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Microvascular Vasodilator Plasticity After Acute Exercise

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[1]Department of Kinesiology and Applied Physiology, University of Delaware, Newark, DE; [2]Department of Physical Therapy; [3]Division of Pulmonary, Critical Care, Sleep, and Allergy, Department of Medicine; [4]Department of Kinesiology and Nutrition; [5]Integrative Physiology Laboratory; and [6]Division of Endocrinology, Diabetes, and Metabolism, Department of Medicine, University of Illinois at Chicago, Chicago, IL.

ROBINSON, A.T., I.S. FANCHER, A.M. MAHMOUD, and S.A. PHILLIPS. Microvascular vasodilator plasticity after acute exercise. *Exerc. Sport Sci. Rev.*, Vol. 46, No. 1, pp. 48–55, 2018. *Endothelium-dependent vasodilation is reduced after acute exercise or after high intraluminal pressure in isolated arterioles from sedentary adults but not in arterioles from regular exercisers. The preserved vasodilation in arterioles from exercisers is hydrogen peroxide (H_2O_2) dependent, whereas resting dilation is nitric oxide (NO) dependent. We hypothesize chronic exercise elicits adaptations allowing for maintained vasodilation when NO bioavailability is reduced.* **Key Words:** high blood pressure, oxidative stress, antioxidants, endothelium, endothelial dysfunction.

Key Points

- Endothelium-dependent vasodilation is reduced after acute exercise or exposure to high intraluminal pressure in isolated arterioles from sedentary adults.
- Endothelium-dependent vasodilation is preserved after acute exercise or high intraluminal pressure in isolated arterioles from regular exercisers.
- Preserved vasodilation is hydrogen peroxide (H_2O_2) dependent, whereas resting dilation is nitric oxide (NO) dependent, suggesting chronic exercise elicits adaptations allowing for maintained vasodilator function when NO bioavailability is reduced.
- Future studies are needed to determine if vasodilation in arterioles from regular exercisers is protected from other "real world" noxious stimuli such as high-fat or high-sugar meals, second hand smoke, mental stress, and excess alcohol consumption.

INTRODUCTION

The endothelium controls vascular function by regulating platelet activation and aggregation, leukocyte adhesion, and proliferation of vascular smooth muscle (1). In response to various chemical (*e.g.*, acetylcholine) and physical (*i.e.*, shear stress) stimuli, the endothelium mediates vasomotor tone of the microcirculation by synthesizing and releasing a number of compounds that act to dilate or constrict adjacent vascular smooth muscle cells. These compounds include nitric oxide (NO), which largely is agreed to be the primary vasodilator (2), prostacyclin (PGI_2) (3), hydrogen peroxide (H_2O_2) (4,5), and other undefined endothelium-derived hyperpolarizing factors (EDHF). Endothelium-derived vascular smooth muscle–contracting agents include endothelin-1 (ET-1), widely considered the most potent constricting molecule (6).

Reduction in either flow-induced dilation or acetylcholine-induced vasodilation (both of which are normally mediated by NO) is a hallmark of the development of cardiovascular (CV) disease and endothelial dysfunction. Endothelial dysfunction is thought be an initiating event in the development of atherosclerosis (1). However, in humans, *in vivo* and *in vitro* studies have demonstrated that relaxing factors other than NO compensate to maintain endothelium-dependent dilation to flow when NO availability is reduced. For example, over the last two decades, several studies have demonstrated that H_2O_2 serves as a compensatory vasodilator in disease (4,5,7) and after certain environmental insults including high arterial pressure and acute aerobic or resistance exercise (4,5,7). We hypothesize that chronic exercise elicits adaptations allowing for maintained vasodilation when NO bioavailability is reduced. This review will outline the normal mechanisms of vasodilation and data supporting the novel observation that H_2O_2 serves as a compensatory vasodilator to maintain vasodilation after acute resistance exercise and exposure to high intraluminal pressure in arterioles from regular exercisers.

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Accepted for publication: August 4, 2017.

Associate Editor: Philip S. Clifford, Ph.D., FACSM.

