Importance of hemodynamic forces as signals for exercise-induced changes in endothelial cell phenotype

M. Harold Laughlin, Sean C. Newcomer, and Shawn B. Bender

Department of Biomedical Sciences, College of Veterinary Medicine, and Dalton Cardiovascular Research Center (DCRC), University of Missouri, Columbia, Missouri

Submitted 12 October 2007; accepted in final form 4 December 2007

Laughlin MH, Newcomer SC, Bender SB. Importance of hemodynamic forces as signals for exercise-induced changes in endothelial cell phenotype. J Appl Physiol 104: 588–600, 2008. First published December 6, 2007; doi:10.1152/japplphysiol.01096.2007.—Current evidence indicates that the ability of physical activity to sustain a normal phenotype of arterial endothelial cells (ECs) plays a central role in the beneficial effects of exercise (Ex) on atherosclerotic disease. Here we evaluate the strength of evidence that shear stress (SS) and/or circumferential wall stress (stretch) are the primary signals, produced by bouts of Ex, that signal altered gene expression in arterial ECs, thereby resulting in a less atherogenic EC phenotype. Current literature indicates that SS is a signal for expression of antiatherogenic genes in cultured ECs, in ECs of isolated arteries, and in ECs of arteries in intact animals. Furthermore, SS levels in the arteries of humans during Ex are in the range that produces beneficial changes. In contrast, complex flow profiles within recirculation zones and/or oscillatory flow patterns can cause proatherogenic gene expression in ECs. In vivo evidence indicates that Ex decreases oscillatory flow/SS in some portions of the arterial tree but may increase oscillatory flow in other areas of the arterial tree. Circumferential wall stress can increase expression of some beneficial EC genes as well, but circumferential wall stress also increases production of reactive oxygen species and increases the expression of adhesion factors and other proatherogenic genes. Interactions of arterial pressure and fluid SS play an important role in arterial vascular health and likely contribute to how Ex bouts signal changes in EC gene expression. It is also clear that other local and circulating factors interact with these hemodynamic signals during Ex to produce the healthy arterial EC phenotype. We conclude that available evidence suggests that exercise signals formation of beneficial endothelial cell phenotype at least in part through changes in SS and wall stretch in the arteries.

Atherosclerotic cardiovascular disease is responsible for over 19 million deaths annually worldwide and coronary artery disease (CAD) remains the leading cause of death among cardiovascular diseases (93). Physical inactivity is recognized as a risk factor for the development of atherosclerotic CAD, and exercise training programs have been shown to be effective in prevention and treatment of CAD (7, 26, 29, 34, 88, 89, 94, 95, 102, 105, 129). Given these facts, there is growing interest in determining the mechanisms whereby physical activity and exercise produce beneficial effects in prevention and treatment of atherosclerosis. The primary mechanisms that underlie the effectiveness of physical activity in prevention and/or treatment of CAD are now being examined on several fronts but have not been fully established. Current evidence indicates that the ability of physical activity to sustain a normal phenotype of arterial endothelial cells plays a central role in the beneficial effects of physical activity (47). The purpose of this paper is to evaluate the strength of evidence that shear stress on the artery wall and/or circumferential wall stress (stretch on the artery wall) (Fig. 1) are the primary signals, produced by bouts of exercise, that signal altered gene expression in arterial endothelial cells, thereby resulting in a less atherogenic endothelial cell phenotype. There are several excellent, recent reviews outlining the general and specific effects of shear stress and stretch on endothelial cells (16, 51, 115, 136). Here we focus on the importance of these two hemodynamic signals in exercise training effects on endothelial cell phenotype. Available literature indicates that endothelial cell phenotype in vivo is determined via an intricate orchestration of many signals that interact with shear stress and cyclic strain in determining endothelial cell gene expression. The central hypothesis of our discussion is that shear stress and stretch, produced by exercise, modulate endothelial cell phenotype in a net antiatherogenic manner.

Before discussing the effects of these hemodynamic forces on endothelial cell phenotype, we first summarize evidence demonstrating the importance of normal endothelial phenotype in artery health and of changes in endothelial cell phenotype associated with development and progression of atherosclerosis. Next, we review evidence that the beneficial effects of

Address for reprint requests and other correspondence: M. H. Laughlin, Dept. of Biomedical Sciences, W102 Veterinary Medicine, 1600 E. Rollins Rd., Univ. of Missouri, Columbia, MO 65211 (e-mail: laughlinm@missouri.edu).
physical activity on atherosclerosis and CAD are the result of exercise training-induced antiatherogenic endothelial cell phenotype. With this background we then discuss evidence in the literature that supports the hypothesis that shear stress and/or circumferential wall stress (stretch) are important signals responsible for exercise-induced expression of antiatherogenic genes in endothelial cells. First we discuss evidence that shear stress can signal antiatherogenic gene expression in cultured endothelial cells. We then summarize evidence indicating that in vivo shear stresses produced in the human arterial tree during exercise are in the range known to cause these beneficial effects on gene expression in endothelial cells. We also discuss how recirculating flow patterns and oscillatory flow can have deleterious effects on endothelial cell phenotype. After the discussion of shear stress we consider evidence that circumferential stress on the walls of arteries can signal antiatherogenic gene expression in arterial endothelial cells. Finally, we evaluate evidence that the interaction of these hemodynamic signals, as occurs in vivo, is critical in producing the antiatherogenic phenotype in endothelium of arteries of physically active subjects.

Importance of Endothelial Cell Phenotype in Artery Health

It is generally accepted that an early event in the pathogenesis of atherosclerosis is endothelial cell dysfunction. Endothelial cell dysfunction can be characterized by one or more of the following features: 1) reduced endothelium-mediated vasodilation, 2) enhanced endothelial cell turnover, 3) increased expression of adhesion molecules and other inflammatory genes of endothelial cells, 4) increased oxidant stress, and 5) increased permeability characteristics of the endothelial barrier. These changes in endothelial function represent the change in endothelial cell phenotype that, in this paper, we will refer to as proatherogenic phenotype.

