NADPH Oxidases, Reactive Oxygen Species, and Hypertension

Clinical implications and therapeutic possibilities

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Reactive oxygen species (ROS) influence many physiological processes including host defense, hormone biosynthesis, fertilization, and cellular signaling. Increased ROS production (termed “oxidative stress”) has been implicated in various pathologies, including hypertension, atherosclerosis, diabetes, and chronic kidney disease. A major source for vascular and renal ROS is a family of nonphagocytic NAD(P)H oxidases, including the prototypic Nox2 homolog-based NAD(P)H oxidase, as well as other NAD(P)H oxidases, such as Nox1 and Nox4. Other possible sources include mitochondrial electron transport enzymes, xanthine oxidase, cyclooxygenase, lipoxygenase, and uncoupled nitric oxide synthase. NAD(P)H oxidase-derived ROS plays a physiological role in the regulation of endothelial function and vascular tone and a pathophysiological role in endothelial dysfunction, inflammation, hypertrophy, apoptosis, migration, fibrosis, angiogenesis, and rarefaction, important processes underlying cardiovascular and renal modeling in hypertension and diabetes. These findings have evoked considerable interest because of the possibilities that therapies against nonphagocytic NAD(P)H oxidase to decrease ROS generation and/or strategies to increase nitric oxide (NO) availability and antioxidants may be useful in minimizing vascular injury and renal dysfunction and thereby prevent or regress target organ damage associated with hypertension and diabetes. Here we highlight current developments in the field of reactive oxygen species and cardiovascular disease, focusing specifically on the recently identified novel Nox family of NAD(P)H oxidases in hypertension. We also discuss the potential role of targeting ROS as a therapeutic possibility in the management of hypertension and cardiovascular disease.

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Abbreviations: NOS, nitric oxide synthase; ROS, reactive oxygen species; SHR, spontaneously hypertensive rat; SOD, superoxide dismutase.

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PRODUCTION AND METABOLISM OF ROS — ROS are produced by all vascular cell types, including endothelial, smooth muscle, and adventitial cells, and can be formed by numerous enzymes. Enzymatic sources of ROS that are important in vascular disease and hypertension are xanthine oxidase, uncoupled nitric oxide synthase (NOS), and NAD(P)H oxidase (Fig. 1).

Xanthine oxidase, which catalyzes the oxidation of hypoxanthine and xanthine to form \( \text{O}_2^- \), is present in the vascular endothelium (17). Although xanthine oxidase–derived \( \text{O}_2^- \) has been studied mainly in the context of cardiac disease, there is evidence suggesting involvement in vascular dysfunction in hypertension. Spontaneously hypertensive rats (SHRs) demonstrate elevated levels of endothelial xanthine oxidase and increased ROS production, which are associated with increased arteriolar tone (18). This may be mediated in part through an adrenal pathway, because adrenalectomy reduces xanthine oxidase expression (19). Endothelial dysfunction in transgenic rats with overexpression of renin and angiotensinogen has also been associated with increased xanthine oxidase activity (20). In addition to effects on the vasculature, xanthine oxidase may play a role in end-organ damage in hypertension. In experimental models of hypertension, xanthine oxidase activity is increased in the kidney. In SHRs, long-term inhibition of xanthine oxidase with allopurinol reduced renal xanthine oxidase activity without lowering blood pressure, indicating that the increased renal ROS production was a consequence of hypertension rather than a contributing factor (21). The finding that allopurinol can improve cardiac and renal hypertrophy in SHRs and slow the progression of renal disease in patients with chronic kidney disease and hypertension (22), while having a minimal impact on blood pressure (23), supports a role for xanthine oxidase in hypertensive end-organ damage rather than in the development of hypertension per se. This may be mediated through direct vascular effects of xanthine oxidase–produced uric acid (24).

