

Review

Molecular mechanisms of vascular adaptations to exercise. Physical activity as an effective antioxidant therapy?

Georg Kojda^{b,*}, Rainer Hambrecht^a

^aHerzzentrum der Universität, 04289 Leipzig, Germany

^bInstitut für Pharmakologie und Klinische Pharmakologie, Medizinische Einrichtungen, Heinrich-Heine-Universität, Moorenstr. 5, 40225 Düsseldorf, Germany

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Abstract

A lack of exercise training and/or regular physical activity is a known risk factor for cardiovascular disease. Exercise training induces marked vascular remodeling by increasing angiogenesis and arteriogenesis. These changes in the architecture of the vascular tree are likely associated with functional changes and improved organ blood flow. Physical forces such as shear stress, transmural pressure and cyclic stretch activate mechanotransduction mechanisms in endothelial and smooth muscle cells that are mediated by integrins and associated RhoA small GTPase. They stimulate various signal transduction pathways involving phosphorylation of kinases such as focal adhesion kinase, c-Src, Akt kinase, phosphatidylinositol 3-kinase, myosin light chain kinase and mitogen-activated protein kinases (MAPK) such as extracellular signal-regulated kinase (ERK). These mechanisms result in upregulation of genes mediating antiatherogenic effects by promoting antiapoptotic and antiproliferative signals, by increasing vascular NO bioavailability and by changing calcium handling and the vascular myogenic response to pressure. Exercise-induced increase of vascular eNOS expression and of eNOS Ser-1177 phosphorylation is most likely an important and potentially vasoprotective effect of exercise training. The underlying mechanisms involve cell membrane proteins such as integrins and products of vascular oxidative stress such as hydrogen peroxide. Exercise-induced eNOS expression is transient and reversible and regulated by factors such as angiogenesis, arteriogenesis and antioxidative effects including upregulation of superoxide dismutases (SOD1, SOD3) and downregulation of NAD(P)H oxidase, which likely blunts the effects of oxidative stress. Based on these observations, it appears reasonable to assume that exercise training can be viewed as an effective antioxidant and antiatherogenic therapy.

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1. Introduction

Regular physical activity is an important factor in the prevention and treatment of cardiovascular diseases. Large clinical studies demonstrated a reduction of morbidity and mortality among physically active individuals as compared to sedentary controls in both health and cardiovascular disease. As for primary prevention, daily walking of 2 miles reduced the mortality of nonsmoking retired men [1] as did

brisk walking for at least 2.5 h/week in postmenopausal women [2]. In the presence of coronary artery disease (CAD), hypertension and heart failure exercise training as secondary prevention or adjunctive therapy was associated with a significant reduction of morbidity and mortality [3–8]. Even in comparison to established interventional strategies, exercise training provided improved event-free survival at lower treatment costs as evidenced in a recently published prospective randomized trial in 101 patients with stable CAD [9]. In addition, lifestyle interventions including exercise training have been shown to be a feasible option to prevent type-2 diabetes in overweight patients with impaired

* Corresponding author. Tel.: +49 211 81 12518; fax: +49 211 81 14781.

E-mail address: kojda@uni-duesseldorf.de (G. Kojda).

glucose tolerance [10]. It should be noted that the reduction of cardiovascular morbidity and mortality achievable with exercise training in primary and secondary prevention is quite similar to the effect of treatment with effective drugs such as inhibitors of the renin angiotensin aldosterone system or with statins [11] but certainly more cost-effective.

A number of factors contribute to the beneficial effects of exercise on maintaining cardiovascular health and slowing the progression of cardiovascular disease [12]. Baseline physical activity plays an important role for the effects of exercise training: It seems plausible that physical activity has greater cardiovascular effects among sedentary as compared to active individuals. In Western societies, exercise training may therefore compensate for a general loss of physical activity. In line with this concept, Booth et al. argued that exercise might simply restore the natural gene expression pattern designed to secure survival in precivilized times [13].

This hypothesis is supported by data from animal experiments, which show that only 5 weeks of forced physical inactivity to mimic a sedentary lifestyle in mice cause a strong reduction of vascular endothelial nitric oxide synthase (eNOS) gene expression accompanied by the development of endothelial dysfunction [14], a well known risk factor for cardiovascular disease [15]. Likewise, lifelong voluntary exercise training is capable of preventing age-related alterations of mouse myocardial mRNA expression and this is associated with an increased mean lifespan [16]. Based on a detailed analysis of gene expression changes at the transcriptional level, the authors conclude that exercise significantly reduces the global and overall upregulation of inflammatory and stress responses of the heart induced by ageing. It appears noteworthy to add that these effects of exercise occurred despite a dramatic reduction of daily exercise in aged mice to less than 10% of that performed by young mice.