Current evidence indicates that endothelial dysfunction is present prior to and throughout all stages of atherosclerosis, suggesting that the change of endothelial cell phenotype from normal to proatherogenic plays a key role in initiating atherosclerosis and in the development of atherosclerosis (20, 27, 32, 77, 130–132). Indeed, it has been proposed that decreased endothelium-dependent dilation and/or nitric oxide (NO) bioavailability are key components of the proatherogenic endothelial cell phenotype as one develops this disease. The progressive atherosclerotic process involving endothelial cell-leukocyte adhesion and transmigration, vessel wall inflammation, lipid accumulation/foam cell formation, and conversion to the synthetic smooth muscle cell phenotype has been reviewed in detail previously (26, 35, 74, 93, 117, 118). Accumulating evidence demonstrates, however, that the progression of atherosclerosis and CAD can be slowed, stopped, or even reversed by various interventions, including regular physical activity, and that these effects are accompanied by improved endothelium-dependent vasodilation and/or NO bioavailability (48, 117, 118).

Several studies have examined the effects of exercise training in patients with advanced atherosclerotic CAD (37, 38, 47, 49, 101). For example, Hambrecht and colleagues (49) reported that 4 wk of exercise training resulted in improved endothelium-dependent dilation in conduit arteries (Fig. 2A) and resistance arteries/arterioles (Fig. 2B) in CAD patients. In conduit arteries intracoronary infusion of acetylcholine (ACh) produced less constriction after 4 wk of exercise training and exercise training increased ACh-induced dilation in the coronary microcirculation as shown by measures of total coronary blood flow obtained with a Doppler wire in the coronary artery (Fig. 2B) (49). Lifestyle changes, including increased physical activity, have also been reported to decrease the rate of progression of CAD and/or reverse CAD in patients (38, 48, 101). In summary, research over the past few years provides substantial and increasing evidence that these beneficial effects of exercise are the result of effects of exercise on endothelial cells (75, 80, 90).

Exercise Training Produces Antiatherogenic Endothelial Cell Phenotype in Atherosclerotic Arteries

The balance of proatherogenic and antiatherogenic factors in artery tissue determine whether or not areas of lipid-laden foam cells in the intima of the artery develop into atherosclerotic lesions (35, 75, 117, 118). As outlined above, the proatherogenic phenotype in arterial endothelial cells is characterized by reduced endothelium-dependent dilation (i.e., endothelial dysfunction), decreased expression of enzymes involved in endothelial cell signaling pathways (i.e., pathways for producing NO, endothelium-derived hyperpolarizing factor (EDHF), and prostacyclin), increased expression of adhesion factors, inflammatory mediators, increased oxidative stress, and increased expression of other atherogenic genes (51, 75, 131, 132). Hypercholesterolemia has been reported to cause signs of proatherogenic endothelial cell phenotype (decreased endothelium-dependent relaxation) in conduit arteries of humans (8, 9), monkeys (52, 76), and pigs (6, 17, 32, 113, 124, 139, 140). In addition, the established risk factors (i.e., hypertension, diabetes, smoking, obesity, etc.) also cause signs of proatherogenic endothelial cell phenotype and decreased endothelium dependent relaxation (51, 75, 131, 132). A growing body of evidence indicates that disruption of the NO synthase (NOS) pathway (26, 86, 95) and/or reduced bioavailability of NO are important components of the proatherogenic endothelial cell phenotype (75, 131, 132).

Sustained physical activity has been shown to improve or maintain normal endothelial function in peripheral arteries (57, 68, 80, 84, 90, 119, 140) and coronary arteries (91, 104, 134) and to have similar effects in the presence of vascular disease (47, 124, 139). This improved endothelial function produced by exercise appears to be partially the result of changes in

Fig. 1. Mechanical effects of hemodynamic forces on the vascular wall. Figure is adapted from and used with permission of Dancu et al. (19).
regulation of the endothelial NOS (eNOS) pathway (68, 71, 73, 91, 139) that involve increased expression of eNOS protein (47, 72) and activation by phosphorylation of eNOS serine 1177 by Akt (47). It is important to emphasize that in the key studies of Hambrecht and colleagues (47, 49) as well as studies in experimental animals (68, 71, 73, 91, 139) outlined above, the increased expression and activity of eNOS was associated with improved endothelial function. Other signs of an antiatherogenic endothelial cell phenotype induced by increased physical activity include increased expression and activity of superoxide dismutases (SOD) (31, 110, 111) and of catalase (40) and decreased oxidative stress (75). These changes would serve to increase NO bioavailability by reducing reactive oxygen species (ROS) that catabolize NO to peroxynitrite leading to the formation of nitrotyrosine (51, 75, 110). Work examining the effects of exercise training on the development of atherosclerosis in rabbit aortas demonstrated that exercise training reduces atherosclerosis and improves endothelial function and phenotype as shown by decreased expression of adhesion molecules (e.g., P-selectin, VCAM-1) as well as inflammatory markers such as MCP-1 (16) and inducible NOS (iNOS) (143, 144). The opposing effects of exercise and hypercholesterolemia on eNOS expression, NO bioavailability, and endothelial function in arteries are consistent with the hypothesis that exercise has its beneficial effects through modulation of endothelial gene expression.

