulus of the upregulation of NADPH oxidase, but ET-1 and urotensin II may also participate to the activation of NADPH oxidase, thereby resulting in increased ROS. It is likely that the most well-known function of NADPH oxidase-derived superoxide is the inactivation of NO in the reaction that forms peroxynitrite, which leads to impaired endothelium dependent vasodilation and the uncoupling of endothelial NO synthase (eNOS) to produce additional superoxide.<sup>16,22</sup> In the vasculature, the activation of NADPH oxidase has been strongly associated with hypertension.<sup>23</sup>

**Uncoupled eNOS:** The primary function of eNOS is NO production, which regulates vasodilation. Nevertheless, deficiency or oxidation of L-arginine and tetrahydrobiopterin (BH<sub>4</sub>), which are two essential cofactors for eNOS action, are associated with the uncoupling of the L-arginine-NO pathway that results in the decreased formation of NO and increased eNOS-mediated generation of superoxide. NADPH oxidase is the initial source of ROS. Superoxide combines with NO, which is synthesized by eNOS, to form peroxynitrite.<sup>24</sup> In turn, peroxynitrite oxidizes and destabilizes eNOS to produce additional superoxide.<sup>22,25</sup> Superoxide also leads to BH<sub>4</sub> oxidation (in fact, BH<sub>4</sub> is highly sensitive to oxidation), which promotes eNOS uncoupling and further ROS production.

**Xanthine oxidase:** Xanthine oxidase is also an important source of ROS in the vascular endothelium.<sup>23,26</sup> It catalyzes the last two steps of

purine metabolism. During this process, oxygen is reduced to superoxide. There is evidence that suggests the involvement of this enzyme in hypertension. Spontaneously hypertensive rats demonstrated elevated levels of endothelial xanthine oxidase and increased ROS production, which is associated with increased arteriolar tone.<sup>21</sup> In addition to effects on the vasculature, xanthine oxidase may have a function in end-organ damage in hypertension.<sup>27</sup>

**Mitochondria:** Mitochondria are a major source and target of ROS. Some of the superoxide produced in the intermembrane space may be carried to the cytoplasm.<sup>28</sup> Ubiquinol or coenzyme Q produces superoxide when partially reduced (semiquinone form) and an antioxidant when fully reduced.<sup>29</sup> Complex I produces most of the superoxide that is generated by mammalian mitochondria *in vitro*. Complexes II and IV are not normally significant sites of ROS production. Mild uncoupling very effectively decreases the high superoxide production that occurs from complex I. A reduction in antioxidant enzymatic activity in patients with hypertension has been reported.<sup>30</sup>

### Role of the vascular wall components

The endothelium senses mechanical and hormonal stimuli. In response, it releases agents that regulate vasomotor function. There is no doubt that the endothelium has a regulatory and protective role by generating vasorelaxing substances. Under some pathophysiological circumstances, endothelium-derived vasoconstricting factors, such as ET-1, AT-II, urotensin II, superoxide anions, vasoconstrictor prostaglandins and thromboxane A<sub>2</sub>, can be released and contribute to the paradoxical vasoconstrictor effects.

VSMCs function not only in the short-term regulation of blood vessel diameter and, therefore, blood pressure but also participate in long-term adaptation via structural remodeling. ROS mediate many of these pathophysiological processes.

The adventitia can contribute to hypertension by either reducing NO bioavailability or participating in vascular remodeling through ROS.

**Role of vascular hormones and factors.** NO: NO has an important role as a key paracrine regulator of vascular tone. Physiologically, NO inhibits leukocyte-endothelial cell adhesion, VSMC proliferation and migration, and platelet aggregation to maintain the health of the vascular endothelium. Therefore, it has many beneficial effects. The decrease in bioavailability of NO in the vasculature reduces vasodilatory capacity and contributes to hypertension. The enzyme that catalyzes the formation of NO from oxygen and arginine is NOS, which, in fact, is an entire family of enzymes. eNOS is the predominant NOS isoform in the vessel wall. Receptor-mediated agonist stimulation leads to rapid enzyme activation. Furthermore, shear stress and allosteric modulators are important modulators of eNOS activity.<sup>31</sup> In addition to its vasorelaxing and antiproliferative roles, NO has an important role in antagonizing the effects of AT-II, endothelins and ROS. NO diffuses as a gas to the adjacent smooth muscle in which it interacts with different receptor molecules, such as the soluble guanylyl cyclase.