Exercise training leads to a variety of changes in cardiovascular function including reduced heart rate, reduced blood pressure, increased maximal myocardial oxygen uptake and adaptations involving skeletal muscle, cardiac muscle, circulating blood volume and various metabolic modifications. Of these beneficial training effects, reduction of blood pressure and inhibition of atherogenesis are mainly mediated by changes of vascular biology [17–20]. The aim of this review is to summarize the molecular mechanisms underlying the vascular adaptations to exercise training. To accomplish this, the results of animal experiments giving insights into the underlying molecular mechanism are discussed and, whenever possible, completed by clinical data.

2. Effects of physical activity on vascular formation of NO

More than a decade ago it was observed that exercise training increases endothelium-dependent vasodilation in

dog epicardial arteries [21]. This observation was confirmed many times, and accumulating evidence suggest that exercise-induced activation of the NO/cGMP pathway is an important mechanism mediating beneficial vascular effects of exercise training.

2.1. Clinical effects of physical activity on vascular endothelial function

In vivo studies in animals and humans have shown that exercise results in an increased vascular expression of endothelial cell eNOS [22–25]. The functional relevance of improved endothelial function for coronary blood flow was confirmed in patients with stable CAD who underwent a vigorous exercise protocol consisting of 4 weeks of 6 bicycle ergometer exercise units of 10 min/day at 80% of the heart rate the patients had reached during peak oxygen uptake in an initial exercise test [26]. These patients responded to intracoronary acetylcholine with a significantly attenuated paradox vasoconstriction and had a 29% greater coronary blood-flow reserve as compared to sedentary control patients. A similar beneficial training response was observed in patients with chronic heart failure (CHF): Among these patients 6 months of home-based ergometer exercise training for 20 min/day at 70% of peak oxygen uptake were effective in reducing peripheral vascular resistance and resulted in small but significant improvements in stroke volume and a reduction in cardiomegaly [27]. Endothelium-dependent vasodilation of the radial artery was significantly improved after 4 weeks of daily hand-weight training and this effect was enhanced by concomitant supplementation of oral L-arginine, the precursor of endogenous NO production [28].

2.2. Mechanisms underlying vascular eNOS expression induced by physical activity

2.2.1. Shear stress

The regulation of eNOS expression is highly complex. A variety of factors such as shear stress, lysophosphatidylcholine, cGMP analogs, lipoproteins, inhibitors of protein kinase C and different cytokines are known to alter eNOS expression [22,29]. However, there are several lines of evidences suggesting that the molecular mechanism of exercise-induced upregulation of vascular eNOS expression is closely related to the changes of frequency and magnitude of physical forces in the vasculature, in particular fluid shear stress. Exercise increases heart rate, which in turn increases blood flow and vascular shear stress. Besides its role as the most important physiologic activator of endothelial NO production, shear stress has been shown to increase vascular eNOS expression [22]. For example, when cultured endothelial cell were exposed to laminar shear, a robust upregulation of eNOS mRNA and protein was observed [30]. Further studies suggested that this process is dependent on c-Src [31]. Exercise training increased eNOS protein >2-

fold in the aorta and 1.7-fold in the heart in C57Blk/6 mice but had no effect on eNOS protein levels in c-Src^{+/-} mice. These data are consistent with a study in bovine aortic endothelial cells, where c-Src plays a central role in modulation of eNOS expression in response to shear stress via divergent pathways involving a short-term increase in eNOS transcription and a longer-term stabilization of eNOS mRNA [32]. Thus, an increased intensity of physiological shear stress, as expected in exercise training, might increase vascular eNOS expression by increasing the shear stress-dependent activity of c-Src in endothelial cells.

2.2.2. Oxidative stress

Another mechanism that might contribute to exercise-induced upregulation of vascular eNOS is a triggering function of vascular oxidative stress. Exercise increases not only oxygen consumption but also the generation of reactive oxygen species such as superoxide and hydrogen peroxide [33]. It is well known that superoxide generation is increased in a non-enzymatic fashion during ATP-synthesis by an electron transfer from coenzyme Q to molecular oxygen [34]. Furthermore, and likely more important, shear stress has been shown to increase the vascular generation of reactive oxygen species by an endothelium-dependent mechanism [35]. A later analysis of this phenomenon showed an activation of endothelial NADPH oxidase as a possible underlying cause [36]. While superoxide barely traverses cell membranes and is rapidly converted by superoxide dismutases (SOD), the resulting product hydrogen peroxide can diffuse through the vascular wall and is much more stable [15]. Data obtained in endothelial cells have shown that hydrogen peroxide can increase the expression and activity of eNOS by phosphorylation of Ca²⁺/calmodulin-dependent protein kinase II/janus kinase 2 [37,38]. By generating transgenic mice with an endothelial specific overexpression of catalase, it was recently shown that hydrogen peroxide contributes to exercise-induced upregulation of eNOS [39]. In striking contrast to normal C57Bl/6 mice, 3 weeks of exercise had no effect on vascular eNOS expression in catalase-overexpressing transgenic mice. These data suggest that endogenous hydrogen peroxide plays a key role in the endothelial adaptation to exercise training by stimulating an upregulation of eNOS. The effect of hydrogen peroxide on exercise-induced eNOS expression is likely supported by the concomitant increase of SOD1 and SOD3 expression [23,40], which facilitates the generation of hydrogen peroxide from superoxide.