Thus there is substantial epidemiological and clinical evidence indicating that exercise training has beneficial effects in prevention and treatment of CAD (75) and, as discussed above, that these effects are associated with changes in endothelial cell phenotype. As shown in Fig. 3, we interpret currently available data as support for the hypothesis that the effects of exercise on expression of endothelial genes making the cells antiatherogenic (reflected as improvement of endothelial phenotype) represents a major mechanism whereby exercise limits or reverses atherosclerosis. The preponderance of evidence in this regard supports the widely held contention that the exercise related signal for antiatherogenic endothelial gene expression is increased shear stress produced by the increased cardiac output and regional blood flows required to provide oxygen to active cardiac and skeletal muscle during exercise (75). Locomotory exercise at intensities that produce maximal levels of oxygen consumption is associated with dramatic stress on the cardiovascular system. The demands for muscle blood flow cause dramatic increases in cardiac output (5–6 l/min to 25 l/min) and increases in shear stress, produced by movement of blood along the walls of the arteries. Also, high-intensity exercise increases stretch on the artery walls associated with increases in systolic arterial pressures and increases in heart rate that alter the magnitude and frequency of systolic stretch. Because of the magnitude of these hemodynamic effects of exercise on the conduit arteries, many have proposed that the beneficial supports the widely held contention that the exercise related signal for antiatherogenic endothelial gene expression is increased shear stress produced by the increased cardiac output and regional blood flows required to provide oxygen to active cardiac and skeletal muscle during exercise (75). Locomotory exercise at intensities that produce maximal levels of oxygen consumption is associated with dramatic stress on the cardiovascular system. The demands for muscle blood flow cause dramatic increases in cardiac output (5–6 l/min to 25 l/min) and increases in shear stress, produced by movement of blood along the walls of the arteries. Also, high-intensity exercise increases stretch on the artery walls associated with increases in systolic arterial pressures and increases in heart rate that alter the magnitude and frequency of systolic stretch. Because of the magnitude of these hemodynamic effects of exercise on the conduit arteries, many have proposed that the beneficial supports the widely held contention that the exercise related signal for antiatherogenic endothelial gene expression is increased shear stress produced by the increased cardiac output and regional blood flows required to provide oxygen to active cardiac and skeletal muscle during exercise (75). Locomotory exercise at intensities that produce maximal levels of oxygen consumption is associated with dramatic stress on the cardiovascular system. The demands for muscle blood flow cause dramatic increases in cardiac output (5–6 l/min to 25 l/min) and increases in shear stress, produced by movement of blood along the walls of the arteries. Also, high-intensity exercise increases stretch on the artery walls associated with increases in systolic arterial pressures and increases in heart rate that alter the magnitude and frequency of systolic stretch. Because of the magnitude of these hemodynamic effects of exercise on the conduit arteries, many have proposed that the beneficial supports the widely held contention that the exercise related signal for antiatherogenic endothelial gene expression is increased shear stress produced by the increased cardiac output and regional blood flows required to provide oxygen to active cardiac and skeletal muscle during exercise (75). Locomotory exercise at intensities that produce maximal levels of oxygen consumption is associated with dramatic stress on the cardiovascular system. The demands for muscle blood flow cause dramatic increases in cardiac output (5–6 l/min to 25 l/min) and increases in shear stress, produced by movement of blood along the walls of the arteries. Also, high-intensity exercise increases stretch on the artery walls associated with increases in systolic arterial pressures and increases in heart rate that alter the magnitude and frequency of systolic stretch. Because of the magnitude of these hemodynamic effects of exercise on the conduit arteries, many have proposed that the beneficial supports the widely held contention that the exercise related signal for antiatherogenic endothelial gene expression is increased shear stress produced by the increased cardiac output and regional blood flows required to provide oxygen to active cardiac and skeletal muscle during exercise (75). Locomotory exercise at intensities that produce maximal levels of oxygen consumption is associated with dramatic stress on the cardiovascular system. The demands for muscle blood flow cause dramatic increases in cardiac output (5–6 l/min to 25 l/min) and increases in shear stress, produced by movement of blood along the walls of the arteries. Also, high-intensity exercise increases stretch on the artery walls associated with increases in systolic arterial pressures and increases in heart rate that alter the magnitude and frequency of systolic stretch. Because of the magnitude of these hemodynamic effects of exercise on the conduit arteries, many have proposed that the beneficial supports the widely held contention that the exercise related signal for antiatherogenic endothelial gene expression is increased shear stress produced by the increased cardiac output and regional blood flows required to provide oxygen to active cardiac and skeletal muscle during exercise (75). Locomotory exercise at intensities that produce maximal levels of oxygen consumption is associated with dramatic stress on the cardiovascular system. The demands for muscle blood flow cause dramatic increases in cardiac output (5–6 l/min to 25 l/min) and increases in shear stress, produced by movement of blood along the walls of the arteries. Also, high-intensity exercise increases stretch on the artery walls associated with increases in systolic arterial pressures and increases in heart rate that alter the magnitude and frequency of systolic stretch. Because of the magnitude of these hemodynamic effects of exercise on the conduit arteries, many have proposed that the beneficial supports the widely held contention that the exercise related signal for antiatherogenic endothelial gene expression is increased shear stress produced by the increased cardiac output and regional blood flows required to provide oxygen to active cardiac and skeletal muscle during exercise (75). Locomotory exercise at intensities that produce maximal levels of oxygen consumption is associated with dramatic stress on the cardiovascular system. The demands for muscle blood flow cause dramatic increases in cardiac output (5–6 l/min to 25 l/min) and increases in shear stress, produced by movement of blood along the walls of the arteries. Also, high-intensity exercise increases stretch on the artery walls associated with increases in systolic arterial pressures and increases in heart rate that alter the magnitude and frequency of systolic stretch. Because of the magnitude of these hemodynamic effects of exercise on the conduit arteries, many have proposed that the beneficial supports the widely held contention that the exercise related signal for antiatherogenic endothelial gene expression is increased shear stress produced by the increased cardiac output and regional blood flows required to provide oxygen to active cardiac and skeletal muscle during exercise (75). Locomotory exercise at intensities that produce maximal levels of oxygen consumption is associated with dramatic stress on the cardiovascular system. The demands for muscle blood flow cause dramatic increases in cardiac output (5–6 l/min to 25 l/min) and increases in shear stress, produced by movement of blood along the walls of the arteries. Also, high-intensity exercise increases stretch on the artery walls associated with increases in systolic arterial pressures and increases in heart rate that alter the magnitude and frequency of systolic stretch. Because of the magnitude of these hemodynamic effects of exercise on the conduit arteries, many have proposed that the beneficial supports the widely held contention that the exercise related signal for antiatherogenic endothelial gene expression is increased shear stress produced by the increased cardiac output and regional blood flows required to provide oxygen to active cardiac and skeletal muscle during exercise (75). Locomotory exercise at intensities that produce maximal levels of oxygen consumption is associated with dramatic stress on the cardiovascular system. The demands for muscle blood flow cause dramatic increases in cardiac output (5–6 l/min to 25 l/min) and increases in shear stress, produced by movement of blood along the walls of the arteries. Also, high-intensity exercise increases stretch on the artery walls associated with increases in systolic arterial pressures and increases in heart rate that alter the magnitude and frequency of systolic stretch. Because of the magnitude of these hemodynamic effects of exercise on the conduit arteries, many have proposed that the beneficial supports the widely held contention that the exercise related signal for antiatherogenic endothelial gene expression is increased shear stress produced by the increased cardiac output and regional blood flows required to provide oxygen to active cardiac and skeletal muscle during exercise (75). Locomotory exercise at intensities that produce maximal levels of oxygen consumption is associated with dramatic stress on the cardiovascular system. The demands for muscle blood flow cause dramatic increases in cardiac output (5–6 l/min to 25 l/min) and increases in shear stress, produced by movement of blood along the walls of the arteries. Also, high-intensity exercise increases stretch on the artery walls associated with increases in systolic arterial pressures and increases in heart rate that alter the magnitude and frequency of systolic stretch. Because of the magnitude of these hemodynamic effects of exercise on the conduit arteries, many have proposed that the beneficial supports the widely held contention that the exercise related signal for antiatherogenic endothelial gene expression is increased shear stress produced by the increased cardiac output and regional blood flows required to provide oxygen to active cardiac and skeletal muscle during exercise (75). Locomotory exercise at intensities that produce maximal levels of oxygen consumption is associated with dramatic stress on the cardiovascular system. The demands for muscle blood flow cause dramatic increases in cardiac output (5–6 l/min to 25 l/min) and increases in shear stress, produced by movement of blood along the walls of the arteries. Also, high-intensity exercise increases stretch on the artery walls associated with increases in systolic arterial pressures and increases in heart rate that alter the magnitude and frequency of systolic stretch. Because of the magnitude of these hemodynamic effects of exercise on the conduit arteries, many have proposed that the beneficial
effects of exercise on endothelial phenotype are the result of these hemodynamic, mechanical signals (36, 75).

