The Impact of a 12-week High-Intensity Interval Training Program on Popliteal Vascular Responses to Prolonged Sitting

by

Haoxuan Liu

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Abstract

Prolonged, uninterrupted sitting (≥1-h) impairs lower-limb flow-mediated dilation (FMD), nitroglycerin-mediated dilation (NMD), and resistance vessel responses. The benefits of regular aerobic exercise on vascular health are well established. However, conflicting evidence exists regarding the influence of aerobic fitness on sitting induced-reductions in lower-limb arterial function. To explore the impact that 12-weeks of high-intensity interval training (HIIT, 3 sessions/week) had on popliteal FMD, NMD, and reactive hyperemic responses to a bout of prolonged sitting. Twenty-one healthy adults were randomly assigned to HIIT (n=7, 24±8 years, 7♀) or Control groups (n=10, 22±1 years, 6♀). Relative FMD responses (% peak increase from baseline diameter) to 5-min distal cuff occlusion (250 mmHg) and relative NMD responses (% peak increase from baseline diameter) to sublingual nitroglycerin administration (0.4 mg), as well as post-occlusive peak red cell velocity (RBCv) were assessed via duplex ultrasonography before and after a ~3-h bout of uninterrupted sitting. Peak oxygen consumption (relative $\dot{V}O_2$peak, indirect calorimetry) using a maximal cycle ergometer protocol graded cycle ergometry was also assessed. These assessments were repeated following the HIIT (2×20-min bouts of alternating between 15-s intervals at 100% of peak aerobic power and passive recovery) or Control (habitual physical activity) periods. 12-week HIIT improved relative $\dot{V}O_2$peak (35.4±7.8 to 39.5±6.1 ml/kg/min, P=0.005), with no changes observed in the Control group (P=0.306). Sitting-induced changes in popliteal FMD (HIIT: -1.4±2.6 to -1.7±1.9%; Control: -2.9±2.2 to -2.0±2.3%), NMD (HIIT: -3.1±2.9 to -3.2±2.5%; Control: -3.0±2.9 to -3.8±3.7%), or peak hyperemic responses (HIIT: -12.8±6.1 to -12.5±11.0 cm/s; Control: -26.0±14.4 to -9.5±24.7 cm/s) did not change at follow-up in either group (all, P≥0.105). These results indicate that a 12-week HIIT intervention did not provide protection against prolonged sitting-induced lower-limb vascular dysfunction.

List of Abbreviations Used

AUC – Area under the curve

Ca^{2+} – Calcium

cGMP – cyclic guanosine monophosphate

CVD – Cardiovascular disease

DBP – Diastolic blood pressure

HIIT – High-intensity interval training

FMD – Flow-mediated dilation

NADPH – Nicotinamide adenine dinucleotide phosphate

NO – Nitric oxide

NMD – Nitroglycerin-mediated dilation

OONO – Peroxynitrate

PAP – Peak aerobic power

PBF – Popliteal blood flow

RBCv – Red blood cell velocity

RM-ANOVA – Repeated measures analyses of variance

SBP – Systolic blood pressure

SR_{AUC} – Shear rate area under the curve

$\dot{V}CO_2$ – The volume rate of carbon dioxide

$\dot{V}O_2peak$ – Peak oxygen consumption

VSMCs – Vascular smooth muscle cells

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Chapter 1. INTRODUCTION

Excessive time spent in sedentary postures (e.g., sitting) has been associated with cardiovascular disease (CVD) incidence and mortality (1). Recent evidence suggests that the harmful effects of prolonged sitting on vascular health are likely responsible for the increased CVD risk associated with sedentary behavior (2, 3). In contrast, regular aerobic exercise training is well-known to improve vascular health and is an evidence-based strategy for decreasing CVD risk (4). However, the interaction between the acute cardiovascular effects of sedentary behaviours and aerobic exercise training remains unclear. Specifically, it is unknown whether aerobic exercise training can afford protection against the detrimental effects of prolonged sedentary bouts on cardiovascular health.

The flow-mediated dilation (FMD) technique provides a measure of conduit artery endothelium-dependent health by quantifying the increase in lumen diameter in response to an acute rise in blood flow (i.e., reactive hyperemia) and shear stress provoked by a prior period of distal ischemia (5). The elevated shear stress stimulates endothelial nitric oxide synthase to increase nitric oxide (NO) production, which relaxes the underlying vascular smooth muscle cells (VSMCs) to cause vasodilation (6). In addition, the magnitude of the reactive hyperemia also serves as an index of downstream resistance vessel (e.g., arterioles) function (7, 8). The FMD and reactive hyperemia responses are traditionally assessed in the brachial artery as they are predictive of adverse cardiovascular events (9, 10). However, upper- and lower-limb arteries exhibit heterogeneous FMD responses (11, 12). Furthermore, lower-limb arteries are more susceptible to atherosclerosis development and are directly exposed to local

hemodynamic changes during sedentary activities (e.g., sitting) and lower body modes of aerobic exercise (e.g., cycling) compared with upper-limb arteries (e.g., brachial artery). As such, the importance of assessing the impact of sedentary bouts and aerobic training on lower-limb FMD and reactive hyperemia response is warranted.

Prolonged uninterrupted sitting (i.e., ≥ 1-h) markedly attenuates reactive hyperemic and FMD responses in lower-limb arteries (e.g., popliteal artery), as reviewed by Paterson et al. (2). Habitual prolonged bouts of sedentary time are also associated with lower popliteal FMD responses (13). Sitting-induced declines in FMD are typically attributed to decreased blood flow-induced shear stress, diminished NO bioavailability (14), increased production of vasoconstrictors (e.g., endothelin-1), and corresponding attenuations in the shear stress stimulus for FMD (15, 16). The seated posture also leads to arterial bending created by hip and knee flexion. Arterial tortuosity not only contributes to decreased blood flow, but also creates an area of disturbed/turbulent flow that may cause endothelial dysfunction (17). Additionally, reductions in VSMCs sensitivity to NO may also be involved in sitting-induced declines in FMD (18). We have previously reported that prolonged sitting also attenuates popliteal nitroglycerin-mediated dilation (NMD) responses (19), which is indicative of diminished vascular smooth muscle sensitivity to an exogenous NO donor (18). Importantly, NMD responses also serve as an index of VSMCs function and are attenuated in individuals with elevated CVD risk (20–24). As such, prolonged sitting-induced vascular smooth muscle dysfunction may be involved in the mechanisms linking sedentary behaviour to increased CVD risk.

It is well established that aerobic exercise training, particularly high-intensity interval training (HIIT), defined as repeated short (<45-s) to long (2-4 min) bouts of high-intensity (i.e., ≥90% of $\dot{V}O_2$peak) exercise interspersed with recovery periods (25, 26), can augment FMD responses (27). Additionally, HIIT is a safe and effective exercise strategy to elicit improvements in aerobic fitness (28–30), which is directly related to more favorable endothelium-dependent vasodilatory function (31). However, the effects of aerobic training on prolonged sitting-induced vascular dysfunction remain unknown. To date, conflicting reports exist regarding whether higher aerobic fitness *per se* attenuates (32), exacerbates (33), or has no impact (34) on lower-limb FMD responses to a 3-h bout of prolonged sitting. A major limitation of these studies is they all used a cross-sectional design, which is susceptible to between-subject factors that may have contributed to the divergent findings. Accordingly, randomized, controlled exercise training intervention studies are warranted to determine the impact of aerobic fitness on the vascular consequences to uninterrupted sitting. Mekari et al. (35) have shown that a 6-week, high-volume HIIT protocol did not improve aerobic fitness (but did increase peak aerobic power output) in young healthy adults. However, O'Brien et al. (29) found that the same 6-week HIIT protocol was effective at increasing aerobic fitness in older adults. This indicates that younger, healthy adults may require a longer training period, and/or progressive increases in intensity and session durations, to achieve improvements in aerobic fitness compared with older adults who likely have lower baseline fitness levels.

Collectively, the primary objective of the present study was to explore the impact of a 12-week HIIT program on baseline popliteal health outcomes, as well as vascular responses to prolonged sitting in younger, healthy adults. It was hypothesized

that: 1) popliteal FMD, NMD, and reactive hyperemic responses would be attenuated after prolonged sitting before the 12-week HIIT program, and 2) aerobic fitness and pre-sitting popliteal FMD would be improved after the 12-week HIIT program. However, no directional hypothesis regarding the impact of HIIT on popliteal vascular responses to sitting should be assumed due to the conflicting evidence from the three cross-sectional studies highlighted above (32–34).

Chapter 2. LITERATURE REVIEW

2.1 Impact of Aerobic Exercise and Aerobic Fitness on Cardiovascular Health

Cardiovascular diseases (CVD) are the leading cause of death worldwide (36). Engagement in regular physical activity (37) and aerobic exercise are strongly associated with reduced CVD risk (4). The benefits of aerobic exercise on cardiovascular health include bradycardia (lowered resting heart rate), reductions in blood pressure, and enhanced myocardial contractility (38). Furthermore, the protective effects of aerobic exercise extend to the vasculature, especially the arterial system (39).

A key outcome of aerobic exercise training is an improvement in aerobic or cardiorespiratory fitness (30), which refers to the maximal capacity of the respiratory and circulatory systems to deliver oxygen, as well as for the active tissues (e.g., skeletal muscles) to extract and utilize oxygen for aerobic energy metabolism (40). Aerobic fitness can be measured as peak oxygen uptake ($\dot{V}O_2$peak) via indirect calorimetry during a graded, maximal exercise test that incorporates large muscle groups (e.g., cycling) (40). Peak $\dot{V}O_2$ can be expressed in absolute units (L/min), which is directly related to body mass. As such, absolute values should not be used to compare aerobic fitness between individuals of varying sizes (40). A better alternative is to also express $\dot{V}O_2$peak in units relative to body mass (ml/kg/min) (40). Relative $\dot{V}O_2$peak is better for comparing fitness levels between different individuals of different sizes.

There is accumulating evidence that maintaining or improving aerobic fitness over the lifespan reduces modifiable risk factors (e.g., hypertension, dyslipidemia) for CVD (41). In contrast, low aerobic fitness is an important risk factor for CVD (41) and all-cause mortality [**Figure 2.1**, (42)]. For example, Laukkanen et al. (43) conducted an

11-year follow-up study that demonstrated a 9% relative risk reduction (i.e., the division of absolute risk reduction by absolute risk of the group) of all-cause mortality for every 1 ml/kg/min increase in relative $\dot{V}O_2$peak among 579 middle-older adults (aged 42-60 years). As reviewed by Kodama et al. (44), lower aerobic fitness can be used as a clinical predictor of future CVD events and all-cause mortality risk. Therefore, improving aerobic fitness through aerobic exercise training is particularly important for preventing CVD and promoting cardiovascular health.

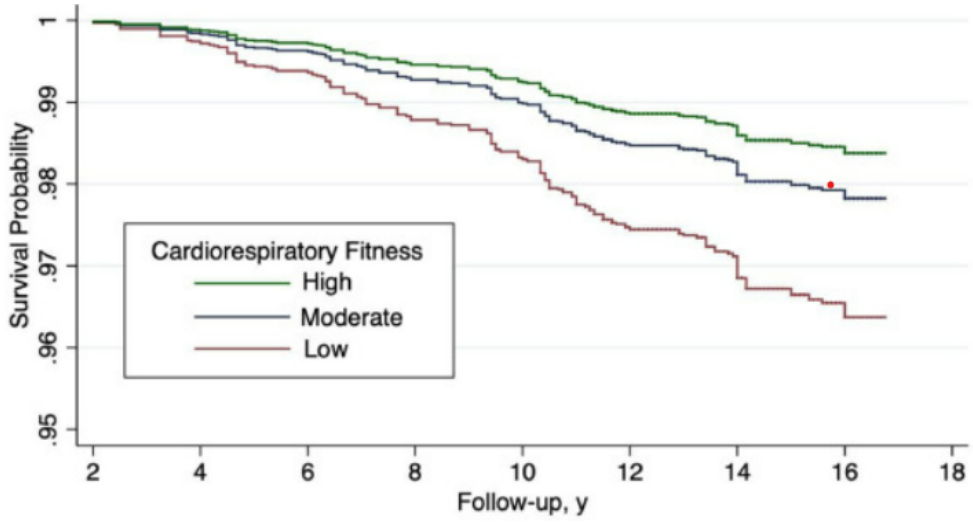

Figure 2.1. Survival curves of all-cause mortality stratified by cardiorespiratory fitness levels (42). Higher levels of cardiorespiratory fitness were associated with reduced all-cause mortality rates. Cut-off values: Low fitness: 32.5 ml/kg/min (male) and 26.4 ml/kg/min (female); Moderate fitness: 39 ml/kg/min (male) and 31.9 (female); High fitness: 49.9 ml/kg/min (male) and 42.2 ml/kg/min (female).

2.2 Impact of Sedentary Behaviour on Cardiovascular Health

Habitual sedentary behaviors (i.e., any waking behavior characterized by an energy expenditure of ≤ 1.5 metabolic equivalents while in a sitting, lying, or reclining position) is among the leading behavioral risk factors for CVD (45). Epidemiological studies have indicated a direct dose-response relationship between daily sedentary behavior time versus all-cause and CVD-related mortality, independent of physical

activity levels (1). In addition to total sedentary time, the accumulation pattern of sedentary behaviors has recently been postulated to influence cardiovascular health (13, 46–48). Interestingly, more breaks in sedentary time are beneficially associated with CVD risk factors including waist circumference, triglycerides, body mass index, plasma glucose, and inflammatory biomarker (i.e., C-reactive protein), independent of total sedentary time (46, 47). Furthermore, our lab recently demonstrated that more frequent sedentary breaks and/or a lower number of prolonged sedentary bouts, but not lower total sedentary time, were positively related to healthy vagal-mediated blood pressure regulation (48) and lower-limb arterial function (13). Despite these cross-sectional studies, well-controlled laboratory-based research has shown that an acute bout of prolonged, uninterrupted sitting (e.g., 1-6 h) elicits elevations in postprandial blood glucose, blood pressure, and aortic stiffening, as well as impairments in lower-limb artery health (2, 49, 50). The Canadian 24-hour movement guidelines also suggest that adults break up long periods of sitting as often as possible to maximize health benefits (51). Collectively, the available evidence reveals that prolonged, uninterrupted sedentary behaviors are a significant risk factor for CVD. Recent evidence has shown that the link between sedentary behavior and CVD risk is partially attributed to the harmful effects of prolonged sitting on lower-limb vascular health (2, 3).

2.3 Overview of Peripheral Artery Structure and Function

2.3.1 Conduit Artery and Resistance Vessels

The role of conduit arteries is to provide a low resistance path for effectiveness blood flow distribution to the organs and tissues. The downstream resistance vessels (i.e., arterioles) constitute a major site for the generation of peripheral vascular resistance and

are responsible for the regulation of blood pressure and capillary blood flow (52). According to Poiseuille's Law (Resistance = $\frac{8*\eta*l}{\pi*r^4}$, where 'η' is viscosity, 'l' is the length of the vessel, and 'r' is the radius of the vessel lumen), vascular resistance is inversely proportional to the fourth power of blood vessel radius, which indicates that a small decrease in the lumen size can markedly increases vascular resistance (52). As such, relatively small changes in the lumen diameter of resistance vessels via vasodilation (i.e., increased diameter) or vasoconstriction (i.e., decreased diameter) can effectively regulate blood pressure. In addition to blood pressure regulation, resistance vessels also play a vital role in distributing blood within tissues according to local metabolic needs (e.g., oxygen demand) (53). Resistance vessels are very sensitive to local metabolic stimulus and can respond to metabolic demands of the tissue via adjustment of lumen diameter to alter capillary blood flow (53).

As shown in **Figure 2.2** (52), conduit arteries and resistance vessels are composed of 3 discrete layers: *tunica adventitia* (or *externa*), the *tunica media*, and the *tunica intima* (52). The *tunica adventitia* is the outermost layer of the blood vessel that provides shape and structural support (52). It is composed of connective tissue (e.g., elastin and collagen), along with post-ganglionic sympathetic nerve terminals (i.e., *nervi vasorum*) and blood supply (i.e., *vaso vasorum*) (52). The *tunica media* is the middle layer, composed of vascular smooth muscle cells (VSMCs), collagen, elastin, and glycoproteins (52). It is primarily responsible for adjusting vascular tone (i.e., degree of vasoconstriction or vasodilation) and blood flow regulation (52). Finally, the *tunica intima* consists of a single layer of endothelial cells that line the entire vascular tree and are in direct contact with the blood (i.e., it is a selectively permeable membrane) (52).

The endothelial cells produce potent vasoactive substances that regulate smooth muscle contraction and relaxation (52).

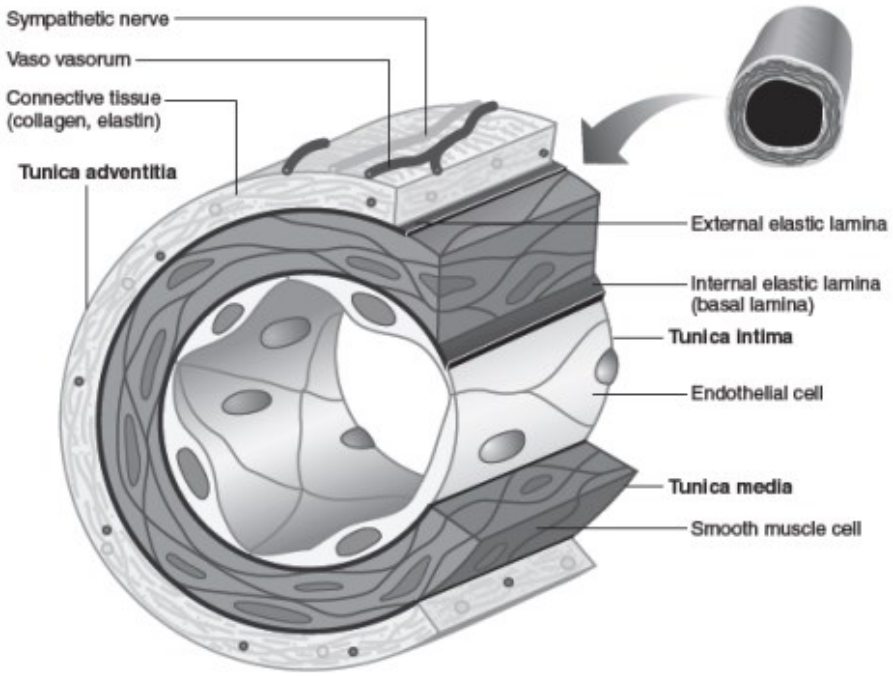

Figure 2.2. The structure of a conduit artery or resistance vessel (52). The *tunica adventitia* is composed of connective tissue (i.e., collagen and elastin), post-ganglionic sympathetic nerve endings, and the vaso vasorum (i.e., small blood vessels delivering nutrients and oxygen to the vessel wall). The *tunica media* is composed of vascular smooth muscle that regulates lumen diameter and blood flow through relaxation and contraction. The *tunica intima* is composed of the vascular endothelium that produces vasoactive substances (e.g., nitric oxide).

2.3.2 Vascular Endothelium

The endothelium is sensitive to hemodynamic changes (i.e., changes in blood flow) and regulates vascular tone via the production and release of vasoactive substances (52). These chemicals can be either vasodilatory [e.g., nitric oxide (NO), endothelium-derived hyperpolarizing factors, and prostacyclin), or vasoconstrictor substances [e.g., endothelin-1] (52). In addition to the regulation of vascular tone, the endothelium exerts anticoagulant, fibrinolytic, anti-inflammatory, and antiplatelet properties (52). As such, the endothelium plays a critical role in maintaining vascular homeostasis.

2.3.2.1 Endothelium-Dependent Nitric Oxide Production

Alteration in the release and action of endothelium-derived vasoactive factors, particularly NO, contributes to attenuated vascular reactivity in the early stages of vascular diseases (e.g., atherosclerosis) (54). Nitric oxide is not only a potent vasodilator, but also exerts powerful anti-inflammatory, antioxidant, and antithrombotic effects (52). Physiologically, increases in blood flow and shear stress (i.e., the tangential, frictional force generated by red blood cells on the endothelial surface) are the primary mechanism of NO production and subsequent vasodilation (5). Shear stress is sensed by the deformation of mechanosensitive structures (e.g., glycocalyx, primary cilia) on the endothelial cell membrane, which interacts with ion channels and the cytoskeleton (55). For example, shear stress can be detected by the primary cilia that extend into the blood vessel lumen and sense changes in blood flow (56). When there is a sudden increase in blood flow, the cilia are bent and calcium (Ca^{2+}) channels on the cilia open via mechanosensitive polycystin-1 membrane proteins and the polycystin-2 channel (56). As shown in **Figure 2.3** (57), this results in an influx of Ca^{2+} into the endothelial cells, which binds to calmodulin (i.e., a calcium modulating protein). Calmodulin activates the enzyme endothelial nitric oxide synthase (eNOS) that converts the amino acid L-arginine to NO and L-citrulline (6, 57, 58). Of note, the production of NO by eNOS also requires various co-factors such as tetrahydrobiopterin and nicotinamide adenine dinucleotide phosphate (57, 58).