The normal production of NO has a crucial role in the maintenance of the physiological conditions within the cardiovascular system. L-arginine, which is a substrate for eNOS, seems to be a promising molecule in the preservation of NO formation. However, L-arginine failed to prevent blood pressure increases and left ventricle remodeling due to chronic treatment with methyl ester of N-nitro-L-arginine, which is an inhibitor of eNOS.<sup>32</sup> The ACE inhibitor captopril completely prevented NO-deficient hypertension without improving NOS activity. NO also has an ACE down-regulating effect. Thiols

protect NO from oxidation by scavenging ROS and by forming nitrosothiols; both of these effects prolong NO half-life and the duration of NO action.<sup>33,34</sup>

Reduced NO levels can be attributed to elevated levels of ROS. Superoxide combines with NO to form peroxynitrite that oxidizes BH<sub>4</sub> and destabilizes eNOS to produce more superoxide<sup>22,24,25</sup> thus, further enhancing the development of oxidative stress. The balance between NO and AT-II in the vasomotor centers is important for the regulation of sympathetic tone.

**Renin–Angiotensin system:** The renin–angiotensin system has a key role in the development of cardiovascular disease. AT-II is a potent vasoactive peptide that can be formed in vascular beds that are rich in ACE. When AT-II production increases above normal levels, it induces vascular remodeling and endothelial dysfunction in association with increases in the levels of blood pressure. As a potent activator of NADPH oxidase, AT-II contributes to the production of ROS.<sup>35,36</sup> In rats and mice in which hypertension is induced by AT-II infusion, the expression of NADPH oxidase subunits, oxidase activity and the generation of ROS are all increased.<sup>37</sup> AT-II not only increases NADPH oxidase activity but also upregulates superoxide dismutase activity, possibly to compensate the increased levels of ROS. In situations where this compensatory effect is efficient, ROS levels may appear normal even under prooxidant conditions. However, when ROS production becomes overwhelming, compensatory mechanisms are inadequate, and pathophysiological consequences occur.<sup>38</sup>

Captopril and enalapril prevented increases in blood pressure in young, spontaneously hypertensive rats by inhibiting ACE. Captopril, probably because of the antioxidant role of its thiol group, had a more effective hypotensive effect than enalapril.<sup>40</sup> In contrast, NO not only antagonizes the effects of AT-II on vascular tone, cell growth and renal sodium excretion but also downregulates the synthesis of ACE and the expression of AT<sub>1</sub> receptors. However, ACE inhibition upregulates eNOS expression. The ability of AT-II to induce endothelial dysfunction is also due to its ability to downregulate soluble guanylyl cyclase, thereby leading to impaired NO/cGMP signaling.

**Acetylcholine:** In vascular vessels, acetylcholine induces endothelium-dependent dilation via the production of endothelial factors, mainly NO, which then diffuses to the underlying VSMC, in which it induces VSMC relaxation. The decrease in NO bioavailability will lead to significantly reduced acetylcholine-mediated vasodilation.<sup>39,40</sup> The consequence of an overall increase in ROS is a reduction in the bioavailability of NO.

**ET-1:** Endothelins are potent vasoconstrictor isopeptides that are produced in different vascular tissues, including the vascular endothelium. ET-1 is the main endothelin generated by the endothelium and is the most important endothelin in the cardiovascular system. When ET-1 is administered in high concentrations, it behaves as a potent vasoconstrictor that is capable of exerting an array of physiological effects, including the potential to alter arterial pressure. ET-1 mediates its effects through two receptors, ET<sub>A</sub> and ET<sub>B</sub>. The ET<sub>A</sub> receptor mediates contractions via the activation of NADPH oxidase, xanthine oxidase, lipoxygenase, uncoupled NOS and mitochondrial respiratory chain enzymes. The ET<sub>B</sub> receptor induces relaxation in endothelial cells.<sup>41</sup> Many factors that normally stimulate ET-1 synthesis, (for example, thrombin and AT-II) also cause the release of vasodilators, such as prostacyclin (PGI<sub>2</sub>) and/or NO, which oppose the vasoconstricting function of ET-1. It has been reported that essential hypertension is characterized by increased ET-1-mediated vasoconstrictor tone, which is an effect that seems to be dependent on decreased endothelial ET<sub>B</sub>-mediated NO production that is attributable to the impaired NO bioavailability.

