VASCULAR ADAPTATION TO EXERCISE IN HUMANS: THE ROLE OF HEMODYNAMIC STIMULI

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ABSTRACT

On the 400th anniversary of Harvey's Lumleian lectures, this review focusses on the impact of physical exercise on "hemodynamic" forces associated with the movement of blood through arteries in humans and the functional and structural adaptations that result from repeated episodic exposure to such stimuli. The late 20th century discovery that endothelial cells modify arterial tone via paracrine transduction, provoked studies exploring the direct mechanical effects of blood flow and pressure on vascular function and adaptation *in vivo*. In this review, we address the impact of distinct hemodynamic signals that occur in response to exercise, the inter-relationships between these signals, the nature of the adaptive responses that manifest under different physiological conditions and the implications for human health. Exercise modifies blood flow, luminal shear stress, arterial pressure and tangential wall stress, all of which can transduce changes in arterial function, diameter and wall thickness. There are important clinical implications of the adaptation that occurs as a consequence of repeated hemodynamic stimulation associated with exercise training in humans, including impacts on atherosclerotic risk in conduit arteries, the control of blood pressure in resistance vessels, oxygen delivery and diffusion, and microvascular health. Exercise training studies have demonstrated that direct hemodynamic impacts on the health of the artery wall contribute substantially to the well-established decrease in cardiovascular risk attributed to physical activity.

TABLE OF CONTENTS

I. INTRODUCTION: EXERCISE AND ARTERY HEALTH IN HUMANS
A. Impact of exercise and physical activity on cardiovascular risk
B. The risk factor gap: Traditional risk factors do not fully explain risk reduction
C. Evidence for a role of direct impacts of hemodynamic forces on vascular health
D. Integrative aspects of vascular adaptation to training

II. WHAT HEMODYNAMIC FORCES ARE RELEVANT IN THE VASCULATURE?
A. Pressure effects
i. Detection of cyclic circumferential strain by the endothelium
ii. Relevance of the pattern of cyclic circumferential strain
B. Endothelial shear stress
i. Detection of shear stress by the endothelium
ii. Relevance of the pattern of shear
C. Importance of interaction between hemodynamic forces

III. HEMODYNAMIC STIMULI DURING ACUTE EXERCISE
A. Effects of exercise on hemodynamic forces
i. Shear stress in vascular territories perfusing active areas
ii. Shear stress in vascular territories perfusing inactive areas: start of exercise
iii. What are the mechanisms for changes in shear stress at the start of exercise?
iv. Shear stress in vascular territories perfusing inactive areas: continuation of exercise
v. Non-shear stress hemodynamic stimuli mediating artery vasomotion during exercise
B. Impact of different shear stress patterns on artery function

IV. VASCULAR ADAPTATIONS TO EXERCISE *TRAINING*: ROLE OF HEMODYNAMIC FACTORS
A. Adaptations in vascular function
i. Conduit arteries
ii. Resistance arteries
iii. Microcirculation
iv. Cutaneous microcirculation: an active vessel bed during exercise
B. Adaptation in vascular structure
i. Conduit arteries
ii. Resistance arteries
iii. Microcirculation
C. Changes in vascular cell gene expression induced by exercise training

V. WHICH FACTORS MODERATE THE ADAPTATION TO TRAINING?
A. Distinct adaptations to different forms of exercise training
B. Distinct time-course in adaptation in different vascular properties
C. Interaction between changes in function and structure
D. Impact of cardiovascular disease on hemodynamic stimuli and vascular adaptation to exercise

VI. SUMMARY AND IMPLICATIONS FOR EXERCISE SCIENCE AND HEALTH

We in the West are the first generation in human history in which the mass of the population has to deliberately exercise to be healthy.

– Professor Jeremiah Morris (216) –

I. INTRODUCTION: EXERCISE AND ARTERY HEALTH IN HUMANS

Recent technological "advances" have fundamentally altered the vocational and lifestyle behaviours of humans in the space of a few generations. Profound changes associated with ubiquitous exposure to television, mobile communication devices and the internet have rapidly accelerated an underlying trend in sedentary behaviour related to urbanisation, automation and widespread use of the automobile (272). In global terms, it was recently estimated that physical inactivity caused 6–10% of all deaths from the major non-communicable diseases (coronary disease, type 2 diabetes, breast and colon cancers), or more than 5.3 of the 57 million deaths that occurred worldwide. This equates to the number of deaths attributable to tobacco (112).

Approximately 1/3rd of the global population do not meet minimum physical activity (PA) requirements to sustain health (112). In the West, the impact of technological change on PA levels and cardiovascular health is occurring on a background of unprecedented demographic shifts associated with population ageing, raising the spectre of individuals experiencing more years of frailty and compromised life quality, with associated increases in healthcare costs (229). There has never been a more sedentary population of humans than the 21st century Western society, prompting some to suggest that the positive historical trend in life expectancy may soon be threatened (231). These observations reinforce the critical importance of increasing physical activity levels and primary prevention is now a global policy agenda (137).

Although exercise programs may be regarded as an effective strategy to "compensate" for loss of routine physical activity, better insight is required into the physiological adaptations to distinct stimuli associated with exercise. This review focuses on the impact of exercise on the vasculature, in particular, the direct effects mediated by physical, mechanical and/or hemodynamic forces on arterial function, structure and adaptation in humans.

A. Impact of exercise and physical activity on cardiovascular risk

Retrospective studies strongly suggested that regular physical activity is associated with lower risk for primary CV mortality and morbidity (197, 241). Subsequent prospective studies provided direct evidence that adopting a physically active lifestyle delays all-cause mortality, extends longevity (242) and reduces risk for CV mortality by 42-44%, compared to persistently unfit men (28, 180). Furthermore, the relationship between physical activity and CV risk exhibits a curvilinear dose-response pattern (319) with increasing, but diminishing, returns at higher activity levels (210). It is important to acknowledge that, whilst fitness has been regarded as a surrogate for habitual physical activity, these factors have independent and overlapping roles in the prevention of cardiovascular disease (63). In those with heart disease, exercise-based rehabilitation is associated with a reduction in CV mortality and fewer hospital admissions (9). These benefits, in the context of both primary and secondary prevention of CVD, approximate and may exceed those associated with antihypertensive (308) or lipid lowering drugs (47, 203). Indeed, meta-epidemiological evidence (205 randomized controlled trials, n=339,274) found equal effectiveness of exercise training and contemporary drug interventions (220), in terms of mortality reduction.

B. The risk factor gap: Traditional risk factors do not fully explain risk reduction

Until recently, the rationale for the promotion of exercise, and methods of prescribing it, were based on the assumption that exercise exerted its benefits by virtue of "secondary" effects. That is, exercise benefit was judged by its capacity to modify CV risk factors such as BP, lipids, insulin resistance, smoking and obesity (303). Indeed, studies linking exercise to changes in CV risk factors report significant improvement in individual CV risk factors (106, 155), although the magnitude of such change is typically modest compared to pharmacological interventions (303). Importantly, the cardioprotective effects of exercise training remain after statistical correction for traditional and novel CV risk factors (28, 159). Mora *et al.* (211) assessed the contribution of changes in CV risk factors as a result of physical activity to the occurrence of CVD in 27,055 women (10.9 year follow-up) and reported that established and novel risk factors explained only part of the beneficial impact of exercise on CVD risk. Others have reported that CV risk factors explained only 27-41% of the cardioprotective benefits of exercise training (48, 120, 286). The beneficial impacts of exercise on CV risk therefore exceed that expected from changes in CV risk factors alone: A risk factor gap exists in explaining the benefits of exercise in humans (106, 155).

Exercise exerts direct effects on the vasculature via the impact of repetitive exposure to hemodynamic stimuli, such as shear stress and transmural pressure. Consequently, exercise transduces functional and structural adaptations in the vascular wall, providing a plausible contribution to the risk factor gap described above (106, 155). Improvements in flow-mediated dilation (FMD), a validated surrogate for CV health and disease risk (103, 145, 252, 287), can occur as a result of exercise training in the absence of changes in CV risk factors (109) reinforcing the notion that (196) exercise exerts some of its benefit by virtue of impacts distinct from those on traditional risk factors (104, 296). This proposition is supported by

epidemiological evidence, presented above, that ~50% or more of the beneficial impact of exercise on CV endpoints cannot be explained by risk factor modification (211). Although alternative explanations exist (32) including modulation of autonomic tone (106, 155), there is a strong basis to propose that exercise-induced hemodynamic changes induce anti-atherogenic adaptations in vascular function and structure that contribute to the CV benefits of exercise training.

C. Evidence for a role of direct impacts of hemodynamic forces on vascular health

At the end of the 19th century Thoma noted, in observations of chick embryos, that many branches developed in blood vessels in which blood flow was rapid, whilst no branches developed in blood vessels where blood flow was slower (301). This early observation suggested that hemodynamic forces, broadly defined as mechanical forces associated with flowing blood (i.e. shear stress and/or pressure), were important in the adaptation of the vasculature. More recently the endothelium has provided a focus for research, given its strategic placement between the flowing blood and artery wall and crucial role in the progression and development of atherosclerosis (201). It is now understood that vascular adaptation is dependent upon an intact, functional endothelium (257, 326) and that hemodynamic stimuli induce functional and structural changes in the arterial wall via endothelial cell signal transduction.

D. Integrative aspects of vascular adaptation to training

Clausen (50) noted nearly 4 decades ago in this journal that exercise *training* improves oxygen uptake and cardiac output during maximal exercise (**Figure 1**), whereas mean arterial pressure remains unaffected. These findings infer that the increase in cardiac output as a result of exercise training is accommodated by a corresponding rise in vascular conductance,

the latter mediated by functional and/or structural adaptations in conduit, resistance, and microvessels. Changes in the vasculature are associated with decreased cardiac afterload at rest and during submaximal exercise, which enhances ventricular function and myocardial oxygen demand (118). This integrative physiological perspective emphasises the key role played by changes in the vasculature in response to exercise training.

II. WHAT HEMODYNAMIC FORCES ARE RELEVANT IN THE VASCULATURE?

A. Pressure effects

Exercise increases systolic pressure, whilst diastolic pressures remain at resting levels or may decrease (127). As the arterial pressure waves propagate, pulse pressure changes due to interactions between the segmental arterial compliance and pressure wave harmonics, such that systolic and diastolic pressures in peripheral arteries (brachial, femoral) can be significantly different from those measured in the aorta(260).

Blood pressure can influence vascular cells in at least two ways. First, cell culture experiments have demonstrated that exposure of endothelial cells to pressure affects their growth rate; pressures of 20 to 100 mmHg increase growth compared to no pressure (173). Second, pressure distends arteries, thereby stretching vascular cells in the wall. Because arteries are compliant, changes in pressure consequently produce circumferential stress (i.e. strain). Because of the pulsatile nature of arterial blood pressure, this circumferential strain results in cyclic circumferential strain (**Figure 2**).

i. Detection of cyclic circumferential strain by the endothelium

In response to cyclic circumferential strain, endothelial cells respond morphologically with alignment of cells perpendicular to the force vector, subsequently followed by phenotypic changes (52). The mechanism by which the endothelium recognizes and transduces mechanical stimuli involves various signalling systems (i.e. integrins, ion channels, G proteins-coupled receptors and receptor tyrosine kinases). This complex system of mechanosensors converts mechanical stimuli into chemical signals that lead to the activation of intracellular signalling cascades (**Figure 2**). The latter can alter transcription factors and

activation of genes that regulate the fate of the endothelial cells and smooth muscle cells: i.e. proliferation, migration and/or apoptosis. In this process, cyclic circumferential strain eventually modifies matrix proteins [i.e. metalloproteinases (MMPs)] that can affect components of the extracellular matrix, but also influence non-matrix substrates (e.g. growth factors and receptors) (52).

ii. Relevance of the pattern of cyclic circumferential strain

Increases in arterial pressure that distend arteries and increase transmural pressure also induce increased cyclic circumferential strain on endothelial cells (67). Cyclic circumferential strain can also increase as a result of relaxation of vascular smooth muscle, which induced vasodilation and stretching of the endothelial cells lining (**Figure 2**). Endothelial cells are experience cyclic circumferential strain across the cardiac cycle in vivo. In humans, cyclic circumferential strain has been measured in the aorta using MRI, with peak strain occurring after peak flow (67). During exercise, the distention in the aorta increases because of increases in heart rate and systolic pressure. Increased exposure to cyclic circumferential strain has been reported to alter vascular cell gene expression, such as increased expression/activity of endothelium-dependent dilator pathways endothelial nitric oxide synthase (eNOS) and endothelium-derived hyperpolarizing factor (EDHF) synthase (CYP450), as well as increase release of reactive oxygen species (ROS), expression of adhesion molecules such as intercellular adhesion molecule (ICAM), selectin, and monocyte chemoattractant protein-1 (MCP-1) (173). Consistent with these observations, chronic increases in blood pressure are associated with impaired endothelial function and progression of atherosclerosis (71) (**Figure 2**).

The effects of cyclic circumferential strain are complex and variable. Because cyclic circumferential strain can have direct effects on endothelial cell gene expression, but also increases superoxide, other forms of ROS and adhesion molecules (e.g. VCAM-1), the direct effects of stretch on gene expression are not easily predicted (173). ROS produced by cyclic circumferential strain may indirectly alter vascular cell phenotypes. The major effect of increased cyclic circumferential strain on endothelial cells appears to be pro-atherogenic, even though the changes in eNOS expression alone would be anti-atherogenic. The pro-atherogenic effect of increased cyclic circumferential strain is likely explained because the increased ROS-production and expression of adhesion molecules override the effects of increased eNOS-expression (124). Increased eNOS-expression, although not sufficient to preserve function, may compensate for the loss of bioactive NO caused by superoxide and other ROS. Whilst exercise bouts are usually <2-h duration, many studies of the effects of cyclic circumferential strain on endothelial cell phenotype have used 24 h/day exposure to a similar level of pressure. These data suggest that the *pattern* of change in cyclic circumferential strain is relevant as transient increases in blood pressure and ROS, associated with exercise bouts, may increase eNOS-expression and other beneficial effects of exercise, whereas chronic increases in blood pressure may chronically elevate ROS, causing mal-adaptations.

It is clear that circumferential strain can influence vascular smooth muscle cell phenotype through effects of stretch on vascular smooth muscle cells. While not the focus of this review, **Figure 2** illustrates many known effects of exercise on vascular smooth muscle cells. The classic pressure-induced myogenic response in smooth muscle is initiated by a stretch-induced depolarization due to activation of cation channels (167). This depolarization activates Ca^{2+} influx through Cav1.2 and subsequent Ca-induced contraction. In addition,

both stretch and Ca^{2+} activate BK channels, while depolarization activates Kv channels to hyperpolarize and limit depolarization in a negative-feedback manner (167). The net result, other than contraction, is a dominant Cav1.2 mediated calcium influx which increases expression of smooth muscle-specific genes (a.k.a. differentiation markers) such as smooth muscle specific myosin heavy chain (SMMHC) and smooth muscle alpha actin (SMaA) (32,167). The transcription factors that drive smooth muscle specific gene expression, myocardin and to a lesser extent MEF, are also increased by pressure-induced Ca influx (32,167). Pressure/stretch is known to regulate smooth muscle synthesis of connective tissue growth factor (CTGF), collagen and fibronectin indicating that pressure can influence vessel wall matrix composition through smooth muscle (30-32, 167)(**Figure 2**). As highlighted elsewhere, the seminal studies of Folkow infer that changes in arterial wall thickness can exacerbate effects on arterial pressure *in vivo* (74, 75).

B. Endothelial shear stress

In 1933, Schretzenmayr exposed cat femoral arteries to an increase in blood flow via stimulation of the hind leg motor nerves and observed a gradual increase in femoral artery diameter (268). This may be the first study to demonstrate that conduit arteries are able to react to forces exerted by the circulating blood. The role of endothelial cells in the 'detection' of changes in flow, and their production of vasoactive second messengers, was only recognized later.

In 1975, Rodbard published a prescient account of endothelial transducer function (256). He proposed four steps (**Figure 3**), starting with exposure of the endothelium to an increase in viscous drag (step 1), or "shear stress". Any increase in flow that increases this drag (step 2), subsequently triggers acute dilation (step 3), a functional change that tends to homeostatically

modify the initial increase in shear. When exposed to prolonged periods of change in flow and shear, vessel remodelling can occur, whereby local drag is returned toward the norm by virtue of structural arterial modification (step 4). In 1980, Furchgott published his famous experiment, which provided elegant evidence for the importance of the endothelium in mediating vasodilation in response to acetylcholine through the release of a vasodilator substance, EDRF, later described as nitric oxide (NO) (79). This *Nature* paper was preceded by another, which observed that vasodilator prostaglandins were produced by the endothelium (208), although the role of shear stress in this transduction process was demonstrated somewhat later (160, 263).

Studies in the early 1980s provided experimental evidence for Rodbard's assumptions that an increase in flow induces artery dilation (134), most likely through the release of a dilator signal from the endothelium (263). Pohl and colleagues performed a series of experiments (246) in which they demonstrated that *in vivo* infusion of acetylcholine and augmentation of arterial flow elicited remarkably similar dilation, whereas mechanical removal of the endothelial layer abolished these responses (**Figure 4**). In the same year, Rubanyi and colleagues reinforced the ability of increases in flow to induce an endothelium-dependent dilation, and also found that, in addition to prostacyclin, flow triggers the release of another relaxing substance to mediate vasodilation (263). Subsequently, Berdeaux *et al.* (21) (**Figure 4**) showed exercise-intensity dependent vasodilation of canine epicardial coronary arteries was converted to vasoconstriction after mechanical endothelial denudation. Taken together, these studies provided further evidence for the importance of the functional integrity of the endothelium in the integrated control of arterial diameter, with the endothelium being hypothesized to protect against the vasoconstrictive effects of catecholamines released during exercise (21).

In 1989, two human-based studies reported that increases in brachial artery flow induced by distal blood pressure cuff inflation around the forearm were followed by dose-dependent dilation of the artery (8, 275). These observations prompted introduction of the "flow-mediated dilation (FMD)" approach as an in vivo bioassay of endothelium-dependent vascular smooth muscle relaxation in humans. This non-invasive approach uses a 5-minute cuff occlusion of the forearm to induce increased shear stress in the brachial artery and consequent dilation (44), quantified by non-invasive high resolution ultrasound. Human data support the endothelium- and NO-dependence of brachial FMD (98), and radial artery FMD is significantly reduced after endothelial denudation *in vivo* (61) (**Figure 4**).

The endothelium is also essential in mediating structural arterial adaptation. A decrease in the common carotid artery size was observed in response to chronic reductions in blood flow, whilst such adaptation was abolished when the endothelium were removed (167) (**Figure 4**). A subsequent study further explored the role of endothelium-derived nitric oxide (NO) in adaptive changes in diameter (307) (**Figure 4**). Blood flow through the carotid artery was chronically increased by an arteriovenous fistula, which caused the diameter to increase (causing normalisation of shear stress). However, animals in which NO-synthesis was pharmacologically blocked showed attenuated adaptation of carotid diameter. These findings suggest that the endothelium, through NO-dependent pathways, plays a role in remodelling of vessel diameters in response to increases in shear stress.

i. Detection of shear stress by the endothelium

Mechanotransduction at the luminal surface of the endothelium is initiated by shear stress detection by ion channels (K^+, Ca^{2+}, Na^+, Cl^-), cell membrane receptors (tyrosine kinase

receptors), G-proteins, caveolae, and the plasma membrane lipid bilayer (10) (**Figure 2**). Furthermore, the lumen is lined with glycocalyx, a glycoprotein-polysaccharide structure that is specifically responsible for shear stress-induced NO production (10). There is evidence for primary cilia that are linked to shear stress-mediated production of NO (10) and shear has also been proposed to be detected by the cytoskeleton of the endothelial cells, largely through its connection to integrins (VE-cadherin and integrin) and a mechanosensory complex (platelet endothelial cell adhesion molecule-1). A possible explanation for the involvement of multiple, distinct types of mechanotransduction is that shear stress, in contrast with pressure, is a relatively weak force (10). Therefore, highly sensitive mechanisms seem necessary to sense shear stress (**Figure 2**), including the detection of complex patterns of shear.

ii. Relevance of the pattern of shear

Correlational studies. In 1969, Caro and colleagues studied the incidence of atherosclerosis in low *versus* high shear regions and branch points in the coeliac, mesenteric and renal arteries. They found that, relative to areas of higher shear (i.e. inner wall of branch points), regions of low shear (i.e. outer wall of daughter vessels) revealed greater burden of atherosclerotic lesions. The authors proposed that local hemodynamics play a fundamental role in the etiology of atherosclerotic disease (38). Of relevance to the present review, Caro *et al.* were likely the first to articulate that *"physical exercise involving increase of cardiac output, and hence increased shear rate, might retard the development of atheroma"* (38). Along these lines, post-mortem human carotid artery specimens exhibit the greatest atherosclerotic burden at the outer wall of the vessel, a region subjected to low levels of shear stress and substantial flow reversal (325). Studies in large animals also support the idea that atherosclerosis preferentially develops in susceptible regions of the vasculature. Indeed, low

and oscillatory shear regions occur in porcine coronary and peripheral conduit arteries at geometrically irregular sites (i.e. branch points, bifurcations and curvatures), and these regions are highly atherosclerosis-prone (49, 57, 110, 139, 165, 245). Furthermore, it was recently reported in a pig model of familial hypercholesterolemia (251) that the distal portion of the aorta, subjected to disturbed blood flow profiles, presents ~3 fold greater levels of atherosclerotic burden relative to the proximal portion of descending aorta, which is exposed to more unidirectional flow (236).

In vitro studies. Intricate mechanotransduction and signaling mechanisms operate in concert to alter endothelial gene expression and function, and adaptation depends upon the characteristics of the shear stress stimulus (148). Initial *in vitro* studies demonstrated that oscillatory shear promotes endothelial inflammation, activation, and abnormal alignment of endothelial cells (58), whereas laminar shear reduces inflammation-related events such as leukocyte-endothelial adhesion (179). Using sophisticated technology, more recent studies showed that expression of pro-atherogenic genes is increased in cultured endothelial cells subjected to shear patterns replicating *in vivo* patterns in atheroprone regions, in contrast to cells exposed to patterns characteristic of protected regions (35, 126, 228). Pro-atherogenic shear patterns induce production of NADPH oxidase- and mitochondria-derived superoxide radicals, augment production of endothelin-1, and upregulate VCAM-1 and ICAM-1 (51, 54, 142, 143, 228, 284, 318), all critical early events in the development of atherosclerosis.

