Vascular nitric oxide: effects of physical activity, importance for health

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Abstract

NO (nitric oxide), formed in the vascular endothelium and derived from a biochemical reaction catalysed by eNOS (endothelial NO synthase), appears to play a role in exercise-induced dilation of blood vessels supplying cardiac and skeletal muscle. Endothelium-dependent, NO-mediated vasodilation is augmented by exercise training. Increases in eNOS gene transcription, eNOS mRNA stability and eNOS protein translation appear to contribute to increased NO formation and, consequently, enhanced NO-mediated vasodilation after training. Enhanced endothelial NO formation may also have a role(s) in the prevention and management of atherosclerosis because several steps in the atherosclerotic disease process are inhibited by NO. A growing body of work suggests that exercise training, perhaps via increased capacity for NO formation, retards atherosclerosis. This has significant implications for human health, given that atherosclerosis is the leading killer in Western society.

Introduction

In the 25 years following the discovery of NO (nitric oxide)-mediated dilation of blood vessels by Furchgott and Zawadski [1], a wealth of knowledge has accumulated concerning vascular NO. The driving force for this study

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was the discrepancy between vascular responses to acetylcholine in vivo (vasodilation) and those observed when vessels were isolated from an animal and acetylcholine administered in vitro (frequently vasoconstriction). Furchgott and Zawadski astutely hypothesized that the latter was due to unintentional vessel damage during isolation. These investigators demonstrated that vasodilation induced by the agent acetylcholine requires an intact endothelium (Figure 1). The endothelium consists of a single layer of endothelial cells, with one or more layers of smooth muscle cells underlying it in the vascular wall. The requirement for endothelium led to the term endothelium-dependent vasodilation. Subsequent research revealed that acetylcholine administration leads to formation of NO from the amino acid L-arginine in the endothelium which, in turn, results in cGMP formation in vascular smooth muscle cells and relaxation of those cells. In addition, a host of other pharmacological agents, as well as physiological stimuli, were subsequently reported to induce NO formation in the vascular endothelium [2].

Given that vasodilation is a key component of the cardiovascular response to acute exercise (i.e. a single bout of exercise [3]), there has been considerable interest in determining whether endothelium-dependent, NO-mediated vasodilation occurs during exercise. Animal studies are generally supportive of such a role [4], whereas human studies are more equivocal [5]. Further, given that exercise training (i.e. regular physical activity) induces numerous

![Figure 1. Effect of removing endothelium from rabbit aortic rings by rubbing](image)

Aortic rings were studied using myograph preparations. The ordinate represents isometric tension development by rings. NA (noradrenaline) was used to induce a contractile response. Ach (acetylcholine) was used to induce vasorelaxation. The concentrations of NA and ACh are given in log molar units. W indicates the washing out of the vasoactive agent. Note that the rubbed aortic ring fails to relax in response to ACh, in contrast to the unrubbed ring with intact endothelium. Reproduced by permission from Macmillan Publishers Ltd: Nature (1980) vol. 288, pages 373–376. Copyright (1980); www.nature.com/nature.
adaptations in the cardiovascular system [3], it has also been of interest to
determine whether training augments endothelium-dependent vasodilation. A
principal focus of this review will be on exercise-training-induced adaptations
in endothelium-dependent, NO-mediated dilation of the coronary and skeletal
muscle circulations. These two vascular beds would benefit from enhanced
endothelium-dependent dilation because the tissues they supply with blood
flow, cardiac and skeletal muscle, are the most metabolically active tissues during
exercise. Augmented vasodilation would serve to deliver more oxygen and
other nutrients to these tissues. Although we will primarily refer to exercise
training studies involving humans or animals, some reference will be made to
studies involving cultured endothelial cells. Such studies, although limited by
their in vitro nature, involve tight experimental control and therefore permit
more definitive conclusions. These studies have typically applied laminar shear
stress, a physiological stimulus for the endothelium to generate NO, to mono-
layers of endothelial cells.