NOS can also contribute to ROS production, since all three NOS isoforms have been shown to be susceptible to the “uncoupling” that leads to the formation of \( \text{O}_2^- \) (rather than NO) (25). For endothelial NOS, this process is triggered in vitro through the absence of the cofactors L-arginine and tetrahydrobiopterin (26). Uncoupling of endothelial NOS has been demonstrated in deoxycorticosterone acetate (DOCA)-salt–induced hypertension and in SHRs (27,28). Treatment with tetrahydrobiopterin improves blood pressure in both DOCA-salt hypertension and SHRs (27,28). Whether uncoupled NOS effects are due to changes in production of \( \text{O}_2^- \) or NO remain unclear. To address this, blood pressure and endothelial function in mice with endothelium-targeted transgenic eNOS overexpression (eNOS-Tg) were compared with littermates in which eNOS coupling was rescued by additional endothelium-targeted overexpression of GTP cyclohydrolase 1 (eNOS/GCH-Tg) to increase endothelial BH4 levels (29). Blood pressure was equally reduced in both genotypes, compared with wild-type animals. Furthermore, both eNOS-Tg and eNOS/GCH-Tg mice exhibited similarly impaired endothelial-dependent vasorelaxation, demonstrating that reduced vasorelaxation responses result from desensitization of cGMP-mediated signaling and are associated with increased NO production rather than changes in superoxide production (29). However, others have demonstrated that vascular effects of eNOS uncoupling are due to enhanced \( \text{O}_2^- \) production. Increased vascular ROS itself may induce eNOS uncoupling as a consequence of increased oxidation of tetrahydrobiopterin and inhibition of dimethylarginine dimethylaminohydrolase (30). In fact, NAD(P)H oxidase has been shown to cause endothelial NOS uncoupling and to promote xanthine oxidase–dependent superoxide production (31).

NAD(P)H OXIDASE — NAD(P)H oxidase is a multi-subunit enzyme that catalyzes \( \text{O}_2^- \) production by the 1-electron reduction of \( \text{O}_2 \) using NADPH or NADH [hence the parentheses in NAD(P)H] as the electron donor: \( 2\text{O}_2 + \text{NAD(P)H} \rightarrow 2\text{O}_2^- + \text{NAD(P)}^+ + \text{H}^+ \). The prototypical NAD(P)H oxidase is that found in neutrophils and has five subunits: p47phox ("phox" stands for phagocyte oxidase), p67phox, p40phox, p22phox, and the catalytic subunit gp91phox (also termed "Nox2") (32,33).

In unstimulated cells, p47phox, p67phox, p40phox, and p22phox exist in the cytosol, whereas p22phox and gp91phox are in the membrane, where they occur as a heterodimeric flavoprotein, cytochrome b558. On stimulation, p47phox becomes phosphorylated and the cytosolic subunits form a complex that translocates to the membrane, where it associates with cytochrome b558 to assemble the active oxidase, which transfers electrons from the substrate to \( \text{O}_2 \), forming \( \text{O}_2^- \) (34). Activation also requires participation of Rac 2 (or Rac 1) and Rap 1A (35).

Although NAD(P)H oxidases were originally considered as enzymes expressed only in phagocytic cells involved in host defense and innate immunity, re-
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cent evidence indicates that there is an entire family of NAD(P)H oxidases, based on the discovery of gp91phox homologs (36,37). The new homologs, along with gp91phox, are now designated the Nox family of NAD(P)H oxidases. The family comprises seven members, including Nox1, Nox2 (formerly termed “gp91phox”), Nox3, Nox4, Nox5, Duox1, and Duox2 (38). They are expressed in many tissues and mediate diverse biological functions. Nox1 is found in colon and vascular cells and plays a role in host defense and cell growth; Nox2 is the catalytic subunit of the respiratory burst oxidase in phagocytes, but is also expressed in vascular, cardiac, renal, and neural cells; Nox3 is found in fetal tissue and the adult inner ear and is involved in vestibular function; Nox4, originally termed “Renoxx” (renox oxidase), because of its abundance in the kidney, is also found in vascular cells and osteoclasts; and Nox5 is a Ca2+-dependent homolog, found in testis and lymphoid tissue, but also in vascular cells. Duox1 and -2 are thyroid Noxes involved in thyroid hormone biosynthesis. While all Nox proteins are present in rodents and humans, the mouse and rat genome does not contain the nox5 gene. The regulation and function of each Nox remains unclear, but it is evident that Nox enzymes are critical for normal biological responses and that they contribute to cardiovascular and renal disease, including hypertension and atherosclerosis.