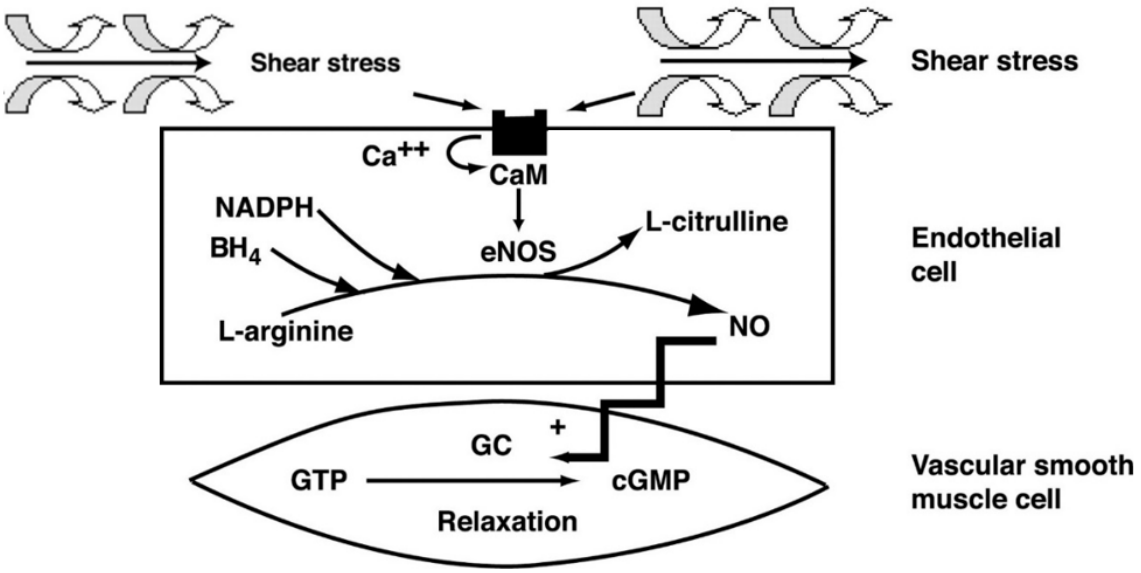

Figure 2.3. Shear stress-induced endothelial nitric oxide (NO) production via a calcium-dependent pathway (57). Shear stress activates mechanoreceptors (e.g., glycocalyx) on the endothelial cell membrane, which causes an increase in the intracellular concentration of calcium (Ca^{++}). Calcium binds to calmodulin (CaM) and results in the activation of the calmodulin-binding domain of endothelial nitric oxide synthase (eNOS) leading to phosphorylation of eNOS and subsequent conversion of L-arginine into NO. This reaction requires the co-factors nicotinamide adenine dinucleotide phosphate (NADPH) and tetrahydrobiopterin (BH_4). Once produced, NO diffuse into the vascular smooth muscle cell and stimulates soluble guanylate cyclase (sGC), thereby increasing cyclic guanosine-3', 5'-monophosphate (cGMP), and finally leading to calcium depletion from the cytosolic space and smooth muscle relaxation.

The shear stress-mediated activation of eNOS and NO production can also be achieved via Ca^{2+}-independent pathways [**Figure 2.4**, (58)]. Specifically, shear stress increases the concentration of 3',5'-cyclic adenosine monophosphate, which stimulates phosphorylation of protein kinase A, which in turn, phosphorylates eNOS at the activation sites Serine1177 and Serine 635, increasing the rate of NO production (58). The production of NO is also regulated via the protein kinase B pathway, activated by phosphoinositide-3-kinase-dependent mechanisms.

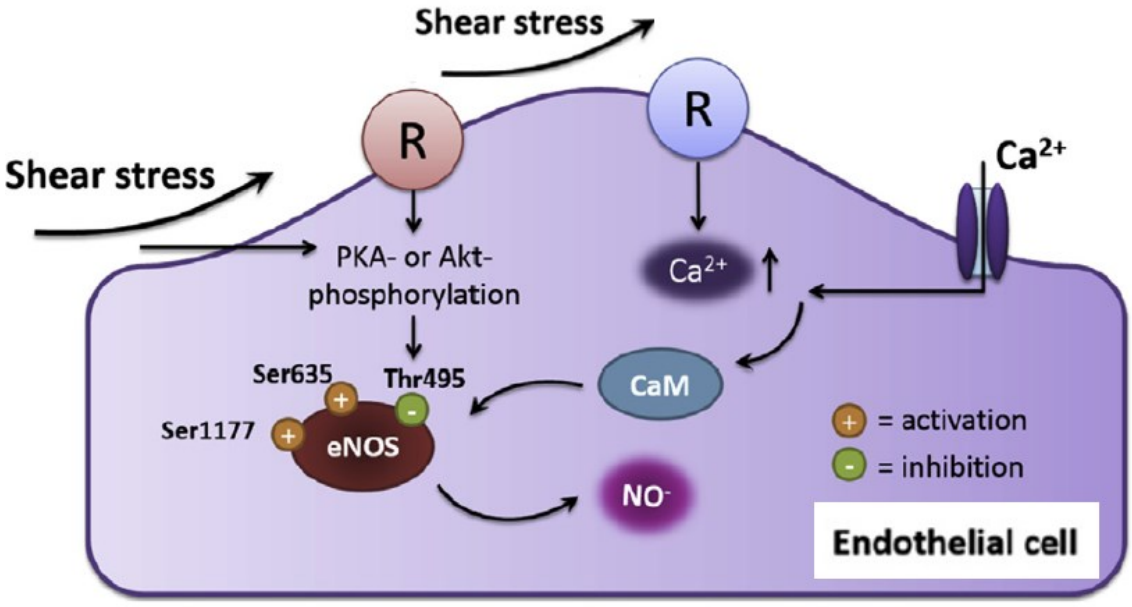

Figure 2.4. Shear stress-induced endothelial nitric oxide (NO) production via a calcium (Ca^{2+})-independent pathway (58). Shear stress stimulates phosphorylation of protein kinase A (PKA) and/or protein kinase B (Akt) at the activation sites Serine 1177 and Serine 635, which induces the phosphorylation of endothelial nitric oxide synthase (eNOS) and subsequent production of NO. Threonine 495 is an inhibitory site for eNOS. Exposure to high oxidative stress induces phosphorylation of threonine 495 that inhibits NO production. The production of NO is also regulated in a calcium-dependent manner via the calcium-calmodulin (CaM) pathway. R: receptors on the endothelial cell membrane.

Although shear stress activates the production of NO, NO bioavailability can be substantially diminished under conditions of high oxidative stress [**Figure 2.5**, (52)], which is defined by the overproduction and accumulation of reactive oxygen species (e.g., superoxide O_2^-) relative to antioxidant defenses (59). Reactive oxygen species are primarily generated by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, which catalyzes the transfer of electrons from NADPH to oxygen to produce superoxide radicals (58, 60). Once produced, reactive oxygen species react with NO and produce peroxynitrate ($OONO^-$) (**Figure 2.5**) (52, 58). As a result, less NO diffuses into VSMCs to induce vasodilation. Furthermore, $OONO^-$ directly promotes eNOS uncoupling, resulting in less NO production and endothelial dysfunction (58). Specifically, uncoupled

eNOS becomes a superoxide-generating enzyme that produces low amounts of NO and high levels of superoxide radicals (52, 57). This is partially mediated by $OONO^-$-induced oxidation of the eNOS cofactor tetrahydrobiopterin (58). Encouragingly, chronic aerobic exercise training appears to upregulate antioxidant enzymes, which can counteract these deleterious effects of reactive oxygen species (61).

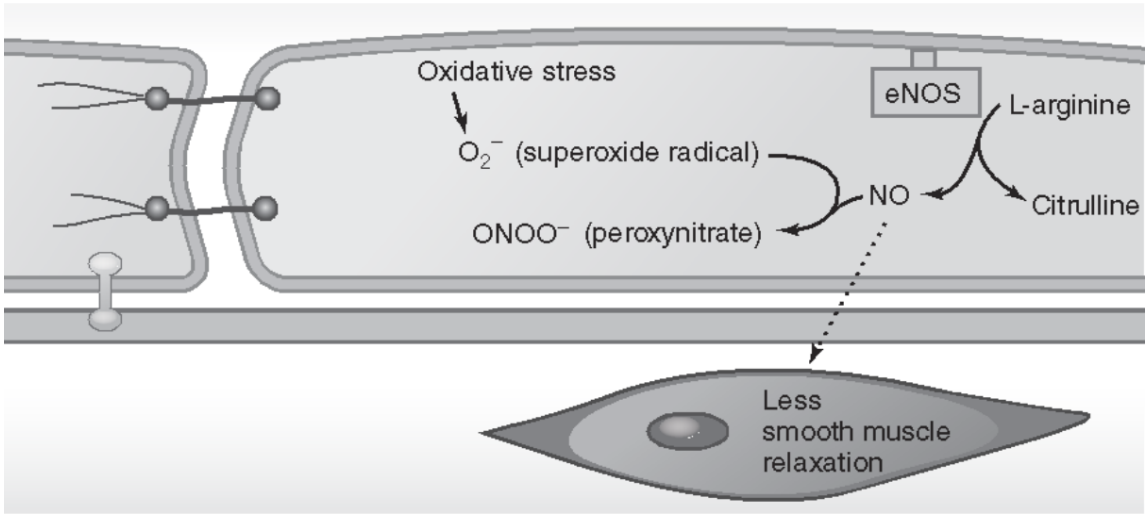

Figure 2.5. Impaired endothelium-derived nitric oxide (NO) production under conditions of high oxidative stress (52). Oxidative stress is caused by an imbalance between production and accumulation of reactive oxygen species such as superoxide radical (O_2^-) versus their detoxification by a biological system. When high levels of oxidative stress exist, NO produced from endothelial nitric oxide synthase (eNOS) reacts with superoxide radical, producing peroxynitrate ($ONOO^-$). Therefore, less NO can diffuse into smooth muscle, leading to attenuated smooth muscle relaxation.

2.3.3 Vascular Smooth Muscle

As highlighted above, VSMCs located in the *tunica media* directly mediate vasoconstriction and vasodilation, which is important for controlling blood pressure and blood flow distribution (62). As shown in **Figure 2.6** (63), VSMCs contain the contractile

protein filaments actin and myosin. The actin filaments are connected via dense bodies within the interior of the cell and anchored to the cell membrane by dense bands (52). The myosin filaments are interwoven between actin filaments and when they attach to form an actin-myosin complex, smooth muscle contraction/vasoconstriction occurs (see below) (52). Adjacent VSMCs are interconnected to increase the surface area for mechanical coupling via tight junctions that allow the transfer of intercellular tension (52). Electric and chemical coupling between VSMCs is achieved via gap junctions (52).

The contraction and relaxation of VSMCs directly determines artery lumen diameter [**Figure 2.6**, (63)]. When the cross-bridges formed by the actin and myosin filaments shorten, they pull the dense bodies towards the dense bands at the cell surface so that the VSMCs become “puffed up”, which encroaches into the lumen to decrease the diameter (52). In contrast, when these cross-bridges lengthen duration relaxation, the VSMCs are “flattened”, which increases lumen diameter (52).

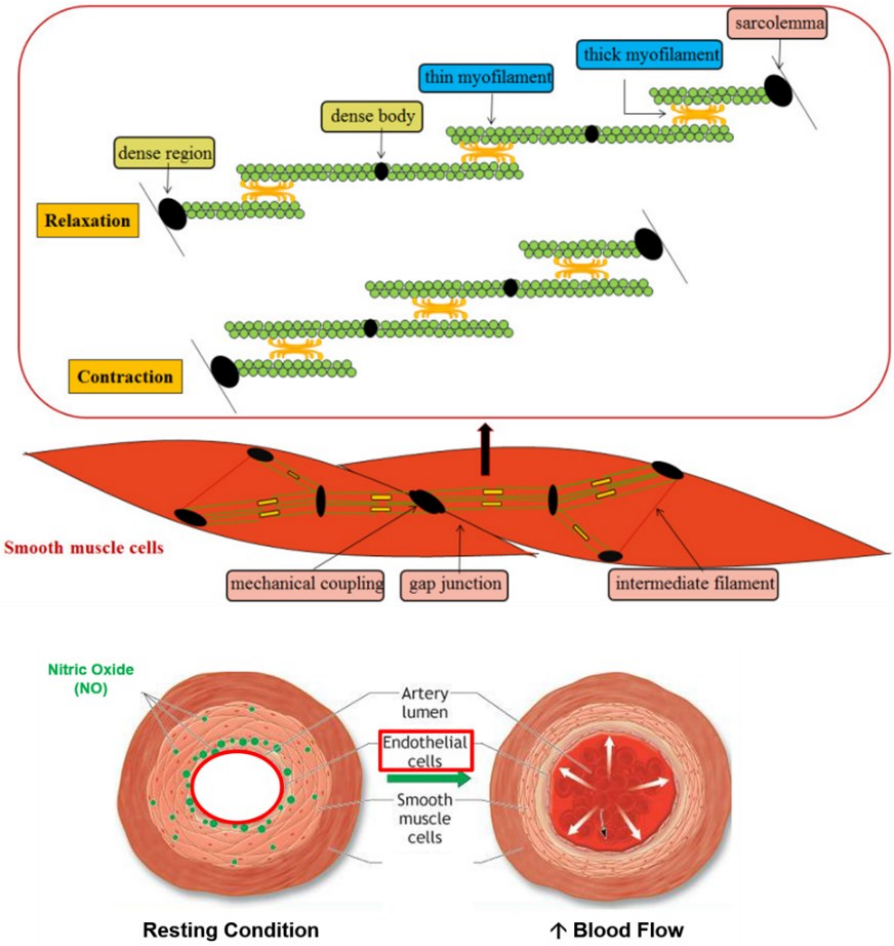

Figure 2.6. The structure of vascular smooth muscle cells (63). Vascular smooth muscle cells are connected to each other via gap junctions. Thin myofilaments (i.e., actin) are anchored to dense bands at the cell surface. Dense bodies are responsible for connecting actin filaments within the interior of the cell. Think myofilaments (i.e., myosin) are interwoven in between the actin filaments. Actin-myosin complexes are responsible for the action of muscle contraction or relaxation. Specifically, muscle contraction is caused by the shortening of actin-myosin filaments. In contrast, when nitric oxide diffuses into vascular smooth muscle cells, the actin-myosin filaments lengthen so that the cell is in a "flattened state" and relaxes causing an increase in lumen diameter.

2.3.3.1 Nitric Oxide-Dependent Vascular Smooth Muscle Relaxation

As shown in **Figure 2.7**, NO diffuses into VSMCs and binds to activate soluble guanylate cyclase (52, 58), which converts guanosine triphosphate into cyclic 3'5'-guanosine monophosphate (cGMP). This second messenger then stimulates the cell membrane Ca^{2+} pump to promote the efflux of Ca^{2+} and increases the activity of protein kinase G to promote the reuptake of cytosolic Ca^{2+} into the sarcoplasmic reticulum (via ↑

Ca^{2+}-ATPase activity) and reduce the influx of extracellular calcium (via closure of voltage-gated calcium channels) (52, 58). As a result, calmodulin is inactivated which reduces the activity of myosin light chain kinase (52, 58). Myosin light chain kinase is responsible for the phosphorylation of the light chain of myosin in response to increased Ca^{2+} and calmodulin thereby activating myosin to interact with actin (52, 58). As such, reduced activity of myosin light chain kinase leads to the dephosphorylation of the regulatory light chain of myosin and hence inhibits cross-bridge formation (52, 58). Furthermore, decreased intracellular Ca^{2+} increases the activity of myosin light-chain phosphatase, which removes the phosphate group from the myosin light chain leading to the detachment of myosin from actin. Collectively, the actin-myosin cross-bridges are broken due to decreased activity of myosin light chain kinase and increased activity of myosin light chain phosphatase, and eventually VSMCs relaxation occurs (52, 58).

The responsiveness of VSMCs to NO is susceptible to high oxidative stress. Firstly, $OONO^-$ inactivates soluble guanylate cyclase (64, 65). In addition, soluble guanylate cyclase exists in a physiological equilibrium between two redox states: reduced (NO-sensitive) and oxidized (NO-insensitive) (64, 65). However, the equilibrium is shifted toward the oxidized state under conditions of high oxidative stress, which impairs NO-cGMP pathway signaling (64, 65). Banday & Lokhandwala (66) suggested that oxidative stress also reduces NO-mediated protein kinase G activation, thus blunting the decrease in VSMCs intracellular Ca^{2+} concentration. Collectively, oxidative stress not only impairs endothelium-derived NO production, but also attenuates VSMCs responsiveness to NO (i.e., impairs both endothelium-dependent and endothelium-independent vasodilation).

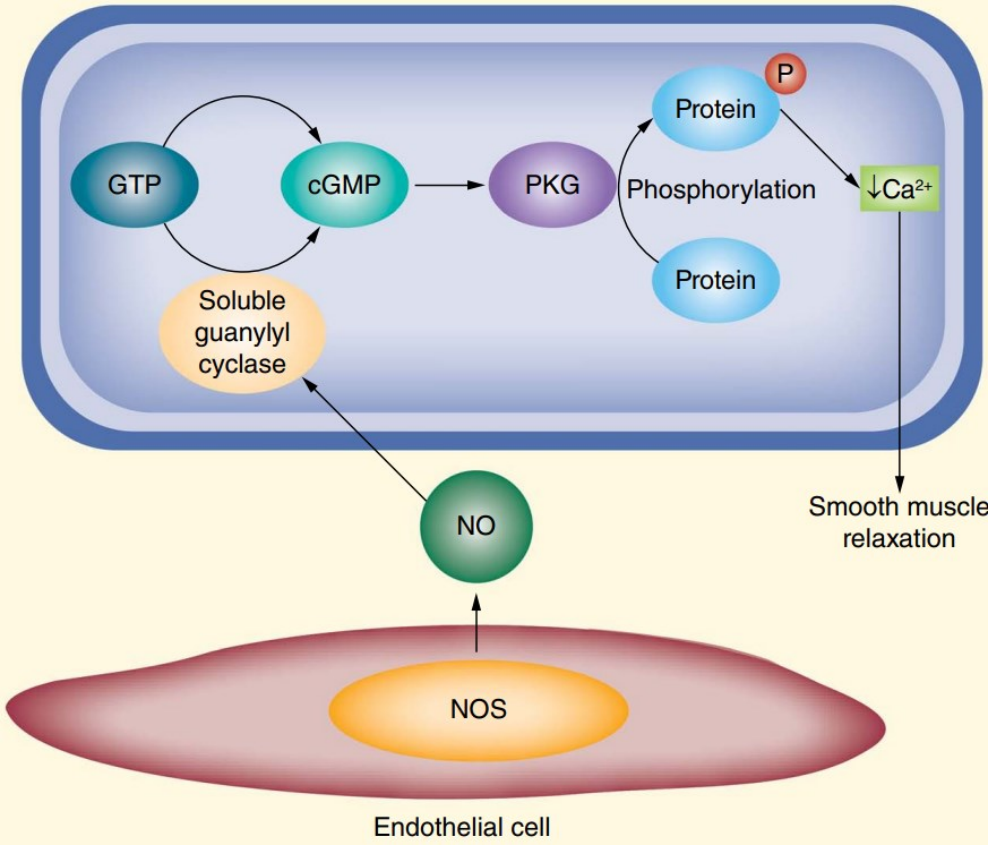

Figure 2.7. Vascular smooth muscle relaxation mediated by nitric oxide (NO) production from endothelial cells. Nitric oxide diffuses into the vascular smooth muscle cell and binds to its intracellular receptor ([soluble guanylate (or guanylyl) cyclase], which converts guanosine triphosphate (GTP) into cyclic guanosine-3',5'-monophosphate (cGMP). The production of cGMP activates protein kinase G (PKG) that decreases intracellular calcium (Ca^{2+}) concentration and causes vascular smooth muscle relaxation (vasodilation).

2.3.4 Non-Nitric Oxide-Dependent Endothelial-Vascular Smooth Muscle Pathway

In addition to the endothelium- and VSMCs-dependent vasodilatory pathways highlighted above, there are additional NO independent mechanisms involved in both conduit and resistance vessel. Ion channels located on the cell membrane of endothelial cells and VSMCs also play a role mediating vasodilation. For example, as shown in **Figure 2.8** (67), shear stress (not depicted in the figure) and agonists (e.g., acetylcholine, adenosine triphosphate) can activate the influx of Ca^{2+} and the release of Ca^{2+} from the endoplasmic reticulum (68). The Ca^{2+} signals activate small- and intermediate-

conductance Ca^{2+}-activated potassium channels, which cause endothelial hyperpolarization. This hyperpolarization activates inward rectifier potassium channels, which in turn, amplifies the degree of hyperpolarization (67, 68). The endothelial hyperpolarization signal is then transmitted to underlying VSMCs via myoendothelial gap-junctions (67, 68). There are also some diffusible factors (i.e., endothelium-derived hyperpolarizing factors) generated by the endothelial cell due to the increased levels of intracellular Ca^{2+} (67, 68). These factors diffuse into the VSMCs and cause hyperpolarization via the activation of Na^{+}/K^{+}-ATPase and/or opening of potassium channels (e.g., big-conductance potassium channels, inward rectifier potassium channels, and ATP-sensitive potassium channels) (67, 68).

2.4 Ultrasound Assessment of Popliteal Artery Function

2.4.1 Principle of Duplex Ultrasonography

The evaluation of peripheral vascular function can be achieved using high-resolution duplex ultrasonography [i.e., the simultaneous recording of artery lumen diameter, via brightness-mode (B-mode) and red blood cell velocity (RBCv) via pulsed-wave Doppler mode] (5). A high-resolution, multi-frequency linear transducer (probe) with two arrays of piezoelectric crystals is required for imaging peripheral arteries (69). One array of piezoelectric crystals transmits ultrasound beams at a frequency range (in megahertz, MHz) that is inversely proportional to the depth of penetration (70). The popliteal artery typically requires a transmission frequency between 8-10 MHz for B-mode imaging and ~5-MHz for the pulsed-wave Doppler collection of RBCv. The 2-dimensional artery image (**Figure 2.9**) obtained from B-mode is generated by the reflection of ultrasound beams from tissue boundaries (e.g., endothelium) (70).

