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Vascular dysfunction and exercise

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Abbreviations:

ACh	acetylcholine
c AMP	cyclic adenosine monophosphate
CAD	coronary artery disease
cGMP	cyclic guanosine monophosphate
COX	cyclooxygenase enzyme
EDCF	endothelium-derived contracting factor
EDHF	endothelium-derived hyperpolarizing factor
EDRF	endothelium-derived relaxing factors
EETs	epoxyeicosatrienoic acids
eNOS	endothelial nitric oxide synthase
ET-1	endothelin-1
FID	flow-induced dilation
FMD	flow-mediated dilation
H_2O_2	hydrogen peroxide
LDL	low-density lipoprotein
NADPH	nicotinamide adenine dinucleotide phosphate
NO	nitric oxide
O_2^-	superoxide
$OH^·$	hydroxyl radicals
$ONOO^-$	peroxynitrite
PGI_2	prostaglandin I_2
ROS	reactive oxygen species

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Abstract

The endothelium regulates vascular permeability and actively controls the exchange of molecules in response to environmental and molecular signals. Endothelial cells regulate vascular tone by releasing a number of soluble mediators, including nitric oxide, prostaglandin I_2, and endothelium-derived hyperpolarizing factor. Misbalance between pro-oxidants and antioxidants results in increased production of reactive oxygen species, increased oxidative stress and endothelial dysfunction. Endothelium dysfunction is characterized by an impaired nitric oxide bioavailability due to reduced production of nitric oxide, reduced endothelium-dependent hyper-polarization and enhanced production of contracting factors. Endothelium dysfunction leads to vascular remodeling by vascular smooth muscle cells proliferation, migration and extracellular matrix remodeling. The presence of impaired endothelium-dependent vasodilation is a marker of endothelial dysfunction. The benefits of exercise on the cardiovascular system have long been recognized. Most clinical and experimental studies have reported beneficial effects of regular physical activity in increasing nitric oxide bioavailability and reducing oxidative stress. Therefore, regular physical exercise may be a protective factor against vascular dysfunction induced by acute exertion.

INTRODUCTION

The endothelium plays an important role in maintaining vascular homeostasis. Endothelial cells control vascular function by responding to various hormones, neurotransmitters and vasoactive factors which affect vasomotion, thrombosis, platelet aggregation and inflammation *(1, 2).* The endothelium mediates the vasomotor tone of the microcirculation in response to various chemical (acetylcholine, ACh) or physical (shear stress) stimuli *(3),* by synthesizing and releasing different vasodilator and vasoconstrictor mediators. There are endothelium–derived relaxing factors (EDRF), including nitric oxide (NO), prostaglandin I_2 (PGI_2), and endothelium-derived hyperpolarizing factor (EDHF), but also endothelium–derived contracting factors (EDCFs), including endothelin–1 (ET-1) representing the most potent molecule. In healthy blood vessels, EDCFs are released dependent on the presence of NO and EDHFs *(4).*

Mechanisms of vasodilator function

Numerous studies have shown that NO and PGI_2 play a key role in tone control of large conduit arteries, whereas EDHFs play a major role in the resistance arteries *(5).* Different EDHFs could exist depending on

species, blood vessels and the size of blood vessels *(6),* and the major EDHFs are epoxyeicosatrienoic acids (EETs), metabolites of arachidonic P450 epoxygenase pathway, K ions, NO itself, reactive oxygen species (H_2O_2), and electrical coupling through myoendothelial gap junctions *(7).*

Various agonists, but also shear stress, can activate the endothelial nitric oxide synthase (eNOS) to produce NO, and also activate phospholipase A_2 to release arachidonic acid. NO activates soluble guanylate cyclase, produces cyclic guanosine monophosphate (cGMP), and relaxes vascular smooth muscle. PGI_2 is produced from arachidonic acid by cyclooxygenase (COX) and relaxes vascular smooth muscle in a *cyclic adenosine monophosphate* (cAMP)-dependent manner. EDHF hyperpolarizes vascular smooth muscle by opening K channels and then stimulates vasodilatation *(4).* NO has been shown to play an important role in the maintenance of basal vasodilator tone of the blood vessels *(8)* and plays a key role in vasodilation *(9).* It also prevents platelet adhesion and aggregation, as well as leukocyte adhesion and migration into the arterial wall and inhibits smooth muscle cell proliferation and intimal migration, oxidation of *low-density lipoprotein* (LDL) cholesterol, apoptosis of smooth muscle cells, all key events in the development of atherosclerosis *(10-14).*