0091-6331/4601/48–55

Exercise and Sport Sciences Reviews

DOI: 10.1249/JES.0000000000000130

Normal Mechanisms of Vasodilation

As noted in the preceding section, NO is regarded as the primary vasodilator in the vasculature. NO production in the endothelium is catalyzed by the activation of endothelial nitric oxide synthase (eNOS), a constitutively Ca^{2+}-dependent member of the NOS family of enzymes. NOS isozymes catalyze the oxidation of the amino acid L-arginine to L-citrulline and gaseous NO in the presence of nicotinamide adenine dinucleotide phosphate (NADPH) and the cofactors flavin adenine dinucleotide, flavin mononucleotide, and tetrahydrobiopterin (BH_4) (2). Vascular NO production is contingent on eNOS phosphorylation of serine (S) residues, predominately S117 (2,6). When NO is formed in the vascular endothelium, it diffuses rapidly into adjacent vascular smooth muscle cells binding to a heme ion within guanylyl cyclase stimulating an increase in the hydrolysis of guanosine triphosphate to cyclic guanosine monophosphate (cGMP), thus increasing intracellular cGMP. Cyclic GMP is a second messenger that promotes vasodilation via activation of protein kinase G, which causes the concentration of Ca^{2+} to decrease through inhibiting Ca^{2+} mobilization from the sarcoplasmic reticulum and increasing the conductance of Ca^{2+}-activated K^+ (K_{Ca}) channels with subsequent hyperpolarization and reduction in Ca^{2+} influx through voltage-gated channels leading to relaxation of the vascular smooth muscle cells (2).

Inhibition of NOS via N^G-Nitro-L-arginine Methyl Ester (L-NAME) or N^G- Monomethyl-L-arginine, monoacetate (L-NMMA) blocks the majority of endothelium-dependent dilation under resting conditions (4,5). A litany of clinical studies has used brachial artery flow-mediated dilation (FMD), a noninvasive assessment of endothelial function first described by Anderson and Mark (8) and popularized by Celermajer *et al.* (9). This assessment includes measuring endothelial function via ultrasonographic imaging of arteries at rest and after a cuff-induced ischemia and release used to generate an increase in shear stress and a subsequent endothelium-dependent vasodilation (10).

Initially, brachial artery FMD was thought to serve as a bioassay for NO. However, a recent study demonstrated that inhibition of NO production via L-NMMA did not abolish brachial artery FMD, which is in agreement with previous studies showing NO inhibition studies in the radial artery (10). Cuff placement of the occlusion cuff during the FMD procedure has been shown to alter the NO dependency of FMD, with a distal placement leading to less NO-dependent dilation compared with a proximal cuff placement (11). Taken together, these findings suggest that other factors contribute to endothelium-dependent vasodilation. Nonetheless, NO does play an essential role in normal endothelial health. Disease states associated with impaired vascular function have been shown to be associated with impaired NO bioavailability.

Effect of Cardiovascular Disease on Vasodilator Mechanisms

Patients with CV risk factors such as hypertension, hypercholesterolemia, insulin resistance, and smoking exhibit endothelial dysfunction, which is characterized mainly by insufficient production of NO, and impaired flow-induced dilation (9,11). Several mechanisms have been proposed to explain the impaired NO production associated with endothelial dysfunction, including increased production of reactive oxygen species (ROS), suppression of the upstream eNOS activator-phosphatidylinositol 3-kinase (particularly in the case of insulin resistance) (6), and increased levels of asymmetric dimethyl-L-arginine (ADMA), a competitive inhibitor of L-arginine that results in reduced NO production (12). Of the mechanisms thought to contribute to endothelial dysfunction and the etiology of CV disease, excessive ROS has gained the most attention likely because of the complex nature of ROS in regulating vascular tone in disease.

There are several sources of ROS production in the endothelium. These include the NADPH oxidase (NOX) family of enzymes, xanthine oxidase, enzymes of the mitochondrial respiratory chain, lipoxygenase, and uncoupled eNOS (2,4,13–15). ROS production is associated with increased risk of CV disease (11). Furthermore, a commonality of most conditions that precede CV disease such as insulin resistance, hyperglycemia, hypercholesterolemia, smoking, etc., is increased ROS production. Biomarkers of increased oxidative stress such as oxidized-LDL, myeloperoxidase, and plasma F2-Isoprostanes are detected at higher levels in patients with coronary artery disease (CAD), atherosclerosis, ischemic heart disease, and heart failure (3). Hypertension, which is a major risk factor for stroke and myocardial infarction, also is accompanied by elevated levels of ROS (13,16).

There are several mechanisms through which ROS interfere with eNOS activity and NO bioavailability. Paradoxically, ROS can convert eNOS from an NO-producing enzyme to a superoxide (O_2^-)-producing enzyme via eNOS uncoupling. This process is mediated via oxidation of eNOS cofactor BH_4 to BH_2, accumulation of endogenous ADMA, or S-glutathionylation of eNOS (17). ROS also interfere with NO availability through reacting with the free NO resulting in the formation of the highly reactive oxidant, peroxynitrite (2). Our group has demonstrated that even an acute transient elevation of intraluminal pressure is sufficient to increase ROS generation, reduce NO bioavailability, and impair flow-induced dilation in isolated adipose arterioles from healthy humans (5) and mice (7). High pressure-induced reductions in endothelium-dependent dilation is prevented by sepiapterin, a precursor of BH_4, thus preventing uncoupling of eNOS (17). Collectively, our findings along with other clinical and experimental studies demonstrate the detrimental effects of oxidative stress on vascular function.

