Nitric oxide-mediated metabolic regulation during exercise: effects of training in health and cardiovascular disease

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ABSTRACT **Accumulating data suggest that nitric oxide (NO) is important for both coronary and peripheral hemodynamic control and metabolic regulation during exercise. Although still controversial, NO of endothelial origin may potentiate exercise-induced hyperemia. Mechanisms of release include both acetylcholine derived from the neuromuscular junction and elevation in vascular shear stress. A splice variant of neuronal nitric oxide synthase (NOS), nNOSμ, is expressed in human skeletal muscle. In addition to being a potential modulator of blood flow, NO from skeletal muscle regulates muscle contraction and metabolism. In particular, recent human data indicate that NO plays a role in muscle glucose uptake during exercise independently of blood flow. Exercise training in healthy individuals elevates NO bioavailability through a variety of mechanisms including increased NOS enzyme expression and activity. Such adaptations likely contribute to increased exercise capacity and cardiovascular protection. Cardiovascular risk factors including hypercholesterolemia, hypertension, diabetes, and smoking as well as established disease are associated with impairment of the various NO systems. Given that NO is an important signaling mechanism during exercise, such impairment may contribute to limitations in exercise capacity through inadequate coronary or peripheral perfusion and via metabolic effects. Exercise training in individuals with elevated cardiovascular risk or established disease can increase NO bioavailability and may represent an important mechanism by which exercise training conveys benefit in the setting of secondary prevention.—Kingwell, B. A. Nitric oxide-mediated metabolic regulation during exercise: effects of training in health and cardiovascular disease. *FASEB J.* 14, 1685–1696 (2000)**

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THERE IS INCREASING evidence that nitric oxide (NO) is an important hemodynamic and metabolic regulator during performance of physical activity. Furthermore, there are adaptations in this system as a result of exercise training that are likely to contribute to increased functional capacity and the cardioprotective effects associated with higher fitness levels. Exercise has particular efficacy in restoring dysfunction of the vascular endothelial NO system, which is becoming established as a precursor to the atherosclerotic process. Recent data also suggest that this benefit may extend to skeletal muscle NO, which appears *inter alia* to mediate glucose uptake during exercise (1–4).

The production of NO from L-arginine is catalyzed by the dioxygenase, nitric oxide synthase (NOS), which closely resembles cytochrome P450. Three isoforms of NOS, termed NOS I (neuronal), NOS II (inducible), and NOS III (endothelial), have been recognized. NO has been implicated in such diverse processes as vasodilation, inhibition of platelet aggregation, immune function, cell growth, neurotransmission, metabolic regulation, and excitation-contraction coupling. This review will focus predominantly on the constitutively expressed, low-output neuronal (n) and endothelial (e) NOS isoforms that produce NO as a signaling mechanism. In humans, in addition to its expression in endothelial cells, endothelial NOS is found in smooth and cardiac muscle, male and female reproductive tract and brain, whereas neuronal NOS is expressed in brain, spinal cord, sympathetic ganglia, peripheral nerves, pancreas, epithelial cells of the stomach, lung, uterus, and skeletal muscle. From a functional perspective, emphasis will be placed on the role of NO in hemodynamic and metabolic control during exercise, the adaptations that occur with training, and how these may contribute to the preventative and therapeutic effects associated with physical activity and fitness.

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NO AS A METABOLIC REGULATOR DURING EXERCISE

Matching tissue oxygen and substrate supply to demand during physical activity is controlled both by blood delivery and the capacity of cells to extract these substrates. As discussed below, NO appears to play a role in both of these processes. Empirical evidence to support a role for NO during exercise includes elevation in exhaled NO (5) as well as increased urinary excretion of the NO second messenger cyclic GMP and the NO metabolite nitrate during performance of exercise in athletes (6). The potential mechanisms of NO release and its specific actions are discussed in the following section.

Skeletal muscle perfusion

The mechanisms controlling skeletal muscle blood flow during exercise are complex and involve neural, metabolic, endothelial, myogenic, and muscle pump control. These mechanisms modulate blood flow via effects on perfusion pressure and the caliber of resistance vessels. Traditionally, vessel caliber has been thought to represent a balance between vasodilation mediated directly by production of metabolites from the exercising muscle and sympathetic activation via muscle metabo- and mechanoreceptor stimulation. NO derived from both the endothelium (endothelial NOS, type III) and skeletal muscle (neuronal NOS, type I) may, however, play an important role in matching tissue perfusion to demand. In support of this argument, Roberts et al. have recently shown that a 45 min exhaustive exercise bout increases both neuronal and endothelial NOS activity in rats (7)

Even though it is undisputed that stimuli such as adenosine, acidity, temperature, pO_2, pCO_2, magnesium, and potassium ions contribute to dilation of the microvessels, it has become clear that other mechanisms mediate upstream dilation of larger 'feed' arteries. Vascular shear stress that is determined by blood flow and viscosity is now a well-established stimulus for elevation of intraendothelial Ca^{2+} levels and release of NO from the vascular endothelium. NO formed from this reaction then diffuses to underlying vascular smooth muscle cells, where it activates guanylate cyclase to produce cGMP from GTP and ultimately vasodilation. Thus, microvessel dilation in response to accumulation of vasodilatory metabolites creates a pressure gradient that stimulates flow-mediated dilation of upstream arteries by shear stress-induced release of NO from the endothelium (**Fig. 1,** 2a and 2b) (8). This hypothesis is consistent with the lag time of several seconds between metabolic dilation and flow-induced dilation of feed arteries (9).