The role of shear stress *patterns* in modulating vascular health is also supported by studies in isolated and pressurized arteries. For example, isolated rat soleus feed arteries exposed to high flow and shear stress for 4 hrs exhibited increased expression of eNOS mRNA and enhanced endothelium-dependent dilation (321). Conversely, exposure of rat carotid arteries

to low levels of shear stress for 4 hrs promoted the upregulation of adhesion molecules such ICAM-1 and VCAM-1 (236). Others have shown that pig carotid arteries subjected to oscillatory shear for three days exhibited a marked impairment in endothelium-dependent vasorelaxation (80), an effect that was accompanied by reduced eNOS mRNA and protein expression. Also, evidence exists indicating that flow reversal significantly reduces NO bioavailability in isolated porcine femoral arteries due to increased superoxide production (188), a known molecular mediator of atherogenesis (234). NADPH oxidase appears to be the principal source of retrograde flow-induced reactive oxygen species generation in isolated arteries (89, 188). Taken together, the data from *in vitro* cell culture and isolated vessel preparations firmly support the view that disturbed shear stress patterns stimulate a pro-atherogenic endothelial cell phenotype.

In vivo studies. Data demonstrating that disturbed shear stress profiles produce detrimental vascular effects are also available from *in vivo* studies in animals. The partial carotid artery ligation model has provided a valuable experimental model for the *in vivo* study of disturbed shear stress profiles (162, 221). Due to ligation of all but one of the distal branches, and a consequent increase in downstream vascular resistance, the proximal portion of the carotid artery is chronically exposed to disturbed blood flow. This model leads to substantial wall thickening with leukocyte infiltration and smooth muscle proliferation within 2 weeks in normal mice (162) and endothelial dysfunction and advanced atherosclerotic lesions in ApoE-/- mice (221). These functional alterations are preceded by rapid molecular phenotypic changes. In this regard, expression of more than 500 genes is altered within 2 days following ligation (226). This experimental model has provided considerable insights related to the mechanisms underlying disturbed shear stress-induced atherosclerosis. Leukocytes have been reported to promptly accumulate into the arterial wall during initiation and progression of

disturbed flow-induced atherosclerosis (3). In addition, eNOS uncoupling (184), NADPH oxidase-derived superoxide radicals (221), fibronectin polymerization (46), IL-17 signaling (189), and adhesion molecules such as PECAM-1 (45) all are involved in the development of atherosclerosis caused by partial carotid ligation in mice. Of note, studies in which the partial ligation model was superimposed onto models of obesity (181) and reno-vascular hypertension (147) indicate that the pro-atherogenic effects of disturbed shear stress may be more prominent when accompanied by cardiovascular risk factors.

C. Importance of interaction between hemodynamic forces

Exercise has complex effects on hemodynamics that result in increased blood flow and shear stress, increased frequency of pulsatile changes in pressures and flows, and increased arterial systolic and pulse pressures. These complex hemodynamic effects of exercise can contribute to the expression of pro-atherogenic vascular phenotypes, especially when the hemodynamics are asynchronous. Dancu *et al.* showed that synchronous pulsatile changes in diameter, flow and blood pressure have different effects on silicon tubes lined by endothelium, compared to the effects of asynchronous changes (55). Synchronous hemodynamics exist when flow, heart rate and pressures have the same time courses, i.e. peak pressure, peak flow, and peak diameter occur at nearly the same time. Synchronous hemodynamics are often seen in the aorta, whereas asynchronous hemodynamics are common in the coronary arteries. Indeed, peak coronary flow is observed in early diastole, when pressure and coronary artery diameter are low. Therefore, asynchronous hemodynamics are normal for coronary arteries (56). When synchronous and asynchronous changes in pressure, tube diameter, and gene expression in endothelial cells cultured in silicon tubes are examined in more detail, results demonstrate that asynchronous pulsations in shear stress and circumferential strain result in decreased eNOS expression, but increased ET-1 expression in endothelial cells (55, 56). The

fact that coronary arteries are exposed to asynchronous hemodynamics may therefore explain, in part, the propensity of these arteries to develop atherosclerosis. During exercise, however, coronary blood flow becomes pulsatile with positive flow in both systole and diastole. The relatively greater exercise-induced increase in shear stress and blood flow (4-6 fold increase), combined with small increase in systolic pressures and non-oscillatory coronary blood flow during exercise, results in net anti-atherogenic signals (171, 173).

III. HEMODYNAMIC STIMULI DURING ACUTE EXERCISE

A. Effects of exercise on hemodynamic forces

i. Shear stress in vascular territories perfusing active areas

At the onset of exercise, blood flow and shear stress markedly increases in the active regions in an exercise intensity-dependent manner, to meet the increased metabolic demand (95, 270, 290). For example, handgrip exercise, which causes minor changes in blood pressure and cardiac output, induces large hyperemic responses, suggesting that vasodilation in downstream resistance vessels is the major cause of increased blood flow during handgrip exercise (95). Lower limb exercise, which engages a larger muscle volume and consequently increases blood pressure and cardiac output, is associated with increases in femoral artery blood flow that result from changes in downstream resistance vessel dilation in concert with the increases in central driving pressure. Studies that have examined local vasodilator mechanisms contributing to exercise-hyperemia have generally found a redundancy of vasodilator mechanisms (153), which means that blocking single pathways does not importantly impair exercise-hyperemia. This redundancy ensures that blood flow to exercising muscle is highly protected, even in the absence, or attenuated presence, of key vasodilator pathways. Therefore, local vasodilator mechanisms along with increases in arterial pressure and cardiac output contribute to exercise hyperemia, leading to significant increases in shear stress in the active areas during exercise.

Whilst a large number of studies have explored exercise hyperemic mechanisms, little attention has been paid to the impact of exercise on the *pattern* of blood flow and shear stress. This likely relates to the technical difficulty and limitations associated with contemporary techniques to validly assess shear stress patterns in physically active limbs, such as in the lower limbs during cycling or running exercise. However, some studies have examined

brachial artery shear stress pattern during local handgrip exercise. In agreement with studies adopting techniques to assess bulk blood flow, Green *et al.* found that brachial artery blood flow and shear rate increased with incremental levels of handgrip exercise in healthy subjects (95). An intensity-dependent increase in antegrade shear, with negligible levels of retrograde shear rate, was observed during handgrip exercise (95). This study also explored the role of NO during incremental handgrip exercise. Largely in agreement with observations regarding redundancy and blockade effects during exercise-hyperemia (153), blood flow and shear rate patterns of the brachial artery during incremental handgrip exercise were modestly altered in the presence of a NO synthase blocker (95). These data demonstrate that increases in mean shear rate to an active limb are largely mediated through increases in antegrade shear rate, with negligible changes in retrograde shear rate, whilst NO is not obligatory to mediate these changes in shear patterns.

The increase in antegrade shear induced by handgrip exercise is not stable, but consists of a highly fluctuating pattern of antegrade shear, at least partly induced by muscle contractions (**Figure 5**). Previous work examined whether such fluctuating pattern of shear stress (due to handgrip exercise), compared to gradual elevation in shear stress, affects the ability of arteries to dilate (248). It was demonstrated that, when matched for mean shear, comparable conduit artery dilation was achieved during handgrip exercise and heating. This indicates that muscle contractions *per se*, are not obligatory for the impact of shear on conduit artery dilation.

Despite a significant amount of work exploring exercise hyperemia and its underlying mechanisms, surprisingly little is known about the relative impact of distinct changes in shear stress *pattern* during different forms of exercise in the active limbs. This knowledge may

importantly contribute to our understanding of adaptations in vascular function and structure in the physically active regions. It is also important to consider that distinct forms of exercise have impacts on vascular compression and the transduction of forces related to transmural pressure (14). Given evidence that these factors have impacts of arterial adaptation, the differential impact of exercise involving sustained (e.g. rowing) versus cyclical (e.g. rhythmic handgrip) muscular contraction warrants further investigation (261, 262).

ii. Shear stress in vascular territories perfusing inactive areas: start of exercise

Early studies indicating that predominant lower limb exercise training induced adaptation in upper limb vascular function (186, 193, 194) (previously reviewed (104)) stimulated interest in the patterns of shear stress occurring in vascular territories other than those feeding active musculature.

Historical evidence suggests that blood flow to the inactive upper limbs decreases during the initial stages of lower limb cycle exercise, and is subsequently restored as exercise continues (25). This study, involving indirect quantification of blood flow using oxygen saturation levels of axillary venous blood, reported a biphasic response in total arm blood flow during the lower limb exercise, characterised by initial decreases from resting values, with subsequent increase in flow. More recently, novel imaging technologies have confirmed this pattern. Small increases in antegrade flow are accompanied by substantial increases in retrograde flow (92, 93) through the brachial artery during the initial stages of cycle ergometer exercise. The increase in retrograde flow may relate to activation of the sympathetic nervous system and an increase in downstream vascular resistance (43, 240). Alternatively, the immediate increase in retrograde shear at the onset of exercise may be caused by an immediate increase in microvascular critical closing pressure (113). This

immediate increase in brachial artery retrograde shear at the start of cycle exercise remains relatively stable as exercise workloads increase. In contrast, higher cycle exercise intensities are associated with larger increases in antegrade shear, likely due to increases in cardiac output. Consequently, brachial artery mean blood flow shows a biphasic pattern, with a decrease at lower exercise intensities and an increase in blood flow at higher exercise intensity levels (92, 93).

The pattern of shear stress described above depends on the type of exercise performed (290). Cycling and walking, both representing rhythmic lower limb exercise, result in the typical oscillatory shear rate pattern. Blood flow patterns were markedly different during leg kicking, which was linked to a systolic blood pressure-driven increase in antegrade shear rate, without changes in retrograde shear. The different shear patterns suggest that distinct stimuli are responsible for the resulting change in shear stress during exercise. It should be acknowledged that pulsatile pressure and heart rate (and therefore cyclic circumferential strain) also differ markedly between these types of exercise. Whilst leg kicking exercise is associated with small changes in heart rate and is typically sustained for 5-10 minutes, rhythmic exercise can be sustained for prolonged periods at relatively high heart rates and significant elevation in blood pressure. These differences likely contribute to the distinct shear stress patterns between different types of exercise, which have implications for vascular cell signal transduction and consequent arterial adaptation in humans.

iii. What are the mechanisms for changes in shear stress at the start of exercise?

Blocking NO-synthase causes a significant drop in brachial artery mean blood flow during cycling, especially at higher workloads (93, 95). These observations suggest that upper limb blood flow during lower limb cycle exercise is, at least partly, mediated through

endothelium-mediated release of NO. To better understand the hemodynamic stimuli responsible for NO-production under these circumstances, the role of increases in heart rate was explored in the absence of exercise-induced changes in pulse pressure (92). Heart rate was increased in patients with implanted pacemakers to levels similar to those observed during lower limb exercise. In the absence of increases in pulse pressure, isolated increases in pulsatility induced no change in brachial artery blood flow or the contribution of NO to the blood flow response. This suggests that pulse pressure, rather than pulse frequency, may be important for NO-production in the upper limb during lower limb cycle exercise *in vivo* (92).

iv. Shear stress in vascular territories perfusing inactive areas: continuation of exercise

During prolonged lower limb exercise, brachial artery blood flow and shear stress patterns in the inactive upper limbs undergo marked changes. In addition to central factors (i.e. cardiac output, arterial pressure, sympathetic nervous system), marked dilation of resistance arteries and skin microcirculation occur during prolonged exercise, mainly as a thermoregulatory response to facilitate heat exchange. This thermoregulatory dilation leads to marked decreases in peripheral vascular resistance, which subsequently affect the upstream conduit artery blood flow and shear stress patterns.

Simmons *et al.* examined the time-course of changes in skin perfusion and brachial artery shear stress patterns during prolonged cycle exercise (274). At the start of cycle exercise, the increase in brachial artery retrograde shear rate was accompanied with a modest decrease in cutaneous vascular resistance. This suggests that forearm cutaneous resistance does not mediate the initial changes in brachial artery blood flow patterns during lower limb exercise. More likely, these changes in shear pattern are mediated through increases in downstream skeletal muscle vascular resistance. Continuation of moderate-intensity cycle exercise

decreased vascular resistance, as the cutaneous microcirculation dilated to subserve thermoregulation. Hence, initial retrograde flow patterns, observed at the onset of exercise due to peripheral vasoconstriction under the effects of the SNS, eventually resolve as vascular resistance diminishes as a consequence of thermoregulatory dilation (239, 274). Indeed, forearm cooling at the end of the exercise bout significantly increased forearm and skin vascular resistance and, subsequently, increased retrograde shear (274). These data indicate the importance of integrative changes in human physiological responses to exercise. Changes in blood flow response to exercise *per se*, along with thermoregulatory modification of systemic blood flow distribution and hemodynamics, both contribute to the ultimate pattern of blood flow and shear stress through human arteries *in vivo.*

Another relevant question is whether exercise *per se* is essential to the modulation of arterial diameter in response to changes in shear stress. Carter and colleagues reported a dose-dependent dilation of the brachial artery in response to step-wise increases in shear stress which were exercise-independent (39). The hypothesis tested was that, if artery dilation during exercise is a consequence of changes in arterial shear stress, then similar changes in shear stress in the absence of exercise should induce a similar magnitude of dilation. It was observed that heating of the forearm (39, 239) or legs (41) at rest caused comparable arterial dilation in response to increases in brachial artery shear stress. More importantly, some of these studies have performed bilateral assessment of the brachial artery, with unilateral cuff inflation to effectively attenuate the heat- or exercise-induced increase in blood flow and shear in one arm, leaving the contra-lateral arm unaffected. Abolishing the exercise- or heat-induced increase in blood flow and shear stress prevented brachial artery dilation under these experimental conditions. Such within-subject designs involving simultaneously derived measurements, controls for systemic factors and subject variability, strongly imply that shear

stress is an important stimulus to acutely dilate conduit arteries in humans. These studies provide insight into the observation that repeated whole body heating (e.g. sauna) may confer clinical benefits in terms of vascular function and health (37, 178).

v. Non-shear stress hemodynamic stimuli mediating artery vasomotion during exercise

Exercise causes marked increases in transmural pressure, a stimulus that reduces arterial diameter in studies using isolated preparations and animals (59, 151). Examining the impact of transmural pressure in humans *in vivo* is challenging due to the confounding influence of concurrent changes in shear stress which typically accompany alterations in pressure. In a recent study, Atkinson and co-workers utilized 30-min unilateral handgrip exercise to induce systemic elevation in blood pressure (14). This approach was not associated with changes in shear rate in the contra-lateral arm, or changes in sympathetic nervous system activation, providing a model to isolate the impact of transmural pressure from that of shear rate *in vivo*. Unilateral handgrip exercise caused a step-wise *decrease* in contra-lateral brachial artery diameter in the resting limb, whereas these decreases in diameter were mitigated in the active limb by exercise-induced elevation in shear stress (14). This work supports the role of transmural pressure in the regulation of vascular tone and suggests active competition in distinct vascular beds between the effects of transmural wall pressure changes and changes in localized shear stress.

Taken together, the local hemodynamic stimulus, involving shear stress and transmural pressure, markedly differ between vessels supplying active *versus* non-active areas, but also differ between various types of exercise. Functional and structural characteristics of the cardiovascular system also affect the hemodynamic responses to exercise (20). The various factors influencing shear stress patterns in conduit arteries during exercise are summarized

(**Figure 5**), highlighting the complex, integrative nature of the exercise stimulus. Insight into the different hemodynamic stimuli may improve our understanding of the impact of exercise training on adaptations in vascular function and structure and the consequent implications for vascular health (107).

B. Impact of different shear stress patterns on artery function

Studies have demonstrated that acute exercise can lead to an immediate increase in endothelium-mediated dilation (60). To examine the relative importance of shear stress in these functional changes, Tinken *et al.* examined brachial artery vasodilator function, using the FMD test, before and after 30-minutes handgrip exercise (i.e. metabolically driven), cycle exercise (i.e. thermoregulatory-driven), and forearm heating (i.e. non-exercise driven) (305). After successfully increasing shear stress levels, FMD significantly improved. Given the marked differences between the 3 interventions in pulse pressure and pulse frequency, these results highlight the importance of shear stress in mediating acute changes in endothelium-mediated dilation. Indeed, unilaterally attenuating the shear stress stimulus, with preservation of the pulse pressure and frequency, abolished the improvement in FMD (305). This suggests that elevation in shear stress, independent of exercise, directly impacts vascular function in humans.

Given the intensity-dependent relationship between exercise and hyperemia, higher intensity exercise (and therefore larger shear stress) may lead to incremental increases in post-exercise vascular function. However, most studies that have explored this relationship have reported a decrease in vascular function immediately after high-intensity cycle exercise (60), that may be followed by a rebound recovery of function one or more hours after the cessation of the bout. For example, cycle exercise at 70-85% impaired FMD post exercise, a response not

observed following exercise at 50% of maximal heart rate (22). In addition to increases in shear stress, strenuous exercise also mediates other effects such as the production of ROS and activation of the sympathetic nervous system (90). These potentially detrimental effects may mitigate beneficial shear stress effects of exercise (60). To address these competing impacts, Atkinson *et al.* (13) examined the effect of incremental levels of handgrip exercise on brachial artery vascular function. Such exercise increases shear stress in the brachial artery, without producing the same degree of reflex sympathetic activation or hormonal change associated with exercise using a larger muscle group, such as lower limb exercise. The dose-dependent increase in brachial artery blood flow and shear stress in response to hand gripping was associated with post-exercise improvement in vascular function following 1 hour of recovery from the bout, at the highest exercise only. These data provide further evidence that increases in shear stress, *per se,* can improve vascular function, possibly in a dose-dependent manner.

To explore the relevance of the pattern of shear stress, one study compared the effects of 30-minute forearm heating, handgrip exercise and leg cycle exercise (305). Mean shear levels under each condition were matched by manipulating the exercise intensities. Comparable improvements in vascular function were observed under each condition. Unilateral forearm cuff inflation reduced mean blood flows in the contra-lateral arm under each of the experimental conditions, causing distinct shear patterns. Vascular function did not change after heating or handgrip exercise in the cuffed arm, whereas FMD decreased after cycle exercise in the cuffed arm, a condition associated with much greater retrograde flow and shear stress. This study therefore suggested that decreases in FMD occur particularly after exercise that induces a retrograde shear component, such as is evident in the upper limbs during leg cycling. A subsequent study utilized sub-diastolic cuff inflation (25, 50 and 75 mmHg) in resting subjects to explore the impact of 30-minute exposures to incremental levels

of retrograde shear rate, with matched levels of antegrade shear rate (291). Manipulation of the magnitude of retrograde shear in a dose-dependent manner leads to a step-wise decrease in FMD. Taken together, these data are largely in agreement with previous work in animals (see section IIB*ii*), and support a role for shear stress in the alteration of endothelial function, with distinct shear patterns potentially leading to different changes **(Figure 6)**.

If the pattern of shear stress is important, as these studies suggest, the return of brachial artery retrograde shear to baseline values and increase in antegrade shear during prolonged exercise may be advantageous. These changes reflect conversion from a potentially pro-atherogenic stimulus (patterns dominated by a retrograde component) to an anti-atherogenic stimulus. This likely represents the predominant stimulus to which arteries are exposed during prolonged exercise. The differential impacts of exercise intensity on shear patterns may also be a relevant consideration, with lower intensities potentially inducing less detrimental patterns. The protective effects of shear in this regard should be considered in the context of the epidemiological evidence, which indicates that the greatest impact on cardiovascular events occurs from adoption of lower levels of physical activity and that the benefits trail off as the volume of PA increases. The relevance of the pattern of shear stress for adaptation in vascular function and structure to exercise training is further discussed below.

IV. VASCULAR ADAPTATIONS TO EXERCISE *TRAINING*: ROLE OF HEMODYNAMIC FACTORS

A. Adaptations in vascular function

i. Conduit arteries

Studies in subjects who exhibit impaired endothelial function, such as those possessing CV risk factors (e.g. hypertension, hypercholesterolemia, type 2 diabetes mellitus, obesity) or with established CVD (e.g. heart failure, peripheral artery disease), have typically revealed improvement in conduit artery function (measured as the FMD) following exercise training (85, 104, 244, 296). Indeed, a recent meta-analysis of randomized controlled trials (12) confirmed earlier proposals (104, 196), that exercise training improves FMD, with larger improvements in populations with cardiometabolic disorders. These findings also confirm observations from our group, in which data on 182 subjects who underwent supervised centre-based exercise training were pooled. The strong inverse relation between pre-training FMD and improvement in FMD (100) suggested that conduit artery endothelial function is highly amenable to improvement, especially in subjects with the presence of CV disease and/or risk. Similarly, exercise training is able to improve coronary artery diameter, coronary blood flow responses to intra-coronary administration of acetylcholine and coronary blood flow reserve to adenosine infusion (119, 170).

To understand the role of hemodynamic stimuli on vascular adaptation to training, Hambrecht *et al.* studied the impact of 4 weeks of cycle exercise training on the internal mammary artery of CAD patients (114) (**Figure 7**). Data from the harvested arteries indicated a 2-fold increase in endothelial nitric oxide synthase (eNOS) expression and 4-fold higher eNOS Ser^{1177} phosphorylation after 4-weeks training (114). The upregulation of eNOS Ser^{1177} is of particular relevance, since phosphorylation of eNOS at position Ser^{1177} is linked to shear

stress transduction. Moreover, a correlation was present between improvement in endothelial function *in vivo* and shear-dependent eNOS phosphorylation. These data suggest that exercise causes activation of eNOS, through a shear stress-induced/Akt-dependent increase in eNOS phosphorylation on Ser^{1177}, ultimately leading to improvement in endothelial function. These results are supported by reports that exercise training improved endothelium-dependent dilation in peripheral and coronary arteries in humans (114, 115, 117) and a porcine model of early stage atherosclerotic disease (302, 322, 323).

