# NADPH Oxidases, Reactive Oxygen Species, and Hypertension

## Clinical implications and therapeutic possibilities

TAMARA M. PARAVICINI, PHD
RHIAN M. TOUYZ, MD, PHD

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 remodeling 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.

*Diabetes Care* 31 (Suppl. 2):S170–S180, 2008

Reactive oxygen species (ROS) play an important role in the development of cardiovascular disease, including hypertension, atherosclerosis, diabetes, cardiac hypertrophy, heart failure, ischemia-reperfusion injury, and stroke. This is due, in large part, to excess production of oxidants, to decreased nitric oxide (NO) bioavailability, and to decreased antioxidant capacity in the vasculature and kidneys (1–3). The ROS family comprises many molecules that have divergent effects on cellular function, such as regulation of cell growth and differentiation, modulation of extracellular matrix production and breakdown, inactivation of NO, and stimulation of many kinases and proinflammatory genes (4–6). Importantly, many of these actions are associated with pathological changes observed in cardiovascular disease.

The term "oxidative stress" describes conditions involving increased ROS levels. Reactive oxygen species, also termed "oxygen-derived species" or "oxidants," are produced as intermediates in reduction-oxidation (redox) reactions leading from $O_2$ to $H_2O$. ROS are reactive chemical entities comprising two major groups: free radicals (e.g., superoxide [$\cdot O_2^-$], hydroxyl [OH·], nitric oxide [NO·]) and nonradical derivatives of $O_2$ (e.g., $H_2O_2$, $ONOO^-$) (7,8). A free radical is any species capable of independent existence (thus the term "free") that contains one or more unpaired electron. The unpaired electron imparts high reactivity and renders the radical unstable. Nonradical derivatives are less reactive and more stable with a longer half-life than free radicals. The sequential univalent reduction of $O_2$ is as follows:

$$O_2 \xrightarrow{e^-} \cdot O_2^- \xrightarrow{e^-} H_2O_2 \xrightarrow{e^-} OH\cdot \xrightarrow{e^-} \cdot H_2O + O_2$$

Of the ROS generated in cardiovascular cells, $\cdot O_2^-$ and $H_2O_2$ appear to be particularly important.

In biological systems, $\cdot O_2^-$ is short-lived owing to its rapid reduction to $H_2O_2$ by superoxide dismutase (SOD) (9). The charge on the superoxide anion makes it unable to cross cellular membranes, except possibly through ion channels. In contrast, $H_2O_2$ has a longer biological lifespan than $\cdot O_2^-$, is relatively stable, and is easily diffusible within and between cells. The main source of $H_2O_2$ in vascular tissue is the dismutation of $\cdot O_2^-$: $2\cdot O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$. This reaction can be spontaneous or it can be catalyzed by SOD, of which there are three mammalian isoforms: copper/zinc SOD (SOD1), mitochondrial SOD (Mn SOD, SOD2), and extracellular SOD (ecSOD, SOD3) (10,11). The major vascular SOD is eSOD.

The distinct properties between $\cdot O_2^-$ and $H_2O_2$ and their different sites of distribution mean that different species of ROS can activate different signaling pathways, which lead to divergent, and potentially opposing, functional responses. For example, increased $\cdot O_2^-$ levels inactivate the vasodilator NO leading to endothelial dysfunction and vasoconstriction, characteristic of many vascular diseases, including hypertension (12,13). On the other hand, $H_2O_2$ acts as a vasodilator in some vascular beds, including cerebral, coronary, and mesenteric arteries (14–16).

From the Kidney Research Centre, Ottawa Health Research Institute, University of Ottawa, Ottawa, Ontario, Canada.

Address correspondence and reprint requests to Rhian M. Touyz, MD, PhD, Canada Research Chair in Hypertension, Kidney Research Centre, OHRI/University of Ottawa, 451 Smyth Rd., Ottawa, K1H 8M5, Ontario, Canada. E-mail: rtouyz@uottawa.ca.

The authors of this article have no relevant duality of interest to declare.

This article is based on a presentation at the 1st World Congress of Controversies in Diabetes, Obesity and Hypertension (CODHy). The Congress and the publication of this article were made possible by unrestricted educational grants from MSD, Roche, sanofi-aventis, Novo Nordisk, Medtronic, LifeScan, World Wide, Eli Lilly, Keryx, Abbott, Novartis, Pfizer, Generx Biotechnology, Schering, and Johnson & Johnson.

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

DOI: 10.2337/dc08-s247

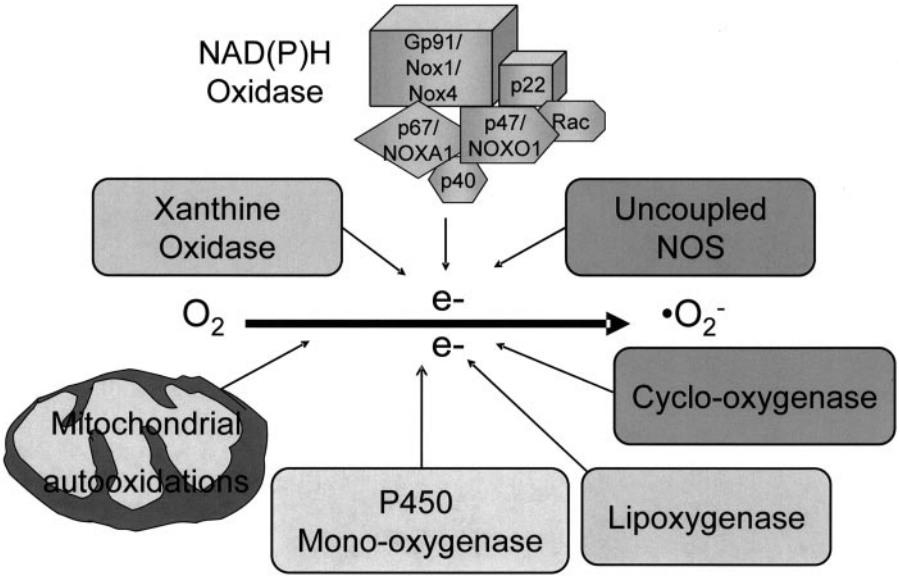


**Figure 1—***Enzymatic sources of superoxide anion ($\cdot O_2^-$). The major enzymes responsible for ROS generation in the vasculature include NAD(P)H oxidase, xanthine oxidase, and uncoupled NOS. NAD(P)H oxidase is a multisubunit enzyme, comprising gp91phox (or its homologs, Nox1 and Nox4), p22phox, p47phox (or NOXO1), p67phox (or NOXA1), and p40phox.*

