Oxidative Stress and Vascular Disease

Nageswara R. Madamanchi, Aleksandr Vendrov, Marschall S. Runge

Abstract—Growing evidence indicates that chronic and acute overproduction of reactive oxygen species (ROS) under pathophysiologic conditions is integral in the development of cardiovascular diseases (CVD). These ROS can be released from nicotinamide adenine dinucleotide (phosphate) oxidase, xanthine oxidase, lipoxygenase, mitochondria, or the uncoupling of nitric oxide synthase in vascular cells. ROS mediate various signaling pathways that underlie vascular inflammation in atherogenesis: from the initiation of fatty streak development through lesion progress to ultimate plaque rupture. Various animal models of oxidative stress support the notion that ROS have a causal role in atherosclerosis and other cardiovascular diseases. Human investigations also support the oxidative stress hypothesis of atherosclerosis. Oxidative stress is the unifying mechanism for many CVD risk factors, which additionally supports its central role in CVD. Despite the demonstrated role of antioxidants in cellular and animal studies, the ineffectiveness of antioxidants in reducing cardiovascular death and morbidity in clinical trials has led many investigators to question the importance of oxidative stress in human atherosclerosis. Others have argued that the prime factor for the mixed outcomes from using antioxidants to prevent CVD may be the lack of specific and sensitive biomarkers by which to assess the oxidative stress phenotypes underlying CVD. A better understanding of the complexity of cellular redox reactions, development of a new class of antioxidants targeted to specific subcellular locales, and the phenotype-genotype linkage analysis for oxidative stress will likely be avenues for future research in this area as we move toward the broader use of pharmacological and regenerative therapies in the treatment and prevention of CVD. (Arterioscler Thromb Vasc Biol. 2005;25:29-38.)

Key Words: reactive oxygen species • NAD(P)H oxidase • mitochondria • atherosclerosis • antioxidants

Cardiovascular diseases (CVD)—coronary artery disease, hypertension, congestive heart failure, and stroke—are the leading cause of death and disability in the Western world. In the United States, the CVD death toll is nearly one million each year, and in 2002 the estimated cost of CVD treatment was $326.6 billion. To provide early prognosis and better therapies for preventing and curing these diseases, an understanding of the basic pathophysiologic mechanisms of CVD is essential.

Atherosclerosis: An Overview

The majority of cardiovascular disease results from complications of atherosclerosis. An important initiating event for atherosclerosis may well be the transport of oxidized low-density lipoprotein (Ox-LDL) across the endothelium into the artery wall. This is likely to occur at the sites of endothelial damage which are caused by Ox-LDL itself as well as physical or chemical forces and infection (Figure 1). Endothelial cells, smooth muscle cells (SMCs), and macrophages are the sources of oxidants for the oxidative modification of phospholipids. Ox-LDL can damage endothelial cells and induce the expression of adhesion molecules such as P-selectin and chemotactic factors such as monocyte chemoattractant protein-1 and macrophage colony stimulating factor (CSF). These processes lead to the tethering, activation, and attachment of monocytes and T lymphocytes to the endothelial cells. Endothelial cells, leukocytes, and SMCs then secrete growth factors and chemoattractants which effect the migration of monocytes and leukocytes into the subendothelial space. Monocytes ingest lipoproteins and morph into macrophages; macrophages generate reactive oxygen species (ROS), which convert Ox-LDL into highly oxidized LDL, which is, in turn, taken up by macrophages to form foam cells. Foam cells combine with leukocytes to become the fatty streak, and as the process continues foam cells secrete growth factors that induce SMC migration into the intima. SMC proliferation, coupled with the continuous influx and propagation of monocytes and macrophages, converts fatty streaks to more advanced lesions and ultimately to a fibrous plaque that will protrude into the arterial lumen. Later, calcification can occur and fibrosis continues, yielding a fibrous cap that surrounds a lipid-rich core. This formation may also contain dead or dying SMCs. In acute coronary syndromes (eg, myocardial infarction), when fibrous plaques rupture (Figure 1), the formation and release of thrombi may ultimately occlude vessels.