2.3. Factors influencing exercise-induced vascular eNOS expression

Although increased vascular expression of eNOS has been repeatedly demonstrated in animals and man, there are studies showing no effect of exercise on eNOS expression, particularly in healthy subjects [19]. Many factors may contribute to this apparent contradiction, but there are

specific conditions that appear to be of significant importance, e.g. the time of exercise training after which eNOS expression and/or endothelium-dependent vasorelaxation is measured, the baseline physical activity of the animal and human study subjects undergoing exercise, and existing polymorphisms of the eNOS gene including the eNOS promoter.

2.3.1. Effects of training time

Studies in our own laboratory have shown that there was no increase of aortic and myocardial expression of eNOS in C57Bl/6 mice undergoing 9 weeks of training (unpublished data), while 3 weeks of training resulted in a strong upregulation of eNOS mRNA and protein expression in the aorta and the left ventricular myocardium associated with increased eNOS activity [23,41]. Thus, upregulation of eNOS expression and activity by exercise training appears to be a transient effect. In their excellent review on the effect of exercise training on endothelium-derived functions of NO in humans, Green et al. hypothesized that in long-term exercise training the acute exercise-induced increase in shear stress is counter-regulated by an NO-dependent vascular remodeling process leading to an increased vessel caliber and a “structural normalization” of shear stress [19]. If this hypothesis were correct, it would not be surprising that long-term exercise training does not elicit the same response of vascular gene expression than does medium-term exercise training.

2.3.2. Effects of baseline physical activity

In individuals with a normal level of physical activity, the effects of exercise training on vascular expression and activity of eNOS should be much smaller compared to sedentary individuals. A recent study compared the effect of 3 weeks of running wheel exercise training in sedentary mice living alone in small cages with normally moving mice living in large cages in groups of 5 mice [14]. The difference in physical activity was biochemically quantified by measuring citrate synthase activity. While sedentary mice responded to the exercise training with a strong increase of aortic and left ventricular eNOS expression, normally moving mice showed only a small response. These data suggest that a low intensity of physical activity may be sufficient to maintain normal endothelial function in young healthy individuals. Additional exercise training appears to have little further effect on endothelium-dependent vasodilation and eNOS expression in normally active individuals.

2.3.2.1. Clinical findings. In the clinical context, these findings might explain why an identical reduction in the rate of cardiovascular events was observed in women who simply walked for exercise and those who exercised vigorously [2]. Given the above considerations, it seems reasonable to assume that the change in shear stress is the most important signal initiating the increase in eNOS expression and activity induced by exercise training.

2.3.3. Effect of eNOS polymorphisms

Studies with eNOS-deficient mice have suggested that the upregulation of eNOS in response to exercise training seems to require both intact eNOS genes [41]. Heterozygotic eNOS-deficient mice that display no specific phenotype and have normal blood pressure, heart rate and aortic endothelium-dependent vasodilation [42] were unable to upregulate aortic and myocardial eNOS expression in response to exercise [24]. Interestingly, genomic regulation of NO bioavailability from neuronal NOS (nNOS) in cardiac autonomic ganglia in response to exercise training appears to be also dependent on both alleles of the gene [43].

2.3.3.1. Clinical findings. To investigate the effects of hereditary eNOS mutations on training-induced vascular adaptations, Erbs et al. found that a promotor polymorphism (T-786C) of eNOS might attenuate the exercise training-induced improvement of endothelial function [44]. These findings are consistent with another recent study on the T-786C promotor polymorphism that showed shear stress-induced eNOS mRNA and protein upregulation was present in human TT and CT genotype endothelial cells but absent in cells with CC genotype [45]. Likewise, the eNOS exon polymorphism Glu298Asp attenuated the exercise training-induced decrease of systolic and diastolic blood pressure during training in a gene dose-dependent manner (homozygotes > heterozygotes), while having no effect on resting blood pressure [46]. Taken together, these findings suggest that individuals with eNOS polymorphisms may have a normal vascular reactivity under basal conditions but may be unable to adapt their vascular reactivity in response to exercise training.