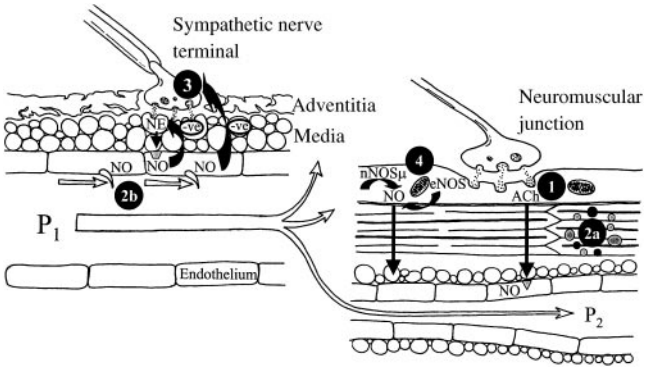

Figure 1. Schematic representation of a feed artery (left) and microvessel (right) illustrating potential NO mechanisms mediating exercise-induced hyperemia. *1)* Acetylcholine (ACh) from the neuromuscular junction may diffuse to the vascular endothelium where it activates muscarinic receptors (gray inverted triangle), promoting endothelial NO release, local smooth muscle cell relaxation, and conducted dilation via endothelial cell hyperpolarization. *2)* Accumulation of metabolites in actively contracting muscle (*2a*) induces dilation in the microvessels, promoting a pressure gradient (P1>P2) with upstream feed arteries. The resultant increase in flow elevates shear stress and release of endothelially derived NO (*2b*) although the precise signal transduction mechanism is yet to be determined. *3)* Central and ergoreflex-generated elevation in sympathetic activity to feed arteries promotes NO release via stimulation of endothelial α2 receptors (gray trapezoid). NO may also inhibit norepinephrine (NE) release prejunctionally and α2-mediated constriction of vascular smooth muscle. *4)* NO of skeletal muscle origin (nNOSμ), possibly produced in response to contraction-induced elevation of intracellular calcium, may also diffuse into arteriole smooth muscle and promote vasodilation.

NO-mediated dilation of feed arteries permits increased microvascular flow without reduction in muscle perfusion pressure. In addition, this system allows a greater capacity for regulation of systemic vs. local perfusion demands since the feed arteries are a major site of sympathetic nervous control of vascular tone. NO may exert control on sympathetic function peripherally at the level of the feed arteries (10) but also in the central nervous system (11), inducing functional sympatholysis (Fig. 1, 3). In the periphery, catecholamine stimulation of endothelial α2-adrenoreceptors causes NO release (12). α2-Adrenoreceptor-induced vasoconstriction is particularly sensitive to the inhibitory effects of NO (12). Prejunctional inhibition of norepinephrine release by NO is also known to occur (13)

Acetylcholine may represent another mechanism mediating NO-dependent dilation during physical activity. Although acetylcholine has been widely used to activate endothelial muscarinic receptors to study endothelium-dependent NO-mediated vascular reactivity, a physiological role for acetylcholine is more controversial. The neuromuscular junction of motor nerves that synthesize, store, and release acetylcholine may represent a physiological source of acetyl-

choline during exercise that stimulates endothelial release of NO, triggering vasorelaxation and increased blood flow (Fig. 1, 1). Segal and colleagues have also postulated that during physical activity, acetylcholine released from the neuromuscular junction triggers hyperpolarization, which is conducted along the endothelial cell layer via gap junctions between cells (14), causing vasodilation of the arteriole network. This effect does not, however, appear to be dependent on NO. Acetylcholine of neuronal origin may also be important in the myocardium, particularly during exercise-induced ischemia, when NO production from the heart is increased (15) and the vagally mediated Bezold-Jarisch reflex is thought to be activated (16). Non-neuronal acetylcholine has also been demonstrated in platelets and leukocytes as well as endothelial cells from human skin (17) and rat (18) and in porcine brain vessels (19). Although the role of such non-neuronal acetylcholine has not been fully elucidated, acetylcholine is released from endothelial cells under basal conditions and in response to flow (20). Thus, acetylcholine is likely to play a role in control of vascular tone both at rest and during exercise.

