Vascular Nitric Oxide and Oxidative Stress: Determinants of Endothelial Adaptations to Cardiovascular Disease and to Physical Activity

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Abstract/Résumé

Cardiovascular disease is the single leading cause of death and morbidity for Canadians. A universal feature of cardiovascular disease is dysfunction of the vascular endothelium, thus disrupting control of vasodilation, tissue perfusion, hemostasis, and thrombosis. Nitric oxide bioavailability, crucial for maintaining vascular endothelial health and function, depends on the processes controlling synthesis and destruction of nitric oxide as well as on the sensitivity of target tissue to nitric oxide. Evidence supports a major contribution by oxidative stress-induced destruction of nitric oxide to the endothelial dysfunction that accompanies a number of cardiovascular disease states including hypertension, diabetes, chronic heart failure, and atherosclerosis. Regular physical activity (exercise training) reduces cardiovascular disease risk. Numerous studies support the hypothesis that exercise training improves vascular endothelial function, especially when it has been impaired by preexisting risk factors. Evidence is emerging to support a role for improved nitric oxide bioavailability with training as a result of enhanced synthesis and reduced oxidative stress-mediated destruction. Molecular targets sensitive to the exercise training effect include the endothelial nitric oxide synthase and the antioxidant enzyme superoxide dismutase. However, many fundamental details of the cellular and molecular mechanisms linking exercise
to altered molecular and functional endothelial phenotypes have yet to be discovered. The working hypothesis is that some of the cellular mechanisms contributing to endothelial dysfunction in cardiovascular disease can be targeted and reversed by signals associated with regular increases in physical activity. The capacity for exercise training to regulate vascular endothelial function, nitric oxide bioavailability, and oxidative stress is an example of how lifestyle can complement medicine and pharmacology in the prevention and management of cardiovascular disease.

La première cause de mortalité et de morbidité chez les Canadiens est la maladie cardiovasculaire. La dysfonction de l’endothélium vasculaire, qui est la caractéristique principale de la maladie, entraîne un dérèglement du contrôle de la vasodilatation, de la perfusion des tissus, de l’hémostasie, et de la coagulation. Le maintien de la fonction et de la santé de l’endothélium vasculaire, assuré par la biodisponibilité du monoxyde d’azote, dépend des processus de contrôle de la synthèse et de la dégradation du monoxyde d’azote et de la sensibilité des tissus ciblés par le monoxyde d’azote. Selon de solides études, la destruction du monoxyde d’azote attribuable au stress par oxydation contribue à la dysfonction de l’endothélium observée dans diverses conditions pathologiques dont l’hypertension, le diabète, l’insuffisance cardiaque chronique, et l’athérosclérose. La pratique régulière de l’activité physique (entraînement physique) réduit le risque de maladie cardiovasculaire. De nombreuses études appuient la thèse que l’entraînement physique améliore la fonction de l’endothélium vasculaire, notamment quand ce dernier a été déréglé par des facteurs de risque en place. Il appert en outre que l’entraînement améliore la biodisponibilité du monoxyde d’azote en favorisant la synthèse aux dépens de la dégradation due au stress par oxydation. Les cibles moléculaires sensibles à l’entraînement physique comprennent le monoxyde d’azote synthase endothéliale et la superoxyde dismutase. Cependant, il reste à identifier les aspects fondamentaux des mécanismes moléculaires et cellulaires reliant l’exercice physique aux diverses modifications moléculaires et fonctionnelles des phénomènes endothéliaux. L’hypothèse de travail est la suivante: des signaux associés à l’augmentation de la pratique régulière de l’activité physique contribuent à cibler et à corriger les mécanismes cellulaires de la dysfonction endothéliale. L’entraînement physique utilisé à des fins de régulation de la fonction de l’endothélium vasculaire, de la biodisponibilité du monoxyde d’azote, et du stress par oxydation est un exemple de complémentarité des saines habitudes de vie à la médecine et à la pharmacologie dans la prévention et le traitement de la maladie cardiovasculaire.

Introduction

In Canada, cardiovascular disease (CVD) causes ~35% of all deaths, contributes significantly to morbidity, and accounts for ~20 billion dollars in annual direct and indirect health care costs (Health Canada, 1997; Heart and Stroke Foundation of Canada, 1999). Major independent risk factors for CVD include hypertension, dyslipidemias, smoking, obesity, diabetes mellitus, and physical inactivity. Increasing habitual physical activity can reduce CVD risk. Indeed, a dose-response relationship has been found between the amount of exercise performed and the reduction in CVD mortality in middle-aged and elderly populations (Blair et al., 1995; Lee et al., 1995). The effect is multi-tiered; in addition to eliminating physical inactivity as an independent CVD risk factor, habitual endurance exercise may also have a beneficial effect on other independent risk factors including blood
lipid profiles, blood pressure, body weight, and insulin sensitivity (American Heart Association, 1996; Booth et al., 2000; Health Canada, 1997; King et al., 1988; Manson et al., 1999; Rosenthal et al., 1983; Tran and Weltman, 1985; U.S. Dept. Health and Human Services, 1996; Williams, 1996; Wood et al., 1991). Thus, aerobic physical activity is a potentially powerful intervention to prevent and/or counteract the development of CVD.

Function of the vascular endothelium is affected by both CVD and exercise training; impairment of function accompanies a number of CVD states while improvement in endothelial function occurs with regular exercise. Over the past two decades, a central role of endothelial cells in regulating vascular homeostasis has been established through the discovery of certain endothelial-derived substances that influence vascular physiology (Behrendt and Ganz, 2002; Verma et al., 2003; Widlansky et al., 2003).

By balancing the release of vasodilators such as nitric oxide (NO\textsuperscript{*}), prostacyclin, and endothelium-derived hyperpolarizing factor (EDHF), and vasoconstrictors such as thromboxane A\textsubscript{2}, endothelin-1, and angiotensin II, the endothelium can alter vascular smooth muscle (VSM) cell contractile state (tone) and thus control blood pressure and tissue perfusion. The endothelium controls the vascular thrombotic state through the production of factors such as NO\textsuperscript{*}, prostaglandins, tissue plasminogen activator, thrombomodulin, plasminogen activator inhibitor-1, tissue factor, and von Willibrand’s factor, which help to regulate platelet activation, the clotting cascade, and the fibrinolytic system. Moreover, the endothelium regulates vascular inflammatory and adhesion processes by producing cytokines and adhesion molecules such as C-reactive protein, interleukins, monocyte chemotactic factor-1, tumor necrosis factors, adhesion molecules, and selectins. Whereas the balanced production of counter-regulatory substances maintains a healthy endothelial phenotype, in a pathophysiological CVD state the endothelium may adopt an alternate phenotype wherein the balance is disrupted and proconstrictory, proinflammatory, and prothrombotic signals prevail, leading to vascular dysfunction.

