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The Effect of Exercise Training on Endothelial Function in Cardiovascular Disease in Humans

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WALTHER, C., S. GIELEN, and R. HAMBRECHT. The effect of exercise training on endothelial function in cardiovascular disease in humans. *Exerc. Sport Sci. Rev.*, Vol. 32, No. 4, pp. 129–134, 2004. *Endothelial dysfunction occurs early in atherosclerosis in response to cardiovascular risk factors. The occurrence of endothelial dysfunction is primarily the result of reduced nitric oxide (NO) bioavailabilty. It represents an independent predictor of cardiovascular events and predicts the prognosis of the patient. Therefore, endothelial function has been identified as a target for therapeutic intervention. Regular exercise training is a nonpharmacological option to improve endothelial dysfunction in patients with cardiovascular disease by increasing NO bioavailability.*
Key Words: Exercise training, endothelial function, independent predictor, cardiovascular disease, NO bioavailability.

INTRODUCTION

The endothelium is a unique structure building up the inner layer of the vasculature, thus forming an interface between circulating blood and the various organ systems. In contrast to previous concepts the endothelium is not just a passive interior lining of the blood vessels but a vital dynamic tissue involved in many other active functions, such as secretion and modification of vasoactive substances or participation in the process of contraction and relaxation of vascular smooth muscle.

Alterations in coronary vascular endothelial function were first described in 1986 when Ludmer *et al.* (10) observed a paradoxical vasoconstriction of atherosclerotic segments after infusing acetylcholine into the left coronary artery of patients with atypical chest pain. This endothelial dysfunction can be observed in different clinical contexts: 1) in the presence of coronary risk factors, such as hypertension, hypercholesterolemia, cigarette smoking, diabetes mellitus and obesity; 2) in manifest coronary artery disease (CAD); 3) in the presence of generalized inflammatory response like sepsis; and 4) in chronic heart failure (Fig. 1). But cardiovascular disease is not the only cause of endothelial dysfunction; aging also can lead to the impairment of endothelial function by different mechanisms. Aging is associated with increased expression of proinflammatory cytokines in the vascular wall (like IL-1, IL-6 and TNF-α), leading to induction of ROS-producing enzymes like NADPH-oxidases. As a consequence, NO-bioavailabilty decreases gradually and a proatherogenic phenotype with endothelial dysfunction develops. In the presence of additional factors (cardiovascular risk factors and physical inactivity), the development of endothelial dysfunction is accelerated and overt atherosclerosis ensues (Fig. 2).

Endothelial dysfunction represents one of the earliest events in the pathogenesis of cardiovascular disease (13). Thus, the preservation of endothelial function should be a major therapeutic goal. Over the last decade the specific effect of physical exercise on endothelial function has been the major focus of a number of research projects. The following overview of endothelial function and its clinical, functional and molecular aspects will analyze the specific effects of exercise training and vascular shear stress.

MEASUREMENT OF ENDOTHELIAL FUNCTION

The endothelium has the ability to individually regulate blood flow in response to various pharmacological agonists (like acetylcholine infusion) or mechanical stimuli (like shear stress). In 1980, Furchgott and Zawadzki (2) discovered the obligatory role of the endothelium in arterial relaxation in response to the administration of acetylcholine. Acetylcholine releases NO from endothelial cells by binding to muscarinergic receptors. In the presence of endothelial

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Accepted for publication: July 16, 2004.