A crucial role for shear stress in mediating vascular adaptation was described in a subsequent series of human *in vivo* studies, which adopted the model of unilateral, sub-diastolic cuff inflation to attenuate shear stress during exercise. By performing simultaneous bilateral assessments of vascular function and structure, this approach provided a within-subject model to explore the importance of shear stress, especially since both arteries were exposed to similar levels of circulating stimuli, reflex activation and pressure-related hemodynamics. Adopting this design, 8 weeks of bilateral handgrip exercise training (306) and cycle exercise training (22) resulted in significant, time-dependent changes in vasodilator function and structure of the brachial artery in the non-cuffed arm. In marked contrast, these exercise training-related adaptations across 8 weeks of exercise training were non-existent in the arm devoid of shear stress due to cuff inflation.

To further evaluate the importance of shear stress, subsequent studies induced repeated episodic increases in brachial artery shear stress using an exercise-*independent* heating stimulus (39, 222). Exposure to forearm heating increases brachial artery shear stress, whereas inflating a blood pressure cuff around the forearm abolishes such changes (39, 222). Eight weeks of exposure to forearm heating caused a time-dependent improvement in

brachial artery vasodilator function and structure, whilst no adaptation was apparent in the cuffed arm (222). Both arms were directly exposed to heat, which may impact the findings. Therefore, experiments were repeated using 8-weeks episodic submersion of the lower limbs in warm water, leading to forearm hyperemia subserving thermoregulation (40). In keeping with previous studies, adaptations in brachial artery function occurred in the uncuffed arm, but not in the cuffed arm in which shear stress was not elevated. These observations support the idea that increases in shear, independent of the method, induce vascular adaptations.

It is important to emphasise that the relative contribution of different vasodilator and constrictor pathways to the improvement in conduit artery function following training remains largely unknown. Specifically, whilst some previous work has indicated that training-induced improvements in FMD are largely mediated through NO (98), other less well studied mechanisms are nonetheless likely to contribute (136). In addition, whilst many studies have reported no change in endothelium-independent smooth muscle-mediated dilation following training, most of these adopted a near maximal dose of NO donor, and it is possible that changes in smooth muscle function as a result of training have been overlooked. Indeed, animal studies have often observed changes in artery function that are endothelium-independent as a result of training (170). These research questions require further investigation to fully address the pathways underlying generalized improvement in conduit artery function in response to exercise training in humans.

Another biophysical property of large arteries, arterial wall stiffness, can be reliably measured via pulse wave velocity (PWV), which strongly relates to atherosclerotic disease (313). Measurement of PWV compliments measures of FMD in that PWV captures structural along with functional health of the arterial wall. The increase in risk for CV events is 30% for

every 1SD change in PWV (17) a 1 m/sec increase in PWV leads to a 7% increase of the hazard for CV events (314) and PWV improves 10-yr risk classification by 13%. PWV is therefore a commonly adopted tool to examine conduit artery stiffness, with studies distinguishing 'central' (femoral-carotid PWV) or 'peripheral' stiffness (brachial-ankle PWV). Recent work analysed all randomized controlled studies of the impact of exercise training on both measures of PWV (11). Whilst a generalized effect of exercise training was observed, a somewhat larger effect size was related to longer duration of training and in those with lower *a priori* levels of arterial stiffness. Furthermore, a larger reduction in PWV after exercise training was observed for brachial-ankle PWV compared to carotid-femoral PWV. This suggests that exercise has a larger effect on 'peripheral' conduit artery stiffness compared to aortic stiffness (11). Possibly, more muscular, stiffer peripheral arteries allow for larger adaptations of arterial wall properties in response to exercise training, compared to central, more elastic arteries. In agreement with this hypothesis, one study reported that exercise training improves peripheral artery stiffness (i.e. popliteal artery), in the absence of changes in a central (carotid) artery (249). Few studies have directly examined the importance of distinct hemodynamic factors in mediating changes in the stiffness of arteries in humans.

ii. Resistance arteries

Traditionally, the impact of exercise training in resistance arteries has been studied using forearm strain-gauge plethysmography (154). When combined with intra-brachial infusion of agonists or antagonists, this allows for detailed insight into the mechanisms underlying changes with training (154). Adopting a cross-sectional design, Green *et al.* explored the impact of regular exercise on resistance artery endothelial function, and found no differences in endothelial function (infusion of acetylcholine), or contribution of NO to basal resting tone

(L-NMMA), between the dominant and non-dominant arms of elite tennis players (101). Similarly, there was no impact on NO-mediated vasodilation as a result of 4 weeks on unilateral handgrip exercise training (96). Kingwell *et al.* observed that 4 weeks cycle training did not change forearm resistance artery endothelial function, whereas an improvement in basal NO function was observed (158). Subsequent studies performed in healthy subjects have reported conflicting results regarding the impact of exercise training on resistance artery endothelial function, with some showing improvement (16, 209), but many reporting no change (195, 233). The majority of longitudinal studies in healthy subjects suggest that exercise training does not "supra-normalise" resistance vessel endothelial function (104).

Studies performed in subjects with *a prior* impaired resistance artery endothelial function have been more consistent. Exercise training in middle-aged subjects improved forearm resistance vessel endothelial function (65) and NO bioavailability (281). Exercise training also improves endothelial function and/or increases the contribution of NO to basal tone in subjects with cardiovascular risk including hypertension (131), T2DM (193, 223), obesity (202) and hypercholesterolemia (183, 316) and in subjects with coronary artery disease (114, 315) and heart failure (115, 116, 192, 194). Nonetheless, not all studies uniformly demonstrate improvement in resistance artery endothelial function (5, 16), and this may relate to the short duration and/or insufficient exercise intensity used in some studies. There are also well established impacts of sex hormones on the function of arteries (102, 213) and some preliminary evidence that the impact of training may differ (26, 108, 214), although interactions between hormones and shear stress in terms of arterial adaptation have not been directly addressed in humans. Taken together, these findings strongly support the notion that

exercise training improves resistance artery vascular function in subjects with CV risk or disease, in whom endothelial function is initially impaired (104).

Recent studies have focused on the potential impact of exercise training on the vasodilator prostacyclin (PGI_2). Hellsten and colleagues found that 8 weeks of exercise training in hypertensive participants increased the formation of interstitial adenosine and PGI_2, which may contribute to improved vascular responses after exercise training (129). Work from the same group demonstrated that exercise training increased PGI_2 muscle protein levels and muscle interstitial concentrations in older men (88), whilst training-induced increases in PGI_2 plasma levels were found in postmenopausal women (227) and hypertensive subjects (123). Others found that exercise training can improve the PGI_2-pathway in humans (330), further supporting a role for upregulation of the prostanoid system to improve endothelial function after exercise training.

Vasoconstrictors ET-1 and Ang II do not importantly contribute to the regulation of baseline vascular tone in healthy volunteers (111, 294, 312) and aerobic exercise training does not alter these vasoconstrictor pathways in healthy volunteers. In contrast, vasoconstrictor pathways are upregulated in subjects with cardiovascular disease or risk, and aerobic training is able to partly reverse the contribution of ET-1 to baseline vascular tone in older humans (297, 310). Exercise training is also associated with decreased plasma and muscle levels of ET-1 (191, 227). Regarding Ang II, training in CAD patients caused a 49% reduction in Ang II-induced vasoconstriction (2). Taken together, exercise training improves vasoconstrictor pathways in individuals who, *a priori*, demonstrate an increased contribution of vasoconstrictors to vascular tone.

The sympathetic nervous system (SNS) is a highly relevant vasoactive pathway, particularly in the context of exercise and training. Heart rate variability, a measure of autonomic balance, improves as a result of exercise training (219, 243), especially in those with autonomic disorders (243). Others have found that plasma noradrenaline decreases following training in heart failure patients. This effect may differ between healthy subjects and those with elevated noradrenaline (34). Consistent with these findings, training decreases age-related impairment in baroreflex function (207). Furthermore, muscle sympathetic nerve activity decreases after a period of exercise training, especially in subjects with elevated SNS activity (42, 232, 258). Finally, exercise training induces cyclic activation of brainstem centres, including the rostral ventrolateral medulla, which may modify central sympathetic output and vasoconstriction (217). Generally, these studies support the notion that exercise training decreases SNS activity level and SNS-mediated vasoconstriction. In contrast, some studies performed in healthy volunteers provide compelling evidence that exercise training does *not* lower SNS activity (258, 259). In fact, Sugawara and colleagues demonstrated that aerobic training in healthy volunteers increased basal SNS vasoconstrictor tone (using ∝-adrenoceptor blockade) (281). This observation concurs with some evidence for elevated sympathetic tone following training in healthy subjects (6). Despite this apparent increase in resting tone, basal blood flows were similar after training, probably as a consequence of compensatory increases in NO-mediated vasodilator function (281). In keeping with this, exercise training can increase NO-mediated vascular tone, despite preserved resting blood flow (158). These lines of evidence support the contention that increased training-induced increases in vasodilator function or arterial remodelling may be counteracted by increased sympathetic tone, with no resultant change in *resting* blood flow or arterial diameter despite enhanced vasodilator capacity.

iii. Microcirculation

Coronary arterioles from exercise trained pigs exhibit enhanced myogenic constriction compared to arterioles from sedentary pigs (218) and similar results were found in exercise trained rats (121). This enhanced tone may be due to altered calcium-dependent PKC-signalling in the coronary smooth muscle cells (163) and increased voltage-gated calcium currents in smooth muscle of large arterioles through L-type calcium channels (31) (**Figure 2**). The increased constriction in response to stretch (myogenic reactivity) is not accompanied by changes in receptor-mediated vasoconstriction (ET-1, acetylcholine) or to direct stimulation of voltage-gated calcium channel activation with the L-type calcium channel agonist Bay K8644 or by K^+ (172). Exercise training may increase activity of K_v and K_{Ca} channels of coronary vascular smooth muscles and/or alter calcium control by sarcoplasmic reticulum (33, 128, 169).

Exercise training increases the maximal adenosine-induced increase in coronary blood flow per gram of myocardium in both dogs and miniature swine *in vivo*. Although these results demonstrate that coronary blood flow capacity is increased by exercise training, resting blood flow and blood flow during submaximal exercise (same absolute intensities) is equal or slightly lower after exercise training. At similar levels of cardiac work, coronary blood flow is not changed by exercise training, suggesting a minimal effect on the coupling between myocardial metabolism and coronary blood flow (170).

Exercise training has also been reported to increase endothelium-dependent dilation in response to intra-coronary serotonin (30) and bradykinin in coronary arterioles (64-157 μm in diameter) isolated from exercise trained swine (218). The increased bradykinin-induced dilation appeared to be the result of increased NO release from eNOS, because L-NMMA

inhibited dilation to a greater extent in arterioles from exercise trained pigs and eliminated the difference between trained and sedentary groups suggesting exercise training enhances NO production by NOS (218). Consistent with this interpretation, subsequent work revealed increased endothelial NOS expression in coronary arterioles of exercise trained swine (176). The observation that cytosolic copper/zinc superoxide dismutase (SOD-1) was upregulated in coronary arterioles of trained pigs (265) suggests that the increased endothelium-dependent vasodilator responses were, at least in part, the result of decreased quenching of NO by superoxide. In both exercise trained and control arterioles, indomethacin decreased vasodilator responses without altering the exercise effect. Importantly, the sodium nitroprusside response did not differ between sedentary and trained swine (218), implying that exercise increased NO production in the endothelium. Indeed, Laughlin *et al.* (176) demonstrated increases NO synthase content in the coronary endothelium of exercise trained normal pigs.

In coronary arterioles isolated from animal models of vascular disease, exercise training is reported to increase basal myogenic tone and endothelium-dependent dilation (73, 130). Fogarty *et al.* (73) vascular endothelial growth factor (VEGF165) mediated vasodilation was enhanced by exercise training via elevated NO bioavailability. So, available evidence indicates that in animal models of coronary artery disease and in patients with coronary disease, exercise training increases endothelium-dependent dilation in coronary arterioles (86, 185, 253).

iv. Cutaneous microcirculation: an active vessel bed during exercise

During exercise in humans, when a given threshold is reached, cutaneous vasodilation increases linearly with increases in core temperature until a plateau is achieved. Exercise

training modifies this response, causing a leftward shift of the relation between cutaneous vasodilation and core temperature (i.e. vasodilation at a lower threshold) and a higher plateau (i.e. larger blood volume to the skin for heat dissipation) (254). In understanding these adaptations, a previous study linked responses to changes in blood volume (144). Ikegawa and colleagues trained healthy men for 5 days and reinforced the presence of a leftward shift for the temperature threshold for skin vasodilation, increased plateau and expansion in plasma volume (~10%). When these tests were repeated after removal of the increase in plasma volume, the leftward shift in temperature threshold for cutaneous vasodilation and increase in plateau were eliminated. These results suggest that initial training-induced adaptations in cutaneous blood flow importantly depend on expansion of circulating blood or plasma volume (144). Whilst these adaptations seem essential for systemic thermoregulatory purposes, exercise training may also affect intrinsic microvascular function. These intrinsic adaptations may be particularly relevant to the prevention of microvascular disease and its manifestations.

Studies investigating intrinsic cutaneous microvascular function have utilized skin laser-Doppler to assess local skin flux responses to substances such as acetylcholine (administered using iontophoresis or microdialysis), local heating and/or reactive hyperemia. Local heating is often applied, especially since the plateau phase after sustained local heating is largely NO-mediated (204) and can provide an index of NO-mediated microvascular function. A cross-sectional study found that the heating plateau phase was significantly larger in exercise trained individuals compared to their sedentary peers (255), suggesting that exercise training is associated with improved NO-availability in the skin, a finding that supports observations in the studies that adopted iontophoresis or microdialysis (164, 317). Studies have also explored the effects of exercise training on cutaneous reactive hyperemia. Although the

technique and data analysis differ between studies, cross-sectional comparisons report larger skin hyperemia responses in favour of the trained participants (76, 182, 311). Since these skin responses are correlated with nitrite/nitrate concentration (76) and plasma antioxidant capacity (77), larger skin microcirculatory responses to heat or ischemia observed with training may be related to the NO-pathway and/or oxidative capacity.

Studies adopting longitudinal, prospective designs confirm these cross-sectional observations, in that regular exercise training improves cutaneous vascular function. These studies indicated that cutaneous responsiveness to both local heating stimuli and acetylcholine microdialysis were enhanced in response to exercise training in young (164, 317) and older humans (27, 132). Black and colleagues also explored the role of the NO-pathway in these adaptations by blocking NO-production before and after training. They found that improvement in cutaneous microvascular responsiveness to exercise training was, in large part, due to improvements of the NO-pathway (27).

The stimulus responsible for the intrinsic cutaneous vascular function adaptations to exercise training may relate to the hemodynamic impact of repeated exposure to increases in skin blood flow. To test this hypothesis, the impact of repeated episodic elevation in cutaneous blood flow, achieved by directly heating both forearms, was examined (42 °C, 8 wks, 3 session/wk), whilst unilateral manipulation using cuff inflation attenuated cutaneous dilation in one arm (97). After 8 weeks of this conditioning, cutaneous vasodilator responses to local heating were enhanced in the uncuffed arm, whereas this adaptation was not observed in the cuffed arm. A similar study found that abolishing forearm cutaneous vasodilation in response to 8 weeks of repeated lower limb heating also prevented adaptation in the skin microvascular observed in the arm exposed to repeated increases in flow (40). In these studies, cutaneous

vasodilation was accompanied by increases in forearm skin temperature during lower limb heating (40) and direct forearm heating (97). Therefore, heat application *per se* may represent a stimulus for adaptation, possibly by virtue of interactions between NO and heat shock proteins (81). Indeed, when the increase in skin temperature in response to lower limb heating were "clamped" by submersion of one forearm in thermoneutral water, skin microvascular adaptations differed from those observed in the "unclamped" limb, in which both temperature and blood flow increased (40). These results support an evolving hypothesis that repeated increases in skin blood flow induce intrinsic skin microvascular function, whilst changes in skin temperature may contribute to the nature of the adaptation. The relationship between these findings and recent elegant observations by Alexander and colleagues that impaired skin blood flow responses in subjects with cardiovascular risk factors may be dependent upon tetrahydrobiopterin coupling of NOS, remains to be determined (4).

B. Adaptation in vascular structure

i. Conduit arteries

An early observation of enlargement of vessels in response to intense exercise training dates to 1961 and relates to the autopsy of Clarence DeMar who ran 34 marathons (including 7 wins of the Boston Marathon) (53) and in whom 'unusually large coronary arteries' were described post-mortem. More recently, ultrasound- and MR-based techniques have confirmed the idea that regular exercise training is associated with enlarged coronary artery size (125, 225) and dilator capacity. This type of remodelling is analogous to the concept, originally introduced by Morganroth and colleagues, that regular (endurance) exercise training involves repeated hemodynamic stimuli that remodel the heart (215). Larger coronary arteries are found after exercise training and may facilitate the increased oxygen demand associated with cardiac hypertrophy and increased cardiac workloads during exercise (170).

Athletes also exhibit increased diameter in large peripheral arteries (i.e. aorta, carotid, subclavian arteries), relative to matched sedentary controls (327) and exercise training studies have revealed remodelling of conduit arteries (66, 224, 280, 293), providing direct evidence that regular exercise increases conduit artery lumen diameter. To better understand the process of remodelling, studies have explored whether remodelling occurs regionally (i.e. related to local processes) or consistently across vascular beds (i.e. related to systemic processes). Huonker *et al.* found that wheelchair athletes (engaged with upper body exercise) possess enhanced dimensions in the aortic arch and subclavian artery, but smaller diameters of the femoral artery, compared to able-bodied controls (140, 141). In line with these observations, Rowley and co-workers found largest brachial artery diameters in canoeists and kayakers, whilst within-subject differences were present between the dominant and non-dominant brachial arteries of elite squash players (261, 262). Longitudinal studies involving unilateral leg cycle (206) or unilateral upper arm exercise (329), combined with bilateral assessment of diameter, provided further evidence that exercise training leads to localized adaptation in conduit artery diameter. Taken together, these studies strongly suggest that exercise training induces localized adaptation of conduit artery diameter, typically supplying the physically active limb.

Studies performed in animals demonstrate that experimentally increasing blood flow leads to significant outward remodelling. Inhibition of NO synthesis, either by administration of NO-synthesis inhibitors (307) or in eNOS knockout mice (264), results in no changes in conduit artery diameter in response to chronic increases in shear stress (**Figure 4**). The importance for the NO-pathway was confirmed in subsequent work, in which changes in eNOS gene-expression strongly correlated with the magnitude of change in shear stress levels (309). Studies in humans also suggest that repeated exposure to shear stress, associated with

exercise training, represents a key stimulus for remodelling. When exploring bilateral brachial artery adaptations to handgrip (306) or cycle (23) exercise training, unilateral shear manipulation with sub-diastolic cuff inflation prevented increases in peak brachial artery diameter observed in the arm exposed to episodic increases in shear. Hence, data from animals and humans support an important role for shear stress in the mediation of structural outward remodelling of conduit arteries.

A widely adopted hypothesis to explain remodelling of conduit arteries is that changes in diameter represent an attempt to normalise shear stress (156). Unfortunately, most previous studies in humans have not documented shear stress, limiting insight into the concept that shear stress is 'regulated' by structural arterial adaptation. In a cross-sectional study, larger femoral artery diameter and blood flow in endurance trained athletes were reported, compared to controls, whilst mean and peak shear stress levels were comparable (266). Furthermore, 8 weeks cycle exercise training resulted in an increase in the diameter *and* blood flow of the ascending and abdominal aorta, whilst blood velocity and shear stress were preserved (205). Whilst studies are not conclusive, this work provides some support for the notion that shear stress is regulated via remodelling, (66, 140). It is important to emphasise that most of the interventional exercise training studies have been undertaken over relatively brief time periods (4-12 weeks), whereas many of the classic structural adaptation findings relate to cross-sectional comparison of controls to athletes who have trained for decades. Nonetheless, those studies that have involved longer training interventions have generally produced results consistent with the cross-sectional findings (280).

To understand the requirement for artery remodelling, it is important to appreciate the relationship between blood flow and oxygen consumption. For example, one-legged exercise

training caused a significant increase in femoral artery diameter that was strongly related to the increase in the one-legged maximal oxygen uptake (r=0.86) (206). These findings support observations in healthy volunteers that conduit artery diameter is related to lean mass (135, 329). Indeed, the marked differences in femoral artery diameter between subjects with paraplegia and controls disappeared after correcting for differences in thigh lean mass (230). In agreement with these findings, a similar time-course of changes in femoral artery diameter and leg muscle volume is reported following a spinal cord injury (62) or during a period of functional electrical stimulation-assisted exercise training in spinal cord-injured individuals (295). Therefore, regional increases in blood flow, tightly coupled with the metabolic demand of the distal muscle, are associated with training-induced arterial remodelling and facilitate the ability to perform aerobic work.

Conduit arteries (wall thickness). When directly comparing endurance-trained and sedentary populations, most studies found no significant differences in carotid wall thickness between trained and untrained cohorts (288). In contrast, Rowley *et al.* found a smaller carotid artery wall thickness in elite athletes compared to sedentary controls (261, 262). One explanation for these differences in findings is that elite athletes are exposed to a larger volume, duration and intensity of exercise than recreationally active subjects (212, 247, 285). A recent study reported a significant decrease in carotid artery wall thickness after 6 months of endurance or resistance exercise training (279). This longitudinal work provides further evidence that carotid artery wall thickness is modifiable and another recent study showed that 8-weeks of exercise training induced comparable changes in wall thickness in the popliteal (i.e. supplying the active lower limbs) and the carotid (i.e. supplying a non-active area) arteries (108). Therefore, cross-sectional and longitudinal training studies both suggest potent effects

of training that can mediate reductions in conduit artery wall thickness supplying the active and non-active areas (289).