**REGULATION OF NAD(P)H OXIDASE ACTIVITY** — How the NAD(P)H oxidase subunits interact in cardiovascular cells and how they generate O2 is not fully known. All Noxes appear to have an obligatory need for p22phox (39,40). Whereas Nox2 requires p47phox and p67phox for its activity, Nox1 may interact with the recently identified homologs of p47phox and p67phox, namely NAD(P)H oxidase organizer 1 (NOXO1) and NAD(P)H oxidase activator 1 (NOX1A1), respectively (41,42). Vascular NAD(P)H oxidase is responsive to several growth factors (platelet-derived growth factor, epidermal growth factor, and transforming growth factor β), cytokines (tumor necrosis factor-α, interleukin-1, and platelet aggregation factor), mechanical forces (cyclic stretch, laminar, and oscillatory shear stress), and metabolic factors (hyperglycemia, hyperinsulinenia, free fatty acids, advanced glycation end products, and G protein–coupled receptor agonists (serotonin, thrombin, bradykinin, endothelin, and Ang II) (43–47). Ang II, via AT1 receptors, is an important and potent regulator of cardiovascular NAD(P)H oxidase that activates NAD(P)H oxidase through stimulation of signaling pathways involving c-Src p21ras, protein kinase C, phospholipase D, and phospholipase A2 (48–50). Ang II also influences NAD(P)H oxidase activation through transcriptional regulation of oxidase subunits (51).

**ANTIOXIDANT DEFENSES** — Antioxidants are defined as substances that, when present at low concentrations relative to an oxidizable substrate, significantly delay or prevent oxidation of that substrate. Living organisms have evolved a number of antioxidant defenses to maintain their survival against oxidative stress. These mechanisms are different in the intracellular and extracellular compartments and comprise enzymatic and nonenzymatic types. The major vascular enzymatic antioxidants are SOD, catalase, and glutathione peroxidase (52–54). SOD catalyzes the dismutation of O2 into H2O2 and O2. Of the three SOD isoforms, extracellular SOD is the main vascular SOD. It is produced and secreted by vascular smooth muscle cells and binds to glycosaminoglycans in the vascular extracellular matrix on the endothelial cell surface and plays an important role in the regulation of the oxidant status in the vascular interstitium (55). Reduced glutathione plays a major role in the regulation of the intracellular redox state of vascular cells by providing reducing equivalents for many biochemical pathways (56). Glutathione peroxidase reduces H2O2 and lipid peroxides to water and lipid alcohols, respectively, and in turn oxidizes glutathione to glutathione disulfide. The glutathione peroxidase/glutathione system may be important in low-level oxidative stress. Catalase is an intracellular antioxidant enzyme that is mainly located in cellular peroxisomes and to some extent in the cytosol, which catalyzes the reaction of H2O2 to water and molecular oxygen (57). Catalase is very effective in high-level oxidative stress and protects cells from H2O2 produced within the cell. The enzyme is especially important in the case of limited glutathione content or reduced glutathione peroxidase activity. Thioredoxin reductase is an antioxidant enzyme that participates in thiol-dependent cellular reductive processes (58). Numerous nonspecific antioxidants, such as α-tocopherol (vitamin E) and ascorbic acid (vitamin C), scavenge OH· as well as other radicals (59). Low antioxidant bioavailability promotes cellular oxidative stress and has been implicated in oxidative damage associated with hypertension (53).

