

Adaptation of endothelium to exercise training: Insights from experimental studies

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1. ABSTRACT

Endothelial dysfunction is one of the hallmarks of cardiovascular disease and serves as a prognostic marker for forecasting the development and outcome of the disease process. Current pharmacological treatment strategies only incompletely repair endothelial dysfunction whereas exercise training corrects this dysfunction, primarily due to improved production and/or bioavailability of nitric oxide, the main endothelium-derived vasodilator. This type of treatment also improves the function of healthy endothelium. The focus of this review is to discuss the underlying biological factors involved in improved endothelial function after exercise training in healthy individuals as well as those with cardiovascular disease or a metabolic syndrome. The ability to sustain the bioavailability of nitric oxide (NO) in the endothelium is probably the most important factor in restoring normal endothelial function by exercise training.

2. ENDOTHELIAL FUNCTION

The innermost cell layer lining the cardiovascular system, the endothelium, was initially regarded as an inert, static layer in the circulatory system. However, Furchgott and Zawadzki discovered the importance of the endothelium in the regulation of vascular tone (1). The endothelium is now acknowledged as an organ with important autocrine and paracrine functions. A large number of vasoconstrictive and vasodilating substances are produced in endothelial cells to act on the underlying vascular smooth muscle cells. NO; probably the most important endothelial-derived relaxing factor, is produced by the endothelial isoform of NO synthase (eNOS). In addition to relaxing vascular smooth muscle, NO counteracts the formation of atherosclerosis through inhibition of leukocyte adhesion and invasion, smooth muscle cell proliferation, platelet aggregation, and inflammation (2). Abnormalities in one or more of the