**Urotensin-II:** Urotensin-II is a potent vasoactive peptide<sup>42</sup> and indeed is the most potent identified vasoconstrictor. It functions through the activation of NADPH oxidase. The role of urotensin-II in disease is not well understood. The constrictor response to urotensin-II appears to be variable and highly dependent on the vascular bed examined. Vasoconstriction is not its only effect because UT receptors have been found in other organs.<sup>43,44</sup> Urotensin-II has also been shown to function as a potent vasodilator in some isolated vessels.<sup>45</sup>

**Norepinephrine:** VSMC are primarily innervated by the sympathetic nervous system through adrenergic receptors. Three types of adrenoceptors are present within VSMC namely:  $\alpha$ 1,  $\alpha$ 2 and  $\beta$ 2. Norepinephrine stimulates VSMC proliferation. In addition, over-expression of inducible NOS increases blood pressure via central activation of the sympathetic nervous system, which is mediated by an increase in oxidative stress.<sup>5</sup>

**Prostaglandins:** PGI<sub>2</sub>, which is another endothelium-dependent vasodilator, relaxes the VSMC. PGI<sub>2</sub> is released in higher amounts in response to the binding of ligands, such as thrombin, arachidonic acid, histamine or serotonin. The enzyme prostaglandin H<sub>2</sub> synthase uses arachidonic acid as a substrate to produce prostaglandin H<sub>2</sub>. Prostaglandin H<sub>2</sub> is converted to vasoactive molecules, such as PGI<sub>2</sub>. The enzyme isoform, prostaglandin H<sub>2</sub> synthase-2, may mediate vascular dysfunction under oxidative stress conditions. Thus, peroxynitrite inhibits the enzymatic activity of PGI<sub>2</sub> synthase, thereby impairing PGI<sub>2</sub>-mediated vasodilation.

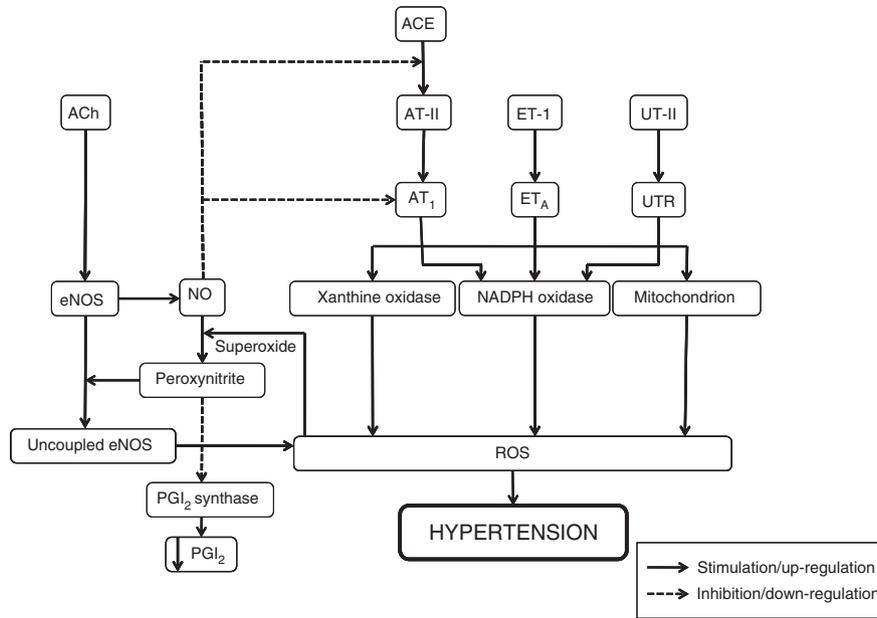
**Homocysteine:** This molecule may have an important role in the pathogenesis of essential hypertension.<sup>3</sup> Elevated homocysteinemia diminishes the vasodilation by NO, increases oxidative stress, stimulates the proliferation of VSMC and alters the elastic properties of the vascular wall. Thus, homocysteine contributes to the elevation of blood pressure.<sup>46</sup> Additionally, elevated homocysteine levels could lead to oxidant injury of the endothelium.<sup>3</sup> The correction of elevated homocysteinemia by the administration of the vitamins B12, B6 and folic acid could be a useful adjuvant therapy for hypertension.<sup>3,47</sup> However, further controlled randomized trials are necessary to establish the efficacy of these therapeutic agents.