Mechanism of flow-induced vasodilation

Flow–induced dilation (FID) is a physiologically important stimulus regulating vascular tone and homeostasis of the peripheral circulation. This important endothelial mechanism of vasodilation occurs in virtually every vascular bed. In large arteries, FID may be critical for preventing atherosclerosis through the release of NO and PGI_2, the endothelium-derived antiproliferative compounds. Animal studies have reported that the contribution of NO to FID is reduced as oxidative stress increases in the presence of risk factors for cardiovascular disease *(15).* In humans, *in vivo* and *in vitro* studies have demonstrated that relaxant factor(s), other than NO, compensate to maintain FID when NO availability is reduced *(16).* Altered endothelium-dependent FID is a hallmark of the development of cardiovascular disease and is an initiating event in the development of atherosclerotic heart disease *(17).* During coronary artery disease, arterioles exhibit altered endothelium-dependent vasodilation. For example, Kuo et al demonstrated that the pathophysiological manifestations of atherosclerosis in a pig model extend into the microcirculation and manifest as abnormal endothelium-dependent pharmacological responses to agonists and abolish flow-mediated vasodilation in coronary arterioles *(18).* In humans, Phillips *et al.* have shown that hydrogen peroxide (H_2O_2) replaces NO as the mediator of endothelium-dependent FID in resistance arteries of visceral fat in the presence of coronary artery disease *(19).* Recently, we have found that flow stimulates H_2O_2 in isolated resistance arteries from bariatric patients without any known cardiovascular disease (Grizelj et al unpublished result). Miura *et al.* provided evidence that shear stress induces endothelial release of H_2O_2, a result that is consistent with the idea that H_2O_2 is an EDHF that contributes to FID in human coronary arteries from patients with heart disease *(20).* An increase in oxidative stress appears to be a major mechanism underlying the development of vascular endothelial dysfunction. The dominant mechanism responsible for endothelial dysfunction is the decrease in bioavailable NO, as well as the increase in reactive oxygen species (ROS) production and apoptosis. The generation of ROS in the endothelium includes anions (O_2^-), hydroxyl radicals ($OH^\cdot$) and hydrogen peroxide (H_2O_2). ROS modulate vascular tone by several mechanisms; directly act as EDCF or indirectly potentiate EDCF mediated responses by reducing the bioavailability of NO. ROS might interact with NO and reduce its bioavailability via different pathways: direct NO inactivation by superoxide with peroxynitrite ($ONOO^-$) formation; reduction in NO synthase expression; and activity due to changes in their substrate or cofactors, and also endothelial NOS uncoupling *(21,22).*

Interaction between NO and ROS mechanisms of vasodilation

The endothelium -dependent vasorelaxation caused by H_2O_2 in large vessels depends on eNOS. Indeed, H_2O_2 can acutely stimulate eNOS production of NO *(23).* When eNOS was uncoupled to produce $O2^-$ rather than NO in hypertensive or atherosclerotic large vessels, endothelium-derived H_2O_2 mediated compensatory relaxation via unknown mechanisms. One possibility is direct polarization of vascular smooth muscle. Indeed, H_2O_2 was found to be an endothelium-derived hyperpolarizing factor in small arteries *(24, 25)* and an activator of potassium channel in large cerebral arteries *(26).* In human coronary arterioles, flow-induced vasodilatation is mediated by endothelium-derived H_2O_2 (27). In these arterioles, the enzymatic source of H_2O_2 appears to be the mitochondrial respiratory chain whereas in large vessels, vascular nicotinamide adenine dinucleotide phosphate (NADPH) oxidases are responsible for H_2O_2 production *(25).* This seems consistent with previous observations that NO plays a lesser role in vasodilatation of small arteries. Of note, in coronary arterioles, H_2O_2 induced endothelium-dependent vasorelaxation is NO independent involving activation of cyclooxygenase-1 and smooth muscle potassium channel *(26).* Therefore, it is clear that H_2O_2 is capable of mediating endothelium-dependent vasorelaxation, NO-dependently or independently since H_2O_2 can also stimulate endothelium-independent vasorelaxation *(27).* However, the exact mechanisms of H_2O_2 release and signaling are unknown.

Effect of acute exercise on endothelial function

Reduced bioavailability of NO is an important consequence of endothelial damage and is thought to contribute to the development of cardiovascular disease. Moreover, studies have shown that physical exercise is associated with lower morbidity and mortality from coronary heart disease *(28).* Exercise training has been shown to improve peripheral vascular endothelial function *(29).* However, certain exercise modes (such as weight lifting) can induce large, transient increases in arterial pressure *(30, 31),* which is known to impair endothelial function. Jurva *et al.* observed impaired vascular endothelial function following a single weight lifting session in sedentary subjects but not in conditioned weight lifters *(30).* In another study, Phillips *et al.* showed that sedentery subjects not performing regular exercise programs have impaired brachial flow-mediated dilation (FMD) following acute hypertension induced by a relatively short weight lifting session *(32).* The effects of transient elevations in arterial pressure on impaired endothelial function can last at least 2.5 hours *(33),* making it important to understand the relationships between chronic exercise and the protection against the risk of reduced vascular endothelial function after acute exercise.