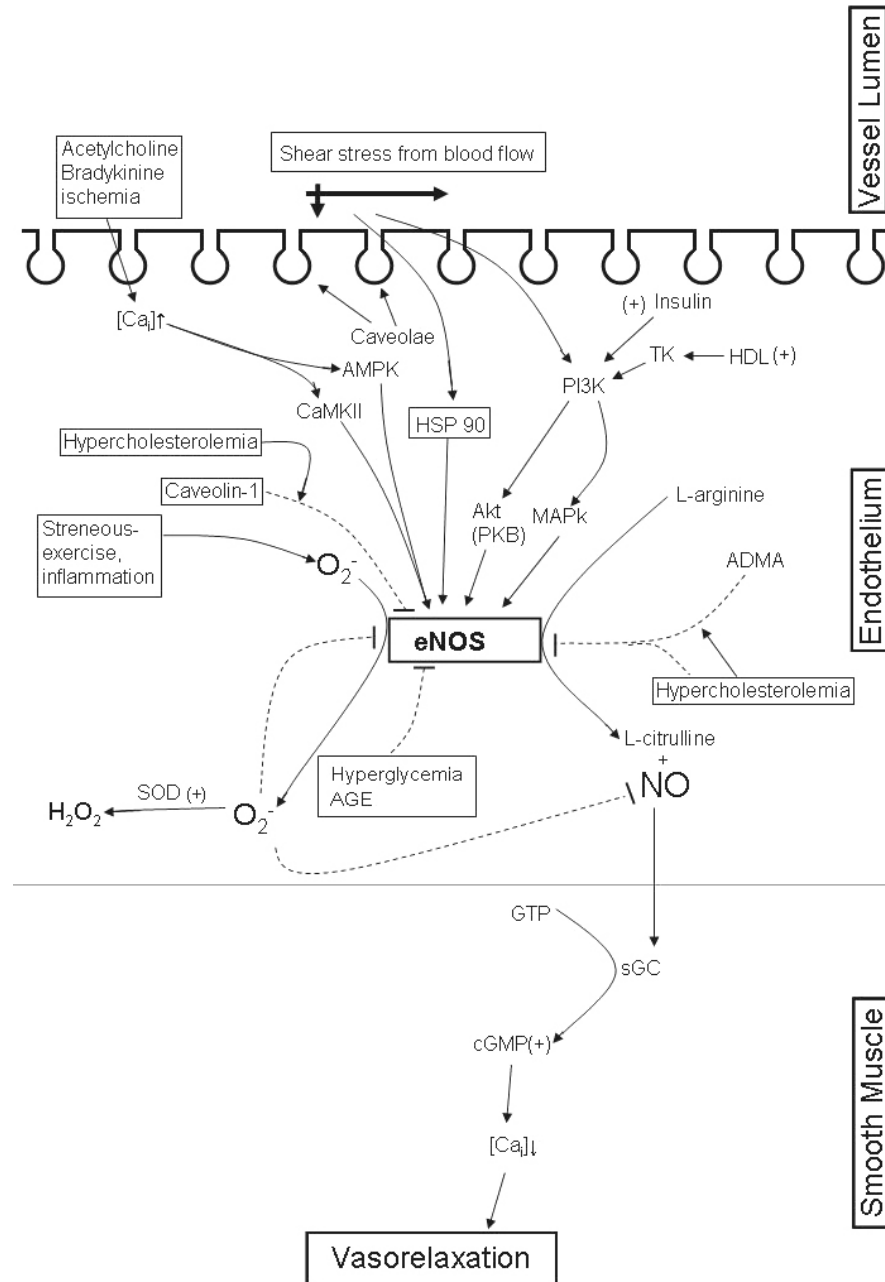


Figure 1. Factors influencing the bioavailability of nitric oxide (NO) in the endothelium, arrows define stimulating pathways, while bars define inhibitory pathways. See text for further details. AMPK, AMP-activated protein kinase; CaMKII, Ca^{2+} -calmodulin-dependent protein kinase II; TK, tyrosine kinase; HDL, high density lipoprotein; PI3K, phosphatidylinositol 3-kinase; ADMA, asymmetric dimethylarginine; SOD, super oxide dismutase; O_2^- , superoxide anion; MAPK, mitogen activated protein kinase; eNOS, endothelial nitric oxide synthase; AGE, advanced glycosylation end products; NO, nitric oxide; GTP, guanosine tri-phosphate; sGC, soluble guanosine cyclase; cGMP, cyclic guanylyl monophosphate; $[Ca^{2+}]$, Ca^{2+} concentration; PKB, protein kinase B (Akt); HSP 90, heat shock protein 90; H_2O_2 , hydrogen peroxide.

pathways that ultimately regulate the availability of NO may lead to dysfunction of the endothelium (Figure 1). Endothelial dysfunction is characteristic of cardiovascular disease and often found in conjunction with other coronary risk factors, such as hypertension, thrombosis, sepsis, hypercholesterolemia, cigarette smoking, diabetes mellitus,

and obesity. Importantly, endothelial dysfunction, as defined by impaired endothelial-dependent vasorelaxation, has been identified as an independent risk factor and a strong prognostic marker of long-term cardiovascular morbidity and mortality in latent and manifest cardiovascular disease (3, 4). Furthermore, impaired

endothelial function has been observed several years ahead of traditional markers of cardiovascular disease, and is thus linked to pathogenesis of atherosclerosis (3, 4). Therefore, the preservation of endothelial NO-production and NO-bioavailability should be a major therapeutic goal.

3. EXERCISE AND THE ENDOTHELIUM

Physical activity was found to be associated with protection against coronary artery disease already in 1953 (5), whereas Kramsch (6) in 1981 found that moderate exercise in monkeys on an atherogenic diet reduced the incidence of coronary atherosclerosis. Also “certain lifestyles” including regular exercise were suggested by Eichner (7) in 1984 to be protective against thrombosis. The evidence for the endothelium and the effect of exercise upon the endothelium mediating this became however evident only later. A wealth of pharmaceutical agents have been found to improve endothelial function (8), but exercise was first found to be beneficial for the endothelium in 1993, when Delp (9) reported that relaxation in response to acetylcholine (Ach), an endothelium-dependent dilator, was enhanced by 12 weeks of exercise training in rats. Training-induced alterations appeared to be specific to the endothelium and to be due to increased production of NO, since endothelium-independent dilation was not altered by exercise training.