A hypothesis for the role of vascular oxidative stress in hypertension is depicted in Figure 1.

This review has discussed the importance of ROS in the vasculature and its relation to hypertension; however, it is important to also emphasize the evidence that hypertensive stimuli, such as high salt and AT-II, promote the production of ROS not only in the vasculature but also in the kidney and the central nervous system. In addition, each of these sites contributes either to hypertension or to the adverse sequelae of this disease.<sup>47</sup>

### Role of oxidative stress in the kidney

Evidence suggests a key role for ROS in the pathophysiological processes of several renal diseases; these diseases are considered causes and consequences of hypertension. Regarding glomerular alterations, ROS mediate lipoprotein glomerulopathy and other inflammatory glomerular lesions.<sup>48</sup> A recent study demonstrated that NADPH oxidase activation and the production of ROS through lipid raft clustering is an important molecular mechanism that triggers homocysteine-mediated oxidative injury of podocytes. This injury may represent an early event that initiates glomerulosclerosis during hyperhomocysteinemia.<sup>49</sup> One of the underlying mechanisms of ROS-mediated tubulointerstitial injury is the exposure of tubular cells to LDL, which may result in tubulointerstitial damage due to NADPH oxidase-mediated ROS production.<sup>50</sup> AT-II has a pivotal role



**Figure 1** Schematic summary of the role of vascular oxidative stress in the pathogenesis of hypertension. ACE, angiotensin converting enzyme; AT-II, angiotensin II; AT<sub>1</sub>, type 1 angiotensin II receptor; ET-1, endothelin 1; ETA, type A endothelin receptor; UT-II, urotensin II; UTR, urotensin-II receptor; ACh, acetylcholine; NO, nitric oxide; eNOS, nitric oxide synthase; PGI<sub>2</sub>, prostacyclin; ROS, reactive oxygen species.

not only in the progression of tubulointerstitial injury but also in obstructive nephropathy.<sup>51,52</sup> It activates NADPH oxidase and, subsequently, generates superoxide that leads to hypertrophy of the renal tubular cells.<sup>53</sup>

There is evidence which suggests that a high-fat diet induces renal inflammation and aggravation of blood pressure via ROS in spontaneously hypertensive rats.<sup>54</sup> Additionally, metabolic syndrome is a risk factor for chronic kidney disease (CKD) that is at least in part independent of diabetes and hypertension and probably mediated by ROS. Moreover, the onset and maintenance of renal damage may worsen metabolic syndrome features, such as hypertension, leading to potential vicious cycles.<sup>55</sup>

There are several oxidative stress-mediated mechanisms involved in endothelial dysfunction in CKD.<sup>56</sup> ROS are elevated in CKD and are related to endothelium-dependent vascular reactivity and systolic blood pressure.<sup>57</sup> High ROS and increased levels of the endogenous asymmetric dimethylarginine were reported to be novel risk factors for endothelial dysfunction.<sup>58</sup> Moreover, high levels of asymmetric dimethylarginine were reported in CKD and were associated with increased intima-media thickness and cardiovascular events.<sup>59</sup> In renovascular hypertension, oxidative stress in the ischemic kidney has a major role in the maintenance of hypertension in two kidney-one clip rats.<sup>60</sup>

#### Role of oxidative stress in the central nervous system

In addition to the kidney and the vasculature itself, the sympathetic nervous system, which is regulated in the central nervous system, is involved in the pathogenesis of hypertension.<sup>61</sup> Recent studies strongly suggest that central sympathetic outflow is increased in hypertension.<sup>62</sup> There is also evidence that increased ROS generation in the brainstem contributes to the neural mechanisms of hypertension in hypertensive rats.<sup>63</sup>