Apart from ROS, individuals at increased CV risk also exhibit a higher basal level of ET-1 (6). Previous studies have shown that blocking the ET-1 vasoconstrictor pathway might improve the response to aerobic exercise in at-risk populations. For example, Schreuder *et al.* (18) demonstrated that ET-1 receptor blockade increased aerobic exercise–induced brachial artery blood flow in type 2 diabetics, and Barrett-O'Keefe *et al.* (19) demonstrated that ET-1 receptor blockade increased leg kicking exercise-induced leg blood flow in the elderly. Interestingly, both of these studies found that ET-1 vasoconstrictor pathway did not influence exercise blood flow in healthy individuals. The increased reliance to ET-1 in the elderly may have to do with impaired α-adrenergic and myogenic vasoconstriction that occurs with aging (20). However, regular exercise has been shown to partially restore normal α-adrenergic and myogenic vasoconstriction in a rodent model of aging (20). Several of the studies highlighted in this review focus on young

and middle-aged humans and animal models. How these findings apply to the elderly and other at-risk populations such as diabetics and prehypertensives remain to be investigated.

Effect of Acute Exercise on Vasodilation in Human Microcirculation

The pluripotent, beneficial health effects of regular exercise are indisputable and have been thoroughly reviewed (21). Vascular-specific adaptations to chronic exercise also will be discussed in a later section of this review. Here, the effects of acute bouts of resistance exercise on the vasculature will be reviewed. Observational studies suggest acute strenuous physical exertion increases the risk of CV events in sedentary adults (22). The experimental evidence on this matter is equivocal (for a thorough review see (23)). Some studies demonstrate that acute aerobic or resistance exercise impairs vascular function, some demonstrate no effect, and some demonstrate an increase in vascular function after acute aerobic or resistance exercise. The divergent findings are due to multiple factors including differences in participant sex, age and health/fitness status, varied intensities of the acute exercise stimulus, and assessments being taken at varied time points (23). It should be noted that most of these studies investigated the effects of acute exercise on conduit artery function. In regard to the microcirculation, our group has found that acute resistance exercise impairs endothelium-dependent dilation in arterioles in sedentary individuals, but not exercisers (4,5), which mirrors our findings using brachial artery FMD (4,5,7,24).

Dating back to 2006, Jurva *et al.* (24) hypothesized that impaired brachial artery FMD after acute resistance exercise was mediated via resistance exercise-induced elevations in arterial pressure. These findings were later corroborated by Phillips *et al.* (25), who demonstrated an immediate reduction in brachial artery FMD after a bout of acute resistance exercise in untrained subjects, but not in regular exercisers. Brachial blood pressures of more than 200 mm Hg were reported in both studies. These early findings led our group to develop the high intraluminal pressure model we currently use in isolated *ex vivo arterioles*. Lending further support to the notion of high pressure mediating endothelial dysfunction, MacDougall *et al.* reported arterial pressures reach up to 400 mm Hg during lower body resistance exercise using beat-to-beat measures of blood pressure (24). Finally, a recent eloquent study by Buchanan *et al.* (26) demonstrated that restricting elevation in brachial blood pressure with the use of a proximal pressure cuff inflated to 100 mm Hg prevented reduced endothelium-dependent dilation after acute lower body resistance exercise. Several other studies have since found that regular exercisers are protected from acute resistance exercise-induced endothelial dysfunction as well (4,5).

Interestingly, in the microcirculation, we have demonstrated that a shift from NO-mediated to H_2O_2-mediated dilation preserves endothelium-dependent dilation after acute resistance exercise using *ex vivo* arterioles from exercise-trained individuals (4,5) (see switching pattern outlined in Fig. 1). This paradoxical phenomenon indicates an increased production of ROS during acute bouts of exercise which, in turn, elicits alternative vasodilation mechanisms similar to those previously observed in patients with CAD (27,28). Durand *et al.* (5) were the first to demonstrate that H_2O_2 contributed to the maintained vasodilation to acetylcholine in isolated adipose arterioles obtained from exercise-trained subjects after acute resistance exercise in contrast to NO-mediated dilation at rest. Robinson *et al.* (4) demonstrated this same pattern after 8 wk of aerobic exercise training in overweight and obese participants (*i.e.*, switch from NO-mediated to H_2O_2-mediated dilation). Important mechanistic insights also were gleaned from these studies including that treating arterioles with the angiotensin type I receptor blocker losartan prevented an increase in ROS and restored endothelium-dependent dilation in arterioles from sedentary individuals (5). This suggests that acute resistance exercise elicited activation of the local renin angiotensin system