Nitric oxide is a particularly important endothelial-derived substance in light of its multiplex vascular functions. As well as being a potent vasodilator, NO\textsuperscript{*} inhibits the synthesis of proinflammatory cytokines and chemokines, the expression of leukocyte adhesion molecules, the activation and aggregation of platelets, and the proliferation of VSM cells (Eberhardt and Loscalzo, 2000; Ganz and Vita, 2003). Vascular endothelial NO\textsuperscript{*}-dependent vasomotor dysfunction is often found in persons with overt CVD or when one or more risk factors are present (Behrendt and Ganz, 2002; Celermajer et al., 1994; Ludmer et al., 1986; Verma et al., 2003; Vita et al., 1990; Widlansky et al., 2003). Studies of adaptations in NO\textsuperscript{*} bioavailability (determined by synthesis and destruction of NO\textsuperscript{*} and target tissue sensitivity to NO\textsuperscript{*}, details below) in CVD indicate that oxidative stress-mediated NO\textsuperscript{*} destruction is commonly associated with this loss of endothelial vasomotor function (Cai and Harrison, 2000; Kojda and Harrison, 1999).

In contrast, regular physical activity (exercise training) can improve endothelial NO\textsuperscript{*}-mediated vasomotor function and tissue blood flow control (Delp et al., 1993; Graham and Rush, 2004; Hambrecht et al., 1998; 2000; Higashi et al.,
The mechanisms responsible for these improvements with chronic exercise have not been fully elucidated, though recent data suggest that improvements in the management of vascular oxidative stress may provide a significant contribution to the enhanced NO bioavailability. This review will outline the role of NO and oxidative stress in endothelial vasomotor function and adaptations to CVD and exercise training as identified through integrative studies in humans and animal models.

Assessment of Nitric Oxide-Dependent Endothelial Vasomotor Function

Efforts to understand the vascular biology of NO began with the discovery that the endothelium plays a major role in controlling VSM tone. Using isolated rabbit aorta mounted for isometric tension recordings in vitro (vascular myography; Figure 1A), Furchgott and Zawadzki (1980) demonstrated that acetylcholine (Ach) caused relaxation so long as care was taken not to denude the endothelium of the excised vascular segments. Disruption of the endothelium eliminated the relaxing effect of Ach (Furchgott and Zawadzki, 1980). These researchers concluded that endothelial cells were releasing an endothelium-derived relaxing factor (EDRF) in response to Ach that was somehow responsible for arterial smooth muscle relaxation.

Subsequent studies by Furchgott (1988), Ignarro et al. (1987; 1988), Murad and colleagues (Rapport and Murad, 1983), Gryglewski et al. (1986), and Palmer et al. (1987; 1988) were instrumental in characterizing EDRF as NO, and demonstrating that NO: (a) is enzymatically synthesized from the amino acid L-arginine; (b) stimulates VSM soluble guanylate cyclase (sGC); and (c) is rapidly inactivated by superoxide anion (O2•−). Moncada and contemporaries, using the arginine analogs NG-monomethyl-L-arginine (L-NMMA) and L-NG-arginine methyl ester (L-NAME) to inhibit EDRF/NO production, demonstrated that loss of tonic EDRF/NO synthesis resulted in significant vasoconstriction of a variety of vascular beds in animals and humans (Amezcua et al., 1989; Lahera et al., 1991; Persson et al., 1990; Vallance et al., 1989; Wiklund et al., 1990), as well as an elevation in arterial blood pressure (Rees et al., 1989).

Most experimental and clinical assessments of endothelial function make use of either blood flow manipulations or drugs such as Ach to stimulate vasodilatory and/or blood flow responses. Schertzenmayr (1933) provided the first experimental evidence that increases in blood flow cause large arteries to dilate. This phenomenon, termed flow-mediated dilation (FMD), was confirmed over the next several decades (for review, see Rubanyi, 1995) and shown to be endothelium-dependent (Holz et al., 1983; 1984; Smiesko et al., 1983; 1985). This dilatory response can also be observed in vitro using isolated segments of either a conduit or resistance vessel mounted on perfusion pipettes on a microscope stage, allowing for precise manipulation of pressure and flow while continuously measuring diameter (isolated vessel flow-mediated dilation technique; Figure 1B). Studies using this methodology, together with pharmacological inhibitors of vasodilatory pathways, have shown that NO released from the endothelium in response to elevated flow is a major mechanism of FMD (Bagi et al., 2001; Sun et al., 2001).
Figure 1. Methods to assess endothelial function. Endothelial vasomotor function can be assessed (A) in vitro using wire-mounted vessel rings by measuring isometric tension development, or (B) in mounted, pipette-perfused vessel segments by monitoring changes in diameter by video microscopy. Human in vivo endothelial vasomotor function, assessed as a change in vessel diameter or flow in response to vasoactive drug infusion or elevated shear stress, can be measured (C) in the coronary circulation by quantitative angiography, or (D) in the forearm circulation by plethysmography or Doppler ultrasound. Black arrowheads represent sites of catheter placement for infusion of vasoactive drugs; inset panels represent imaging of vessels and data acquisition. (E) Thrombotic, inflammatory, and damage/apoptotic aspects of human and animal endothelial function can be assessed by measuring blood-borne biomarkers such as high-sensitivity C-reactive protein (CRP), vascular cell adhesion molecule (VCAM), intercellular adhesion molecule (ICAM), circulating endothelial microparticles (EMPs), and circulating endothelial progenitor cells (EPCs). For further details of these methods and their applications, see text under Assessment of Nitric Oxide-Dependent Endothelial Vasomotor Function.
Quantitative angiography can be used to assess epicardial conduit artery function in vivo by imaging changes in vascular diameter in response to graded concentrations of endothelium-dependent vasodilators (e.g., Ach) delivered through an intracoronary arterial catheter, or in response to elevated blood flow caused by vasodilator drug-induced dilation of downstream resistance vessels (quantitative coronary angiography technique; Figure 1C). In this same type of invasive coronary catheterization protocol, the endothelial vasomotor function of coronary resistance vessels can be assessed by intracoronary Doppler ultrasound measurements of blood flow responses to vasoactive agents (Zeiher et al., 1991). The first studies to demonstrate clinical endothelial dysfunction employed quantitative coronary angiography and showed that patients with coronary artery disease had “paradoxical vasoconstriction” in response to graded Ach infusions (Ludmer et al., 1986) and impaired dilation in response to elevated flow (Cox et al., 1989), likely owing to a reduced NO• bioavailability in these arteries.