The rostral ventrolateral medulla is the major vasomotor center and is essential for the maintenance of basal vasomotor tone.<sup>64,65</sup> Some findings strongly indicate that ROS in the rostral ventrolateral medulla

is increased in stroke-prone spontaneously hypertensive rats and thereby contributes to the neural mechanisms of hypertension through activation of the sympathetic nervous system.<sup>64</sup> The paraventricular nucleus of the hypothalamus is most likely also involved in the ROS-mediated neural mechanism of hypertension.<sup>60,66</sup> There is evidence that other regions of the brain are likewise involved in ROS-mediated hypertension. These investigations suggest that increased intracellular superoxide production in the subfornical organ is critical to the development of AT-II-induced hypertension.<sup>67</sup>

#### ANTIOXIDANTS IN HYPERTENSION

This section discusses the antihypertensive role of endogenous and exogenous antioxidants that have demonstrated an ability to alter the function of blood vessels and participate in the main redox reactions involved in the pathophysiology of hypertension.

##### Endogenous antioxidants

The main endogenous antioxidant enzyme systems involve superoxide dismutase, catalase and glutathione peroxidase (GSH-Px). GSH-Px reduces both hydrogen peroxide and organic hydroperoxides into water and alcohols, respectively.<sup>68,69</sup> Catalase catalyzes the breakdown of hydrogen peroxide to water and molecular oxygen. Superoxide dismutase is a major superoxide scavenger in humans and converts the superoxide anion to hydrogen peroxide, which is a substrate for catalase and GSH-Px.<sup>70</sup> The decrease in enzymatic antioxidant activity in hypertensive humans and in animal models of hypertension has been widely reported, which suggests that reduced antioxidant defenses are involved in this disease.<sup>71-76</sup>

Another endogenous antioxidant is glutathione, which displays direct antioxidant activity by providing the hydrogen at the sulfur atom to transform a free radical into a stable molecule.<sup>77</sup> An impairment of the glutathione system in hypertension has been demonstrated.<sup>78,79</sup> Recent studies have demonstrated the importance of the thioredoxin system in the maintenance of a reduced redox state and in the pathophysiology of hypertension.<sup>80</sup> It has been reported that

impaired thioredoxin expression in spontaneously hypertensive rats<sup>81</sup> and in endothelial cells negatively regulates ROS production in VSMC through thioredoxin upregulation.<sup>82</sup> Thus, thioredoxin could be an important therapeutic target for the prevention and treatment of oxidative stress and hypertension.<sup>83–85</sup>

Recent studies have shown that the antioxidant effects of mildly elevated serum bilirubin levels may protect against diseases that are associated with oxidative stress<sup>86</sup> through mechanisms that decrease oxidative stress and increase NO levels.<sup>87,88</sup> A mild increase within the physiological range of serum bilirubin concentration is negatively correlated with hypertension.<sup>89</sup> Finally, uric acid has long been recognized for its antioxidant properties and is the most abundant scavenger of free radicals in humans. High concentrations of uric acid are associated with increased serum antioxidant capacity and reduced oxidative stress.<sup>90,91</sup> However, there is evidence of a potential association between the plasma concentration of uric acid, blood pressure and cardiovascular risk.<sup>92–96</sup> This situation can be explained by the hyperactivity of xanthine oxidase found in hypertension.<sup>21,27</sup>

### Exogenous antioxidants

**Vitamin C.** Vitamin C is a potent water-soluble antioxidant. In the vascular wall, it functions as an enzyme modulator that upregulates eNOS and downregulates NADPH oxidase.<sup>97</sup> Most studies have demonstrated an inverse relationship between plasma ascorbate levels and blood pressure in both normotensive and hypertensive populations.<sup>15</sup> Treatment with antioxidants has been shown to improve vascular function and reduce blood pressure in animal models<sup>98,99</sup> and in human hypertension.<sup>100,101</sup> Vitamin C may have favorable effects on vascular dilation, possibly through its antioxidant effects on NO.<sup>102–104</sup>