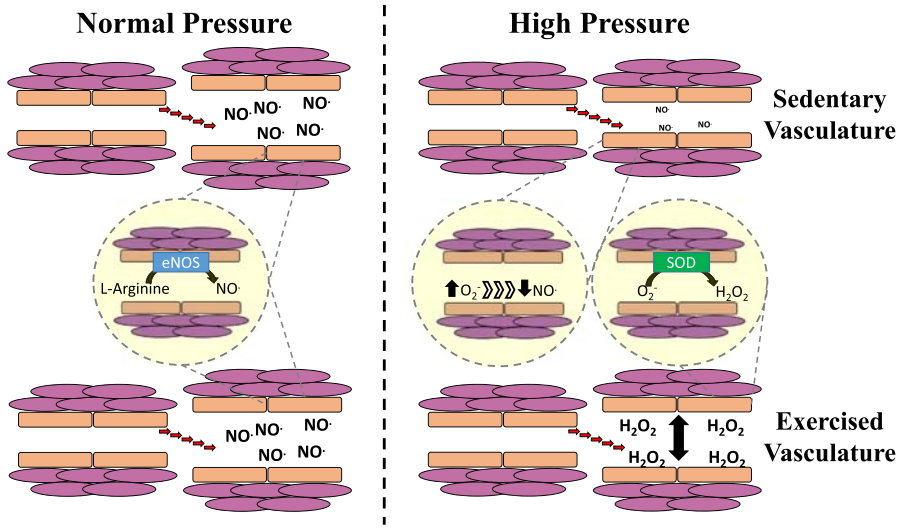

Figure 1. Divergent dilatory signaling between sedentary individuals and exercisers in response to high pressure. At rest, arterioles from both sedentary individuals and regular aerobic or resistance exercisers exhibit nitric oxide (NO)-dependent vasodilation (left panel). This is supported by several studies demonstrating that this dilation is nearly abolished by the nitric oxide synthase (NOS) inhibitor L-NAME (4,5,7). After exposure to high intraluminal pressure or acute resistance exercise, arterioles from sedentary individuals demonstrate reduced vasodilation and reduced sensitivity to NG-Nitro-L-arginine Methyl Ester (L-NAME) and the hydrogen peroxide (H_2O_2) scavenger catalase. In contrast, arterioles from regular aerobic and resistance exercisers demonstrate preserved vasodilation and an enhanced response to catalase, suggesting a greater reliance on H_2O_2-dependent dilation (4,5,7).

(RAS) resulting in increased ROS production and resultant endothelial dysfunction. Our studies also indicate differential detoxification capacity of ROS via the superoxide dismutase (SOD) antioxidant system between regular exercisers and sedentary individuals likely determines the microvascular response to acute exercise (covered in detail in the following section). To date, no studies have investigated if this phenotypic switch to H_2O_2-mediated dilation plays a role in conduit artery function after acute aerobic or resistance exercise.

The Effect of High Vascular Pressure on Vasodilation

In 1990, Panza *et al.* (29) demonstrated, for the first time, that hypertensive individuals display impaired endothelium-dependent vasodilation. Larger cross-sectional studies have since confirmed these findings (30). However, the temporal relation between hypertension and endothelial dysfunctions remains to be fully understood. In the large-cohort (Multi-Ethnic Study of Atherosclerosis; MESA) study, impaired FMD was not an independent predictor of future hypertension (30). This finding suggests impaired endothelial function is not a predictor of hypertension. However, the effect of high pressure on vasodilation has been studied using several models, and it seems high arterial pressure does induce endothelial dysfunction. Using a whole-body approach, Millgard *et al.* (31) assessed endothelium-dependent forearm blood flow with methacholine before, and immediately after, a 1-h norepinephrine infusion used to elevate blood pressure (DBP ≥95 mm Hg). High pressure did elicit a decrease in endothelium-dependent blood flow, although it must be noted that this model could have been confounded by norepinephrine infusion increasing ROS independent of blood pressure. As previously noted, acute bouts of resistance exercise increase blood pressure and result in transient impairment of endothelium-dependent dilation (4,5,24,25). However, there is evidence to suggest other mediators play a role in endothelial dysfunction after acute cycling exercise, particularly increased sympathetic nerve activity (32). Hence, our laboratory and others have used isolated arteries to investigate the role of high pressure alone in eliciting endothelial dysfunction.

To isolate the effects of high pressure on vascular function, several studies have used *in vitro* models of high pressure to induce endothelial dysfunction in vessels from both animal models and humans. Mouse carotid arteries subjected to 30 min of high pressure (180 mm Hg) demonstrate impaired vasodilation to acetylcholine and increased O_{2-} production, both of which are rescued via inhibition of NOX II signaling (33). In an *in situ* model using open-chest anesthetized dogs, subjecting the left anterior descending coronary artery to 30 min of hypertension (200 mm Hg) augmented endothelium-dependent constrictor responses to serotonin (34). In time course studies, as little as 1–5 min of high pressure evoked increased serotonin constrictor sensitivity for up to 2½h.