0091-6331/3204/129–134
Exercise and Sport Sciences Reviews

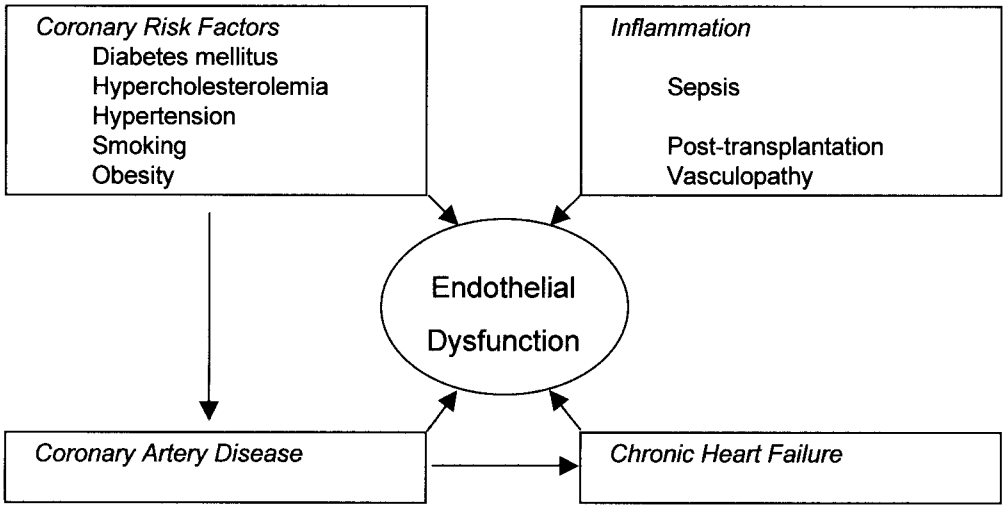

Figure 1. This chart represents four major causes of endothelial dysfunction and their interrelations. (Reprinted from Gielen, S., and R.Hambrecht. Effects of exercise training on vascular function and myocardial perfusion. Exercise in secondary prevention and cardiac rehabilitation. *Cardiol. Clin.* 19:357–368, 2001. Copyright © 2001 Elsevier Science. Used with permission.)

dysfunction, acetylcholine fails to cause any significant vasodilation when infused into the affected vessel. In fact, when the endothelial layer is completely destroyed, acetylcholine may even induce paradoxical vasoconstriction by binding to cholinergic receptors on the surface of smooth muscle cells (10).

Endothelial function can be assessed with a variety of invasive and noninvasive methods. Assays measuring NO activity as an indirect surrogate marker of endothelial function in plasma and urine exist, but are heavily affected by dietary habits. Therefore, a direct measure of endothelial function is preferred. Noninvasive or minimally invasive measurements of endothelial function consist of forearm plethysmography and brachial artery ultrasound scanning to assess increased flow in response to NO stimuli. There is some concern that forearm plethysmographic measurements exhibit considerable day-to-day variability, potentially limiting their use in long-term studies. Therefore the most widely used technique for noninvasive assessment of endothelial function is brachial ultrasound scanning. With this method the ability of the endothelium to dilate in response to shear-induced increase in NO is tested. Brachial artery diameter and flow velocity are measured at baseline and after reactive hyperemia produced by upper or lower arm occlusion. Healthy arteries typically show an increase in diameter in the range of 5% to 15%; brachial artery flow generally increases five- to six-fold. However, this noninvasive technique comprises some technique- and patient-related limitations (specially trained technicians are necessary, image timing can be difficult, the location of occlusion cuff may affect measurements, and the intima can be difficult to visualize).