**Shear Stress as a Signal for Altered Endothelial Cell Phenotype**

Regular exposure to increased “shear stress” on endothelial cells that results from increases in blood flow during bouts of exercise is considered by many investigators in this field to be the primary signal for exercise training-induced adaptations of endothelial function and phenotype (50, 87, 92, 112). Acute changes in shear stress on the wall of arteries are a well-known signal for flow-induced vasodilation (62, 65, 66, 109). Wall shear stress is increased by an increase in blood flow (Q) as described by the following equation: \( SS = 4\eta Q / \pi R_i^3 \) (where SS is shear stress, \( \eta \) is viscosity, and \( R_i \) is internal radius of the artery).

Accordingly, at a given vessel radius, when flow increases, shear stress at the vessel wall also increases (Fig. 1). Acutely, flow-induced dilation of the artery increases radius and thereby returns shear stress toward normal levels. It appears that substances that signal remodeling and altered phenotype of endothelial and coronary smooth muscle cells are also released in response to increased shear stress (50, 75, 77).

There is a growing body of evidence that increased shear stress is a signal for increased eNOS, decreased endothelin-1 (ET-1) and decreased VCAM-1 gene expression in endothelial cells. Early studies involving cultured endothelial cells suggested that this may underlie training-induced increases in endothelium-dependent vasodilation. In 1992, Harrison and colleagues (96) reported that increases in eNOS mRNA and eNOS protein were observed when cultured endothelial cells were exposed to 24 h of a sustained increase in shear stress. These investigators subsequently demonstrated that as little as 3 h of increased shear stress promoted increased eNOS gene transcription (125, 127, 128) and that eNOS mRNA expression exhibited a dose-response relationship with shear. These findings were later confirmed by other investigators (97, 107). Several intracellular signaling mechanisms have been identified that mediate increased transcription of the eNOS gene in endothelial cells when exposed to shear stress (51). These mechanisms include G proteins (82), Ca\(^{2+}\) (82), and c-Src (21, 22, 51). c-Src is a proto-oncogene product that acts as an intracellular signaling molecule via its tyrosine kinase activity. In addition to an increase in transcription of the eNOS gene, exercise training may lead to elevated eNOS mRNA levels via posttranscriptional mechanisms. One such mechanism is increased mRNA stability as shown in Fig. 4. Recent work by Harrison and colleagues (51) has shown that cultured endothelial cells exhibit increased poly(A) tail length in response to shear stress, inferring a prolonged half-life on eNOS mRNA.