There are several studies in human vasculature that have studied the impact of high pressure on vascular function. For example, isolated human saphenous vein segments and internal thoracic artery segments subjected to 170 mm Hg of intraluminal pressure demonstrated reduced stimulated $NO^{\cdot}$ release and increased immunocyte adhesion (35). To determine the effects of high pressure alone on the microcirculation, our group has undertaken several studies using experimentally induced high intraluminal pressure in *ex vivo* arterioles from humans and mice. Similar to acute resistance exercise, Durand *et al.* (5) demonstrated that high intraluminal pressure elicits endothelial dysfunction in arterioles from sedentary individuals but not arterioles from exercise-trained individuals, which undergo a phenotypic switch to H_2O_2 -mediated dilation. Robinson *et al.* (7) recently demonstrated this same pattern in mice that underwent 2 wk of wheel running. Furthermore, exercised mice demonstrated increased arterial SOD expression (SOD generates H_2O_2 from O_{2-}, discussed in detail in a later section). These findings corroborate our previous finding that 8 wk of aerobic exercise prevented endothelial dysfunction in the overweight and obese after acute resistance exercise via H_2O_2-mediated dilation and increased plasma SOD levels (4).

Chronic Exercise Effects on the Mechanisms of Vasodilation After Acute Exercise

Shear stress is a primary factor in eliciting vascular adaptations to regular exercise in humans. Several studies have used local heating and compression cuffs to manipulate vascular shear stress in human participants and found that shear alone can induce beneficial adaptations (36,37). Furthermore, recent studies suggest that a reduction in popliteal artery FMD that occurs with prolonged sitting is prevented by lower limb heating (37) and *fidgeting* (36), both of which increase shear stress through the popliteal artery.

At the cellular level, signaling kinases including protein kinase B, protein kinase A, and adenosine monophosphate–activated protein kinase (AMPK) are all phosphorylated in response to shear stress in isolated microvessels (38). Furthermore, AMPK promotes the increased expression of SOD enzymes (16) (Fig. 2). All SOD enzymes catalyze the dismutation of O_{2-} into H_2O_2, thus increasing NO bioavailability and providing H_2O_2 to act as an alternative vasodilator. SOD I localizes in the cytoplasm, SOD II is found in the mitochondria, and SOD III is extracellular (Fig. 3). SOD I and SOD III complex with copper and zinc, whereas SOD II complexes with manganese. Elevated shear stress has been found to increase SOD I gene expression as well as SOD I, SOD II, and total SOD protein expression in addition to increased NOS levels in endothelial cells (14) (Fig. 2). Aerobic exercise training increases SOD I protein expression in primary aortic endothelial cells (39) and coronary arterioles (15) in addition to reducing NOX II subunit expression (39).

We recently showed that regular aerobic exercise reduces NOX II expression while increasing SOD expression (7). Interestingly, we found zinc diethyldithiocarbamate, an inhibitor of SOD I and SOD III, did not block dilatory responses to the same extent as catalase, which targets H_2O_2 from all SOD isozymes (see inset, Fig. 3). This finding indicates that there may be a particularly important role for mitochondrial SOD in preserving dilation, which is in line with previous studies suggesting that shear stress modulates mitochondrial physiology (40,41). Shear stress increases expression of genes related to mitochondrial biogenesis including NRF-2 and the mitochondrial complexes involved in oxidative phosphorylation in endothelial cells, and shear reduces mitochondria membrane hyperpolarization (41). This is of importance because RAS induces mitochondrial membrane hyperpolarization leading to excessive oxidative stress and impaired endothelial function (13). In support of the hypothesis that regular