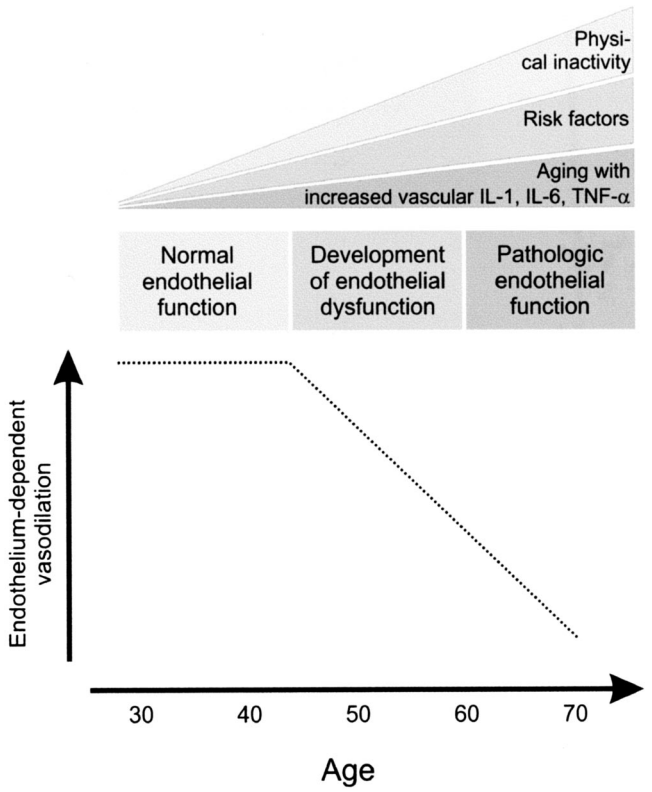

Figure 2. Coherence between aging and the development of endothelial dysfunction in the presence of cardiovascular risk factors.

Quantitative coronary angiography (QCA) is the most invasive technique for assessing endothelial function, and probably the most accurate. Measurement of vessel diameter by QCA and coronary blood flow by intravascular ultrasound directly reflects endothelial function. Because it was used in most of the studies discussed here, this method will be explained in more detail.

For QCA, angiographic images are digitized, and automatic edge detection algorithms are used to determine internal vascular diameter. To measure coronary blood flow velocity, a 0.014-inch intracoronary Doppler flow velocity guidewire, with a 12-MHz pulsed Doppler ultrasound crystal at the tip, is positioned approximately 1 cm distal to the end of the infusion catheter, close to an anatomic landmark, to continuously record maximum and average peak velocity

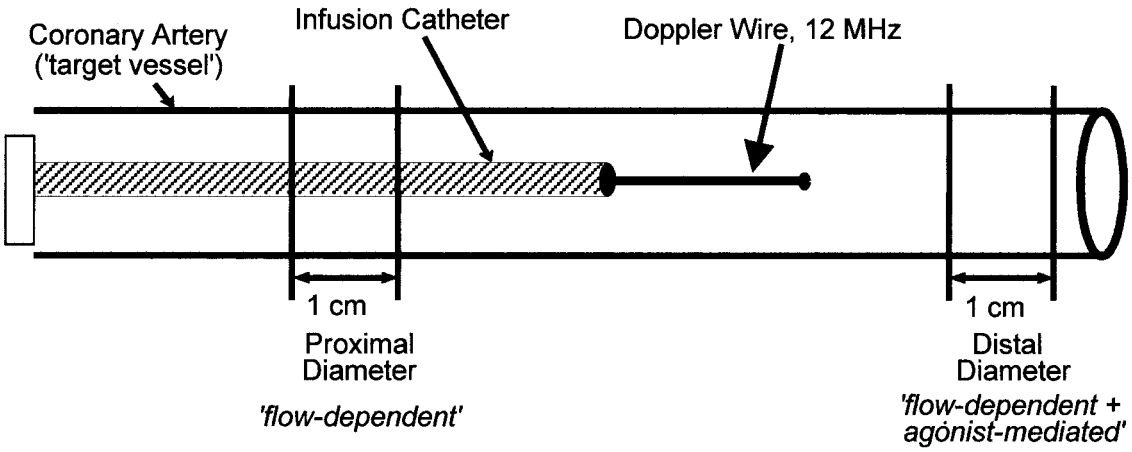

Figure 3. Method for measurement of coronary endothelial function *in vivo*. A 2.5 F infusion catheter is introduced into the target vessel over a 0.014-inch Doppler guidewire. Flow-dependent vasodilation can be determined proximal to the infusion site, whereas the sum effect of agonist + flow dependent dilation is assessed distal to the infusion catheter. (Reprinted from Gielen, S., G. Schuler, and R. Hambrecht. Exercise training and clinical events in coronary artery disease: role of the coronary endothelium. *J. Ir. Coll. Physicians Surg.* 31:158–162, 2002. Copyright © 2002 Royal College of Surgeons of Edinburgh. Used with permission.)

(Fig. 3). Acetylcholine is then infused in ascending concentrations to measure endothelium-dependent vasodilation.

PROGNOSTIC IMPLICATIONS OF ENDOTHELIAL FUNCTION

Over the past few years the prognostic importance of endothelial function has grown increasingly clear. Several groups have recently addressed the important question of whether endothelial dependent vasomotion provides prognostic information in humans.