Individuals with endothelial dysfunction have an impaired ability to carry out maximal exercise, as maximal oxygen uptake (VO_{2max}) is reduced by at least 20% compared with control subjects of similar age and physical activity level (10, 11). On the other end of the scale, highly trained endurance athletes may also have an endothelial insufficiency that limits exercise capacity, as VO_{2max} and cardiac output can reach values of more than $80 \text{ mL} \cdot \text{kg} \cdot \text{min}^{-1}$ and $40 \text{ L} \cdot \text{min}^{-1}$, respectively. If conduction cannot match the skeletal muscle demand, the endothelial function may become limiting for exercise capacity (12, 13).

4. PRODUCTION OF NO

NO is synthesized from L-arginine by eNOS following stimulation by either shear stress or endothelial agonists such as bradykinin or Ach. Agonist occupation leads to increased endothelial Ca^{2+} concentration ($[\text{Ca}^{2+}]$), which activates AMP-activated protein kinase (AMPK) and Ca^{2+} -calmodulin-dependent kinase II (CaMKII) that phosphorylate eNOS at the serine 1177 residue (in humans). Phosphorylation of the same residue can also occur independent of Ca^{2+} , by mechanical stimulation with shear stress as a consequence of the sequential activation of phosphatidylinositol 3-kinase (PI3K) (14) (Figure 1). Furthermore, a complex reaction occurs involving the transfer of electrons from nicotinamide-adenine dinucleotide phosphate (NADPH), via flavins in the carboxy-terminal reductase domain, to the heme in the amino-terminal oxygenase domain, where the substrate L-arginine is oxidised to L-citrulline and NO (15). Besides eNOS, two other NOS isoforms; inducible (iNOS) and neuronal (nNOS) exist, of which eNOS and nNOS reside in the endothelium. NOS functions as a dimer consisting of

two single monomers, each with a separate C-terminal reductase domain, an N-oxidase domain and a calmodulin site responsible for Ca^{2+} -activation. For appropriate function of the enzyme, the monomers are connected, depending upon heme and tetrahydrobiopterin (BH4). High abundance of BH4 and the eNOS-associated heat shock protein 90 (Hsp90) (16, 17) makes NOS a pure NO-synthesizer, while decreasing availability of BH4 and Hsp90 ultimately transforms NOS into a producer of superoxide anions (O_2^-) (18).

4.1. Regulation of eNOS expression

Both shear stress and regular exercise upregulate eNOS expression. Furthermore, the shear stress-induced increase in eNOS mRNA in bovine aortic endothelial cells has been shown to depend upon Ca^{2+} (19) and G-proteins (20). Other stimuli that upregulate eNOS are hypoxia (21), vascular endothelial growth factor (VEGF) (22), HMG-CoA reductase inhibitors (23) and hyperthyroidism (24). eNOS can also be downregulated by factors such as tumor necrosis factor ($\text{TNF-}\alpha$) (25), oxidized low density lipoprotein (LDL) (26), hypothyroidism (24), hypertension (27) and by distortions in lipid metabolism (28, 29). Partial deletion of the eNOS gene increases susceptibility to high-fat diet-mediated arterial hypertension (30), while mice with double knock-out of the eNOS gene produce a phenotype that mimics the metabolic syndrome, including insulin resistance, hyperinsulinemia, dyslipidemia, and hypertension (31, 32). Also, caloric restriction increased mitochondrial biogenesis in wild type, but not eNOS null mice (33). This suggests that lower abundance of eNOS might be a molecular link between cardiovascular and metabolic diseases. The cause-effect relationship in this process is not clear, but it has been suggested that perturbation of lipid metabolism causes early abnormalities in Ach-dependent relaxation and decreased eNOS expression in arteries (28, 29).