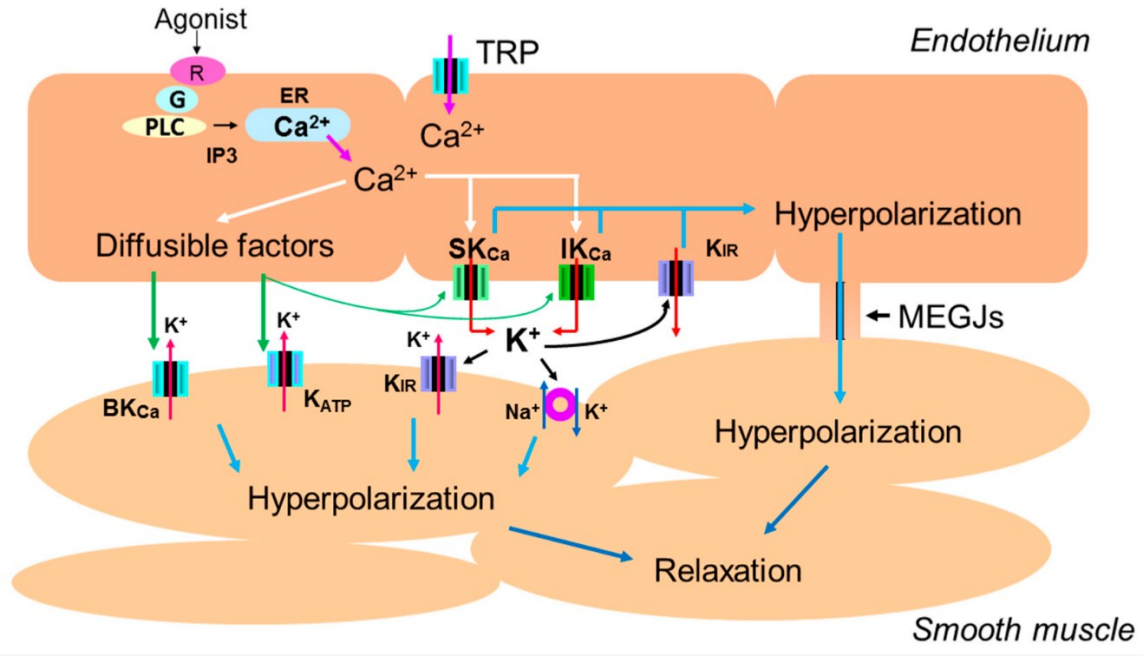

Figure 2.8. An endothelial cell is stimulated by shear stress or agonists binding to cell membrane receptors (R), which activates a G protein (G) leading to the activation of phospholipase C (PLC). This results in the production of inositol triphosphate (IP_3) that is responsible for the release of calcium (Ca^{2+}) from the endoplasmic reticulum (ER) and Ca^{2+} influx from the nonselective cation channels of the transient receptor potential (TRP) family. The elevated levels of Ca^{2+} activates small- (SK_{Ca}) and intermediate- (IK_{Ca}) conductance potassium (K^+) channels. These channels allow K^+ to rush out of the endothelial cell down its electrochemical gradient and leads to endothelium-dependent hyperpolarization, which spreads via myoendothelial gap-junctions (MEGJs) into the vascular smooth muscle cell. The endothelium-dependent hyperpolarization also activates the inward rectifier K^+ channels (K_{IR}), which further facilitate the hyperpolarization induced by SK_{Ca} and IK_{Ca}. In addition, diffusible factors generated by the endothelial cell contribute to vascular smooth muscle hyperpolarization via activation of Na^+/K^+-ATPase and/or opening of K^+ channels, including big-conductance K^+ channel (BK_{Ca}), K_{IR}, and ATP-sensitive K^+ channel (K_{ATP}). Eventually, hyperpolarization of the vascular smooth muscle causes vasodilation.

In pulsed-wave Doppler mode (**Figure 2.9**), the emitted high-frequency sound beams are reflected off circulating red blood cells to determine RBCv (69, 70). Specifically, the measurement of RBCv is based on the Doppler shift principle: any moving object alters the characteristics (e.g., frequency) of sound waves (69, 70). The Doppler shift frequency (Δf) is known as the difference between the transmitted frequency (f_t; set at 5 MHz) and that of the received 'echoes' (f_r) (69, 70). The measurement of RBCv is achieved via rearranging the Doppler shift equation: $\Delta f = \frac{2*ft*\cos\theta*v}{c}$ [**Figure 2.10**, (71)], where 'c' is the speed of sound in soft tissues (1540 m/s), 'v' is RBCv, 'cos' is cosine, and 'θ' is the angle of insonation between the ultrasound beam and the velocity vector (i.e., the direction of blood flow) (69, 70). The ultrasound machine automatically calculates RBCv via the rearranged equation: $V = \frac{C*\Delta f}{2*ft*\cos\theta}$.

The artery typically runs parallel to the skin surface; hence an optimal artery image is obtained by placing the ultrasound probe perpendicular on the skin surface, which allows the ultrasound beam to bisect the vessel at 90° (70). However, as per the Doppler equation ($V = \frac{C*\Delta f}{2*ft*\cos\theta}$), this angle results in no signal for the Doppler mode as the cosine of 90° equals 0, whereas the signal is strongest when the insonation angle is 0° (i.e., cosine of 0 = 1) (69, 70). Therefore, the "angle steer" function of the ultrasound machine is used to alter the angle of the ultrasound beam without the need to move the transducer (probe) (69, 70). However, an insonation angle greater than 60° substantially increases the error associated with the calculation of RBCv. As such, an insonation angle of ≤60° has been recommended (69, 70). The ultrasound machine can "steer" the ultrasound beam by 30° to create an insonation angle of 60° while the sonographer

maintains the transducer perpendicular to the skin surface and does not have to manually move the transducer for achieving an optimal RBCv signal.

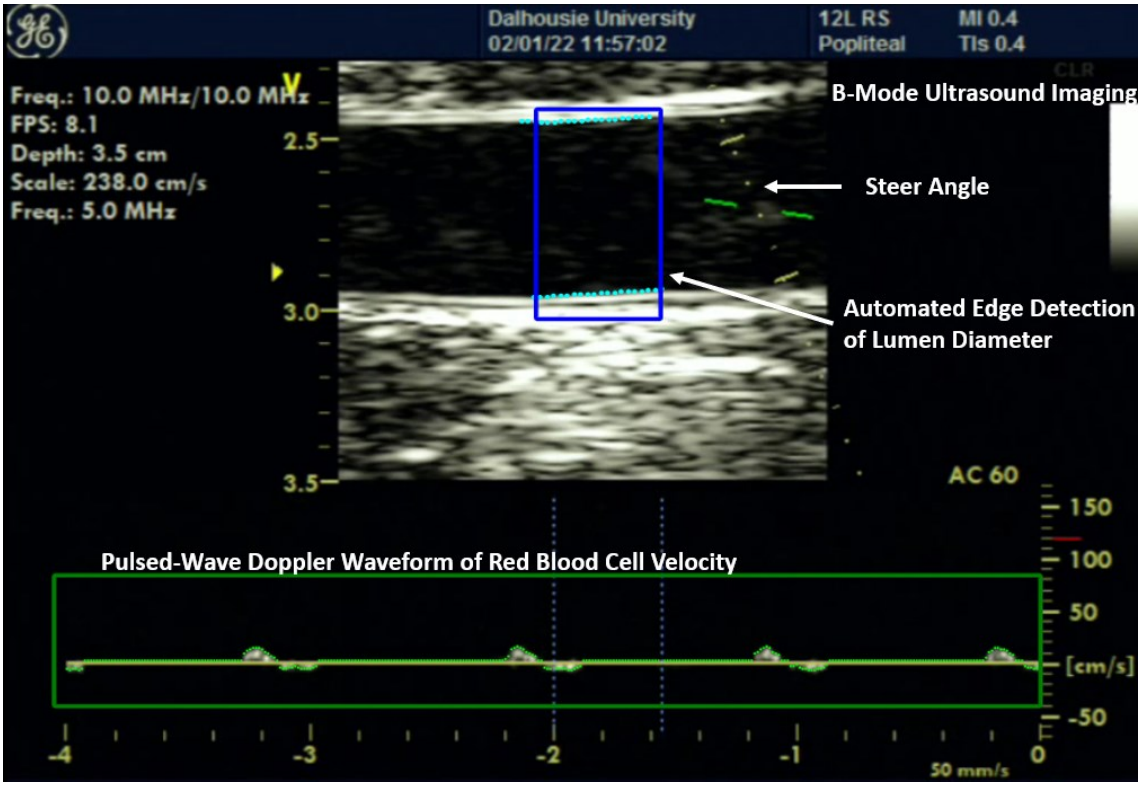

Figure 2.9. An example of popliteal artery duplex ultrasound imaging with Brightness-mode (upper) and pulsed-wave Doppler waveforms (lower). A 2-dimensional image of the popliteal artery is obtained using Brightness-mode. Automated edge-detection software (Cardiovascular Suite, Quipu) is used for analyzing the lumen diameter. The red blood cell velocity signal is measured via pulsed-wave Doppler mode.

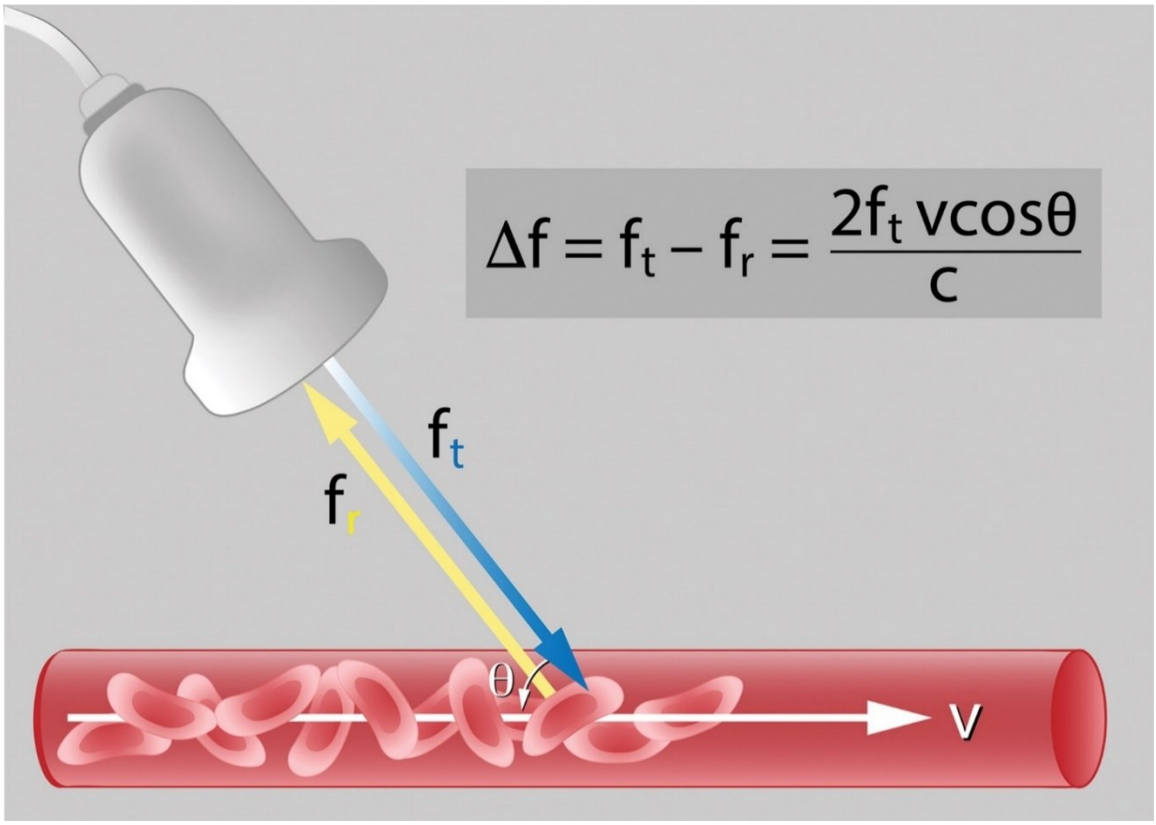

Figure 2.10. The illustration of the Doppler principle, where 'Δf' is the difference between the transmitted frequency (f_t; always set at 5-MHz for popliteal imaging) and the frequency of the returned waves (f_r). 'C', speed of sound constant (1540 m/s); cos, cosine; v, red blood cell velocity; θ, angle of insonation between the ultrasound beam and the velocity vector (i.e., the direction of blood flow) (71). The equation of the Doppler frequency shift can be rearranged to calculate red blood cell velocity: $V = \frac{C*\Delta f}{2*ft*cos\theta}$.

2.4.2 Assessment of Popliteal Artery Endothelium-Dependent Vasodilation

Conduit artery endothelial function is typically examined via the non-invasive flow-mediated dilation (FMD) technique using high-resolution duplex ultrasonography (5). Flow-mediated dilation examines endothelium-dependent, or NO-mediated, dilation in response to an acute increase in blood flow and associated shear stress (5). The FMD test starts with a 2-min baseline measurement of resting lumen diameter and RBCv, followed by a 5-min ischemic stimulus induced by distal cuff occlusion (i.e., pressure inflated to ~250 mmHg). The cuff is deflated after the ischemic period, which elicits a

sudden increase in blood flow and shear stress, which provides the stimulus for endothelial NO production and subsequent vasodilation. Lumen diameter and RBCv are continuously measured for an additional 5-min following cuff deflation to capture the peak diameter response.

The FMD test has traditionally been assessed in the brachial artery, which is largely NO-mediated and predictive of adverse cardiovascular events (72). However, the brachial artery is less susceptible to obstructive atherosclerosis, compared with the lower-limb vasculature (73). Furthermore, lower-limb arteries are directly exposed to lower blood flows and elevated hydrostatic pressures during the seated posture, as well as more prone to reductions in FMD following prolonged bouts of sitting (74). Therefore, assessment of lower-limb FMD provides more meaningful information for understanding the impact of sitting on vascular function and cardiovascular health. However, only superficial femoral artery FMD responses have been demonstrated to be primarily NO-mediated (75). Although FMD of other lower-limb arteries (e.g., popliteal artery) are also assumed to be NO-mediated, this requires confirmation by future studies.

The magnitude of FMD is reflective of endothelial health, but it is highly impacted by the magnitude of the imposed reactive hyperemia-induced shear stress stimulus. Shear stress is calculated using the equation: shear stress = blood viscosity × RBCv / lumen diameter. However, a common practice in the literature is to determine "shear rate", which is an estimate of shear stress that assumes there is a constant level of blood viscosity (5). Specifically, shear rate (SR) can be calculated as 8 × (RBCv / lumen diameter).

Reactive hyperemia and associated shear stress are influenced by various parameters such as CVD risk factors (7, 8), age (76), and ethnicity (77). As such, it is critical to determine whether small FMD responses are the consequence of endothelial dysfunction or the result of a smaller shear stress stimulus (78). As such, it has been recommended that FMD be normalized to the SR area under the curve [$SR_{AUC,}$ between the time of distal cuff deflation to when the peak vasodilatory response occurred; **Figure 2.11,** (79)] if the following 2 assumptions are met: 1) there is a positive linear correlation between FMD and SR_{AUC}, and 2) the y-intercept of this regression is not statistically different from zero (5, 78).

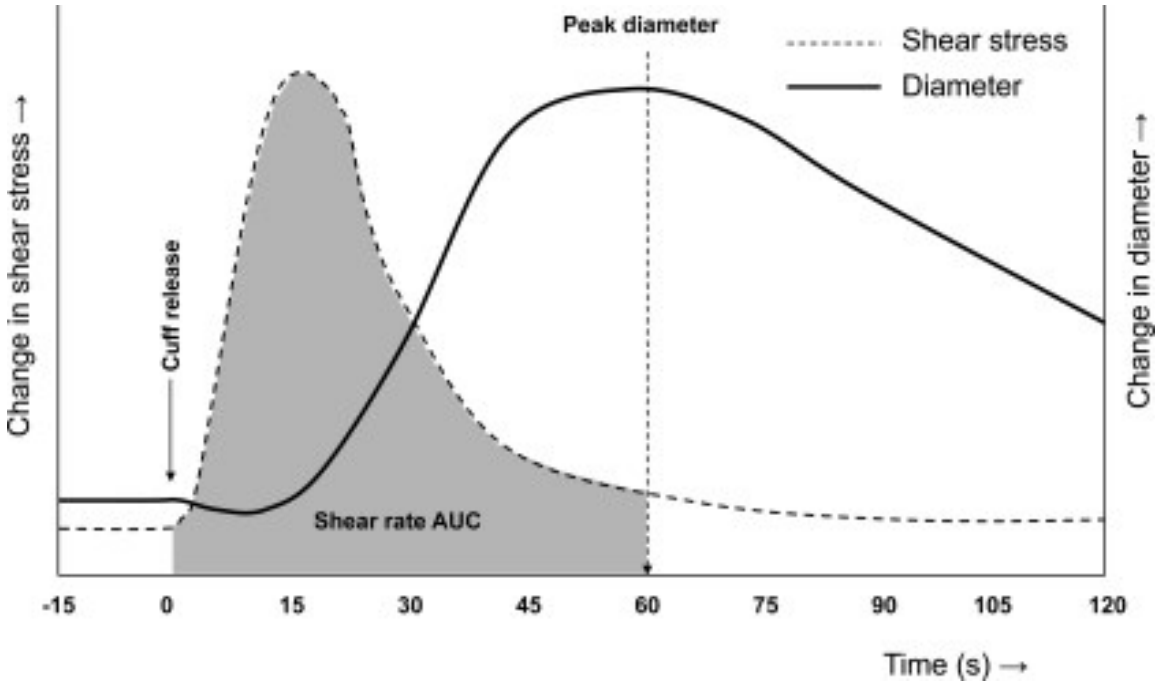

Figure 2.11. Schematic of artery diameter and shear stress/rate responses following a 5-min period of distal cuff occlusion (80). Published guidelines (5) recommend that relative FMD (% increase in diameter above baseline) be normalized to the shear rate area under the curve (AUC, grey area in the figure) if it represents the primary stimulus for the peak diameter response (78).

Both inter- and intra-individual (e.g., with ageing or long-term aerobic training) differences in baseline lumen diameter may also influence the magnitude of FMD responses. Specifically, Atkinson & Batterham (81) found that the calculation of FMD may be biased, whereby smaller arteries exhibited greater FMD response than larger arteries. Furthermore, baseline diameter also inversely influences the magnitude of the shear stress/rate stimulus for a given level of RBCv (and blood viscosity) according to the above-mentioned equations. As such, a smaller artery may have a greater shear stress stimulus following cuff deflation than a larger artery and thus exhibit greater FMD responses. Hence, Atkinson et al. (82) suggested the use of the allometric scaling approach to reduce the potential dependency of FMD responses on baseline diameter, which could improve the specificity of FMD for indicating endothelial function. The allometric scaling approach is particularly important for the design of exercise training studies, which have demonstrated an increase in baseline artery diameter (83). The assumption required to perform allometric scaling is that the slope of the linear relationship between the logarithmically transformed baseline and peak diameters differs from '1' and has an upper 95% confidence interval <1 (82). Linear regression analysis was performed to check whether the data met the assumptions for normalization of FMD to SR_{AUC} and/or allometric scaling (see Methods for details).

2.4.3 Assessment of Popliteal Artery Endothelial-Independent Vasodilation

Conduit artery VSMCs sensitivity to an exogenous NO donor can be non-invasively assessed by the nitroglycerin-mediated dilation (NMD) test (5). The NMD test serves as an index of VSMCs-dependent vasodilatory function in response to sublingual (i.e.., under the tongue) nitroglycerin administered (18). Nitroglycerin acts directly on

VSMCs by activating soluble guanylate cyclase to induce vasodilation (18). As such, the NMD test is endothelium-independent and has been utilized as a control test for FMD to determine if increases or decreases in FMD responses are due to corresponding changes in VSMC sensitivity to NO versus the capacity of endothelial cells to produce and release NO (or conversion to $OONO^-$ by elevated oxidative stress) following the reactive hyperemic stimulus (5).

The NMD test starts with a 1-min baseline measurement of arterial lumen diameter prior to the sublingual nitroglycerin administration (0.4-mg dosage), followed by 10-min of continuous arterial diameter imaging. The peak dilation is typically observed ~3-4 min following the nitroglycerin administration. A previous study has documented the rapid disappearance rates of nitroglycerin from the blood after sublingual administration, as well as in total body clearance (84). Specifically, the elimination half-life of a 0.6-mg sublingual nitroglycerin dose was ~4.5-min and barely detectable in the blood after 20-min (84). In the current study, there is ~3-hrs between pre- and post-sitting popliteal FMD and NMD assessments (see Experimental Design section below). As such, the first nitroglycerin administration should not interfere with the post-sitting assessments.

Vascular responsiveness to NO is attenuated in individuals with cardiovascular risk factors such as aging (20), obesity (21, 22), excessive alcohol consumption (23), and smoking (24). Furthermore, Adams et al. (85) found that high cholesterol, smoking, diabetes mellitus, aging, and impaired FMD responses are predictors of reduced NMD. The NMD response is also impaired in patients with clinical disorders including coronary artery disease/atherosclerosis (86), chronic obstructive pulmonary disease (87), and

essential hypertension (88). Collectively, NMD may be an important predictor of future cardiovascular events and has high clinical relevance.

2.5 Assessment of Lower-Limb Resistance Vessel Function

Resistance vessel dysfunction plays a vital role in the pathogenesis of hypertension because of the profound impact of resistance vessels on peripheral vascular resistance (89). Recent studies have demonstrated that the post-occlusive reactive hyperemic response induced during the FMD assessment is a predictor of adverse cardiovascular events and can discriminate individuals with increased CVD risk (10, 90, 91). Interestingly, Mitchell et al. (92) and Philpott et al. (90) both provided evidence that CVD risk factors (e.g., age, systolic blood pressure, low-density lipoprotein cholesterol, body mass index) are more closely associated with reactive hyperemia than with FMD *per se*. Collectively, reactive hyperemia is an important indicator of vascular function and should be systematically studied independent of FMD.

Reactive hyperemia is a well-established technique to assess resistance vessel function and can be measured after the ischemic period of ischemia during the FMD test. Although reactive hyperemia is captured within the popliteal artery, it should not be confused as a conduit artery-specific outcome. Furthermore, unlike the FMD response, the increase in blood flow is not an endothelium-dependent, or predominantly NO-mediated response (7, 8). The stimulus driving reactive hyperemia is thought to be related to tissue hypoxia and an accumulation of metabolic vasodilators (e.g., adenosine, carbon dioxide, hydrogen ions, potassium) that are subsequently ‘washed out’ of the resistance vessels during the increase in blood flow (7, 8).