Although quantitative coronary angiography is considered the benchmark for human endothelial vasomotor function testing, it is an invasive technique associated with risk and expense, and is therefore essentially restricted to use in patients who have a clinical diagnostic need for cardiac catheterization. These limitations led to the development of surrogate indicators of coronary vascular endothelial vasomotor function utilizing less invasive technologies in easily accessible peripheral arteries, such as those in the arm/forearm.

Human forearm resistance-vessel endothelial vasomotor function can be assessed using strain-gauge venous occlusion plethysmography to detect volume, or Doppler ultrasound to detect velocity (forearm blood flow technique; Figure 1D), to quantify the change in forearm blood flow after intraarterial (brachial or radial artery) infusion of vasoactive drugs. This approach is valuable in that it can provide dose-response relationships and can be used to study the basic mechanisms underlying endothelial vasomotor function utilizing less invasive technologies with appropriate drugs/blockers. The major drawback of this technique is the requirement for arterial catheterization which increases invasiveness and risk, thus limiting its widespread use.

A solution to this limitation that does not require arterial catheterization or drug administration was introduced by Celermajer and colleagues in their assessment of FMD in the human conduit brachial artery imaged using echo Doppler ultrasound (forearm blood flow technique; Figure 1D; Celermajer et al., 1992; Corretti et al., 2002). To create a flow stimulus through the brachial artery, a blood pressure cuff placed about the arm or forearm is inflated to a suprasystolic pressure to elicit arterial occlusion for several minutes. The resultant ischemia causes downstream resistance vessels to dilate in response to the accumulation of metabolic byproducts released by skeletal muscle. Subsequent rapid cuff deflation brings about a rapid increase in blood flow through the brachial artery because of the lowered downstream resistance. This response, termed reactive hyperemia, is characterized by a peak brachial artery flow that is sustained for a few seconds before slowly decaying back toward resting level as metabolites are washed out and arterioles regain their basal tone. The elevation in flow/shear stress causes the brachial artery to dilate, with a maximal response typically occurring 45 to 90 seconds after cuff deflation.
The correlation between FMD assessed in the brachial artery and Ach-mediated dilation in epicardial arteries (Anderson et al., 1995) indicates that peripheral conduit arteries may serve as reasonable surrogate marker for coronary endothelial vasomotor responses. This observation has added to the legitimacy of the brachial artery FMD test as a practical and widely used technique for assessing endothelial vasomotor function in humans. Additional benefits include anatomical accessibility, noninvasiveness, time- and cost-efficiency, and use of a physiological stimulus (flow) as opposed to pharmacological agonists. There are several technical limitations to this approach, however, including the expertise required to image the brachial artery using Doppler ultrasound, a relatively poor signal-to-noise ratio (poor resolution relative to artery size), and no standardization among centers with respect to an occlusion protocol to stimulate flow and subsequent dilation (Betik et al., 2003; Corretti et al., 2002).

The latter could be very important to interpretation of FMD data, as recent studies suggest there may be protocol-dependent effects on the mechanism mediating FMD. For instance, studies assessing FMD in the brachial/radial artery following arginine analog infusion have demonstrated that with brief periods of hyperemia, conduit vessel dilation is almost exclusively mediated by NO• (Joannides et al., 1995; Lieberman et al., 1996; Mullen et al., 2001), whereas with more prolonged periods of hyperemia the dilation appears to be much less dependent on NO• (Bellien et al., 2003; Mullen et al., 2001). For the reasons described above there is continued effort to develop better means of noninvasively assessing NO•-mediated endothelial vasomotor function (Ganz and Vita, 2003; Widlansky et al., 2003). There is also increasing emphasis on supplementing functional assessments with blood markers of endothelial phenotype including proinflammatory/prothrombotic biomarkers such as high-sensitivity C-reactive protein, vascular cell adhesion molecules, circulating endothelial progenitor cells, and circulating endothelial microparticles, thought to result from endothelial damage/apoptosis (Figure 1E; Horstman et al., 2004; Hristov et al., 2003; Verma et al., 2003; Willerson and Ridker, 2004).

Factors Controlling NO• Bioavailability In Vivo

Through 20 years of vascular cellular and molecular studies, the mechanism of NO• action has been well defined and the factors that affect NO• bioavailability are being examined with a high degree of sophistication including gene/protein manipulation and complex pharmacology. Through this integrative physiology approach, molecular defects responsible for endothelial dysfunction and potential targets for improving endothelial function are being identified.

The generally accepted sequence of events of NO•-mediated vasodilation is as follows: in response to a number of physical and chemical stimuli to the endothelium, NO• is generated in the cytosol by the endothelial isoform of NO• synthase (eNOS; NOS3) and diffuses to underlying VSM cells. Within the media of the vessel wall, NO• may be transported as NO• per se or as an S-nitrosothiol form moving between VSM cells by simple diffusion across cell membranes and through cytosol and/or diffusion through gap junctions between VSM cells. The mechanism of NO• transport in the media of specific vessels likely depends on the num-
ber of layers of VSM cells that must be targeted simultaneously for vasodilation to occur, and thus on the vessel caliber (i.e., arterioles vs. larger arteries). This complexity is not shown in Figure 2, for the sake of simplifying the illustration to the general determinants of NO’ bioavailability in all vessels. NO’-mediated activation of sGC in the cytosol of VSM cells leads to cGMP accumulation, protein kinase G (PKG) activation, and PKG-mediated phosphorylation of a number of Ca^{2+} regulatory and contractile proteins. The result is a lowering of VSM sarcoplasmic Ca^{2+} concentration (Cohen, 2000), relaxation of VSM, dilation of the blood vessel, decreased vascular resistance, and increased flow through the vessel (Figure 2).

Two important principles of cell signaling are identifiable in this simplified NO’ mechanism of action: amplification of the original endothelial signal through

![Figure 2](image-url)
second messenger and protein kinase systems; and redundancy in the multiple molecular targets of PKG, all of which act to decrease cytoplasmic Ca\(^{2+}\) and the contractile state of VSM. Although the amplification inherent in this signaling system allows for responsiveness to even nanomolar NO• concentrations, micromolar concentrations of NO• are present physiologically (Malinski and Taha, 1992; Malinski et al., 1993). At physiological NO• concentrations there is also evidence for cGMP-independent mechanisms of action mediated by the direct action of NO• on redox-sensitive thiol groups of ion-regulatory proteins (Bolotina et al., 1994).