4.2. Oxidative stress and bioavailability of NO

Endothelial function is dependent upon the balance of oxidant and antioxidant mechanisms. An imbalance in redox state where pro-oxidants overwhelm anti-oxidant capacity results in oxidative stress (34). O_2^- will then decrease the function of eNOS and reduce the half-life of NO by increasing the production of peroxynitrite from NO and O_2^- . This reaction is associated with pathological conditions, whereas in normal conditions, O_2^- is quenched by super-oxide dismutase (SOD). Reactive oxygen species (ROS) also regulate vascular function by modulating cell growth, apoptosis, migration, inflammation, secretion, and extracellular matrix protein production (35). Oxidative stress and the associated oxidative damage are mediators of vascular injury and inflammation in many cardiovascular diseases, especially when complicated with hypertension, hyperlipidemia, and diabetes. The major source of oxidative stress in the arterial wall is NADPH oxidase. In addition, xanthine oxidase, uncoupled NOS, and mitochondrial leakage of ROS during oxidative reactions may also cause stress. Obesity and the metabolic syndrome is associated with raised oxidative stress, and recent studies have revealed that inflammatory and stress-response genes are among the most abundantly

regulated genes in adipose tissue of obese animals (35). To illustrate the high impact of oxidative stress on NO-availability, the antioxidant ascorbic acid is able to reverse endothelial dysfunction in patients with coronary disease (36). Exercise is also found to effectively improve antioxidant status (37, 38), even only after one bout of exercise (38, 39). However, the high oxidant load after a single bout of high intensity exercise is capable of inducing a brief period of endothelial dysfunction before a salutary endothelial function prevails a few hours later (39).

4.3. Shear stress

Blood vessels are not homogenous. However, the endothelium remains largely unchanged throughout the cardiovascular system. It is constantly exposed to hemodynamic forces varying in magnitude and direction, depending upon the anatomy of the blood vessel and of the viscous drag from the blood flow. The blood flow-linked forces that act on the arterial wall can be divided into two principal vectors; one perpendicular to the wall and the other parallel to the wall. Together, they create a frictional force that exerts shear stress on the surface of the endothelium (40, 41, 42). This is an important stimulus to the endothelium, and it is involved in stimulation of NO production, vascular remodelling and blood vessel formation (43). Vascular shear stress increases during exercise. To study the impact of the shear stress, one may apply shear stress to cultured endothelial cells. This approach has provided an important technique for the study of exercise-mediated shear stress in the endothelial cell (40, 41, 43).

4.4. Sensing of shear stress and intercellular signaling

The mechanotransduction pathways transforming mechanical shear stress into alterations in gene expression are not yet fully understood, but several pathways have been proposed (40, 41). A central hypothesis concerns the caveolae, the numerous 50-100 nm invaginations of the endothelial plasma membrane. The caveolae, consisting of the governing protein caveolin-1, and phospholipids, sphingolipids and cholesterol, have been suggested to be important for regulating NO production (44, 45). Proposed functions of caveolae are vesicular transport and cholesterol homeostasis, but in recent years, the “caveolae signaling hypothesis” (46, 47) has suggested that caveolins serve as oligomeric docking sites for organizing and concentrating signaling molecules within caveolae membranes (48). This includes eNOS, which is bound to and inhibited by caveolin-1 in the caveolae. Studies with the caveolin knock-out mice (49, 50) further support this by exhibiting unstable basal vessel tone and vigorous Ach-mediated NO production that indicates a lack of the inhibiting caveolin-1. Chronic shear stress stimulates caveolae formation by translocating caveolin-1 from the Golgi-apparatus to the luminal plasma membrane (51), which leads to enhanced sensitivity to shear stress by increasing phosphorylation of eNOS (43). Previous studies have shown no effects of exercise or chronic heart failure on the expression of caveolin-1 in aortic tissue (44), but an increase linked to a high-fat diet (54), in experiments involving aortacaval shunts (55) and exposure to shear stress in endothelial cell cultures (53, 56). Disturbances of