Suwaidi (15) and coworkers measured endothelial function in the coronary vessel, monitoring 157 patients with moderate nonstenotic CAD for an average of 28 months. Only patients showing the lowest tertile of coronary responses to acetylcholine experienced cardiovascular events. Schächinger et al. (14) found that impaired endothelial-dependent and endothelium-independent coronary vasoreactivity were associated with a significantly higher incidence of cardiovascular events over a 7.7-yr period. Similar findings by Halcox et al. (4) identified epicardial and microvascular coronary endothelium-dependent vasodilator function as independent predictors of cardiovascular events (11).

In addition to the prognostic impact of coronary endothelial function on cardiovascular events, peripheral vascular function may also serve as a prognostic marker. Of the eight clinical studies that addressed this question, the majority demonstrated that peripheral endothelial-dependent vasodilation, measured in response to acetylcholine or as flow-dependent vasodilation, has profound and independent prognostic implications, at least in patients with underlying coronary artery disease or cardiovascular risk factors (11).

EXERCISE TRAINING AND ENDOTHELIAL FUNCTION—CLINICAL DATA

With the growing knowledge of the importance of endothelial function, the endothelium has become a major target for therapeutic interventions. Apart from pharmacological interventions with ACE inhibitors and statins, exercise training has evolved as an accepted therapy to improve endothelial function. A number of studies have been performed on animals and humans with respect to exercise training and its effects on endothelial function in different clinical contexts, especially in the presence of cardiovascular risk factors. It could be demonstrated that, even with most of these risk factors (hypertension, diabetes, smoking, obesity), regular exercise training improves endothelial dysfunction. Similar effects were observed in heart transplant recipients with post transplantation vasculopathy. However, the main concern of this review is endothelial dysfunction in cardiovascular disease.

Effects of Exercise Training in Patients with Coronary Artery Disease

In a prospective clinical study, the effect of intensive exercise training on endothelium-dependent vasodilation of coronary conduit and resistance vessels was investigated in patients with CAD (6). Nineteen patients with coronary endothelial dysfunction, as documented by acetylcholine-induced coronary vasoconstriction, were prospectively randomized to a training group (10 patients) or to a control group (9 patients). At baseline both groups had similar constrictive responses to acetylcholine. After 4 wk of intensive physical training, acetylcholine-induced coronary artery constriction was significantly reduced by 54% from -0.41 ± 0.05 mm to -0.19 ± 0.07 mm ($P < 0.05$). Coronary flow velocity increased by $142 \pm 28\%$ ($P < 0.01$ vs control) in the target vessel during acetylcholine infusion at 7.3 μg/min, indicating a remarkable improvement of acetylcholine-induced endothelial NO release. Coronary flow reserve (CFR) as an indicator of intramyocardial resistance vessel function was measured after application of adenosine. CFR increased by 29% after 4 wk of exercise training, whereas it remained virtually unchanged in the control group ($P < 0.01$).

These results showed, for the first time, that exercise training leads to an attenuation of paradoxical vasoconstriction

and an improvement in endothelial function in patients with stable coronary artery disease.

As exercise interventions have become good clinical practice in patients with manifest ischemic heart disease, with documented positive effects on physical performance, angina threshold, and myocardial perfusion, we continued to compare exercise training as a first-line treatment strategy with percutaneous coronary intervention (PCI; *e.g.*, balloon angioplasty and stentimplantation) in patients with stable coronary artery disease. In this recently published trial, 101 male patients with stable CAD, at least one coronary artery stenosis amenable for PCI, and no prognostic indication for invasive treatment were included. The patients were randomly assigned to either 12 months of exercise training ($N = 51$) or to PCI ($N = 50$). Exercise training was associated with a higher event-free survival (88% vs 70% in the PCI group, $P = 0.023$) (Fig. 4) and increased maximal oxygen uptake by 16% (from 22.7 ± 0.7 to 26.2 ± 0.8 $mL \cdot kg^{-1} \cdot min^{-1}$, $P < 0.001$ vs baseline and vs PCI after 12 months) at lower costs (8). This study adds an important piece of evidence to the rationale for exercise training in patients with stable CAD: it documents that an optimized medical therapy, along with exercise training as a lifestyle intervention, can be an alternative approach to an interventional strategy in selected motivated patients with stable CAD.