**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 $\cdot O_2^-$, is present in the vascular endothelium (17). Although xanthine oxidase–derived $\cdot O_2^-$ 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 $\cdot O_2^-$ (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 $\cdot O_2^-$ 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 endothelium-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 $\cdot O_2^-$ 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 $\cdot O_2^-$ production by the 1-electron reduction of $O_2$ using NADPH or NADH [hence the parentheses in NAD(P)H] as the electron donor: $2O_2 + NAD(P)H \rightarrow 2\cdot O_2^- + NAD(P)^+ + H^+$. The prototypical NAD(P)H oxidase is that found in neutrophils and has five subunits: p47phox ("phox" stands for *ph*agocyte *ox*idase), p67phox, p40phox, p22phox, and the catalytic subunit gp91phox (also termed "Nox2") (32,33). In unstimulated cells, p47phox, p67phox, and p40phox 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 cyto-chrome b558 to assemble the active oxidase, which transfers electrons from the substrate to $O_2$, forming $\cdot O_2^-$ (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-

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 "Renox" (renal oxidase), because of its abundance in the kidney, is also found in vascular cells and osteoclasts; and Nox5 is a $Ca^{2+}$-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 $\cdot O_2^-$ 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 (NOXA1), 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, hyperinsulinemia, 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 $AT_1$ 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 $p21^{Ras}$, protein kinase C, phospholipase D, and phospholipase $A_2$ (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 $\cdot O_2^-$ into $H_2O_2$ and $O_2$. 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 $H_2O_2$ 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 $H_2O_2$ to water and molecular oxygen (57). Catalase is very effective in high-level oxidative stress and protects cells from $H_2O_2$ 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 $OH\cdot$ as well as other radicals (59). Low antioxidant bioavailability promotes cellular oxidative stress and has been implicated in oxidative damage associated with hypertension (55).

## 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 ($H_2O_2$ may have vasodilator actions) or indirectly by decreasing NO bioavailability through quenching by $\cdot O_2^-$ to form $ONOO^-$ (58,59). ROS, through the regulation of hypoxia-inducible factor 1 (HIF-1), are also important in $O_2$ sensing (60), which is essential for maintaining normal $O_2$ 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 $Ca^{2+}$ (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 $F_{2\alpha}$-isoprostanes, tissue concentrations of $\cdot O_2^-$ and $H_2O_2$, and activation of NAD(P)H oxidase and xanthine oxidase,

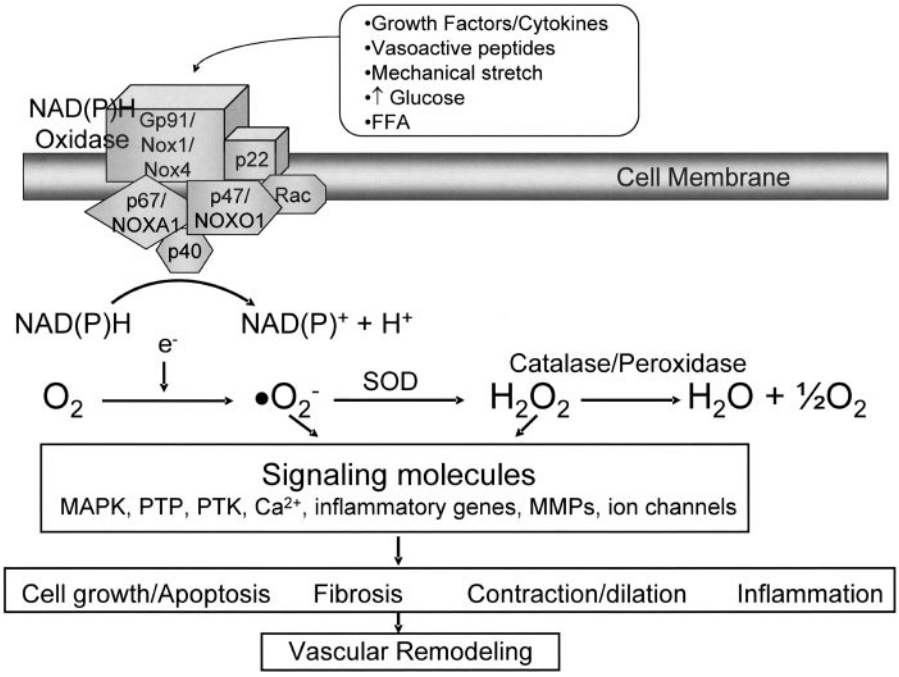


**Figure 2—***Activation of vascular NAD(P)H oxidase by multiple factors results in generation of ROS, which in turn influence signaling molecules involved in vascular growth, fibrosis, contraction/dilation, and inflammation. These redox-sensitive processes contribute to vascular damage and remodeling in hypertension and other cardiovascular diseases. FFA, free fatty acid; MAPK, mitogen-activated protein kinase; MMPs, matrix metalloproteinases; PTK, protein tyrosine kinases; PTP, protein tyrosine phosphatases.*

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), oxidase 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 (Nox2) 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 diphenylene iodinium, 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 SHRs, 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/$ET_A$ 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 piperidinoxyl), free radical scavengers, or tetrahydrobiopterin ($BH_4$), 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 $H_2O_2$

**Table 1—*Evidence supporting a role for oxidative stress in human hypertension***

| |
|---|
| 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 $H_2O_2$ 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 $H_2O_2$ 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 ~20–35% of the observed variance in $H_2O_2$ 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 (115), 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 $\cdot O_2^-$-generating enzymes (121). Gene therapy targeting oxidant 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 Gruppo Italiano per lo Studio della Sopravvivenza 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 end points. 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 Supplémentation en Vitamines et Mineraux 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 $Mg^{2+}$, 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 $H_2O_2$, which may be more important than $\cdot O_2^-$ 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 $\cdot O_2^-$ 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, $AT_1$ receptor antagonists, and $Ca^{2+}$-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 $\cdot O_2^-$ and $H_2O_2$, 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$\cdot$ can be enhanced. Such therapies could have potential in the management of diseases associated with vascular damage, including hypertension.

**Acknowledgments** — Work from the author's laboratory was supported by grants 44018 and 57886, both from the Canadian Institutes of Health Research. Tamara M. Paravicini is supported by a Ralph Reader Overseas Research Fellowship (O 04M 1727) from the National Heart Foundation of Australia.