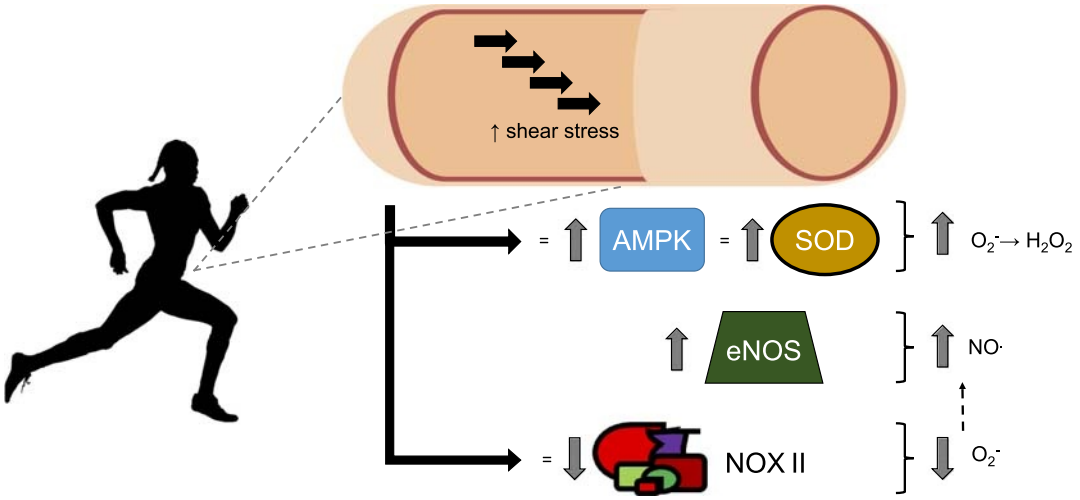

Figure 2. Exercise and shear-induced vascular adaptations. Aerobic exercise and shear stress result in increased vascular adenosine monophosphate-activated protein kinase (AMPK) (16) and superoxide dismutase (SOD), resulting in greater conversion of (O_{2-}) to hydrogen peroxide (H_2O_2) (15). By reducing O_{2-}, there is likely a resultant increase nitric oxide (NO) bioavailability. In addition, some studies have demonstrated an increase in nitric oxide synthase (NOS) expression (14), which also would result in increased NO. Aerobic exercise also has been shown to reduce Nicotinamide adenine dinucleotide phosphate oxidase (NOX) II subunit expression, which results in less O_{2-}, which theoretically also should yield increased NO bioavailability because superoxide quenches NO to form peroxynitrite ($ONOO^-$) (7,14). Adenosine monophosphate-activated protein kinase, AMPK; endothelial nitric oxide synthase, eNOS.

exercise counteracts RAS and vascular mitochondrial dysfunction, Kim *et al.* (40) demonstrated shear stress mimics the effects of regular exercise in prehypertensives. Specifically, shear increased mitochondrial biogenesis markers, and these effects were abolished using siRNA to abolish sirtuin 1 signaling. This may explain the potential insulin sensitizing effect of shear stress. Collectively, these findings indicate regular aerobic exercise induces adaptations that improve the microcirculatory redox environment (Fig. 2), which may manifest in preserved vasodilation in the face of RAS-induced oxidative stress (activated by acute resistance exercise and high intraluminal pressure).

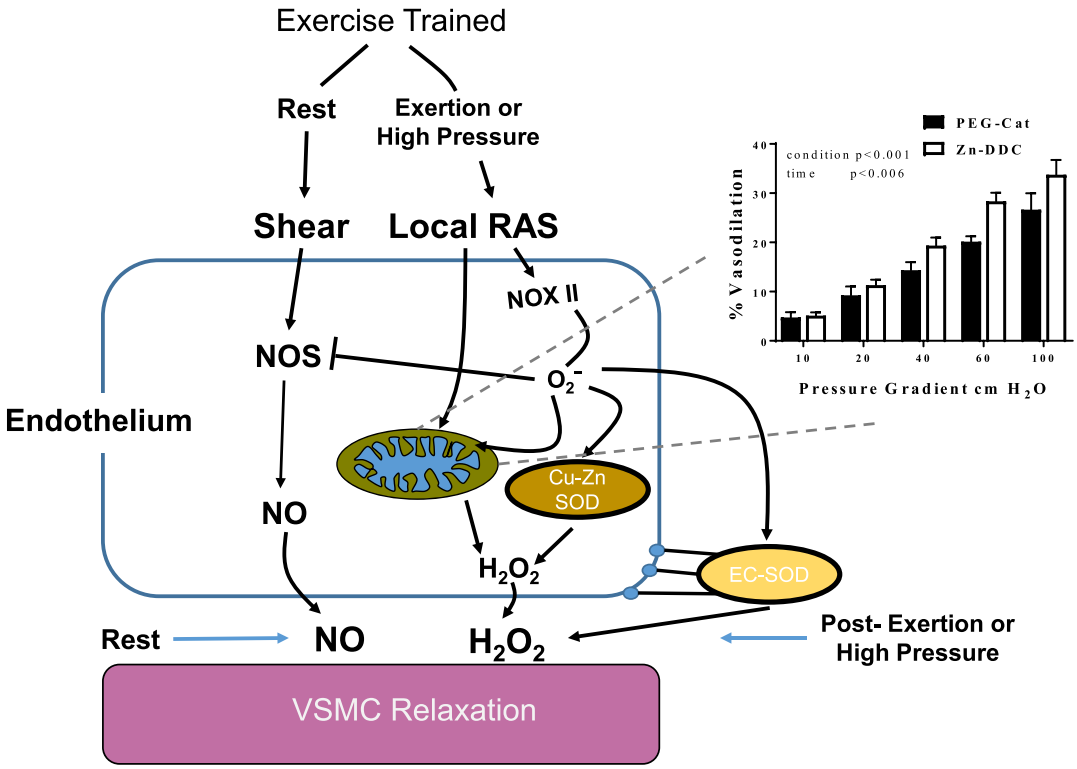

Figure 3. Cellular microenvironment at rest and during high pressure in the exercise-trained vasculature. Activation of local renin angiotensin system (RAS) results in increased NOX II activity and subsequent production of O_{2-}. Increased superoxide dismutase (SOD) expression allows the exercised vasculature to convert this superoxide to hydrogen peroxide (H_2O_2), which can be used for vasodilation when nitric oxide (NO) bioavailability is reduced. Regular exercise results in several beneficial adaptations to the mitochondria (40,41). Mitochondrial H_2O_2 seems to play a significant role in this maintained dilation because blockade of SOD I and SOD III does not reduce dilation to the same extent as catalase, which scavenges H_2O_2 from all three SOD isozymes (7). Nicotinamide adenine dinucleotide phosphate oxidase, NOX; vascular smooth muscle contraction, VSMC.