Effects Of Exercise Training in Patients With Chronic Heart Failure

Exercise also has a profound impact on peripheral endothelial dysfunction in patients with chronic heart failure. In several studies it has been demonstrated that exercise enhances endothelium-dependent peripheral vasodilation. The close correlation between the increase in endothelium-dependent dilation and the change in peak oxygen uptake ($\dot{V}O_2max$) ($r = 0.64$; $P < 0.005$) suggests a causal relationship between the correction of peripheral endothelial dysfunction and the improvement of functional work capacity.

In addition to improved endothelial function, exercise training has beneficial effects on hemodynamic parameters in chronic heart failure. After 6 months of regular aerobic training program, a small but significant improvement of left ventricular ejection fraction was observed (0.35 ± 0.09 vs 0.30 ± 0.08 at baseline, $P = 0.003$), accompanied by a reduction in left ventricular end-diastolic diameter (-4 ± 6 mm in the training group versus +1 ± 4 mm in the control group, $P < 0.001$) (5).

Is this beneficial effect on cardiac function an intrinsic effect of exercise, or is it secondary to changes in heart rate or peripheral resistance? In fact, resting heart rate declined by an average of 8 bpm in the training group. Both total peripheral resistance (TPR) (TPR) and pulmonary resistance declined in training patients, whereas they increased in the control group. The changes in TPR were inversely related to stroke volume at rest and during peak exercise, indicating that improved cardiac function may at least partially be interpreted as secondary to the afterload reduction caused by improved endothelium-dependent peripheral vasodilation.

The effects of exercise training on afterload reduction by improved endothelial function can be compared to the effects of established pharmacological therapies (*e.g.*, ACE inhibition). Thus it is not surprising that long-term studies of training in patients with chronic heart failure documented beneficial effects on exercise capacity, quality of life, and mortality.

MOLECULAR BIOLOGY OF ENDOTHELIAL FUNCTION/DYSFUNCTION

How can these clinical observations and functional changes be explained on the molecular level? With the production of multiple vasoactive substances, the normal endothelium modulates the tone of the underlying vascular smooth muscle. These substances include endothelium-derived relaxing factors such as prostacyclin (PG1 (2)), nitric oxide (NO), and endothelium-derived hyperpolarizing factor (EDHF), and vasoconstrictors such as endothelin-1 and angiotensin II. NO acts mostly through an elevation of cyclic guanosine monophosphate in vascular smooth muscle, whereas prostacyclin stimulates adenylate cyclase. The mode

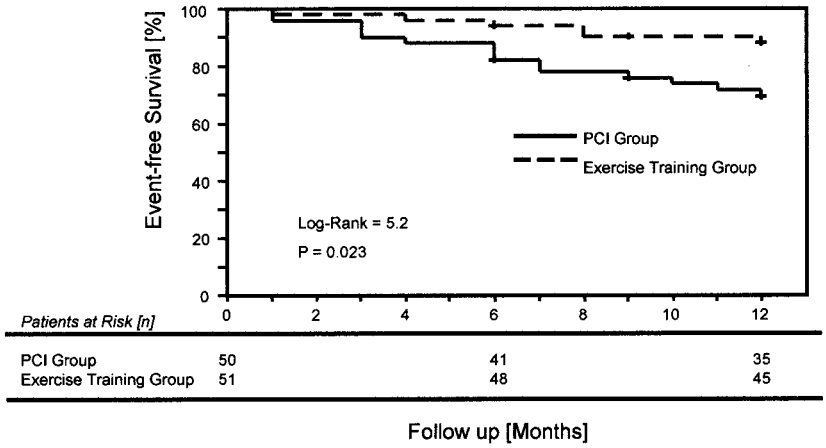

Figure 4. Event-free survival after 12 months was significantly superior in the exercise training group versus PCI group ($P = 0.023$ by log-rank test). (Reprinted Hambrecht, R., C. Walther, S. Mobius-Winkler, S. Gielen, A. Linke, K. Conradi, S. Erbs, R. Kluge, K. Kendziorra, O. Sabri, P. Sick, G. Schuler. Percutaneous coronary angioplasty compared with exercise training in patients with stable coronary artery disease. *Circulation* 109: 1371–1378, 2004. Copyright © 2004 Lippincott Williams & Wilkins. Used with permission.)