Oxidative Stress and Atherosclerosis

Oxygen is an abundant molecule in biological systems. Despite being a radical, it is sparingly reactive because its two
unpaired electrons are situated in different molecular orbitals and demonstrate parallel spins. Thus, oxygen undergoes univalent reduction to form superoxide ($O_2^-$) by means of enzymes such as the nicotinamide adenine dinucleotide (phosphate) (NADH/NAD(P)H) oxidases and xanthine oxidases (XO) (Figure 2). Nonenzymatically, oxygen can also become $O_2^-$ by reacting with redox active compounds such as semiquinone of the mitochondrial electron transport chain. Superoxide anion is dismutated enzymatically to become hydrogen peroxide ($H_2O_2$) through the action of superoxide dismutases (SODs). In biological tissues, $O_2^-$ can also undergo nonenzymatic transformation into $H_2O_2$ and singlet oxygen ($^1O_2$). $H_2O_2$ can react with other radicals such as transition metal $Fe^{2+}$ to produce highly reactive hydroxyl radicals (OH); this is known as the Fenton reaction (Figure 2). These radicals are capable of destroying biomolecules through oxidation. When $Fe^{3+}$ initially oxidizes $O_2^-$, molecular oxygen and $Fe^{2+}$ are generated; the $Fe^{2+}$ initiates the Fenton reaction and this regenerates $Fe^{3+}$ which perpetuates the production of OH. Myeloperoxidase, a heme protein secreted by phagocytes, can amplify the oxidative potential of $H_2O_2$. At physiological concentrations of Cl-, hypochlorous acid (HOCl) is the major oxidant generated by the myeloperoxidase–$H_2O_2$–Cl$^-$ system, and HOCl can react with $O_2^-$ to produce OH.

**Figure 2.** Sources of ROS in vascular cells. Activated NAD(P)H oxidase, 12/15-LO and XO generate superoxide ($O_2^-$). NOS switches from a coupled state to an uncoupled state and generates $O_2^-$ with decreased availability of 5,6,7,8-tetrahydrobiopterin (BH$_4$) or L-arginine. Dysfunctional mitochondrial respiratory chain is another source of $O_2^-$ generation. SOD isoforms, Mn SOD, and CuZn SOD dismutate $O_2^-$ to produce hydrogen peroxide ($H_2O_2$). Myeloperoxidase generates HOCl from $H_2O_2$ in the presence of Cl$^-$. $H_2O_2$ reacts with transition metals to produce hydroxyl radicals (OH).

**NAD(P)H Oxidase**

Recent evidence indicates that membrane-bound NAD(P)H oxidases are the major source of $O_2^-$ generation, and both NAD(P)H oxidase–derived $O_2^-$ and mitochondrial-derived $O_2^-$ constitute the bulk of this radical in the vasculature. However, the vascular-derived NAD(P)H oxidase has a similar but distinct structure from the phagocytic enzyme. The phagocytic oxidase contains the membrane-bound subunits gp91phox (Nox2) and p22phox, the catalytic site of the oxidase and the cytosolic components p47phox, p67phox, and G-protein rac1 or rac2. Endothelial cells and adventitial fibroblasts possess all the components of phagocytic oxidase.

**Figure 1.** Development of atherosclerosis. ROS produced by endothelial cells, SMCs, and macrophages oxidize LDL in the subendothelial space, at the sites of endothelial damage, initiating events that culminate in the formation of a fibrous plaque. Rupture of fibrous plaque leads to thrombus formation and occlusion of the vessel.
cytic cells and VSMCs is that the latter do not appear to possess p67phox.25 The comprehensive term “vascular NAD(P)H oxidase” will be used henceforth to include the NAD(P)H oxidases present in VSMCs, endothelial cells, and adventitial fibroblasts. Vascular oxidase is active during normal metabolism, and a sustained activation of this enzyme occurs in response to agonists. Also, vascular oxidase produces much less superoxide (by several orders of magnitude) than does phagocytic oxidase.26 One of the early steps in the activation of the NAD(P)H oxidase is the phosphorylation and translocation of p47phox.27