**ROLE OF NAD(P)H OXIDASE-DERIVED ROS IN VASCULAR BIOLOGY** — ROS influence vascular cell growth, migration, proliferation, and activation (56,57). Physiologically, NAD(P)H oxidase–derived ROS have been implicated in the regulation of vascular tone by modulating vasodilation directly (H2O2 may have vasodilator actions) or indirectly by decreasing NO bioavailability through quenching by O2·− to form ONOO− (58,59). ROS, through the regulation of hypoxia-inducible factor 1 (HIF-1), are also important in O2 sensing (60), which is essential for maintaining normal O2 homeostasis. In pathological conditions, ROS are involved in inflammation, endothelial dysfunction, cell proliferation, migration and activation, extracellular matrix deposition, fibrosis, angiogenesis, and cardiovascular remodeling, important processes contributing to cardiovascular and renal remodeling in hypertension, atherosclerosis, diabetes, cardiac failure, and myocardial ischemia-reperfusion injury (61,62) (Fig. 2). These effects are mediated through redox-sensitive regulation of multiple signaling molecules and second messengers including mitogen-activated protein kinases, protein tyrosine phosphatases, tyrosine kinases, proinflammatory genes, ion channels, and Ca2+ (63–65).

**NAD(P)H OXIDASE, OXIDATIVE STRESS, AND HYPERTENSION**

Oxidative stress in experimental models of hypertension

The relationship between oxidative stress and increased blood pressure has been demonstrated in many models of experimental hypertension. Increased ROS formation precedes development of hypertension in SHRs, suggesting that ROS participate in the development and maintenance of hypertension (67,68). Markers of oxidative stress, such as thiobarbituric acid reactive substances and F2-isoprostanes, tissue concentrations of ·O2− and H2O2, and activation of NAD(P)H oxidase and xanthine oxidase,
Ang II–dependent hypertension is particularly sensitive to NAD(P)H oxidase–derived ROS. In rats and mice made hypertensive by Ang II infusion, expression of NAD(P)H oxidase subunits (Nox1, Nox2, Nox4, p22phox), oxidative activity, and generation of ROS are increased (72–75). To support a role for oxidative activity, and generation of ROS are increased, whereas levels of NO and antioxidant enzymes are reduced in experimental hypertension (69–71).

Ang II–dependent hypertension is particularly sensitive to NAD(P)H oxidase–derived ROS. In rats and mice made hypertensive by Ang II infusion, expression of NAD(P)H oxidase subunits (Nox1, Nox2, Nox4, p22phox), oxidative activity, and generation of ROS are increased (72–75). To support a role for NAD(P)H oxidase–derived ROS production in the pathogenesis of Ang II–sensitive hypertension, various mouse models with altered NAD(P)H oxidase subunit expression have been studied. In p47phox knockout mice and in gp91phox knockout mice, Ang II infusion fails to induce hypertension, and these animals do not show the same increases in \( \cdot O_2^- \) production, vascular hypertrophy, and endothelial dysfunction observed in Ang II–infused wild-type mice (75–77). In Ang II–infused mice treated with siRNA targeted to renal p22phox, renal NAD(P)H oxidase activity was blunted, ROS formation was reduced, and blood pressure elevation was attenuated, suggesting that p22phox is required for Ang II–induced oxidative stress and hypertension (78). On the other hand, overexpression of vascular p22phox was associated with increased oxidative stress and vascular dysfunction but no significant increase in blood pressure (79). Treatment with apocynin or diphenylethylammonium, pharmacological inhibitors of NAD(P)H oxidase, or gp91ds-tat, a novel specific inhibitor of NAD(P)H oxidase, reduced vascular \( \cdot O_2^- \) production, prevented cardiovascular remodeling, and attenuated development of hypertension in Ang II–treated mice (74,80,81). In most of these models, Ang II was infused for a short time period (1–3 weeks), inducing an acute hypertensive response. In a model of chronic Ang II–dependent hypertension, where we crossed transgenic mice expressing human renin (which exhibit an Ang II–sensitive hypertensive phenotype) with Nox2–/– mice, development of hypertension was not prevented even though oxidative stress was reduced, suggesting that other Nox homologs, such as Nox1, may be important (82). To support this, recent studies in Nox1-deficient mice demonstrated that vascular \( \cdot O_2^- \) production is reduced and blood pressure elevation in response to Ang II is blunted (83,84), whereas in transgenic mice in which Nox1 is overexpressed in the vascular wall, Ang II–mediated vascular hypertrophy and blood pressure elevation are enhanced (85).