of action of EDHF involves the activation of K+ channels. The multiplicity of the factors released by the endothelium, as well as the complexity of the interactions among these factors and those with other nonendothelial mediators, determine the extent of vasomotor control exerted locally by the endothelium. However, the L-arginine-nitric oxide (NO) pathway is still thought to be the most important enzymatic vasodilator source. Therefore, this section will focus primarily on the vasoactive substance NO.

The amount of NO available depends on several key factors involved in the NO pathway: 1) the availability of the NO precursor molecule L-arginine (12); 2) the activity of the endothelial nitric oxide synthase (eNOS), which is influenced by expression levels, posttranslational modifications, phosphorylation status of the enzyme, and genetic polymorphism; and 3) NO degradation, which depends on the intrinsic half life and the reaction with reactice oxygen species (ROS) (Fig. 5) (9).

L-Arginine

The availability of L-arginine, the substrate for the eNOS, depends on several factors: 1) L-arginine intake with food and endogenous synthesis, 2) intracellular storage and degradation of L-arginine, and 3) the presence of asymmetrical dimethyl arginine (ADMA), which works as a competitive inhibitor reducing eNOS activity.

Regulation of eNOS Activity

The rate at which NO is synthesized by eNOS depends on the expression of eNOS and the functional activity of the eNOS enzyme. In atherosclerosis, eNOS expression is significantly attenuated. This downgrade may be an important factor for the pathogenesis of endothelial dysfunction. Exposure to high levels of TNF-α, oxidized low density lipoprotein (LDL), and hypoxia have been shown to lower eNOS expression in cultured endothelial cells.

eNOS activity may be altered *constitutively* (*i.e.*, as a result of mutations/polymorphisms in the eNOS gene) or *reversibly* (*i.e.*, by posttranslational modifications). The posttranslational regulation of eNOS activity is highly complex, and depends on intracellular targeting of the enzyme to plasma membranes, on its binding with the calcium/calmodulin complex, and on its phosphorylation state.

Various genetic polymorphisms of the eNOS gene have been reported as susceptibility genes in a number of cardiovascular diseases. However, whether polymorphisms of eNOS result in alterations of enzyme activity remains controversial.

Nitric Oxide Breakdown

Endothelial dysfunction has been associated with increased extracellular degradation of secreted NO in the presence of reactive oxygen species by formation of peroxynitrite. NADPH oxidases localized in the adventitia produce superoxide in quantities high enough to affect endothelial function. This mechanism has been confirmed in CAD in intervention studies with antioxidants (3). However, the underlying mechanism is more complex. Vascular smooth muscle cells produce and release a potent antioxidative enzyme, the extracellular superoxid dismutase (ecSOD). It has been postulated that endothelium-derived NO stimulates the expression and release of ecSOD from vascular smooth muscle cells. It has been confirmed that atherosclerosis is associated with increased levels of ROS. It may be that despite constant or even slightly increased SOD, the prevalence of large amounts of ROS in atherosclerosis leads to a mismatch between oxidative stress and antioxidative enzyme capacity, resulting in decreased NO half-life.

EXERCISE TRAINING AND ENDOTHELIAL FUNCTION—MOLECULAR DATA

Many studies have investigated the effects of mechanical stimulation of endothelium by shear stress (*e.g.*, exercise training). Experimental and animal studies have demonstrated a significant increase in eNOS expression after exposure to shear stress.