Reactive hyperemia can be expressed as a peak increase in RBCv, or as a total RBCv volume (i.e., area under the curve, $RBCv_{AUC}$) increase quantified over an extended period (e.g., 60-s) following the distal cuff deflation period. Interestingly, although the peak and $RBCv_{AUC}$ reactive hyperemic responses share some common mechanisms, there are also some independent mechanisms associated with each outcome. For example, inhibition of inwardly rectifying potassium channels and Na^+/K^+-ATPase substantially attenuated both the peak (~60%) and volume (AUC) (~90%) reactive hyperemic responses (93). In contrast, blockade of ATP-sensitive potassium channels modestly reduced the reactive hyperemia AUC (15-25%), but did not alter the peak response (94, 95). Similarly, blockade of NO had no impact on peak reactive hyperemia, but modestly attenuated the AUC outcome (75, 96). Collectively, the metric that is most reflective of resistance vessels remains unknown. Accordingly, published guidelines recommended that reactive hyperemia outcomes be presented in multiple ways to be comprehensive and allow for comparisons with other studies (7). Therefore, both the peak and 60-s $RBCv_{AUC}$ reactive hyperemic responses are reported in the present study to reflect lower-limb resistance vessel function.

2.6 Impact of Prolonged Sitting on Lower-Limb Vascular Function

The detrimental vascular effects of prolonged sitting are well-established (2, 3). An acute bout (1-6 hrs) of prolonged sitting attenuates popliteal and superficial femoral artery FMD responses (2, 3). According to a recent meta-analysis by Paterson et al. (2), the popliteal artery exhibited a greater decrease in FMD responses compared with the superficial femoral artery. One potential mechanism linked with attenuated FMD responses following prolonged sitting is decreased resting blood flow-induced shear

stress (97). Blood flow-induced shear stress is essential for maintaining vascular health. It is well known that low shear stress diminishes NO bioavailability, promotes oxidative stress, and eventually leads to early signs of atherosclerosis (e.g., endothelial dysfunction) (98). Importantly, preventing sitting-induced reductions in leg blood flow and shear stress by immersing the lower foot/ankle into warm water or leg fidgeting have been shown to preserve FMD responses (15, 16). Therefore, sitting-induced reductions in resting leg blood flow and shear stress have been suggested as a key contributor to impaired FMD responses (97). Moreover, it has been suggested that low shear stress promotes oxidative stress (99, 100). Thosar et al. (101) demonstrated that having participants administered the antioxidant vitamin C during sitting also prevented the decline in superficial femoral FMD, suggesting that oxidative stress may also contribute to sitting-induced impairments in endothelial function.

Among the lower limb arteries, the popliteal artery located behind the knee is most susceptible to disturbed/turbulent flow due to arterial 'kinking' during knee-bent sitting (102). Endothelial cells exposed to disturbed/turbulent flow experience various cellular stresses (e.g., oxidative stress) and exhibit lower transcription of the eNOS gene (17). Chronic exposure to disturbed/turbulent flow also induces a proatherogenic endothelial cell phenotype resulting in less NO production and impaired endothelium-dependent dilation (17, 103). Moreover, release of the potent endothelial-derived vasoconstrictor endothelin-1 is increased in response to low (and disturbed/turbulent) vascular shear stress induced by prolonged sitting (104, 105). Additionally, endothelin-1 can further enhance oxidative stress by increasing the formation of vascular reactive oxygen species contributing to impaired endothelium-dependent dilatory function (106).

Another potential mechanism behind sitting-induced endothelial dysfunction that has been proposed is an increase in sympathetic vasoconstrictor activity directed towards skeletal muscle resistance vessels (97). Unfortunately, sympathetic nerve activity has yet been directly measured (or has not been reported) in laboratory-based prolonged sitting studies with the exception that Iwase et al. (107) made continuous seated sympathetic activity measurements during parabolic flight on a plane. However, muscle sympathetic nerve activity is assumed to increase based on the evidence that sympathetic activity is greater in the sitting versus supine posture (108) and blood pressure is elevated following sitting (49). Elevated muscle sympathetic nerve activity has been shown to acutely promotes proatherogenic retrograde and oscillatory shear patterns that can acutely impair FMD responses (109). Indeed, Hijmering et al. (110) demonstrated that acute sympathetic stimulation attenuated FMD responses via an alpha-adrenergic vasoconstriction mechanism. Collectively, attenuated FMD responses following bouts of prolonged sitting are perhaps due, in part, to elevated sympathetic activity during sitting. However, this theory needs to be confirmed by directly measuring muscular sympathetic nerve activity in future laboratory-based prolonged sitting studies. One more potential mechanism linking prolonged sitting to diminished FMD responses is an attenuated post-occlusive reactive hyperemia stimulus after sitting (74, 111). As previously mentioned, reactive hyperemia is not only the stimulus for conduit artery FMD responses but is an indicator of downstream resistance vessel function (7). As such, prolonged sitting results in impairments in both lower-limb conduit artery endothelium-dependent and resistance vessel functions.

Despite the well-established prolonged sitting-induced endothelial and resistance vessel dysfunction, the corresponding impact on VSMCs sensitivity to NO is largely unexplored. As mentioned above, the NMD test provides an index of this and is evaluated to determine whether changes in FMD are reflective of endothelial NO production versus underlying VSMCs reactivity to NO (5). I have recently reported that a bout of prolonged sitting also attenuates popliteal NMD responses (19). However, the exact mechanisms responsible for sitting-induced impairments in VSMCs vasodilatory function are unknown. It has been speculated that low blood flow-induced oxidative stress during sitting reduces soluble guanylate cyclase sensitivity to NO via the oxidation of the heme iron (Fe^{3+}) (112). The dysfunction of guanylate cyclase could lead to decreased production of cGMP and hence impaired vasodilatory responses (52). Future studies are still needed to confirm or refute these proposed mechanisms. Regardless, decreased NMD responses following acute sitting are indicative of vascular smooth muscle dysfunction, which provides additional insight into the link between sedentary behaviour and CVD risk.

2.7 Impact of Aerobic Training and Aerobic Fitness on Lower-Limb Vascular Function

Aerobic fitness is positively associated with popliteal FMD in both younger (33) and older adults (12, 113). Furthermore, Morishima et al. (32) observed greater popliteal FMD responses in young males with higher versus lower aerobic fitness. The rationale for the direct association between aerobic fitness and lower-limb endothelial-dependent vasodilatory function is based on evidence that $\dot{V}O_2$peak is largely dependent upon maximal cardiac output and blood flow to active muscles (31, 114). Elevated cardiac output during exercise is partially caused by peripheral vasodilation in the active tissues

(e.g., upstream of and/or within lower extremity muscles during cycling or running), which results in increased venous return and decreased cardiac afterload (114). Despite the well-established relationship between aerobic fitness and lower-limb FMD response, there is a paucity of data on resistance vessel function and VSMCs sensitivity to NO.

The above-mentioned cross-sectional studies established the preliminary evidence for a causal relationship between aerobic fitness and lower-limb endothelial function. Exercise-training intervention studies further confirmed the beneficial effects of improving aerobic fitness on lower-limb endothelial function. Specifically, Rakobowchuk (30) found that 6-weeks of sprint-interval (4-6 × 30-s Wingates separated by 4.5-min of recovery for 3 days/week) and moderate-intensity continuous training (40-60 min of cycling at 65% of $\dot{V}O_2$peak for 5 days/week) improved aerobic fitness and popliteal FMD in young, healthy adults. Furthermore, Scholten et al. (115) demonstrated that 12-weeks of continuous training (40-min of cycling at 70-80% of $\dot{V}O_2$peak for 2-3 days/week) improved aerobic fitness and superficial femoral FMD, as well as NMD in formerly pre-eclamptic females and healthy control females. Additionally, Gokce et al. (116) reported that 10-weeks treadmill- or stationary cycle-based aerobic training (3×30-min sessions/week) improved functional capacity and posterior tibial FMD, but not NMD in patients with coronary artery disease. Similarly, our laboratory reported that 6-weeks of high-intensity interval training (HIIT, 2 × 20-min cycling bouts, alternating between 15-s intervals at 100% of peak aerobic power and passive recovery) and moderate-intensity continuous training (34-min cycling at 60% peak aerobic power) improved aerobic fitness and popliteal FMD, but did not increase NMD in healthy older adults (29). Collectively, the endothelial health-enhancing influence of improving aerobic fitness via

exercise training is well-established, but training effects on VSMCs sensitivity to NO are inconsistent.

The key mechanism linking aerobic exercise training to improved FMD has been suggested to be the repeated increase in blood flow-induced shear stress to active tissues (e.g., muscles of the lower extremity during cycling or running) (83, 117). The repeated increases in shear stress play a crucial role in mediating both acute and chronic vascular adaptation (83, 117). Specifically, increases to the expression of eNOS (98), reduced vascular oxidative stress (100), and improved antioxidant defenses have been reported (118). These factors collectively enhance NO bioavailability and thus improve the vasodilatory capacity of conduit arteries (98, 100, 118). Altogether, it seems plausible that training at a higher intensity may result in a larger enhancement in vascular function due to a correspondingly greater exercise-induced shear stimulus to the lower-limb active muscles.

Exercise training also induces increase in blood volume, which is primarily derived by an increased plasma volume (119, 120). The link between training-indued increased plasma volume and peripheral vascular functional adaptation is largely unknown. Blood volume expansion results in a decreased hematocrit (i.e., concentration of red blood cells), which is commonly observed in endurance-trained athletes (121). Decreased hematocrit is directly associated with lower blood viscosity (121). According to the equation for calculating shear stress: shear tress = SR × viscosity, blood viscosity is directly proportional to shear stress stimulus. Therefore, training-induced increases in blood volume may eventually result in lower vascular shear stress. As previously mentioned, shear stress is a key modulator of NO production (83, 117). Interestingly,

elevated blood viscosity has been linked with cardiovascular risk factors and elevated in many pathologies (122, 123). Moreover, conflicting evidence has been reported regarding whether increases in blood viscosity improves, attenuates, or has no change on FMD (124–126). Unfortunately, most human studies assumed that blood viscosity does not differ between participants and thus did not measure blood viscosity and adopted alternate approaches to calculate SR (e.g., SR = 8 × RBCv/diameter) depending on the sample volume size and placement for pulsed-wave Doppler (5). Given the complex interplay between blood volume, blood viscosity, shear stress and endothelial function, more studies are required to examine the impact of exercise training-induced increases in blood volume on vascular adaptations.

High-intensity (i.e., ≥90% of $\dot{V}O_2$peak) interval training has been considered one of the most effective exercise training protocols for vascular health benefits (e.g., versus moderate-intensity continuous training, 60-75% of $\dot{V}O_2$peak) (27, 127, 128). The superior effects of HIIT on vascular function may be primarily attributed to greater vascular shear stress. For example, Padilla et al. (129) demonstrated that walking on a treadmill at high-intensity (75% of $\dot{V}O_2$max) elicited larger post-exercise brachial artery shear stress responses compared with low (25% of $\dot{V}O_2$max) and moderate intensity (50% of $\dot{V}O_2$max). Similarly, Montalvo et al. (130) also observed greater carotid artery blood flow and shear stress during high-intensity exercises versus low- or moderate-intensity exercises. Collectively, HIIT appears to be more effective than other forms of aerobic training at improving vascular function and was chosen as the training protocol for the present study.

2.8 Aerobic Fitness and Lower-Limb Vascular Responses to Prolonged Sitting

Given the well-established cardiovascular benefits of aerobic exercise, it seems plausible that improving aerobic fitness via performing HIIT can afford protection against prolonged sitting-induced vascular dysfunction. However, the interaction between aerobic fitness and prolonged sitting on vascular health is very complex and remains controversial.

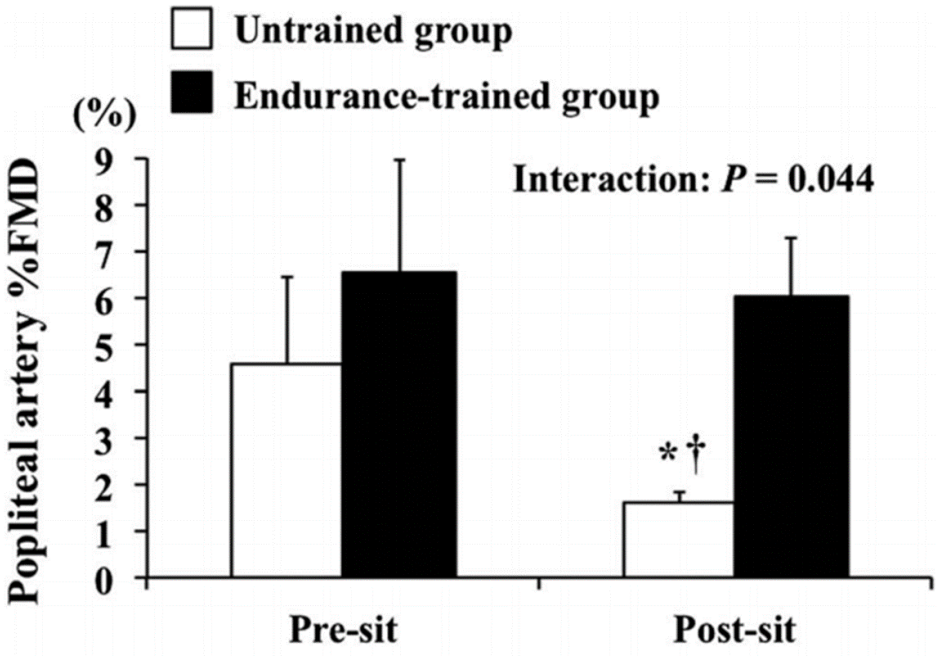

Figure 2.12. Popliteal relative flow-mediated dilation (FMD) response to 3-h of sitting in Untrained versus Endurance-trained males reported by Morishima et al. (32). Unlike the Untrained males, the Endurance-trained group did not observe a post-sitting reduction in popliteal FMD. *,P<0.05 versus pre-sitting (Pre-sit); †, P< 0.05 versus Endurance-trained group.

Specifically, Morishima et al. (32) found that an acute bout of sitting attenuated popliteal FMD in untrained but not endurance-trained males (Untrained ΔFMD: -2.7% versus Endurance-trained ΔFMD: -0.5%; **Figure 2.12**). However, Garten et al. (34) observed similar prolonged sitting-induced impairments in common femoral artery

function, assessed by the passive leg movement technique, between aerobically-trained and untrained individuals (**Figure 2.13**).

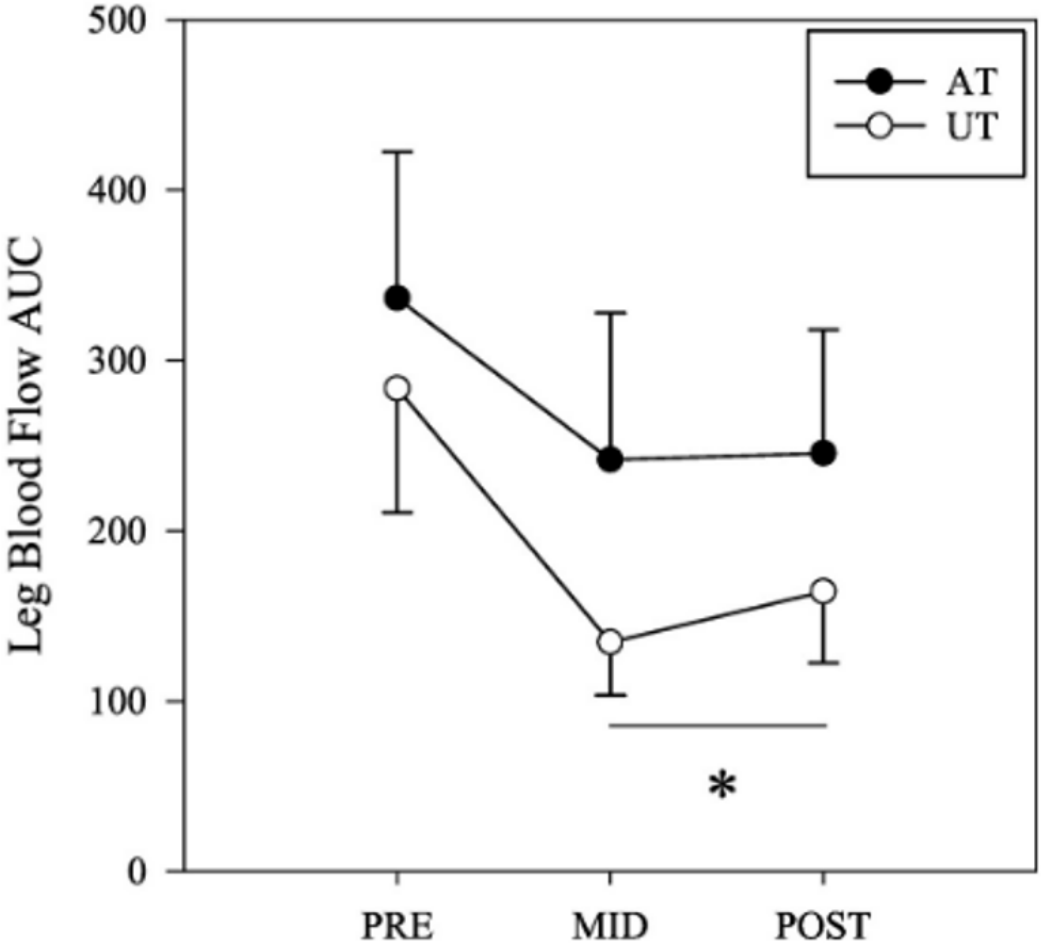

Figure 2.13. Common femoral artery hyperemic response induced by 60-s of repetitive passive leg movements at 0-h (PRE), 1.5-h (MID), and 3-h (POST) of sitting in aerobically trained (AT) and untrained (UT) groups (34). Similar sitting-induced reductions in lower-limb vascular function were observed for both groups *, $P<0.05$ versus PRE in both groups.

Given the conflicting evidence from these 2 studies, our laboratory examined the relationship between aerobic fitness and sitting-induced declines in popliteal FMD (33). Interestingly, we found that higher aerobic fitness was associated with greater declines in FMD after sitting (**Figure 2.14**) (33). Now, all possible options regarding the effect of aerobic fitness on lower-limb vascular responses to acute sitting have been reported in

these cross-sectional studies [i.e., a beneficial effect (101), no effect (122) or a larger reduction with sitting (98)]. As such, an exercise training intervention study is warranted to help uncover the impact of increasing aerobic fitness on sitting-induced FMD responses and hopefully solve this discrepancy.

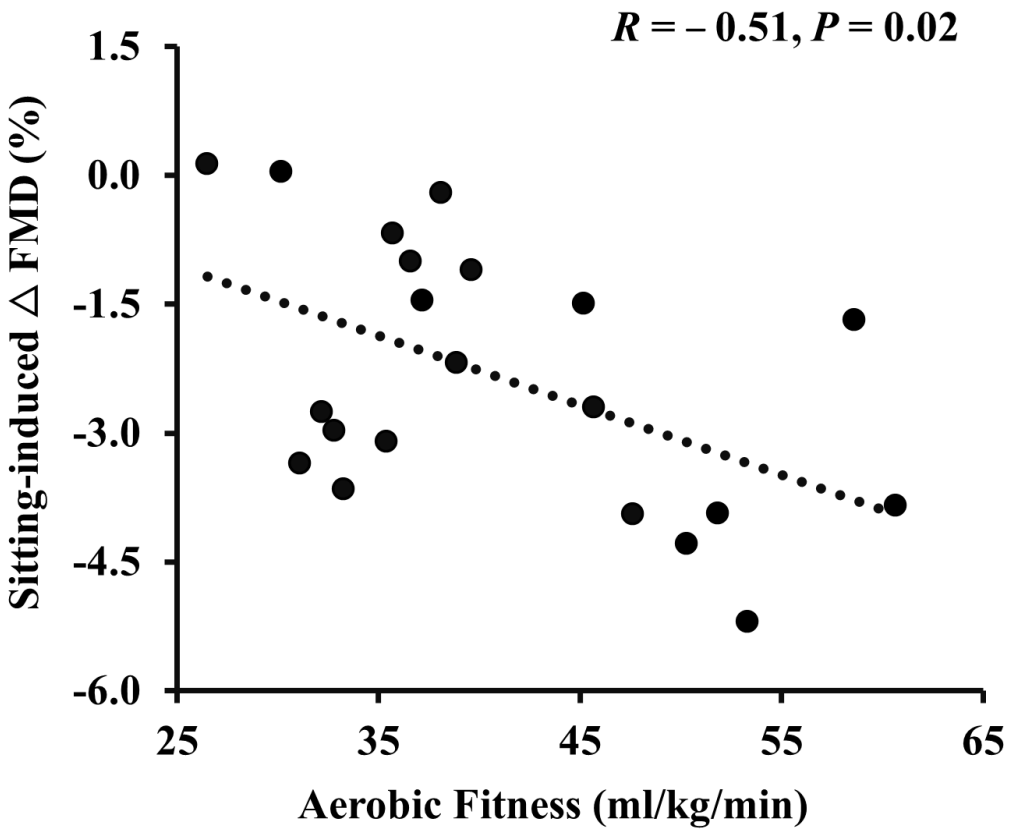

Figure 2.14. The inverse relationship observed between aerobic fitness (relative $\dot{V}O_2$peak) versus prolonged sitting-induced declines in popliteal flow-mediated dilation (FMD) reported by Liu et al. (98).

2.9 Research Question and Hypothesis

The leg vasculature is highly susceptible to atherosclerosis compared to upper-limb arteries (73). This may be due, in part, to the adverse vascular effects of prolonged sitting in the lower-limb. As such, it is important to explore strategies that may offset the detrimental effects of sitting. Given the well-established cardiovascular benefits of

aerobic exercise training, particularly HIIT, this study explored the impact of a 12-week HIIT program on popliteal vascular responses (i.e., FMD, NMD, reactive hyperemia) to an acute bout of prolonged sitting. The primary outcome measures were: 1) aerobic fitness, 2) prolonged sitting-induced declines in FMD, NMD, and reactive hyperemia. It was hypothesized that: 1) popliteal FMD, NMD and reactive hyperemia would decrease after prolonged sitting before the 12-week HIIT program started, and 2) aerobic fitness and pre-sitting popliteal FMD would improve after training. However, no directional hypothesis was included regarding the impact of HIIT on popliteal responses to sitting due to the conflicting evidence from the cross-sectional studies highlighted above regarding the impact of aerobic fitness (or lack thereof) on sitting-induced lower-limb vascular function (32–34).