the caveolae system have also been linked to the metabolic paradigm in vascular diseases such as atherosclerosis, leading to a failure of Ach-mediated vasorelaxation, similar to the one reported in rats with low aerobic exercise capacity and metabolic syndrome (27, 46, 47, 57). Since decreased VO_{2max} is closely linked to decreased cardiac output and therefore also decreased shear stress (13, 42, 43), we hypothesized that decreased VO_{2max} would be associated with less shear stress-responsive elements, such as caveolae in the rats with low aerobic capacity and metabolic syndrome. Consistently, we observed a decrease in the density of caveolae along with a 2-fold decrease in caveolin-1. Exposing cultured endothelial cells to shear stress has been shown to increase the density of caveolae as well as the level of eNOS and NO (53, 56). Thus, we also hypothesized that the arterial shear stress developed during exercise would increase the caveolae density along with improvements in endothelial function. Indeed, exercise training increased caveolae density and expression of caveolin-1, indicating that this pathway is a key element in the exercise-induced improvement of endothelial function in rats with low aerobic capacity and metabolic syndrome. One may also hypothesize that the sequestration of eNOS in caveolae together with the muscarinic cholinergic receptor and the other members of its regulatory pathway may be the critical point as the dissociation of caveolin-1 and eNOS is augmented during agonist mediated increase in $[Ca^{2+}]$ (46, 58), although this remains to be studied.

Shear stress is also associated with a rapid upregulation of eNOS mRNA and protein expression levels (59, 60). Protein kinase A (PKA) (61) and the serine/threonine protein kinase Akt (62), have both been demonstrated to be upregulated in cultured endothelial cells subjected to shear stress, independently of Ca^{2+} . Hambrecht and co-workers (63) found double and triple enhanced expression of total eNOS and eNOS phosphorylated at the serine residue 1177 (peNOS), respectively, in the left mammary artery of patients with atherosclerotic disease who underwent regular exercise. The total expression of Akt was not upregulated, but the level of phosphorylated Akt (pAkt) was upregulated by 90%. Moreover, there was a close correlation between pAkt and peNOS. The output of NO during exposure to NO is however only 1/3 of the output during agonist-mediated production, but the shear stress-mediated output is maintained for hours, whereas the output after mediation by agonists only lasts for minutes (64).

4.5. Exercise and NO: shear stress or metabolic factors?

As previously mentioned, exercise can improve the endothelial function by solely intervening on metabolic factors such as hyperglycemia, hyperlipidemia and hypertension. A reduction in these risk-factors can be achieved by dietary or low- intensity exercise interventions, as shown in animal models of diabetes and hyperlipidemia (29, 39). A reduction in hyperglycemia, TNF- α , LDL-cholesterol, asymmetric dimethylarginine (ADMA), and pro-oxidant status or an increase in high density lipoprotein (HDL) and antioxidant status will increase the bioavailability of NO and improve endothelial function. Moreover, and as outlined above, increasing shear stress

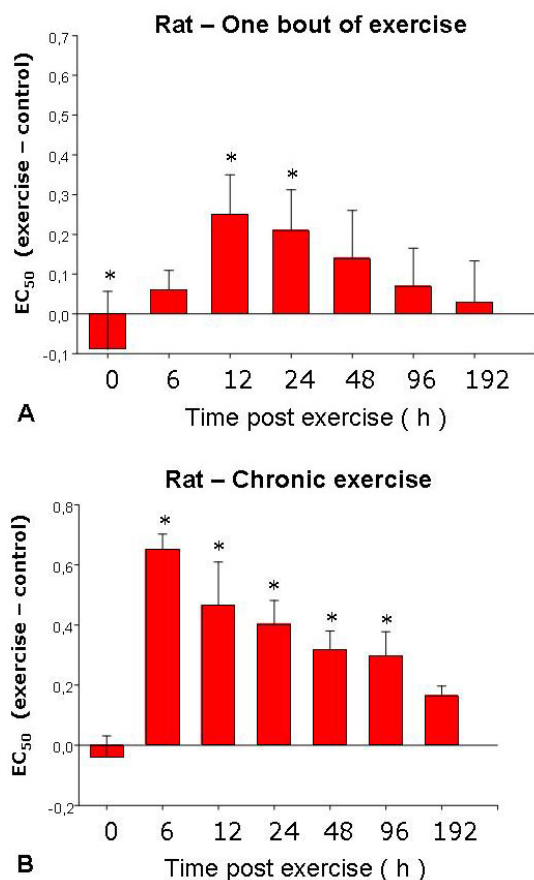


Figure 2. Endothelial function determined by acetylcholine induced vasodilation in rat (panel A and B). After one bout of exercise in rat (panel A) a transient decrease was followed by a rapid increase in endothelial function. Chronic high intensity exercise in rat (panel B) also showed increased absolute relaxation. * for $p < 0.05$.