Despite the plausibility of these hypotheses and the associated *in vitro* evidence, there is still much controversy regarding the role of NO in exercise hyperemia, due largely to methodological difficulties associated with the study of exercise hyperemia in intact animals and humans. There have been six recent studies in humans examining the effects of NOS inhibition during exercise (4, 21–25). Three have examined responses in small (arm) muscle groups, with one showing no effect (22), one a small effect (24) and the other a moderate effect (21). All three studies measured blood flow using venous occlusion plethysmography, which necessitates cessation of exercise during measurement, thus limiting blood flow measurements to the period immediately after exercise. Hickner and colleagues showed a significant effect of NOS inhibition, but measured leg blood flow using microdialysis probes, a technique that requires further validation and at best measures only blood flow localized to the area immediately surrounding the probe (23). In contrast, Radegran and Saltin found no effect of NOS inhibition on femoral blood flow assessed using noninvasive Doppler during knee extensor exercise (25). In an attempt to overcome the methodological limitations of small muscle exercise and indirect blood flow measurement techniques, leg blood flow was measured using constant infusion thermodilution during dynamic cycling exercise with intrafemoral infusion of either saline or L-NMMA (4). During an exercise bout of 30 min duration performed at 60% of each subject's maximum capacity, there was no effect of NOS inhibition on leg blood flow or oxygen consumption (**Fig. 2**, lower panel) (4). These data suggest that either NO plays no role in the hyperemic response to exercise or that there are redundancies in the vasodilatory mechanism such that NO does not have an obligatory role in exercise hyperemia if other mechanisms are functional. Resolution of these two possibilities will be difficult but may be possible with inhibition of other major metabolic dilator mechanisms including adenosine, prostacyclin, and K^+ATP channels.

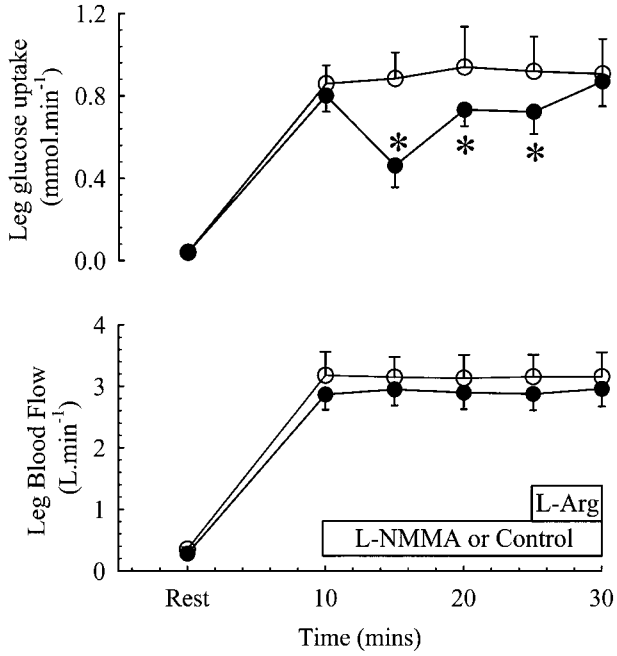

Figure 2. Leg glucose uptake (upper panel) and leg blood flow (lower panel) in 7 healthy male subjects during 30 min supine cycling. Subjects performed both the L-NMMA protocol and the control protocol in a counterbalanced, double-blind, crossover design separated by ~90 min of rest. Mean ± SE. L-NMMA protocol (filled circles); control protocol (open circles). L-arginine (L-Arg) was infused during the final 5 min of both protocols. *$P<$ 0:05 between trials (4)

Coronary perfusion

Similar controversy surrounds the mechanisms controlling coronary vasodilation during exercise. It is well established that hyperpolarization of vascular smooth muscle cell membranes brought about by opening of K^+ATP channels is very important for metabolic coronary vasodilation (26, 27). Blockade of vascular smooth muscle K^+ATP channels in chronically instrumented dogs, however, did not attenuate the coronary blood flow response to exercise (27), although both adenosine and K^+ATP channel blockade reduced the exercise-induced increase in coronary flow by half (28). When NOS blockade was added to adenosine and K^+ATP channel blockade, coronary flow was reduced to levels below that of resting control conditions (29). In the

presence of adenosine and K^+ATP channel blockade, NO can produce approximately one-quarter of the coronary vasodilation normally produced during exercise with all systems intact. However, NOS inhibition alone or combined with adenosine receptor blockade did not influence either resting or exercise coronary flow, indicating that when K^+ATP channels are intact, neither NO nor adenosine-dependent mechanisms are obligatory for maintaining coronary flow. Thus, all three systems are important for coronary vasodilation during exercise, but like metabolic skeletal muscle, vasodilation there is certainly substantial redundancy in the coronary system.

In addition to the obvious relationship between coronary vessel vasodilation and flow, coronary perfusion may also be influenced by aortic properties, which in turn are affected by vascular reactivity to dilators and constrictors, including NO. Simulated aortic stiffening induced in dogs by aortic bandaging (30) limits subendocardial perfusion, particularly in the setting of a coronary occlusion, in response to a reduction in diastolic blood pressure. At the same time, aortic stiffening increases systolic pressure and cardiac afterload, further stressing the relationship between cardiac systolic performance and myocardial perfusion. The NOS inhibitor L-NAME increases vascular impedance in rats (31) whereas organic nitrates improve arterial wall viscoelasticity in mini pigs (32), together indicating that NO reduces arterial stiffness. The most likely mechanism involves NO-induced vasodilation, which in the physiological pressure range transfers wall stress from the stiffer collagen fibers to the more distensible elastin matrix. Although there are currently no studies that have directly examined a role for NO in modulation of large artery stiffness in the context of exercise, the known relationship between vascular properties and vasodilation plus the fact that NO is released during exercise suggests that NO is probably an important contributor to ventriculo-vascular coupling and to coronary perfusion via this mechanism during physical activity. In support of this hypothesis, large artery compliance is increased immediately after an acute exercise bout (33)