To address the hemodynamic stimuli contributing to adaptation in wall thickness, Rowley *et al.* compared brachial artery wall thickness between the dominant and non-dominant arm of squash players (261). This model was based on the assumption that the brachial artery in the dominant arm is exposed to higher levels of shear stress, whereas systemic hemodynamic stimuli are similarly present in both arms. No differences in brachial artery wall thickness were found between the arms. In a follow-up study, brachial and femoral artery wall thickness were compared between elite athletes engaged in lower limb *versus* upper limb exercise, healthy controls, wheelchair controls and athletes (262). All athletes (able-bodied and wheelchair-bound) showed a smaller brachial and femoral artery wall thickness compared to their physically inactive peers. These findings suggest that shear stress is not the sole or key stimulus responsible for arterial wall remodelling in humans. A further study provided more direct evidence for this hypothesis by performing bilateral handgrip exercise, with unilateral cuff manipulation to manipulate the shear stress stimulus (292). Despite successfully manipulating the shear stress response between the limbs, brachial artery wall thickness showed a small, but significant gradual decline in both arms as a result of training. These findings suggest that adaptations in wall thickness in response to exercise training may occur largely independently of localized elevations in shear stress.

In interpreting studies that address the impact of exercise training on arterial wall thickness, it should be acknowledged that the arteries are constantly modifying their vasomotor tone. Thijssen *et al.* demonstrated that reducing vascular tone through sublingual administration of glyceryl trinitrate leads to a generalized and marked acute decrease in wall thickness in both

young and older humans (298). Changes in arterial wall thickness may therefore be mediated, at least in part, by changes in functional tone, along with structural remodelling in the vessel wall. This is particularly relevant in the case of exercise training, since training impacts vascular tone and function.

According to Laplace's Law, an increase in diameter leads to an increase of the circumferential wall stress, typically followed by an increased wall thickness to lower wall stress (given the inverse relation between wall thickness and wall stress). Indeed, such adaptations have been described in the process of atherosclerosis. Regular exercise training also leads to an increase in diameter and, therefore, will lead to an increase in circumferential wall stress. However, as described above, exercise training has been associated with a *decrease* in wall thickness. Accordingly, one hypothesis is that exercise training leads to remodelling of the arterial wall and tissue organisation that result in the ability to sustain higher wall tension in the presence of decreased thickness.

The ability of exercise training to alter arterial wall characteristics may be relevant for atherosclerotic plaque development. Recent animal studies suggest that exercise training is associated with stabilisation of atherosclerotic plaque and increased content of collagen and elastin (273). In a retrospective analysis in humans, higher physical fitness levels were associated with high fibrous volume and thick fibrous cap thickness of coronary plaques (324). Recently, 12-weeks of exercise training in patients with CAD decreased the necrotic plaque core (190). These effects of exercise training on conduit artery wall characteristics may have important clinical implications in terms of both plaque stabilisation, the evolution of plaque volume and rupture.

ii. Resistance arteries

A traditional approach to assess resistance artery "structure" involves creating a stimulus that leads to peak blood flow, often through prolonged ischemia (>15 minutes) or ischemia combined with exercise. An inability to further increase flow suggests that peak blood flow reflects a "structural ceiling" or capacity measure of the vascular bed. Using this technique, Sinoway *et al.* compared the dominant and non-dominant forearms of recreational tennis players to assess the impact of prolonged intensive exercise. They found that the preferred limb exhibited higher peak vasodilator responses than the non-preferred limbs, whilst no bilateral differences in forearm peak blood flows were observed in controls (276). Comparable findings were later observed when comparing vascular responses between both arms in elite tennis players (101) and elite squash players (261), but also when comparing leg peak blood flow between elite athletes and controls (277). The presence of a higher peak blood flow after exercise training, either adopting between- or within-subject comparisons, supports the ability of exercise training to cause remodelling of resistance arteries.

Resistance artery remodelling is a localized adaptation. Studies involving cycle exercise training found no changes in forearm peak blood flow (23, 261, 262). These observations suggest that dominant local hemodynamic factors, such as shear stress, contribute to remodelling of resistance arteries (as is the case in conduit arteries, described above). Some support for this was provided by Tinken *et al*., who found that the increase in brachial artery peak blood flow after 8-weeks handgrip exercise training was abolished when exercise-induced increases in blood flow were clamped using a unilateral cuff manipulation (306). Interestingly, 8-week repeated elevation in brachial shear stress through cycle exercise training (23) or heat exposure (222) did not alter brachial artery peak blood flow. Therefore, resistance artery remodelling in response to exercise training is tightly coupled to metabolic

work performed by the muscle area perfused by the vascular bed. Indeed, lower limb peak blood flow, but not forearm peak blood flow, is coupled with whole body peak oxygen consumption (152, 278). These data suggest that repeated increases in shear stress represent an important stimulus for enlargement of resistance arteries. That is, structural enlargement of skeletal muscle resistance vessels seems to require an increase in metabolic work which leads to the increased skeletal muscle blood flow and shear stress stimulus.

iii. Microcirculation

The enhanced intrinsic vasodilator capacity of the muscle microvasculature following training may conceivably result from the increase in capillary density that occurs with training (7). Histochemical analyses of muscle biopsies, often taken from the quadriceps muscle, assess capillary density and the capillary-to-fibre ratio. The majority of studies examining the impact of exercise training, either adopting cross-sectional (athletes vs controls) or longitudinal design, demonstrate significant and marked increases in the total number of capillaries, capillary-to-fibre ratio and capillary density in response to different training regimes (mode, intensity and duration) and across various age groups (133). These structural changes in skeletal muscle microvasculature have been strongly linked to improvements in local and whole body peak oxygen uptake. For example, a previous study found that increases in capillary density represent an early adaptation during exercise training that precedes the improvement in peak oxygen uptake (70). Therefore, growth of capillaries represents an important adaptation to regular exercise training that enables sufficient diffusion capacity, even under highly demanding conditions during which muscle blood flow is profound. However, studies have suggested that an increase in capillary density may not necessarily affect muscle blood flow supplying the skeletal muscle (198). More likely, the increase in capillary density may prolong transit time of red blood cells through the muscle

capillaries, leading to an increased time frame for gas exchange within the muscle capillaries (36, 69, 177). These beneficial adaptations are hypothesized to contribute to an improved microvascular *milieu* that allows for more efficient diffusion of oxygen from the capillaries to (muscle) cells (87), whereas the locus of control of muscle blood flow lies further upstream from these small vessels, in feed arterioles and resistance vessels (271).

Few studies have explored the importance of hemodynamic stimuli in mediating microvascular adaptation, mainly because these hemodynamic stimuli are extremely difficult to quantify and manipulate, particularly in humans. Some studies, however, have provided indirect insight into factors that may contribute to microvascular adaptation to training. Esbjornsson *et al.* examined whether skeletal muscle capillarization in response to 4-week one-legged cycle training is different when performed under local ischemia (72). By performing leg cycle exercise in a sealed, +50 mmHg pressure chamber, local blood flow to the exercising limb was impaired. Consequently, the limb exposed to the impaired blood flow (and ischemia) during exercise showed a larger increase in capillary-to-fibre ratio after training compared to the contra-lateral limb that underwent the same amount of work under normoxic conditions (72). In agreement with this study, exercise training performed under hypoxia showed a larger improvement in capillary density (83) or capillary-to-fibre ratio (161) compared to exercise training under normoxia. These findings suggest that (localized) hypoxia, possibly mediated by exercise, represents an important modulator for structural adaptation in the skeletal microvasculature (36, 133).

In addition to hypoxia, mechanical forces that are present during muscle activity, such as shear stress and passive stretch, contribute to adaptation in muscle capillary growth. Angiogenic factors involved in mediating these adaptations include vascular endothelial

growth factor (VEGF). During muscle contractions, VEGF increases in the muscle and binds to VEGF-receptors on the capillary endothelium. As a direct consequence, VEGF triggers an angiogenic process that contributes to remodelling of the capillary vascular bed. Furthermore, exercise-induced release of VEGF-containing vesicles in the circulation leads to rapid replenishment of the VEGF stores. Consequently, this allows for VEGF secretion upon exposure of a subsequent bout of exercise (133). To reiterate, growth in capillaries, whilst important for gas exchange, may not be a significant determinant of blood pressure regulation *in vivo* (271).

iv. Cutaneous microcirculation: an active vessel bed during exercise

Atkinson *et al.* recently reported a significant decrease in peak cutaneous flux-responses to local heating after training (15). Although the lower peak flux to local heating seems somewhat counterintuitive, these observations may support the presence of enlargement of the capillary bed. As a result of a larger capillary bed, peak blood velocity (or flux; measured using laser-Doppler) will decrease for the same volume of blood to pass through the capillaries. This may lead to prolongation of red cell transit time through the skin microcirculation, akin to the prolongation of transit time as a result of exercise training in skeletal muscle (133).

C. Changes in vascular cell gene expression induced by exercise training

A recent series of studies evaluated the effects of regular exercise on the vascular transcriptome in pigs (238) and rats (174, 175, 235). A common finding is that the effects of exercise are heterogeneous across vascular beds. In a recent and comprehensive study (174), next-generation, transcriptome-wide RNA sequencing (RNA-Seq) technology was used to assess the effects of exercise training on transcriptional profiles in skeletal muscle arterioles

isolated from the soleus and gastrocnemius muscles of Otsuka Long Evans Tokushima Fatty (OLETF) rats, a model of obesity and type 2 diabetes. In this study rats underwent a 12-week endurance exercise training program, interval sprint training program, or remained sedentary. Endurance exercise caused the greatest number of changes in gene expression in the soleus and white gastrocnemius 2a arterioles, with little to no changes in the feed arteries. In contrast, interval sprint training produced considerable changes in gene expression in the feed arteries. Ingenuity-pathway analysis revealed 18 pathways with significant changes in gene expression when analyzed across vessels (174). From this comprehensive analysis it was concluded that training-induced changes in arteriolar gene expression patterns differ by muscle fiber type composition and along the arteriolar tree. It should also be noted that the effects of exercise on gene expression may manifest to a greater extent in the arteries perfusing the working muscles; however, effects of exercise beyond the active muscle beds are also apparent (238), providing evidence that the effects of vascular exercise are systemic but not homogenous. Studies in humans introduced the notion of a systemic impact of training on vascular adaptation and follow-up studies investigated the potential contribution of hemodynamic stimuli to this adaptation (93-95, 290, 305). In addition, reviews summarized the mechanisms related to vascular adaptations beyond active muscle beds (104, 196, 237).

In addition, using RNA-sequencing, the extent to which the effects of obesity (i.e. differences between obese OLETF rats and lean-counterparts rats) on aortic endothelial gene expression could be reversed by endurance exercise was recently reported (146, 235). Exercise altered expression of 324 endothelial genes but only partially or totally restored expression of 8.6% of 396 genes affected by obesity (146, 235). This finding, that only a small fraction of endothelial transcriptional changes produced by obesity can be offset by regular exercise,

further supports the notion that exercise exerts direct effects on the artery wall, independent of reductions in obesity and other related co-morbidities.

Finally, it is important to emphasize that studies of the myriad factors that impact vasomotor function in humans have emphasized the notion of compensatory redundancy in control (267). Conclusions based on gene expression data should be informed by functional assessment of the relative importance of different pathways to the integrated adaptive response to training, particularly given that few pathways appear to be obligatory in the acute functional response to exercise (187).

V. WHICH FACTORS MODERATE THE ADAPTATION TO TRAINING?

A. Distinct adaptations to different forms of exercise training

Type of exercise. Most studies focused on the differential impact of distinct types (modalities) of exercise have compared athletes and controls or fit and unfit individuals, but subject differences introduce significant bias in terms of the true impact of exercise training, limiting the validity of implications regarding the impacts of exercise *per se* (279). Spence *et al.* directly compared the impact of endurance *versus* resistance exercise training within-subjects on conduit artery vascular adaptation (279). Six months upper limb-dominant resistance training improved brachial, but not femoral, artery resting and peak diameter (indicative of structural remodelling) and vascular function. In contrast, lower limb endurance exercise training increased resting and peak femoral, but not brachial, artery diameter and vascular function (279). These observations of distinct adaptations between resistance and endurance exercise may be linked to site specific elevation in blood flow (and shear stress) in the active limbs during exercise (i.e. the hemodynamic stimulus), rather than the type of exercise *per se*. Future studies, adopting direct comparison between different types of exercise within subjects, are required to understand the differences between exercise types, but also the importance of the magnitude of exercise-induced elevation in blood flow and shear rate.

Exercise intensity. Few studies have performed direct comparisons between the same *mode* of exercise training, performed at different *intensity* levels, on vascular function and structure. Goto *et al.* performed an elegant study which randomized subjects to perform 12-week cycle exercise training at mild, moderate or high intensity (90). Only moderate-intensity exercise was associated with improvement in NO-mediated endothelial function and a decrease in markers of oxidative stress. The absence of adaptation after high-intensity exercise training was hypothesized to be the result of the induction of significant oxidative stress during each

bout of intense exercise, potentially mitigating the effects of exercise on the endothelium. Some evidence for this hypothesis was provided in a follow-up study, where these authors demonstrated that high-intensity, but not mild- or moderate-intensity exercise, caused increases in markers for oxidative stress (91). These observations are largely in line with studies examining the acute effects of exercise on vascular function. Whilst low-to-moderate exercise intensity shows somewhat conflicting results (24, 305), an increase in exercise intensity is typically associated with a (larger) decrease in vascular function (60) immediately post-exercise. Taken together, these studies support the notion that a dose-response relationship exists in terms of functional responses the vasculature to exercise and training, and that higher intensities of exercise may truncate benefits of training via impacts on inflammation and oxidative stress. It is important to reiterate that the epidemiological evidence suggests that the largest impact on vascular risk occurs from the adoption of lower volumes and intensities of physical activity.

Somewhat in contrast to this paradigm, studies on the effects of high-intensity interval training (HIIT), which is characterized by repeated exposure to short bouts of exercise (1-4 minutes) performed at near or supra-maximal level (84, 320), suggest potentially superior effects compared to traditional endurance exercise. Some studies have compared the impact of HIIT to more conventional endurance training on various outcome measures, including vascular function and structure. Recent work suggested that HIIT leads to superior improvements in vascular function compared to endurance training (250), although selection bias and inconsistencies in the FMD protocol may contribute to these findings. Little work has explored the potential (superior) effects of HIIT on resistance arteries and/or microvessels, whilst concerns about the potential health risks of HIIT have not yet been ruled out (157). Taken together, this relatively new field on HIIT requires further work to better

understand the potential benefits, if any, over more traditional and graduated approaches to intensity prescription, particularly in higher risk populations.

B. Distinct time-course in adaptation in different vascular properties

Animal and human data support the existence of different time-course effects of adaptation in artery function and structure as a consequence of exercise training (104). In healthy animals, 1-4 weeks of exercise training improved vasodilator function in conduit arteries (200), muscle arterioles (282) and the aorta (64) and was associated with increased eNOS expression in pulmonary arteries (150). In marked contrast, studies adopting 16-20 weeks exercise training have not consistently shown augmented endothelial function (199) or changes in eNOS expression (149). Nonetheless, longer training duration is associated with enlargement of arterial diameters. Based on these cross-sectional observations, Laughlin proposed that initial improvements in vascular dilatory function contribute to normalise shear stress during exercise bouts, whereas continuing exercise will result in a more "permanent" normalisation of shear stress (168). As a consequence of structural enlargement, initial improvement in vascular function returns towards baseline **(Figure 8)**.

A human study designed to test this proposal utilized repeated assessments of the time-course of adaptation of vascular function and structure in response to exercise training. Tinken *et al.* examined both brachial and popliteal artery function and structure across 8-weeks of exercise training in healthy volunteers (304). The results confirm the hypothesis that exercise training leads to an initial improvement in vasodilator function, which returns toward baseline once structural remodelling occurs. Comparable findings of time-dependent adaptation in vasodilator function and structure have been reported in subsequent studies involving cycle exercise (23), handgrip exercise (306), resistance training with blood flow restriction (138),

but also local (222) and systemic (41) heating in the absence of an exercise stimulus. Taken together, these data in humans provide strong evidence for time-dependent adaptations in vascular dilatory function and structure across a period of exercise training, although the impacts of cardiovascular ageing and/or the presence of endothelial dysfunction may modulate the relative time course (65).

C. Interaction between changes in function and structure

Seminal work on the impact of structural characteristics on the function of arteries was performed by Folkow in the mid-1950s (75). His work focused on the arterial structural adjustments observed in hypertension, and explored the impact of a thickened arteriolar wall on vascular resistance and BP. Based on calculations, Folkow revealed that an increased wall-to-lumen ratio would produce exaggerated luminal changes to any vasoactive stimulus (74, 75). Contraction of smooth muscle also causes increased wall tissue mass that has impact on lumenal dimensions. Thickening of the arterial wall, therefore, is accompanied by vascular hyper-reactivity, even in the absence of changes in vasoactive signal transduction. Recently, this hypothesis regarding interaction between structural and functional characteristics was supported in conduit arteries in humans *in vivo* (300). Shear-dependent and –independent vasodilation was strongly and positively related to wall-to-lumen ratio, with a larger dilation observed in conduit arteries that exhibit a larger wall-to-lumen ratio. Therefore, structural changes of the arterial wall likely impact functional responses and adaptation.

D. Impact of cardiovascular disease on hemodynamic stimuli and vascular adaptation to exercise

Hemodynamic stimuli during exercise. CV risk and disease may alter blood flow responses to exercise. Early studies performed in the 1940s revealed (1, 25) differences between healthy

and diseased populations in terms of changes in blood flow to the hands. In agreement with these observations, others reported impaired ability to lower vascular resistance during exercise in subjects with CV disease or risk (29). These distinct vascular responses between healthy subjects and subjects with CV disease potentially contribute to altered shear stress patterns during exercise.

HF patients exhibit impaired vasodilator responses to passive heat exposure, the mechanism for which was at least partly due to impaired NO-mediated dilator function (105). During exposure to 38 °C in a heat chamber, HF patients demonstrated attenuated heating-induced vasodilation of the skin and controls exhibited elevated NO dilator function. Also during cycle exercise, HF is associated with attenuated skin temperature responses compared to healthy controls (18). Interestingly, a recent study compared cycle exercise-induced brachial artery shear stress between HF patients and healthy age-matched controls (19). It was observed that HF was associated with an exaggerated exercise-induced increase in retrograde shear stress and attenuated increase in antegrade shear stress, which both remained present throughout the 30-minute cycle bout. Previous work has linked cutaneous vasodilation to attenuation of retrograde shear during prolonged cycle exercise in healthy volunteers (274). Accordingly, attenuated cutaneous vasodilatory responses to passive heat and exercise in HF patients may contribute to the distinct antegrade and retrograde shear stress patterns during exercise in HF. Understanding these interactions is very important as these observations suggest that exercise may have untoward effects in HF patients because it these hemodynamic effects would be expected to blunt the beneficial effects of exercise on vascular structure and function.

Exercise in subjects with CV disease/risk is also associated with exaggerated exercise-induced blood pressure responses (29) which may relate to the presence of endothelial dysfunction, oxidative stress and/or impaired neuro-hormonal activation. Subjects with exaggerated exercise-induced blood pressure responses also exhibit transient post-exercise *hyper*tension, rather than the *hypo*tension; normally present in healthy volunteers after exercise (82). Therefore, subjects with CV disease and/or risk may be exposed to higher pressure responses during and after exercise, potentially impacting subsequent vascular adaptation.

Adaptation to exercise. Studies in animals have provided some evidence that CV risk factors impair shear stress mechanotransduction (328) and attenuate NO-release upon elevation in shear stress (283). The presence of impaired ability to detect and respond to hemodynamic stimuli, in combination with altered hemodynamics during exercise (see above), may affect vascular adaptation to exercise training. In contrast to this hypothesis, studies in humans have typically found improvement in vascular function after exercise training in populations with CV disease or risk (104). Moreover, a recent study found larger improvement in vascular function in those with *a priori* impaired function (99). Therefore, despite exposure to unfavourable hemodynamic stimuli, there is sufficient evidence for improvement of vascular function as a result of exercise training in subjects with CV risk or disease.

Some studies suggest that CV risk factors may impair vascular structural adaptation to training. For example, studies in animals have demonstrated that expansion of resistance arteries occurred in young animals after 1-2 weeks exposure to high flow, whilst such adaptation was absent in older animals (68, 78). Interestingly, the capacity for arterial expansion in older animals was restored under co-infusion of drugs directly impacting

vasodilator function of these arteries (68, 78). In young and older humans, our laboratories explored vascular changes in response to increases in brachial artery retrograde shear stress (269, 299). We found that 30-minute and 2-week elevation in retrograde shear stress caused a decrease in endothelial function and smaller diameter in young subjects, whilst such adaptations were not observed in older individuals (299). Furthermore, Hansen *et al.* found that hypertension is associated with an attenuated exercise-induced release of VEGF, an important angiogenic factor that is linked to capillarisation (see above) (122). The attenuated release of VEGF after exercise was also associated with a limited effect on capillary density after training. In another exercise training study, 6-week exercise training caused a significant increase in the capillary-to-fiber ratio in healthy controls, whilst such adaptation were absent in heart transplant recipients (166).

Summarizing this work, subjects with CV disease or risk may demonstrate less favourable hemodynamic stimuli during exercise and/or impaired angiogenic or adaptive stimuli in response to training. Despite these unfavourable hemodynamic stimuli in subjects with CV risk and/or disease, arteries remain highly adaptive for improvement in vascular function. Nonetheless, some evidence suggests that structural adaptations are less likely to occur in those with CV risk and/or disease. These data highlight the complexity of the integrative stimuli evoked by exercise and their ultimate impacts on vascular adaptation.

SUMMARY AND IMPLICATIONS FOR EXERCISE SCIENCE AND HEALTH

Exercise is anti-atherogenic and increasing physical activity has a profound impact on cardiovascular risk. Whilst some of this is due to exercise-mediated modification of traditional cardiovascular risk factors, exercise is a relatively weak poly-pill for changes in CV risk factors compared to the impacts of pharmacological agents (303). In contrast, the beneficial impacts of exercise on CV risk exceed that expected from changes in CV risk factors alone and this *risk factor gap* (106, 155) may be filled, at least in part, by the direct impacts of exercise on the artery wall. On the evidence presented in this review, it is clear that the hemodynamic impacts of exercise on blood flow and pressure transduce acute changes in vascular function and that repeated exercise leads to arterial adaptation in humans. Exercise can therefore be considered an evolutionary stimulus to maintaining human vascular health (106, 155). In the same way that exercise is accepted as a stimulus to the maintenance of musculoskeletal function in the face of ageing, frailty and disease, exercise and associated hemodynamic forces are a direct form of vascular medicine in humans.