**References**

1. Touyz RM: Reactive oxygen species in vascular biology: role in arterial hypertension. *Expert Rev Cardiovasc Ther* 1:91–106, 2003
2. Tain YL, Baylis C: Dissecting the causes of oxidative stress in an in vivo model of hypertension. *Hypertension* 48:828–829, 2006
3. Vaziri ND, Rodriguez-Iturbe B: Mechanisms of disease: oxidative stress and inflammation in the pathogenesis of hypertension. *Nat Clin Pract Nephrol* 2:582–593, 2006
4. Harrison DG, Widder J, Grumbach I, Chen W, Weber M, Searles C: Endothelial: mechanotransduction, nitric oxide and vascular inflammation. *J Intern Med* 259:351–363, 2006
5. Touyz RM: Molecular and cellular mechanisms in vascular injury in hypertension: role of angiotensin II. *Curr Opin Nephrol Hypertens* 14:125–131, 2005
6. Mueller CF, Laude K, McNally JS, Harrison DG: ATVB in focus: redox mechanisms in blood vessels. *Arterioscler Thromb Vasc Biol* 25:274–278, 2005
7. Droge W: Free radicals in the physiological control of cell function. *Physiol Rev* 82:47–95, 2002
8. Fridovich I: Superoxide anion radical (O2-.), superoxide dismutases, and related matters. *J Biol Chem* 272:18515–18517, 1997
9. Johnson F, Giulivi C: Superoxide dismutases and their impact upon human health. *Mol Aspects Med* 26:340–352, 2005
10. Faraci FM, Didion SP: Vascular protection: superoxide dismutase isoforms in the vessel wall. *Arterioscler Thromb Vasc Biol* 24:1367–1373, 2004
11. Mendez JI, Nicholson WJ, Taylor WR: SOD isoforms and signaling in blood vessels: evidence for the importance of ROS compartmentalization. *Arterioscler Thromb Vasc Biol* 25:887–888, 2005
12. Cai H, Harrison DG: Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. *Circ Res* 87:840–844, 2000
13. Tabet F, Savoia C, Schiffrin EL, Touyz RM: Differential calcium regulation by hydrogen peroxide and superoxide in vascular smooth muscle cells from spontaneously hypertensive rats. *J Cardiovasc Pharmacol* 44:200–208, 2004
14. Paravicini TM, Chrissobolis S, Drummond GR, Sobey CG: Increased NAD(P)H-oxidase activity and Nox4 expression during chronic hypertension is associated with enhanced cerebral vasodilatation to NAD(P)H in vivo. *Stroke* 35:584–589, 2004
15. Liu Y, Zhao H, Li H, Kalyanaraman B, Nicolosi AC, Gutterman DD: Mitochondrial sources of H2O2 generation play a key role in flow-mediated dilation in human coronary resistance arteries. *Circ Res* 93:573–80, 2003
16. Matoba T, Shimokawa H, Nakashima M, Hirakawa Y, Mukai Y, Hirano K, Kanaide H, Takeshita A: Hydrogen peroxide is an endothelium-derived hyperpolarizing factor in mice. *J Clin Invest* 106:1521–1530, 2000
17. Lacy F, Gough DA, Schmid-Schönbein GW: Role of xanthine oxidase in hydrogen peroxide production. *Free Radic Biol Med* 25:720–727, 1998
18. Suzuki H, DeLano FA, Parks DA, Jamshidi N, Granger DN, Ishii H, Suematsu M, Zweifach BW, Schmid-Schonbein GW: Xanthine oxidase activity associated with arterial blood pressure in spontaneously hypertensive rats. *Proc Natl Acad Sci U S A* 95:4754–4759, 1998
19. DeLano FA, Parks DA, Ruedi JM, Babior BM, Schmid-Schonbein GW: Microvascular display of xanthine oxidase and NADPH oxidase in the spontaneously hypertensive rat. *Microcirculation* 13:551–566, 2006
20. Mervaala EM, Cheng ZJ, Tikkanen I, Lapatto R, Nurminen K, Vapaatalo H, Muller DN, Fiebeler A, Ganten U, Ganten D, Luft FC: Endothelial dysfunction and xanthine oxidoreductase activity in rats with human renin and angiotensinogen genes. *Hypertension* 37:414–418, 2001
21. Laakso J, Mervaala E, Himberg JJ, Teravainen TL, Karppanen H, Vapaatalo H, Lapatto R: Increased kidney xanthine oxidoreductase activity in salt-induced experimental hypertension. *Hypertension* 32:902–906, 1998
22. Siu YP, Leung KT, Tong MK, Kwan TH: Use of allopurinol in slowing the progression of renal disease through its ability to lower serum uric acid level. *Am J Kidney Dis* 47:51–59, 2006
23. Laakso JT, Teravainen TL, Martelin E, Vaskonen T, Lapatto R: Renal xanthine oxidoreductase activity during development of hypertension in spontaneously hypertensive rats. *J Hypertens* 22:1333–1340, 2004
24. Corry DB, Tuck ML: Uric acid and the vasculature. *Curr Hypertens Rep* 8:116–119, 2006
25. Andrew PJ, Mayer B: Enzymatic function of nitric oxide synthases. *Cardiovasc Res* 43:521–531, 1999
26. Vasquez-Vivar J, Kalyanaraman B, Mar-