Nevertheless, several small and short-term clinical trials on the effect of vitamin C supplements on blood pressure have yielded inconsistent findings.<sup>105–111</sup> The lack of antihypertensive efficacy observed in studies using supplementation with vitamin C alone could be due to the decreased bioavailability of NO under conditions of oxidative stress. It was shown that these effects are mediated in part by the ability of vitamin C to protect BH<sub>4</sub> from oxidation and thereby increase the enzymatic activity of eNOS.<sup>112</sup> In addition, this uncertain clinical beneficial effect of vitamin C as an antihypertensive agent could be due to the lack of consideration of its pharmacokinetic properties. It was experimentally determined that the antihypertensive effect of vitamin C is expected to occur at a concentration of 10 mM,<sup>103</sup> which is an unattainable plasma level in humans through oral administration; that concentration is required for vitamin C to compete efficiently with the reaction of NO with superoxide. The renal ascorbic acid threshold occurs at daily vitamin C doses between 60 and 100 mg. The plasma is completely saturated at doses of 400 mg daily and higher, producing a steady-state plasma concentration of approximately 80 μM.<sup>113</sup> Thus, the antihypertensive effect may only be active in plasma following high doses of vitamin C infusion.

**Vitamin E.** This major lipid-soluble antioxidant has received considerable attention for its antioxidant potential. Epidemiological data support an association between high dietary vitamin E intake and a reduced incidence of cardiovascular disease.<sup>56</sup> Increasing evidence indicates that vitamin E can function as a biological modifier independently of its antioxidant activity. Available experimental evidence shows that vitamin E is capable of dose-dependently regulating mitochondrial generation of superoxide and hydrogen peroxide.

However, intervention trials have not been convincing, and several studies have demonstrated no beneficial effect of vitamin E on

cardiovascular disease outcomes.<sup>114–117</sup> Moreover, a study using supplementation with vitamin E, either as α-tocopherol or mixed tocopherols, showed a significant increase in blood pressure, pulse pressure and heart rate in individuals with type 2 diabetes.<sup>118</sup> It should be noted that sufficiently high concentrations in the vascular microenvironment that would enable vitamin E to interfere effectively with all components of oxidative stress is unlikely to be achieved.<sup>119</sup>

**Association of Vitamins C and E.** Ascorbic acid may reduce the α-tocopheroxyl radical and may be required for the beneficial vascular effects of α-tocopherol.<sup>120</sup> In fact, the effect of α-tocopherol seems to be dependent on tissue saturation with vitamin C, and both vitamins may function synergistically to provide optimal conditions for endothelial NO formation.<sup>121</sup> Thus, the association of vitamins C and E is expected to have an antihypertensive effect, probably because this combined therapy provides a reinforcement of their individual properties through a complementary effect.<sup>122</sup>

Despite the biological effects of both vitamin C and E, long-term clinical trials have failed to consistently support their antihypertensive effects in high-risk cardiovascular patients. Some short-term trials have shown that supplemental antioxidant vitamin intake lowers blood pressure,<sup>106,123–125</sup> but the majority of long-term clinical trials did not demonstrate any antihypertensive effects of antioxidant vitamins. However, most of these studies lacked rigorous exclusion criteria in the selection of subjects to avoid the influence of confounders.<sup>126</sup> It should be noted that most of the subjects in cohorts of large trials had irreversible cardiovascular disease. A study performed with newly diagnosed hypertension, without end-organ damage, showed an association between oxidative-stress-related parameters and blood pressure thus, suggesting a role for oxidative stress in the pathogenesis of essential hypertension.<sup>127,128</sup> The available data suggest that there might be a beneficial antihypertensive effect of vitamins C and E if they are administered during the phase of endothelial dysfunction, which precedes established vascular damage. In contrast, an antihypertensive effect in patients with significant cardiovascular disease should not be expected because the chronic damaging effects of oxidative stress may be irreversible. Thus, it seems that ROS have a more important role in the induction rather than in the maintenance of hypertension.

**Allopurinol.** Xanthine oxidase is an important source of ROS in the vascular endothelium.<sup>24</sup> It catalyzes the last two steps of purine metabolism and produces uric acid. Xanthine oxidase activity is associated with an increasing arteriolar tone and, therefore, hypertension.<sup>129,130</sup> Xanthine oxidase inhibitors, such as allopurinol, have shown marked improvements in endothelial function in various cohorts that were at risk for cardiovascular events. Treatment with allopurinol results in the reduction in blood pressure in adolescents,<sup>92</sup> spontaneously hypertensive rats<sup>93</sup> and patients with CKD.<sup>131</sup> Nevertheless, most of the evidence gathered to date was derived from smaller mechanistic studies, and the few large randomized controlled trials have not shown a significant mortality benefit of using these agents.<sup>132</sup>