Recent evidence obtained with chip array analysis indicates that “...mean shear stress significantly affects the expression of about 3,000 endothelial cell genes” (53). Space does not allow us to discuss all the endothelial cell genes for which shear stress has been shown to influence expression. For the remainder of this discussion we focus on expression of eNOS and SODs as markers of an antiatherogenic endothelial phenotype and on expression of ET-1, adhesion molecules (VCAM-1, ICAM-1, selectins), and ROS as markers of a proatherogenic endothelial cell phenotype. Shear stress has been demonstrated to signal increased expression of antioxidant genes (96, 97, 107), increased production of prostacyclin (30, 39, 147), and increased expression of COX-1 and PGI\(_2\) synthase (98) in cultured endothelial cells, in addition to increased expression of eNOS. Also, increased shear stress in isolated perfused coronary arterioles produced increased eNOS and SOD-1 expression, demonstrating that it is not just cultured endothelial cells that exhibit these effects of increased shear stress.
shear stress (138). There is limited evidence from in vivo experiments evaluating the effects of shear stress on endothelial phenotype. There is evidence that chronic increases in blood flow produced by arteriovenous fistulas in dogs result in increased endothelium dependent relaxation, decreased production of endothelin and altered vasomotor responses to endothelin (87). Also, in rats arteriovenous fistulas have been reported to cause increased eNOS activity, mRNA levels, and protein content (92). Although the results of these experiments support the hypothesis that shear stress may signal antiatherogenic endothelial cell phenotype in vivo, they did not evaluate the effect of shear stress on other anti- or proatherogenic genes.

As summarized above, shear stress has been shown to produce increases in eNOS expression in cultured endothelial cells, in a dose-related manner, across the range of shear stress from 4 to 15 dyn/cm² (128). Although it is clear that mean wall shear stress levels are not uniform in the arterial tree of mammals (11), recent results indicate that shear stress levels are generally in the range of 1 to 15 dyn/cm² in human arteries. Areas prone to atherosclerosis in the arterial circulation generally exhibit low shear stress that can be caused by low velocity of laminar flow due to low blood flow levels and/or secondary recirculating flow patterns (separation, reattachment, and recirculation) around branch points and distal to stenoses (136). During exercise both causes of low shear in arteries appear to be decreased. For example, MR imaging allows measurement of shear stress in human arteries at rest and during exercise (12, 25, 122) and indicate that at rest, shear stress is in the range of 1 to 2 dyn/cm² in the human abdominal aorta and increases to an average of 7 to 20 dyn/cm² during moderate exercise (122). Also, Cheng et al. (11) report that review of the literature indicates that in nonatherosclerotic human arteries mean wall shear stress levels are in the range of 2 to 15 dyn/cm² and that wall shear stress is inversely related to the diameter of the artery. Thus the magnitude of shear stress in humans during exercise spans the range of values that produce antiatherogenic endothelial gene expression in cultured cells, suggesting that shear stress could be an exercise-induced signal in humans and large mammals.

The effects of body size on cardiovascular hemodynamics should be considered as one evaluates available literature relative to the magnitude of shear stress produced in the arteries of the mammal. For example, in contrast to the values of shear stress in the aorta of humans outlined above, values of shear stress are much greater in the aortas of rats and mice. Greve et al. (45) report that shear stress values range between 60 and 120 dyn/cm² in rodent aortas. Also, Cheng et al. (11) report that average mean wall shear stress levels in the common carotid artery of small rodents (rat and mouse) are in the range of 50 to 70 dyn/cm² whereas larger mammals (human and dog) are in the range of 10 to 20 dyn/cm². Given these observations, exercise-induced changes in shear stress may have a relatively different effect on endothelium in rodents than in arteries of large mammals.

Considering effects of exercise on shear stress, it is important to remember that increases in blood flow do not necessarily produce increased shear stress if radius of the artery also increases. This may explain some reports in the literature of no increase in eNOS expression in some arteries after exercise training (58, 83, 99, 100, 103). Another important effect of exercise on shear stress may be that exercise can influence the frequency and magnitude of pulsations in flow (unidirectional) and/or oscillatory flow (ante- rgrade and retrograde flow) and resulting shear stresses. It is well known that under some circumstances coronary blood flow can be retrograde and that retrograde flow also occurs in other areas of the cardiovascular system (12, 13, 43, 122). Oscillatory shear stress, as occurs with retrograde and ante- grade flow, has been reported to produce the opposite effects on expression of many endothelial genes as does unidirectional shear stress (46). For example, unidirectional shear stress decreases expression of ET-1 (64, 81) and VCAM-1 (53) whereas oscillatory shear stress produces increased expression of ET-1 (147), increases expression of adhesion molecules (i.e., VCAM-1) (10, 53), decreases eNOS expression (even below zero shear stress levels) (55), increases expression of enzymes that produce ROS (i.e., NAPDH oxidase) (23, 55), and increases the release of superoxide (85). As summarized in Table 1, current literature clearly demonstrates that oscillatory shear stress produces an increased expression of proathero- genic genes in cultured endothelial cells and a decrease in antiatherogenic genes (51).

The effects of exercise on oscillatory flow and shear stress appear to be heterogeneous in different segments of the arterial tree. For example, in the human aorta, available information indicates that exercise decreases oscillatory flow and shear stress as total shear increases (122). Indeed, in human subjects, mild exercise was reported to abolish retrograde flow and shear in the supraceliac and infrarenal segments of the abdominal aorta (12). This is important because, whereas both oscillatory and steady shear stress stimulate increased NO production and eNOS expression in cultured cells, oscillatory shear stress produces substantial increases in the production of ROS whereas steady shear stress does not (51, 85). The effects of exercise on oscillatory flow in conduit arteries that perfuse active skeletal muscle are very complicated because exercise effects are dependent on interactions between the cardiac cycle and skeletal muscle contractions. For instance, Lutjemeier et al. (78) demonstrated that the amount of antegrade and retrograde blood flow during single-leg knee extension exercise was dependent on the point in the cardiac cycle where muscle contraction was initiated. Blood flow became oscillatory during contractions as the contraction would impede flow followed by augmented flow during skeletal muscle relaxation. As a result of these interactions of different combinations of timing of cardiac cycle and muscle contraction, blood flow patterns vary substantially during rhythmic exercise (78). Available evidence indicates that when going from rest to exercise antegrade flow increases to a greater extent than retrograde flow in arteries perfusing exercising skeletal muscle. The effects of exercise on oscillatory flow in conduit arteries that are not providing blood flow to exercising muscle tissue are also complex. For example, exercise has been reported to increase retrograde flow in the brachial arteries of humans performing leg exercise (42), in contrast to the decrease in oscillatory shear rate produced by exercise in the abdominal aorta.