Skeletal muscle metabolism

Both neuronal and endothelial NOS isoforms are constitutively expressed in rat skeletal muscle fibers whereas in humans, nNOS is found in skeletal muscle fibers and eNOS is present in the endothelium of vessels perfusing muscle (34–36). The neuronal NOS isoform expressed in human skeletal muscle is a splice variant incorporating an additional 102 bp, and termed nNOSμ (37). In rodent muscle, neuronal NOS is predominantly found in type II (fast twitch) muscle fibers (38), whereas in humans and subhuman primates, neuronal NOS is more homogeneously distributed between type I and II fibers (for review, see ref 39). Immunoreactivity is predominantly found near the sarcolemma of muscle spindle fibers, particularly nuclear bag fibers (which are type I), and localized to motor end plates (38). The dystrophin–glycoprotein complex mediates the association of nNOS with the sarcolemma, either directly or linked to α1-syntrophin through PDZ domain (PSD-95 (postsynaptic density protein, M_r 95K), D1g (*discs-large* protein), and ZO-1 (zonula occludens-1) interactions (39).In rodents the endothelial NOS isoform is homogeneously distributed between fast and slow twitch fibers, with immunohistochemical staining showing a strong correlation between expression and mitochondrial content (visualized histochemically by succinate dehydrogenase) in skeletal muscle (35). Type II or inducible NOS is not expressed constitutively in skeletal muscle but can be induced in response to inflammation.

Balon and Nadler have demonstrated that NO production is increased more than twofold during electrical stimulation of isolated rat extensor digitorum longus muscle (1). NO production from skeletal muscle has been implicated in metabolic control via effects on blood delivery, glucose uptake, oxidative phosphorylation, contractility, and excitation-contraction coupling. The immunohistochemical association of nNOS with the sarcolemma suggests that it is this isoform that influences blood delivery, glucose uptake, and excitation-contraction coupling. Expression of eNOS is seen in association with the mitochondria in rodents and correlates with mitochondrial respiration, and therefore most likely influences oxidative phosphorylation (40).

Blood delivery and glucose uptake

A role for nNOS in control of blood flow during exercise comes from studies of dystrophin-deficient (mdx) mice in which expression of this enzyme is greatly reduced (Fig. 1, 4). During contraction, arteriolar dilation was less in contracting skeletal muscle from mdx mice than controls (41). It is possible that reduced blood flow via this mechanism may contribute to impaired exercise capacity in the early stages of Duchenne muscular dystrophy prior to the occurrence of muscle wasting.

The blood flow effects of NO are consistent with those on muscle metabolism that work to preserve intracellular energy stores by promoting glucose uptake and by inhibiting glycolysis, mitochondrial respiration, and phosphocreatine breakdown. Experimentally, NO modulates carbohydrate metabolism through enhancement of glucose uptake (1) and inhibition of glyceraldehyde-3-phosphate dehydrogenase, and therefore glycolysis (42). More re-

cent rat studies using isolated muscle (2) and sarcolemmal vesicular preparations (3) indicate that NO-mediated glucose uptake occurs during exercise. The first evidence that NO-mediated glucose uptake during exercise is important in humans was published recently (4). In this study, the NOS inhibitor L-NMMA infused into the femoral artery during cycling reduced glucose uptake by 48% compared with a control, saline infusion (Fig. 2, upper panel). This effect occurred in the absence of any changes in blood flow, suggesting that L-NMMA directly affected the ability of skeletal muscle to extract glucose from the blood (Fig. 2, lower panel). The precise mechanism for this action has not been elucidated, but nNOS stimulation may be linked to the rise in intracellular calcium associated with contraction (43). Furthermore, since insulin and NO have additive effects on glucose uptake and insulin-mediated glucose uptake is not affected by NOS inhibition, insulin and NO appear to recruit discrete pools of the glucose transporter GLUT-4 (44)

Oxygen consumption

It is well known that large local concentrations of NO produced in response to inducible NOS activation inhibit cellular respiration in a pathophysiological setting. Studies in conscious dogs, however, support the notion that tissue oxygen consumption is modulated physiologically *in vivo* by constitutively produced NO (45, 46). In these studies, NO synthase inhibition significantly increased whole-body and skeletal muscle oxygen extraction above levels expected purely as a result of vasoconstriction, so that under normal physiological conditions NO inhibits oxygen extraction. More recent data from dogs also confirm this hypothesis in the heart (47).

The mechanism by which NO reduces oxygen extraction appears to relate to effects on enzymes involved in oxidative phosphorylation (48) and transfer of high-energy phosphates (49) as well as c-GMP-dependent pathways (50). Of these mechanisms, perhaps the most well described is the reversal inhibition of cytochrome *c* oxidase by NO or its derivative peroxy nitrite ($ONOO^-$) at low (nanomolar) concentrations through competition with oxygen (51). At higher concentrations, NO inhibits other respiratory chain enzymes through nitrosylation and oxidation (50). The expected reduction in contractility as a result of inhibition of these processes by NO has been observed in the heart (47) and skeletal muscle (52). Definitive *in vivo* evidence for regulation of mitochondrial respiration by NO is still lacking, particularly in the setting of exercise. Indeed, in the only animal study examining oxygen extraction during exercise, NO favored aerobic rather than anaerobic metabolism in horses, as evidenced by higher plasma lactate during NOS inhibition with L-NAME (53). Whether such effects related to reduced oxygen delivery as a result of lower blood flow is unclear. NOS inhibition has not been shown to affect oxygen uptake during exercise in humans (4).