**Selenium.** Selenium is an essential trace element and an integral part of many proteins with catalytic and structural functions. It exerts an antioxidant function mainly in the form of selenocysteine residues, which are an integral constituent of ROS-detoxifying selenoenzymes, such as GSH-Px, thioredoxin reductases and selenoprotein P.<sup>133</sup> Maintenance of full GSH-Px and thioredoxin reductase activity by adequate dietary intake of selenium has been proposed to be useful in the prevention of several cardiovascular disorders.<sup>112</sup> In addition,

selenium is capable of preventing the binding of nuclear factor kappa B to its nuclear response elements in DNA.<sup>134</sup> Nuclear factor kappa B has a key role in inflammation and the production of ROS. The inhibition of nuclear factor kappa B is presumed to be the result of the binding of the selenium to the essential thiols of this transcription factor.<sup>135</sup>

The antioxidant properties of selenium have been documented in several trials.<sup>134,136–141</sup> At low doses, selenium can provide significant protection of the human coronary artery endothelium against oxidative stress-mediated damage.<sup>133</sup> In an animal model, dietary

**Table 1 Mechanisms whereby exogenous antioxidants can ameliorate hypertension**

Antioxidant	Mechanisms	References
Vitamin C	eNOS upregulation	50
	NADPH oxidase downregulation	50
	Protection against BH <sub>4</sub> oxidation	33
	ROS scavenging	50,64,66,67
Vitamin E	eNOS upregulation	50
	NADPH oxidase downregulation	50
	Regulation of mitochondrial ROS synthesis	57–60
	ROS scavenging	50,64,66,67
N-acetylcysteine	Protection against BH <sub>4</sub> oxidation	100
	ROS scavenging	101,102
Polyphenols	Production of NO	104,105
	ROS scavenging	65,103
	Iron chelation	65,103
	Enzyme modulation	65,103
Allopurinol	Inhibition of xanthine oxidase	75–77
Selenium	Cofactor of GSH-Px, TR and SeP	55,86
	Inhibition of NF-κB activation	88
Diet		108,109,129,131

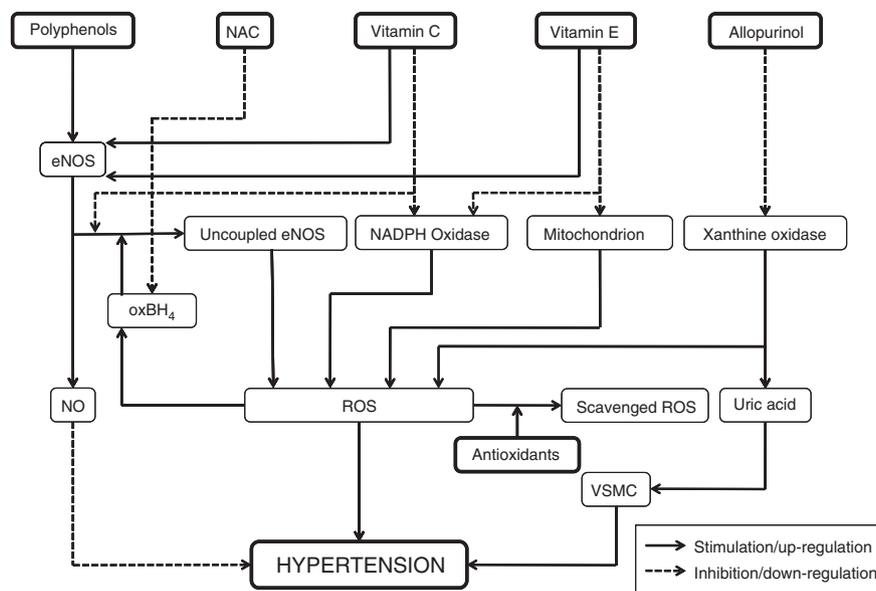
Abbreviations: BH<sub>4</sub>, tetrahydrobiopterin; eNOS, endothelial nitric oxide synthase; GSH-Px: glutathione peroxidase; NADPH, nicotinamide adenine dinucleotide phosphate; NF-κB, nuclear factor kappa-B; NO, nitric oxide; ROS: reactive oxygen species; SeP: selenoprotein P; TR: thioredoxin reductases.