Currently literature also suggests that oscillatory flow and shear stress may have different effects on endothelium in vivo than those observed in cell culture experiments (Table 1). For example, it has been reported that leg exercise produces in-
increased blood flow and increased contribution of NOS-generated NO release in brachial arteries of humans (42-44). Green et al. (44) demonstrated that leg cycling exercise produced oscillatory, antegrade/retrograde, blood flow patterns in the brachial artery and, importantly, that these conditions were associated with a larger contribution of NOS generated NO release from the brachial circulation. Green et al. concluded that these results may suggest an important role of oscillatory shear stress-mediated release of NO from the endothelium of the brachial circulation during leg exercise. On the basis of the literature summarized above, it is reasonable to propose that repetitive enhancement of NO production by brachial artery endothelial cells produced by leg exercise training would lead to an upregulation of eNOS expression, i.e., an antiatherogenic endothelial cell phenotype. However, at this time it is not possible to establish for sure that the increased NO production seen under these conditions in the arm vasculature is the result of the oscillatory shear, as proposed, or due to other stimuli in the arms during leg exercise, i.e., systemic effects of exercise on endothelial cell phenotype. Therefore, it is important that future work determine whether the increase in NO production in the arm, reported to occur during leg exercise, is evidence that oscillatory shear stress in the arm vasculature is a signaling mechanism underlying production of an antiatherogenic endothelial cell phenotype. More work is needed to understand these hemodynamic interactions and to pinpoint the impact of oscillatory flow and shear on endothelial cell phenotype in different arteries in vivo.

In summary, review of the current literature indicates that shear stress produces increased expression of antiatherogenic genes and decreased expression of proatherogenic genes in cultured endothelial cells and in endothelial cells of arteries in vivo. Also, the magnitudes of shear stress required for these alterations in gene expression are in the range of shear stress values reported in arteries during moderate levels of exercise in conscious humans. These observations indicate that shear stress during exercise is sufficient to induce antiatherogenic gene expression and are consistent with the hypothesis that shear stress is an important signal for exercise induced changes in endothelial cell gene expression. In cultured endothelial cells, oscillatory shear stress produces proatherogenic gene expression. Conversely, exercise appears to reduce retrograde flow in some arteries, thus decreasing oscillatory shear stress signals, whereas exercise increases retrograde flow in other arteries. More focused research in humans is needed to establish the importance of oscillatory flow in the effects of exercise on endothelial cell phenotype. Despite this current limitation in our knowledge, we conclude that available evidence strongly supports the hypothesis that shear stress is one signal for exercise-induced expression of antiatherogenic endothelial cell phenotype in arteries.

Pressure and Circumferential Stress (Stretch) as a Signal for Altered Endothelial Cell Phenotype

It is well known that arterial pressure fluctuates with systolic pressures of 120 mmHg and diastolic pressures of 80 mmHg at rest. During exercise peak systolic pressures can approach 200 mmHg and diastolic pressures remain normal or decrease (108). Arterial pressure waves pass through the arterial tree, and the magnitude of pulse pressure is determined by interactions between the compliance of the segments of the arterial
Tree and harmonics of the pressure waves. Thus arterial systolic and diastolic pressures in the femoral arteries can be significantly different from those measured in the aorta (108). Blood pressure can influence endothelial cells in at least two manners. First, cell culture experiments have demonstrated that exposure of endothelial cells to pressure affects growth rate of endothelial cells. Pressures in the range of 20 to 100 mmHg cause increased growth rates and higher pressures (>130 mmHg) cause inhibition of endothelial cell growth (115, 133). In addition to these effects on growth rates, pressure can signal altered endothelial cell gene expression. For example, exposure of endothelial cells in culture to pressures of 135 mmHg results in increased eNOS and iNOS protein content (133).

A second manner whereby changes in pressure can alter gene expression of endothelial cells results from stretch of the arteries. Because arteries are compliant, changes in pressure can stretch endothelial cells producing circumferential stress (strain) and the pulsatile nature of arterial blood pressure results in cyclic strain on the arteries. It appears that cyclic strain applied to endothelial cells produces even more dramatic effects on endothelial cell phenotype than does just compression (direct effects of pressure on the cells), as discussed in detail below.