would also increase NO production. In the absence of metabolic distortions such as diabetes or hyperlipidemia that decreases the bioavailability of NO, shear stress augmenting the production of NO is probably the most important contributor to improved endothelial function. Improvements in endothelial function are therefore not commonly seen in patients with only a relatively few metabolic distortions undergoing an exercise regime of mild intensity (62, 63). In addition to upregulating eNOS, shear stress can also regulate other enzymes such as SOD (64), which thus would reduce NO degradation and hence affect the bioavailability of NO. A shear stress-dependent augmentation of endothelial function therefore improves NO balance by more factors than only those that increase NO production.

5. TIME COURSE OF EXERCISE-INDUCED ENDOTHELIAL ADAPTATION

The endothelium is highly dynamic and responds rapidly to exercise. We have shown that a single bout of high intensity treadmill running (90% of VO_{2max}) is followed by a brief period of reduced endothelial function

(Figure 2, Panel A). However, improvement was present 12-24 hours post-exercise, followed by a rapid return to baseline 48 hours after the exercise bout. The transient reduction of endothelial function following one bout of exercise was prevented by incubating the vessels with the O_2^- scavenger SOD, suggesting that oxidative radicals produced during strenuous exercise decrease the half-life of NO and therefore the bioavailability thereof immediately after exercise. The beneficial effect of one bout of exercise has also been documented in humans at moderate intensity (60% of VO_{2max}) (66). Interestingly, we have also recently shown in humans with the metabolic syndrome that a single bout of high intensity interval training (90% of VO_{2max}), but not moderate-intensity exercise (60% of VO_{2max}), improves flow-mediated endothelial function, with an effect that lasts up to a week (66) (Figure 2, Panel B). The fact that a single bout of exercise is able to initiate a substantially improved endothelium-dependent vasodilatation may affect exercise prescriptions for prevention, treatment and rehabilitation of cardiovascular disease. However, no effects of a single exercise bout have also been reported (70), although one should note that there was no control of exercise-intensity in this study, which might have caused the lack of effect.

A daily exercise training program for weeks and months induces a more pronounced improvement in endothelial function than a single bout of exercise; after 10 weeks of exercise training at a high aerobic intensity (90% of VO_{2max}), the [Ach] evoking half-relaxation (EC_{50}) decreased ~4-fold (39, 69). This might be due to a higher gain in the eNOS-NO-cGMP pathway. In contrast, the exercise training-improved endothelial function returned to baseline levels within 1-2 weeks of inactivity (39, 69). Interestingly, this decline is closely associated with regression of VO_{2max} , suggesting a link between them. Notwithstanding, the data also suggest that even in highly trained individuals, it is not possible to “store” exercise-induced improvements in endothelial function for prolonged periods of time, as a week of inactivity is enough to abolish the effect of 6-10 weeks of endurance training. Thus, regular exercise is necessary for long term preservation of improved endothelial function.

6. EXERCISE INTENSITY-DEPENDENCE OF ENDOTHELIAL IMPROVEMENT

It has been suggested that high exercise intensity is necessary to enhance endothelial function in healthy individuals, whereas lower exercise intensity may be sufficient in those that present with endothelial dysfunction when the exercise program commences (66). We have shown in healthy rats that although endothelium-mediated vasorelaxation was indicated better after high intensity exercise training, the effect was not statistically different from moderate intensity training (90% versus 60% of VO_{2max}) (68). However, no consensus has been reached yet as to whether or not improvements in endothelial function are intensity-dependent. Other studies have reported that aerobic high exercise intensity may have both detrimental (70) and positive (39, 63, 66, 68) effects on endothelial function. The reason for this discrepancy is not clear, but

might have to do with the timing of the measurements (39). We observed a biphasic endothelial response to both acute and chronic exercise, first being reduced immediately after exercise, but increased 6-12 hours after exercise (39). Interestingly, we have also recently shown that a single bout of high intensity, but not moderate, exercise improves flow-mediated endothelial function in humans with the metabolic syndrome, and that this effect may last up to a week after the exercise session (66).