Chapter 3. METHODS

3.1 Participants

Healthy adults (i.e., free of chronic disease) were recruited to participate in this study. All participants were non-smokers, non-hypertensive (systolic blood pressure, SBP <139 mmHg and diastolic blood pressure, DBP <89 mmHg), non-obese (body mass index <30 kg/m^2) and had no contraindications for maximal exercise testing or HIIT. The study eligibility was assessed via the Canadian Society for Exercise Physiology - Get Active Questionnaire (Appendix A) and a Healthy History Questionnaire (Appendix B). Participants were informed of the methods and study design verbally and in writing before providing written informed consent (Appendix C). All protocols and procedures were approved by the Dalhousie University Health Sciences Research Ethics Board (#2021-5645; Appendix D) in accordance with the standards set by the Declaration of Helsinki, except for registry in a public database.

3.2 Experimental Measures

3.2.1 Anthropometrics and Aerobic Fitness

Body mass and height were measured using a calibrated stadiometer and physician's scale (Health-O-Meter, McCook IL, USA) to the nearest 0.1 kg and 0.5 cm, respectively. Body mass and height were used to calculate the body mass index (kg/m^2).

To assess aerobic fitness, participants completed an incremental and maximal exercise test on a cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands) via a mixing chamber-based commercial metabolic system (TrueOne 2400, Parvo Medics Inc., Sandy, UT). The test started with a 5-min warm-up at 50 watts. The intensity was then step increased to 70 watts and gradually increased by 20 watts/min until voluntary

exhaustion (29). Heart rate (HR) was continuously monitored via a wireless monitor (Polar, H9, Lachine, QC, Canada) worn across the sternum. Ratings of perceived exertion were determined every 2-min via the Borg 6-20 scale (131). The respiratory exchange ratio was calculated as the volume rate of carbon dioxide ($\dot{V}CO_2$) divided by $\dot{V}O_2$. Upon completion of the test, the workload was immediately reduced to 50W for a 5-min cool-down period.

The $\dot{V}O_2$ data were averaged every 15-s over the duration of the test and presented in both absolute units (L/min), as well as relative to body mass (ml/kg/min). $\dot{V}O_2$peak was considered as the greatest, consecutive 30-s average. The attainment of a $\dot{V}O_2$max was based upon the criteria of achieving a plateau in relative $\dot{V}O_2$ (increase <1.5 ml/kg/min) despite an increase in workload (i.e., between successive 15-s averages). In summary, 15/21 participants at baseline, 8/11 at mid-testing, and 17/21 participants at follow-up achieved a $\dot{V}O_2$max. If a plateau in $\dot{V}O_2$ was not achieved, the attainment of $\dot{V}O_2$peak was evaluated based upon meeting 2 of the following criteria: 1) a respiratory exchange ratio ≥ 1.10 (19/21 participants at Baseline, all participants at Mid, and 18/21 participants at Follow-up), 2) an age-predicted peak HR (i.e., 208 – 0.7 × age) ≥ 90% (achieved by all participants at Baseline, Mid, and Follow-up) (132), and/or 3) a peak rating of perceived exertion ≥18 (met by all participants at Baseline, and Mid, as well as 20/21 at Follow-up) (133). As the criteria for $\dot{V}O_2$peak was achieved in all participants across all timepoints, this term was used to describe aerobic fitness in this study.

The ventilatory threshold was determined by the V-slope method to identify the breakpoint where $\dot{V}CO_2$ increases more rapidly than $\dot{V}O_2$ and was expressed as a percentage of absolute $\dot{V}O_2$peak (134). Additionally, peak aerobic power output (PAP,

watts) was recorded for each test. Peak metabolic equivalents of task (Peak METs) were calculated by dividing relative $\dot{V}O_2$peak (ml/kg/min) by a standardized resting relative $\dot{V}O_2$ value of 3.5 ml/kg/min (135). Relative $\dot{V}O_2$peak data were used to determine aerobic fitness percentiles using a continuous, sex- and age-specific model of $\dot{V}O_2$max percentile rank developed based on the percentile rank published by the American College of Sports Medicine (136).

3.2.2 Systemic Hemodynamics

Resting HR was determined from successive cardiac intervals obtained from a 3-lead, bipolar electrocardiogram. Systolic and DBP were measured using automated vital signs monitor (Carescape v100; General Electric Healthcare, Mississauga, ON, Canada). The electrocardiogram was recorded using a PowerLab (PL3508 PowerLab 8/53; ADInstruments, Sydney, NSW, Australia) data acquisition system sampled at 1000 Hz. Recordings of the electrocardiogram were displayed in real-time and analyzed offline using LabChart software (Version 8, ADInstruments, Sydney, Australia). Mean arterial pressure was calculated using the equation ⅓SBP + ⅔DBP.

3.2.3 Popliteal Vascular Function

3.2.3.1 Popliteal Flow-Mediated Dilation

As previously described (137, 138), the popliteal artery was imaged using a 12-MHz multifrequency linear array probe attached to a high-resolution ultrasound system (Vivid i; General Electric Healthcare, Mississauga, ON, Canada) proximal to the bifurcation, at or slightly above the popliteal fossa, with participants in a seated position. Red blood cell velocity (RBCv) was obtained using a pulsed-frequency of 5-MHz and corrected with an insonation angle of 60°. As recommended in published guidelines (5),

the sample volume was adjusted to encompass the entire lumen. Video signals from the ultrasound were exported to an external laptop via a video graphics array converter (Epiphan Systems Inc., VGA 2 USB, Ottawa, Canada) for offline analysis. Analysis of popliteal lumen diameter and RBCv were performed using automated commercial edge detection and wall-tracking software (FMD Studio, Cardiovascular Suite; Quipu, Pisa, Italy). At least 2-min of popliteal data were averaged at the pre-, mid- (1.5-h), and post-sitting periods to determine resting lumen diameter and mean RBCv. Shear rate (SR, /s) was calculated as [(8 × mean RBCv (cm/s) / baseline diameter (cm)]. Popliteal blood flow (PBF, mL/min) was determined as [mean RBCv (cm/s) × 60 (s/min) × π × lumen radius^2 (cm^2)]. All vascular measurements were blindly analyzed.

A pressure cuff connected to an automatic rapid Inflation system (E20 and AG101; Hokanson, Bellevue, WA, USA) was placed around the largest circumference of the calf (~10 cm distal to the popliteal fossa) and marked on the skin before the pre-sitting FMD assessment to ensure consistency of cuff placement for the post-sitting assessment. Popliteal lumen diameter and RBCv were measured for a minimum of 2-min before cuff inflation to obtain baseline values. The pressure cuff was then rapidly inflated to 250 mmHg for 5-min. Upon cuff deflation, lumen diameter and RBCv were recorded continuously for an additional 5-min. The hyperemic SR area under the curve (SR_{AUC}, the stimulus for the FMD response) was calculated between the start of cuff deflation to the time that peak diameter occurred. The time-to-peak dilation (s) from the moment of cuff deflation was recorded.

Absolute FMD (mm) was calculated as the difference between baseline diameter and peak diameter, while relative FMD was calculated using the equation: FMD (%) =

[(peak diameter – baseline diameter)/baseline diameter × 100%]. It has been recommended that relative FMD be normalized to SR_{AUC} if the following statistical assumptions are met: 1) the relationship between FMD and SR_{AUC} is linear, and 2) the y-intercept (i.e., unstandardized β-coefficient for the constant) for the regression slope of this relationship is zero (i.e., the 95% confidence intervals for the constant includes '1' or $P > 0.05$) (78). In the present study, there was a moderate positive relationship between the pooled FMD and SR_{AUC} responses ($R = 0.607$, $P < 0.001$), but the y-intercept for the regression slope of this relationship was not zero ($P < 0.001$). As such, there was no requirement to normalize the FMD outcomes to SR_{AUC}.

In addition, allometric scaling can be used to account for the potential influence of baseline lumen diameter on FMD responses (i.e., smaller baseline diameters may produce larger shear stress and FMD responses). There was a linear relationship between the logarithmically transformed peak FMD and baseline diameters ($R = 0.983$, $P < 0.001$), which yielded an unstandardized β-coefficient that deviated from 1 and had an upper 95% confidence interval <1 for the pooled FMD (β = 0.89, 95% CI: 0.86-0.93) (81). As such, allometric scaling was required and applied to the FMD responses.

3.2.3.2 Popliteal Reactive Hyperemia (Assessment of Resistance Vessel Function)

The $RBCv_{AUC}$ and PBF_{AUC} were calculated for 60-s following cuff deflation during the FMD test. The $RBCv_{AUC}$, PBF_{AUC}, peak RBCv, and peak PBF responses were used to quantify lower-limb resistance vessel function (7, 8).

3.2.3.3 Popliteal Nitroglycerin-Mediated Dilation

The NMD response was determined by measuring the peak increase in lumen diameter following a single, sublingual dose of nitroglycerin spray (0.4 mg). This test

provided an assessment of endothelium-independent dilation and an index of VSMCs sensitivity to NO (139). Lumen diameter was continuously measured for 1-min before and 10-min following the nitroglycerin administration. Absolute NMD (mm) was calculated as the difference between the baseline and peak diameters. Relative NMD was quantified as a percentage change in peak diameter (from baseline) achieved following administration of nitroglycerin. The elimination half-life of an 0.6 mg sublingual dose of nitroglycerin is ~4-min (84) As such, it was anticipated that the NMD test should not have interfered with the anticipated negative vascular effects of prolonged sitting (e.g., reduced popliteal blood flow/shear stress). Throughout the course of the study, a posture change (from seated to reclined) was required for the NMD test (see **section 3.3.1** for details).

3.2.4 Habitual Physical and Sedentary Activity Monitoring

Habitual physical activity levels may influence lower-limb vascular function (13, 113). To properly document the activity levels of our participants and examine whether changes in habitual physical and/or sedentary activity may have confounded the HIIT-induced effects on vascular function, participants wore an activPAL monitor (Pal Technologies Ltd, Glasgow, UK) 24-h per day for 7-d. The activPAL was waterproofed and secured using transparent medical dressing (Tegaderm, 3M, London, ON, Canada) to the midline of the right thigh, one-third of the way between the hip and the knee. The activPAL inclinometer is a valid measure of time spent on standing, stepping, and sitting/lying down (i.e., sedentary) (140, 141). Physical activity intensities (i.e., light, moderate, and vigorous activity) were determined using step rate thresholds, which were

calculated from a cross-validated curvilinear cadence-intensity equation individualized for body height (142, 143).

Participants self-reported their waking hours and non-stride-based activities (e.g., swimming, cycling) to assist with activPAL analysis. A customized LabVIEW program (LabVIEW 2013; National Instruments, Austin, TX) that confirmed waking hours and summarized daily averages of time spent awake, standing, and sitting/lying down was used to analyze the activPAL data.

3.3 Experimental Design

The experimental design is outlined in **Figure 3.1**. At Baseline, both groups completed 2 laboratory visits. During the first visit, participants provided written informed consent and completed the Get Active Questionnaire (144) and a Health History Questionnaire. The remainder of visit 1 was dedicated to anthropometric measurements (i.e., body mass and height), followed by the pre- and post-sitting popliteal FMD/Resistance Vessel Function and NMD assessments (see details below). During visit 2, participants completed the incremental and maximal cycling exercise test to determine $\dot{V}O_2$peak and PAP. At the end of this visit, participants were equipped with the activPAL physical and sedentary activity monitor. The order of visits 1 and 2 varied depending on participant and equipment availability. If participants completed the $\dot{V}O_2$peak test during visit 1, visit 2 was scheduled at least 24 hours afterwards to avoid the effects of vigorous physical activity on vascular function (See **section 3.3.1**) (145).

The HIIT group then started the 12-week exercise training. There was a 1-week cessation of training after 6-weeks to accommodate the mid-training assessment of aerobic fitness and PAP. The Control group was asked to maintain their habitual physical

activity levels for 13-weeks. After the 13-week period, both groups repeated the habitual monitoring, aerobic fitness, and prolonged sitting protocol assessments. Two participants from the Control group and 1 participant from the HIIT group did not repeat the habitual activity monitoring due to time constraints.

3.3.1 Prolonged Sitting Protocol

All study visits were performed in a thermoneutral environment (20-22 °C). Vascular assessments were completed under standardized conditions. Specifically, participants were well-hydrated and avoided vigorous physical activity, as well as the consumption of any products known to acutely influence vascular function (e.g., caffeine, alcohol, saturated fats, chocolate, antioxidant, and multivitamin supplements) for 24-h prior to all testing sessions (5).

Prior to the prolonged sitting protocol, participants were positioned in the prone posture (i.e., lying on their front) while the optimal imaging site of the popliteal arteries in both legs were identified. Resting popliteal hemodynamics were recorded from each leg for ~5-min. Participants then moved to a laboratory chair for the start of the sitting protocol. Popliteal FMD and NMD responses were assessed in opposite legs with the leg order randomized between participants but kept consistent within each participant for all pre- and post-sitting assessments. The FMD test was performed first with the contralateral leg kept straight until ready for the NMD assessment (**Figure 3.2**). All FMD assessments were performed with participants in the seated position. In addition to the pre- and post-sitting FMD assessments, popliteal RBCv and lumen diameter were continuously recorded for 5-min at the 1.5-h timepoint of sitting (i.e., mid-sitting) in the same leg.

The NMD assessments were initially performed in the seated position for the first 7 participants. However, 3 participants experienced negative side effects of nitroglycerin administration (e.g., fainting, light-headedness, dizziness). For ethical and safety considerations, the posture for subsequent NMD assessments was changed to a semi-recumbent position (i.e., the backrest of the chair was lowered such that the participant was passively placed into a reclining posture) (19). Before all ultrasound assessments, study personnel carefully positioned the participants' leg in a slightly extended position (~30° knee flexion) to allow for imaging of the popliteal artery. The leg was then positioned back to a flexed position (~90° knee flexion).

3.3.2 HIIT Protocol

The 12-week HIIT protocol is outlined in **Figure 3.3** below. All HIIT sessions were supervised and conducted 3-days per week (Mondays, Wednesdays, and Fridays). Throughout the protocol, warm-up and cool-down involved 5-min of cycling at 25% PAP. During the first 3-weeks of training, there were 2 sets of 40 repeated 15-s cycling intervals at 100% PAP interspersed with 15-s of passive recovery (i.e., sitting on ergometer without pedaling) per session. There was a 5-min passive recovery period separating the 2 sets (i.e., 40-min of exercise in total).

Considering anticipated improvements in aerobic fitness, PAP and exercise tolerance, the duration of each training session was increased during weeks 4 and 5 to 46-min (i.e., 2 × 23-min sets or 2 sets of 46 intervals). During week 6, the duration of each training session was decreased back to 40-min (i.e., 2 × 20-min sets or 2 sets of 40 intervals) to provide a tapering period and ensure appropriate recovery before the mid-training assessment of aerobic fitness during week 7. There were no HIIT sessions during

this week. The highest PAP achieved between the pre- and mid-training tests were compared, and the highest PAP used to prescribe intensity for the second half of training. Six participants achieved higher PAP, 4 had equivocal, and 1 produced a lower PAP at the mid-training test. During weeks 7 and 8, the intensity was set at 100% PAP and the duration of each session increased to 52-min (i.e., 2 × 26-min sets or 2 sets of 52 intervals). The intensity was then increased to 110% PAP for weeks 9 and 10, then increased again to 115% PAP for week 11. Each training session remained at 52-min during weeks 9-11. As during week 6, the duration of each session was decreased to 40-min, and the intensity reduced back to 100% PAP for the last week of training (i.e., week 12). To remain in the study, HIIT participants were required to complete ≥80% of all training sessions (i.e., ≥29 of 36 sessions) and could not miss 3 consecutive sessions.

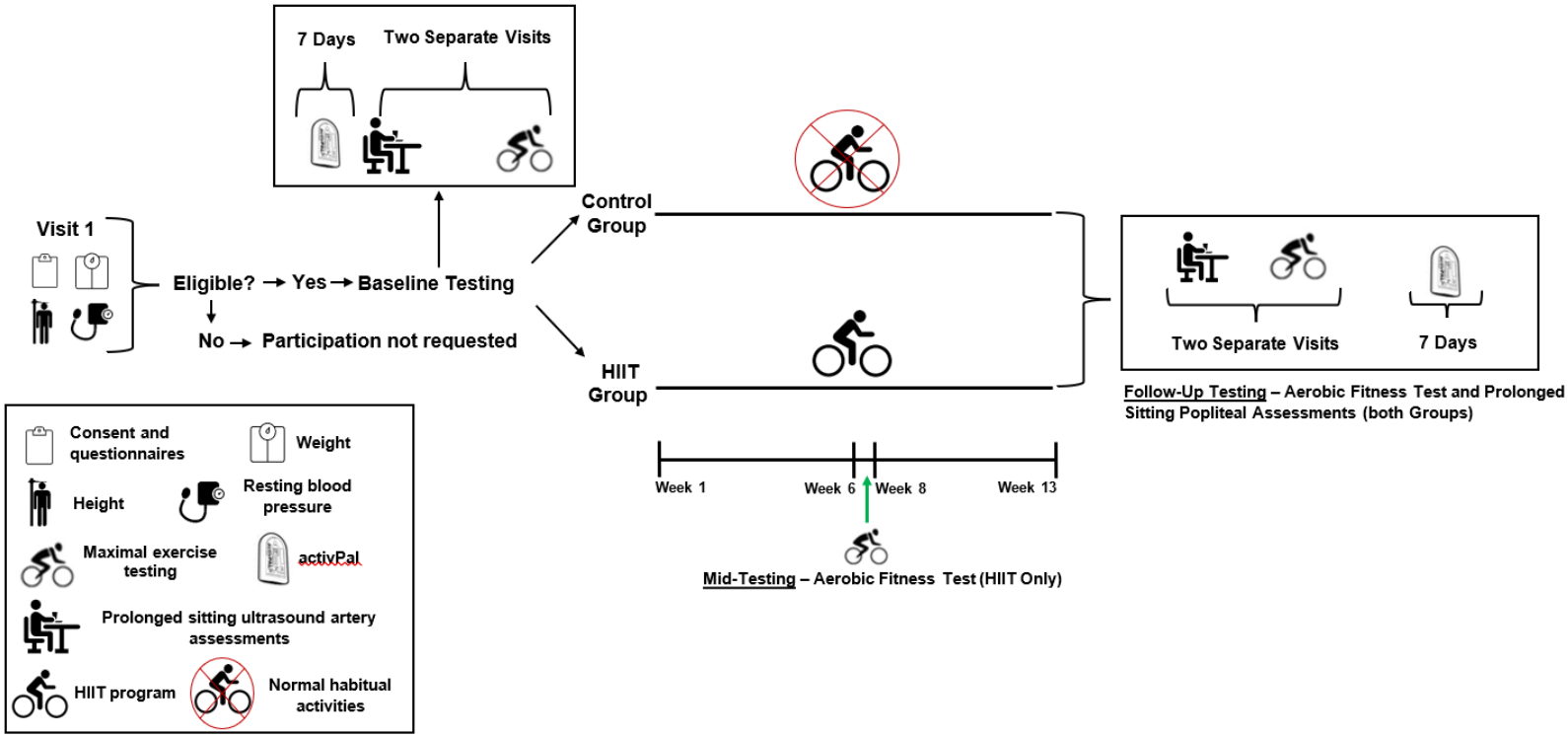

Figure 3.1. Schematic of the 12-week high-intensity interval training (HIIT) and Control study design. Questionnaires include a Canadian Society for Exercise Physiology - Get Active Questionnaire (Appendix A) and a Healthy History Questionnaire (Appendix B). Anthropometrics and aerobic fitness assessments, prolonged sitting protocol and habitual activity monitoring were completed at Baseline and Follow-up in both groups. Anthropometrics and aerobic fitness were also assessed at week 7 (Mid-testing) for the HIIT group only.