Contractility

Endogenous NO production decreases submaximal skeletal muscle force by modulating excitation-contraction coupling (38). In addition, NO donors and cGMP depress contractile function whereas NOS inhibition, extracellular NO chelation, and guanylyl cyclase inhibition augment contractile function (38, 40). Although these findings support a cGMP-dependent inhibition of contraction, NO may also modulate contractile function directly through inhibition of opening of calcium release channels of the sarcoplasmic reticulum (54). On the other hand, NO may be an essential component for active shortening by facilitating cross-bridge cycling (55). These opposing actions of NO on contractile function must be interpreted in the light of studies showing that contraction induces a decline in muscle NOS activity, which if localized to the mitochondria might represent a compensatory mechanism through which muscle contractility and mitochondrial function are protected from the inhibitory influence of NO (56).

Cardiac muscle function

In addition to the role of endothelially derived NO in the coronary vasculature, human cardiac muscle expresses both eNOS (57) and nNOS (58) whereas iNOS is inducible in disease states, including cardiomyopathy (59). Recent studies suggest that nNOS is expressed in cardiac sarcoplasmic reticulum and that the expressed enzyme may be a novel isoform (58). As in skeletal muscle, NO appears to inhibit contractile function and oxygen consumption (60), particularly in the setting of endotoxin exposure and iNOS expression (61). In contrast to skeletal muscle, however, NO appears to inhibit glucose uptake in the myocardium at rest (62). The contractile effects are consistent with myocardial relaxation and reduced diastolic tone (61) and are mediated in part by inhibition of respiratory chain enzymes and creatine kinase (60). However, findings are not homogeneous, and there are many examples where NO acts as a mild inotrope (for review, see ref 63).The reasons for these discrepancies are currently unresolved but may relate to methodological (*in vivo* vs. *in vitro*) and species differences.

Although few studies have examined the role of NO in the myocardium during exercise, Bernstein and colleagues have shown that acute treadmill

exercise in dogs increases NO production from the coronary circulation (64). Blockade of NO prevented NO release, increased myocardial oxygen consumption, and reduced myocardial free fatty acid consumption for comparable levels of coronary blood flow and work. These metabolic changes occurred in the absence of alterations in myocardial glucose or lactate consumption.

Summary

NO potentially affects metabolic control during exercise via multiple mechanisms, including:

- Elevation in skeletal muscle and cardiac blood flow and increased delivery of oxygen, substrates, and regulatory hormones (e.g., insulin);
- Preservation of intracellular skeletal muscle energy stores by promoting glucose uptake, inhibiting glycolysis, mitochondrial respiration, and phosphocreatine breakdown;
- Depression of contractile function.

Together, these actions of NO on blood flow, substrate utilization, and contractile function appear to be directed toward protection from ischemia. Although increased blood and substrate delivery to active muscle, including the myocardium, will clearly contribute to enhanced exercise performance, some other actions of NO may actually limit exercise capacity. Since many of the NO actions discussed relate to local intracellular levels of NO, it is important to discuss the role of this important signaling molecule during exercise in the proper context.

EFFECTS OF EXERCISE TRAINING ON NO FUNCTION IN HEALTHY INDIVIDUALS

From the preceding section it can be seen that NO has multiple roles in the circulatory and metabolic response to an acute bout of exercise. It is not surprising, therefore, that this system adapts in response to training and that such adaptations may contribute to enhanced exercise capacity and reduced cardiovascular disease risk. To date, most studies of the effects of exercise training on NO function have focused on the regulation of vascular tone and blood flow rather than metabolic or other effects. Studies have typically examined basal release of NO inferred from the effects of NOS inhibition and/or NO released in response to agonists such as bradykinin or acetylcholine. There are few human studies but a large body of work in animals including rats, rabbits, dogs, and pigs. All published studies have examined adaptations at rest, with no reports of the effects of training on the hemodynamic role of NO during performance of exercise.

Animal studies

The effects of training on resting NO release and reactivity in isolated vessels have been reviewed extensively elsewhere and will not be expanded on in this review (65). In brief, vessels exposed to elevated flow during exercise, including the aorta, coronaries, and vessels from the active muscle bed, generally show evidence of increased NO production, gene expression, and/or reactivity (66). In dog models, exercise training enhanced reactivity to NO-dependent agonists in both proximal coronary arteries (66, 67) and coronary microvessels (66), but the opposite was true in rats (68) and pigs (69). There are clear species and regional differences in the NO response to training, highlighting the importance of human studies.