Cell culture studies and animal models provide evidence for the critical role of NAD(P)H oxidase–derived oxidative stress in atherosclerosis. NAD(P)H oxidase and O$_2^-$ production are increased in vascular cells by a number of agonists associated with the pathogenesis. These agonists include angiotensin II (Ang II), thrombin, platelet-derived growth factor (PDGF), and tumor necrosis factor (TNF)-α.25,28–30 Depending on vascular flow conditions, temporal regulation of NAD(P)H oxidase also occurs. Cell culture studies have shown that the atheroprotective laminar shear stress down-regulates NAD(P)H oxidase activity, whereas atherogenic oscillatory shear stress induces a sustained increase in the oxidase activity.31 In hypercholesterolemic rabbits prone to atherosclerosis, enhanced AT1 receptor regulation and increased NADH-dependent vascular O$_2^-$ production were associated with endothelial dysfunction.32 AT1 receptor antagonists not only inhibited the oxidase and improved endothelial function but also reduced early plaque formation, suggesting that oxidative stress plays a central role in the early stages of atherosclerosis. Recently, using NAD(P)H oxidase–deficient mice, we reported evidence for this.33 Mice lacking the p47phox gene not only had lower levels of aortic O$_2^-$ production compared with wild-type mice, but also, when in a hypercholesterolemic apolipoprotein E–deficient (apoE(−/−)) background, had significantly fewer lesions in their descending aortas compared with apoE (−/+/-) mice. Further support for NAD(P)H oxidase–induced oxidative stress in neointimal hyperplasia comes from studies of balloon-injured porcine34 and rat35 coronary arteries. In the rat balloon injury model, Nox1 and p22phox were upregulated for 3 to 15 days, gp91phox for 7 to 15 days, and Nox4 at only 15 days after injury, suggesting not only a spatiotemporal but also a dynamic and differential regulation of the NAD(P)H-oxidase isoforms. In the rat carotid artery model, use of the gp91 ds-Tat peptide, a chimeric peptide consisting of a fragment from the Tat peptide of the HIV virus plus a fragment of gp91phox that prevents the interaction of p47phox with Nox subunits in cell free systems, provided direct evidence for the role of this oxidase in angioplasty-induced neointimal hyperplasia.36 The Tat fragment allowed the ready uptake of the peptide inhibitor into the cells and blocked both ROS production and neointima/media area and thickness. After angioplasty in these animals, the Tat peptide also inhibited peroxynitrite formation and stretch-induced ROS generation in distended vessels. Together these data clearly show that NAD(P)H oxidase–induced oxidative stress is causal in atherosclerosis.

In human studies there is also considerable evidence to show the importance of NAD(P)H oxidase–derived oxidative stress in atherosclerosis. High levels of vasoactive agonists that induce oxidative stress in vitro were observed in human atherosclerotic plaques. Ang II was observed at the shoulder regions—the presumed rupture site of atherosclerotic plaques in human coronary arteries—in acute myocardial infarction; Ang II also colocalizes with the AT1 receptor.37 Similarly, increased expression of the thrombin receptor was observed in human atheroma.38 Recently, Azumi et al39 reported that in atherosclerotic human coronary arteries ROS production and Ox-LDL are spatially associated with NAD(P)H oxidase subunit p22phox. This suggests that ROS catalyze the formation of Ox-LDL, which leads to its uptake by macrophages and thus results in forming activated foam cells.40 These investigators also reported that ROS production was significantly higher in unstable versus stable angina pectoris, which suggests that ROS might also modulate plaque stability. These results are further supported by the observation that intimal SMCs, but not medial SMCs and macrophages, express high levels of NAD(P)H oxidase subunits.41 Plaque instability is initiated when ROS induce the expression of matrix-degrading enzymes such as matrix metalloproteinase (MMP)-2 and MMP-9.42 ROS also induce apoptosis in endothelial and SMCs through Ox-LDL.43

Other Oxidase Systems

XO generates O$_2^-$ by catalyzing hypoxanthine and xanthine to uric acid. Under pathophysiologic conditions, this is another major source of vascular oxidative stress.44 Xanthine oxidase exists in plasma and endothelial cells but not in SMCs.18 In hypercholesterolemic rabbits, atherosclerosis resulting from diet was ascribed to XO activation–induced oxidative stress.45 In hypercholesterolemic patients, vasodilation is improved by using the XO inhibitor oxypurinol. The role for XO in atherosclerosis is further corroborated by the following observations:46

1. In the coronary arteries of patients with coronary artery disease (CAD), electron spin resonance studies show significant activation of both NAD(P)H oxidase and XO;
2. In these same patients, endothelial XO is inversely proportional and positively related to the effect of vitamin C on endothelium-dependent vasodilation; and
3. In asymptomatic young individuals with familial hypercholesterolemia, the increase of vascular XO activity is an early event.