tasek P, Hogg N, Masters BS, Karoui H, Tordo P, Pritchard KA Jr: Superoxide generation by endothelial nitric oxide synthase: the influence of cofactors. *Proc Natl Acad Sci U S A* 95:9220–9225, 1998
27. Landmesser U, Dikalov S, Price SR, McCann L, Fukai T, Holland SM, Mitch WE, Harrison DG: Oxidation of tetrahydrobiopterin leads to uncoupling of endothelial cell nitric oxide synthase in hypertension. *J Clin Invest* 111:1201–1209, 2003
28. Hong HJ, Hsiao G, Cheng TH, Yen MH: Supplementation with tetrahydrobiopterin suppresses the development of hypertension in spontaneously hypertensive rats. *Hypertension* 38:1044–1048, 2001
29. Adlam D, Bendall JK, De Bono JP, Alp NJ, Khoo J, Nicoli T, Yokoyama M, Kawashima S, Channon KM: Cardiovascular control: relationships between nitric oxide-mediated endothelial function, eNOS coupling and blood pressure revealed by eNOS-GTP cyclohydrolase 1 double transgenic mice. *Exp Physiol* 92: 119–126, 2007
30. Widder JD, Guzik TJ, Mueller CF, Clempus RE, Schmidt HH, Dikalov SI, Griendling KK, Jones DP, Harrison DG: Role of the multidrug resistance protein-1 in hypertension and vascular dysfunction caused by angiotensin II. *Arterioscler Thromb Vasc Biol* 27:762–768, 2007
31. Landmesser U, Harrison DG, Drexler H: Oxidant stress: a major cause of reduced endothelial nitric oxide availability in cardiovascular disease. *Eur J Clin Pharmacol* 62 (Suppl. 1):13–19, 2006
32. Babior BM: NADPH oxidase. *Curr Opin Immunol* 16:42–47, 2004
33. Chabrashvili T, Tojo A, Onozato ML, Kitiyakara C, Quinn MT, Fujita T, Welch WJ, Wilcox CS: Expression and cellular localization of classic NADPH oxidase subunits in the spontaneously hypertensive rat kidney. *Hypertension* 39:269–274, 2002
34. Touyz RM, Yao G, Schiffrin EL: c-Src induces phosphorylation and translocation of p47phox: role in superoxide generation by angiotensin II in human vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol* 23:981–987, 2003
35. Bokoch GM, Zhao T: Regulation of the phagocyte NADPH oxidase by Rac GTPase. *Antioxid Redox Signal* 8:1533–1548, 2006
36. Geiszt M: NADPH oxidases: new kids on the block. *Cardiovasc Res* 71:289–299, 2006
37. Cave AC, Brewer AC, Panicker AN, Ray R, Grieve DJ, Walker S, Shah AM: NADPH oxidases in cardiovascular health and disease. *Antiox Redox Sig* 8:691–727, 2006
38. Griendling KK: NADPH oxidases: new regulators of old functions. *Antioxid Redox Signal* 8:1443–1445, 2006
39. Ambasta RK, Kumar P, Griendling KK, Schmidt HH, Busse R, Brandes RP: Direct interaction of the novel Nox proteins with p22phox is required for the formation of a functionally active NADPH oxidase. *J Biol Chem* 279: 45935–45941, 2004
40. Kawahara T, Ritsick D, Cheng G, Lambeth JD: Point mutations in the proline-rich region of p22phox are dominant inhibitors of Nox1- and Nox2-dependent reactive oxygen generation. *J Biol Chem* 280:31859–1869, 2005
41. Ambasta RK, Schreiber JG, Janiszewski M, Busse R, Brandes RP: Noxa1 is a central component of the smooth muscle NADPH oxidase in mice. *Free Radic Biol Med* 41:193–201, 2006
42. Cheng G, Lambeth JD: Alternative mRNA splice forms of NOXO1: differential tissue expression and regulation of Nox1 and Nox3. *Gene* 356:118–126, 2005
43. Bayraktutan U, Blayney L, Shah AM: Molecular characterization and localization of the NAD(P)H oxidase components gp91-phox and p22-phox in endothelial cells. *Arterioscler Thromb Vasc Biol* 20:1903–1911, 2000
44. Lassegue B, Clempus RE: Vascular NAD(P)H oxidases: specific features, expression, and regulation. *Am J Physiol Regul Integr Comp Physiol* 285:R277–R297, 2003
45. Li JM, Shah AM: Intracellular localization and preassembly of the NAD(P)H oxidase complex in cultured endothelial cells. *J Biol Chem* 277:19952–19960, 2002
46. Miller AA, Drummond GR, Sobey CG: Novel isoforms of NADPH-oxidase in cerebral vascular control. *Pharmacol Ther* 111:928–948, 2006
47. Takeya R, Sumimoto H: Regulation of novel superoxide-producing NAD(P)H oxidases. *Antioxid Redox Signal* 8:1523–1532, 2006
48. Seshiah PN, Weber DS, Rocic P, Valppu L, Taniyama Y, Griendling KK: Angiotensin II stimulation of NAD(P)H oxidase activity: upstream mediators. *Circ Res* 91:406–413, 2002
49. Ushio-Fukai M, Alexander RW, Akers M, Griendling KK: p38 mitogen-activated protein kinase is a critical component of the redox-sensitive signaling pathways activated by angiotensin II: role in vascular smooth muscle cell hypertrophy. *J Biol Chem* 273:15022–15029, 1998
50. Griendling KK, Sorescu D, Lassegue B, Ushio-Fukai M: Modulation of protein kinase activity and gene expression by reactive oxygen species and their role in vascular physiology and pathophysiology. *Arterioscler Thromb Vasc Biol* 20:2175–2183, 2000
51. Touyz RM, Chen X, Tabet F, Yao G, He G, Quinn MT, Pagano PJ, Schiffrin EL: Expression of a functionally active gp91phox-containing neutrophil-type NAD(P)H oxidase in smooth muscle cells from human resistance arteries: regulation by angiotensin II. *Circ Res* 90: 1205–1213, 2002
52. Gongora MC, Qin Z, Laude K, Kim HW, McCann L, Folz JR, Dikalov S, Fukai T, Harrison DG: Role of extracellular superoxide dismutase in hypertension. *Hypertension* 48:473–481, 2006
53. Sindhu RK, Ehdaie A, Farmand F, Dhaliwal KK, Nguyen T, Zhan CD, Roberts CK, Vaziri ND: Expression of catalase and glutathione peroxidase in renal insufficiency. *Biochim Biophys Acta* 1743: 86–92, 2005
54. Sui H, Wang W, Wang PH, Liu LS: Effect of glutathione peroxidase mimic ebselen (PZ51) on endothelium and vascular structure of stroke-prone spontaneously hypertensive rats. *Blood Press* 14:366–372, 2005
55. Wassmann S, Wassmann K, Nickenig G: Modulation of oxidant and antioxidant enzyme expression and function in vascular cells. *Hypertension* 44:381–386, 2004
56. Touyz RM, Tabet F, Schiffrin EL: Redox-dependent signalling by angiotensin II and vascular remodelling in hypertension. *Clin Exp Pharmacol Physiol* 30:860–866, 2003
57. Cai H: Hydrogen peroxide regulation of endothelial function: origins, mechanisms, and consequences. *Cardiovasc Res* 68:26–36, 2005
58. Kajiya M, Hirota M, Inai Y, Kiyooka T, Morimoto T, Iwasaki T, Endo K, Mohri S, Shimizu J, Yada T, Ogasawara Y, Naruse K, Ohe T, Kajiya F: Impaired NO-mediated vasodilation with increased superoxide but robust EDHF function in right ventricular arterial microvessels of pulmonary hypertensive rats. *Am J Physiol Heart Circ Physiol* 292:H2737–H2744, 2007
59. Shimokawa H, Matoba T: Hydrogen peroxide as an endothelium-derived hyperpolarizing factor. *Pharmacol Res* 49:543–549, 2004
60. Goyal P, Weissmann N, Grimminger F, Hegel C, Bader L, Rose F, Fink L, Ghofrani HA, Schermuly RT, Schmidt HH, Seeger W, Hanze J: Upregulation of NAD(P)H oxidase 1 in hypoxia activates hypoxia-inducible factor 1 via increase in reactive oxygen species. *Free Radic Biol Med* 36:1279–1288, 2004
61. San Martin A, Du P, Dikalova A, Lassegue B, Aleman M, Gongora MC, Brown K, Joseph G, Harrison DG, Taylor WR, Jo H, Griendling KK: Reactive oxygen species-selective regulation of aortic inflammatory gene expression in type 2 diabetes. *Am J Physiol Heart Circ Physiol* 292:H2073–H2082, 2007