The mechanism of NO• action identifies key components of the responsiveness or sensitivity of VSM to NO•. The factors influencing NO• synthesis and destruction must also be considered in order to appreciate the many potential sites for molecular-level alterations that can result in depression or enhancement of NO•-mediated vasodilation.

**ENDOTHELIAL NO• SYNTHESIS**

Synthesis of NO• in endothelial cells is mediated by eNOS (Figure 2). In response to a number of physical (e.g., flow/shear stress) and chemical (e.g., Ach) stimuli, cell signaling events lead to elevation in endothelial cell cytosolic Ca\(^{2+}\), which binds to the regulatory protein calmodulin (CaM). In turn, Ca\(^{2+}\)-CaM binds to and activates eNOS.

The acute regulation of endothelial NO• synthesis is much more complex than the Ca\(^{2+}\)-CaM influences alone and involves: availability of substrate (L-arginine), endogenous inhibitor (asymmetric dimethyl arginine), and cofactors (FAD, FMN, NADP\(^+/\)NADPH and tetrahydrobiopterin); non-Ca\(^{2+}\)-dependent eNOS activation; proteins that regulate eNOS localization and activity through protein-protein interactions (HSP-90, caveolin); and covalent modifications of eNOS including phosphorylation/dephosphorylation by multiple kinases/phosphatases and acylation (Cohen, 2000; Dillon and Vita, 2000; Feron et al., 1999; Feron and Michel, 2000; Forstermann et al., 1995; Garcia-Cardeña et al., 1998; Hambrecht et al., 2003; Sessa et al., 1994; Wang and Marsden, 1995). Elucidating the details of localization and phosphorylation effects on eNOS are topics of recent reviews (Boo and Jo, 2003; Fleming and Busse, 2003; Govers and Rabelink, 2001; Shaul, 2002). In addition to the acute factors, chronic regulation of eNOS is achieved in part through adaptations in its level of expression, which is modulated by a number of stimuli. There are also excellent reviews of the regulation of eNOS activity and expression (Boo and Jo, 2003; Li et al., 2002).

Recent results add to the understanding of vascular NO• production by illustrating that NO• released from neuronal nitric oxide synthase (nNOS) localized in neurons lining coronary and pial arteries can mediate flow- and agonist-induced dilations in eNOS knockout mice (Huang et al., 2002; Lamping et al., 2000; Meng et al., 1998). This suggests a compensatory interaction between eNOS and nNOS that could offset eNOS deficiencies. However, as the convincing results are currently limited to knockout models lacking the eNOS gene, it will require much more work to clarify the relative role of nNOS-derived NO• in vasomotor control in vessels with competent eNOS, and its physiological and pathophysiological importance.
DESTRUCTION OF NO\(^•\) BY OXIDATIVE STRESS

Although the term oxidative stress is frequently used to indicate an excess exposure to reactive oxygen species (ROS), it should be kept in mind that oxidative stress is a relative term. While increasing evidence suggests that low levels of ROS contribute to normal physiological cell signaling (Buetler et al., 2004), excessive production or impaired buffering of ROS leads to increased oxidative stress that can have pathophysiologic consequences (Buetler et al., 2004; Griendling et al., 2000; Kunsch and Medford, 1999; Taniyama and Griendling, 2003).

Local NO\(^•\) degradation in the artery wall in vivo occurs predominantly through the interaction of NO\(^•\) with ROS, such as O\(_2\)\(^•\), to form peroxynitrite (\(\cdot\)ONOO\(^-)\) (Figure 2). Since the rate of interaction between NO\(^•\) and O\(_2\)\(^•\) is diffusion-limited, a fine balance between NO\(^•\) and ROS must be maintained in the vascular wall in order to preserve adequate NO\(^•\) bioavailability (Darley-Usmar et al., 1995) (Figure 2). Support for a major contribution of oxidative stress to the regulation of NO\(^•\) has come from several fronts: exposure to endogenous or exogenous O\(_2\)\(^•\) reduces endothelium-dependent dilation to Ach (Gryglewski et al., 1986; Katusic and Vanhoutte, 1989); experimental inhibition of superoxide dismutase (SOD) impairs agonist-evoked endothelium-dependent NO\(^•\)-mediated dilation to Ach (Gryglewski et al., 1986; Katusic and Vanhoutte, 1989); inclusion of SOD or chemical antioxidants in vitro protects NO\(^•\) against O\(_2\)\(^•\) (Laursen et al., 1997; Zalba et al., 2000); and enhancement of vascular wall SOD by gene or protein transfer in vivo restores the NO\(^•\) action previously impaired by overproduction of ROS (Chu et al., 2003; Fennell et al., 2002; Mugge et al., 1991b; Schnackenberg et al., 1998).

The conclusion stemming from these and other observations is that oxidative stress-induced destruction of NO\(^•\) is a major mechanism in the regulation of NO\(^•\) bioavailability. It is thus important to consider the chemical and enzymatic pro- and antioxidant influences in the vascular wall.

In vascular endothelial and smooth muscle cells the main pro-oxidant influences are the enzymes NAD(P)H oxidase, xanthine oxidase, and eNOS (Cai and Harrison, 2000; Darley-Usmar et al., 1995; Dillon and Vita, 2000; Kojda and Harrison, 1999), with NAD(P)H oxidase recognized as the predominant source of O\(_2\)\(^•\) (Figure 2; Griendling et al., 1994; 2000; Mohazzab-H et al., 1994; Mohazzab-H and Wolin, 1994; Pagano et al., 1995; Rajagopalan et al., 1996).

The NAD(P)H oxidase enzyme complex is present in both endothelial and VSM cells, although its molecular composition varies slightly between cell types. Activation of this enzyme involves recruitment of at least three regulatory cytosolic subunits to the heterodimeric membrane-bound catalytic complex to form the holoenzyme complex. A recent and excellent review of the structure and function of this enzyme and its role in CVD provides much more detail (Griendling et al., 2000).

Several chemical and enzymatic antioxidant systems exist in the vascular environment. Cellular chemical antioxidants include glutathione and other thiols, as well as antioxidant vitamins such as vitamins C and E, and β-carotene. Enzymatic antioxidants in the vascular wall include three isoforms of SOD: the cytosolic Cu/Zn-dependent SOD-1 isoform and the mitochondrial Mn-dependent SOD-2
isoform, both of which are present in endothelial cells and VSM cells, and the Cu/Zn-dependent SOD-3 isoform (ecSOD) located in the extracellular matrix. In addition, the H$_2$O$_2$-reducing enzymes glutathione peroxidase (GPx) and catalase are present in both endothelial and VSM cells (Figure 2).