In addition, lipoxygenases are another important source of ROS production in the vascular wall; these non-heme containing dioxygenases oxidize polyunsaturated fatty acids to hydroperoxy fatty-acid derivatives.47 Leukocyte-type 12/15-lipoxygenase (LO) and its products, 12(S)-hydroxyeicosatetraenoic acid[12(s)-HETE] and 15(S)-HETE, are also implicated in atherogenesis. In addition, homoyzous deletion of the 12/15-LO gene caused remarkable inhibition of early atherosclerosis in apoE-deficient mice.48 Inhibiting 12/15-LO reduced blood pressure in hypertensive rats49 and blocked intimal hyperplasia in balloon-injured rat carotid arteries.47 These findings bolster support for the role of this enzyme in vascular pathology. Furthermore, 12/15-LO activation leads
to SMC growth, hypertrophy, and inflammatory gene expression; SMCs deficient in the enzyme show reduced generation of mitogen-induced ROS.46,49

**Uncoupling of NOS**

Nitric oxide synthases (NOSs), and in particular endothelial NOS (eNOS), can be potential sources of O2− under certain pathophysiologic conditions.18 Under normal conditions, these enzymes transfer electrons from a heme group in the oxygenase domain to the substrate l-arginine to form l-citrulline and nitric oxide (NO); 5,6,7,8-tetrahydrobiopterin (BH4) serves as a cofactor in this process.50 If the availability of either BH4 or l-arginine decreases, eNOS switches from a coupled state (generates NO) to an uncoupled state (generates O2−) because the electrons from the heme reduce oxygen to form O2− (Figure 2). Increased vascular O2− production not only alters endothelium-dependent vascular relaxation through interaction with NO, but the resultant peroxynitrite can also oxidize BH4. This causes a deficiency of BH4 and the pathogenic uncoupling of NOS.51 It has been further reported that NAD(P)H oxides are necessary for BH4 oxidation; this process does not occur in p47phox (−/−) mice that are hypertensive.52 The majority of studies report that NO induction decreases atherosclerosis in hypercholesterolemic animals and improves vascular function in hypercholesterolemic or hyperhomocysteinemic humans (see Cooke and Sydow, 2003).53

**Mitochondrial ROS**

Mitochondria provide energy (adenosine triphosphate; ATP) to the cell through oxidative phosphorylation. Oxidative phosphorylation is the process by which ATP is formed as electrons are transferred from NADH or FADH2 (generated through the Krebs cycle) to molecular oxygen. This occurs through a series of electron transport carriers localized in the inner mitochondrial membrane. The electron transport carriers include: complex I (NADH-ubiquinone oxidoreductase), complex II (succinate-ubiquinone oxidoreductase), complex III (ubiquinol-cytochrome c reductase), and complex IV (cytochrome c oxidase) (Figure 3). The transfer of more than 98% of electrons by the electron transport carriers/chains is coupled with the production of ATP. Only 1% to 2% of electrons leak out to form O2−, and this is scavenged by manganese SOD (MnSOD/SOD2). However, during mitochondrial oxidative phosphorylation under pathophysiologic conditions, the electron transport chain may become uncoupled, leading to increased O2− production.54

Mitochondrial DNA (mtDNA) is prone to oxidative damage because of several factors. These include the proximity of mtDNA to the sources of ROS generation in the mitochondrial inner membrane, the lack of protective histone-like proteins, and poor DNA damage-repair activity.55 MtDNA damage eventually results in reduced mtRNA transcription and, therefore, a loss of function.56 In accordance with this, we have shown that exogenous ROS-mediated mtDNA damage decreases mtDNA-encoded gene transcription in a dose-dependent manner.57 We have also demonstrated that the extent of atherosclerosis correlated well with mtDNA damage in the aortas from humans and apoE (−/−) mice and preceded atherogenicity in young apoE (−/−) mice.58 Furthermore, apoE (−/−) mice deficient in SOD2 showed increased mtDNA damage at earlier stages as well as an accelerated atherogenesis phenotype at arterial branch points. Together, the above reports indicate that mitochondrial ROS are clearly associated with enhanced susceptibility to atherosclerosis. Future and clinical trials are imperative to ascertain whether mtDNA damage can be used as a diagnostic oxidative phenotypic marker for atherosclerosis.