62. Pawlak K, Naumnik B, Brzosko S, Pawlak D, Mysliwiec M: Oxidative stress: a link between endothelial injury, coagulation activation, and atherosclerosis in haemodialysis patients. *Am J Nephrol* 24: 154–161, 2004
63. Hool LC, Corry B: Redox control of calcium channels: from mechanisms to therapeutic opportunities. *Antioxid Redox Signal* 9:409–435, 2007
64. Kimura S, Zhang GX, Nishiyama A, Shokoji T, Yao L, Fan YY, Rahman M, Abe Y: Mitochondria-derived reactive oxygen species and vascular MAP kinases: comparison of angiotensin II and diazoxide. *Hypertension* 45:438–444, 2005
65. Yoshioka J, Schreiter ER, Lee RT: Role of thioredoxin in cell growth through interactions with signaling molecules. *Antioxid Redox Signal* 8:2143–21451, 2006
66. Touyz RM: Reactive oxygen species as mediators of calcium signaling by angiotensin II: implications in vascular physiology and pathophysiology. *Antioxid Redox Signal* 7:1302–1314, 2005
67. Kitiyakara C, Wilcox CS: Antioxidants for hypertension. *Curr Opin Nephrol Hypertens* 7:531–538, 1998
68. Houston MC: Nutraceuticals, vitamins, antioxidants, and minerals in the prevention and treatment of hypertension. *Prog Cardiovasc Dis* 47:396–449, 2005
69. Touyz RM, Schiffrin EL: Reactive oxygen species in vascular biology: implications in hypertension. *Histochem Cell Biol* 122: 339–352, 2004
70. Welch WJ: Intrarenal oxygen and hypertension. *Clin Exp Pharmacol Physiol* 33: 1002–1005, 2006
71. Redon J, Oliva MR, Tormos C, Giner V, Chaves J, Iradi A, Saez GT: Antioxidant activities and oxidative stress byproducts in human hypertension. *Hypertension* 41:1096–1101, 2003
72. Rajagopalan S, Kurz S, Munzel T, Tarpey M, Freeman BA, Griendling KK, Harrison DG: Angiotensin II-mediated hypertension in the rat increases vascular superoxide production via membrane NADH/NAD(P)H oxidase activation: contribution to alterations of vasomotor tone. *J Clin Invest* 97:1916–1923, 1996
73. Fukui T, Ishizaka N, Rajagopalan S, Laursen JB, Capers QT, Taylor WR, Harrison DG, de Leon H, Wilcox JN, Griendling KK: p22phox mRNA expression and NAD(P)H oxidase activity are increased in aortas from hypertensive rats. *Circ Res* 80:45–51, 1997
74. Virdis A, Neves MF, Amiri F, Touyz RM, Schiffrin EL: Role of NAD(P)H oxidase on vascular alterations in angiotensin II-infused mice. *J Hypertens* 22:535–542, 2004
75. Landmesser U, Cai H, Dikalov S, McCann L, Hwang J, Jo H, Holland SM, Harrison DG: Role of p47(phox) in vascular oxidative stress and hypertension caused by angiotensin II. *Hypertension* 40:511–515, 2002
76. Li JM, Wheatcroft S, Fan LM, Kearney MT, Shah AM: Opposing roles of p47phox in basal versus angiotensin II-stimulated alterations in vascular O2- production, vascular tone, and mitogen-activated protein kinase activation. *Circulation* 109:1307–1313, 2004
77. Jung O, Schreiber JG, Geiger H, Pedrazzini T, Busse R, Brandes RP: gp91phox-containing NADPH oxidase mediates endothelial dysfunction in renovascular hypertension. *Circulation* 109:1795–1801, 2004
78. Modlinger P, Chabrashvili T, Gill PS, Mendonca M, Harrison DG, Griendling KK, Li M, Raggio J, Wellstein A, Chen Y, Welch WJ, Wilcox CS: RNA silencing in vivo reveals role of p22phox in rat angiotensin slow pressor response. *Hypertension* 47:238–244, 2006
79. Laude K, Cai H, Fink B, Hoch N, Weber DS, McCann L, Kojda G, Fukai T, Schmidt HH, Dikalov S, Ramasamy S, Gamez G, Griendling KK, Harrison DG: Hemodynamic and biochemical adaptations to vascular smooth muscle overexpression of p22phox in mice. *Am J Physiol Heart Circ Physiol* 288:H7–H12, 2005