Superoxide dismutase is essential for normal NO$^+$ function because of its action in converting O$_2^-•$ to H$_2$O$_2$, thus limiting the interaction of NO$^+$ and O$_2•^-$(Darley-Usmar et al., 1995). Much less is known regarding the roles of GPx and catalase in controlling NO$^+$ bioavailability. Recent evidence suggests that H$_2$O$_2$ may contribute to the chronic regulation of NO$^+$ bioavailability, however, as it induces eNOS expression at both transcriptional and posttranscriptional levels in cultured bovine endothelial cells (Drummond et al., 2000).

The preceding information suggests that oxidative stress-induced destruction of NO$^+$ contributes to the endothelial vasomotor dysfunction. A tempered ability of antioxidant enzyme systems to buffer O$_2•^-$ results in NO$^+$ destruction and impaired NO$^+$ bioavailability whereas supplemental SOD and chemical antioxidant treatments can buffer excess O$_2•^-$ production and restore endothelium-dependent dilation (Carneado et al., 2002; Fukui et al., 1997; Gokce et al., 1999; Hattori et al., 1991; Horie et al., 1998; Jackson et al., 1998; Kinlay et al., 1999; Kinouchi et al., 1991; Laursen et al., 1997; Mugge et al., 1991b; Nakazono et al., 1991; Raja-gopalan et al., 1996; Taddei et al., 1998; Ting et al., 1996; 1997; Zalba et al., 2000). With the main vascular pro- and antioxidant influences introduced, attention will now shift to specific evidence supporting oxidative stress-induced reductions in NO$^+$ bioavailability as a pathophysiological mechanism in CVD.

Reduced NO$^+$ Bioavailability in Cardiovascular Disease

A transition in endothelial cell phenotype toward increased vascular constriction, thrombosis, and inflammation occurs at the earliest stages of CVD (Figure 3). Numerous recent prospective and retrospective studies have shown that testing of NO$^+$-mediated endothelial vasomotor function has prognostic value for clinical cardiovascular events including myocardial infarction and ischemic stroke (Behrendt and Ganz, 2002; Ganz and Vita, 2003; Verma et al., 2003; Widlansky et al., 2003). Considering this, a detailed analysis of vascular NO$^+$ bioavailability and how it is manipulated by risk factors and various interventions is essential to understanding CVD.

Although there are undoubtedly contributions of diminished VSM responsiveness to NO$^+$ (Adachi et al., 2002; Adams et al., 1998; Bauersachs et al., 1998; Creager et al., 1990; Kojda et al., 1998; Weisbrod et al., 1997), and depressed synthesis of NO$^+$ resulting from multiple deficiencies in the NO$^+$ synthesis pathway (Blair et al., 1999; Crabos et al., 1997; Creager et al., 1992; Feron et al., 1999; Stroes et al., 1997; Yoshizumi et al., 1993), the overwhelming evidence supports oxidative stress-induced destruction of NO$^+$ as a major mechanism of the reduced NO$^+$ bioavailability leading to vascular dysfunction in CVD. Indeed, elevated ROS has been causatively linked to endothelial vasomotor dysfunction in atherosclerosis, hypertension, diabetes, and chronic heart failure (CHF) (Berry et al., 2000; Cai and Harrison, 2000; Crabos et al., 1997; Darley-Usmar et al., 1995; Dillon and Vita, 2000; Fujita et al., 1995; Fukui et al., 1997; Gil-Longo et al., 1996; Griendling
Elevations in the expression and activity of NAD(P)H oxidase are likely involved in the vascular pathogenesis of coronary artery disease, hypertension, diabetes, and CHF (Cai and Harrison, 2000; Fukui et al., 1997; Griendling et al., 1994; Rajagopalan et al., 1996; Rudd et al., 2000; Taddei et al., 1993; Treasure et al., 1992).}

CORONARY ARTERY DISEASE

O$_2^{-}$ production and oxidative NO$^-$ destruction is elevated several-fold in the aortic wall of hypercholesterolemic and atherosclerotic animals (Mugge et al., 1991b; Ohara et al., 1993) and this is associated with impairment in NO$^-$-mediated, endo-
thelium-dependent vasodilation assessed in vitro using vascular myography (Mugge et al., 1991b; Figure 3). A landmark study by Ohara et al. (1993) identified endothelial cells as the major contributor of excess O$_2^•$ in hypercholesterolemia, while subsequent studies have revealed an involvement of macrophage and VSM cell O$_2^•$ production in the later stages of atherosclerosis (Miller et al., 1998).

Several investigations have shown that providing a more robust vascular enzymatic/chemical antioxidant capacity can improve NO$^•$-mediated endothelial vasomotor function in hypercholesterolemia (Huang and Keaney, 2000; Figure 3). For instance, Mugge and co-workers doubled the aortic wall SOD enzyme activity in hypercholesterolemic rabbits using polyethylene glycol-conjugated SOD injections, and this reversed endothelium-dependent NO$^•$-mediated aortic vasomotor dysfunction (Mugge et al., 1991b). Furthermore, intraarterial infusion of the chemical antioxidant, vitamin C, at 1-10 mM improved endothelial vasomotor function in the forearm resistance arteries of human hypercholesterolemic patients assessed in vivo using strain-gauge venous occlusion plethysmography (Ting et al., 1997).

Conversely, oral consumption of chemical antioxidants at more reasonable dietary concentrations daily for one month (1,000 mg of vitamin C; 800 IU of vitamin E; 30 mg of β-carotene) had no effect on vasomotor function, although the treatment was associated with a reduction in the susceptibility of LDL cholesterol to oxidation ex vivo (Gilligan et al., 1994). Notably, this latter study demonstrates that nonpharmacological doses of chemical antioxidants in humans may not always be sufficient to combat vascular oxidative stress-induced endothelial vasomotor dysfunction in hypercholesterolemia, though they may exert other vascular health benefits such as reducing circulating oxidized LDL cholesterol.

**HYPERTENSION**

Both human and animal models of hypertension are associated with elevated O$_2^•$ production and NO$^•$-mediated vascular dysfunction (Duffy et al., 1999; 2001; Laursen et al., 1997; Mehta et al., 1994; Schnackenberg et al., 1998; Sherman et al., 2000; Solzbach et al., 1997; Taddei et al., 1993; 1998; Figure 3). As in hypercholesterolemia, manipulation of artery SOD content affects the NO$^•$-mediated dilatory response in arteries from hypertensive animals; treatment with liposome-encapsulated SOD for 8 days increased aortic SOD activity by ~30% and partially prevented angiotensin-II-induced O$_2^•$ release, elevation of blood pressure, and endothelial vasomotor dysfunction assessed by vascular myography (Laursen et al., 1997). In addition, the SOD mimetic tempol (4-hydroxy-2,2,6,6,-tetramethyl piperidinoxyl) reduces blood pressure and renal vascular resistance while preserving renal blood flow in spontaneously hypertensive rats (SHR; Schnackenberg et al., 1998).