7. ENDOTHELIAL PROGENITOR CELLS, NEOANGIOGENESIS, AND ENDOTHELIAL FUNCTION

Improvements in endothelial function not only depend on the cells that reside in the vessel wall, but can also be modified by bone marrow-derived endothelial progenitor cells (EPCs) (73). It has been shown that blood cells with anti-CD34 or anti-Flk-1 properties can differentiate into endothelial cells and contribute to angiogenesis (74). Although both myocardial infarction and bypass surgery acutely increase the number of EPCs, accumulation of cardiovascular risk factors in contrast reduces the number of these cells, suggesting that vascular health and repair processes after injury require increased numbers of this potentially beneficial population of cells (75-79). Amongst other factors, exercise too appears to stimulate the release of EPCs. The increased NO production during exercise has been proposed to be the main stimulator for the release of EPCs, as treating exercising animals with the L-arginine antagonist L-NAME significantly decreases the number of EPCs (76). In line with improved endothelial function (39), one bout of exercise also increases the number of EPCs (78).

8. ATHLETE'S ARTERIES?

A close link between VO_{2max} , regular endurance training, and endothelial function has now been established in humans (63, 72, 83). It is also widely accepted that the endothelial benefits from exercise are most pronounced in (and perhaps even limited to) subjects with pre-existing endothelial dysfunction (66). For example, exercise has been shown to improve endothelial function in humans and rats with metabolic syndrome (80, 81) and chronic heart failure (82, 83). In contrast, studies of healthy subjects reveal conflicting data, suggesting improved (87), unaltered (85) or even depressed (86) endothelial function. Furthermore, it seems that endothelial function is well preserved in young, healthy women and men (89) and that a high aerobic training status does not increase the dilating capacity. Nonetheless, athletes have larger artery diameters compared to untrained counterparts, and thus have a larger functional capacity of their blood vessels (88, 89). A large resting brachial artery is known to be an independent predictor of coronary artery disease (90). However, in athletes with a high cardiac output, shear stress and endothelial function is preserved despite the larger arterial diameter (87). This may be an analogue to physiological hypertrophy (athlete's heart) with improved function versus the pathological hypertrophy (i.e. that observed in patients with heart failure or volume or pressure overloaded hearts).

Increased arterial diameter on the basis of an exaggerated production of NO in athletes (87) may suggest that a structural enlargement of the artery has taken place. The mechanisms responsible for mediating structural enlargements of the vascular system are not fully understood, but evidence points to shear stress-mediated NO release playing an important part (91).

9. ENDOTHELIAL FUNCTION IN RATS WITH DIVERGING AEROBIC CAPACITY

After eleven generations of selective breeding based upon aerobic treadmill running capacity, we obtained contrasting rat lines of Low Capacity Runners (LCR) and High Capacity Runners (HCR) (92). HCR were superior to the LCR for distance run to exhaustion (350%) and VO_{2max} (60%). LCR demonstrated a cluster of risk factors for cardiovascular disease, such as higher body mass, visceral adiposity, hypertension, and pathological levels of insulin, glucose, free fatty acids, and triglycerides, whereas HCR had better running economy, cardiac function, NO-induced vascular dilation, and improved adaptation to regular exercise training. Moreover, the impaired mitochondrial status in LCR may link reduced fitness to cardiovascular and metabolic disease, and further strengthen the argument that the LCR rats have developed the metabolic syndrome. In rats with low inherited VO_{2max} (LCR), arterial eNOS expression was markedly decreased, in line with the impaired NO-mediated vasorelaxation. Although exercise improved endothelial function in LCR up to the level of sedentary HCR, the eNOS expression was not upregulated to the same extent. This strongly suggests that exercise promoted countermeasures that restored endothelial function rather than reversing a primary defect in eNOS of LCR. Failure to increase eNOS levels is consistent with the notion that exercise does not always increase eNOS levels (93), and that when it does, it exerts its effect in conjunction with restoration of lipid metabolism (29). Endurance training did not fully restore triglyceride levels, but whether this underlies the failure of exercise to restore eNOS levels remains to be determined. A possibility is that one or more shear stress-responsive elements responsible for the transcription of the eNOS sequence are dysfunctional (59) and that the eNOS mRNA was unstable in the LCR rats.