2.4. Training effects on eNOS phosphorylation

It has been demonstrated that shear stress-induced eNOS activation does not depend on a rise of intracellular calcium but is directly dependent on phosphorylation of the enzyme [47]. This posttranscriptional modification was shown to occur at serine 1177 and to be mediated by the serine/threonine protein kinase Akt (protein kinase B) [48]. It alters the sensitivity of the enzyme to Ca^{2+} , rendering its activity maximal at subphysiological concentrations of Ca^{2+} . It has been reported that eNOS phosphorylation might also be mediated by other kinases. Chen et al. provided evidence for a Ser-1177 phosphorylation of eNOS in the presence of Ca^{2+} /calmodulin by rat skeletal and cardiac muscle AMP-activated protein kinase (AMPK), an enzyme that is activated by vigorous exercise and ischemic stress [49]. In addition, Boo et al. suggested that a coordinated interaction between Akt and PKA may be an important mechanism regulating eNOS activity in response to shear stress [50]. These data suggest that phosphorylation of eNOS, which is clearly dependent on shear stress, might be induced by exercise.

2.4.1. Clinical findings

The clinical relevance of these signal transduction pathways was confirmed in patients with stable CAD who underwent 4 weeks of supervised exercise training for a total of 60 min/day. A significant 4-fold increase of eNOS Ser-1177 phosphorylation levels in specimens of the left internal mammary artery was confirmed [25]. This was associated with a 2-fold increase of eNOS and a significantly increased endothelium-dependent vasodilation in this artery. Taken together, current evidence suggests that shear stress-induced phosphorylation contributes to the improvement of endothelium-dependent vasodilation induced by exercise.

3. Effects of physical activity on vascular oxidative stress

3.1. Effects of acute exercise training in humans

Acute aerobic and anaerobic exercise training increases vascular oxidative stress and subsequent damage to cellular proteins, lipids and nucleic acids as well as changes to the glutathione system [33]. For example, very hard exercise training (overload training) for 4 weeks induced an increase of plasma glutathione peroxidase activity and a decrease of plasma total antioxidant status under resting, pre-exercise conditions, and these effects were more pronounced under post-exercise conditions and accompanied by a decreased ratio of reduced glutathione (GSH) and oxidized glutathione (GSSG, GSH/GSSG ratio) and an increase in plasma thiobarbituric acid reactive substances (TBARS) [51]. Furthermore, a small study in participants of the Tour de France 2001 provided evidence for an involvement of xanthine oxidase in tissue damage induced by exhaustive exercise in trained athletes [52]. The activity of both creatine kinase and aspartate aminotransferase, which were measured to estimate tissue damage, was significantly reduced by more than 3-fold (each) in athletes treated with the xanthine oxidase inhibitor allopurinol. Thus, acute bouts of exhaustive exercise obviously represent a challenge to the antioxidative capacity of tissues such as skeletal muscle and vascular cells. On the other hand, chronic exercise training appears to induce activities of antioxidant enzymes such as vascular eNOS and perhaps stimulate levels of reduced glutathione in body fluids [53]. In addition, endurance training has been shown to reduce measures of oxidative stress such as lipid peroxidation in erythrocyte membrane in response to exhaustive exercise in young, untrained male subjects [54].

3.2. Effects of endurance training

While acute and exhaustive exercise leave little time for cellular adaptation reactions based on altered gene expression, these adaptation reactions have been shown to occur in moderate exercise training for several weeks, e.g. the

upregulation of eNOS as discussed above. Interestingly, induction of eNOS expression by exercise training was shown to be followed by an induction of SOD3 protein [23]. Studies in isolated human vascular smooth muscle cells showed that NO increases the expression of SOD3 and normal C57Bl/6 mice that underwent 3 weeks of exercise training had 3-fold higher levels of vascular SOD3. This effect was completely blunted in two different strains of eNOS^{-/-} mice and these mice had also lower vascular SOD3 expression in the first place. It should be noted that upregulation of vascular SOD by exercise not only provides a more efficient detoxification of superoxide but also effectively reduces the generation of peroxynitrite, a strong oxidant with pathophysiological importance [11]. Another more recent study provided evidence for an upregulation of SOD1 in the aortic endothelium of trained Yucatan miniature pigs [40]. While protein levels of manganese superoxide dismutase (SOD2) were not altered, protein levels of p67^{phox}, a subunit of the pro-oxidant enzyme NAD(P)H oxidase, were reduced by exercise training. The latter observation demonstrates that antioxidant effects of exercise may not only be mediated by increased expression of antioxidant enzymes but also include a reduced expression of pro-oxidant enzymes.