Human studies

Evidence in humans for chronic changes in the NO system with training is accumulating. Recent work suggests that endothelium-dependent dilation may be altered by training in the rest period between exercise bouts and that the effect may not be restricted to the trained muscle bed (70–72). Moderate cycle training for 4 wk increased basal NO production in the forearm (**Fig. 3**, center panel; ref 71) and a 10 wk program increased forearm flow-mediated dilation (72). These data indicate first that whole-body dynamic exercise may represent a powerful stimulus for adaptations in the NO system, and second that increased vascular shear stress as a result of elevation in heart rate, pulse pressure, blood viscosity, and blood flow may alter NO function in nonexercising muscle beds (71). In contrast, athletes who were predominantly leg trained exhibited no changes in basal NO production (Fig. 3; left panel; ref 70). This finding may reflect differences between cross-sectional (70) and longitudinal studies (71), the latter being more specific for exercise, with an alternate explanation being that although NO may play a role in the short term adaptations to exercise (i.e., over a few weeks), long-term exercise over a period of years is not associated with changes. It is possible that adaptations to meet metabolic demands with training evolve from vasodilation mediated at least in part by NO in the short term, to longer term adaptations such as metabolic enzyme changes and vascular restructuring. This is consistent with the hypothesis advanced by McAllister and Laughlin, who speculated that vascular endothelial function is enhanced after just a few days of training and that such adaptation could serve to buffer the increase in shear stress experienced during exercise. NO may be involved in the signaling cascade that subsequently triggers the structural changes (includ-

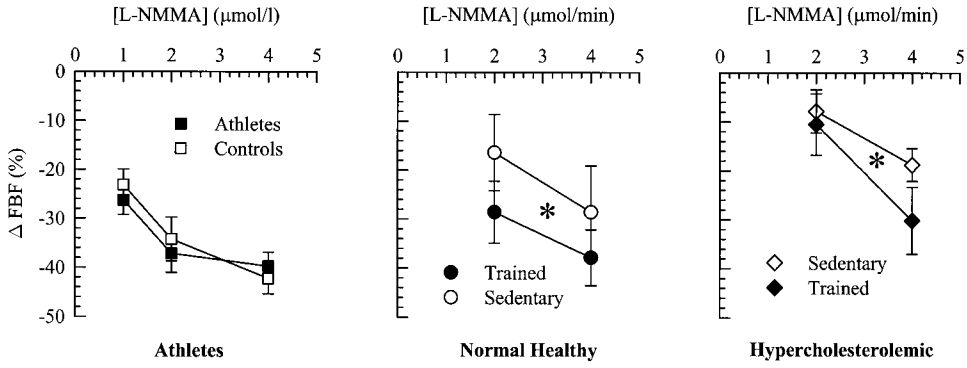

Figure 3. Basal nitric oxide production may be inferred from the vasoconstrictor responses to the nitric oxide synthase inhibitor N^G-monomethyl L-arginine (L-NMMA). The figure shows percentage change in forearm blood flow from baseline after infusion of L-NMMA. Left panel: highly trained athletes (solid squares) and matched sedentary controls (open squares) (70). Center panel: normal healthy individuals in the sedentary state (open circles) and after 4 wk of moderate cycle training (solid circles) (71). Right panel: hypercholesterolemic patients in the sedentary state (open diamonds) and after 4 wk of moderate cycle training (solid diamonds) (100). Error bars are SEM; *significant difference from baseline, $P < 0.05$.

ing increased vessel diameter) that minimize or even eliminate the need for enhanced release of NO (73)

Whereas basal NO production appears unaffected at rest by long-term training, acetylcholine-stimulated release is increased, possibly relating to lower total cholesterol in athletes (70). This effect implies a greater endothelium-dependent vasodilator reserve in athletes, which would increase capacity to perform localized exercise not limited by cardiac considerations. In addition, if such adaptations are present in the coronary circulation, the enhanced dilator reserve capacity observed in response to nitroglycerin in ultradistance runners (74) could be evoked physiologically by endothelial mechanisms. This may in part underlie the enhanced ischemic threshold induced by training in the setting of coronary disease. Finally, enhanced NO-dependent dilator reserve in the aorta may contribute to improved coronary perfusion and reduced afterload via the increased aortic compliance we have documented in athletes (75) and after 4 wk of moderate intensity cycling in previously sedentary subjects (76)

Summary

The vast animal literature together with more recent human studies indicates that endurance exercise training for a period ranging from days to several weeks enhances basal release of nitric oxide from the aorta, active and inactive muscle, and coronary arteries. This adaptation may contribute to the reduction in resting blood pressure that can be observed after as little as 4 wk of training (77, 78). Increased vascular NO production appears to be a transitory response to training that progresses to structural and other sustained adaptations. Training also enhances agonist-induced, endothelium-dependent dilation in these same vascular beds, but is associated with training durations ranging from weeks to months. Such adaptations would be expected to enhance blood and substrate delivery to cardiac and active skeletal muscle, thus contributing to enhanced exercise capacity. The effects of training on the other purported metabolic actions of NO have not been investigated.

IMPLICATIONS OF IMPAIRED NO FUNCTION FOR EXERCISE CAPACITY

Endothelium

Impaired release and/or bioavailability of endothelial NO are associated with a growing list of cardiovascular disease risk factors including hypercholesterolemia, hypertension, smoking, and diabetes and in established coronary disease and cardiac failure. Impaired release may be due to down-regulation of NOS expression or defects in the shear stress or agonist-linked receptor mechanisms that activate NOS. Once released, NO activity is also inversely related to the presence of oxygen-derived radicals, which combine with NO to form $ONOO^-$, a less biologically active vasodilator than NO.