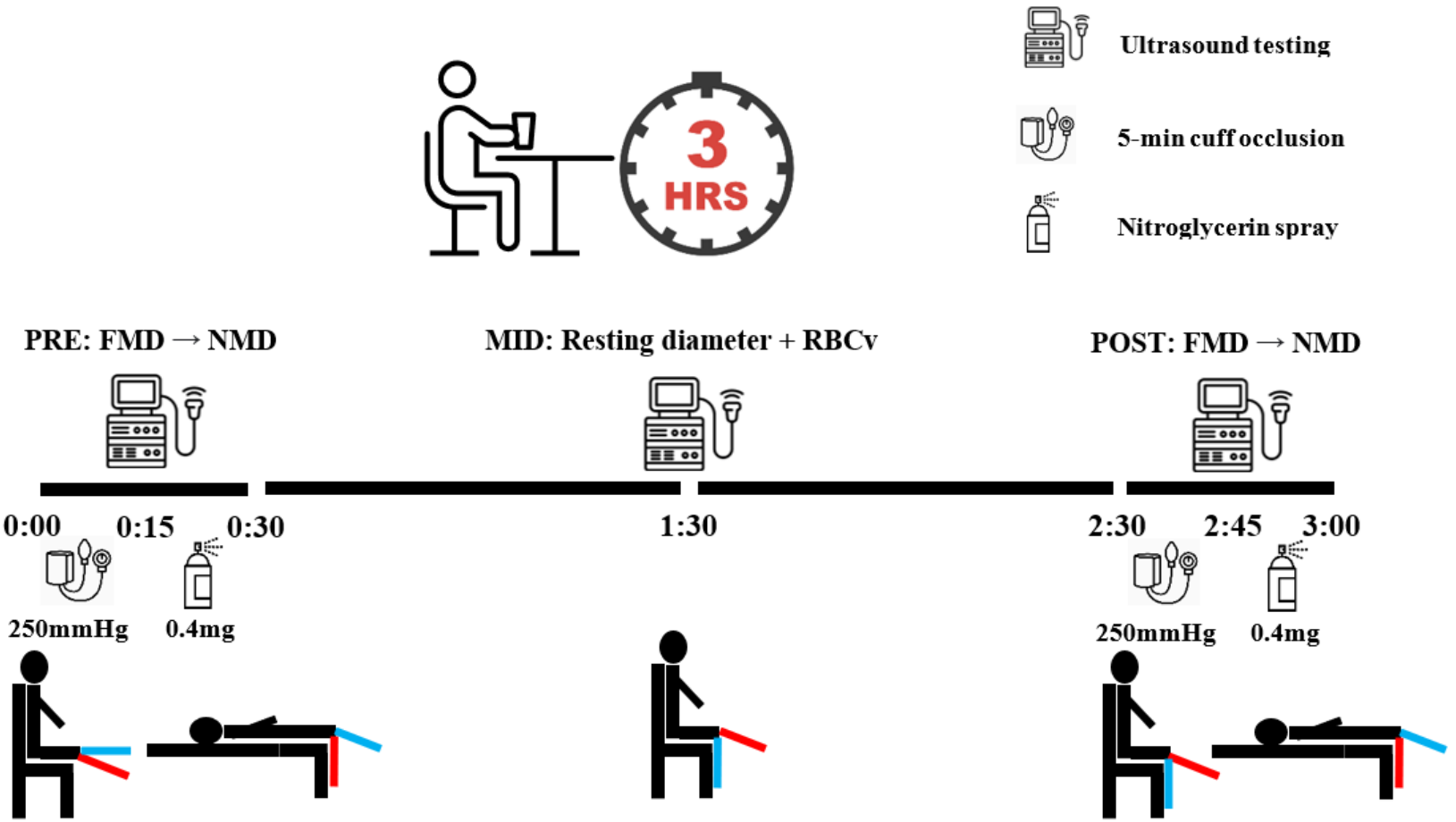

Figure 3.2. Schematic of the prolonged sitting protocol including pre-, mid-, and post-sitting popliteal assessments, as well as the timepoints for each assessment. Flow-mediated dilation (FMD) assessments are represented by the red leg, performed at 0-h and 2.5-h of sitting. Popliteal lumen diameter and red blood cell velocity (RBCv) were measured prior to each FMD assessment, as well as after 1.5-h of sitting in the same (red) leg. Nitroglycerin-mediated dilation (NMD) assessments are represented by the blue leg and were performed at 0.25-h and 2.75-h of sitting. The leg order used for FMD versus NMD was randomized between participants but kept consistent for pre- and post-sitting assessments. Note: NMD assessments were performed in the seated posture for the first 7 participants.

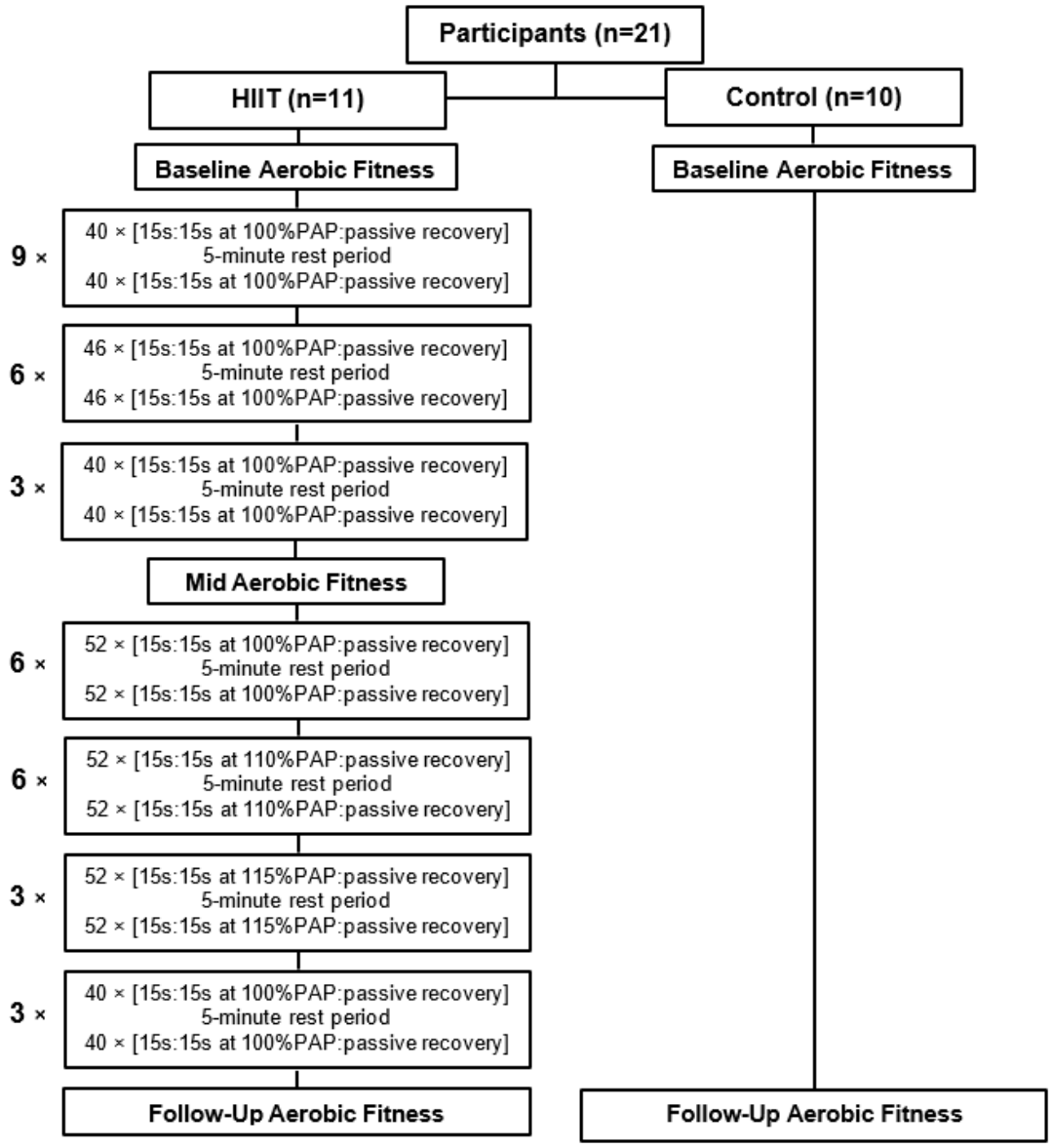

Figure 3.3. Schematic overview of the 12-week high-intensity interval training (HIIT) protocol, as well as timepoints for aerobic fitness assessments in both the HIIT and Control groups. PAP, peak aerobic power.

3.4 Statistical Analyses

All dependent variables were assessed for normality using a Shapiro Wilk test. All non-normally distributed data (i.e., peak rating of perceived exertion; light, moderate, vigorous, and moderate-to-vigorous physical activity; resting RBCv, PBF, and SR; relative FMD; time-to-peak dilation; SR_{AUC}; peak RBCv, peak PBF and PBF_{AUC}) were logarithmically transformed prior to statistical analysis. Non-parametric tests were used for $\dot{V}O_2$peak percentile rank and sitting-induced changes in FMD, NMD and reactive hyperemia variables.

Participant descriptive characteristics, aerobic fitness, as well as habitual physical and sedentary activity data were analyzed using a Group (Control/HIIT) × Time × (Baseline/Follow-up) repeated measures analyses of variance (RM-ANOVA). A 3-way (Group ×Time × Pre-/Post-Sitting) RM-ANOVA compared all popliteal outcomes. A separate 1-way RM-ANOVA was conducted to account for the mid-training aerobic fitness test within the HIIT group. An analysis of covariance (ANCOVA) was used to examine the impact of Group, Time and Sitting on popliteal relative FMD responses to control for the effects of baseline diameter (i.e., allometric scaling). Specifically, the difference between the logarithmically transformed peak and baseline diameters (lnD_{peak}–$lnD_{baseline}$) was set as the dependent variable, while Group, Time, and Pre-/Post-Sitting were set as the fixed factors, with logarithmically transformed baseline diameter ($lnD_{baseline}$) as the covariate. Sitting-induced reductions (post-sitting – pre-sitting) in popliteal outcomes were analyzed using a Group × Time RM-ANOVA. The variance of differences was assessed using Mauchly's test of sphericity for all ANOVAs. When assumptions of sphericity were violated, the Greenhouse-Geisser correction factor to the

degrees of freedom was used. Bonferroni *post hoc* testing was conducted on statistically significant ANOVAs. Effect sizes for ANOVAs were calculated as Partial Eta Squared (η_p^2). Effect sizes for pairwise comparisons were calculated as Cohen's d for aerobic fitness, systemic hemodynamics, and popliteal outcome measures between Baseline and Follow-up for both groups. Small, medium, and large ANOVA effect sizes were calculated as Partial Eta Squared (η_p^2) were defined as 0.01-0.06, 0.06-0.14, and ≥0.14, respectively (146). Small, medium, and large Cohen's d effect sizes were defined as 0.2, 0.5, and 0.8, respectively (147). All statistics were completed in SPSS Version 26.0 (IBM). Statistical significance was accepted as $P \leq 0.05$. All data were expressed as means ± standard deviations (SD).

Chapter 4: RESULTS

4.1 Participant Characteristics, Aerobic Fitness, and Habitual Activity

Twenty-nine participants (17♀) were recruited for the project [HIIT: *n*=15 (8♀), Control: *n*=14 (9♀)]. Two (1♀) were removed from the Control group due to NMD-induced dizziness/light-headedness. Two other female Control participants were removed due to their unavailability for Follow-up testing. As such, complete data sets were collected from 10 participants in the Control group (22±1 years, 6♀). In the HIIT group, 4 participants (1♀) were excused due to an inability to complete ≥80% of the training sessions. In total, 11 participants (24±8 years, 7♀) completed the 12-week HIIT protocol. Adherence to the 36 HIIT sessions was 93±3% [i.e., 33±1 (32-35) sessions].

Participants descriptive characteristics, aerobic fitness, and habitual activity are presented in Table 4.1. There was no change in weight or body mass index in either group from Baseline to Follow-up (both, $P \geq 0.794$; Table 4.1). Group × Time interaction effects were observed such that PAP (Table 4.1), absolute $\dot{V}O_2$peak (Figure 4.1A), relative $\dot{V}O_2$peak (Figure 4.1B), peak METs (Table 4.1), and the ventilatory threshold (% of $\dot{V}O_2$peak; Table 4.1) did not change in the Control group at Follow-up (all, $P \geq 0.114$), but improved after 12-weeks of HIIT (all, $P \leq 0.005$). Furthermore, the HIIT group had a greater ventilatory threshold than the Control group at Follow-up (P=0.009). Within the HIIT group, absolute and relative $\dot{V}O_2$peak, as well as peak METs, also increased after 6-weeks of HIIT (all, $P \leq 0.004$; $d \geq 1.35$). However, PAP and the ventilatory threshold were unchanged after 6-weeks of training (both, $P \geq 0.285$; $d \leq 0.56$). No differences in PAP, absolute $\dot{V}O_2$peak, relative $\dot{V}O_2$peak or peak METs were observed between the Mid- and Follow-up timepoints (all, $P \geq 0.075$; $d \leq 0.79$). However, the ventilatory threshold was higher after 12-weeks versus 6-weeks of HIIT (P=0.016; d=1.07, Table 4.1). $\dot{V}O_2$peak

percentile was not different between Baseline and Follow-up within the Control group (40 ± 30 vs 35 ± 24, P = 0.594, Table 4.1). Within the HIIT group, $\dot{V}O_2$peak percentile increased after 6-weeks of HIIT (P=0.003; d=1.18) and remained higher at Follow-up compared to Baseline (P=0.026; d=0.81). There was no difference in $\dot{V}O_2$peak percentile between Mid and Follow-up within the HIIT group (P=0.512; d=0.21). No between-group difference was observed for $\dot{V}O_2$peak percentiles at Baseline or Follow-up (both, P≥0.205; d≤0.58). There were no interactions observed for any habitual activity outcomes (all, P≥0.155; Table 4.1).

4.2 Systemic and Popliteal Hemodynamics

All systemic hemodynamics data are presented in Table 4.2. No Group × Time × Pre-/Post-Sitting interaction effects were observed for HR, SBP, DBP, or mean arterial pressure (all, P≥0.575). However, Group × Pre-/Post-Sitting interaction effects were observed for DBP and mean arterial pressure (both, P≤0.013) such that DBP was higher post-sitting (versus pre- and mid-sitting) in the HIIT group at Baseline (both, P≤0.012; d ≥0.89). Furthermore, the HIIT group had a higher post-sitting DBP compared to the Control group at Baseline and Follow-up (both, P≤0.040). In addition, mean arterial pressure increased during the mid- and post-sitting timepoints in the HIIT group at Follow-up (P=0.006, d=0.99, Table 4.2).

4.3 Popliteal Flow-Mediated Dilation Outcomes

Popliteal hemodynamic and FMD outcomes are presented in Table 4.3. No Group × Time × Pre-/Post-Sitting interaction effects were observed for resting diameter, RBCv, PBF, or SR (all, P≥0.383). However, resting RBCv, PBF, and SR decreased similarly at

the mid- and post-sitting timepoints (all, $P \leq 0.035$, $d \geq 0.99$). There were no between-group differences at any timepoint for resting diameter, RBCv, PBF, or SR (all, $P \geq 0.06$).

Popliteal FMD (and reactive hyperemia) data were obtained from all 11 HIIT participants and 8/10 Control participants. Data from the 2 Control participants were removed from the FMD analysis due to poor image quality or file corruption. No differences in resting or FMD peak popliteal diameter were observed between Baseline and Follow-up for either group (Table 4.3; all, $P \geq 0.383$). Furthermore, resting and peak popliteal diameters did not change following sitting in either group (all, $P \geq 0.059$). Absolute popliteal FMD decreased after sitting at Baseline and Follow-up within the Control group (both, $P \leq 0.017$, $d \geq 0.92$; Table 4.3). However, absolute FMD was attenuated following sitting at Follow-up ($P=0.014$, $d=0.84$) but not at Baseline ($P=0.078$, $d=0.55$; Table 4.3).

As depicted in Figure 4.2A, pre-sitting popliteal relative FMD did not change at Follow-up in either the HIIT (ΔFMD = -0.1±2.7%; $P=0.548$, $d=0.03$), or Control (ΔFMD = -1.2±0.9%; $P=0.173$, $d=1.3$) groups. Controlling for the effects of resting popliteal diameter did not alter the results (Time effect: $P= 0.264$, Group × Time interaction effect: $P=0.380$).

Table 4.1. Participant characteristics, aerobic fitness, and habitual activity

	HIIT			CONTROL		Interaction *P*-Value	Effect size (η_p^2)
	BASELINE	MID	FOLLOW-UP	BASELINE	FOLLOW-UP		
Participant Characteristics							
Body Mass (kg)	73.2 ± 14.4	72.4 ± 15.1	73.1 ± 13.2	66.9 ± 10.2	66.6 ± 9.6	0.898	0.001
Body Mass Index (kg/m^2)	25.1 ± 3.5	24.8 ± 3.5	25.1 ± 3.5	23.5 ± 2.3	23.4 ± 2.3	0.794	0.004
Aerobic Fitness							
Peak Aerobic Power (watts)	213 ± 55	230 ± 56	241 ± 55*	218 ± 45	206 ± 55	**<0.001**	0.447
Absolute $\dot{V}O_2$peak (L/min)	2.58 ± 0.68	2.83 ± 0.70	2.90 ± 0.70*	2.59 ± 0.68	2.48 ± 0.63	**0.004**	0.364
Relative $\dot{V}O_2$peak (ml/kg/min)	35.4 ± 7.8	39.1 ± 6.7	39.5 ± 6.1*	38.6 ± 7.6	37.1 ± 7.0	**0.008**	0.312
Peak METs	10.1 ± 2.2	11.2 ± 2.0	11.6 ± 1.6*	11.0 ± 2.2	10.6 ± 2.0	**0.008**	0.316
$\dot{V}O_2$peak Percentile Rank	33 ± 33	46 ± 30	49 ± 28*	40 ± 30	35 ± 24	-	-
Peak RER ($\dot{V}CO_2/\dot{V}O_2$)	1.19 ± 0.06	1.22 ± 0.04	1.19 ± 0.07	1.22 ± 0.11	1.23 ± 0.11	0.856	0.002
Peak RPE	20 ± 1	20 ± 1	20 ± 1	20 ± 1	19 ± 1	0.104	0.133
Peak HR (beats/min)	192 ± 9	192 ± 10	192 ± 9	193 ± 8	189 ± 8	0.222	0.077
%Age-predicted HRmax	103 ± 4	102 ± 5	103 ± 5	102 ± 4	100 ± 5	0.243	0.071
VT (%$\dot{V}O_2$peak)	71 ± 4	72 ± 4	76 ± 4*$^{\#}$	71 ± 4	71 ± 3	**0.022**	0.245
Heart Rate at VT (beats/min)	165 ± 10	167 ± 12	171 ± 8	165 ± 16	171 ± 11	**0.016**	0.269
Habitual Activity							
Step Count (steps/day)	11170 ± 4449	–	11412 ± 4687	11574 ± 4449	11671 ± 4245	0.902	0.001
Sedentary Time (hours/day)	8.8 ± 1.3	–	9.0 ± 1.5	8.7 ± 1.5	9.2 ± 0.8	0.648	0.013
Standing Time (hours/day)	6.2 ± 1.4	–	6.0 ± 1.5	6.2 ± 1.4	5.8 ± 1.5	0.798	0.004
LPA (mins/week)	574 ± 135	–	572 ± 198	634 ± 234	575 ± 127	0.648	0.013
MPA (mins/week)	304 ± 104	–	267 ± 142	277 ± 164	278 ± 148	0.524	0.026
VPA (mins/week)	63 ± 67	–	46 ± 54	41 ± 44	58 ± 53	0.155	0.122
MVPA (mins/week)	367 ± 161	–	313 ± 180	319 ± 201	336 ± 186	0.355	0.054

Data are presented as Means ± SDs. Participant characteristics and aerobic fitness data were obtained from 11 HIIT (7♀) and 8 Control (5♀) participants. Habitual activity data were obtained from 10 HIIT (6♀) and 8 Control (4♀) participants. $\dot{V}O_2$peak, peak volume rate of oxygen consumption; $\dot{V}CO_2$, volume rate of carbon dioxide production; METs, metabolic equivalents; RER, respiratory exchange ratio; RPE, rating of perceived exertion; VT, ventilatory threshold; LPA, light physical activity; MPA: moderate physical activity; VPA: vigorous physical activity; MVPA, moderate-to-vigorous physical activity. Group × Time interaction effects were assessed using a repeated measures analysis of variance with Bonferroni *post hoc* pairwise comparisons to determine between and within group differences. Peak RPE, LPA, MPA, VPA, and MVPA were logarithmically transformed before analysis. Between- (Mann-Whitney U) and within-participants (Wilcoxon singed-rank) non-parametric tests were used to assess $\dot{V}O_2$peak percentile rank. *, $P < 0.05$ versus Baseline within the same group. $^{\#}$, $P < 0.05$ versus Control group at the same timepoint.

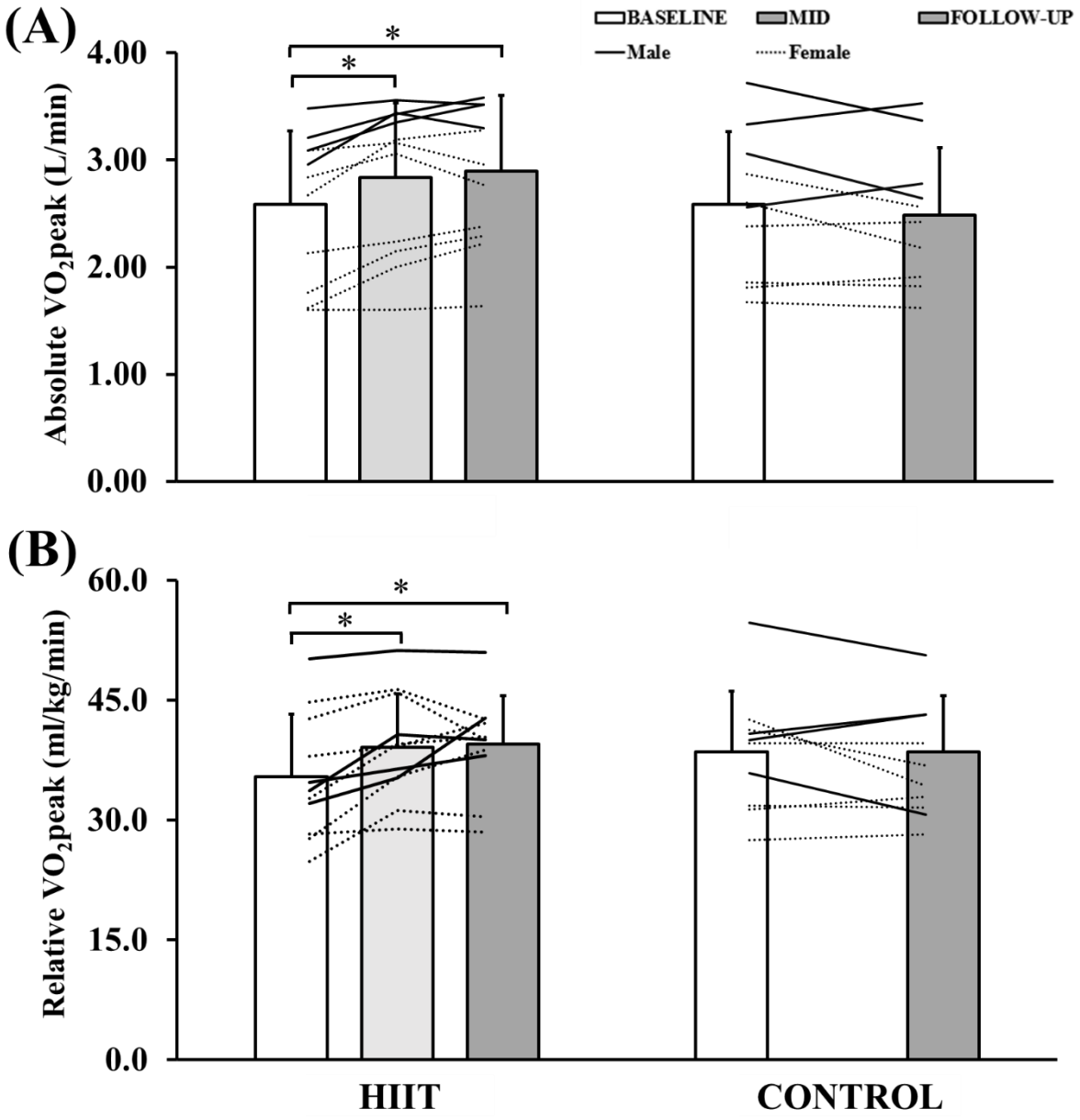

Figure 4.1. (A) Absolute V̇O2peak (L/min) and (B) Relative V̇O2peak between Baseline, 6-Week, and Follow-up for the HIIT group (n = 11, 7♀) and between Baseline and Follow-up for Control group (n = 10, 6♀). Group × Time interaction effects were determined using a 2-way repeated measures analysis of variance (RM-ANOVA) with Bonferroni post hoc pairwise comparisons for Baseline and Follow-up. Differences with in the HIIT group between Mid versus Baseline and Follow-Up were assessed using a 1-way RM-ANOVA with Bonferroni post hoc pairwise comparisons. Means ± SDs for each group, as well as individual data are presented. Females represented as dashed lines and males as solid lines. *, $P < 0.05$ versus Baseline.