A second focus of this review will be on the potential role of NO in
preventing and/or managing chronic disease. Atherosclerosis, specifically its
associated events such as myocardial infarction, is the leading killer in Western
society. There is, however, evidence that vascular NO retards several aspects
of the atherosclerotic process [6]. Our focus will be, therefore, on how exercise
training may modulate this specific disease process. Given that atherosclerosis
in the coronary and skeletal muscle vascular beds (coronary artery disease and
peripheral arterial disease respectively) constitute a majority of human patients
with this disease, we will again focus on these two circulations.

Biochemistry of NO formation in the endothelium

Superficially, NO formation appears to be a simple biochemical reaction in
which L-arginine is converted into L-citrulline and NO. It is, however, a more
complex reaction (Figure 2) catalysed by multiple isoforms of a protein. Three
isoforms of the protein NOS (NO synthase), including eNOS (endothelial
NOS), nNOS (neuronal NOS) and iNOS (inducible NOS), catalyse NO
formation in various tissues/cell types. It appears that iNOS, expressed in
macrophages, is only present in the vasculature under pathological conditions.

Figure 2. Detailed representation of the NOS reaction
Note that the conversion of the guanidino group on L-arginine into a carbonyl group (relevant
atoms bolded) results in the formation of L-citrulline, with the evolution of NO. Reproduced
from Methods in Nitric Oxide Research (Feelisch, M. and Stamler, J.S., eds.), 1999, with permission
from John Wiley & Sons.
The nNOS isoform, whilst reported to be present in normal vascular smooth muscle [7], has an uncertain role in vascular function. We will therefore restrict our discussion to the eNOS isoform.

Many enzymes require a coenzyme(s) for their function, and NOS is such an enzyme as reflected by the number of participants in Figure 2. A key coenzyme of the NO-generating reaction is NADPH. Oxidation of NADPH is coupled to conversion of the guanidino group of L-arginine into a carbonyl group and, consequently, evolution of L-citrulline and NO. Several additional coenzymes are required, primarily for electron shuttling from NADPH to the haem iron of NOS, including FAD, FMN and tetrahydrobiopterin (BH$_4$). Calmodulin, a Ca$^{2+}$-binding protein, is yet another requisite coenzyme by virtue of its delivery of the cofactor Ca$^{2+}$ to NOS [9]. Both eNOS and nNOS are Ca$^{2+}$-dependent isoforms of NOS.

Although we will restrict this review to NO production, it is important to recognize that NO availability is also governed by its inactivation. ROS (reactive oxygen species), such as the superoxide anion, quench NO, rendering it inactive [10]. Several endogenous antioxidant systems oppose these ROS, and there is emerging evidence that certain nutritional supplements and exercise training can bolster these endogenous antioxidants, thereby increasing NO availability. This is an area of intense research interest [11].

Adaptations of endothelium-dependent vasodilation to exercise training

Exercise training is a potent stimulus for adaptations of the cardiovascular system [3]. Numerous studies in both humans [12] and animals [13] have demonstrated augmented endothelium-dependent dilation of both conductance (i.e. larger, low-resistance) and resistance (i.e. smaller, high-resistance) blood vessels, specifically those supplying skeletal muscle with blood flow, following a period of endurance exercise training. Endurance training consists of lower intensity, more prolonged sessions of physical activity such as walking, running or swimming, and is that typically used in preventive and rehabilitative programmes with humans. Studies have also demonstrated an augmentation of endothelium-dependent dilation of vessels in the coronary circulation [14]. The majority of these studies concluded, based on pharmacological blockade experiments, that enhanced NO formation accounted for increased vasodilatory responses in the coronary and skeletal muscle circulations.

Adaptations of eNOS to exercise training

Since it appears that augmented endothelium-dependent dilation associated with exercise training is primarily due to enhanced NO formation in the vascular endothelium, most mechanistic research has focused on eNOS. An increase in NO formation could result from increased activity of pre-existing
eNOS and/or an increase in the amount of eNOS protein present in the endothelium. As detailed below, there is considerable evidence for the latter possibility; that is, regular physical activity leads to increases in expression of the eNOS gene and, subsequently, eNOS protein.