**Oxidative Stress and Hypertension**

More than 50 million Americans experience systolic hypertension,59 and hypertension is a risk factor for many other vascular diseases including atherosclerosis and stroke. The molecular basis of hypertension is complex; more than 50 genes have been implicated in the regulation of blood pressure.60 However, in the recent past, the role of the AT1 receptor in regulating hypertension has been the subject of intense investigation in both in vitro and animal models. Ang II modulates hypertension through its effect on the renin-angiotensin system, and the stimulation of AT1 receptors in the vascular wall leads to activation of NADH/NAD(P)H oxidase in vascular cells. The resultant oxidative stress is considered a unifying mechanism for hypertension and atherosclerosis.61,62 In addition to its indirect effect on NAD(P)H oxidase activation through AT1 receptor stimulation, Ang II can also directly regulate NAD(P)H activation by inducing a rapid translocation of small GTPase rac1 to the cell membrane.63 or by phosphorylating and translocating p47phox to cell membrane (Figure 4).64 Mechanical stretch, a hallmark of arterial hypertension, was recently shown to induce p47phox membrane translocation and NAD(P)H oxidase activation in VSMC.24 Stretch-stimulated NAD(P)H oxidase activation was absent in p47phox (−/−) cells and might play an
important role in hypertension-induced vessel wall remodeling through activation of MMP-2.

The critical role for oxidative stress in hypertension was demonstrated in animal models: when spontaneously hypertensive rats were treated with statins, $\text{O}_2^-$ production and AT1-receptor activation decreased with a concomitant reduction in blood pressure.65 As mentioned above, the chimeric gp91ds-Tat peptide that inhibits NAD(P)H oxidase activation also attenuated Ang II-induced ROS production and blood pressure increases in mice.66 Similarly, a hypertensive response to Ang II infusion was blunted in p47phox (−/−) mice.67 Transgenic mice that expressed constitutively active rac1 in VSMCs exhibited a hypertensive phenotype; increased ROS production and treatment with antioxidants reversed hypertension.68 Oxidative stress induced by elevated 12-LO has also been implicated in hypertension in rat models.69 This suggests that multiple oxidative mechanisms are involved in hypertension.69 Consistent with this, increased levels of 12-LO and its product, 12-hydroperoxyeicosatetraenoic acid [12-(S)-HETE], were observed in patients with essential hypertension.70 Treatment of hypertensive patients with AT1 receptor blockers not only reduced blood pressure and diminished levels of malondialdehyde (MDA), a marker of oxidative stress,71 but also reversed hypertension-induced wall-structural changes in resistance arteries.72 Together, these observations strongly suggest that oxidative stress is a modulator of hypertension, a risk factor for atherosclerosis.

Oxidative Stress and Heart Failure

The importance of oxidative stress in chronic heart failure can be gauged by the fact that antioxidants prevent the progression of several pathological processes—such as cardiac hypertrophy, cardiac myocyte apoptosis, ischemia-reperfusion, and myocardial stunning—which lead to heart failure in animal models.73 In rat cardiac myocytes, TNF-α and Ang II induced hypertrophy in a ROS-dependent manner; antioxidant use, including butylated hydroxyanisole, vitamin E, and catalase, prevented this.74 Overexpression of catalase significantly reduced Ang II–induced hypertrophy, and transfection with antisense p22phox inhibited Ang II–induced H$_2$O$_2$ production. This suggests that NAD(P)H oxidase–induced oxidative stress led to the hypertrophy.75 During compensated hypertrophy in a guinea pig model, NAD(P)H oxidase–dependent ROS production significantly and progressively increased to a peak at the level of decompensated heart failure. This indicates that ROS may be important mediators of heart failure.76 Other sequelae in the failing heart which may result from XO77 and mitochondria78 include structural damage and contractile dysfunction. Increased production of ROS may decrease NO bioavailability and impair diastolic function.79 In addition, increased peroxynitrite may cause cytokine-induced myocardial contractile failure by inactivating sarcomplasmatic Ca$^{2+}$-ATPase and dysregulating Ca$^{2+}$ homeostasis.80,81

Emerging evidence demonstrates that oxidative stress in general and NAD(P)H oxidase–derived ROS in particular are important in human cardiac failure. In the failing myocardium of patients with ischemic or dilated cardiomyopathy, NAD(P)H oxidase–derived ROS were upregulated.82 Plasma TNF-α levels and platelet-derived NAD(P)H oxidase activity were also elevated in patients with heart failure.83 In addition, NAD(P)H oxidase activation and increased translocation of regulatory p47phox from the cytosol to the sarcolemmal membrane were recently observed in failing human myocardium.84 These combined results suggest that oxidative stress has a role in the pathophysiologic cardiac dysfunction in heart failure.