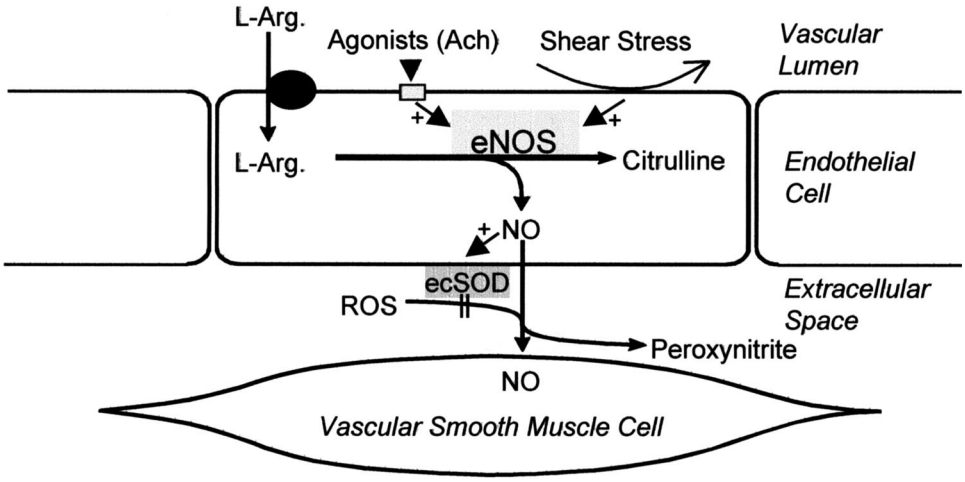

Figure 5. The basic pathways of L-arginine (L-Arg.) transport, endothelial nitric oxide (NO) generation, and extracellular NO breakdown. Ach, Acetylcholine; eNOS, endothelial NO synthase; ecSOD, extracellular superoxide dismutase; ROS, reactive oxidative species. (Reprinted from Gielen, S., and R.Hambrecht. Effects of exercise training on vascular function and myocardial perfusion. Exercise in secondary prevention and cardiac rehabilitation. *Cardiol. Clin.* 19:357–368, 2001. Copyright © 2001 Elsevier Science. Used with permission.)

Recently, these molecular changes after exercise training were confirmed for the first time in the human vascular system (7). Patients with stable coronary artery disease scheduled for CABG were included in this study, and the left internal mammarial artery (LIMA) was used as a target vessel for both functional *in vivo* analyses and *ex vivo* molecular studies. 35 male patients were randomly assigned to a training group (17 patients) or a sedentary control group (18 patients). Patients of the training group stayed in the hospital for 4 wk and exercised under close supervision 6 times daily for 10 min on a bicycle and row ergometer. Invasive measurement of endothelial function of the LIMA was assessed initially and after 4 wk of exercise training. Patients of the training group showed significantly improved endothelial function: acetylcholine led to a significantly greater increase in LIMA mean peak blood flow velocity compared with baseline by 56 ± 8%. Along with this functional improvement, a two-fold increase in eNOS mRNA expression and a 3.2-fold increase of eNOS phosphorylation on the Ser^{1177} residue was observed. There were neither significant functional nor molecular changes observed in the control group.

Therefore exercise training seems to have a dual positive effect: it increases the protein expression of eNOS, which may take a couple of hours, and it enhances the phosphorylation of eNOS at Ser^{1177} within minutes of shear stress application, thereby leading to increased vascular NO production. The correlation ($r = 0.59$, $P < 0.05$) between the proportion of eNOS phosphorylated on Ser^{1177} and the change of endothelium-dependent blood flow induced by exercise training is consistent with the hypothesis that the increase in eNOS protein expression and phosphorylation leads to improved endothelium function in patients with CAD.

Besides increasing eNOS expression and phosphorylation, exercise training can also positively influence the NO half life by reducing NO degradation. Exercise training led to an increase in both eNOS and ecSOD in wild-type mice, whereas ecSOD remained unchanged in mice lacking eNOS, suggesting that the effect of training on ecSOD is mediated via endothelium-derived NO. Since ecSOD leads to superoxide detoxification, extracellular degradation of NO is effectively reduced (1).

In summary, exercise training leads, through repetitive increase of laminar shear stress, to an increase of NO-bioavailability. This is a result of an increased NO-production and a reduced NO-inactivation through ROS.