Further studies have found that acute aerobic exercise testing reduced brachial FMD in obese and overweight subjects who were inactive *(34).* However, active patients demonstrated increased FMD after a single exercise test *(34).* These studies outline the importance of other cardiovascular risk (i.e. obesity and lack of physical activity) factors on the endothelial responses to exercise. Recent studies by Currie *et al. (35)* showed that high intensity interval exercise improved FMD acutely in patients with coronary artery disease (CAD). The dose, intensity, and disease status of the patient may have important implications for the responses of the endothelium to acute exercise.

Effects of exercise training on mechanisms of endothelial function

The regular exposure to exercise, physical training, promotes a set of morphological and functional adaptations that increase body`s capacity to respond to the exercise stress *(28).* However, the mechanism(s) by which exercise training alters endothelial function has not been fully elucidated. Exercise intensity is an important consideration of exercise effects on vascular function. For example, Goto *et al.* showed that moderate-intensity aerobic exercise augments endothelium-dependent vasodilation in humans through the increased production of NO *(36).* In another study, Tengchaisri *et al.* have shown that exercise training did not enhance vascular smooth muscle function, suggesting that the endothelium is the key modulator of exercise effects on vascular function *(26).*

Many recent studies show an increase in biosynthesis of NO in exercise-induced enhancement of vasodilation *(37, 38).* One possible mechanism, by which long-term aerobic exercise augments NO release, is an increase in vascular shear stress resulting from increased blood flow. Augmentation of NO bioavailability induced by exercise could be the result of the increased activity/expression of eNOS. Hambrecht *et al.* performed one of the first studies demonstrating the positive effects of exercise training in the form of cardiac rehabilitation of vascular function and eNOS expression in the isolated human blood vessels of patient with cardiac disease *(39).* Further studies showed that the same intervention reduced NADPH oxidase and reactive oxygen species in the vascular wall of patients participating in cardiac rehabilitation compared to patients with no exercise *(40).*

Others demonstrated that, in the epicardial coronary arteries of dogs, the increase in shear stress from 10 days of treadmill exercise enhanced the expression of the vascular endothelial constitutive NO synthase gene *(41).* Some investigators have shown that H_2O_2 is importantly involved in the mechanisms of enhanced vascular relaxation and that differential roles may be played by NO and H_2O_2 in the vascular adaptations to chronic coronary occlusion and exercise training. For example, increased levels of H_2O_2 may compensate for decreased NO production during NOS activation suggesting the concept of rapid switching between H_2O_2 and NO production *(26).* Our group found a similar switch from NO to H_2O_2 mechanism involved in the ACh vasodilation of isolated resistance arteries from exercise trained subjects after acute exertion *(42).* Chronic exercise also leads to the upregulation of the endothelium's antioxidant defense mechanism, which helps minimize oxidative stress *(43).* These data suggest mechanistic differences in the mechanisms for endothelium-dependent vasodilation after an acute stressor such as exercise.

In addition, chronic increases in shear stress have been shown to lead to functional and histological alterations of vascular endothelium, resulting in enhanced vascular structure and function *(44).* Jurva *et al.* has shown that acute resistance exercise associated with hypertension impairs endothelial function in unconditioned subjects and that chronic resistance training protects against this vascular dysfunction *(30).* It is possible that maintained endothelium-dependent vasodilation in conditioned individuals could protect against altered vascular and hemodynamic responses to other physiological perturbations.

Clinical relevance and perspectives

Physical activity and exercise prescription is an important component of cardiovascular disease and risk management. In terms of moderate and intense exercise, evidence suggests that exercise is associated with reduced risk

of atherosclerotic cardiovascular disease *(45).* In addition, higher exercise capacity (in terms of VO_2) is closely associated with a reduction in mortality and morbidity *(46).* However, it is well recognized that sudden death and cardiovascular events occur at high frequency during or soon after vigorous exercise *(47).* Endothelial function is an early prognostic indicator of cardiovascular disease and events. In terms of athletic populations, engaged in high intensity exercise training programs, there appears to be a protection from endothelial dysfunction induced by acute exertion *(48).* Exercise-induced up-regulation of antioxidants and protection of NO bioavailability may be an important mechanistic link between exercise and cardiovascular protection from other physiologic causes of endothelial damage from other stressors such as alcohol, diet, and sudden physical exertion. Since peripheral endothelial function correlates well with coronary function *(49),* these studies suggest that the exercise response may extend to predict and reduce cardiovascular risk as well. Further, these data are consistent with the observation that exertion-related acute myocardial infarction occurs more frequently in habitually inactive individuals with multiple cardiac risk factors *(50).* This "exercise paradox" of chronic exercise that maintains endothelium-dependent vasodilation and cardiovascular health on the one hand and where acute, sudden exercise increases cardiovascular risk on the other hand may be an important marker that identifies risk for vascular dysfunction in populations without overt cardiovascular disease.

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