**mRNA for eNOS**

The question of whether an increase in mRNA for eNOS is associated with exercise training is important in understanding the mechanism(s) responsible for enhancement of endothelial NO formation. An increase in eNOS mRNA quantity could indicate that exercise training induces increased transcription of the eNOS gene. Early studies involving cultured endothelial cells suggested that this possibility underlies training-induced increases in endothelium-dependent vasodilation. In 1992, Nishida et al. [15] reported that when cultured endothelial cells were exposed to 24 h of a sustained increase in shear stress, increased eNOS mRNA levels were observed. Increases in eNOS transcription have been attributed to a shear stress-responsive element that has been demonstrated to be present in the promoters of several endothelial-specific genes [16]. In addition to increasing transcription of the eNOS gene, exercise training may lead to elevated eNOS mRNA levels via post-transcriptional mechanisms. One such mechanism is increased mRNA stability. Recent work by Harrison et al. has shown that cultured endothelial cells increase poly(A) tail length in response to shear stress, conferring a prolonged half-life on eNOS mRNA [17].

The first study to demonstrate that these endothelial adaptations occurred with exercise training was that of Sessa et al., who reported that a brief period of treadmill run training (10 days) led to increased eNOS mRNA levels in canine aorta [18]. This finding has since been replicated in murine aorta [19], as well as resistance vessels from both rat skeletal muscle [20] and the porcine coronary circulation [21].

Collectively, these studies indicate that exercise training, perhaps due to increases in shear stress on the vascular endothelium during exercise training sessions, induces increased transcription of the eNOS gene and increases in stability of eNOS mRNA. These events would be predicted to lead, in turn, to increased translation of eNOS protein.

**eNOS protein**

Studies utilizing cultured endothelial cells have also been helpful in understanding how exercise training modulates eNOS protein expression. The 1992 study by Nishida et al. [15] that showed increases in eNOS mRNA with shear stress (see above) also demonstrated increased eNOS protein content. An increase in translation of eNOS protein would be an anticipated outcome of increased eNOS mRNA availability. Exercise training studies examining the aorta have uniformly reported that increased eNOS protein content is
associated with training [19,22,23]. This adaptation has been extended to the resistance vasculature of the coronary [24] and skeletal muscle circulations [20,25], although it does not appear to be uniformly distributed in either vascular bed (Figure 3). In the gastrocnemius muscle, 2A through 5A arterioles in the red section, but not the white section, exhibit increased eNOS content. This is likely because endurance exercise training sessions would be predicted to increase blood flow through vessels supplying the red, but not white, section of this muscle. These findings for resistance vessels are important because these vessels play a large role in determining tissue blood flow.

**Figure 3. eNOS protein content of various orders of arterioles from rat gastrocnemius muscle**

Rats either underwent endurance exercise training (ex) or remained sedentary (sed). FA, feed artery; 1A–5A, various orders of arterioles from proximal to distal. Values above bars are P-values for ex versus sed (sed=1.00). Note that arterioles from red, but not white, sections of muscle exhibit significant increases in eNOS protein content. Reproduced from *Journal of Applied Physiology* (2005) vol. 98, pages 753–761, with permission from The American Physiological Society.
**eNOS activity**
An expected outcome of increased eNOS gene and protein expression would be increased eNOS activity and, consequently, greater capacity for NO formation in vascular endothelium. Selected studies have confirmed that exercise-trained animals with an increase in eNOS protein expression exhibit increased eNOS activity [18,19].

**Post-translational mechanisms**
It has become appreciated in recent years that eNOS activity can be acutely modulated by interactions with other endothelial proteins, most notably protein kinase B (also known as Akt) and Hsp (heat shock protein) 90 [9]. It appears that increased eNOS activity can be realized upon phosphorylation of certain amino acid residues; this phosphorylation is mediated by Akt, with Hsp90 playing a facilitating role. Several studies have demonstrated that cultured endothelial cells respond to shear stress by increasing the extent of eNOS phosphorylation, with unchanged eNOS protein content [26]. It is unknown whether acute exercise, like shear stress, induces eNOS phosphorylation; further, effects of exercise training on eNOS phosphorylation are uncertain. One study in which human patients with coronary atherosclerosis were subjected to an exercise training programme demonstrated an increase in phosphorylated eNOS content of the internal mammary artery [27]. This study also reported increased total eNOS protein content in the same artery. Importantly, this study demonstrated augmented endothelium-dependent dilation of internal mammary arteries from exercise-trained patients, suggesting that increases in phosphorylated eNOS and total eNOS protein were of functional significance.