80. Hu L, Zhang Y, Lim PS, Miao Y, Tan C, McKenzie KU, Schyvens CG, Whitworth JA: Apocynin but not L-arginine prevents and reverses dexamethasone-induced hypertension in the rat. *Am J Hypertens* 19:413–418, 2006
81. Rey FE, Cifuentes ME, Kiarash A, Quinn MT, Pagano PJ: Novel competitive inhibitor of NAD(P)H oxidase assembly attenuates vascular O(2)(−) and systolic blood pressure in mice. *Circ Res* 89: 408–414, 2001
82. Touyz RM, Mercure C, He Y, Javeshghani D, Yao G, Callera GE, Yogi A, Lochard N, Reudelhuber TL: Angiotensin II-dependent chronic hypertension and cardiac hypertrophy are unaffected by gp91phox-containing NADPH oxidase. *Hypertension* 45:530–537, 2005
83. Gavazzi G, Banfi B, Deffert C, Fiette L, Schappi M, Herrmann F, Krause KH: Decreased bloodpressure in NOX1-deficient mice. *FEBS Lett* 580:497–504, 2006
84. Matsuno K, Yamada H, Iwata K, Jin D, Katsuyama M, Matsuki M, Takai S, Yamanishi K, Miyazaki M, Matsubara H, Yabe-Nishimura C: Nox1 is involved in angiotensin II-mediated hypertension: a study in Nox1-deficient mice. *Circulation* 112:2677–2685, 2005
85. Dikalova A, Clempus R, Lassegue B, Cheng G, McCoy J, Dikalov S, San Martin A, Lyle A, Weber DS, Weiss D, Taylor WR, Schmidt HH, Owens GK, Lambeth JD, Griendling KK: Nox1 overexpression potentiates angiotensin II-induced hypertension and vascular smooth muscle hypertrophy in transgenic mice. *Circulation* 112:2668–2676, 2005
86. Zhang Y, Griendling KK, Dikalova A, Owens GK, Taylor WR: Vascular hypertrophy in angiotensin II-induced hypertension is mediated by vascular smooth muscle cell-derived H2O2. *Hypertension* 46:732–737, 2005
87. Zalba G, Beaumont FJ, San Jose G, Fortuno A, Fortuno MA, Etayo JC, Diez J: Vascular NADH/NAD(P)H oxidase is involved in enhanced superoxide production in spontaneously hypertensive rats. *Hypertension* 35:1055–1061, 2000
88. Akasaki T, Ohya Y, Kuroda J, Eto K, Abe I, Sumimoto H, Iida M: Increased expression of gp91phox homologues of NAD(P)H oxidase in the aortic media during chronic hypertension: involvement of the renin-angiotensin system. *Hypertens Res* 29:813–820, 2006
89. Somers MJ, Mavromatis K, Galis ZS, Harrison DG: Vascular superoxide production and vasomotor function in hypertension induced by deoxycorticosterone acetate-salt. *Circulation* 101: 1722–1728, 2000
90. Callera GE, Touyz RM, Teixeira SA, Muscara MN, Carvalho MH, Fortes ZB, Nigro D, Schiffrin EL, Tostes RC: ETA receptor blockade decreases vascular superoxide generation in DOCA-salt hypertension. *Hypertension* 42:811–817, 2003
91. Park JB, Touyz RM, Chen X, Schiffrin EL: Chronic treatment with a superoxide dismutase mimetic prevents vascular remodeling and progression of hypertension in salt-loaded stroke-prone spontaneously hypertensive rats. *Am J Hypertens* 15:78–84, 2002
92. Callera GE, Tostes RC, Yogi A, Montezano AC, Touyz RM: Endothelin-1-induced oxidative stress in DOCA-salt hypertension involves NADPH-oxidase-independent mechanisms. *Clin Sci (Lond)* 110:243–253, 2006
93. Elmarakby AA, Loomis ED, Pollock JS, Pollock DM: NAD(P)H oxidase inhibition attenuates oxidative stress but not hypertension produced by chronic ET-1. *Hypertension* 45:283–287, 2005
94. Amiri F, Virdis A, Neves MF, Iglarz M, Seidah NG, Touyz RM, Reudelhuber TL, Schiffrin EL: Endothelium-restricted overexpression of human endothelin-1 causes vascular remodeling and endothelial dysfunction. *Circulation* 110: 2233–2240, 2004
95. Chen X, Touyz RM, Park JB, Schiffrin EL: Antioxidant effects of vitamins C and E are associated with altered activation of vascular NADPH oxidase and superoxide dismutase in stroke-prone SHR. *Hypertension* 38:606–611, 2001
96. Fortuno A, Olivan S, Beloqui O, San Jose