3.2.1. Clinical findings

This hypothesis was recently confirmed by a clinical study in which exercise-induced changes in the expression of subunits of the ROS-producing enzyme NAD(P)H oxidase (gp91^{phox}, p22^{phox} and NOX4) were determined in left internal mammary artery segments of male patients with stable coronary artery disease [55]. Exercise training significantly reduced the expression of all pro-oxidant proteins, while angiotensin II receptor type 2 protein expression was increased. These exercise-induced expression changes were accompanied by a reduced generation of

vascular reactive oxygen species and an improvement of endothelium-dependent vasodilation. Taken together, these results suggest that antioxidant effects of medium-term exercise training appear to be closely related to increased expression of vascular eNOS and are mediated at least in part by increasing the expression of antioxidant proteins such as SOD3, SOD1 and angiotensin II receptor type 2 and by decreasing the expression of pro-oxidant proteins such as NAD(P)H oxidase and angiotensin II receptor type 1 (Fig. 1).

The increase in vascular oxidative stress initiated by exercise training, even if this occurs only transiently during the acute bout of exercise, gives rise for serious concerns when exercise is prescribed to patients with cardiovascular diseases such as coronary artery disease, heart failure or stroke, and demonstrates the importance of prescribing individualized exercise programs combined with patient education about this particular program. Unfortunately, the frequency and magnitude of weekly exercise necessary to achieve beneficial prognostic effects is still uncertain so that recommendations vary between 2.5 h of walking and exercise training equivalent to 10 km of running per week [2,12]. Thus, more experimental and randomized controlled clinical studies are needed to clarify this important question.

3.3. Exercise training as an effective antioxidant therapy

It appears that exercise-induced oxidative stress that might initially induce tissue damage contributes to favorable changes of vascular gene expression observed after a few weeks of exercise training. In view of the various mechanisms discussed in this review, it is interesting to speculate whether the antioxidant effects of exercise training are dependent on intermittent vascular oxidative stress. The repetition of short-term generation of increased vascular oxidative stress during an acute bout of exercise seems to

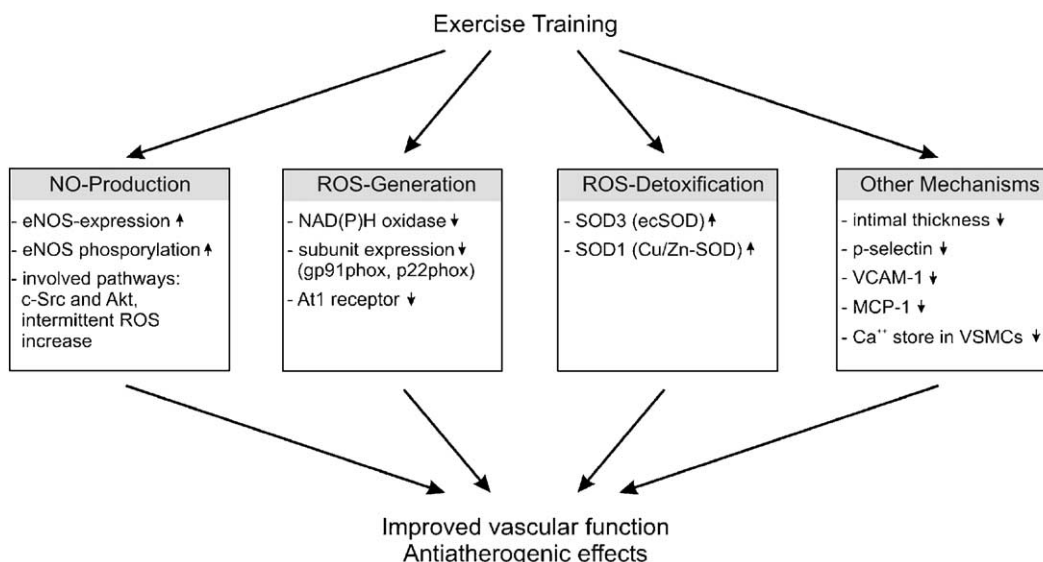


Fig. 1. Exercise-induced changes of the expression and the activity of vascular endothelia and smooth muscle cell protein.

significantly contribute to the changes of vascular gene observed in medium-term (e.g. 3 weeks) exercise training. Vascular hydrogen peroxide has been shown to increase vascular eNOS expression *in vitro* and *in vivo* [37,39]. Furthermore, medium-term exercise training has been shown to reduce oxidative stress as measured by lipid peroxidation in the erythrocyte membrane in response to exhausting exercise in young, untrained male subjects [54], and eNOS activity was demonstrated to be a crucial factor for the vascular expression of the antioxidant enzyme SOD3. Likewise, 4 weeks of exercise training reduced the expression of potentially pro-oxidant proteins such as NAD(P)H oxidase and angiotensin II receptor type 1, while the expression of vascular antioxidant proteins such as angiotensin II receptor type 2 is reduced [55]. Finally, there is evidence that some of the potentially beneficial effects of exercise and/or regular physical activity such as increased eNOS expression is reversible by a sedentary lifestyle as induced by forced physical inactivity [14].