In some but not all reports, treatment of hypertensive humans with chemical antioxidants can partially restore endothelial vasomotor function and reduce blood pressure (Duffy et al., 1999; 2001; Sherman et al., 2000; Solzbach et al., 1997; Taddei et al., 1998). A study by Duffy et al. (1999) demonstrated that a relatively small dosage of vitamin C taken chronically (500 mg/day for 1 month) was able to significantly reduce blood pressure in otherwise healthy patients with essential hypertension. However, experiments assessing forearm vascular function using FMD and strain-gauge venous occlusion plethysmography report that it took a relatively large (pharmacological) dosage of vitamin C delivered intraarterially to
improve endothelial vasomotor function in the forearm arteries of hypertensive patients (Duffy et al., 2001; Sherman et al., 2000; Solzbach et al., 1997; Taddei et al., 1998).

Thus, as was the case for the chemical antioxidant treatment experiments with hypercholesterolemic patients, these studies demonstrate that nonpharmacological doses of chemical antioxidants in humans may be insufficient to combat vascular oxidative stress-induced endothelial vasomotor dysfunction associated with hypertension—though they may exert other vascular health benefits such as reducing blood pressure itself in certain cases. These results are consistent with those of the seven large-scale primary and secondary prevention trials conducted in humans in the last decade demonstrating little or no benefit of chronic chemical antioxidant dietary supplementation at reasonable doses in preventing or treating CVD (for review, see Shihabi et al., 2002).

Recognizing the established role of oxidative stress in endothelial dysfunction, from an antioxidant supplementation perspective it seems necessary and worthwhile to put significant effort into the development of more powerful chemical antioxidants in order for dietary, nutraceutical, or pharmacological supplementation to be effective in treating this root cause of vascular pathophysiology. In this regard, cell-permeable mimetics of SOD are a promising possibility (Muscoli et al., 2003). An alternative and parallel strategy is to establish physiological or pharmacological mechanisms to enhance the endogenous vascular enzymatic antioxidant capacity and/or to dampen the enzymatic pro-oxidant capacity in order to temper oxidative stress-induced reductions in NO• bioavailability and vasomotor dysfunction and thus contribute to the prevention and treatment of CVD.

Sedentary lifestyle is also a CVD risk factor. Recent investigations of the vascular endothelial adaptations to exercise confirm that, in general, endothelial function is poorer in sedentary individuals than in those who exercise regularly (Higashi et al., 1999a; Kingwell et al., 1996; Figure 3). There is substantial evidence that increased NO• bioavailability could be of key importance to the improved endothelial vasomotor function that results from exercise training (Delp et al., 1993; Delp and Laughlin, 1997; Graham and Rush, 2004; Hambrecht et al., 1998; 2000; Higashi et al., 1999a; 1999b; Kingwell et al., 1996; Muller et al., 1994; Rush et al., 2000; 2003; Sessa et al., 1994; Woodman et al., 1997; 1999). However, the mechanisms controlling changes in NO• bioavailability in response to exercise training are not completely understood. The potential roles of adaptations in eNOS and oxidative stress in the functional adaptations of arteries to exercise training will now be considered. The working hypothesis is that some of the very cellular mechanisms that contribute to endothelial dysfunction in well-defined cases of CVD could be targeted and reversed by signals associated with regular increases in physical activity.

Adaptations Associated With Exercise Training

Numerous studies using humans and other animal models have demonstrated improved NO•-dependent endothelial function as a result of aerobic exercise training in otherwise healthy sedentary subjects, and in those with preexisting endothelial dysfunction associated with conditions such as hypertension, coronary artery disease/hyperlipidemia, and CHF (Chen et al., 1996; Delp et al., 1993; Delp and
Resistance artery endothelial function, assessed as the maximal forearm blood flow response to intrabrachial artery Ach infusion in sedentary humans, was observed to be ~40% lower in hypertensives vs. normotensives. However, 12 weeks of low-intensity aerobic exercise training (brisk walking 6 bouts/week, 30 min/bout @ ~55% VO_2max) improved the response in hypertensive patients to ~85% of sedentary normotensive values (Higashi et al., 1999a). The same training program also improved the Ach-induced forearm blood flow response in normotensive subjects by ~35% (Higashi et al., 1999a). Animal models of hypertension confirm this observation; maximal Ach-induced dilation of aortic rings from sedentary SHR was approximately half as much as the dilation of rings from sedentary, normotensive Wistar Kyoto rats (WKY) in myography experiments, but aortic rings from SHR that had undergone 6 weeks of moderate intensity exercise training demonstrated similar Ach-induced vasodilatory responses as those from WKY rats (Graham and Rush, 2004). Thus, exercise training can reverse endothelial vasomotor dysfunction associated with hypertension, at least in some vessel types and experimental conditions (Figure 3).

In coronary artery disease patients, exercise training at a heart rate of ~110 bpm (80% of maximal achievable heart rate in these patients, most of whom were taking β-blockers), 6 bouts per day for 10 minutes each bout over a study period of 4 weeks, improved endothelium-dependent dilation both in epicardial vessels and in coronary resistance vessels (Hambrecht et al., 2000). This resulted in an average doubling of the peak flow velocity in response to intracoronary Ach, and a 30% increase in coronary blood flow reserve. Similarly, feeding a high fat/high cholesterol diet to miniature swine resulted in a blunting of endothelium-dependent vasodilation in isolated coronary arteries, and exercise training was shown to attenuate these functional effects (Thompson et al., 2004; Woodman et al., 2004).

Six months of moderate exercise training in CHF patients (70% individual maximal heart rate, ~25 min/day, 5 days/week) resulted in improved exercise capacity associated with a 25% increase in VO_2max and a 200% increase in the femoral artery flow response to Ach, indicating improved endothelial-dependent dilation of human skeletal muscle resistance vessels (Hambrecht et al., 1998). Animal studies have confirmed that CHF is accompanied by impairment of endothelium-dependent vasodilation of systemic vessels (Buikema et al., 1993; Drexler and Lu, 1992; Kaiser et al., 1989; Kiuchi et al., 1993; Lindsay et al., 1992; Mulder et al., 1996; Nasa et al., 1996; Ontkean et al., 1991; Teerlink et al., 1993; Varin et al., 1999) that in turn compromises peripheral tissue perfusion (Drexler and Lu, 1992; Kiuchi et al., 1993; Ueno et al., 1994).