Oxidative Stress and Stroke

Results from a number of studies implicate oxidative stress in brain injury after ischemia and reperfusion. ROS produced during cerebral ischemia induce lipid peroxidation, protein oxidation, and DNA damage.85,86 In rodents, manipulating the genes responsible for antioxidant enzyme production provided the clearest evidence for oxidative stress in ischemia-induced brain injury. In SOD2 knockout mice, exacerbated infarct size and upregulated oxidative stress markers such as increased mitochondrial cytochrome c release and DNA fragmentation were observed after permanent focal cerebral ischemia.87,88 In contrast, mice that overexpress SOD2 showed neuronal protection.89 Similarly, a reduction in blood-brain barrier disruption and infarct size along with decreased oxidative DNA damage and DNA fragmentation were observed after photothrombotic ischemia in copper/zinc
SOD (SOD1) transgenic mice as compared with wild-type mice. In a mouse ischemia-reperfusion model, deficiency of the antioxidant enzyme GPx-1 resulted in a threelfold increase in brain infarct volume, early activation of caspase-3 expression, and enhanced apoptosis when compared with the wild-type mouse. Consistent with this, infusion of ebselel, a GPx mimic, before and during middle cerebral artery occlusion in rats conferred significant protection against ischemic damage. Support for the role of oxidative stress in cerebral ischemia was also obtained from mice deficient in Nos isoforms. In separate studies of mice deficient for neuronal (nNOS) and inducible NOS (iNOS) isoforms, reduced infarct size was observed after permanent focal cerebral ischemia. In endothelial-type Nos isoform (eNOS) knockout mice, increases in lesion volume were observed. In the brains of stroke-prone spontaneously hypertensive rats, electron spin resonance spectroscopy revealed high oxidative stress when compared with Wistar-Kyoto rats. Glutathione concentrations decreased before cerebral infarction in severe transient focal cerebral ischemia in rats which suggests that early oxidative stress might contribute to cerebral damage in stroke.

Significant increases in plasma homocysteine, lipid peroxide, and NO, and decreased ascorbate levels were observed in stroke patients as compared with healthy controls. Also compared with healthy individuals, the circulating phagocytes of patients with ischemic stroke showed increased ROS production through mechanisms that were both opsonin receptor–dependent and –independent. These observations offer cumulative evidence that oxidative stress plays a distinct role in the pathogenesis of ischemic brain injury.

**CVD and Oxidative Stress Risk Factors**

Diabetes is a risk factor for atherosclerosis and, like atherosclerosis, is progressive and associated with enhanced oxidative stress. In diabetic patients, enhanced oxidative stress in hyperglycemia is indicated by increased levels of the urinary isoprostane 8-epi-PGF2α; with vitamin E treatment, it decreased significantly. In the recently concluded Diabetes Control and Complications Trial, a 6-year follow-up study revealed a significant increase in intima-media thickness (IMT) in diabetic patients compared with healthy subjects. After six years, intensive diabetes treatment decreased IMT progression, which clearly underlines the association of diabetes and atherosclerosis.