**Substrate and coenzyme availability**
No data are available concerning either substrate (i.e. L-arginine) or coenzyme (e.g. BH4) availability following a period of exercise training. It is an important question, however, because of the so-called arginine paradox. The essence of this paradox is that although the intracellular concentration of L-arginine is well in excess of that required for the eNOS reaction to proceed, L-arginine supplementation is frequently effective in augmenting endothelium-dependent vasodilation [28]. This effect has been reported for patient populations; it is uncertain whether exercise-trained humans and/or animals also exhibit the L-arginine paradox. It is conceivable that a relative lack of substrate is present, given the exercise training-induced increase in eNOS protein expression (see above).

**Exercise training, NO and the prevention and management of atherosclerosis**
Exercise training is a powerful measure for both prevention and management of atherosclerosis. In a recent study, Myers et al. [29] divided approx. 2500
individuals without cardiovascular disease into quintiles based on exercise capacity, which presumably reflected exercise training status. At the end of a 6 year follow-up period, risk of death in the individuals comprising the lowest quintile of exercise capacity was more than four times that of individuals in the highest quintile (Figure 4). These findings were paralleled by approx. 3500 individuals with cardiovascular disease (Figure 4). It is important to note that risk of death was arbitrarily set to 1.0 in the groups of healthy individuals and those with cardiovascular disease; the latter were, of course, at higher risk of death than the former in any given quintile. These epidemiological data suggest that exercise training confers both preventive and rehabilitative effects on atherosclerosis. The mechanisms underlying these beneficial effects are poorly understood, but as explained below it is likely that NO is involved.

It is well-established that the endothelium is dysfunctional in atherosclerosis, at least in part due to decreased NO availability [30]. In contrast to normal vessels, in which endothelium-dependent dilation is augmented by exercise training (see above), it appears that training merely normalizes dilation of atherosclerotic vessels in humans and animals [14]. This effect may not be trivial, however, given that dysfunctional endothelium has been proposed to play a central role in atherosclerosis [30].
Evidence for modulation of the atherosclerotic disease process by exercise training is available in the areas of adhesion molecule expression, smooth muscle cell migration and proliferation, and platelet aggregation. Regarding adhesion molecules, tethering of leukocytes to the vascular endothelium via these molecules and the subsequent migration of leukocytes into the subendothelial space are important preliminary events in foam cell formation [31]. Foam cells are cholesterol-laden macrophages that are significant constituents of atherosclerotic plaques; macrophages are derived from monocytes, a subset of the leukocyte population. Vascular smooth muscle cell migration to or proliferation in the subendothelium is a later event in the atherosclerotic process [31]. Platelet aggregation is a late and often terminal event (in the case of fatal myocardial infarction) in this disease process [31].

Leukocyte adhesion is promoted by expression of endothelial proteins such as P-selectin and VCAM-1 (vascular cell adhesion molecule-1) [31]. NO inhibits leukocyte adhesion [6] and raises the possibility that greater NO formation, an outcome of exercise training-induced increases in eNOS expression, inhibits this aspect of atherosclerosis. Recent findings from Chen and co-workers lend support for this hypothesis [32]. In this study, cholesterol-fed rabbits were assigned to either a sedentary or an exercise-trained group. Exercise training consisted of treadmill walking for up to 6 weeks. Cholesterol feeding led to areas of atherosclerosis in the aortic wall of sedentary rabbits (approx. one-third of the wall area after 6 weeks), along with expression of adhesion molecules such as P-selectin and VCAM-1. Exercise training retarded both the extent of atherosclerosis and expression of the adhesion molecules, P-selectin and VCAM-1. These investigators did not examine eNOS protein expression or activity, but they did determine aortic endothelium-dependent dilation, a functional surrogate of eNOS expression. Endothelium-dependent vasodilation was significantly greater in the exercise-trained rabbits compared with their sedentary counterparts, suggesting that reduced atherosclerosis in the trained animals was, at least in part, due to enhanced NO availability.