Endothelial cells are exposed to cyclic circumferential stress (strain) from distention of arteries caused by increased blood pressure (which increases transmural pressure across the arterial wall) (25) and/or by relaxation of smooth muscle in the wall, which allows the blood vessel to increase in diameter, producing stretch of the endothelial cells lining the vessel. In vivo, arterial endothelial cells are exposed to cyclic distention during the cardiac cycle. Cyclic strain and wall movement have been measured in human thoracic aorta with MRI, and results demonstrate that peak strain usually occurs after peak flow in the aorta (25). The frequency and magnitude of this distention increases during exercise because of increased systolic pressure and heart rate (108). As summarized in Table 2, there is a growing body of evidence that cyclic strain alters endothelial cell gene expression patterns. Awolesi et al. demonstrated that cyclic strain increased transcription of eNOS in cultured endothelial cells (3) and increased eNOS enzyme activity of cultured endothelial cells (2). Since these observations, it has been proposed that distention (increased intraluminal pressure) of arteries is a signal for increased expression of the eNOS gene (148). There is also evidence that stretch increases expression of EDHF synthase (CYP450) (106), suggesting that cyclic strain may increase endothelium-dependent dilator pathways in general. Considering factors that may be proatherogenic, however, as summarized in Table 2, cyclic strain has also been reported to increase release of ROS (15) and to increase expression of adhesion molecules including ICAM (14, 145), selectin (145), and MCP-1 (141, 142, 145). These observations are consistent with many reports in the literature that indicate that chronic increases in blood pressure are associated with impaired endothelial function and increased progression of atherosclerosis (28). Also, Dancu et al. (19) reported that cyclic strain applied to cultured endothelial cells grown in silicon tubes caused a decrease in eNOS expression, in contrast to reports of studies using other methods of inducing stretch on cultured cells (3).

It is clear that the effects of cyclic strain on endothelial cells are complex and can be modified by a number of important variables. One important consideration in interpreting cyclic strain literature is evaluation of direct and indirect effects. For example, when endothelial cells in an intact artery are “stretched” because of increased pressure, there is an increase in superoxide and other forms of ROS in the artery (5) and an increase in adhesion molecules, such as VCAM-1 (126). The ROS produced by cyclic strain may indirectly cause increased expression of eNOS (4, 15). The major effect of rhythmic circumferential strain on endothelial cells appears

Table 2. Cyclic strain effects on markers of endothelial cell inflammation, adhesion, and phenotype

<table>
<thead>
<tr>
<th>Study</th>
<th>Model</th>
<th>Cyclic Strain, %</th>
<th>Duration, h</th>
<th>Cyclic Strain Effects (vs. static)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Awolesi et al. (2)</td>
<td>BAEC on flexible membrane</td>
<td>10 and 24%</td>
<td>24</td>
<td>No change in eNOS mRNA</td>
</tr>
<tr>
<td>Awolesi et al. (3)</td>
<td>BAEC on flexible membrane</td>
<td>6 and 10%</td>
<td>0–24</td>
<td>No change in eNOS mRNA</td>
</tr>
<tr>
<td>Wung et al. (141)</td>
<td>HUVEC on flexible membrane</td>
<td>12%</td>
<td>0.5, 1, and 24</td>
<td>No change in eNOS mRNA</td>
</tr>
<tr>
<td>Wung et al. (142)</td>
<td>HUVEC on flexible membrane</td>
<td></td>
<td>0.5 and 2</td>
<td>No change in ICAM-1 mRNA</td>
</tr>
<tr>
<td>Cheng et al. (14)</td>
<td>HUVEC on flexible membrane</td>
<td>9, 11, and 12%</td>
<td>24–48</td>
<td>No change in soluble ICAM-1 release</td>
</tr>
<tr>
<td>Cheng et al. (15)</td>
<td>HUVEC on flexible membrane</td>
<td>12%</td>
<td>0.25–20</td>
<td>No change in surface ICAM-1 expression</td>
</tr>
<tr>
<td>Yun et al. (145)</td>
<td>HUVEC on flexible membrane</td>
<td>15 and 25%</td>
<td>0.5–24</td>
<td>No change in ICAM-1 mRNA</td>
</tr>
<tr>
<td>Ziegler et al. (148)</td>
<td>Perfused BAEC silicon tubes</td>
<td>4%</td>
<td>24</td>
<td>No change in eNOS promoter activation</td>
</tr>
<tr>
<td>Ziegler et al. (147)</td>
<td>Perfused BAEC silicon tubes</td>
<td>4%</td>
<td>1, 4, and 24</td>
<td>No change in ICAM-1 expression</td>
</tr>
</tbody>
</table>
to be proatherogenic, even though the changes in eNOS expression alone would be antiatherogenic because the increased ROS production and expression of adhesion molecules override the effects of increased eNOS expression (51). The increased eNOS expression, although insufficient to correct function, may be a compensation for the loss of bioactive NO through the actions of superoxide and other ROS species. Consistent with the proposal that increased eNOS expression may be secondary to ROS effects are the reports that increased blood pressure increases eNOS expression in rat aorta (4) in association with increased oxidant stress in the aorta as well (5). Exercise bouts are usually of 1-2 h duration. Conversely, many studies of the effects of cyclic strain on endothelial cell phenotype have been designed so that the cells are exposed to cyclic strain 24 h/day. These different exposure times should be considered when interpreting results. For example, brief increases in blood pressure and ROS associated with bouts of exercise may signal increased eNOS expression and other beneficial effects of exercise, whereas chronic increases in blood pressure may elevate ROS chronically. Hence chronic elevations in blood pressure may trigger different changes in endothelial phenotype than do episodic increases in pressure and wall stretch. This hypothetically may explain the paradoxical enhancement in endothelial function associated with exercise, relative to the impaired endothelial function associated with primary hypertension.

In summary, cyclic strain on endothelial cells causes increased expression of eNOS. However, cyclic strain also signals upregulation of proatherogenic genes including adhesion molecules, MCP-1 expression, and markers of oxidant stress as summarized in Table 2. Also, chronic increases in blood pressure have been shown to result in proatherogenic endothelial cell phenotypes, and increased blood pressure is recognized as a risk factor for development of atherosclerosis (28). Therefore, we conclude that at this time it is unclear whether increased wall strain participates in signaling an antiatherogenic endothelial cell phenotype in physically active subjects. Given the duration of exposure to cyclic strain produced by exercise for 1 or 2 h/day, future research is required to determine whether increases in systolic blood pressures and heart rate produce important signals for the beneficial changes in endothelial cell phenotype associated with exercise training.