supplementation with selenium was associated with low levels of cardiac oxidative damage and increased antioxidant expression, as well as a reduction in disease severity and mortality in spontaneously hypertensive rats.<sup>142</sup> A reduced selenium concentration in hypertensive pregnancies has been associated with a decrease in GSH-Px activity.<sup>143</sup> Thus, it is reasonable to conclude that a deficiency in selenium might be an underestimated risk factor for the development of high blood pressure.<sup>144</sup>

*N-acetylcysteine.* The antioxidant *N*-acetylcysteine, which is a sulfhydryl group donor, improves renal dysfunction and decreases arterial pressure and renal injury in salt-sensitive hypertension.<sup>145</sup> The inhibition of oxidative stress in hypertensive states probably contributes to the therapeutic effects of *N*-acetylcysteine, which are likely mediated via a NO-dependent mechanism.<sup>146</sup> This protective mechanism is exerted by the prevention of BH<sub>4</sub> oxidation by the increased superoxide.<sup>147</sup> In addition, this molecule can protect against oxidative damage by inhibiting lipid peroxidation and scavenging ROS.<sup>148,149</sup>

*Polyphenols.* Polyphenols are the most abundant antioxidants in the human diet. They can function as ROS scavengers, iron chelators and enzyme modulators<sup>150,151</sup> and may possibly enhance the production of NO.<sup>152,153</sup> In humans, circulating NO concentration increases after the consumption of polyphenols.<sup>154</sup> However, some studies have shown increased blood pressure that is mediated by the association of polyphenols with vitamin C.<sup>155</sup>

*Diet.* There is sufficient evidence to suggest that dietary approaches may help to prevent and control high blood pressure. There are dietary approaches to the prevention and management of hypertension, that is, the moderate use of sodium and alcohol, as well as the increased intake of potassium, plant fibers, calcium and foods such as salmon, nuts and wine.<sup>156</sup> In a Mediterranean population with an elevated fat consumption, a high fruit and vegetable intake is inversely associated with blood pressure levels.<sup>157</sup> Short-term studies indicated that specialized diets may prevent or ameliorate mild hypertension; most notable are the Dietary Approaches to Stop Hypertension



**Figure 2** Schematic summary of the exogenous antioxidant mechanisms likely accounting for anti-hypertensive effects. NO, nitric oxide; eNOS, nitric oxide synthase; ROS, reactive oxygen species; BH<sub>4</sub>, tetrahydrobiopterin; oxBH<sub>4</sub>, oxidized tetrahydrobiopterin; NAC, *N*-acetylcysteine; VSMC, vascular smooth muscle cells.

(DASH) diet, which is high in fruits, vegetables and low-fat dairy products.<sup>158</sup> It has been reported that a low sodium DASH diet is effective in reducing blood pressure in hypertensive patients, particularly in those receiving antihypertensive medications.<sup>159</sup> In addition, the DASH diet showed significant beneficial effects on cardiovascular risk.<sup>160–162</sup> In overweight or obese persons with above-normal blood pressure, the addition of exercise and weight loss to the DASH diet resulted in even larger blood pressure reductions, greater improvements in vascular and autonomic function and reduced left ventricular mass.<sup>163,164</sup>

A summary of the exogenous antioxidant mechanisms that likely account for their anti-hypertensive effects is shown in Table 1 and Figure 2.

## CONCLUSIONS AND PERSPECTIVES

There is considerable evidence supporting the view that oxidative stress is involved in the pathophysiology of hypertension. ROS are mediators of the major physiological vasoconstrictors that increase intracellular calcium concentration. In addition, superoxide reduces the bioavailability of NO and enhances superoxide production via uncoupled eNOS; this further enhances oxidative stress, which is a major factor in hypertension.

Antioxidant therapy can curtail the development of hypertension in animal models, but remains controversial in humans. Possible confounding factors in patients include co-existing pathologies and treatments, lack of selection of treatments according to ROS levels, among others. However, the dietary intake of antioxidants and polyphenols could have an effect in the primary prevention or reduction of hypertension. Although the role of antioxidant therapy remains an open question, these interventions are expected to be more efficient in the prevention rather than in the reduction of established hypertension.

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