Finally, it is timely, on the 400th anniversary of the lectures which revolutionized science by revealing the importance of the movement of the heart and blood in livings beings, to reflect that the introduction to *Exercitatio Anatomica de Motu Cordis et Sanguinis in Animalibus* states *"**Very many maintain that all we know is still infinitely less than all that still remains unknown…."***, a statement that remains as true in the age of high resolution non-invasive imaging, as it was in the time of Harvey's anatomical exercises.

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REFERENCES

1. **Abramson DI, Fierst SM, and Flachs K**. Effect of muscular exercise upon the peripheral circulation in patients with valvular heart disease. *The Journal of clinical investigation* 21: 747-750, 1942.
2. **Adams V, Linke A, Krankel N, Erbs S, Gielen S, Mobius-Winkler S, Gummert JF, Mohr FW, Schuler G, and Hambrecht R**. Impact of regular physical activity on the NAD(P)H oxidase and angiotensin receptor system in patients with coronary artery disease. *Circulation* 111: 555-562, 2005.
3. **Alberts-Grill N, Rezvan A, Son DJ, Qiu H, Kim CW, Kemp ML, Weyand CM, and Jo H**. Dynamic immune cell accumulation during flow-induced atherogenesis in mouse carotid artery: an expanded flow cytometry method. *Arterioscler Thromb Vasc Biol* 32: 623-632, 2012.
4. **Alexander LM, Kutz JL, and Kenney WL**. Tetrahydrobiopterin increases NO-dependent vasodilation in hypercholesterolemic human skin through eNOS-coupling mechanisms. *Am J Physiol Regul Integr Comp Physiol* 304: R164-169, 2013.
5. **Allemann Y, Vetter C, Kartal N, Eyer S, Stengel SM, Saner H, and Hess OM**. Effect of mild endurance exercise training and pravastatin on peripheral vasodilatation of forearm resistance vessels in patients with coronary artery disease. *Eur J Cardiovasc Prev Rehabil* 12: 332-340, 2005.
6. **Alvarez GE, Halliwill JR, Ballard TP, Beske SD, and Davy KP**. Sympathetic neural regulation in endurance-trained humans: fitness vs. fatness. *J Appl Physiol (1985)* 98: 498-502, 2005.
7. **Andersen P, and Henriksson J**. Capillary supply of the quadriceps femoris muscle of man: adaptive response to exercise. *The Journal of physiology* 270: 677-690, 1977.

8. **Anderson EA, and Mark AL**. Flow-mediated and reflex changes in large peripheral artery tone in humans. *Circulation* 79: 93-100, 1989.
9. **Anderson L, Oldridge N, Thompson DR, Zwisler AD, Rees K, Martin N, and Taylor RS**. Exercise-Based Cardiac Rehabilitation for Coronary Heart Disease: Cochrane Systematic Review and Meta-Analysis. *Journal of the American College of Cardiology* 67: 1-12, 2016.
10. **Ando J, and Yamamoto K**. Flow detection and calcium signalling in vascular endothelial cells. *Cardiovascular research* 99: 260-268, 2013.
11. **Ashor AW, Lara J, Siervo M, Celis-Morales C, and Mathers JC**. Effects of exercise modalities on arterial stiffness and wave reflection: a systematic review and meta-analysis of randomized controlled trials. *PLoS One* 9: e110034, 2014.
12. **Ashor AW, Lara J, Siervo M, Celis-Morales C, Oggioni C, Jakovljevic DG, and Mathers JC**. Exercise modalities and endothelial function: a systematic review and dose-response meta-analysis of randomized controlled trials. *Sports Med* 45: 279-296, 2015.
13. **Atkinson CL, Carter HH, Dawson EA, Naylor LH, Thijssen DH, and Green DJ**. Impact of handgrip exercise intensity on brachial artery flow-mediated dilation. *European journal of applied physiology* 115: 1705-1713, 2015.
14. **Atkinson CL, Carter HH, Naylor LH, Dawson EA, Marusic P, Hering D, Schlaich MP, Thijssen DH, and Green DJ**. Opposing effects of shear-mediated dilation and myogenic constriction on artery diameter in response to handgrip exercise in humans. *J Appl Physiol (1985)* 119: 858-864, 2015.
15. **Atkinson CL, Thijssen DH, Birk GK, Cable NT, Low DA, Kerstens F, Meeuwis I, Kirk R, Gong P, Samson DD, McLaughlin R, Dawson EA, Carter HH, and**

Green DJ. Cutaneous microvascular adaptation to exercise training in humans. *The Journal of physiology* (In Revision): 2016.

16. **Bank AJ, Shammas RA, Mullen K, and Chuang PP**. Effects of short-term forearm exercise training on resistance vessel endothelial function in normal subjects and patients with heart failure. *J Card Fail* 4: 193-201, 1998.
17. **Ben-Shlomo Y, Spears M, Boustred C, May M, Anderson SG, Benjamin EJ, Boutouyrie P, Cameron J, Chen CH, Cruickshank JK, Hwang SJ, Lakatta EG, Laurent S, Maldonado J, Mitchell GF, Najjar SS, Newman AB, Ohishi M, Pannier B, Pereira T, Vasan RS, Shokawa T, Sutton-Tyrell K, Verbeke F, Wang KL, Webb DJ, Willum Hansen T, Zoungas S, McEniery CM, Cockcroft JR, and Wilkinson IB**. Aortic pulse wave velocity improves cardiovascular event prediction: an individual participant meta-analysis of prospective observational data from 17,635 subjects. *Journal of the American College of Cardiology* 63: 636-646, 2014.
18. **Benda N, Eijsvogels T, Van Dijk A, Bellersen L, Thijjssen DH, and Hopman MTE**. Altered core and skin temperature responses to endurance exercise in heart failure patients and heatlhy controls. *Eur J Prev Cardiol* In Press: 2015.
19. **Benda NM, Seeger JP, van Lier DP, Bellersen L, van Dijk AP, Hopman MT, and Thijssen DH**. Heart failure patients demonstrate impaired changes in brachial artery blood flow and shear rate pattern during moderate-intensity cycle exercise. *Exp Physiol* 2015.
20. **Benda NM, Seeger JP, van Lier DP, Bellersen L, van Dijk AP, Hopman MT, and Thijssen DH**. Heart failure patients demonstrate impaired changes in brachial artery blood flow and shear rate pattern during moderate-intensity cycle exercise. *Experimental physiology* 100: 463-474, 2015.

21. **Berdeaux A, Ghaleb B, Dubois-Rande JL, Vigue B, Drieu La Rochelle C, Hittinger L, and Giudicelli JF**. Role of vascular endothelium in exercise-induced dilation of large epicardial coronary arteries in conscious dogs. *Circulation* 89: 2799-2808, 1994.

22. **Birk GK, Dawson EA, Atkinson C, Haynes A, Cable NT, Thijssen DH, and Green DJ**. Brachial artery adaptation to lower limb exercise training: role of shear stress. *J Appl Physiol* 112: 1653-1658, 2012.

23. **Birk GK, Dawson EA, Atkinson C, Haynes A, Cable NT, Thijssen DH, and Green DJ**. Brachial artery adaptation to lower limb exercise training: role of shear stress. *J Appl Physiol (1985)* 112: 1653-1658, 2012.

24. **Birk GK, Dawson EA, Batterham AM, Atkinson G, Cable T, Thijssen DH, and Green DJ**. Effects of exercise intensity on flow mediated dilation in healthy humans. *International journal of sports medicine* 34: 409-414, 2013.

25. **Bishop JM, Donald KW, Taylor SH, and Wormald PN**. The blood flow in the human arm during supine leg exercise. *The Journal of physiology* 137: 294-308, 1957.

26. **Black MA, Cable NT, Thijssen DH, and Green DJ**. Impact of age, sex, and exercise on brachial artery flow-mediated dilatation. *American journal of physiology Heart and circulatory physiology* 297: H1109-1116, 2009.

27. **Black MA, Green DJ, and Cable NT**. Exercise prevents age-related decline in nitric-oxide-mediated vasodilator function in cutaneous microvessels. *The Journal of physiology* 2008.

28. **Blair SN, Kohl HW, 3rd, Barlow CE, Paffenbarger RS, Jr., Gibbons LW, and Macera CA**. Changes in physical fitness and all-cause mortality. A prospective study of healthy and unhealthy men. *Jama* 273: 1093-1098, 1995.

29. **Bond V, Jr., Franks BD, Tearney RJ, Wood B, Melendez MA, Johnson L, Iyriboz Y, and Bassett DR, Jr.** Exercise blood pressure response and skeletal muscle

vasodilator capacity in normotensives with positive and negative family history of hypertension. *Journal of hypertension* 12: 285-290, 1994.

30. **Bove AA, and Dewey JD**. Proximal coronary vasomotor reactivity after exercise training in dogs. *Circulation* 71: 620-625, 1985.
31. **Bowles DK, Hu Q, Laughlin MH, and Sturek M**. Exercise training increases L-type calcium current density in coronary smooth muscle. *The American journal of physiology* 275: H2159-2169, 1998.
32. **Bowles DK, and Laughlin MH**. Mechanism of beneficial effects of physical activity on atherosclerosis and coronary heart disease. *J Appl Physiol (1985)* 111: 308-310, 2011.
33. **Bowles DK, and Wamhoff BR**. Coronary smooth muscle adaptation to exercise: does it play a role in cardioprotection? *Acta Physiol Scand* 178: 117-121, 2003.
34. **Braith RW, Schofield RS, Hill JA, Casey DP, and Pierce GL**. Exercise training attenuates progressive decline in brachial artery reactivity in heart transplant recipients. *J Heart Lung Transplant* 27: 52-59, 2008.
35. **Brooks AR, Lelkes PI, and Rubanyi GM**. Gene expression profiling of human aortic endothelial cells exposed to disturbed flow and steady laminar flow. *Physiological genomics* 9: 27-41, 2002.
36. **Brown MD**. Exercise and coronary vascular remodelling in the healthy heart. *Experimental physiology* 88: 645-658, 2003.
37. **Brunt VE, Howard MJ, Francisco MA, Ely BR, and Minson CT**. Passive heat therapy improves endothelial function, arterial stiffness and blood pressure in sedentary humans. *The Journal of physiology* 2016.
38. **Caro CG, Fitz-Gerald JM, and Schroter RC**. Arterial wall shear and distribution of early atheroma in man. *Nature* 223: 1159-1161, 1969.

39. **Carter HH, Dawson EA, Birk GK, Spence AL, Naylor LH, Cable NT, Thijssen DH, and Green DJ**. Effect of SR Manipulation on Conduit Artery Dilation in Humans. *Hypertension* 61: 143-150, 2013.

40. **Carter HH, Spence AL, Atkinson CL, Pugh CJ, Cable NT, Thijssen DH, Naylor LH, and Green DJ**. Distinct effects of blood flow and temperature on cutaneous microvascular adaptation. *Medicine and science in sports and exercise* 46: 2113-2121, 2014.

41. **Carter HH, Spence AL, Atkinson CL, Pugh CJ, Naylor LH, and Green DJ**. Repeated core temperature elevation induces conduit artery adaptation in humans. *European journal of applied physiology* 114: 859-865, 2014.

42. **Carter JR, and Ray CA**. Sympathetic neural adaptations to exercise training in humans. *Auton Neurosci* 2014.

43. **Casey DP, Padilla J, and Joyner MJ**. alpha-adrenergic vasoconstriction contributes to the age-related increase in conduit artery retrograde and oscillatory shear. *Hypertension* 60: 1016-1022, 2012.

44. **Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, Lloyd JK, and Deanfield JE**. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet* 340: 1111-1115., 1992.

45. **Chen Z, and Tzima E**. PECAM-1 is necessary for flow-induced vascular remodeling. *Arterioscler Thromb Vasc Biol* 29: 1067-1073, 2009.

46. **Chiang HY, Korshunov VA, Serour A, Shi F, and Sottile J**. Fibronectin is an important regulator of flow-induced vascular remodeling. *Arterioscler Thromb Vasc Biol* 29: 1074-1079, 2009.

47. **Cholesterol Treatment Trialists C, Baigent C, Blackwell L, Emberson J, Holland LE, Reith C, Bhala N, Peto R, Barnes EH, Keech A, Simes J, and Collins R**.

Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170,000 participants in 26 randomised trials. *Lancet* 376: 1670-1681, 2010.

48. **Chomistek AK, Chiuve SE, Jensen MK, Cook NR, and Rimm EB**. Vigorous physical activity, mediating biomarkers, and risk of myocardial infarction. *Medicine and science in sports and exercise* 43: 1884-1890, 2011.
49. **Civelek M, Manduchi E, Riley RJ, Stoeckert CJ, and Davies PF**. Coronary Artery Endothelial Transcriptome In Vivo / Clinical Perspective. *Circulation: Cardiovascular Genetics* 4: 243-252, 2011.
50. **Clausen JP**. Effect of physical training on cardiovascular adjustments to exercise in man. *Physiol Rev* 57: 779-815, 1977.
51. **Conway DE, Williams MR, Eskin SG, and McIntire LV**. Endothelial cell responses to atheroprone flow are driven by two separate flow components, low time-average shear stress and fluid flow reversal. *Am J Physiol Cell Physiol* 298: H367-H374, 2010.
52. **Cummins PM, von Offenberg Sweeney N, Killeen MT, Birney YA, Redmond EM, and Cahill PA**. Cyclic strain-mediated matrix metalloproteinase regulation within the vascular endothelium: a force to be reckoned with. *American journal of physiology* 292: H28-42, 2007.
53. **Currens JH, and White PD**. Half a century of running. Clinical, physiologic and autopsy findings in the case of Clarence DeMar ("Mr. Marathon"). *N Engl J Med* 265: 988-993, 1961.
54. **Dai G, Kaazempur-Mofrad MR, Natarajan S, Zhang Y, Vaughn S, Blackman BR, Kamm RD, Garcia-Cardena G, and Gimbrone MA**. Distinct endothelial phenotypes evoked by arterial waveforms derived from atherosclerosis-susceptible and -resistant regions of human vasculature. *Proceedings of the National Academy of Sciences* 101: 14871-14876, 2004.

55. **Dancu MB, Berardi DE, Vanden Heuvel JP, and Tarbell JM**. Asynchronous shear stress and circumferential strain reduces endothelial NO synthase and cyclooxygenase-2 but induces endothelin-1 gene expression in endothelial cells. *Arteriosclerosis, thrombosis, and vascular biology* 24: 2088-2094, 2004.

56. **Dancu MB, Berardi DE, Vanden Heuvel JP, and Tarbell JM**. Atherogenic Endothelial Cell eNOS and ET-1 Responses to Asynchronous Hemodynamics are Mitigated by Conjugated Linoleic Acid. *Annals of biomedical engineering* 35: 1111-1119, 2007.

57. **Davies PF, Civelek M, Fang Y, Guerraty MA, and Passerini AG**. Endothelial heterogeneity associated with regional athero-susceptibility and adaptation to disturbed blood flow in vivo. *Semin Thromb Hemost* 36: 265-275, 2010.

58. **Davies PF, Remuzzi A, Gordon EJ, Dewey CF, Jr., and Gimbrone MA, Jr.** Turbulent fluid shear stress induces vascular endothelial cell turnover in vitro. *Proceedings of the National Academy of Sciences of the United States of America* 83: 2114-2117, 1986.

59. **Davis MJ, and Hill MA**. Signaling mechanisms underlying the vascular myogenic response. *Physiol Rev* 79: 387-423, 1999.

60. **Dawson EA, Green DJ, Cable NT, and Thijssen DH**. Effects of acute exercise on flow mediated dilatation (FMD) in healthy humans. *Journal of applied physiology (In Press)* 2013.

61. **Dawson EA, Rathore S, Cable NT, Wright DJ, Morris JL, and Green DJ**. Impact of catheter insertion using the radial approach on vasodilatation in humans. *Clin Sci (Lond)* 118: 633-640, 2010.

62. **de Groot PC, Bleeker MW, van Kuppevelt DH, van der Woude LH, and Hopman MT**. Rapid and extensive arterial adaptations after spinal cord injury. *Archives of physical medicine and rehabilitation* 87: 688-696, 2006.

63. **DeFina LF, Haskell WL, Willis BL, Barlow CE, Finley CE, Levine BD, and Cooper KH**. Physical activity versus cardiorespiratory fitness: two (partly) distinct components of cardiovascular health? *Prog Cardiovasc Dis* 57: 324-329, 2015.

64. **Delp MD, and Laughlin MH**. Time course of enhanced endothelium-mediated dilation in aorta of trained rats. *Medicine and science in sports and exercise* 29: 1454-1461., 1997.

65. **DeSouza CA, Shapiro LF, Clevenger CM, Dinenno FA, Monahan KD, Tanaka H, and Seals DR**. Regular aerobic exercise prevents and restores age-related declines in endothelium-dependent vasodilation in healthy men. *Circulation* 102: 1351-1357., 2000.

66. **Dinenno FA, Tanaka H, Monahan KD, Clevenger CM, Eskurza I, DeSouza CA, and Seals DR**. Regular endurance exercise induces expansive arterial remodelling in the trained limbs of healthy men. *The Journal of physiology* 534: 287-295, 2001.

67. **Draney MT, Herfkens RJ, Hughes TJ, Pelc NJ, Wedding KL, Zarins CK, and Taylor CA**. Quantification of vessel wall cyclic strain using cine phase contrast magnetic resonance imaging. *Annals of biomedical engineering* 30: 1033-1045, 2002.

68. **Dumont O, Pinaud F, Guihot AL, Baufreton C, Loufrani L, and Henrion D**. Alteration in flow (shear stress)-induced remodelling in rat resistance arteries with aging: improvement by a treatment with hydralazine. *Cardiovascular research* 77: 600-608, 2008.

69. **Duncker DJ, and Bache RJ**. Regulation of coronary blood flow during exercise. *Physiol Rev* 88: 1009-1086, 2008.

70. **Duscha BD, Robbins JL, Jones WS, Kraus WE, Lye RJ, Sanders JM, Allen JD, Regensteiner JG, Hiatt WR, and Annex BH**. Angiogenesis in skeletal muscle precede improvements in peak oxygen uptake in peripheral artery disease patients. *Arterioscler Thromb Vasc Biol* 31: 2742-2748, 2011.

71. **Dzau VJ, Gibbons GH, Morishita R, and Pratt RE**. New perspectives in hypertension research. Potentials of vascular biology. *Hypertension* 23: 1132-1140, 1994.

72. **Esbjornsson M, Jansson E, Sundberg CJ, Sylven C, Eiken O, Nygren A, and Kaijser L**. Muscle fibre types and enzyme activities after training with local leg ischaemia in man. *Acta Physiologica Scandinavica* 148: 233-241, 1993.

73. **Fogarty JA, Muller-Delp JM, Delp MD, Mattox ML, Laughlin MH, and Parker JL**. Exercise training enhances vasodilation responses to vascular endothelial growth factor in porcine coronary arterioles exposed to chronic coronary occlusion. *Circulation* 109: 664-670, 2004.

74. **Folkow B**. Cardiovascular structural adaptation: Its role in the initiation and maintenance of primary hypertension. *Clin Sci Mol Med* 55(suppl 4): 3s-22s, 1978.

75. **Folkow B, Grimby G, and Thulesius O**. Adaptive structural changes in the vascular walls in hypertension and their relation to the control of peripheral resistance. *Acta Physiologica Scandinavica* 44: 255, 1958.

76. **Franzoni F, Galetta F, Morizzo C, Lubrano V, Palombo C, Santoro G, Ferrannini E, and Quinones-Galvan A**. Effects of age and physical fitness on microcirculatory function. *Clin Sci (Lond)* 106: 329-335, 2004.

77. **Franzoni F, Plantinga Y, Femia FR, Bartolomucci F, Gaudio C, Regoli F, Carpi A, Santoro G, and Galetta F**. Plasma antioxidant activity and cutaneous microvascular endothelial function in athletes and sedentary controls. *Biomed Pharmacother* 58: 432-436, 2004.

78. **Freidja ML, Vessieres E, Clere N, Desquiret V, Guihot AL, Toutain B, Loufrani L, Jardel A, Procaccio V, Faure S, and Henrion D**. Heme oxygenase-1 induction restores high-blood-flow-dependent remodeling and endothelial function in mesenteric arteries of old rats. *Journal of hypertension* 29: 102-112, 2011.

79. **Furchgott RF, and Zawadzki JV**. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 288: 373-376, 1980.

80. **Gambillara V, Chambaz C, Montorzi G, Roy S, Stergiopulos N, and Silacci P**. Plaque-prone hemodynamics impair endothelial function in pig carotid arteries *Am J Physiol Heart Circ Physiol* 290: H2320-H2328, 2006.

81. **Garcia-Cardena G, Fan R, Shah V, Sorrentino R, Cirino G, Papapetropoulos A, and Sessa WC**. Dynamic activation of endothelial nitric oxide synthase by Hsp90. *Nature* 392: 821-824, 1998.

82. **Gaudreault V, Despres JP, Rheaume C, Bergeron J, Almeras N, Tremblay A, and Poirier P**. Exercise-induced exaggerated blood pressure response in men with the metabolic syndrome: the role of the autonomous nervous system. *Blood Press Monit* 18: 252-258, 2013.

83. **Geiser J, Vogt M, Billeter R, Zuleger C, Belforti F, and Hoppeler H**. Training high--living low: changes of aerobic performance and muscle structure with training at simulated altitude. *Int J Sports Med* 22: 579-585, 2001.

84. **Gibala MJ, and McGee SL**. Metabolic adaptations to short-term high-intensity interval training: a little pain for a lot of gain? *Exercise and sport sciences reviews* 36: 58-63, 2008.

85. **Gielen S, Laughlin MH, O'Conner C, and Duncker DJ**. Exercise training in patients with heart disease: review of beneficial effects and clinical recommendations. *Prog Cardiovasc Dis* 57: 347-355, 2015.

86. **Gielen S, Schuler G, and Adams V**. Cardiovascular effects of exercise training: molecular mechanisms. *Circulation* 122: 1221-1238, 2010.

87. **Gliemann L, Buess R, Nyberg M, Hoppeler H, Odriozola A, Thaning P, Hellsten Y, Baum O, and Mortensen SP**. Capillary growth, ultrastructure remodelling and exercise training in skeletal muscle of essential hypertensive patients. *Acta physiologica (Oxford, England)* 214: 210-220, 2015.