There is also evidence for ROS involvement in the pathogenesis of hypertension independent of direct Ang II actions. In SHR, vascular, renal, and cardiac \( \cdot O_2^- \) production is increased compared with normotensive control subjects (15,86,87). In stroke-prone SHRs, aortic expression of Nox1 and Nox4 is significantly increased compared with Wistar-Kyoto (88). In DOCA salt-induced mineralocorticoid hypertension, vascular \( \cdot O_2^- \) production involving elevated NAD(P)H oxidase activity, uncoupling of endothelial NOS, and mitochondrial sources, in part through the endothelin-1/\( \alpha \) receptor pathway, is increased (27,89–92). Infusion of endothelin-1 increases NAD(P)H oxidase-dependent \( \cdot O_2^- \) production; however, preventing this increase in ROS generation does not inhibit development of hypertension in these animals (93). Overexpression of human endothelin-1 in mice also induces vascular remodeling and impairs endothelial function, via activation of NAD(P)H oxidase (94).

To further support a role for oxidative stress in hypertension, many studies have shown that treatment with antioxidant vitamins and superoxide dismutase mimetics, such as tempol (4-hydroxy-2,2,6,6-tetramethyl piperidinyl), free radical scavengers, or tetrahydrobiopterin (BH₄), attenuates or prevents development of hypertension and associated target organ damage (27,28,68,95).

**Oxidative stress and human hypertension**

Although studies in humans have not been as convincing as those in experimental models, there is evidence that oxidative stress is increased in patients with essential hypertension, renovascular hypertension, malignant hypertension, salt-sensitive hypertension, cyclosporine-induced hypertension, and preeclampsia (96–100) (Table 1). These findings are based, in general, on increased levels of plasma thiobarbituric acid reactive substances and 8-epi-isoprostanes, biomarkers of lipid peroxidation and oxidative stress (96–101). Polymorphonuclear leukocyte- and platelet-derived \( \cdot O_2^- \), which also participates in vascular oxidative stress and inflammation, is increased in hypertensive patients (102,103).

Hypertensive patients exhibit a significantly higher production of plasma \( H_2O_2 \).
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Table 1—Evidence supporting a role for oxidative stress in human hypertension

| Phenomenon                                                                 |
|---------------------------------------------------------------------------|
| Increased plasma and urine levels of markers of oxidative stress (e.g., TBARS, isoprostanes) |
| Increased vascular generation of superoxide anion                         |
| Decreased plasma levels of antioxidant vitamins                           |
| Inverse association between plasma ascorbate levels and blood pressure in epidemiological studies |
| Blood pressure–lowering effect of vitamin C in small clinical studies     |
| Antihypertensive drugs reduce ROS production and decrease oxidative stress by inhibiting activation of NADPH oxidase and through intrinsic antioxidant properties |

than normotensive subjects (104). Additionally, normotensive subjects with a genetic risk of hypertension (positive family history of hypertension) have greater \( \text{H}_2\text{O}_2 \) production than blood pressure–matched normotensive subjects without a family history of hypertension, suggesting that there may be a genetic component that leads to elevated production of hydrogen peroxide (104,105). Lacy et al. (104) determined familial correlations for \( \text{H}_2\text{O}_2 \) production as a quantitative trait in a family-based cohort of hypertensive subjects and used these results to estimate the heritability of this trait. Heritability estimates revealed that approximately 20–35% of the observed variance in \( \text{H}_2\text{O}_2 \) production could be attributed to genetic factors, suggesting an important heritable component to the overall determination of this trait.

We reported that production of ROS is increased in vascular smooth muscle cells from resistance arteries of hypertensive patients and that this is associated with upregulation of vascular NAD(P)H oxidase (106,107). The importance of this oxidase in oxidative stress in human cardiovascular disease is supported by studies from Zalba et al. (108), who demonstrated that polymorphisms in NAD(P)H oxidase subunits are associated with increased atherosclerosis and hypertension. In particular, the \(-930(A/G)\) polymorphism in the p22phox promoter may be a novel genetic marker associated with hypertension. The C242T CYBA polymorphism is associated with essential hypertension, and hypertensive patients carrying the CC genotype of this polymorphism exhibit features of NAD(P)H oxidase–mediated oxidative stress and endothelial damage (109). In a Japanese population, the G(−930)A polymorphism of CYBA was confirmed to be important in the pathogenesis of hypertension (110).