There is emerging evidence in humans and more complete data in animals indicating that endothelial dysfunction limits exercise capacity either through cardiac or peripheral mechanisms. The ApoE-deficient mouse that simulates hypercholesterolemia and wild-type mice fed the NOS inhibitor LNA (N^G-nitro-L-arginine) had reduced postexercise nitrate excretion, aerobic capacity, and peak exercise redistribution of cardiac output to running muscles (expressed as a percentage of cardiac output) (79). Such findings indicate that NO contributes significantly to limb blood flow during exercise and that conditions that reduce NO disturb the hyperemic response to exercise, resulting in a reduced exercise capacity. In support of this contention, the forearm blood flow response to handgrip exercise is diminished in cardiac failure patients. Intra-arterial L-arginine infusion had no effect on blood flow re-

sponse in healthy individuals but restored responses to normal in cardiac failure patients (80). Whereas L-arginine is known to have NO-independent vasodilatory actions, the lack of effect in the healthy group where L-arginine is thought to be non rate-limiting to NO production suggests that the mechanisms for restoration of forearm blood flow responses to handgrip exercise in the cardiac failure group was NO related.

In patients with hypercholesterolemia and coronary atherosclerosis, coronary and systemic arteries constrict during exercise (81–83), probably reflecting loss of dilator regulation by the coronary endothelium as a consequence of diminished NO release or increased degradation. Such effects would also be expected to augment responsiveness to vasoconstrictors such as norepinephrine and endothelin. Endothelial dysfunction in the setting of coronary disease is thus likely to be a major contributor to exertion-induced ischemia and a limiting factor to exercise capacity. Indeed, Ishibashi and colleagues have suggested, based on their studies with dogs, that coronary disease patients with impaired small coronary endothelium-dependent dilation are more susceptible to hypoperfusion distal to a coronary artery stenosis, particularly during exertion (84).

NO may also have an important role in pulmonary vasodilation during exercise. NO is increased in exhaled air during exercise in normal subjects (85); at rest, NO concentration in expired air was less in congestive heart failure than normal subjects and increased during exercise by a greater amount in normal subjects (86). This may contribute to blunted pulmonary dilation and thus an abnormally high ventilatory response, which occurs during exercise in cardiac failure. Inhalation of NO normalizes this response and improves exercise capacity in patients with moderate right ventricular failure (87)

Skeletal muscle

Since the potential role of NO in metabolism and glucose uptake has emerged only relatively recently, few studies have examined the effect of cardiovascular disease on skeletal muscle NOS expression or on functional responses; the link with glucose metabolism has led to a predominant focus on diabetes. Skeletal muscle NOS activity is reduced in both obese insulin-resistant Zucker rats (88) and humans (89). Although no study has examined the question directly, our findings with regard to the role of NO in glucose uptake in humans (4) would suggest that reduced skeletal muscle nNOS expression may contribute to the impaired exercise capacity in diabetics (90, 91). However, glucose uptake is often preserved in diabetics during exercise, and further work is clearly warranted to directly examine the role of NO in glucose uptake in this group.

Expression of inducible NOS has also been examined in the setting of cardiac failure as a possible link between myocardial dysfunction and reduction in peripheral exercise tolerance (49, 92). Since there is increased expression of inducible NOS in vastus lateralis muscle of congestive heart failure patients relative to normal controls (92), exercise capacity may thus be limited via the inhibitory effects of NO on enzymes of oxidative phosphorylation (48). Furthermore, Haembrecht and colleagues have observed intracellular NO accumulation in heart failure and an inverse relationship between skeletal muscle iNOS expression and maximum oxygen uptake/mitochondrial creatine kinase (49). These data imply that premature muscle fatigue in cardiac failure may be mediated by both impaired energy production and impaired energy transfer from the mitochondrion to the cytosol.

Summary

Definitive evidence for a pivotal role of NO in the impaired response to exercise in cardiovascular conditions is not yet available. Furthermore, it is difficult to separate the specific limitations of NO dysfunction on exercise capacity from limitations related to other aspects of disease; however, the data cited and mechanistic plausibility support the contention that NO dysfunction limits exercise capacity. The major mechanism appears to be reduced blood delivery to active muscle including the pulmonary and coronary circulations. These limitations may be particularly important in cardiac failure.

EFFECTS OF EXERCISE TRAINING ON NO FUNCTION IN CARDIOVASCULAR DISEASE

The therapeutic potential of training to normalize NO-dependent vasodilation in disease states has been examined in a number of studies, although there are few published studies on NO-related metabolic effects including glucose uptake. With regard to endothelial function, training has the potential to provide benefit via a number of different mechanisms, including:

- increased shear stress-induced release of NO and prostaglandins;
- increased expression of endothelial NOS;
- reduced inactivation of NO by superoxide or other oxygen-derived free radicals (93).

Hypertension

In genetic models of hypertension, training improves acetylcholine responses in aortic and mesenteric rings (94), decreases aortic and carotid respon-

siveness to norepinephrine by increasing NO release (95), and increases plasma nitrate (96). Recent studies in human hypertension have reported that daily walking for 30 min over a 12 wk period augmented endothelium-dependent vasorelaxation (97, 98); there was a negative correlation between the change in forearm reactive hyperemia and LDL during the intervention. The lipid profile changes in these studies, however, were more consistent with dietary modification than exercise training, and since no objective assessment of improved fitness was presented, it is difficult to attribute the changes in endothelial function solely to training.