Both insulin-dependent diabetes mellitus (type 1) and noninsulin-dependent (type 2) diabetes are characterized by hyperglycemia. Hyperglycemia can induce oxidative stress by several different mechanisms. Autoxidation of glucose and the nonenzymatic glycation of proteins generate O2−. Also, hyperglycemia induced by intra-arterial injection of dextrose impaired endothelium-dependent vasodilatation, which was restored in nondiabetic subjects when they were treated with vitamin C. Glucose can also directly react with LDL phospholipids and apolipoprotein B (apoB) lysine groups to form the advanced glycation end products (AGEs) that facilitate lipid peroxidation. Consistent with this, an increased concentration of AGEs was correlated with endothelial dysfunction in patients with type 2 diabetes. In cultured bovine aortic endothelial cells, inhibiting elevated mitochondrial O2− blocked hyperglycemia-induced damage. Inhibition was accomplished through several pathway-specific means: (1) inhibiting electron transport chain complex II; (2) uncoupling oxidative phosphorylation; and (3) overexpression of uncoupling protein-1 or SOD2 (Figure 3). This suggests that in diabetes, mitochondrial ROS act through several independent pathways. Depletion of BH4, an essential cofactor for Nos, or l-arginine, a precursor of Nos, impaired endothelium-dependent vasorelaxation in the aortic rings of streptozotocin-induced diabetic rats. Similarly, endothelium-dependent vasodilatation that had been attenuated in response to acetylcholine was improved in type 2 diabetic patients treated with BH4.

As in atherosclerosis, activation of NAD(P)H oxidase and enhanced O2− production were observed in the aortas of streptozotocin-induced diabetic rats. Inhibition of Nos with Nω-nitro-l-arginine increased O2− levels in control vessels but reduced levels in diabetic vessels, which identifies Nos as a O2− source. As described earlier, Nos (NOSI and NOSIII) may become uncoupled in the presence of low levels of l-arginine or BH4 thus leading to the generation of O2− instead of NO. Depletion of l-arginine occurs in dyslipidemic conditions because Ox-LDL can inhibit endothelial l-arginine uptake. In contrast, peroxynitrite produced from the NO/O2− reaction can oxidize BH4 to inactive dihydrobipterin. Hyperglycemia might also induce the dedifferentiation of aortic SMCs, which then produce more O2− and quench NO even when NO synthetic pathways are enhanced. Atherosclerotic lesions are initiated at reduced or disturbed oscillatory shear stress points in the arterial tree; reduced coronary shear stress was observed in diabetic patients compared with age-matched controls, clearly indicating that uncontrolled hyperglycemia leads to atherosclerosis.

Other risk factors for vascular disease, such as obesity and cigarette smoking, are associated with systemic oxidative stress. In dyslipidemic obese mice, impaired high-density lipoprotein (HDL) defense and increased LDL oxidation were associated with increased atherosclerosis. The HDL-associated enzymes paraoxonase (PON1) and lecithin:cholesterol acyltransferase (LCAT) inhibit the oxidation of LDL. Transient overexpression of LCAT has led to a decrease in oxidative stress as measured by both a reduction in autoantibodies against MDA-modified LDL and a decrease in atherosclerosis. PON1 knockout mice were susceptible to atherosclerosis and exhibited enhanced expression of oxidative stress–related genes. Significantly lower levels of PON1 activity and concentration were observed in CAD patients compared with control subjects, which strongly suggests a role for PON1 in dyslipidemia-associated atherosclerosis.

A correlation between increasing body-mass index and enhanced systemic oxidative stress, as measured by urinary F2-isoprostanes, was observed in 3000 patients involved in the Framingham Heart Study. In addition, android obesity is also associated with elevated urinary F2-isoprostane levels. Cigarette smoke is replete with a number of oxidants, and this, smokers have increased F2-isoprostane levels in both plasma and urine. In animal models, brief exposure to cigarette smoke promoted atherosclerotic plaque development, and the combination of secondhand smoke and...
hypercholesterolemia resulted in a greater extent of mtDNA damage and atherosclerosis. In addition, benzo[a]pyrene, a polycyclic aromatic hydrocarbon present in tobacco smoke, can modulate VSMCs from a quiescent to a proliferative phenotype both in vitro and in vivo. 