Several of the studies highlighted here demonstrate that regular aerobic exercise either directly increases NO bioavailability by modifications to eNOS (38) or acts to indirectly increase NO bioavailability by reducing O_{2-} via a reduction in pro-oxidative enzymes such as NOX II or an increase in O_2^- scavenging enzymes such as the SODs (16,39). However, in the context of the postacute exercise period, we have shown inhibition of eNOS does not impact vasodilation differentially in regular exercisers versus sedentary individuals (*i.e.*, in both populations, there is less sensitivity to eNOS. Inhibition via L-NAME compared with resting condition). Therefore, it seems that the adaptations facilitating H_2O_2-mediated dilation supersede those promoting increased NO bioavailability in this specific context. Non-NO-mediated dilation is discussed at length in a later section.

Non–NO-Mediated Responses to Shear

Shear stress–induced production/release of NO has been extensively studied, and the effects of NO on vascular smooth muscle are well established. Non-NO-mediated response to shear is an area of increasing investigation. In this section, we discuss non-NO shear stress-mediated responses and the mechanisms by which they mediate vascular smooth muscle relaxation. This includes shear responses mediated by PGI_2, H_2O_2, and EDHFs. By name, EDHFs are expected to directly affect smooth muscle membrane potential thereby inducing vascular dilation. Yet, the specific molecule or molecules that comprise EDHF(s) are a topic of debate.

PGI_2 contributes to endothelial cell-mediated vasodilator production, which effect on vascular tone is tightly tied to thromboxane (TX) A2 (3). TXA2 acts as a vasoconstrictor on the vascular smooth muscle, whereas PGI_2 functions as a vasodilator. PGI_2:TXA2 is increased in certain physiological conditions including during acute exercise (42,43) (these studies specifically looked at maximal aerobic exercise). Viinikka *et al.* (44) studied the effect of acute aerobic exercise on PGI_2:TxA2 in a group of well-trained runners and failed to detect significant changes in plasma prostacyclin. This would suggest that the changes of the PGI_2:TxA2 ratio induced by acute aerobic exercise are not enough to explain the protective effect of physical fitness against CVD. However, Feng *et al.* (43) demonstrated that maximal aerobic exercise elicits an increase in plasma PGI_2, whereas patients with endothelial dysfunction demonstrated a reduced PGI_2 response to acute aerobic exercise. These findings are in agreement to that of Wennmalm *et al.*, who reported that both PGI_2 and TXA2 were elevated after acute aerobic exercise in symptom-free patients undergoing a stress test (overall, the PGI_2:TxA2 increased), whereas neither PGI_2 or TxA2 were increased after acute aerobic exercise in those developing chest pain or ST depression during acute aerobic exercise. Taken together, these data suggest a continuum may exist whereby at-risk populations do not exhibit increased PGI2:TXA2 during acute aerobic exercise. Healthy untrained individuals do, and well-trained regular exercisers do not. These findings may relate to NO bioavailability and vascular plasticity, although this topic requires further investigation including responses to acute resistance exercise.

In both large and resistance arteries, shear stress-induced EDHF production is present, although EDHF contributes to vasodilation more so in smaller arteries (45). The contributions of EDHF in vasodilation to shear stress likely also vary between species and vascular beds (45). Mechanosensors including the glycocalyx, integrins, cell surface receptors, caveolae, cell-cell junctions, and ion channels transduce shear forces into signaling cascades involving EDHF and NO (45–47). Many of these mechanosensors are linked to EDHF production, although most mechanisms remain unclear. For instance, members of the K_{Ca} channel family expressed in the endothelium have been linked to EDHF in that pharmacological blockade of these channels, in combination with inhibiting eNOS, abolish dilation to flow (47). How these channels are activated by shear as well as the subsequent role played in EDHF production remains unknown. The location of these channels, particularly small K_{Ca} (SK) and intermediate K_{Ca} (IK) channels, at or near the myoendothelial junction suggests that K^+ ions may serve as EDHF and activate inwardly rectifying K^+ (Kir) channels on vascular smooth muscle. Interestingly, it has been found that the rapid exercise hyperemic response to acute, rhythmic handgrip exercise is mediated by Kir channels *in vivo* (48). However, not all vascular beds express Kir channels in smooth muscle (*e.g.*, mesentery vascular smooth muscle), and SK and IK are still seemingly involved, thus supporting a role for alternative mechanisms such as direct electrical connection of the endothelium to smooth muscle via gap junctions or activation of Na^+/K^+ ATPase (47,49).