CONCLUSION

Exercise training has previously been applied empirically in rehabilitation programs. Today, however, we know that regular physical exercise not only improves clinical symptoms, it also affects the progression of atherosclerosis. These effects are at least partially due to improved endothelial dysfunction through increased NO bioavailability. The increasing knowledge about the role of endothelial dysfunction for cardiac events, and the extent to which training can improve coronary vasomotion, should promote training therapy in coronary artery disease from a symptomatic to a prognostically relevant intervention.

References

1. Fukai, T., M.R. Siegfried, M. Ushio-Fukai, Y. Cheng, G. Kojda, D.G. Harrison. Regulation of the vascular extracellular superoxide dismutase by nitric oxide and exercise training. *J. Clin. Invest.* 105:1631–1639, 2000.
2. Furchgott, R.F., J.V. Zawadzki. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 299:373–376, 1980.
3. Gielen, S., G. Schuler, R. Hambrecht. Exercise Training in Coronary Artery Disease and Coronary Vasomotion. *Circulation* 103:e1-e6, 2001.
4. Halcox, J.P., W.H. Schenke, G. Zalos, R. Mincemoyer, A. Prasad, M.A. Waclawiw, K.R.A. Nour, A.A. Quyyumi. Prognostic value of coronary vascular endothelial dysfunction. *Circulation* 106:653–658, 2002.
5. Hambrecht, R., S. Gielen, A. Linke, E. Fiehn, J. Yu, C. Walther, N. Schoene, G. Schuler. Effects of exercise training on left ventricular function and peripheral resistance in patients with chronic heart failure. A randomized trial. *JAMA* 283:3095–3101, 2000.
6. Hambrecht, R., A. Wolf, S. Gielen, A. Linke, J. Hofer, S. Erbs, N. Schoene, G. Schuler. Effect of exercise on coronary endothelial function in patients with coronary artery disease. *N. Engl. J. Med.* 342:454–460, 2000.
7. Hambrecht, R., V. Adams, S. Erbs, A. Linke, N. Krankel, Y. Shu, Y. Baither, S. Gielen, H. Thiele, J.F. Gummert, F.W. Mohr, G. Schuler. Regular physical activity improves endothelial function in patients with coronary artery disease by increasing phosphorylation of endothelial nitric oxide synthase. *Circulation* 107:3152–3158, 2003.
8. Hambrecht, R., C. Walther, S. Mobius-Winkler, S. Gielen, A. Linke, K. Conradi, S. Erbs, R. Kluge, K. Kendziorra, O. Sabri, P. Sick, G. Schuler. Percutaneous coronary angioplasty compaired with exercise training in patients with stable coronary artery disease: A randomised trial. *Circulation* 109:1371–1378, 2004.
9. Harrison, D.G. Cellular and molecular mechanisms of endothelial cell dysfunction. *J. Clin. Invest.* 100:2153–7, 1997.
10. Ludmer, P.L., A.P. Selwyn, T.L. Shook, R.R. Wayne, G.H. Mudge, R.W. Alexander, P. Ganz. Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. *N. Engl. J. Med.* 315:1046–1051, 1986.
11. Mancini, G.B. Vascular structure versus function: is endothelial dysfunction of independent prognostic importance or not? *J. Am. Coll. Cardiol.* 43:624–628, 2004.
12. Posch, K., K. Schmidt, W.F. Graier. Selective stimulation of L-arginine uptake contributes to shear stress-induced formation of nitric oxide. *Life Science* 64:663–70, 1999.
13. Ross, R. The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature* 362:801–809, 1993.
14. Schächinger, V., M.B. Britten, A. Zeiher. Prognostic impact of coronary vasodilator dysfunction on adverse longterm outcome of coronary artery disease. *Circulation* 101:1899–1906, 2000.
15. Suwaidi, J.A., S. Hamasaki, S.T. Higano, R.A. Nishimura, D.R. Holmes, Jr., A. Lerman. Longterm follow-up of patients with mild coronary artery disease and endothelial dysfunction. *Circulation* 101:948–954, 2000.