88. **Gliemann L, Schmidt JF, Olesen J, Bienso RS, Peronard SL, Grandjean SU, Mortensen SP, Nyberg M, Bangsbo J, Pilegaard H, and Hellsten Y**. Resveratrol blunts the positive effects of exercise training on cardiovascular health in aged men. *J Physiol* 591: 5047-5059, 2013.

89. **Godbole AS, Lu X, Guo X, and Kassab GS**. NADPH oxidase has a directional response to shear stress. *American Journal of Physiology Heart Circulation Physiology* 296: H152-158, 2009.

90. **Goto C, Higashi Y, Kimura M, Noma K, Hara K, Nakagawa K, Kawamura M, Chayama K, Yoshizumi M, and Nara I**. Effect of different intensities of exercise on endothelium-dependent vasodilation in humans: role of endothelium-dependent nitric oxide and oxidative stress. *Circulation* 108: 530-535, 2003.

91. **Goto C, Nishioka K, Umemura T, Jitsuiki D, Sakagutchi A, Kawamura M, Chayama K, Yoshizumi M, and Higashi Y**. Acute moderate-intensity exercise induces vasodilation through an increase in nitric oxide bioavailiability in humans. *Am J Hypertens* 20: 825-830, 2007.

92. **Green D, Cheetham C, Henderson C, Weerasooriya R, and O'Driscoll G**. Effect of cardiac pacing on forearm vascular responses and nitric oxide function. *American journal of physiology* 283: H1354-1360, 2002.

93. **Green D, Cheetham C, Mavaddat L, Watts K, Best M, Taylor R, and O'Driscoll G**. Effect of lower limb exercise on forearm vascular function: contribution of nitric oxide. *American journal of physiology* 283: H899-907, 2002.

94. **Green D, Cheetham C, Reed C, Dembo L, and O'Driscoll G**. Assessment of brachial artery blood flow across the cardiac cycle: retrograde flows during cycle ergometry. *J Appl Physiol* 93: 361-368, 2002.

95. **Green DJ, Bilsborough W, Naylor LH, Reed C, Wright J, O'Driscoll G, and Walsh JH**. Comparison of forearm blood flow responses to incremental handgrip and cycle ergometer exercise: relative contribution of nitric oxide. *The Journal of physiology* 562: 617-628, 2005.

96. **Green DJ, Cable NT, Fox C, Rankin JM, and Taylor RR**. Modification of forearm resistance vessels by exercise training in young men. *J Appl Physiol* 77: 1829-1833, 1994.

97. **Green DJ, Carter HH, Fitzsimons MG, Cable NT, Thijssen DH, and Naylor LH**. Obligatory role of hyperaemia and shear stress in microvascular adaptation to repeated heating in humans. *The Journal of physiology* 588: 1571-1577, 2010.

98. **Green DJ, Dawson EA, Groenewoud HM, Jones H, and Thijssen DH**. Is flow-mediated dilation nitric oxide mediated?: A meta-analysis. *Hypertension* 63: 376-382, 2014.

99. **Green DJ, Eijsvogels T, Bouts YM, Maiorana AJ, Naylor LH, Scholten RR, Spaanderman ME, Pugh CJ, Sprung VS, Schreuder T, Jones H, Cable T, Hopman MT, and Thijssen DH**. Exercise training and artery function in humans: nonresponse and its relationship to cardiovascular risk factors. *J Appl Physiol (1985)* 117: 345-352, 2014.

100. **Green DJ, Eijsvogels T, Bouts YM, Maiorana AJ, Naylor LH, Scholten RR, Spaanderman ME, Pugh CJ, Sprung VS, Schreuder TH, Jones H, Cable T, Hopman MT, and Thijjssen DH**. Exercise Training and Artery Function in Humans: Non-Response and Its Relationship to Cardiovascular Risk Factors. *J Appl Physiol (1985)* 2014.

101. **Green DJ, Fowler DT, O'Driscoll JG, Blanksby BA, and Taylor RR**. Endothelium-derived nitric oxide activity in forearm vessels of tennis players. *J Appl Physiol* 81: 943-948, 1996.

102. **Green DJ, Hopkins ND, Jones H, Thijssen DH, Eijsvogels TM, and Yeap BB**. Sex differences in vascular endothelial function and health in humans: impacts of exercise. *Experimental physiology* 101: 230-242, 2016.

103. **Green DJ, Jones H, Thijssen D, Cable NT, and Atkinson G**. Flow-mediated dilation and cardiovascular event prediction: does nitric oxide matter? *Hypertension* 57: 363-369, 2011.

104. **Green DJ, Maiorana A, O'Driscoll G, and Taylor R**. Effect of exercise training on endothelium-derived nitric oxide function in humans. *The Journal of physiology* 561: 1-25, 2004.

105. **Green DJ, Maiorana AJ, Siong JH, Burke V, Erickson M, Minson CT, Bilsborough W, and O'Driscoll G**. Impaired skin blood flow response to environmental heating in chronic heart failure. *European heart journal* 27: 338-343, 2006.

106. **Green DJ, O'Driscoll G, Joyner MJ, and Cable NT**. Exercise and cardiovascular risk reduction: time to update the rationale for exercise? *J Appl Physiol* 105: 766-768, 2008.

107. **Green DJ, Spence A, Rowley N, Thijssen DH, and Naylor LH**. Vascular adaptation in athletes: is there an 'athlete's artery'? *Experimental physiology* 97: 295-304, 2012.

108. **Green DJ, Swart A, Exterkate A, Naylor LH, Black MA, Cable NT, and Thijssen DH**. Impact of age, sex and exercise on brachial and popliteal artery remodelling in humans. *Atherosclerosis* 210: 525-530, 2010.

109. **Green DJ, Walsh JH, Maiorana A, Best MJ, Taylor RR, and O'Driscoll JG**. Exercise-induced improvement in endothelial dysfunction is not mediated by changes in CV risk factors: pooled analysis of diverse patient populations. *American journal of physiology* 285: H2679-2687, 2003.

110. **Greer SA, Hays VW, Speer VC, and McCall JT**. Effect of dietary fat, protein and cholesterol on atherosclerosis in swine. *J Nutr* 90: 183-190, 1966.

111. **Groothuis JT, Thijssen DH, Rongen GA, Deinum J, Danser AH, Geurts AC, Smits P, and Hopman MT**. Angiotensin II contributes to the increased baseline leg vascular resistance in spinal cord-injured individuals. *Journal of hypertension* 28: 2094-2101, 2010.

112. **Hallal PC, Andersen LB, Bull FC, Guthold R, Haskell W, Ekelund U, and Lancet Physical Activity Series Working G**. Global physical activity levels: surveillance progress, pitfalls, and prospects. *Lancet* 380: 247-257, 2012.

113. **Halliwill JR, and Minson CT**. Retrograde shear: backwards into the future? *American journal of physiology* 298: H1126-1127, 2010.

114. **Hambrecht R, Adams V, Erbs S, Linke A, Krankel N, Shu Y, Baither Y, Gielen S, Thiele H, Gummert JF, Mohr FW, and Schuler G**. 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.

115. **Hambrecht R, Fiehn E, Weigl C, Gielen S, Hamann C, Kaiser R, Yu J, Adams V, Niebauer J, and Schuler G**. Regular physical exercise corrects endothelial

dysfunction and improves exercise capacity in patients with chronic heart failure. *Circulation* 98: 2709-2715, 1998.

116. **Hambrecht R, Gielen S, Linke A, Fiehn E, Yu J, Walther C, Schoene N, and Schuler G**. 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.

117. **Hambrecht R, Hilbrich L, Erbs S, Gielen S, Fiehn E, Schoene N, and Schuler G**. Correction of endothelial dysfunction in chronic heart failure: additional effects of exercise training and oral L-arginine supplementation. *Journal of the American College of Cardiology* 35: 706-713, 2000.

118. **Hambrecht R, Walther C, Mobius-Winkler S, Gielen S, Linke A, Conradi K, Erbs S, Kluge R, Kendziorra K, Sabri O, Sick P, and Schuler G**. Percutaneous coronary angioplasty compared with exercise training in patients with stable coronary artery disease: a randomized trial. *Circulation* 109: 1371-1378, 2004.

119. **Hambrecht R, Wolf A, Gielen S, Linke A, Hofer J, Erbs S, Schoene N, and Schuler G**. Effect of exercise on coronary endothelial function in patients with coronary artery disease. *N Engl J Med* 342: 454-460, 2000.

120. **Hamer M, Ingle L, Carroll S, and Stamatakis E**. Physical activity and cardiovascular mortality risk: possible protective mechanisms? *Medicine and science in sports and exercise* 44: 84-88, 2012.

121. **Hanna MA, Taylor CR, Chen B, La HS, Maraj JJ, Kilar CR, Behnke BJ, Delp MD, and Muller-Delp JM**. Structural remodeling of coronary resistance arteries: effects of age and exercise training. *J Appl Physiol (1985)* 117: 616-623, 2014.

122.**Hansen AH, Nielsen JJ, Saltin B, and Hellsten Y**. Exercise training normalizes skeletal muscle vascular endothelial growth factor levels in patients with essential hypertension. *J Hypertens* 28: 1176-1185, 2010.

123.**Hansen AH, Nyberg M, Bangsbo J, Saltin B, and Hellsten Y**. Exercise training alters the balance between vasoactive compounds in skeletal muscle of individuals with essential hypertension. *Hypertension* 58: 943-949, 2011.

124.**Harrison DG, Widder J, Grumbach I, Chen W, Weber M, and Searles C**. Endothelial mechanotransduction, nitric oxide and vascular inflammation. *Journal of internal medicine* 259: 351-363, 2006.

125.**Haskell WL, Sims C, Myll J, Bortz WM, St Goar FG, and Alderman EL**. Coronary artery size and dilating capacity in ultradistance runners. *Circulation* 87: 1076-1082, 1993.

126.**Hastings NE, Simmers MB, McDonald OG, Wamhoff BR, and Blackman BR**. Atherosclerosis-prone hemodynamics differentially regulates endothelial and smooth muscle cell phenotypes and promotes pro-inflammatory priming. *Am J Physiol Cell Physiol* 293: C1824-C1833, 2007.

127.**Hawley JA, Hargreaves M, Joyner MJ, and Zierath JR**. Integrative biology of exercise. *Cell* 159: 738-749, 2014.

128.**Heaps CL, Bowles DK, Sturek M, Laughlin MH, and Parker JL**. Enhanced L-type Ca2+ channel current density in coronary smooth muscle of exercise-trained pigs is compensated to limit myoplasmic free Ca2+ accumulation. *The Journal of physiology* 528: 435-445, 2000.

129.**Hellsten Y, Jensen L, Thaning P, Nyberg M, and Mortensen S**. Impaired formation of vasodilators in peripheral tissue in essential hypertension is normalized by exercise training: role of adenosine and prostacyclin. *J Hypertens* 30: 2007-2014, 2012.

130. **Henderson KK, Turk JR, Rush JW, and Laughlin MH**. Endothelial function in coronary arterioles from pigs with early-stage coronary disease induced by high-fat, high-cholesterol diet: effect of exercise. *J Appl Physiol (1985)* 97: 1159-1168, 2004.

131. **Higashi Y, Sasaki S, Kurisu S, Yoshimizu A, Sasaki N, Matsuura H, Kajiyama G, and Oshima T**. Regular aerobic exercise augments endothelium-dependent vascular relaxation in normotensive as well as hypertensive subjects: role of endothelium-derived nitric oxide. *Circulation* 100: 1194-1202, 1999.

132. **Hodges GJ, Sharp L, Stephenson C, Patwala AY, George KP, Goldspink DF, and Tim Cable N**. The effect of 48 weeks of aerobic exercise training on cutaneous vasodilator function in post-menopausal females. *Eur J Appl Physiol* 108: 1259-1267, 2010.

133. **Hoier B, and Hellsten Y**. Exercise-induced capillary growth in human skeletal muscle and the dynamics of VEGF. *Microcirculation* 21: 301-314, 2014.

134. **Holtz J, Forstermann U, Pohl U, Giesler M, and Bassenge E**. Flow-dependent, endothelium-mediated dilation of epicardial coronary arteries in conscious dogs: effects of cyclooxygenase inhibition. *Journal of cardiovascular pharmacology* 6: 1161-1169, 1984.

135. **Hopkins ND, Green DJ, Tinken TM, Sutton L, McWhannell N, Thijssen DH, Cable NT, Stratton G, and George K**. Does conduit artery diameter vary according to the anthropometric characteristics of children or men? *American journal of physiology Heart and circulatory physiology* 297: H2182-2187, 2009.

136. **Hornig B, Maier V, and Drexler H**. Physical training improves endothelial function in patients with chronic heart failure. *Circulation* 93: 210-214., 1996.

137. **Hu FB**. Globalization of diabetes: the role of diet, lifestyle, and genes. *Diabetes care* 34: 1249-1257, 2011.

138.**Hunt JE, Galea D, Tufft G, Bunce D, and Ferguson RA**. Time course of regional vascular adaptations to low load resistance training with blood flow restriction. *J Appl Physiol (1985)* 115: 403-411, 2013.

139.**Huo Y, Wischgoll T, and Kassab GS**. Flow patterns in three-dimensional porcine epicardial coronary arterial tree. *Am J Physiol Heart Circ Physiol* 293: H2959-2970, 2007.

140.**Huonker M, Schmid A, Schmidt-Trucksass A, Grathwohl D, and Keul J**. Size and blood flow of central and peripheral arteries in highly trained able-bodied and disabled athletes. *J Appl Physiol* 95: 685-691, 2003.

141.**Huonker M, Schmid A, Sorichter S, Schmidt-Trucksab A, Mrosek P, and Keul J**. Cardiovascular differences between sedentary and wheelchair-trained subjects with paraplegia. *Medicine and science in sports and exercise* 30: 609-613, 1998.

142.**Hwang J, Ing MH, Salazar A, Lassegue B, Griendling K, Navab M, Sevanian A, and Hsiai TK**. Pulsatile versus oscillatory shear stress regulates NADPH oxidase subunit expression: implication for native LDL oxidation. *Circulation research* 93: 1225-1232, 2003.

143.**Hwang J, Saha A, Boo YC, Sorescu GP, McNally JS, Holland SM, Dikalov S, Giddens DP, Griendling KK, Harrison DG, and Jo H**. Oscillatory shear stress stimulates endothelial production of O2- from p47phox-dependent NAD(P)H oxidases, leading to monocyte adhesion. *Journal of Biological Chemistry* 278: 47291-47298, 2003.

144.**Ikegawa S, Kamijo Y, Okazaki K, Masuki S, Okada Y, and Nose H**. Effects of hypohydration on thermoregulation during exercise before and after 5-day aerobic training in a warm environment in young men. *J Appl Physiol (1985)* 110: 972-980, 2011.

145.**Inaba Y, Chen JA, and Bergmann SR**. Prediction of future cardiovascular outcomes by flow-mediated vasodilatation of brachial artery: a meta-analysis. *The international journal of cardiovascular imaging* 26: 631-640, 2010.

146.**Jenkins NT, Padilla J, Thorne P, Martin J, Rector R, Davis J, and Laughlin M**. Transcriptome-wide RNA sequencing analysis of rat skeletal muscle feed arteries. I. Impact of obesity. *J Appl Physiol* 116: 1017-1032, 2014.

147.**Jin SX, Shen LH, Nie P, Yuan W, Hu LH, Li DD, Chen XJ, Zhang XK, and He B**. Endogenous renovascular hypertension combined with low shear stress induces plaque rupture in apolipoprotein E-deficient mice. *Arterioscler Thromb Vasc Biol* 32: 2372-2379, 2012.

148.**Johnson BD, Mather KJ, and Wallace JP**. Mechanotransduction of shear in the endothelium: basic studies and clinical implications. *Vascular medicine (London, England)* 16: 365-377, 2011.

149.**Johnson LR, and Laughlin MH**. Chronic exercise training does not alter pulmonary vasorelaxation in normal pigs. *J Appl Physiol* 88: 2008-2014, 2000.

150.**Johnson LR, Rush JW, Turk JR, Price EM, and Laughlin MH**. Short-term exercise training increases ACh-induced relaxation and eNOS protein in porcine pulmonary arteries. *J Appl Physiol (1985)* 90: 1102-1110, 2001.

151.**Johnson PC**. The Myogenic Response. In: *Comprehensive Physiology* John Wiley & Sons, Inc., 2011.

152.**Jondeau G, Katz SD, Toussaint JF, Dubourg O, Monrad ES, Bourdarias JP, and LeJemtel TH**. Regional specificity of peak hyperemic response in patients with congestive heart failure: correlation with peak aerobic capacity. *Journal of the American College of Cardiology* 22: 1399-1402., 1993.

153.**Joyner MJ, and Casey DP**. Regulation of increased blood flow (hyperemia) to muscles during exercise: a hierarchy of competing physiological needs. *Physiological reviews* 95: 549-601, 2015.

154.**Joyner MJ, Dietz NM, and Shepherd JT**. From Belfast to Mayo and beyond: the use and future of plethysmography to study blood flow in human limbs. *J Appl Physiol* 91: 2431-2441., 2001.

155.**Joyner MJ, and Green DJ**. Exercise protects the cardiovascular system: effects beyond traditional risk factors. *The Journal of physiology* 587: 5551-5558, 2009.

156.**Kamiya A, and Togawa T**. Adaptive regulation of wall shear stress to flow change in the canine carotid artery. *The American journal of physiology* 239: H14-21, 1980.

157.**Keteyian SJ**. Swing and a miss or inside-the-park home run: which fate awaits high-intensity exercise training? *Circulation* 126: 1431-1433, 2012.

158.**Kingwell BA, Sherrard B, Jennings GL, and Dart AM**. Four weeks of cycle training increases basal production of nitric oxide from the forearm. *The American journal of physiology* 272: H1070-1077, 1997.

159.**Kodama S, Saito K, Tanaka S, Maki M, Yachi Y, Asumi M, Sugawara A, Totsuka K, Shimano H, Ohashi Y, Yamada N, and Sone H**. Cardiorespiratory fitness as a quantitative predictor of all-cause mortality and cardiovascular events in healthy men and women: a meta-analysis. *Jama* 301: 2024-2035, 2009.

160.**Koller A, Sun D, and Kaley G**. Role of shear stress and endothelial prostaglandins in flow- and viscosity-induced dilation of arterioles in vitro. *Circulation research* 72: 1276-1284, 1993.

161.**Kon M, Ohiwa N, Honda A, Matsubayashi T, Ikeda T, Akimoto T, Suzuki Y, Hirano Y, and Russell AP**. Effects of systemic hypoxia on human muscular adaptations to resistance exercise training. *Physiol Rep* 2: 2014.

162.**Korshunov VA, and Berk BC**. Flow-induced vascular remodeling in the mouse: a model for carotid intima-media thickening. *Arterioscler Thromb Vasc Biol* 23: 2185-2191, 2003.

163.**Korzick DH, Laughlin MH, and Bowles DK**. Alterations in PKC signaling underlie enhanced myogenic tone in exercise-trained porcine coronary resistance arteries. *J Appl Physiol (1985)* 96: 1425-1432, 2004.

164.**Kvernmo HD, Stefanovska A, Kirkeboen KA, Osterud B, and Kvernebo K**. Enhanced endothelium-dependent vasodilatation in human skin vasculature induced by physical conditioning. *Eur J Appl Physiol Occup Physiol* 79: 30-36, 1998.

165.**LaMack JA, Himburg HA, and Friedman MH**. Distinct profiles of endothelial gene expression in hyperpermeable regions of the porcine aortic arch and thoracic aorta. *Atherosclerosis* 195: e35-41, 2007.

166.**Lampert E, Mettauer B, Hoppeler H, Charloux A, Charpentier A, and Lonsdorfer J**. Skeletal muscle response to short endurance training in heart transplant recipients. *J Am Coll Cardiol* 32: 420-426, 1998.

167.**Langille BL, and O'Donnell F**. Reductions in arterial diameter produced by chronic decreases in blood flow are endothelium-dependent. *Science* 231: 405-407, 1986.

168.**Laughlin MH**. Endothelium-mediated control of coronary vascular tone after chronic exercise training. *Medicine and science in sports and exercise* 27: 1135-1144, 1995.

169.**Laughlin MH**. Joseph B. Wolfe Memorial lecture. Physical activity in prevention and treatment of coronary disease: the battle line is in exercise vascular cell biology. *Medicine and science in sports and exercise* 36: 352-362, 2004.

170.**Laughlin MH, Bowles DK, and Duncker DJ**. The coronary circulation in exercise training. *American journal of physiology* 302: H10-23, 2012.

171.**Laughlin MH, Korthuis RJ, Duncker DJ, and Bache RJ**. Control of blood flow to cardiac and skeletal muscle during exercise. In: *Handbook of Physiology Exercise: Regulation and Integration of Multiple Systems*, edited by APS1996, p. 705-769.

172.**Laughlin MH, and Muller JM**. Vasoconstrictor responses of coronary resistance arteries in exercise-trained pigs. *J Appl Physiol (1985)* 84: 884-889, 1998.

173.**Laughlin MH, Newcomer SC, and Bender SB**. Importance of hemodynamic forces as signals for exercise-induced changes in endothelial cell phenotype. *J Appl Physiol* 104: 588-600, 2008.

174.**Laughlin MH, Padilla J, Jenkins NT, Thorne PK, Martin JS, Rector RS, Akter S, and Davis JW**. Exercise-induced differential changes in gene expression among arterioles of skeletal muscles of obese rats. *Journal of applied physiology (Bethesda, Md : 1985)* 119: 583-603, 2015.

175.**Laughlin MH, Padilla J, Jenkins NT, Thorne PK, Martin JS, Rector RS, Akter S, and Davis JW**. Exercise training causes differential changes in gene expression in diaphragm arteries and 2A arterioles of obese rats. *Journal of applied physiology (Bethesda, Md : 1985)* 119: 604-616, 2015.

176.**Laughlin MH, Pollock JS, Amann JF, Hollis ML, Woodman CR, and Price EM**. Training induces nonuniform increases in eNOS content along the coronary arterial tree. *J Appl Physiol* 90: 501-510, 2001.