Based on these observations, it appears reasonable to assume that exercise training can be viewed as an effective antioxidant therapy that may at least in part mediate its known beneficial effects. In this scenario, regular exercise training would benefit both healthy people and patients with cardiovascular disease. While exercise training may prevent the development of a pro-oxidative vascular gene expression pattern associated with endothelial dysfunction in healthy individuals, it corrects and/or improves already established endothelial dysfunction and increased vascular oxidative stress in cardiovascular disease such as hypertension, diabetes, stroke, coronary artery disease and heart failure [15]. When compared with known antioxidant therapy, e.g. by vitamin E, exercise training appears to be more effective and less costly.

4. Other effects of physical activity on the vascular wall

Exercise training not only affects the expression of genes related to NO production and the antioxidative capacity of the vascular wall protection. In the last part of this review, we describe other types of changes in gene regulation related to exercise training.

4.1. Vascular remodeling in response to exercise training

Exercise training is known to profoundly change the morphology of different blood vessels along the arterial tree, while information on such adaptations of venous vessels is scarce [18,56,57]. Exercise has been shown to increase both the number (angiogenesis) and the diameter (arteriogenesis) of arterial blood vessels in skeletal muscle and the myocardium. These changes of the architecture of the vascular tree are likely associated with functional changes and improved organ blood flow, as extensively reviewed recently [17,58,59].

4.1.1. Angiogenesis

Changes of vascular morphology induced by exercise training in healthy subjects [17,18] are critically dependent on the initial vessel size. An increased number of vessels in response to exercise training, i.e. angiogenesis, appears to occur on the level of capillaries and very small arterioles (<40 μm in diameter), but not in larger arteries [60]. The increase in capillary density occurs shortly after initiation of exercise and is transient. A similar pattern was observed in very small arterioles (<20 μm in diameter) and slightly larger arterioles (20–40 μm in diameter) also increase in number.

The molecular mechanisms underlying angiogenesis induced by exercise training are not fully understood. It has been suggested that growth factors such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF) and angiopoietins (ANG) as well as their corresponding receptors are involved. In addition, proteases necessary for the degradation of the capillary basement membrane such as matrix metalloproteinases (MMPs), urokinase, tissue plasminogen activator and plasminogen likely contribute to the mechanism of sprouting angiogenesis [17]. Interestingly, some of these proteases appear to enable and/or facilitate the mobilization of endothelial progenitor cells (EPCs) from the bone marrow [61]. It has become apparent that exercise can increase the number of circulating EPCs in animals and humans [55,62], and these cells are known to have a great capacity for neovascularization [63] a process that appears to be critically dependent on the protease cathepsin L [64].

4.1.2. Arteriogenesis

Larger arterioles, small arteries and conductance arteries show an increase in size in response to exercise training as evidenced by an expansion of histologically measured vessel diameter [17,18,60]. Other important differences besides increased capillarity are the later onset and the persistence of exercise-induced arteriogenesis. Enlargement of coronary arteries by exercise training is evident in pigs, rats, monkeys and humans [56,60]. The induction of arteriogenesis appears to be an important vascular adaptation [65]. It may lead to the formation of large conductance arteries that are quite capable of compensating for the loss of function of occluded arteries, and this process appears to be more important for the outcome of cardiovascular patients than the growth of new capillaries [66]. Animal experiments have shown that exercise-induced collateral growth unlikely occurs in healthy hearts but becomes evident after narrowing or occluding coronary conductance vessels, suggesting that ischemia is an important predisposing factor [19,56].

As extensively reviewed previously, arteriogenesis is critically dependent on activation of endothelial cells and endothelial adherence of monocytes, T-lymphocytes and bone marrow-derived cells and is mainly dominated by their invasion and production of growth factors in the vascular wall [58]. A number of endothelial factors appear to

contribute to stimulation of arteriogenesis, including vascular cell adhesion molecules (VCAM-1), intercellular adhesion molecules (ICAMs), VEGF, monocyte chemoattractant protein (MCP-1) and β_2 -integrins (Mac-1, LFA-1) mostly signaling via the Ras/Raf and the Rho pathways. Once the process of arteriogenesis is initiated, its progress will ultimately reduce the increased shear stress signal so that arteriogenesis in response to increased shear stress can be viewed as a balanced system where physical forces such as shear increase the resting diameter of an artery and are thereby reduced to normal at the same time.

4.2. Molecular mechanisms depending on physical forces

Exercise training strongly changes the physical forces acting on blood vessels such as shear stress, transmural pressure and cyclic stretch. Each cycle of increased heart rate, blood pressure and myocardial contractility induced by exercise training will transiently increase shear stress due to increased blood flow, transmural pressure due to increased blood pressure and cyclic stretch of blood vessels due to the increased heart rate and the pulsatile nature of blood flow. These physical forces exert significant physiologic effects on endothelial and smooth muscle gene expression and function [20,22] and are considered the most important stimuli of vascular adaptations to exercise training [17,19,56].