However, there was a pre-/post-sitting main effect for popliteal relative FMD (P<0.001, η_p^2=0.637), which remained when allometrically-scaled for resting diameter (P<0.001, η_p^2=0.194; Supplemental Figure, Appendix E). As shown in Figure 4.2A, popliteal relative FMD was attenuated after 3-h of sitting at Baseline (5.2± 2.0% to 2.3 ± 1.3%, P=0.011; d=1.30) and Follow-up (4.0±2.3% to 2.0 ± 1.7%, P=0.004, d=0.88) within the Control group. In the HIIT group, relative FMD also decreased after sitting at Baseline (4.2±3.3 to 2.8±3.0%, P=0.018; d=0.52). However, sitting did not significantly change popliteal relative FMD after 12-weeks of HIIT (4.1±2.3 to 2.4±1.3%, P=0.076; d=0.87). Overall, the sitting-induced changes in relative FMD were not different between Baseline and Follow-up in either group (both, P≥0.327; d≤0.38; Figure 4.2B).

4.4 Popliteal Reactive Hyperemia

No Group × Time × Pre-/Post-Sitting interaction effects were observed for peak RBCv, $RBCv_{AUC}$, peak PBF, or PBF_{AUC} (Table 4.3; all, P≥0.179). $RBCv_{AUC}$ and PBF_{AUC} were attenuated after sitting at Baseline in both groups (all, P≤0.050; d≥0.61), but not at Follow-up in either group (both, P≥0.216; d≤0.76). However, peak RBCv and peak PBF decreased in response to sitting at Baseline in both groups (all, P≤0.001; d≥1.34). At Follow-up, these 2 outcomes were reduced after sitting in the HIIT group (both, P≤0.026; d≥1.13), but not in the Control group (both, P≥0.150; d≤0.39). However, no sitting-induced differences were observed for any reactive hyperemic variables between Baseline and Follow-up in either group (all, P≥0.105; d≤0.62 Figure 4.3).

4.5 Popliteal Nitroglycerin-Mediated Dilation

Popliteal NMD data were obtained from all participants. As shown in Table 4.3 and Figure 4.4, relative NMD was not improved after 12-weeks of HIIT (P=0.558,

d=0.14) nor changed in the Control group (*P*=0.373, *d*=0.38). There was a main effect of sitting for absolute and relative NMD (both, $P \leq 0.001$, $\eta_p^2 \geq 0.667$) such that they were attenuated post-sitting in both groups at Baseline and Follow-up (all, $P \leq 0.049$; Figure 4.4). However, no Group × Time interaction effect were observed for sitting-induced declines in absolute (*P*=0.844, η_p^2=0.002) or relative NMD (*P*=0.609, η_p^2=0.014; Figure 4.4). No between- or within-group differences were observed for sitting-induced declines in relative NMD at any time point (all, $P \geq 0.464$; Figure 4.4).

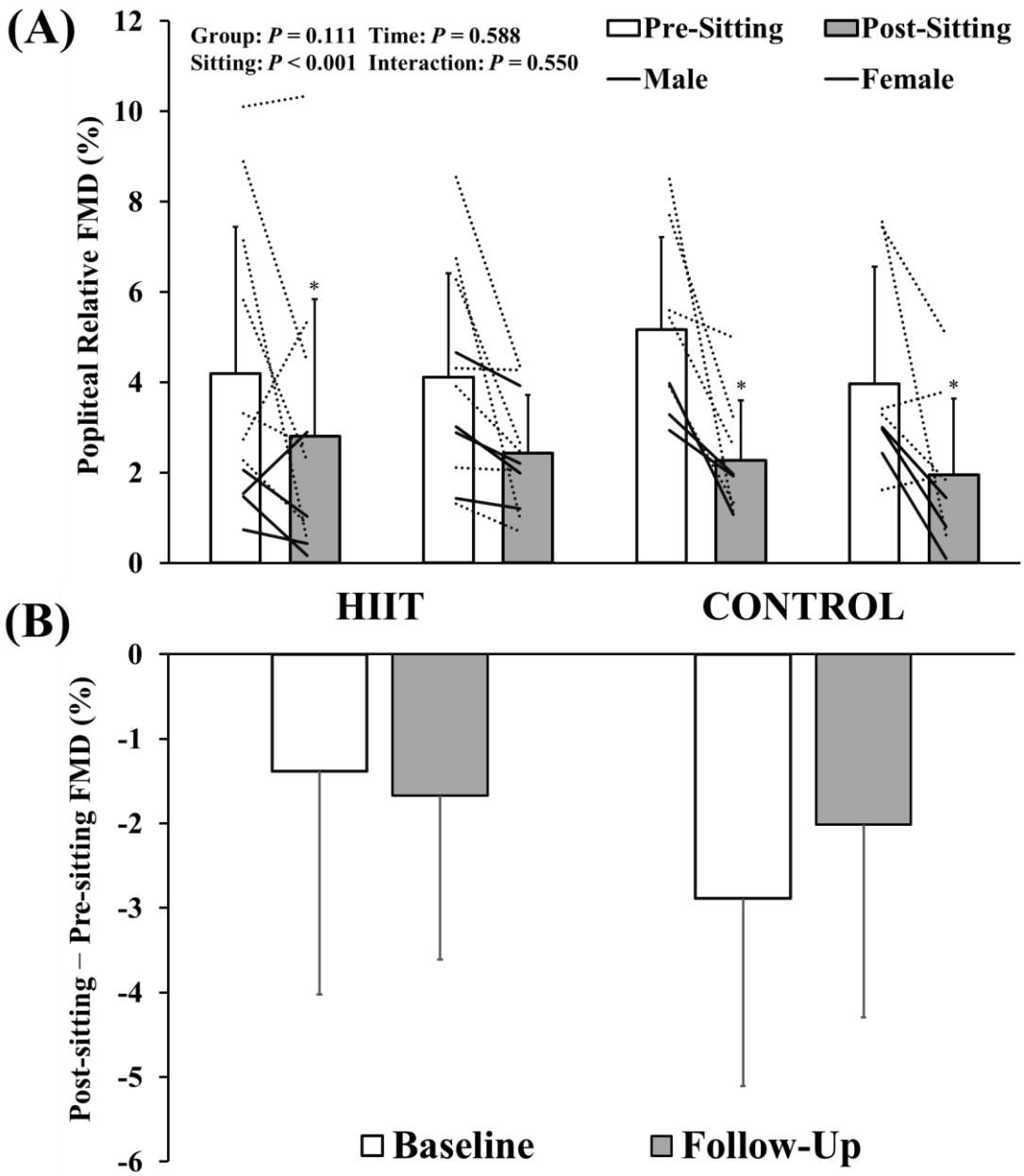

Figure 4.2. (A) Popliteal relative FMD (%) responses to 3-h of prolonged sitting between Baseline and Follow-up for both HIIT (n=11, 7♀) and Control (n=8, 5♀) groups. Group × Time × Pre-/Post-Sitting interaction effects were determined via a 3-way repeated measures analysis of variance with Bonferroni post hoc pairwise comparisons. Means ± SDs for each group, as well as individual data are presented. Females represented as dashed lines and males represented as solid lines. Data were logarithmically transformed before analysis. *, $P < 0.05$ versus Pre-Sitting within the same group. (B) Sitting-induced changes (Post-sitting – Pre-sitting) in relative FMD. Between- (Mann-Whitney U) and within-participants (Wilcoxon signed-rank) non-parametric tests were used. No between- (both, $P \geq 0.497$) or within-group differences were observed (both, $P \geq 0.327$).

Table 4.2. Systemic and popliteal resting hemodynamic responses to prolonged sitting.

	HIIT		CONTROL		Interaction Effect *P*-Value
	BASELINE	**FOLLOW-UP**	**BASELINE**	**FOLLOW-UP**	
Resting Systemic Hemodynamics					
Heart rate (beats/min)					
Pre-Sitting	73 ± 10	72 ± 10	70 ± 7	70 ± 7	Group × Time × Pre-/Post-Sitting: 0.575
Mid-Sitting	74 ± 7	72 ± 9	69 ± 4	69 ± 5	Group × Time: 0.708
Post-Sitting	73 ± 8	71 ± 9	68 ± 3	68 ± 6	Group × Pre-/Post-Sitting: 0.737 Time × Pre-/Post-Sitting: 0.984
Systolic blood pressure (mmHg)					
Pre-Sitting	113 ± 12	113 ± 9	110 ± 9	111 ± 8	Group × Time × Pre-/Post-Sitting: 0.969
Mid-Sitting	113 ± 11	109 ± 12	109 ±8	106 ± 8	Group × Time: 0.591
Post-Sitting	119 ± 10	115 ± 14	112 ±9	110 ± 8	Group × Pre-/Post-Sitting: 0.306 Time × Pre-/Post-Sitting:0.198
Diastolic blood pressure (mmHg)					
Pre-Sitting	62 ± 8	64 ± 6	60 ± 2	63 ± 4	Group × Time × Pre-/Post-Sitting: 0.997
Mid-Sitting	64 ± 7	62 ± 5	65 ± 6	63 ± 5	Group × Time: 0.982
Post-Sitting	70 ± 7$^{*\#\dagger}$	68 ± 10$^{\#\dagger}$	63 ± 7	62 ± 3	**Group × Pre-/Post-Sitting: <0.001** Time × Pre-/Post-Sitting: 0.149
Mean Arterial Pressure (mmHg)					
Pre-Sitting	79 ± 9	81 ± 6	77 ± 3	79 ± 5	Group × Time × Pre-/Post-Sitting: 0.780
Mid-Sitting	80 ± 8	77 ± 6	80 ± 8	78 ± 6	Group × Time: 0.939
Post-Sitting	86 ± 7	84 ± 10$^{\#}$	79 ± 7	77 ± 4	Group × Pre-/Post-Sitting: 0.013 Time × Pre-/Post-Sitting: 0.097
Resting Popliteal Hemodynamics					
Diameter (mm)					
Pre-Sitting	5.28 ± 0.75	5.34 ± 0.67	5.01 ± 0.46	4.96 ± 0.44	Group × Time × Pre-/Post-Sitting: 0.419
Mid-Sitting	5.46 ± 0.72	5.42 ± 0.76	5.20 ± 0.23	5.33 ± 0.42	Group × Time: 0.995
Post-Sitting	5.36 ± 0.83	5.36 ± 0.60	5.16 ± 0.51	5.10 ± 0.42	Group × Pre-/Post-Sitting: 0.507 Time × Pre-/Post-Sitting: 0.836
Red blood cell velocity (cm/s)					
Pre-Sitting	4.1 ± 1.5	4.0 ± 1.3	4.6 ± 2.8	4.8 ± 2.5	Group × Time × Pre-/Post-Sitting: 0.970
Mid-Sitting	2.6 ± 0.6*	2.4 ± 1.2*	2.1 ± 0.7*	2.2 ± 0.6*	Group × Time: 0.211
Post-Sitting	2.7 ± 0.8*	2.7 ± 0.8*	2.0 ± 0.7*	2.4 ± 0.5*	Group × Pre-/Post-Sitting: 0.057 Time × Pre-/Post-Sitting: 0.198

Resting Popliteal Hemodynamics					
Popliteal Blood Flow (mL/min)					
Pre-Sitting	53 ± 18	54 ± 27	53 ± 29	55 ± 25	Group × Time × Pre-/Post-Sitting: 0.780 Group × Time: 0.349 Group × Pre-/Post-Sitting: 0.109 Time × Pre-/Post-Sitting: 0.310
Mid-Sitting	36 ± 10*	35 ± 23*	27 ± 10*	29 ± 8*	
Post-Sitting	37 ± 18*	38 ± 19*	26 ± 12*	29 ± 8*	
Shear rate (/s)					
Pre-Sitting	63 ± 28	60 ± 22	74 ± 48	79 ± 50	Group × Time × Pre-/Post-Sitting: 0.948 Group × Time: 0.186 Group × Pre-/Post-Sitting: 0.055 Time × Pre-/Post-Sitting: 0.196
Mid-Sitting	38 ± 11*	37 ± 17*	33 ± 11*	33 ± 10*	
Post-Sitting	40 ± 12*	41 ± 8*	31 ± 10*	38 ± 9*	

Data are presented as Means ± SDs. Resting systemic hemodynamics data were obtained from 11 HIIT (7♀) and 10 Control (6♀) participants. Resting popliteal hemodynamics data were obtained from 11 HIIT (7♀) and 8 Control (5♀) participants. Group × Time × Pre-/Post-Sitting, Group × Time, Group × Pre-/Post-Sitting, and Time × Pre-/Post-Sitting interaction effects, as well as main effects were assessed using a 3-way repeated measures analysis of variance. *, $P < 0.05$ versus Pre-sitting within the same group. #, $P < 0.05$ versus Mid-Sitting within the same group. †, $P < 0.05$ versus Control at same timepoint.

Table 4.3. Popliteal hemodynamics, flow-mediated dilation, resistance vessel function, and nitroglycerin-mediated dilation outcomes.

	HIIT		CONTROL		Interaction Effect *P*-Value
	BASELINE	**FOLLOW-UP**	**BASELINE**	**FOLLOW-UP**	
Flow-Mediated Dilation Assessment					
Peak Diameter (mm)					
Pre-Sitting	5.49 ± 0.67	5.55 ± 0.65	5.27 ± 0.48	5.14 ± 0.41	Group × Time × Pre-/Post-Sitting: 0.636 Group × Time: 0.552 Group × Pre-/Post-Sitting: 0.621 Time × Pre-/Post-Sitting: 0.980
Post-Sitting	5.49 ± 0.77	5.49 ± 0.59	5.28 ± 0.48	5.20 ± 0.38	
Absolute FMD (mm)					
Pre-Sitting	0.21 ± 0.14	0.21 ± 0.12	0.26 ± 0.10	0.19 ± 0.10	Group × Time × Pre-/Post-Sitting: 0.359 Group × Time: 0.552 Group × Pre-/Post-Sitting: 0.621 Time × Pre-/Post-Sitting: 0.681
Post-Sitting	0.14 ± 0.13	0.13 ± 0.06*	0.12 ± 0.06*	0.10 ± 0.08*	
Pre-Sitting	4.2 ± 3.3	4.1 ± 2.3	5.2 ± 2.0	4.0 ± 2.3	Group × Time × Pre-/Post-Sitting: 0.550 Group × Time: 0.111 Group × Pre-/Post-Sitting: 0.215 Time × Pre-/Post-Sitting: 1.000
Post-Sitting	2.8 ± 3.0*	2.4 ± 1.3	2.3 ± 1.3*	2.0 ± 1.7*	
Allometrically-scaled FMD (%)					
Pre-Sitting	4.3 ± 2.0	4.3 ± 2.0	4.7 ± 1.7	3.4 ± 2.0	Group × Time × Pre-/Post-Sitting: 0.607 Group × Time: 0.034 Group × Pre-/Post-Sitting: 0.370 Time × Pre-/Post-Sitting: 0.992
Post-Sitting	3.0 ± 2.0*	2.7 ± 2.0†	2.2 ± 1.7*	1.7 ± 1.7*	
Time-To-Peak Dilation (s)					
Pre-Sitting	53 ± 17	44 ± 28	43 ± 14	41 ± 17	Group × Time × Pre-/Post-Sitting: 0.748 Group × Time: 0.335 Group × Pre-/Post-Sitting: 0.763 Time × Pre-/Post-Sitting: 0.956
Post-Sitting	53 ± 28	35 ± 13	39 ± 8	38 ± 8	
SR_{AUC} (a.u.)					
Post-Sitting	9881 ± 5098	10666 ± 5099	13679 ± 6027	13165 ± 7191	Group × Time × Pre-/Post-Sitting: 0.892 Group × Time: 0.429 Group × Pre-/Post-Sitting: 0.807 Time × Pre-/Post-Sitting: 0.129
Post-Sitting	7500 ± 5635*	9343 ± 5537	8418 ± 4619*	11735 ± 9036	
Reactive Hyperemia					
Peak RBCv (cm/s)					
Pre-Sitting	43 ± 13	50 ± 11	56 ± 20	51 ± 27	Group × Time × Pre-/Post-Sitting: 0.179 Group × Time: 0.154 Group × Pre-/Post-Sitting: 0.227
Post-Sitting	30 ± 14*	38 ± 11*	30 ± 15*	42 ± 25	

					Time × Pre-/Post-Sitting: <0.001
Reactive Hyperemia					
$RBCv_{AUC}$ (a.u.)					
Pre-Sitting	637 ± 265	745 ± 325	881 ± 307	799 ± 506	Group × Time × Pre-/Post-Sitting: 0.325 Group × Time: 0.477 Group × Pre-/Post-Sitting: 0.608 Time × Pre-/Post-Sitting: 0.208
Post-Sitting	503 ± 352*	643 ± 368	586± 382*	756 ± 517	
Peak PBF (mL/min)					
Pre-Sitting	558 ± 153	701 ± 236	640 ± 144	587 ± 230	Group × Time × Pre-/Post-Sitting: 0.208 Group × Time: 0.264 Group × Pre-/Post-Sitting: 0.690 Time × Pre-/Post-Sitting: 0.138
Post-Sitting	398 ± 155*	494 ±168*	383 ± 230*	483 ± 210	
PBF_{AUC} (a.u.)					
Pre-Sitting	8071 ± 2264	11092 ± 5264	10206 ± 2926	9130 ± 4915	Group × Time × Pre-/Post-Sitting: 0.565 Group × Time: 0.072 Group × Pre-/Post-Sitting: 0.734 Time × Pre-/Post-Sitting: 0.134
Post-Sitting	6307 ± 3620*	8622 ± 3886*#	7247 ± 4976*	8597 ± 4821	
Nitroglycerin-Mediated Dilation Assessment					
Resting Diameter (mm)					
Pre-Sitting	5.15 ± 0.44	5.04 ± 0.60	5.46 ± 0.92	5.12 ± 0.61	Group × Time × Pre-/Post-Sitting: 0.258 Group × Time: 0.655 Group × Pre-/Post-Sitting: 0.869 **Time × Pre-/Post-Sitting: 0.016**
Post-Sitting	5.16 ± 0.57	5.21 ± 0.66	5.30 ± 0.81	5.42 ± 0.67	
Peak Diameter (mm)					
Pre-Sitting	5.60 ± 0.48	5.50 ± 0.58	5.86 ± 0.83	5.55 ± 0.51	Group × Time × Pre-/Post-Sitting: 0.301 Group × Time: 0.662 Group × Pre-/Post-Sitting: 0.807 **Time × Pre-/Post-Sitting: 0.019**
Post-Sitting	5.46 ± 0.63	5.52 ± 0.61	5.54 ± 0.75*	5.68 ± 0.72	
Absolute NMD (mm)					
Pre-Sitting	0.45 ± 0.22	0.46 ± 0.21	0.40 ± 0.13	0.43 ± 0.15	Group × Time × Pre-/Post-Sitting: 0.844 Group × Time: 0.802 Group × Pre-/Post-Sitting: 0.843 Time × Pre-/Post-Sitting: 0.991
Post-Sitting	0.29 ± 0.17*	0.31 ± 0.15*	0.24 ± 0.12*	0.26 ± 0.13*	
Relative NMD (%)					
Pre-Sitting	8.8 ± 4.5	9.4 ± 4.8	7.7 ± 3.5	8.7 ± 3.8	Group × Time × Pre-/Post-Sitting: 0.609 Group × Time: 0.992 Group × Pre-/Post-Sitting: 0.836 Time × Pre-/Post-Sitting: 0.582
Post-Sitting	5.7 ± 3.2*	6.2 ± 3.3*	4.8 ± 2.6*	4.9 ± 2.3*	

Data are presented as Means ± SDs. Resting hemodynamics, flow-mediated dilation (FMD), and reactive hyperemia data were obtained from 11 HIIT (7♀) and 8 Control (5♀) participants. Nitroglycerin-mediated dilation (NMD) data were obtained from 11 HIIT (7♀) and 10 Control (6♀) participants. RBCv, red blood cell velocity; AUC, area under the curve; a.u, arbitrary units;

PBF, popliteal blood flow; SR, shear rate; Group × Time × Pre-/Mid-/Post-Sitting (or Pre-/Mid-Sitting) interaction effect were assessed using a 3-way repeated measures analysis of variance with Bonferroni *post hoc* pairwise comparisons to determine between- and within-group differences. Resting RBCv, resting PBF, resting SR, relative FMD, time-to-peak dilation, SR_{AUC}, peak RBCv, peak PBF and PBF_{AUC}, were logarithmically transformed before analysis. *, $P < 0.05$ versus Pre-Sitting within the same group. $^{\#}$, $P < 0.05$ versus Baseline within the same group. †, $P < 0.05$ versus Control at same timepoint.