Obesity is a major contributing factor to the development of hypertension and subsequent cardiovascular pathology (111). Data from the Framingham Heart Study, a large community-based cohort, showed a positive correlation between obesity and oxidative stress, as assessed by urinary levels of 8-epi-isoprostanes (112). Similar correlations between indexes of obesity (BMI, waist-to-hip ratio) and systemic oxidative stress have also been found in other populations (113). In nondiabetic obese subjects, 4 weeks of dietary restriction in obese subjects reduces both ROS generation by inflammatory cells and markers of systemic oxidative stress without altering plasma levels of antioxidant vitamins (114). Indeed, nutrition may act as a modulator of ROS generation, since fasting causes an acute reduction in ROS production from leukocytes from normal subjects (113), whereas glucose challenge increases ROS production from leukocytes (116). Furthermore, in conditions such as obesity and type 2 diabetes, insulin resistance may also contribute to oxidative stress. In obese nondiabetic patients, administration of insulin suppressed both ROS production and plasma levels of plasminogen-activated inhibitor 1 and intercellular adhesion molecule 1, suggesting an acute anti-inflammatory action of this hormone (117). Similar anti-inflammatory and fibrinolytic actions have also been demonstrated in patients with acute myocardial infarction who received insulin infusion (118).

In addition to excess ROS generation, decreased antioxidant defense mechanisms contribute to oxidative stress in patients with hypertension. Hypertensive patients have reduced activity and decreased content of antioxidant enzymes, including SOD, glutathione peroxidase, and catalase (68,71,119,120). Decreased levels of antioxidant vitamins A, C, and E have been demonstrated in newly diagnosed untreated hypertensive patients compared with normotensive control subjects (120). Moreover, SOD activity has been demonstrated to correlate inversely with blood pressure in patients with hypertension (120).

Therapeutic potential of reducing ROS in human hypertension

Based on experimental evidence of the importance of oxidative stress in vascular damage, there has been enormous interest in developing strategies that target ROS in the treatment of hypertension and other cardiovascular diseases. Therapeutic approaches that have been considered include mechanisms 1) to increase antioxidant capacity, 2) to increase NO bioavailability, and 3) to reduce ROS generation by decreasing activity of \( \text{O}_2^{-} \)-generating enzymes (121). Gene therapy targeting antioxidant systems, such as NOS and hypoxia-inducible factor 1 (HIF-1) (122,123), are also being developed, but their use in clinical hypertension remains unclear.

The potential of antioxidants in treating conditions associated with oxidative stress is supported by experimental investigations, observational findings, small clinical studies, and epidemiological data (124,125). However, findings are inconsistent and clinical trial data are inconclusive. Many large trials have been published regarding antioxidant vitamin effects on risks of cardiovascular disease, including the Cambridge Heart Antioxidant Study (2,002 patients); the Alpha Tocopherol, β-Carotene Cancer Prevention Study (27,271 males); the Group Italiano per lo Studio della Sopravvenza nell’Infarto Miocardico Prevenzione trial (3,658 patients); the Heart Outcomes Prevention Evaluation (HOPE) study (2,545 subjects); the Medical Research Council/British Heart Foundation (MRC/ BHF) Heart Protection Study (20,536 adults); the Primary Prevention Project (4,495 patients); and the Antioxidant Supplementation in Atherosclerosis Prevention (ASAP) study (520 subjects) (126,127). Except for the ASAP study, which demonstrated that 6-year supplementation of daily vitamin E and slow-release vitamin C reduced progression of carotid atherosclerosis, the other studies failed to demonstrate significant beneficial effects of antioxidants on blood pressure or on cardiovascular endpoints. Thus, overall results of clinical trials have been negative.