Hypercholesterolemia

In a mouse model, hypercholesterolemia impaired exercise capacity, possibly due to impaired NO-dependent vasodilatory capacity (99). In this same study, exercise training at 6 days per week for 4 wk increased vascular reactivity and NO synthesis, which in turn correlated with improvement in anaerobic threshold. Hypercholesterolemic patients with impaired forearm reactivity to acetylcholine participated in a randomized crossover study incorporating 4 wk of moderate intensity cycling and 4 wk of normal sedentary activity (100). The training intervention had no effect on lipid profile, although forearm constrictor responses to L-NMMA were augmented (Fig. 3, right panel) and forearm production of the NO metabolites nitrate and nitrite were increased by training, suggesting greater NO production at rest. Responses to acetylcholine were unaffected by the training regimen. These data suggest that for hypercholesterolemic patients, training has benefits in addition to lipid profile modification and may be considered a useful adjunct to conventional lipid lowering therapy.

Diabetes

Otsuka Long-Evans Tokushima fatty rats have impaired aortic dilation to histamine but not sodium nitroprusside. Although exercise training and food restriction both significantly reduced plasma levels of glucose and insulin, lowered serum levels of triacylglycerol and cholesterol, and reduced the accumulation of abdominal fat, only exercise restored responses to histamine and increased urinary excretion of nitrite (101). Furthermore, 8 wk of treadmill training increased skeletal muscle expression of neuronal NOS by fourfold and expression of endothelial NOS by twofold (2). Although there are no human data, the rat studies suggest that modulation of the NO pathway by exercise training could provide a novel approach to improving skeletal muscle glucose uptake.

Coronary disease

The effects of training on NO function have been studied only on an empirical level with regard to coronary disease. A 12 wk cardiac rehabilitation program was associated with a 150% increase in the excretion of NO metabolites. The increase in NO metabolite excretion was in proportion to the increase in functional capacity as a result of training (102).

Cardiac failure

The efficacy of training to restore endothelial dysfunction associated with cardiac failure is controversial. In a coronary artery occlusion heart failure model, 6 wk of treadmill training failed to restore aortic acetylcholine responses (103). Similarly, 4 wk of forearm handgrip exercise did not change responses to acetylcholine, reactive hyperemia, NOS inhibition, or acute forearm exercise, whereas the same intervention augmented acetylcholine and reactive hyperemic responses in healthy controls (104). These data contrast with two earlier studies (105, 106). Hornig and colleagues showed that heart failure patients exhibited impaired forearm dilation to release of both wrist and upper arm occlusion for 4 and 8 min. Patients then underwent daily forearm isometric handgrip training for 4 wk. The training program elevated flow-mediated dilation in the trained arm to levels observed in disease-free subjects, although this improvement regressed to baseline 6 wk after cessation of training. Studies performed in the presence of NOS inhibition with L-NMMA indicated that the restorative effects of training on flow-mediated dilation were related to endothelial release of NO. Second, using plethysmography Katz and colleagues observed augmented responsiveness to acetylcholine after 8 wk of forearm training (105). Training frequency may account for discrepancies between the human studies, with the latter two positive studies incorporating daily training sessions and the former only four sessions per week.

Although the handgrip studies have provided initial support for training-related adaptations in the NO system, only one study has examined the effects of large muscle dynamic endurance exercise in cardiac failure (107). Forty minutes of cycling 5 days per week for 6 months improved femoral blood flow in response to intra-arterial infusion of acetylcholine but not sodium nitroprusside; the vasoconstrictor effect of NOS inhibition with L-NMMA also increased, suggesting enhanced basal release of NO. The increase in peak oxygen uptake was in proportion to the change in endothelium-dependent dilation, so that impaired endothelial NO production

may contribute to peripheral exercise limitation and increased exercise capacity as a result of training may be a consequence of improved NO production from the endothelium.

Summary

The studies discussed indicate that the role of NO in modulating vascular tone after training must be defined in terms of type of training, vascular region, and time course of the training response. Preliminary data indicate that the NO system is modified by training in the setting of cardiovascular disease and that these effects may contribute to increased functional capacity. However, the role of NO in the coronary circulation and skeletal muscle particularly with regard to glucose uptake is yet to be established.

CONCLUSION

NO represents an important molecule for metabolic control during exercise. Impairment, particularly of the vascular endothelial system, may contribute to exercise limitations associated with cardiovascular disease. Dynamic exercise training at moderate levels on the other hand may enhance the activity of various NO systems. The endothelial NO system is up-regulated after relatively short duration training and may be the mechanism that triggers longer term structural changes that ultimately normalize shear stress and return basal NO levels to that of sedentary individuals. Enhanced endothelium-dependent dilator reserve may represent a more sustained response to training that protects the myocardium from exertion-induced ischemia. Animal studies have flagged adaptations in the skeletal muscle NO system as being responsible for some of the metabolic benefits of training. Studies in humans have yet to explore this interesting area that certainly warrants further investigation. FJ

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