G, Moreno MU, Diez J, Zalba G: Association of increased phagocytic NAD(P)H oxidase-dependent superoxide production with diminished nitric oxide generation in essential hypertension. *J Hypertens* 22:2169–2175, 2004
97. Higashi Y, Sasaki S, Nakagawa K, Matsuura H, Oshima T, Chayama K: Endothelial function and oxidative stress in renovascular hypertension. *N Engl J Med* 346:1954–1962, 2002
98. Lip GY, Edmunds E, Nuttall SL, Landray MJ, Blann AD, Beevers DG: Oxidative stress in malignant and non-malignant phase hypertension. *J Hum Hypertens* 16: 333–336, 2002
99. Lee VM, Quinn PA, Jennings SC, Ng LL: Neutrophil activation and production of reactive oxygen species in pre-eclampsia. *J Hypertens* 21:395–402, 2003
100. Ward NC, Hodgson JM, Puddey IB, Mori TA, Beilin LJ, Croft KD: Oxidative stress in human hypertension: association with antihypertensive treatment, gender, nutrition, and lifestyle. *Free Radic Biol Med* 36:226–232, 2004
101. Ide T, Tsutsui H, Ohashi N, Hayashidani S, Suematsu N, Tsuchihashi M, Tamai H, Takeshita A: Greater oxidative stress in healthy young men compared with premenopausal women. *Arterioscler Thromb Vasc Biol* 22:438–442, 2002
102. Minuz P, Patrignani P, Gaino S, Seta F, Capone ML, Tacconelli S, Degan M, Faccini G, Fornasiero A, Talamini G, Tommasoli R, Arosio E, Santonastaso CL, Lechi A, Patrono C: Determinants of platelet activation in human essential hypertension. *Hypertension* 43:64–70, 2004
103. Yasunari K, Maeda K, Nakamura M, Yoshikawa J: Oxidative stress in leukocytes is a possible link between blood pressure, blood glucose, and C-reacting protein. *Hypertension* 39:777–780, 2002
104. Lacy F, Kailasam MT, O'Connor DT, Schmid-Schonbein GW, Parmer RJ: Plasma hydrogen peroxide production in human essential hypertension: role of heredity, gender, and ethnicity. *Hypertension* 36:878–884, 2000
105. Lacy F, O'Connor DT, Schmid-Schönbein GW: Plasma hydrogen peroxide production in hypertensives and normotensive subjects at genetic risk of hypertension. *J Hypertens* 16:291–303, 1998
106. Touyz RM, Schiffrin EL: Increased generation of superoxide by angiotensin II in smooth muscle cells from resistance arteries of hypertensive patients: role of phospholipase D-dependent NAD(P)H oxidase-sensitive pathways. *J Hypertens* 19:1245–1254, 2001
107. Touyz RM, Yao G, Quinn MT, Pagano PJ, Schiffrin EL: p47phox associates with the cytoskeleton through cortactin in human vascular smooth muscle cells: role in NAD(P)H oxidase regulation by angiotensin II. *Arterioscler Thromb Vasc Biol* 25:512–518, 2005
108. Zalba G, San Jose G, Moreno MU, Fortuno A, Diez J: NADPH oxidase-mediated oxidative stress: genetic studies of the p22(phox) gene in hypertension. *Antioxid Redox Signal* 7:1327–1336, 2005
109. Moreno MU, Jose GS, Fortuno A, Beloqui O, Diez J, Zalba G: The C242T CYBA polymorphism of NADPH oxidase is associated with essential hypertension. *J Hypertens* 24:1299–1306, 2006
110. Van Gaal LF, Mertens IL, De Block CE: Mechanisms linking obesity and cardiovascular disease. *Nature* 444:875–880, 2006
111. Keaney JF, Larson MG, Vasan RS, Wilson PW, Lipinska I, Corey D, Massaro JM, Sutherland P, Vita JA, Benjamin EJ: Obesity and systemic oxidative stress: clinical correlates of oxidative stress in the Framingham Study. *Arterioscler Thromb Vasc Biol* 23:434–439, 2003
112. Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, Nakayama O, Makishima M, Matsuda M, Shimomura I: Increased oxidative stress in obesity and its impact on metabolic syndrome. *J Clin Invest* 114:1752–1761, 2004
113. Dandona P, Mohanty P, Ghanim H, Aljada A, Browne R, Hamouda W, Prabhala A, Afzal A, Garg R: The suppressive effect of dietary restriction and weight loss in the obese on the generation of reactive oxygen species by leukocytes, lipid peroxidation, and protein carbonylation. *J Clin Endocrinol Metab* 86:355–362, 2001
114. Dandona P, Mohanty P, Hamouda W, Ghanim H, Aljada A, Garg R, Kumar V: Inhibitory effect of a two day fast on reactive oxygen species (ROS) generation by leucocytes and plasma ortho-tyrosine and meta-tyrosine concentrations. *J Clin Endocrino Metab* 86:2899–2902, 2001
115. Mohanty P, Hamouda W, Garg R, Aljada A, Ghanim H, Dandona P: Glucose challenge stimulates reactive oxygen species (ROS) generation by leucocytes. *J Clin Endocrinol Metab* 85:2970–2973, 2000
116. Kokubo Y, Iwai N, Tago N, Inamoto N, Okayama A, Yamawaki H, Naraba H, Tomoike H: Association analysis between hypertension and CYBA, CLCNKB, and KCNMB1 functional polymorphisms in the Japanese population: the Suita Study. *Circ J* 69:138–142, 2005
117. Dandona P, Aljada A, Mohanty P, Ghanim H, Hamouda W, Assian E, Ahmad S: Insulin inhibits intranuclear factor κB and stimulates IκB in mononuclear cells in obese subjects: evidence for an anti-inflammatory effect? *J Clin Endocrinol Metab* 86:3257–3265, 2001
118. Chaudhuri A, Janicke D, Wilson MF, Tripathy D, Garg R, Bandyopadhyay A, Calieri J, Hoffmeyer D, Syed T, Ghanim H, Aljada A, Dandona P: Anti-inflammatory and profibrinolytic effect of insulin in acute ST-segment-elevation myocardial infarction. *Circulation* 109:849–854, 2004
119. Saez GT, Tormos C, Giner V, Chaves J, Lozano JV, Iradi A, Redon J: Factors related to the impact of antihypertensive treatment in antioxidant activities and oxidative stress by-products in human hypertension. *Am J Hypertens* 17:809–816, 2004
120. Simic DV, Mimic-Oka J, Pljesa-Ercegovac M, Savic-Radojevic A, Opacic M, Matic D, Ivanovic B, Simic T: Byproducts of oxidative protein damage and antioxidant enzyme activities in plasma of patients with different degrees of essential hypertension. *J Hum Hypertens* 20: 149–155, 2006
121. Hamilton CA, Miller WH, Al-Benna S, Brosnan MJ, Drummond RD, McBride MW, Dominiczak AF: Strategies to reduce oxidative stress in cardiovascular disease. *Clin Sci (Lond)* 106:219–234, 2004
122. Dulak J, Zagorska A, Wegiel B, Loboda A, Jozkowicz A: New strategies for cardiovascular gene therapy: regulatable pre-emptive expression of pro-angiogenic and antioxidant genes. *Cell Biochem Biophys* 44:31–42, 2006
123. Kong D, Melo LG, Mangi AA, Zhang L, Lopez-Ilasaca M, Perrella MA, Liew CC, Pratt RE, Dzau VJ: Enhanced inhibition of neointimal hyperplasia by genetically engineered endothelial progenitor cells. *Circulation* 109:1769–1775, 2004
124. Mullan B, Young IS, Fee H, McCance DR: Ascorbic acid reduces blood pressure and arterial stiffness in type 2 diabetes. *Hypertension* 40:804–809, 2002
125. Chen J, He J, Hamm L, Batuman V, Whelton PK: Serum antioxidant vitamins and blood pressure in the United States population. *Hypertension* 40:810–816, 2002
126. Hasnain BI, Mooradian AD: Recent trials of antioxidant therapy: what should we be telling our patients? *Cleve Clin J Med* 71:327–334, 2004
127. Jialal I, Devaraj S: Antioxidants and atherosclerosis: don't throw out the baby with the bath water. *Circulation* 107: 926–928, 2003
128. Czernichow S, Bertrais S, Blacher J, Galan P, Briancon S, Favier A, Safar M, Hercberg S: Effect of supplementation with antioxidants upon long-term risk of hypertension in the SU.VI.MAX study: association with plasma antioxidant levels. *J Hypertens* 23:2013–2018, 2005
129. Duffy SJ, Gokce N, Holbrook M, Huang A, Frei B, Keaney JF Jr, Vita JA: Treatment of hypertension with ascorbic acid. *Lancet* 354:2048–2049, 1999
130. Bates CJ, Walmsley CM, Prentice A,