Unlike the large trials, smaller clinical studies have shown positive responses in hypertensive patients treated with antioxidants, either in combination (zinc,
ascorbic acid, α-tocopherol, β-carotene) of as monotherapy (vitamin C or vitamin E). This has been especially true for vitamin C. Most studies demonstrated an inverse relationship between plasma ascorbate levels and blood pressure in both normotensive and hypertensive populations (68,71). In the Supplementation en Vitamines et Minéraux Antioxydants study, a decreasing trend was observed with vitamin C levels and risk of hypertension in women but not in men (128). Vitamin C supplementation is associated with reduced blood pressure in hypertensive patients, with systolic blood pressure falling by 3.6–17.8 mmHg for each 50 μmol/l increase in plasma ascorbate (71,129,130). However, in a recent study, Ward et al. (131) found that 6-week treatment with vitamin C and grape seed polyphenols was associated with a paradoxical increase in ambulatory blood pressure in hypertensive patients. This was not attributed to increased oxidative stress.

Human studies of vitamin E doses of 400–1,000 IU/day have shown beneficial effects on improving insulin sensitivity, lowering serum glucose levels, increasing intracellular Mg2+, inhibiting thromboxane effects, and reducing vascular resistance (68,71,132). Data from the 1946 British Birth Cohort reported that low vitamin E intake during childhood and adulthood was a good predictor of hypertension at age 43 years (133). However, reductions in blood pressure in hypertensive subjects treated with vitamin E have been inconsistent (68,71). Similar trends have been observed in preeclampsia, where early studies suggested that vitamins C and E may prevent preeclampsia in high-risk patients (134,135), whereas more recent evidence indicates that supplementation with vitamins C and E during pregnancy does not reduce the risk of preeclampsia in nulliparous women (136–138). If vitamin E does in fact have an antihypertensive effect, it is probably small and may be limited to untreated patients or those with vascular disease or other concomitant diseases, such as diabetes (71,139).

High dietary consumption of fruits and vegetables has been shown to significantly reduce blood pressure (140). Diets rich in fruit and vegetables also increase plasma antioxidant capacity in both normal and obese subjects (141–143). This improvement in antioxidant status may partly explain the beneficial effects of high fruit and vegetable consumption on blood pressure, although the accompanying increases in the intake of other micronutrients and fiber, and decreases in saturated and total fat consumption, are also likely to play a role in blood pressure reduction.

**POSSIBLE REASONS FOR NEGATIVE OUTCOMES OF ANTIOXIDANT TRIALS** — Overall results of clinical studies investigating antioxidant effects have been disappointing given the consistent and promising findings from experimental investigations, clinical observations, and epidemiological data. Possible reasons relate to 1) the type of antioxidants used, 2) patient cohorts included in trials, and 3) the trial design itself. With respect to antioxidants, it is possible that agents examined were ineffective and nonspecific and that dosing regimens and duration of therapy were insufficient. For example, vitamins C and E may have pro-oxidant properties with harmful and deleterious interactions. It is also possible that orally administered antioxidants may be inaccessible to the source of free radicals, particularly if ROS are generated in intracellular compartments and organelles (143). Furthermore, antioxidant vitamins do not scavenge H2O2, which may be more important than •O2− in cardiovascular disease. Another factor of importance is that antioxidants do not inhibit ROS production. Regarding cohorts included in large trials, most subjects had significant cardiovascular disease, in which case damaging effects of oxidative stress may be irreversible. Another confounding factor is that most of the enrolled subjects were taking aspirin prophylactically. Because aspirin has intrinsic antioxidant properties (144) additional antioxidant therapy may be ineffective. Moreover, in patients studied in whom negative results were obtained, it was never proven that these individuals did in fact have increased oxidative stress. To date, there are no large clinical trials in which patients were recruited based on evidence of elevated ROS formation. Also, none of the large clinical trials were designed to examine effects of antioxidants specifically on blood pressure.