One of the factors upregulating the activity of eNOS is Hsp90. Hsp90 was downregulated in LCR as compared to HCR, but exercise restored it to the levels of HCR, in line with improvements in endothelial function. Hsp90 binds directly to eNOS, augmenting NO production by inducing conformational adaptation of eNOS that renders it more readily phosphorylated by Akt (95). Decreased Hsp90 is on the other hand associated with a shift from NO to O_2^- and hydrogen peroxide (H_2O_2) production (95).

10. ATTACKING ENDOTHELIAL DYSFUNCTION IN MULTIFACTORIAL DISEASE

If we consider the human genome, it was naturally selected to maximize fitness in the early ancestral

environment, a time in which physical activity was obligatory for survival. Our genome has not changed much the last 100 000 years, and exercise still remains essential for optimal gene expression and avoidance of disease (96, 97, 98). Indeed, physical inactivity is now established as an independent risk factor for cardiovascular morbidity and mortality, an effect that is similar to that of high blood pressure, high levels of blood lipids and smoking combined. The human body is therefore not ideally suited for modern Western lifestyle, where inactivity is the norm with a daily energy expenditure corresponding to 38% of what our Paleolithic ancestors had (96, 97, 98). An inactive lifestyle will therefore alter gene expression, perturb homeostasis, and lead to complex disease scenarios such as the metabolic syndrome and cardiovascular disease.

In this review, we have argued that endothelial dysfunction is associated with, and predictive of cardiovascular disease. Endothelial dysfunction may be corrected with various approaches, such as exercise training, but other options include ascorbic acid (36), allopurinol (99), rosiglitazone (100), and Quyu Xiaoban capsules (101). It is unlikely that restoring or maintaining endothelial function alone would prevent cardiovascular disease, given the multifactorial nature of it. However, for the same reason, we advocate instead exercise training for prevention, treatment, and rehabilitation purposes, because also exercise training has a multifactorial nature (see other papers in this edition of this journal). Exercise is, as opposed to most pharmaceutical interventions, a heterogeneous intervention causing beneficial adaptations to multiple organ system, including the endothelium. For instance, a consequence if this is also that larger cardiac output increases the shear stress to the endothelium, and upregulates shear stress-responsive elements such as Akt (59), PKA (58), eNOS (29), Hsp90 and the antioxidant system (64). Hence, exercise training also stimulates endothelial function and NO-mediated control of arterial tone by an array of pathways that ultimately, at least partly, explain the reduced incidence of cardiovascular disease, and the reduced morbidity and mortality after the onset of disease.

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Abbreviations: Ach, acetylcholine; ADMA, asymmetric dimethylarginine; AMPK, AMP-activated protein kinase; BH4, tetrahydrobiopterin; [Ca²⁺], Ca²⁺ concentration;

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CaMKII, Ca^{2+} -calmodulin-dependent kinase II; eNOS, endothelial nitric oxide synthase; EPC, endothelial progenitor cell; H_2O_2 , hydrogen peroxide; HCR, high capacity runner; HDL, high density lipoprotein; Hsp90, heat shock protein 90; iNOS, inducible nitric oxide synthase; LCR, low capacity runner; LDL, low density lipoprotein; NADPH; nicotinamide-adenine dinucleotide phosphate; nNOS; neuronal nitric oxide synthase; NO, nitric oxide; O_2^- , super oxide anion; pAkt, phosphorylated Akt; peNOS, phosphorylated endothelial nitric oxide synthase; PI3K; phosphatidylinositol 3-kinase; ROS; reactive oxygen species; SOD, superoxide dismutase; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor; $\text{VO}_{2\text{max}}$, maximal oxygen uptake

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