In another study examining the impact of exercise training on atherosclerosis, Indolfi et al. [33] examined vascular wall remodelling consequent to angioplasty in the carotid artery. This intervention induces vascular wall injury, with de-endothelialization being a prominent feature. In this study, some carotid-injured rats remained sedentary whereas other rats were subjected to swim training for up to 4 weeks. Angioplasty-induced vascular smooth muscle cell proliferation was dramatically reduced in trained animals, as was the neointima/media ratio, an index of the extent of smooth muscle cell migration/proliferation. Importantly, carotid eNOS expression and activity were determined and found to be increased in trained rats. Inhibition of eNOS by chronic administration of an eNOS inhibitor eliminated beneficial effects of training on smooth muscle cell proliferation and neointima/media ratio. The authors speculated that accelerated re-endothelialization of the carotid artery subsequent to vascular injury was a mechanism underlying the beneficial effects of exercise training.
These data for expression of adhesion molecules [32] and smooth muscle cell proliferation [33] may contribute to findings from studies of coronary artery disease patients. Exercise training in both American [34] and European populations [35] has been reported to decrease the number of patients exhibiting progression in severity of coronary atherosclerosis; indeed, patients showing regression of atherosclerotic lesions were frequently observed in the trained groups of these studies but only rarely in their respective sedentary groups. These results, whilst confounded by other lifestyle changes made simultaneously (e.g. nutritional), nonetheless hint at a beneficial effect of physical activity on established atherosclerosis.

Platelet aggregation, whilst important for normal haemostatic function, can be lethal in advanced atherosclerosis [31]. It is well-established that aggregation is inhibited by NO via cGMP-mediated actions in platelets [6]. Furthermore, reduced platelet aggregatory potential is associated with exercise training [36]. Swim-trained rats in the study of Indolfi et al. [33] exhibited reduced platelet aggregation, relative to sedentary animals, in an *in vitro* assay. Such changes in platelet aggregation may contribute to a lower cardiac event rate observed in exercise-trained human atherosclerotic patients compared with sedentary control patients [34].

**Conclusion**

Animal-based research is supportive of a role for endothelium-derived NO in mediating exercise-induced vasodilation, although parallel evidence in humans is scarce. The reason(s) for this discordance is unclear, but needs to be elucidated. On the other hand, studies in both animals and humans have demonstrated that endothelium-dependent, NO-mediated vasodilation is greater after exercise training. Transcriptional, post-transcriptional and translational mechanisms leading to increased eNOS expression appear to contribute to enhanced NO-mediated vasodilation in trained individuals. Post-translational modification(s) of eNOS (e.g. phosphorylation) may be an additional mechanism underlying enhanced eNOS function after exercise training; this is, however, a largely uninvestigated possibility. Training-induced adaptations involving eNOS are likely to be clinically relevant, given that NO inhibits several steps of the atherosclerotic disease process. This is another area worthy of additional investigation, given that atherosclerosis is the leading killer in Western society.

**Summary**

- **NO** derived from the eNOS-catalysed reaction appears to play a role in exercise-induced vasodilation.
- Endothelium-dependent, NO-mediated vasodilation is augmented by exercise training.
• Increases in eNOS gene transcription, eNOS mRNA stability and eNOS protein translation likely underlie increased NO formation in the endothelium and, consequently, enhanced endothelium-dependent, NO-mediated vasodilation after training.

• Enhanced endothelial NO formation may also have a role(s) in the prevention and management of atherosclerosis by exercise training because several steps in the atherosclerotic disease process are inhibited by NO. A growing body of work suggests that exercise training, in part via increased capacity for NO formation, retards atherosclerosis.

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expression and activity and reduces restenosis after balloon angioplasty or arterial stenting in rats. Circ. Res. 91, 1190–1197