Interactions of Shear Stress and Circumferential Stress (Stretch) in Altered Endothelial Cell Phenotype

As outlined above, exercise produces complex hemodynamics that 1) increase the magnitude of blood flow and shear stress, 2) the frequency of pulsatile changes in pressures and flows, and 3) increased arterial systolic pressures. Available evidence indicates that the complexity of hemodynamics can contribute to expression of a proatherogenic endothelial cell phenotype especially when these hemodynamic parameters are asynchronous. Dancu et al. demonstrated that when pulsatile changes in diameter, flow and blood pressure occur synchronously these hemodynamic forces have different effects on endothelium lining silicon tubes than when the pulsatile changes are asynchronous (19). Figure 5 illustrates synchron-
Enhanced external counterpulsation is a noninvasive treatment for CAD patients that has been shown to increase diastolic coronary blood flow and total coronary blood flow. Enhanced external counterpulsation increases diastolic pressures above the diaphragm by compressing the tissues below the diaphragm and driving blood toward the heart. In patients who received daily 1-h treatments of enhanced external counterpulsation for 6 wk, it was reported that plasma nitrate and nitrite levels were increased (indicative of increased release of NO in the blood) and ET-1 levels were decreased, suggesting improved endothelial function and phenotype in these patients (1). Similar results have been reported for enhanced external counterpulsation treatment in an animal model of CAD (146). Zhang et al. (146) proposed that enhanced external counterpulsation in a porcine model of CAD applied to the hindlimbs and hips of the pigs would increase shear stress and improve endothelial cell function/phenotype in the coronary and brachial arteries. They found that enhanced external counterpulsation resulted in a doubling of peak diastolic shear stress in the brachial artery. Application of enhanced external counterpulsation for 2 h a day for 7 wk also resulted in a more antatherogenic endothelial cell phenotype in the left anterior descending coronary artery (146). On the basis of the analysis of the effects of hemodynamic stress presented above, it would be expected that the arteries in the hindlimb of these pigs and perhaps in the abdominal aorta, would have retrograde flow during counterpulsation, which drives blood back into the thoracic aorta. If this hypothesis is correct, we would further expect proatherogenic changes in endothelial cell gene expression in these caudal arteries. Zhang et al. did not report whether proatherogenic changes in endothelial cell gene expression were seen in the abdominal aorta and arteries of the hindlimb. Therefore, this hypothesis will have to be tested in future work.

Systemic Distribution of Exercise-Induced Arterial Vascular Adaptations

There are reports of exercise training-induced adaptations in vascular beds providing blood flow to tissues/muscle not heavily involved in training bouts (44, 56, 57, 61, 67, 69, 70) and within skeletal muscle that does not exhibit increased oxidative capacity with exercise training (62, 67, 137). For example, most (24, 79, 120) but not all (123) studies have reported that leg exercise training of healthy and diseased
human populations produces improved endothelium-dependent dilation in conduit arteries of their relatively inactive arms. Such observations may suggest a systemic effect of exercise training on arterial endothelial cell phenotype. However, modest increases in blood flow (42), oscillatory shear rate (42), pulse rate (41, 42), and blood pressure have been reported in the arms of humans during lower extremity exercise (42, 60, 121). These results make it difficult to appreciate the importance of primary hemodynamic signals (shear stress and cyclic strain) that may signal phenotypic changes to endothelial cells in the brachial artery during leg exercise. In addition, it is unclear at this time whether hemodynamic forces or circulating factors are the underlying mechanisms involved in vasculature adaptations in conduit arteries that do not perfuse cardiac or skeletal muscle active during exercise. As a result, it has yet to be determined whether one or a combination of the four hemodynamic forces mentioned above are the underlying mechanism involved in the apparent systemic effects of exercise on endothelial cell function/phenotype.

Conclusions

In conclusion, the available literature reviewed herein shows that shear stress is a signal for expression of antiatherogenic genes in endothelial cells in culture, in isolated arteries, and in arteries of intact animals. Furthermore, shear stress levels in the arteries of humans during exercise are in the range that produces beneficial changes. In contrast, oscillatory flow patterns can cause proatherogenic gene expression in cultured endothelial cells. In vivo evidence indicates that exercise decreases oscillatory flow/shear in some portions of the arterial tree but may increase oscillatory flow in other areas of the arterial tree. We conclude that current evidence indicates that increased shear stress during exercise may be one signal produced by exercise that contributes to an antiatherogenic phenotype of arterial endothelial cells.

Our review of current literature indicates that circumferential wall stress can also increase expression of some beneficial antiatherogenic endothelial cell genes. However, this literature equally provides evidence that circumferential wall stress increases production of ROS and increases the expression of adhesion factors and other proatherogenic genes. These data suggest that circumferential wall stress produced during exercise may contribute to exercise-induced modulation of endothelial cell phenotype. It appears that interactions of arterial pressure and fluid shear stress may play an important role in arterial vascular health and likely contribute to how exercise bouts signal changes in endothelial cell gene expression. Given that the timing of these signals during the cardiac cycle (i.e., whether or not the changes are asynchronous or synchronous) can have major effects on the impact of these signals on endothelial phenotype, more detailed analysis of these hemodynamic variables during bouts of exercise training is required. We conclude that available evidence suggests that exercise signals formation of beneficial endothelial cell phenotype at least in part through these mechanical signals: shear stress and wall stretch. However, it is also clear that other local and circulating factors interact with these hemodynamic signals during exercise to produce the healthy arterial endothelial cell phenotype. Future research promises to reveal these factors and how they interact with hemodynamic forces.

GRANTS

This work was supported by National Institutes of Health Grants RR-18276, HL-56088, HL-52490, and AR-048523.

REFERENCES


Exercise Hemodynamics as Signals for Endothelial Phenotype