4.2.1. Vascular endothelial cells

Vascular endothelial cells form the inner layer of all blood vessels and are therefore the most important cell type exposed to physical forces induced by blood flow, i.e. shear stress. Prolonged laminar shear (e.g. 6–24 h) can regulate the expression of many endothelial genes, including eNOS [22,67,68]. In a systematic investigation using cultured human umbilical vein endothelial cells, Wasserman et al. found 107 endothelial genes that showed at least a 2-fold change in expression when compared to no-flow conditions [69]. Of these, 60 genes were upregulated and 47 were downregulated. These genes were grouped into 9 broad functional classes. Overall, shear induced a downregulation of the majority of genes of the cell cycle/growth group, while genes summarized in the metabolism, signal transduction and transcription groups were predominantly upregulated. This gene expression pattern likely generates a certain phenotype of endothelial cells that is protected from apoptosis, inflammation and oxidative stress [67].

It is unknown whether exercise training induces similarly extensive changes in gene expression, since exercise training does not induce shear per se but intermittently changes the frequency and magnitude of all physical forces in the vascular system, including shear. However, some vascular genes known to be upregulated by shear are also upregulated by exercise training. In particular, exercise training has been shown to increase the expression of potentially atheroprotective vascular proteins such as eNOS, extrac-

ellular superoxide dismutase (ecSOD, SOD3), Co/Zn-SOD (SOD1) and angiotensin receptor type 2 [23,40,55,70], while potentially atherogenic vascular proteins such as subunits of endothelial and vascular smooth muscle NADPH oxidase and angiotensin receptor type I are downregulated by exercise training [40,55].

Mechanical forces activate many intracellular pathways including MAP kinase and induce sequential phosphorylations activating transcription factors and gene expression. Such mechanotransduction mechanisms are mediated by integrins and associated RhoA small GTPase [71]. Integrins are membrane-associated glycoproteins composed of α and β subunits and exist in 22 combinations of the 18 α subunits and the 8 β subunits. These cell-surface receptors, which are known to mediate cell adhesion and cell migration by binding to extracellular matrix proteins [72], are also an essential component regulating angiogenesis [73]. Transduction of signals mediated by shear stress-induced activation of integrins include phosphorylation of kinases such as focal adhesion kinase, c-Src, Akt kinase, phosphatidylinositol 3-kinase, myosin light chain kinase and mitogen-activated protein kinases (MAPK) such as extracellular signal-regulated kinase (ERK). These molecular mechanisms result in shear stress-induced upregulation of genes mediating antiatherogenic effects by promoting antiapoptotic and antiproliferative signals, by increasing vascular NO bioavailability and by vascular remodeling [71].

The increase in physical forces induced by exercise might particularly change the phenotype of endothelial cells in areas of turbulent flow. It has long been known that atherosclerotic lesions develop preferentially in vascular areas of turbulent and/or low shear flow [74]. In striking contrast to laminar shear, oscillatory shear can actually reduce eNOS expression. Recent work has provided evidence that certain blood flow patterns which occur in atherosclerosis-prone areas of human carotid artery (areas with low shear) produce arterial waveforms that initiate only small changes in shear stress and elicit a proinflammatory endothelial phenotype [75]. This phenotype is characterized by increased expression of proinflammatory genes such as interleukin-8 (IL-8), pentaxin-related gene (PTX3), chemokine receptor 4 (CXCR4) and tumor necrosis factor receptor superfamily member 21 (TNFRSF21). Furthermore, low-shear arterial waveforms also upregulate angiogenic factors such as placental growth factor (PGF) and connective tissue growth factor (CTGF). In striking contrast, exposure of endothelial cells to an arterial waveform matching that observed in artery areas with a low susceptibility to develop atherosclerotic lesions (high shear arterial waveforms) showed a completely different gene expression profile. In particular, an increased expression of genes known to be rather atheroprotective such as C-type natriuretic peptide (CNP) and the transcription factor KLF-2 was observed [75].

It is currently not known whether exercise training is able to alter the endothelial gene expression pattern of artery

areas that are particularly susceptible to the development of atherosclerotic lesions. Thus far, only a few studies investigating the effect of exercise training on proinflammatory and antiatherogenic genes expressed in low-shear and high-shear artery areas are available. Data obtained with placenta growth factor (PGF) knockout mice (PGF^{-/-} mice) suggest that the angiogenic factor PGF is not involved in angiogenesis induced by exercise training [76]. A small clinical study showed no effect of exercise training on urinary CNP [77]. However, it has been shown that exercise training can reduce lesion formation in the aortic arch and thoracic aorta of low-density lipoprotein (LDL) receptor-deficient hypercholesterolemic mice [78] and preserves endothelium-dependent relaxation in brachial and coronary arteries from hyperlipidemic pigs [79,80]. Likewise, chronic exercise in hypercholesterolemic rabbits decreased intimal thickening and the aortic expression of P-selectin, VCAM-1, MCP-1 and iNOS [81]. One potentially important molecular mechanism underlying the antiatherosclerotic effects of exercise is the regulation of eNOS expression and activity by fluid shear forces [11,22].