177.**Laughlin MH, and Ripperger J**. Vascular transport capacity of hindlimb muscles of exercise-trained rats. *J Appl Physiol (1985)* 62: 438-443, 1987.

178.**Laukkanen T, Khan H, Zaccardi F, and Laukkanen JA**. Association between sauna bathing and fatal cardiovascular and all-cause mortality events. *JAMA Intern Med* 175: 542-548, 2015.

179.**Lawrence MB, McIntire LV, and Eskin SG**. Effect of flow on polymorphonuclear leukocyte/endothelial cell adhesion. *Blood* 70: 1284-1290, 1987.

180.**Lee DC, Sui X, Artero EG, Lee IM, Church TS, McAuley PA, Stanford FC, Kohl HW, 3rd, and Blair SN**. Long-term effects of changes in cardiorespiratory fitness and body mass index on all-cause and cardiovascular disease mortality in men: the Aerobics Center Longitudinal Study. *Circulation* 124: 2483-2490, 2011.

181.**Lee HY, Youn SW, Oh BH, and Kim HS**. Kruppel-like factor 2 suppression by high glucose as a possible mechanism of diabetic vasculopathy. *Korean Circ J* 42: 239-245, 2012.

182.**Lenasi H, and Strucl M**. Effect of regular physical training on cutaneous microvascular reactivity. *Med Sci Sports Exerc* 36: 606-612, 2004.

183.**Lewis TV, Dart AM, Chin-Dusting JP, and Kingwell BA**. Exercise training increases basal nitric oxide production from the forearm in hypercholesterolemic patients. *Arterioscler Thromb Vasc Biol* 19: 2782-2787, 1999.

184.**Li L, Chen W, Rezvan A, Jo H, and Harrison DG**. Tetrahydrobiopterin deficiency and nitric oxide synthase uncoupling contribute to atherosclerosis induced by disturbed flow. *Arterioscler Thromb Vasc Biol* 31: 1547-1554, 2011.

185.**Linke A, Erbs S, and Hambrecht R**. Exercise and the coronary circulation-alterations and adaptations in coronary artery disease. *Prog Cardiovasc Dis* 48: 270-284, 2006.

186.**Linke A, Schoene N, Gielen S, Hofer J, Erbs S, Schuler G, and Hambrecht R**. Endothelial dysfunction in patients with chronic heart failure: systemic effects of lower-limb exercise training. *Journal of the American College of Cardiology* 37: 392-397, 2001.

187.**Lopez MG, Silva BM, Joyner MJ, and Casey DP**. Roles of nitric oxide and prostaglandins in the hyperemic response to a maximal metabolic stimulus: redundancy prevails. *European journal of applied physiology* 113: 1449-1456, 2013.

188.**Lu X, and Kassab GS**. Nitric oxide is significantly reduced in ex-vivo porcine arteries during reverse flow because of increased superoxide production. *Journal of Physiology* 561: 575-582, 2004.

189.**Madhur MS, Funt SA, Li L, Vinh A, Chen W, Lob HE, Iwakura Y, Blinder Y, Rahman A, Quyyumi AA, and Harrison DG**. Role of interleukin 17 in inflammation, atherosclerosis, and vascular function in apolipoprotein e-deficient mice. *Arterioscler Thromb Vasc Biol* 31: 1565-1572, 2011.

190.**Madssen E, Moholdt T, Videm V, Wisloff U, Hegbom K, and Wiseth R**. Coronary atheroma regression and plaque characteristics assessed by grayscale and radiofrequency intravascular ultrasound after aerobic exercise. *Am J Cardiol* 114: 1504-1511, 2014.

191.**Maeda S, Miyauchi T, Kakiyama T, Sugawara J, Iemitsu M, Irukayama-Tomobe Y, Murakami H, Kumagai Y, Kuno S, and Matsuda M**. Effects of exercise training of 8 weeks and detraining on plasma levels of endothelium-derived factors, endothelin-1 and nitric oxide, in healthy young humans. *Life Sci* 69: 1005-1016., 2001.

192.**Maiorana A, O'Driscoll G, Cheetham C, Collis J, Goodman C, Rankin S, Taylor R, and Green D**. Combined aerobic and resistance exercise training improves functional capacity and strength in CHF. *J Appl Physiol* 88: 1565-1570, 2000.

193.**Maiorana A, O'Driscoll G, Cheetham C, Dembo L, Stanton K, Goodman C, Taylor R, and Green D**. The effect of combined aerobic and resistance exercise training on

vascular function in type 2 diabetes. *Journal of the American College of Cardiology* 38: 860-866, 2001.

194.**Maiorana A, O'Driscoll G, Dembo L, Cheetham C, Goodman C, Taylor R, and Green D**. Effect of aerobic and resistance exercise training on vascular function in heart failure. *American journal of physiology* 279: H1999-2005, 2000.

195.**Maiorana A, O'Driscoll G, Dembo L, Goodman C, Taylor R, and Green D**. Exercise training, vascular function, and functional capacity in middle-aged subjects. *Med Sci Sports Exerc* 33: 2022-2028, 2001.

196.**Maiorana A, O'Driscoll G, Taylor R, and Green D**. Exercise and the nitric oxide vasodilator system. *Sports medicine (Auckland, NZ* 33: 1013-1035, 2003.

197.**Manson JE, Hu FB, Rich-Edwards JW, Colditz GA, Stampfer MJ, Willett WC, Speizer FE, and Hennekens CH**. A prospective study of walking as compared with vigorous exercise in the prevention of coronary heart disease in women. *The New England journal of medicine* 341: 650-658, 1999.

198.**Maxwell LC, White TP, and Faulkner JA**. Oxidative capacity, blood flow, and capillarity of skeletal muscles. *J Appl Physiol Respir Environ Exerc Physiol* 49: 627-633, 1980.

199.**McAllister RM, Kimani JK, Webster JL, Parker JL, and Laughlin MH**. Effects of exercise training on responses of peripheral and visceral arteries in swine. *J Appl Physiol* 80: 216-225, 1996.

200.**McAllister RM, and Laughlin MH**. Short-term exercise training alters responses of porcine femoral and brachial arteries. *J Appl Physiol* 82: 1438-1444, 1997.

201.**McLenachan JM, Williams JK, Fish RD, Ganz P, and Selwyn AP**. Loss of flow-mediated endothelium-dependent dilation occurs early in the development of atherosclerosis. *Circulation* 84: 1273-1278, 1991.

202.**Mestek ML, Westby CM, Van Guilder GP, Greiner JJ, Stauffer BL, and DeSouza CA**. Regular aerobic exercise, without weight loss, improves endothelium-dependent vasodilation in overweight and obese adults. *Obesity (Silver Spring)* 18: 1667-1669, 2010.

203.**Mills EJ, Rachlis B, Wu P, Devereaux PJ, Arora P, and Perri D**. Primary prevention of cardiovascular mortality and events with statin treatments: a network meta-analysis involving more than 65,000 patients. *Journal of the American College of Cardiology* 52: 1769-1781, 2008.

204.**Minson CT, Berry LT, and Joyner MJ**. Nitric oxide and neurally mediated regulation of skin blood flow during local heating. *J Appl Physiol* 91: 1619-1626, 2001.

205.**Miyachi M, Iemitsu M, Okutsu M, and Onodera S**. Effects of endurance training on the size and blood flow of the arterial conductance vessels in humans. *Acta Physiologica Scandinavica* 163: 13-16, 1998.

206.**Miyachi M, Tanaka H, Yamamoto K, Yoshioka A, Takahashi K, and Onodera S**. Effects of one-legged endurance training on femoral arterial and venous size in healthy humans. *J Appl Physiol* 90: 2439-2444, 2001.

207.**Monahan KD, Dinenno FA, Tanaka H, Clevenger CM, DeSouza CA, and Seals DR**. Regular aerobic exercise modulates age-associated declines in cardiovagal baroreflex sensitivity in healthy men. *The Journal of physiology* 529 Pt 1: 263-271, 2000.

208.**Moncada S, Gryglewski R, Bunting S, and Vane JR**. An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. *Nature* 263: 663-665, 1976.

209.**Montero D, Walther G, Diaz-Canestro C, Pyke KE, and Padilla J**. Microvascular Dilator Function in Athletes: A Systematic Review and Meta-analysis. *Medicine and science in sports and exercise* 47: 1485-1494, 2015.

210.**Moore SC, Patel AV, Matthews CE, Berrington de Gonzalez A, Park Y, Katki HA, Linet MS, Weiderpass E, Visvanathan K, Helzlsouer KJ, Thun M, Gapstur SM, Hartge P, and Lee IM**. Leisure time physical activity of moderate to vigorous intensity and mortality: a large pooled cohort analysis. *PLoS Med* 9: e1001335, 2012.

211.**Mora S, Cook N, Buring JE, Ridker PM, and Lee IM**. Physical activity and reduced risk of cardiovascular events: potential mediating mechanisms. *Circulation* 116: 2110-2118, 2007.

212.**Moreau KL, Donato AJ, Seals DR, Dinenno FA, Blackett SD, Hoetzer GL, Desouza CA, and Tanaka H**. Arterial intima-media thickness: site-specific associations with HRT and habitual exercise. *American journal of physiology* 283: H1409-1417, 2002.

213.**Moreau KL, Hildreth KL, Meditz AL, Deane KD, and Kohrt WM**. Endothelial function is impaired across the stages of the menopause transition in healthy women. *The Journal of clinical endocrinology and metabolism* 97: 4692-4700, 2012.

214.**Moreau KL, Stauffer BL, Kohrt WM, and Seals DR**. Essential role of estrogen for improvements in vascular endothelial function with endurance exercise in postmenopausal women. *The Journal of clinical endocrinology and metabolism* 98: 4507-4515, 2013.

215.**Morganroth J, Maron BJ, Henry WL, and Epstein SE**. Comparative left ventricular dimensions in trained athletes. *Annals of internal medicine* 82: 521-524, 1975.

216.**Morris JN**. Foreword. In: Lee I-M, Blair SN, Manson JE, et al. Epidemiologic methods in physical activity studies. edited by Press NYOU2009, p. 3-12.

217.**Mueller PJ**. Physical (in)activity-dependent alterations at the rostral ventrolateral medulla: influence on sympathetic nervous system regulation. *Am J Physiol Regul Integr Comp Physiol* 298: R1468-1474, 2010.

218.**Muller JM, Myers PR, and Laughlin MH**. Vasodilator responses of coronary resistance arteries of exercise-trained pigs. *Circulation* 89: 2308-2314, 1994.

219.**Munk K, Andersen NH, Schmidt MR, Nielsen SS, Terkelsen CJ, Sloth E, Botker HE, Nielsen TT, and Poulsen SH**. Remote Ischemic Conditioning in Patients With Myocardial Infarction Treated With Primary Angioplasty: Impact on Left Ventricular Function Assessed by Comprehensive Echocardiography and Gated Single-Photon Emission CT. *Circ Cardiovasc Imaging* 3: 656-662, 2010.

220.**Naci H, and Ioannidis JP**. Comparative effectiveness of exercise and drug interventions on mortality outcomes: metaepidemiological study. *BMJ (Clinical research ed* 347: f5577, 2013.

221.**Nam D, Ni C-W, Rezvan A, Suo J, Budzyn K, Llanos A, Harrison D, Giddens D, and Jo H**. Partial carotid ligation is a model of acutely induced disturbed flow, leading to rapid endothelial dysfunction and atherosclerosis. *Am J Physiol Heart Circ Physiol* 297: H1535-1543, 2009.

222.**Naylor LH, Carter H, FitzSimons MG, Cable NT, Thijssen DH, and Green DJ**. Repeated increases in blood flow, independent of exercise, enhance conduit artery vasodilator function in humans. *American journal of physiology* 300: H664-669, 2011.

223.**Naylor LH, Davis EA, Kalic RJ, Paramalingam N, Abraham MB, Jones TW, and Green DJ**. Exercise training improves vascular function in adolescents with type 2 diabetes. *Physiol Rep* 4: 2016.

224.**Naylor LH, O'Driscoll G, Fitzsimons M, Arnolda LF, and Green DJ**. Effects of training resumption on conduit arterial diameter in elite rowers. *Medicine and science in sports and exercise* 38: 86-92, 2006.

225.**Nguyen PK, Terashima M, Fair JM, Varady A, Taylor-Piliae RE, Iribarren C, Go AS, Haskell WL, Hlatky MA, Fortmann SP, and McConnell MV**. Physical activity in older subjects is associated with increased coronary vasodilation: the ADVANCE study. *JACC Cardiovasc Imaging* 4: 622-629, 2011.

226.**Ni C-W, Qiu H, Rezvan A, Kwon K, Nam D, Son DJ, Visvader JE, and Jo H**. Discovery of novel mechanosensitive genes in vivo using mouse carotid artery endothelium exposed to disturbed flow. *Blood* 116: e66-73, 2010.

227.**Nyberg M, Seidelin K, Andersen TR, Overby NN, Hellsten Y, and Bangsbo J**. Biomarkers of vascular function in premenopausal and recent postmenopausal women of similar age: effect of exercise training. *Am J Physiol Regul Integr Comp Physiol* 306: R510-517, 2014.

228.**O'Keeffe LM, Muir G, Piterina AV, and McGloughlin T**. Vascular cell adhesion molecule-1 expression in endothelial cells exposed to physiological coronary wall shear stresses. *Journal of Biomechanical Engineering* 131: 081003, 2009.

229.**Odden MC, Coxson PG, Moran A, Lightwood JM, Goldman L, and Bibbins-Domingo K**. The impact of the aging population on coronary heart disease in the United States. *The American journal of medicine* 124: 827-833 e825, 2011.

230.**Olive JL, Dudley GA, and McCully KK**. Vascular remodeling after spinal cord injury. *Medicine and science in sports and exercise* 35: 901-907, 2003.

231.**Olshansky SJ, Passaro DJ, Hershow RC, Layden J, Carnes BA, Brody J, Hayflick L, Butler RN, Allison DB, and Ludwig DS**. A potential decline in life expectancy in the United States in the 21st century. *The New England journal of medicine* 352: 1138-1145, 2005.

232.**Oneda B, Cardoso CG, Jr., Forjaz CL, Araujo TG, Bernardo FR, de Gusmao JL, Pinto LG, Labes E, Abrahao SB, Mion D, Jr., Fonseca AM, and Tinucci T**.

Effects of estrogen therapy and aerobic training on sympathetic activity and hemodynamics in healthy postmenopausal women: a double-blind randomized trial. *Menopause* 21: 369-375, 2014.

233. **Oudegeest-Sander MH, Olde Rikkert MG, Smits P, Thijssen DH, van Dijk AP, Levine BD, and Hopman MT**. The effect of an advanced glycation end-product crosslink breaker and exercise training on vascular function in older individuals: a randomized factorial design trial. *Exp Gerontol* 48: 1509-1517, 2013.

234. **Pacher Pl, Beckman JS, and Liaudet L**. Nitric Oxide and Peroxynitrite in Health and Disease. *Physiological Reviews* 87: 315-424, 2007.

235. **Padilla J, Jenkins N, Thorne P, Martin J, Rector R, Davis J, and Laughlin M**. Transcriptome-wide RNA sequencing analysis of rat skeletal muscle feed arteries. II. Impact of exercise training in obesity. *J Appl Physiol* 116: 1033-1047, 2014.

236. **Padilla J, Jenkins NT, Laughlin MH, and Fadel PJ**. Blood pressure regulation VIII: resistance vessel tone and implications for a pro-atherogenic conduit artery endothelial cell phenotype. *European journal of applied physiology* in press: 2013.

237. **Padilla J, Simmons GH, Bender SB, Arce-Esquivel AA, Whyte JJ, and Laughlin MH**. Vascular effects of exercise: endothelial adaptations beyond active muscle beds. *Physiology* 26: 132-145, 2011.

238. **Padilla J, Simmons GH, Davis JW, Whyte JJ, Zderic TW, Hamilton MT, Bowles DK, and Laughlin MH**. Impact of exercise training on endothelial transcriptional profiles in healthy swine: a genome-wide microarray analysis. *American Journal of Physiology - Heart and Circulatory Physiology* 301: H555-H564, 2011.

239. **Padilla J, Simmons GH, Vianna LC, Davis MJ, Laughlin MH, and Fadel PJ**. Brachial artery vasodilatation during prolonged lower limb exercise: role of shear rate. *Experimental physiology* 96: 1019-1027, 2011.

240.**Padilla J, Young CN, Simmons GH, Deo SH, Newcomer SC, Sullivan JP, Laughlin MH, and Fadel PJ**. Increased muscle sympathetic nerve activity acutely alters conduit artery shear rate patterns. *American journal of physiology* 298: H1128-1135, 2010.

241.**Paffenbarger RS, Jr., Hyde RT, Wing AL, and Hsieh CC**. Physical activity, all-cause mortality, and longevity of college alumni. *The New England journal of medicine* 314: 605-613, 1986.

242.**Paffenbarger RS, Jr., Kampert JB, Lee IM, Hyde RT, Leung RW, and Wing AL**. Changes in physical activity and other lifeway patterns influencing longevity. *Med Sci Sports Exerc* 26: 857-865, 1994.

243.**Pagkalos M, Koutlianos N, Kouidi E, Pagkalos E, Mandroukas K, and Deligiannis A**. Heart rate variability modifications following exercise training in type 2 diabetic patients with definite cardiac autonomic neuropathy. *Br J Sports Med* 42: 47-54, 2008.

244.**Parmenter BJ, Dieberg G, and Smart NA**. Exercise training for management of peripheral arterial disease: a systematic review and meta-analysis. *Sports Med* 45: 231-244, 2015.

245.**Passerini AG, Polacek DC, Shi C, Francesco NM, Manduchi E, Grant GR, Pritchard WF, Powell S, Chang GY, Stoeckert CJ, Jr., and Davies PF**. Coexisting proinflammatory and antioxidative endothelial transcription profiles in a disturbed flow region of the adult porcine aorta. *Proceedings of the National Academy of Sciences of the United States of America* 101: 2482-2487, 2004.

246.**Pohl U, Holtz J, Busse R, and Bassenge E**. Crucial role of endothelium in the vasodilator response to increased flow in vivo. *Hypertension* 8: 37-44, 1986.

247.**Popovic M, Puchner S, Endler G, Foraschik C, Minar E, and Bucek RA**. The effects of endurance and recreational exercise on subclinical evidence of atherosclerosis in young adults. *The American journal of the medical sciences* 339: 332-336, 2010.

248.**Pyke KE, Poitras V, and Tschakovsky ME**. Brachial artery flow-mediated dilation during handgrip exercise: evidence for endothelial transduction of the mean shear stimulus. *American journal of physiology* 294: H2669-2679, 2008.

249.**Rakobowchuk M, Tanguay S, Burgomaster KA, Howarth KR, Gibala MJ, and MacDonald MJ**. Sprint interval and traditional endurance training induce similar improvements in peripheral arterial stiffness and flow-mediated dilation in healthy humans. *Am J Physiol Regul Integr Comp Physiol* 295: R236-242, 2008.

250.**Ramos JS, Dalleck LC, Tjonna AE, Beetham KS, and Coombes JS**. The impact of high-intensity interval training versus moderate-intensity continuous training on vascular function: a systematic review and meta-analysis. *Sports medicine (Auckland, NZ* 45: 679-692, 2015.

251.**Rapacz J, Hasler-Rapacz J, Taylor KM, Checovich WJ, and Attie AD**. Lipoprotein mutations in pigs are associated with elevated plasma cholesterol and atherosclerosis. *Science (New York, NY)* 234: 1573-1577, 1986.

252.**Ras RT, Streppel MT, Draijer R, and Zock PL**. Flow-mediated dilation and cardiovascular risk prediction: A systematic review with meta-analysis. *International journal of cardiology* 168: 344-351, 2013.

253.**Ribeiro F, Alves AJ, Duarte JA, and Oliveira J**. Is exercise training an effective therapy targeting endothelial dysfunction and vascular wall inflammation? *International journal of cardiology* 141: 214-221, 2010.

254.**Roberts MF, Wenger CB, Stolwijk JA, and Nadel ER**. Skin blood flow and sweating changes following exercise training and heat acclimation. *J Appl Physiol Respir Environ Exerc Physiol* 43: 133-137, 1977.

255.**Roche DM, Rowland TW, Garrard M, Marwood S, and Unnithan VB**. Skin microvascular reactivity in trained adolescents. *Eur J Appl Physiol* 108: 1201-1208, 2010.

256.**Rodbard S**. Vascular caliber. *Cardiology* 60: 4-49, 1975.

257.**Ross R, Wight TN, Strandness E, and Thiele B**. Human atherosclerosis. I. Cell constitution and characteristics of advanced lesions of the superficial femoral artery. *Am J Pathol* 114: 79-93, 1984.

258.**Roveda F, Middlekauff HR, Rondon MU, Reis SF, Souza M, Nastari L, Barretto AC, Krieger EM, and Negrao CE**. The effects of exercise training on sympathetic neural activation in advanced heart failure: a randomized controlled trial. *Journal of the American College of Cardiology* 42: 854-860, 2003.

259.**Rowell LB**. *Human Cardiovascular Control*. New York: Oxford University Press, 1993, p. 162-241.

260.**Rowell LB, Brengelmann GL, Blackmon JR, Bruce RA, and Murray JA**. Disparities between aortic and peripheral pulse pressures induced by upright exercise and vasomotor changes in man. *Circulation* 37: 954-964, 1968.

261.**Rowley NJ, Dawson EA, Birk GK, Cable NT, George K, Whyte G, Thijssen DH, and Green DJ**. Exercise and arterial adaptation in humans: uncoupling localized and systemic effects. *J Appl Physiol* 110: 1190-1195, 2011.

262.**Rowley NJ, Dawson EA, Hopman MT, George K, Whyte GP, Thijssen DH, and Green DJ**. Conduit Diameter and Wall Remodelling In Elite Athletes and Spinal Cord Injury. *Medicine and science in sports and exercise* 44: 844-849, 2012.

263.**Rubanyi GM, Romero JC, and Vanhoutte PM**. Flow-induced release of endothelium-derived relaxing factor. *The American journal of physiology* 250: H1145-1149, 1986.

264.**Rudic RD, Shesely EG, Maeda N, Smithies O, Segal SS, and Sessa WC**. Direct evidence for the importance of endothelium-derived nitric oxide in vascular remodeling. *The Journal of clinical investigation* 101: 731-736, 1998.