**Antioxidants and Vascular Diseases**

Despite the preponderance of evidence for the association of increased oxidative stress with various vascular diseases, using antioxidants to prevent CVD has produced mixed outcomes. Of the 12 studies that used antioxidant vitamins at varying concentrations and follow-up times, 5 showed benefit with regard to their respective primary end points. In the Cambridge Heart Antioxidant Study (CHAOS), natural α-tocopherol (RRR-AT) at a dose of either 400 or 800 IU/d caused a significant reduction in the combined primary end point of cardiovascular death and nonfatal myocardial infarction. In the Secondary Prevention with Antioxidants of Cardiovascular disease in End-stage renal disease (SPACE) study, administering 800 IU/d RRR-AT to hemodialysis patients with preexisting cardiovascular disease significantly reduced the composite primary end point, which included fatal and nonfatal myocardial infarction; ischemic stroke; peripheral vascular disease; and unstable angina. An investigation of transplant-associated atherosclerosis with a small sample size (total N=40) revealed that progression of the coronary intimal index (plaque area/vessel area) was inhibited with the combined supplementation of RRR-AT (800 IU/d) and ascorbic acid (AA: 1000 mg/d). In the Antioxidant Supplementation in Atherosclerosis Prevention (ASAP) study (N=440), a combination of RRR-AT (272 IU/d) and slow-release AA (500 mg/d) significantly decreased carotid intima-media thickness in hypercholesterolemic males. During 16 years of follow-up with the women in the Nurses' Health Study (N=85,118), vitamin C intake of >359 mg/d from diet plus supplements or supplement use alone was associated with a significant reduction in nonfatal and fatal myocardial infarction. Contrary to these positive study outcomes, 7 other antioxidant supplementation studies did not show any effect on the primary end points of cardiovascular events (reviewed in Jalal and Devaraj, 2003).

The apparent inability for antioxidants to prevent CVD may be attributable to several reasons. One reason is that although antioxidant supplementation can be effective with a proved methodology for oxidative stress, we are still unable to deduce a priori those subjects who will be responders or nonresponders. Another reason for antioxidant ineffective-ness could relate to the optimum dose and type of antioxidants being used; efficacious threshold doses have been suggested at 800 IU/d of RRR-AT and 500 mg/d of AA. However, a key issue is antioxidant formulation; 5 of 7 antioxidant supplementation trials that reported inefficacy on primary end points used all-racemic-AT (all-rac-AT), but 4 trials described above with successful results used RRR-AT. The third reason why antioxidants are not yet successful in CVD prevention could be the complexity of redox reactions in vivo and the potential for a paradoxical increase in oxidant generation by antioxidants themselves. For instance, vitamin C supplementation exerted prooxidant as well as antioxidant effects in healthy volunteers; however, high doses of this antioxidant increased DNA damage. It was hypothesized that transition metal ions, released from metalloproteins after initial oxidative stress, act as catalysts in the presence of antioxidants to exacerbate the free radical-induced damage. Ongoing research to further elucidate the connection between oxidative stress and CVD is necessary to distinguish the effective use of antioxidant vitamins for the primary prevention of vascular pathologies. In the meantime, we must continue to encourage patients to adopt healthy diets and lifestyles, and to carefully adhere to pharmacological regimens with such agents as statins and ACE inhibitors, to best protect themselves from the potential for vascular disease.

**Conclusions**

In conclusion, growing evidence from investigations of animal models and correlative data from human studies implicate oxidative stress in the development of CVD. However, a better understanding of the ROS-dependent signal transduction mechanisms, their localization, and the integration of both ROS-dependent transcriptional and signaling pathways in vascular pathophysiology is a prerequisite for effective pharmacological interventions for CVD. The development of a new class of antioxidants that are targeted to specific subcellular compartments such as mitochondria may help in combating CVD. Another important step toward effective treatment will be the development of sensitive and specific biomarkers that can be used clinically to assess the oxidative stress phenotypes that underlie various vascular pathologies. Ultimately, a phenotype–genotype linkage analysis that takes advantage of the recent advances in mouse and human genetics will be of immense help in the prognosis of people at risk for CVD. This should be complemented by studies that phenotype vascular cells and their progenitors, which will further contribute to the development of regenerative therapies. For example, regenerative therapies with mitochondria-rich endothelial progenitor cells may enhance myocardial activity and angiogenesis. Ultimately, remedial measures for oxidative stress–induced vascular malfunction will use a combination of preventive and regenerative therapies.

**References**

36 Arterioscler Thromb Vasc Biol. January 2005


128. Frei B. To C or not to C, that is the question! *J Am Coll Cardiol*. 2003;42:253–255.


Oxidative Stress and Vascular Disease
Nageswara R. Madamanchi, Aleksandr Vendrov and Marshall S. Runge

Arterioscler Thromb Vasc Biol. 2005;25:29-38; originally published online November 11, 2004; doi: 10.1161/01.ATV.0000150649.39934.13

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/25/1/29

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/