4.2.2. Vascular smooth muscle cells

Vascular smooth muscle cells are less exposed to shear stress than vascular endothelial cells, but are subjected to transmural pressure and cyclic stretch to a similar degree. Nevertheless, a recent study in rat aortic smooth muscle cells indicates that shear stress-induced smooth muscle cell contraction is dependent on the glycocalyx glucosaminoglycans heparan sulfate and chondroitin sulfate [82]. There are only a few studies that have investigated the effect of exercise training on transduction of biomechanical signals in vascular smooth muscle. In contrast, mechanotransduction has been studied quite frequently *in vitro* by applying mechanical forces such as cyclic strain to cultured smooth muscle cells or isolated arteries and excellent reviews on this topic have been published previously [83,84]. By changing gene expression, vascular smooth muscle cells respond to physical forces in a manner similar to endothelial cells and regulate important cellular functions such as proliferation, apoptosis, calcium handling and the myogenic response to pressure [83]. It was shown that cyclic stretch induces the immediate-early gene transcription factors early growth response gene-1 and c-jun but not c-fos in neonatal rat aortic smooth muscle cells [85]. In another study, stretching of vascular smooth muscle cells activated Jun N-terminal kinase/stress-activated protein kinase by autocrine ATP stimulation of purinoceptors [86].

The transduction of mechanical signals into smooth muscle responses appears to be crucially dependent on integrins such as $\alpha_v\beta_3$ [87], but probably involves other membrane proteins binding to extracellular matrix proteins. For example, it has been shown that cyclic stretch activates the elastin–laminin receptor, which results in a reduction of both expression of the transcription factor c-fos and cell

proliferation [88]. Recently, Cattaruzza et al. described that the focal adhesion protein zyxin is a modulator of cyclic stretch-induced gene expression in vascular smooth muscle cells, which appears to be important for the induction and/or suppression of various smooth muscle proteins [89]. Zyxin depletion by antisense oligonucleotides in stretch-activated cells reduced the induction of endothelin B receptors and cyclooxygenase-1 but enhanced the induction of the matrix protein tenascin-C. In addition, integrins [90] and mechanical forces have been linked to the Rho family GTPases and mechanical forces likely alter biochemical signal transduction by modulating microtubule polymerization [91]. It has been proposed that these proteins mediate the transfer of tension between the cytoskeleton and the extracellular matrix (tensegrity), suggesting that the whole cell is a mechanosensor [84].

Under *in vivo* conditions, vascular smooth muscle cells are constantly exposed to significant physical forces, and exercise training only changes the frequency and magnitude of these forces. As summarized in a recent review [20], the most important finding is that exercise decreases the caffeine-releasable sarcoplasmic Ca^{2+} store of coronary smooth muscle cells (Ca^{2+} unloading), resulting in a decreased contractile response of coronary smooth muscle cells and coronary arteries to endothelin and the thromboxane A_2 derivative U46619. Interestingly, although the release of Ca^{2+} in response to endothelin is diminished, the activity of voltage-operated L-type calcium channels appears to be increased by exercise. One mechanistic link between changes in intracellular Ca^{2+} release and the activity of voltage-operated L-type calcium channels might be an altered signaling via protein kinase C. In addition, current evidence suggests an involvement of K^+ channels. Of these, Ca^{2+} -activated K^+ channels and voltage-dependent K^+ channels might be of particular importance. Further data on exercise-induced reduction in endothelin-stimulated DNA synthesis gives rise to the hypothesis that the overall effect of exercise in coronary smooth muscle is to maintain a stable contractile phenotype [20].

5. Conclusion

The beneficial effects of exercise training on vascular function are well established: long-term physical activity significantly improves endothelium-dependent vasodilation in response to flow or acetylcholine infusion. However, the molecular mechanisms underlying the functional changes are complex and only partially understood. Among the established mechanisms, a shear stress- and hydrogen peroxide-related increase in eNOS expression/activity and a reduction in vascular oxidative stress resulting from both reduced ROS production and improved antioxidative capacity of the vascular wall are most important. While clearly more experimental studies are needed to further elucidate the vascular molecular basis of exercise, the

clinical evidence for its beneficial prognostic effects in both primary and secondary prevention is overwhelming. We have therefore robust scientific evidence to strongly encourage the clinical application of exercise therapy in cardiovascular medicine.

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