The impairment in peripheral artery endothelium-dependent dilation is highly correlated with the degree of exercise intolerance and the severity of CHF in both animals and humans (Demopoulos et al., 1997; Drexler and Lu, 1992; Hambrecht et al., 1998; Katz et al., 1997; Kobayashi et al., 2003; Linke et al., 2001; Nakamura et al., 1996; Vona et al., 2004; Walsh et al., 2003). In contrast, chronic physical activity improves endothelial vasomotor function in numerous vascular beds in
CHF patients/animals (Hambrecht et al., 1998; Hornig et al., 1996; Katz et al., 1997; Kobayashi et al., 2003; Linke et al., 2001; Maiorana et al., 2000; Varin et al., 1999; Walsh et al., 2003; Wang et al., 1997; Figure 3).

Improved NO• bioavailability underlies many of the above listed cases of training-induced improvements in endothelium-dependent dilation, since the adaptations can be reduced or eliminated with NOS inhibitors (Chen and Chiang, 1996; Chen et al., 1996; Delp et al., 1993; Delp and Laughlin, 1997; Graham and Rush, 2004; Hambrecht et al., 1998; Higashi et al., 1999a; 1999b; Hornig et al., 1996; Kingwell et al., 1996; Koller et al., 1995; Muller et al., 1994; Parker et al., 1994; Varin et al., 1999; Wang et al., 1993; 1997; Yen et al., 1995). The roles of adaptations in prostanoid and EDHF pathways to physical activity and disease are less clear at present because, relative to the NO• system, these other endothelium-dependent dilatory pathways have received much less research attention. This should not be confused, however, with a lack of importance of these pathways in the explanation of vascular adaptations to CVD and to exercise. For instance, recent data indicates that the endothelium-dependent dilatory responses in coronary arteries of hyperlipidemic pigs are improved after exercise training as a combined result of both enhanced NO• bioavailability and reduced prostanoid constrictor availability (Thompson et al., 2004; Woodman et al., 2004).

Potential Mechanisms

An important principle to be established prior to discussion of the potential mechanisms responsible for exercise-induced functional adaptations of the endothelium is the possible nonuniversality of the responses. Thus, observed effects of exercise training on vascular endothelial function and gene/protein expression likely depend not only on the characteristics of the exercise training (mode, intensity, duration) but also on the vascular bed examined (coronary, cerebral, skeletal muscle, etc.), and the position in the arterial tree (conduit artery, smaller artery, arteriole, and branch order of arteriole). These issues have been highlighted previously (e.g., Laughlin et al., 1996; 1998; 2003b; 2003c). An additional consideration is the relationship of functional and molecular/cellular adaptations to structural adaptations that also occur in a given vessel and the interaction this has with duration-dependent effects. Early adaptations to physical activity in a given vessel may be quite distinct from those that characterize the steady-state, fully-adapted trained phenotype, and thus it may not be simply a matter of the degree of adaptation that contrasts the early responses from the stably-trained phase responses.

Isolated vessel segment and vessel ring experiments demonstrate increased sensitivity and maximal effect of NO•-mediated endothelium-dependent dilation in some but not all vessel types and calibers after exercise training (Chen and Chiang, 1996; Chen et al., 1996; Delp et al., 1993; Delp and Laughlin, 1997; Graham and Rush, 2004; Koller et al., 1995; Laughlin et al., 1998; 2001; 2003b; 2003c; Muller et al., 1994; Oltman et al., 1995; Parker et al., 1994; Varin et al., 1999; Yen et al., 1995). Increases in vascular eNOS protein levels may play a role in the exercise-induced improvements in NO• bioavailability. For instance, prolonged exercise training for a period of several weeks increased eNOS levels and improved NO•-mediated vasomotor activity of rat aorta and pig coronary arteries and
arterioles (Chen and Chiang, 1996; Chen et al., 1996; Delp et al., 1993; Delp and Laughlin, 1997; Graham and Rush, 2004; Griffin et al., 1999; Laughlin et al., 2001; Muller et al., 1994; Parker et al., 1994; Woodman et al., 1997; Yen et al., 1995).

In contrast, conduit coronary arteries and aortic endothelial cells from pigs exercise trained for several weeks do not exhibit evidence of functional or biochemical improvements in NO•-dependent dilation (Laughlin et al., 2001; Oltman et al., 1995; Rush et al., 2003; Thompson et al., 2004; Woodman et al., 2004). Earlier studies (Sessa et al., 1994; Wang et al., 1993) had concluded that dog conduit coronary arteries and aortic endothelial cells did respond functionally and biochemically with upregulation of NOS and NO• action in response to chronic exercise training. However, these conclusions were based on a 10- to 14-day training period (Sessa et al., 1994; Wang et al., 1993), and recent evidence from a series of studies on pigs demonstrates that the responses of eNOS expression and NO•-dependent dilation of coronary conduit arteries and aortic endothelial cells is different in short-term vs. long-term training; early functional improvements and enhanced eNOS expression are lost later in the training period (Griffin et al., 1999; Laughlin et al., 2001; 2003a; Oltman et al., 1995; Rush et al., 2003; Thompson et al., 2004; Woodman et al., 2004).

One explanation for this phasic response of coronary conduit arteries to exercise training relates to the role of shear stress in controlling eNOS expression. In light of the presence of several shear stress response elements in the eNOS gene promoter region (Venema et al., 1994), one likely contributor to the exercise-induced eNOS response is the elevated flow/shear stress associated with increased blood flow during the exercise bouts. Shear stress has been shown to increase eNOS mRNA and protein in vivo and in cell culture (Nishida et al., 1992; Noris et al., 1995; Topp et al., 1996; Uematsu et al., 1995), and in models of increased flow in vivo (Miller and Vanhoutte, 1988; Nadaud et al., 1996). In addition, it was recently demonstrated that elevated shear stress in isolated, perfused arterioles is capable of increasing eNOS mRNA in as little as 4 hours (Woodman et al., 1999). Thus it is quite conceivable that shear stress is a mediator of the early (days) responses to exercise training, both functional and eNOS upregulation, in conduit coronary arteries.