265.**Rush JW, Laughlin MH, Woodman CR, and Price EM**. SOD-1 expression in pig coronary arterioles is increased by exercise training. *American journal of physiology* 279: H2068-2076, 2000.

266.**Schmidt-Trucksass A, Schmid A, Brunner C, Scherer N, Zach G, Keul J, and Huonker M**. Arterial properties of the carotid and femoral artery in endurance-trained and paraplegic subjects. *J Appl Physiol (1985)* 89: 1956-1963, 2000.

267.**Schrage WG, Joyner MJ, and Dinenno FA**. Local inhibition of nitric oxide and prostaglandins independently reduces forearm exercise hyperaemia in humans. *The Journal of physiology* 557: 599-611, 2004.

268.**Schretzenmayr A**. Uber kreislaufregulatorische vorgange an den grossen arterien bei der muskelarbeit. *Pluegers Arch* 232: 743-748, 1933.

269.**Schreuder TH, Green DJ, Hopman MT, and Thijssen DH**. Impact of retrograde shear rate on brachial and superficial femoral artery flow-mediated dilation in older subjects. *Atherosclerosis* 241: 199-204, 2015.

270.**Schreuder TH, van Lotringen JH, Hopman MT, and Thijssen DH**. Impact of endothelin blockade on acute exercise-induced changes in blood flow and endothelial function in type 2 diabetes mellitus. *Experimental physiology* 99: 1253-1264, 2014.

271.**Segal SS**. Cell-to-cell communication coordinates blood flow control. *Hypertension* 23: 1113-1120, 1994.

272.**Shephard RJ**. A short history of occupational fitness and health promotion. *Preventive medicine* 20: 436-445, 1991.

273.**Shimada K, Mikami Y, Murayama T, Yokode M, Fujita M, Kita T, and Kishimoto C**. Atherosclerotic plaques induced by marble-burying behavior are stabilized by exercise training in experimental atherosclerosis. *International journal of cardiology* 151: 284-289, 2011.

274.**Simmons GH, Padilla J, Young CN, Wong BJ, Lang JA, Davis MJ, Laughlin MH, and Fadel PJ**. Increased brachial artery retrograde shear rate at exercise onset is abolished during prolonged cycling: role of thermoregulatory vasodilation. *J Appl Physiol* 110: 389-397, 2011.

275.**Sinoway LI, Hendrickson C, Davidson WR, Jr., Prophet S, and Zelis R**. Characteristics of flow-mediated brachial artery vasodilation in human subjects. *Circulation research* 64: 32-42, 1989.

276.**Sinoway LI, Musch TI, Minotti JR, and Zelis R**. Enhanced maximal metabolic vasodilatation in the dominant forearms of tennis players. *J Appl Physiol* 61: 673-678, 1986.

277.**Snell PG, Martin WH, Buckey JC, and Blomqvist CG**. Maximal vascular leg conductance in trained and untrained men. *J Appl Physiol (1985)* 62: 606-610, 1987.

278.**Snell PG, Martin WH, Buckey JC, and Blomqvist CG**. Maximal vascular leg conductance in trained and untrained men. *J Appl Physiol* 62: 606-610, 1987.

279.**Spence AL, Carter HH, Naylor LH, and Green DJ**. A prospective randomized longitudinal study involving 6 months of endurance or resistance exercise. Conduit artery adaptation in humans. *J Physiol* 591: 1265-1275, 2013.

280.**Spence AL, Carter HH, Naylor LH, and Green DJ**. A prospective randomized longitudinal study involving 6 months of endurance or resistance exercise. Conduit artery adaptation in humans. *The Journal of physiology* 591: 1265-1275, 2013.

281.**Sugawara J, Komine H, Hayashi K, Yoshizawa M, Otsuki T, Shimojo N, Miyauchi T, Yokoi T, Maeda S, and Tanaka H**. Systemic alpha-adrenergic and nitric oxide inhibition on basal limb blood flow: effects of endurance training in middle-aged and older adults. *American journal of physiology* 293: H1466-1472, 2007.

282.**Sun D, Huang A, Koller A, and Kaley G**. Short-term daily exercise activity enhances endothelial NO synthesis in skeletal muscle arterioles of rats. *J Appl Physiol* 76: 2241-2247, 1994.

283.**Sun D, Huang A, Yan EH, Wu Z, Yan C, Kaminski PM, Oury TD, Wolin MS, and Kaley G**. Reduced release of nitric oxide to shear stress in mesenteric arteries of aged rats. *Am J Physiol Heart Circ Physiol* 286: H2249-2256, 2004.

284.**Takabe W, Jen N, Ai L, Hamilton R, Wang S, Holmes K, Dharbandi F, Khalsa B, Bressler S, Barr ML, Li R, and Hsiai TK**. Oscillatory shear stress induces mitochondrial superoxide production: implication of NADPH oxidase and c-Jun NH2-terminal kinase signaling. *Antioxid Redox Signal* 15: 1379-1388, 2011.

285.**Tanaka H, Seals DR, Monahan KD, Clevenger CM, DeSouza CA, and Dinenno FA**. Regular aerobic exercise and the age-related increase in carotid artery intima-media thickness in healthy men. *J Appl Physiol* 92: 1458-1464, 2002.

286.**Taylor RS, Unal B, Critchley JA, and Capewell S**. Mortality reductions in patients receiving exercise-based cardiac rehabilitation: how much can be attributed to cardiovascular risk factor improvements? *Eur J Cardiovasc Prev Rehabil* 13: 369-374, 2006.

287. **Thijssen DH, Black MA, Pyke KE, Padilla J, Atkinson G, Harris RA, Parker B, Widlansky ME, Tschakovsky ME, and Green DJ**. Assessment of flow-mediated dilation in humans: a methodological and physiological guideline. *American journal of physiology* 300: H2-12, 2011.

288. **Thijssen DH, Cable NT, and Green DJ**. Impact of exercise training on arterial wall thickness in humans. *Clin Sci (Lond)* 122: 311-322, 2012.

289. **Thijssen DH, Cable NT, and Green DJ**. Impact of exercise training on arterial wall thickness in humans. *Clinical science* 122: 311-322, 2012.

290. **Thijssen DH, Dawson EA, Black MA, Hopman MT, Cable NT, and Green DJ**. Brachial artery blood flow responses to different modalities of lower limb exercise. *Medicine and science in sports and exercise* 41: 1072-1079, 2009.

291. **Thijssen DH, Dawson EA, Tinken TM, Cable NT, and Green DJ**. Retrograde flow and shear rate acutely impair endothelial function in humans. *Hypertension* 53: 986-992, 2009.

292. **Thijssen DH, Dawson EA, van den Munckhof IC, Tinken TM, den Drijver E, Hopkins N, Cable NT, and Green DJ**. Exercise-mediated changes in conduit artery wall thickness in humans: role of shear stress. *American journal of physiology* 301: H241-246, 2011.

293. **Thijssen DH, de Groot PC, Smits P, and Hopman MT**. Vascular adaptations to 8-week cycling training in older men. *Acta physiologica* 190: 221-228, 2007.

294. **Thijssen DH, Ellenkamp R, Kooijman M, Pickkers P, Rongen GA, Hopman MT, and Smits P**. A causal role for endothelin-1 in the vascular adaptation to skeletal muscle deconditioning in spinal cord injury. *Arterioscler Thromb Vasc Biol* 27: 325-331, 2007.

295. **Thijssen DH, Ellenkamp R, Smits P, and Hopman MT**. Rapid vascular adaptations to training and detraining in persons with spinal cord injury. *Archives of physical medicine and rehabilitation* 87: 474-481, 2006.

296. **Thijssen DH, Maiorana AJ, O'Driscoll G, Cable NT, Hopman MT, and Green DJ**. Impact of inactivity and exercise on the vasculature in humans. *European journal of applied physiology* 108: 845-875, 2010.

297. **Thijssen DH, Rongen GA, van Dijk A, Smits P, and Hopman MT**. Enhanced endothelin-1-mediated leg vascular tone in healthy older subjects. *J Appl Physiol* 103: 852-857, 2007.

298. **Thijssen DH, Scholten RR, van den Munckhof IC, Benda N, Green DJ, and Hopman MT**. Acute change in vascular tone alters intima-media thickness. *Hypertension* 58: 240-246, 2011.

299. **Thijssen DH, Schreuder TH, Newcomer SW, Laughlin MH, Hopman MT, and Green DJ**. Impact of 2-Weeks Continuous Increase in Retrograde Shear Stress on Brachial Artery Vasomotor Function in Young and Older Men. *J Am Heart Assoc* 4: 2015.

300. **Thijssen DH, Willems L, van den Munckhof I, Scholten R, Hopman MT, Dawson EA, Atkinson G, Cable NT, and Green DJ**. Impact of wall thickness on conduit artery function in humans: is there a "Folkow" effect? *Atherosclerosis* 217: 415-419, 2011.

301. **Thoma R**. *Untersuchungen uber die histogenese und histomechanik des gefassystems*. Stuttgart: 1893.

302. **Thompson MA, Henderson KK, Woodman CR, Turk JR, Rush JW, Price E, and Laughlin MH**. Exercise preserves endothelium-dependent relaxation in coronary

arteries of hypercholesterolemic male pigs. *J Appl Physiol (1985)* 96: 1114-1126, 2004.

303. **Thompson PD, Buchner D, Pina IL, Balady GJ, Williams MA, Marcus BH, Berra K, Blair SN, Costa F, Franklin B, Fletcher GF, Gordon NF, Pate RR, Rodriguez BL, Yancey AK, Wenger NK, American Heart Association Council on Clinical Cardiology Subcommittee on Exercise R, Prevention, American Heart Association Council on Nutrition PA, and Metabolism Subcommittee on Physical A**. Exercise and physical activity in the prevention and treatment of atherosclerotic cardiovascular disease: a statement from the Council on Clinical Cardiology (Subcommittee on Exercise, Rehabilitation, and Prevention) and the Council on Nutrition, Physical Activity, and Metabolism (Subcommittee on Physical Activity). *Circulation* 107: 3109-3116, 2003.

304. **Tinken TM, Thijssen DH, Black MA, Cable NT, and Green DJ**. Time course of change in vasodilator function and capacity in response to exercise training in humans. *The Journal of physiology* 586: 5003-5012, 2008.

305. **Tinken TM, Thijssen DH, Hopkins N, Black MA, Dawson EA, Minson CT, Newcomer SC, Laughlin MH, Cable NT, and Green DJ**. Impact of shear rate modulation on vascular function in humans. *Hypertension* 54: 278-285, 2009.

306. **Tinken TM, Thijssen DH, Hopkins N, Dawson EA, Cable NT, and Green DJ**. Shear stress mediates endothelial adaptations to exercise training in humans. *Hypertension* 55: 312-318, 2010.

307. **Tronc F, Wassef M, Esposito B, Henrion D, Glagov S, and Tedgui A**. Role of NO in flow-induced remodeling of the rabbit common carotid artery. *Arteriosclerosis, thrombosis, and vascular biology* 16: 1256-1262, 1996.

308. **Turnbull F**. Effects of different blood-pressure-lowering regimens on major cardiovascular events: results of prospectively-designed overviews of randomised trials. *Lancet* 362: 1527-1535, 2003.

309. **Tuttle JL, Nachreiner RD, Bhuller AS, Condict KW, Connors BA, Herring BP, Dalsing MC, and Unthank JL**. Shear level influences resistance artery remodeling: wall dimensions, cell density, and eNOS expression. *American journal of physiology* 281: H1380-1389, 2001.

310. **Van Guilder GP, Westby CM, Greiner JJ, Stauffer BL, and DeSouza CA**. Endothelin-1 vasoconstrictor tone increases with age in healthy men but can be reduced by regular aerobic exercise. *Hypertension* 50: 403-409, 2007.

311. **Vassalle C, Lubrano V, Domenici C, and L'Abbate A**. Influence of chronic aerobic exercise on microcirculatory flow and nitric oxide in humans. *Int J Sports Med* 24: 30-35, 2003.

312. **Verhaar MC, Strachan FE, Newby DE, Cruden NL, Koomans HA, Rabelink TJ, and Webb DJ**. Endothelin-A receptor antagonist-mediated vasodilatation is attenuated by inhibition of nitric oxide synthesis and by endothelin-B receptor blockade. *Circulation* 97: 752-756., 1998.

313. **Vlachopoulos C, Aznaouridis K, and Stefanadis C**. Aortic Stiffness for Cardiovarscular Risk Prediction: Just Measure It, Just Do It! *Journal of the American College of Cardiology* 2013.

314. **Vlachopoulos C, Aznaouridis K, and Stefanadis C**. Aortic stiffness for cardiovascular risk prediction: just measure it, just do it! *Journal of the American College of Cardiology* 63: 647-649, 2014.

315. **Walsh JH, Bilsborough W, Maiorana A, Best M, O'Driscoll GJ, Taylor RR, and Green DJ**. Exercise training improves conduit vessel function in patients with coronary artery disease. *J Appl Physiol* 95: 20-25, 2003.

316. **Walsh JH, Yong G, Cheetham C, Watts GF, O'Driscoll GJ, Taylor RR, and Green DJ**. Effects of exercise training on conduit and resistance vessel function in treated and untreated hypercholesterolaemic subjects. *European heart journal* 24: 1681-1689, 2003.

317. **Wang JS**. Effects of exercise training and detraining on cutaneous microvascular function in man: the regulatory role of endothelium-dependent dilation in skin vasculature. *European journal of applied physiology* 93: 429-434, 2005.

318. **Willett NJ, Kundu K, Knight SF, Dikalov S, Murthy N, and Taylor WR**. Redox signaling in an in vivo murine model of low magnitude oscillatory wall shear stress. *Antioxid Redox Signal* 15: 1369-1378, 2011.

319. **Williams PT, and Thompson PD**. Walking versus running for hypertension, cholesterol, and diabetes mellitus risk reduction. *Arteriosclerosis, thrombosis, and vascular biology* 33: 1085-1091, 2013.

320. **Wisloff U, Stoylen A, Loennechen JP, Bruvold M, Rognmo O, Haram PM, Tjonna AE, Helgerud J, Slordahl SA, Lee SJ, Videm V, Bye A, Smith GL, Najjar SM, Ellingsen O, and Skjaerpe T**. Superior cardiovascular effect of aerobic interval training versus moderate continuous training in heart failure patients: a randomized study. *Circulation* 115: 3086-3094, 2007.

321. **Woodman CR, Price EM, and Laughlin MH**. Shear stress induces eNOS mRNA expression and improves endothelium-dependent dilation in senescent soleus muscle feed arteries. *J Appl Physiol* 98: 940-946, 2005.

322. **Woodman CR, Turk JR, Rush JW, and Laughlin MH**. Exercise attenuates the effects of hypercholesterolemia on endothelium-dependent relaxation in coronary arteries from adult female pigs. *J Appl Physiol (1985)* 96: 1105-1113, 2004.

323. **Woodman CR, Turk JR, Williams DP, and Laughlin MH**. Exercise training preserves endothelium-dependent relaxation in brachial arteries from hyperlipidemic pigs. *J Appl Physiol (1985)* 94: 2017-2026, 2003.

324. **Yoshikawa D, Ishii H, Kurebayashi N, Sato B, Hayakawa S, Ando H, Hayashi M, Isobe S, Okumura T, Hirashiki A, Takeshita K, Amano T, Uetani T, Yamada S, and Murohara T**. Association of cardiorespiratory fitness with characteristics of coronary plaque: assessment using integrated backscatter intravascular ultrasound and optical coherence tomography. *Int J Cardiol* 162: 123-128, 2013.

325. **Zarins CK, Giddens DP, Bharadvaj BK, Sottiurai VS, Mabon RF, and Glagov S**. Carotid bifurcation atherosclerosis. Quantitative correlation of plaque localization with flow velocity profiles and wall shear stress. *Circulation research* 53: 502-514, 1983.

326. **Zeiher AM, Drexler H, Wollschlaeger H, Saurbier B, and Just H**. Coronary vasomotion in response to sympathetic stimulation in humans: importance of the functional integrity of the endothelium. *Journal of the American College of Cardiology* 14: 1181-1190, 1989.

327. **Zeppilli P, Vannicelli R, Santini C, Dello Russo A, Picani C, Palmieri V, Cameli S, Corsetti R, and Pietrangeli L**. Echocardiographic size of conductance vessels in athletes and sedentary people. *Int J Sports Med* 16: 38-44, 1995.

328. **Zhang X, Cheng R, Rowe D, Sethu P, Daugherty A, Yu G, and Shin HY**. Shear-sensitive regulation of neutrophil flow behavior and its potential impact on

microvascular blood flow dysregulation in hypercholesterolemia. *Arteriosclerosis, thrombosis, and vascular biology* 34: 587-593, 2014.

329. **Zoeller RF, Angelopoulos TJ, Thompson BC, Wenta MR, Price TB, Thompson PD, Moyna NM, Seip RL, Clarkson PM, Gordon PM, Pescatello LS, Devaney JM, Gordish-Dressman H, Hoffman EP, and Visich PS**. Vascular remodeling in response to 12 wk of upper arm unilateral resistance training. *Med Sci Sports Exerc* 41: 2003-2008, 2009.

330. **Zoladz JA, Majerczak J, Duda K, and Chlopicki S**. Endurance training increases exercise-induced prostacyclin release in young, healthy men--relationship with VO2max. *Pharmacol Rep* 62: 494-502, 2010.

FIGURE LEGENDS

FIGURE 1. Using data from a series of exercise training studies Clausen demonstrated, in his *Physiological Review* (redrawn from (50), that oxygen uptake improved with training (by 0.34 L/min on average), whilst cardiac output also significantly increased (by 2.1 L/min), yet blood pressure did not change, or slightly decreases. This insight highlights the relevance of the peripheral vasculature in accomodating the increase in cardiac output that accomanies training. Vascular adaptations encompass both functional and sturctural changes, which may occur along distinct timecourses (see **Figure 8**).

FIGURE 2. Illustration showing the interactions of hemodynamic signals (Top) (hydrostatic pressure, shear stress and circumferential stretch) that modulate vascular adaptation to exercise. The effects of pressure and/or stretch on the endothelial cells is shown in the middle as described in the text. At the bottom the figure illustrates exercise-induced adaptations of smooth muscle cells (modified from 32,167). The left-center of the smooth muscle figure shows calcium transients with decreased intracellular calcium ($[Ca^{2+}]_i$) response to selective agonists (e.g. endothelin) in exercise trained cells in red (which produces a reduced Ca^{2+}-dependent activation of contraction). This decreased Ca_i occurs despite an increased Ca^{2+}-influx through L-type Ca^{2+} channels ($Ca_v1.2$). Nuclear Ca^{2+} responses ($[Ca^{2+}]n$) are also reduced by exercise training, which may affect Ca^{2+}-dependent transcription factors (CaTF, e.g. CREB, NFAT) and target gene expression. Also illustrated is the increased spontaneous, slow-Ca^{2+} release from the SR into the subscarolemmal space ($[Ca^{2+}]_{ss}$) which may contribute to the increased activation of large conductance, Ca^{2+}-activated (BK) potassium channels caused by exercise. Voltage-gated (K_v) potassium channels are also activated by exercise training.

FIGURE 3. Rendering of Rodbard's prediction of 'flow-dependent, endothelium-mediated dilation' and remodelling. Exposure of the endothelium to an increase in viscous drag or "shear stress" (step 2), triggers dilation (step 3); a functional change that tends to homeostatically modify the initial increase in shear (see also **Figure 8**). When exposed to prolonged periods of change in flow and shear, vessel remodelling can occur, whereby drag forces are "structurally" normalised (step 4). These predictions were ultimately verified in both animals (and humans).

FIGURE 4. Summary of the outcomes of studies that explored the impact of endothelial denudation on conduit artery functional and structural responses. Endothelial removal has typically been achieved using intra-arterial balloon inflation. In endothelium intact arteries, increases in flow and shear induce dilation in the dog hind limb (246), coronary arteries (21) and the human radial artery (61). All functional and structural adaptive responses are abolished or attenuated in the absence of an intact endothelial layer. Remodelling of rabbit carotid arteries in response to chronic decreases in flow and shear induced using unilateral ligation is also endothelium-dependent (167). These studies highlight the importance of the endothelium to mediate (acute and chronic) changes in diameter.

FIGURE 5. Brachial artery Doppler trace during leg cycle exercise (A; brachial artery representing an inactive region) and during handgrip exercise (B: brachial artery representing an active region) at rest, at the start of exercise and during continuation of exercise. The (time-dependent) changes in Doppler patterns are influenced by subject, central and peripheral factors, summarised in the lower panel.

FIGURE 6. Doppler trace (A), blood flow patterns (B) and consequent acute changes in vascular function (measured as the flow-mediated dilation FMD, C). Distinct patterns of blood flow and shear in the brachial artery induced by forearm heating, handgrip exercise, cycle exercise and cuff manipulation have different impacts of the function of the artery, assessed immediately before versus after each intervention. Data are derived from (305) and (291). Taken together, these data are largely in agreement with previous work in animals (see section IIB*ii*), and support a role for shear stress in the alteration of endothelial function, with distinct shear patterns leading to different changes in function.

FIGURE 7. Impact of exercise training in humans with coronary disease. Relative to a non-trained control group (CON), four weeks of exercise training (EX) increased *in vivo* acetylcholine-induced vasodilation of the left internal mammary artery (LIMA) (A), increased endothelial nitric oxide synthase (eNOS) mRNA and protein expression in LIMA (B), as well as increased phosphorylation of Akt at Ser^{473} and eNOS at Ser^{1177} (C), an effect likely mediated by shear stress. Figures redrawn from Hambrecht et al. 2003 (114). Data are mean ± SD. *denotes statistical significance between groups.

FIGURE 8. Time-dependent changes in vascular dilatory function (blue line) and structure (red line) across a period of exercise training in healthy volunteers. Laughlin proposed that initial improvements in vascular dilatory function contribute to normalise shear stress during exercise bouts, whereas continuing exercise will results in more "permanent" normalisation of shear stress (168). Human studies designed to test this proposal confirmed that both brachial and popliteal artery function and structure adapt according to distinct time course across 8-weeks of exercise training in healthy volunteers (304) and that such adaptation is shear dependent (23) (306).