Cytochrome P450 2C9 (CYP2C9) metabolites of arachidonic acid known as epoxyeicosatrienoic acids (EET) are produced by the vascular endothelium in response to chronic shear stress (50). Evidence for EET leading to the activation of endothelial K_{Ca} channels supports the hypothesis that these molecules contribute to the shear-induced EDHF component of vasodilation (51). This is supported by a reduced EDHF component of flow-induced dilation after blockade of TRPV4 or cytochrome CYP450 in the carotid arteries of mice (51). The proposed mechanism involves activation of transient receptor potential (TRP) channel V4 by EET, subsequent Ca^{2+} influx, and, finally, K_{Ca} channel activation (51–53). However, the effects of acute increases in shear stress on CYP2C9 activity have yet to be determined.

Although several reports provide evidence for a role of EET as the EDHF in shear-induced vasodilation, Larsen *et al.* demonstrated that H_2O_2 inhibits CYP2C9 on bradykinin stimulation and acts as an EDHF in lieu of EET production (54). We have shown that flow stimulates H_2O_2 in an endothelium-dependent, yet NO-independent, manner in aerobic or resistance exercise-trained individuals and patients with CAD, thus H_2O_2 may serve as a soluble EDHF in these populations (4,5,28). Furthermore, smooth muscle K^+ channels, such as the large-conductance K_{Ca} channel, are redox sensitive and have been shown to be activated by H_2O_2 (53). Activation of smooth muscle K^+ channels hyperpolarizes membrane potential, thereby reducing Ca^{2+} influx and promoting vasodilation. In regular aerobic or resistance exercisers performing acute resistance exercise, endothelium-dependent dilation switches from NO-mediated to H_2O_2-mediated dilation to maintain blood flow as NO becomes unavailable (4,5,7). However, at rest, trained individuals rely on NO as the main dilator. Patients with CAD almost exclusively rely on H_2O_2 even at rest to sustain vasodilaton, presenting an interesting

comparison between exercise induced mechanisms and disease (28). We recently linked NOX-induced ROS to mitochondrial production of O_2^- and H_2O_2 in arterioles from CAD patients (28). These findings indicate compensatory mechanisms in disease states that may be present in regular exercisers during acute bouts of exercise but exist as the driving vascular mediators in CAD. It is important to note that switching the mediator from NO to ROS in CAD may provide adequate short-term vascular function. However, this likely introduces long-term negative consequences and a direct route to severe CVD.

Implications and Conclusions

Physical activity and exercise prescription is an important component of CV disease and risk management. In terms of moderate and intense exercise training, evidence suggests that exercise is associated with reduced prevalence and onset of CV disease (21). Compelling data show that higher aerobic exercise capacity (*i.e.*, higher $\dot{V}O_{2peak}$) is closely associated with a reduction in mortality and morbidity (22). However, it is well recognized that sudden death and CV events occur at high frequency during, or soon after, vigorous bouts of exercise (22). The assessment of endothelium-dependent vasodilation after perturbations such as intense resistance exercise and high-fat meals is an emerging strategy used 1) to uncover vascular dysfunction in individuals at higher risk for CV disease and 2) disentangle the unaccounted-for protection conferred by regular exercise (vs reduction in risk factors alone) (11). Endothelial dysfunction occurs early in the development of CV disease and occurs in the microcirculation, where changes in function may foster the development of cardiometabolic disease such as hypertension and insulin resistance. Our findings suggest regular aerobic and resistance exercisers are protected from endothelial dysfunction induced by acute exertion (4,5,7). Regular exercise-induced upregulation of antioxidant defenses and protection of NO bioavailability may be an important mechanistic link between exercise and CV protection from endothelial dysfunction to other stressors such as alcohol consumption, poor diet, and sudden physical exertion. Furthermore, the evidence previously reviewed suggests that H_2O_2 may play a more prominent role in protecting against endothelial dysfunction from high pressure induced by acute bouts of resistance exercise. Although the mechanisms of preserved dilation after high pressure and exercise in trained individuals have not been fully elucidated, the available evidence suggests an increase in vascular SOD plays a large role. In addition, shear stress itself may play a critical role in promoting H_2O_2 generation in the microcirculation during acute resistance exercise. This *exercise paradox* of chronic exercise that maintains endothelium-dependent vasodilation in the microcirculation through an H_2O_2-dependent mechanism and CV health on the one hand, and where acute, sudden exertion increases CV risk on the other hand, may be an important marker that identifies risk for vascular dysfunction in populations without overt CV disease.

Acknowledgment

Shane Phillips was supported by grants K23HL085614 and R01HL095701, Austin Robinson was supported by grant R01HL095701-03S1 (Assistantship), Abeer Mahmoud was supported by grant AHA 15POST24480172, and Ibra Francher was supported by grant AHA 16POST27000011.

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