After the initial weeks of exercise training, however, the conduit coronary arteries undergo structural adaptations that result in increased diameter (Bove and Dewey, 1985; Kramsch et al., 1981; Laughlin, 1995; Laughlin and McAllister, 1992; Leon and Bloor, 1968; Windecker et al., 2002; Wyatt and Mitchell, 1978) and a consequent reduction in the shear stress signal associated with a given exercise-induced elevation in blood flow (Laughlin, 1995). This is consistent with the restoration of eNOS (Laughlin, 1995; Laughlin et al., 2001; Thompson et al., 2004) and NO•-dependent dilation (Oltman et al., 1995; Rogers et al., 1991; Thompson et al., 2004; Woodman et al., 2004) to control levels in the steady-state, fully-trained state after their initial elevation in the first days of training.

Shear stress is not the only factor accounting for the exercise effect. In addition to the local and bed-specific signals, it is clear that there must be some neurohumoral influence on the vascular endothelium adaptations to exercise training, as there are profound improvements in forearm artery endothelium dilatory responses after leg-specific exercise training protocols that do not elicit major forearm blood
flow responses during the exercise bout itself (Linke et al., 2001; Maiorana et al., 2000; Walsh et al., 2003). As the field advances it is likely that the neural, humoral, autocrine, paracrine, metabolic, and mechanical signals coordinating vascular adaptions to chronic physical activity will be identified and characterized.

It is not clear whether simple changes in eNOS levels are necessary or sufficient enough to be responsible for exercise training-induced improvements in NO\(^\cdot\) bioavailability and NO\(^\cdot\)-mediated function. As has been established in the discussion of the effects of CVD on NO\(^\cdot\) bioavailability, it appears that regulation of vascular cell oxidative stress can influence NO\(^\cdot\) bioavailability and function. Thus it is possible that favorable adjustments in vascular cell oxidative stress could account in part for improvements in NO\(^\cdot\) bioavailability and NO\(^\cdot\)-mediated vasomotor function that result from chronic exercise training.

Although acute exercise transiently increases oxidative stress because of accelerated ROS generation (Ji, 1995; Liu et al., 2000), recent preliminary evidence suggests that exercise training can result in an increased availability of enzymatic antioxidant defenses in vascular tissue (Fukai et al., 2000; Rush et al., 2000; 2003). Increased mRNA, protein, and enzymatic activity of SOD-1 in coronary arterioles, aortic endothelium, and whole aortic homogenates from exercise-trained pigs compared to sedentary controls are among the recent data suggesting that exercise has positive effects on vascular antioxidant enzyme pathways (Rush et al., 2000; 2003). Another recent study demonstrated that extracellular SOD-3 is also increased as a result of exercise training, and that this response depends in part on NO\(^\cdot\)-mediated SOD-3 gene expression (Fukai et al., 2000). Furthermore, endothelial cell culture studies have recently demonstrated that H\(_2\)O\(_2\) is a potent inducer of eNOS expression (Drummond et al., 2000), and it is therefore conceivable that the elevations in ROS accompanying exercise training bouts could contribute to eNOS expression via this mechanism. Thus, the autocrine and paracrine control of eNOS and antioxidant enzymes by NO\(^\cdot\) and ROS involves extensive biochemical cross-talk and is an exciting new area of interest in the context of exercise training (Drummond et al., 2000; Fukai et al., 2000; Rush et al., 2000; Woodman et al., 1997).

Physical signaling mechanisms cannot be ignored as possible contributors to exercise training-induced responses of vascular antioxidant enzymes, as flow/shear stress-induced increases SOD-1 mRNA in cultured human aortic endothelial cells and in isolated, perfused porcine coronary arterioles have been observed (Inoue et al., 1996; Woodman et al., 1999). The noted increases in vascular cell antioxidant enzymes (Fukai et al., 2000; Rush et al., 2000; 2003) suggest a role in the improved NO\(^\cdot\)-mediated endothelium-dependent function that accompanies exercise training in some vascular beds and in some levels of the arterial tree (Delp et al., 1993; Hambrecht et al., 2000; Higashi et al., 1999a; 1999b; Muller et al., 1994; Sessa et al., 1994; Wang et al., 1993).

Identification of the cellular factors that control vascular dose-response effects to exercise, the differential adaptations among vascular beds, and the response of different types of arterial vessels (large and small arteries and branch orders of arterioles) within a vascular bed are all important to understanding the effects of exercise in vascular wall plasticity, the control of oxidative stress and NO\(^\cdot\) bioavailability, and ultimately the role of exercise in the treatment and pre-
vention of CVD. The observations of exercise-induced increases in SOD-1 and SOD-3 in vascular cells could be particularly interesting in the case of hypertension, since this risk factor is known to be associated with increased \( \text{O}_2^- \) production from NADPH oxidase in vascular cells (Azumi et al., 1999; Berry et al., 2000; Fukui et al., 1997; Görlach et al., 2000; Griendling et al., 1994; Laursen et al., 1997; Mohazzab-H et al., 1994; Nakazono et al., 1991; Rajagopalan et al., 1996; Tschudi et al., 1996).

In addition, recent preliminary data also support a possible reduction in aortic NADPH oxidase expression associated with improved NO•-dependent aortic vasodilation in exercise-trained vs. sedentary SHR (Graham and Rush, 2004). Thus, a possible basis for improvements in blood pressure management and in NO•-mediated endothelial function resulting from regular aerobic exercise could involve improved balance of \( \text{O}_2^- \) and NO•. However, the precise functional impact of exercise-induced increases in vascular antioxidant enzyme levels and reductions in pro-oxidant enzyme levels need to be defined more precisely, and a more thorough assessment must be made of the changes in all the parameters affecting ROS production and management in response to regular physical activity.

**Conclusion**

Sedentary lifestyle and other cardiovascular disease risk factors are associated with dysfunction of the vascular endothelium. Oxidative stress-mediated destruction of nitric oxide appears to be a common mechanism mediating this dysfunction. Exercise training can improve endothelial function and reverse dysfunction associated with cardiovascular disease. Exercise training-induced improvements in endothelial function are associated with increased nitric oxide bioavailability. This holds great promise for rigorously describing one of the molecular mechanisms by which exercise improves endothelial function and cardiovascular health. The challenges that lie ahead include identifying the specific molecular adaptations that result in an improved functional endothelial phenotype as a result of exercise training. Preliminary evidence suggests an interaction of eNOS regulatory adaptations with modifications in the expression level of antioxidant enzymes, although many details remain to be discovered.

Even when more of the specifics regarding the molecular mechanisms are elucidated, identifying the universality of adaptations throughout the vascular tree, in different vascular beds, and in different preexisting physiological and pathophysiological states in humans and a variety of useful animal models present significant but surmountable challenges to our complete understanding of the molecular etiology of functional benefits of exercise training. As progress is made in this and other exercise research, a better evidence-based model of the mechanisms of physical activity and lifestyle in the prevention and treatment of cardiovascular disease will emerge.

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References


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