With the recent advances in our understanding of the complexity of oxidative stress and redox signaling in the vascular system, there is growing interest regarding therapeutic possibilities to target ROS in the management of hypertension and other cardiovascular diseases. Theoretically, agents that reduce oxidant formation should be more efficacious than nonspecific inefficient antioxidant scavengers in ameliorating oxidative stress. This is based on experimental evidence where it has been clearly demonstrated that inhibition of NAD(P)H oxidase–mediated •O2− generation, using pharmacological and gene-targeted strategies, leads to regression of vascular remodeling, improved endothelial function, and lowering of blood pressure (44,74,81). In fact, vascular NAD(P)H oxidase, specifically gp91phox (Nox2) homologs such as Nox1, may be novel therapeutic targets for vascular disease (143).

**RECOMMENDATIONS TO DECREASE OXIDATIVE STRESS IN PATIENTS** — In view of current data and the lack of evidence to prove the benefits from use of antioxidant vitamins to prevent cardiovascular disease (145), it is suggested that the general population consumes a diet emphasizing antioxidant-rich fruits and vegetables and whole grains. Presently, antioxidant supplementation is not recommended for the prevention or treatment of hypertension. This advice, which is consistent with the guidelines of the American Heart Association (146) and the Canadian Hypertension Society (147), considers the role of the total diet in influencing disease risk and is supported by findings from the Dietary Approaches to Stop Hypertension (DASH) study (142) and a recent trial from the U.K. that demonstrated that subjects consuming high fruit and vegetable diets had significantly reduced blood pressure (141). Another important lifestyle modification that may have cardiovascular protective and blood pressure–lowering effects by reducing oxidative stress is exercise. In experimental models of hypertension and in human patients with coronary artery disease, exercise reduced vascular NAD(P)H oxidase activity and ROS production, ameliorated vascular injury, and reduced blood pressure (148–150).

Some of the beneficial effects of classic antihypertensive agents such as β-adrenergic blockers, ACE inhibitors, AT1 receptor antagonists, and Ca2+–channel blockers may be mediated in part by decreasing vascular oxidative stress (151–157). Indeed, angiotensin receptor blockade appears to be particularly effective at reducing ROS generation and markers of oxidative stress independent of blood pressure lowering. These effects
have been attributed to direct inhibition of NAD(P)H oxidase activity and to intrinsic antioxidant properties of the drugs.

**CONCLUSIONS** — Compelling experimental evidence indicates that ROS, particularly \( \text{O}_2^- \) and \( \text{H}_2\text{O}_2 \), function as second messengers activating numerous signaling molecules, which play an important role in vascular biology and cardiovascular disease. In hypertension, activation of pro-oxidant enzymes such as NAD(P)H oxidase, NOS, xanthine oxidase and mitochondrial enzymes, or altered thioredoxin and glutathione systems results in increased ROS formation, which have damaging actions on the vasculature. Recent data indicate that the Nox family of NAD(P)H oxidases, particularly Nox1 and Nox4, may be important in vascular generation of ROS in pathological conditions. Stimuli that activate pro-oxidant systems to generate ROS involve vasoactive agents, growth factors, metabolic factors, and mechanical forces. Oxidative stress contributes to vascular damage by promoting cell growth, extracellular matrix protein deposition, activation of matrix metalloproteinases, inflammation, endothelial dysfunction, and increased vascular tone, characteristic features of the vascular phenotype in hypertension.

From a clinical viewpoint, current data are less conclusive with respect to the pathophysiological role of oxidative stress in hypertension. This may relate to heterogeneity of populations studied, inappropriate or insensitive methodologies to evaluate oxidative state, and incorrect antioxidant therapies used. Further research in the field of oxidative stress and human hypertension is warranted. There is an urgent need for the development of sensitive and specific biomarkers to assess the oxidant status of patients. Also needed are clinical trials designed to specifically address the role of oxidative stress in the development of hypertension. With a better understanding of mechanisms regulating ROS metabolism and identification of processes that promote oxidative excess, it should be possible to target therapies more effectively so that detrimental actions of vascular oxygen free radicals can be reduced and beneficial effects of NO can be enhanced. Such therapies could have potential in the management of diseases associated with vascular damage, including hypertension.

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