Finch S: Does vitamin C reduce blood pressure? Results of a large study of people aged 65 or older. *J Hypertens* 16:925–932, 1998
131. Ward NC, Hodgson JM, Croft KD, Burke V, Beilin LJ, Puddey IB: The combination of vitamin C and grape seed polyphenols increases blood pressure: a randomized, double-blind, placebo-controlled trial. *J Hypertens* 23:427–434, 2005
132. Barbagallo M, Dominguez LJ, Tagliamonte MR, Resnick LM, Paolisso G: Effects of vitamin E and glutathione on glucose metabolism: role of magnesium. Hypertension 34:1002–1006, 1999
133. Mishra GD, Malik NS, Paul AA, Wadsworth ME, Bolton-Smith C: Childhood and adult dietary vitamin E intake and cardiovascular risk factors in mid-life in the 1946 British Birth Cohort. *Eur J Clin Nutr* 57:1418–1425, 2003
134. Poston L, Raijmakers M, Kelly F: Vitamin E in preeclampsia. *Ann N Y Acad Sci* 1031:242–248, 2004
135. Rumiris D, Purwosunu Y, Wibowo N, Farina A, Sekizawa A: Lower rate of preeclampsia after antioxidant supplementation in pregnant women with low antioxidant status. *Hypertens Pregnancy* 25:241–253, 2006
136. Poston L, Briley AL, Seed PT, Kelly FJ, Shennan AH: Vitamins in Pre-eclampsia (VIP) Trial Consortium. Vitamin C and vitamin E in pregnant women at risk for pre-eclampsia (VIP trial): randomised placebo-controlled trial. *Lancet* 367: 1145–1154, 2006
137. Rumbold AR, Crowther CA, Haslam RR, Dekker GA, Robinson JS: ACTS Study Group: Vitamins C and E and the risks of preeclampsia and perinatal complications. *N Engl J Med* 354:1796–1806, 2006
138. Beazley D, Ahokas R, Livingston J, Griggs M, Sibai BM: Vitamin C and E supplementation in women at high risk for preeclampsia: a double-blind, placebo-controlled trial. *Am J Obstet Gynecol* 192:520–521, 2005
139. Skyrme-Jones RA, O'Brien RC, Berry KL, Meredith IT: Vitamin E supplementation improves endothelial function in type I diabetes mellitus: a randomized, placebo-controlled study. *J Am Coll Cardiol* 36:94–102, 2000
140. Appel LJ, Moore TJ, Obarzanek E, Vollmer WM, Svetkey LP, Sacks FM, Bray GA, Vogt TM, Cutler JA, Windhauser MM, Lin PH, Karanja N: A clinical trial of the effects of dietary patterns on blood pressure: DASH Collaborative Research Group. *N Engl J Med* 336:1117–1124, 1997
141. John JH, Ziebland S, Yudkin P, Roe LS, Neil HAW: Effects of fruit and vegetable consumption on plasma antioxidant concentrations and blood pressure: a randomized controlled trial. *Lancet* 359: 1969–1973, 2002
142. Lopes HF, Martin KL, Nashar K, Morrow JD, Goodfriend TL, Egan BM: DASH diet lowers blood pressure and lipid-induced oxidative stress in obesity. *Hypertension* 41:422–430, 2003
143. Cai H, Griendling KK, Harrison DG: The vascular NAD(P)H oxidases as therapeutic targets in cardiovascular diseases. *Trends Pharmacol Sci* 24:471–478, 2003
144. Wu R, Lamontagne D, de Champlain J Antioxidative properties of acetylsalicylic acid on vascular tissues from normotensive and spontaneously hypertensive rats. *Circulation* 105:387–392, 2002
145. Huang HY, Caballero B, Chang S, Alberg AJ, Semba RD, Schneyer CR, Wilson RF, Cheng TY, Vassy J, Prokopowicz G, Barnes GJ 2nd, Bass EB: The efficacy and safety of multivitamin and mineral supplement use to prevent cancer and chronic disease in adults: a systematic review for a National Institutes of Health state-of-the-science conference. *Ann Intern Med* 145:372–385, 2006
146. Tribble DL: Antioxidant consumption and risk of coronary heart disease: emphasis on vitamin C, vitamin E and β-carotene: a statement for the healthcare professionals from the American Heart Association. *Circulation* 99:591–595, 1999
147. Touyz RM, Campbell N, Logan A, Gledhill N, Petrella R, Padwal R: Canadian Hypertension Education Program: The 2004 Canadian recommendations for the management of hypertension: Part III. Lifestyle modifications to prevent and control hypertension. *Can J Cardiol* 20:55–83, 2004
148. Wang JS, Lee T, Chow SE: Role of exercise intensities in oxidized low-density lipoprotein-mediated redox status of monocyte in men. *J Appl Physiol* 101:740–744, 2006
149. Adams V, Linke A, Krankel N, Erbs S, Gielen S, Mobius-Winkler S, Gummert JF, Mohr FW, Schuler G, Hambrecht R: Impact of regular physical activity on the NAD(P)H oxidase and angiotensin receptor system in patients with coronary artery disease. *Circulation* 111:555–562, 2005
150. Pan YX, Gao L, Wang WZ, Zheng H, Liu D, Patel KP, Zucker IH, Wang W: Exercise training prevents arterial baroreflex dysfunction in rats treated with central angiotensin. *Hypertension* 49:519–527, 2007
151. Godfraind T: Antioxidant effects and the therapeutic mode of action of calcium channel blockers in hypertension and atherosclerosis. *Philos Trans R Soc Lond B Biol Sci* 360:2259–2272, 2005
152. Oliveira PJ, Goncalves L, Monteiro P, Providencia LA, Moreno AJ: Are the antioxidant properties of carvedilol important for the protection of cardiac mitochondria? *Curr Vasc Pharmacol* 3:147–158, 2005
153. Cifuentes ME, Pagano PJ: Targeting reactive oxygen species in hypertension. *Curr Opin Nephrol Hypertens* 15: 179–186, 2006
154. Dandona P, Kumar V, Aljada A, Ghanim H, Syed T, Hofmayer D, Mohanty P, Tripathy D, Garg R: Angiotensin II receptor blocker valsartan suppresses reactive oxygen species generation in leukocytes, nuclear factor-kappa B, in mononuclear cells of normal subjects: evidence of an antiinflammatory action. *J Clin Endocrinol Metab* 88:4496–4501, 2003
155. Khan BV, Navalkar S, Khan QA, Rahman ST, Parthasarathy S: Irbesartan, an angiotensin type 1 receptor inhibitor, regulates the vascular oxidative state in patients with coronary artery disease. *J Am Coll Cardiol* 38:1662–1667, 2001
156. Dandona P, Karne R, Ghanim H, Hamouda W, Aljada A, Magsino CH: Carvedilol inhibits reactive oxygen species generation by leukocytes and oxidative damage to amino acids. *Circulation* 101:122–124, 2000
157. Flammer AJ, Hermann F, Wiesli P, Schwegler B, Chenevard R, Hürlimann D, Sudano I, Gay S, Neidhart M, Riesen W, Ruschitzka F, Lüscher TF, Noll G, Lehmann R: Effect of losartan, compared with atenolol, on endothelial function and oxidative stress in patients with type 2 diabetes and hypertension. *J Hypertens* 25:785–791, 2007