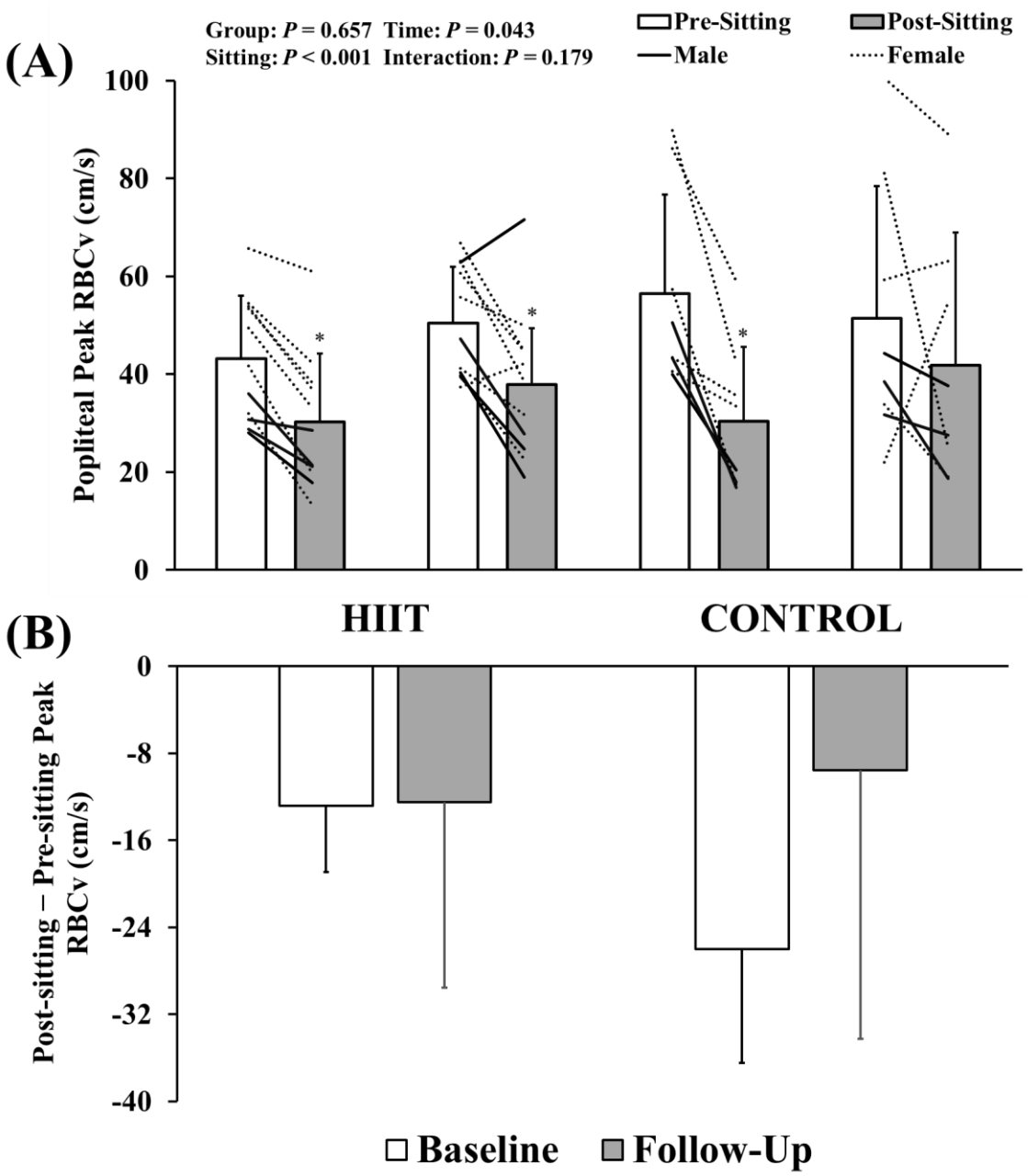

Figure 4.3. (A) Popliteal peak red blood cell velocity (RBCv) responses to 3-h of prolonged sitting between Baseline and Follow-up for both HIIT (n = 11, 7♀) and Control (n = 8, 5♀) groups. Group × Time × Pre-/Post-Sitting interaction effects were determined via a 3-way repeated measures analysis of variance (RM-ANOVA) with Bonferroni post hoc pairwise comparisons. Means ± SDs for each group, as well as individual data are presented. Females represented as dashed lines and males represented as solid lines. Data were logarithmically transformed before analysis. *, $P < 0.05$ versus Pre-Sitting within the same group. (B) Sitting-induced changes (Post-sitting – Pre-sitting) in peak RBCv. Group × Time interaction effect was determined using 2-way RM-ANOVA. No interaction or main effects were observed (all, $P \geq 0.131$).

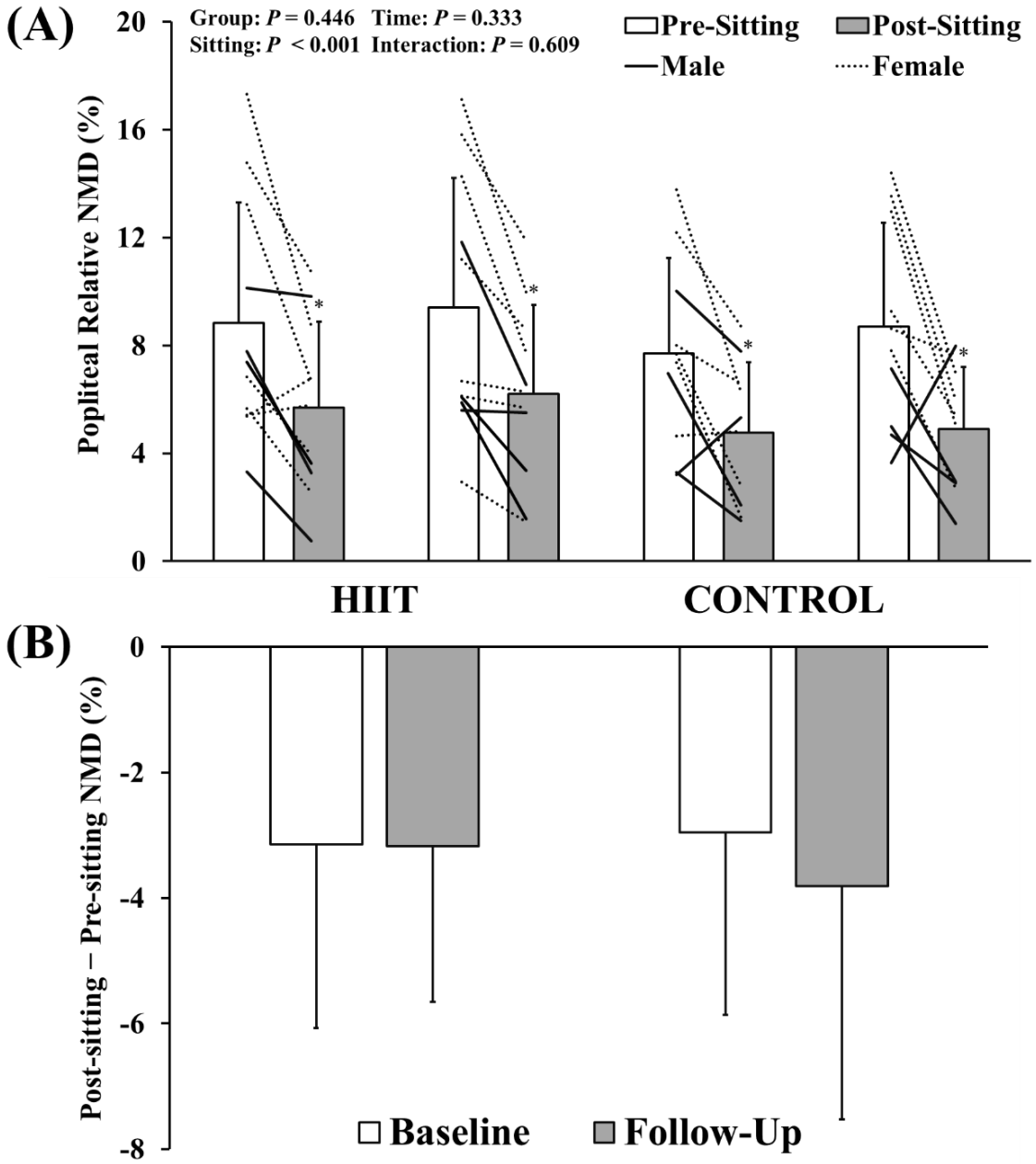

Figure 4.4. (A) Popliteal relative NMD (%) responses to 3-h of prolonged sitting between Baseline and Follow-up for both HIIT (n = 11, 7♀) and Control (n = 10, 6♀) groups. Group × Time × Pre-/Post-Sitting interaction effects were determined via a 3-way repeated measures analysis of variance (RM-ANOVA) with Bonferroni post hoc pairwise comparisons. Means ± SDs for each group, as well as individual data are presented. Females represented as dashed lines and males represented as solid lines. Data were logarithmically transformed before analysis. *, $P < 0.05$ versus Pre-Sitting within the same group. (B) Sitting-induced changes (Post-sitting – Pre-sitting) in relative NMD. Group × Time interaction effect was determined using a 2-way RM-ANOVA. No interaction or main effects were observed (all, $P \geq 0.581$).

Chapter 5. DISCUSSION

The primary purpose of this study was to examine the impact of a 12-week HIIT program on aerobic fitness, as well as the popliteal vascular responses before and following an acute bout of prolonged sitting. It was hypothesized that: 1) popliteal FMD, NMD and reactive hyperemic responses would be attenuated after prolonged sitting before training and 2) HIIT would improve aerobic fitness and pre-sitting popliteal FMD. Due to the conflicting available evidence regarding the impact of aerobic fitness (or lack thereof) on sitting-induced lower-limb vascular outcomes, no directional hypothesis was included regarding the impact of HIIT on popliteal responses to prolonged sitting. Consistent with the first hypothesis, popliteal FMD, reactive hyperemia (i.e., lower-limb resistance vessel function), and NMD responses were attenuated after 3-h of sitting in both groups at Baseline. The second hypothesis was only partially supported as HIIT increased aerobic fitness (Figure 4.1A and B) but did not improve pre-sitting FMD (Figure 4.2A). Furthermore, there were also no changes in reactive hyperemic (Figure 4.3A) or NMD responses (Figure 4.4A) following training. Finally, HIIT did not alter sitting-induced declines in popliteal vascular function. This study marked the first known attempt at uncovering whether an aerobic training intervention impacts prolonged sitting-induced lower-limb vascular dysfunction.

5.1 Impact of 12-Week HIIT on Aerobic Fitness

The observation of improved aerobic fitness following 6- and 12-weeks of HIIT is not unique. A recent meta-analysis by Batacan et al. (148) reported moderate to large effect sizes (i.e., $d \geq 0.74$) regarding the increases in relative $\dot{V}O_2peak$ from short- (< 12-weeks) and long-term (≥12-weeks) HIIT interventions, respectively. Similarly, we

observed large effect sizes for absolute and relative $\dot{V}O_2$peak rises after 6- and 12-weeks of HIIT (versus Baseline), despite no further increases in aerobic fitness observed after the first 6-weeks of training. Furthermore, PAP also improved after 6-weeks of HIIT and remained higher after 12-weeks, which supports the $\dot{V}O_2$peak outcomes. Previously, Mekari et al. (35) observed a similar (~9%) increase in PAP utilizing a 6-week version of the same HIIT protocol as the current study. However, they did not report an improvement in relative $\dot{V}O_2$peak (35). Of note, our HIIT group had a lower Baseline relative $\dot{V}O_2$peak compared to their participants (35.4±7.8 versus 39.7±8.7 ml/kg/min), which may partially explain the improvement in aerobic fitness observed after 6-weeks of training in the current cohort. Specifically, it has been demonstrated that higher fit individuals typically require a greater training stimulus (e.g., higher intensity, duration and/or frequency) to gain similar improvement in $\dot{V}O_2$peak than lower fit individuals (149).

The present study was not designed to uncover the specific physiological mechanisms responsible for the HIIT-induced elevation in $\dot{V}O_2$peak, but may be related to a combination of central (e.g., ↑ blood volume, peak stroke volume/cardiac output) and/or peripheral factors (e.g., ↑ active skeletal muscle mitochondrial volume, angiogenesis), as well as corresponding improvements in the efficiency of oxygen transportation (150, 151). Specifically, aerobic exercise training results in an increase in blood volume, enhanced diastolic filling, increased left ventricular mass, and improved myocardial contractility, which augments peak stroke volume and cardiac output (152). According to a recent meta-analysis by Rosenblat et al. (151), HIIT appears to produce a greater improvement in left ventricular mass (mean difference: 4.5%, 95% confidence

interval: 0.3-8.7) compared with moderate-intensity continuous training. In addition, aerobic exercise training can induce mitochondrial adaptations (e.g., increases in mitochondrial volume density) that improve oxygen extraction and result in greater maximal arterial-venous oxygen differences (153) Maclnnis et al. (154) has shown that HIIT elicits greater increases in skeletal muscle mitochondrial content compared to moderate-intensity continuous training, which is indicative of the superior effects of HIIT on mitochondrial adaptations. Collectively, the increases in $\dot{V}O_2$peak after 12-weeks of HIIT are likely attributed to high-intensity training-induced higher maximal cardiac output and greater arterial-venous oxygen differences.

5.2 Impact of 12-Week HIIT on Popliteal Vascular Function

5.2.1 Endothelial function

Despite the HIIT-mediated benefits to aerobic fitness, no corresponding improvements in popliteal FMD were observed in the present study. Rakobowchuk et al. (30) previously reported that 6-week of sprint-interval training (6 × 30-s Wingate tests separated by 4.5-min active recovery, 3 days/week) improved popliteal FMD in young, healthy adults. The conflicting results between the current study and Rakobowchuk et al. (30) might be attributable to the longer intervention used in the present study. To this point, Tinken et al. (155) conducted an 8-week exercise training (15-min of running + 15-min of cycling at 80% heart rate reserve, 3 days/week) study in healthy young males and assessed popliteal FMD every 2-weeks. Interestingly, they observed an increase in popliteal FMD after 2-, 4-, and 6-weeks of exercise training, which returned to near baseline values after 8-weeks (155). In contrast, arterial remodeling, assessed as the vasodilatory response to an ischaemic exercise stimulus, did not change after 2-weeks of

training but showed progressive increases between the 4- to 8-week timepoints (155). Furthermore, Green et al. (156) provided more direct evidence regarding exercise training-induced arterial remodeling when they found that 12-weeks of exercise training reduced popliteal wall thickness and wall-to-lumen ratio, with no significant change in resting diameter (156). Importantly, the arterial wall-to-lumen ratio is positively related to FMD responses in young, healthy adults (157). In the present study, it is possible that the best time window (2-6 week) for measuring training-induced functional adaptations was missed. Collectively, these findings suggest that the lack of change in FMD following 12-weeks of HIIT may be a result of training-induced popliteal artery structural modification.

5.2.2 Vascular Smooth Muscle Function

The magnitude of FMD response is also influenced by the sensitivity of VSMCs to NO, which can be non-invasively assessed via the NMD test. No increases in popliteal NMD were observed following HIIT. This finding is consistent with a previous study that did not find any change in popliteal NMD following 6-week of HIIT in older adults (158). A meta-analysis of the impact of exercise training on NMD also reported only marginal or no improvement following training (128). Similarly, a more recent meta-analysis concluded that exercise training only had small beneficial effects on VSMCs function in adults >40 years or when the duration of the intervention is longer than 12 weeks (159). Collectively, the current results demonstrated that popliteal NMD was not improved by 12-weeks of HIIT in a group of healthy, young adults. Future studies are encouraged to investigate whether a longer duration of training is effective at improving popliteal VSMCs function. Additionally, the above-mentioned decreased vascular wall-

to-lumen ratio following training is also associated with smaller NMD responses (157). It has been suggested that arteries with a smaller wall-to-lumen ratio have less smooth muscle in the artery wall, leading to attenuated responses to exogenous NO stimuli (157). As such, the lack of change in popliteal NMD responses following 12-week HIIT may also be a consequence of training-induced vascular remodeling.

5.2.3 Resistance Vessel Function

The impact of aerobic fitness and exercise training on popliteal resistance vessel function in young adults is still largely unknown. In this study, no increases in any reactive hyperemia outcomes were observed after 12-weeks of HIIT. These results indicate that popliteal resistance vessel function is not impacted by aerobic training in healthy, physically active adults. Our data are consistent with Tinken et al. (155), who demonstrated that popliteal reactive hyperemia (10-s average hyperemic flow) induced by 5-min of distal cuff occlusion followed by 3-min of isotonic plantar flexion exercise was not altered after 2-, 4-, or 8-weeks of aerobic exercise training (15-min of running + 15-min of cycling at 80% heart rate reserve, 3 days/week) in young, healthy males. Similarly, Rakobowchuk et al. (30) found that 6-weeks of sprint interval training did not improve popliteal post-occlusive reactive hyperemia, as quantified by 25-s average hyperemic velocity and flow. Other studies have also reported that 12-weeks of aerobic training (40-min of cycling at 70-80% heart rate reserve) did not change superficial femoral artery SR_{AUC} in young, healthy females (115). Although SR_{AUC} is not a direct measure of resistance vessel function, it has been utilized to quantify reactive hyperemia (160), and is highly correlated with $RBCv_{AUC}$ (R=0.963 in this study) and PBF_{AUC} (R=0.779 in this study). Besides the longitudinal evidence, Walther et al. (161) showed

that superficial femoral artery peak reactive hyperemic flow is greater in highly-trained swimmers and cyclists compared to their sedentary counterparts. This potentially highlights the benefits of maintaining a high level of aerobic fitness for a long-term on lower-limb resistance vessels. Thus, it is plausible that the 12-week duration of training in the current study was too short to induce functional adaptations of lower-limb resistance vessels, especially in a cohort of healthy, young, and physically active individuals. Additionally, Herrod et al. (162) observed that 6-weeks of HIIT (5 × 1-min high-intensity cycling at 100-110% PAP separated by 1.5-min of unloaded cycling, 3 days/week) improved lower-limb resistance vessel responses to a single bout of muscle contractions in older adults. Similarly, our lab has shown that 6-weeks of HIIT improved popliteal SR_{AUC} in older adults. Collectively, these results suggest that perhaps microvascular benefits of HIIT would have been observed in older and less healthy/aerobically fit populations. Thus, it is plausible that the 12-week duration of training in the current study was too short to induce functional adaptations of lower-limb resistance vessels in a cohort of healthy, young, and physically active adults.

5.3 Impact of Prolonged Sitting on Popliteal Vascular Function

As expected, prolonged sitting attenuated Baseline popliteal FMD and reactive hyperemia in both groups (Figure 4.2 and 4.3). These results are consistent with previous studies conducted in young, healthy adults (74, 111, 138). The mechanisms contributing to lower-limb endothelial and resistance vessel dysfunction following an acute bout of prolonged sitting are still not well-established. Previous studies have hypothesized that local hemodynamic disturbances due to “arterial bending” at the knee and hip joints, elevated hydrostatic pressure, and venous pooling may play a key role (163). Blood flow

and shear stress responses are attenuated after sitting continuously for as little as 15-min (164) and reached nadir following 1-h of sitting (138). Exposures to sitting-induced low shear stress and arterial bending-induced turbulent blood flow upregulate proinflammatory signaling and endothelium generation of reactive oxygen species, which downregulate eNOS expression and thus NO bioavailability (100, 165, 166). In addition, previous studies proposed that elevated sympathetic vasoconstrictor activity during the upright seated posture also leads to proatherogenic retrograde and oscillatory shear patterns, increased peripheral vascular resistance, and therefore endothelial and resistance vessel dysfunction (109, 110). These proposed contributing mechanisms still need to be tested in future studies. Nonetheless, prolonged sitting-induced impairments in lower-limb FMD and reactive hyperemia responses have been well reported.

The impact of prolonged sitting on arterial VSMCs function is still relatively understudied. In the current study, both groups exhibited lower NMD responses after sitting, which demonstrates that an acute bout of prolonged sitting also blunts popliteal endothelium-independent vasodilation (i.e., VSMCs sensitivity to NO). It has been proposed that elevated levels of oxidative stress and/or inflammation induced by reduced/disturbed local blood flow may be a key contributor to diminished bioconversion of nitroglycerin to NO (167, 168) and reduced VSMCs sensitivity to NO (64, 65). Future studies are needed to confirm or refute this hypothesis.

5.4 Impact of 12-Week HIIT on Vascular Responses to Sitting

Despite the increase in aerobic fitness following HIIT, the sitting-induced declines in popliteal FMD were not different between Baseline and Follow-up. Despite the post-sitting reduction in relative FMD not reaching statistical significance (P=0.076, d=0.87;

Figure 4.2A) after HIIT, 8 of 11 participants exhibited a markedly lower relative FMD following sitting, and absolute FMD was significantly attenuated after sitting (P=0.014, d=0.84). These results indicated that improving aerobic fitness had no impact on FMD responses to prolonged sitting. This contradicts a previous cross-sectional observation from our lab that higher aerobic fitness worsened the decline in popliteal FMD after sitting (33). This report also observed a strong negative correlation between pre-sitting popliteal FMD and corresponding sitting-mediated FMD reductions (33). As aerobic fitness was also directly associated with greater pre-sitting FMD responses, it has been proposed that more aerobically fit individuals, with higher baseline FMDs, may have had a larger capacity to decrease after sitting (33). This phenomenon may explain the lack of change in FMD responses to sitting in the present study, since the pre-sitting FMD was not improved after 12-weeks of HIIT.

The current findings also contrast with the study by Morishima et al. (32), who observed no sitting-induced popliteal FMD reductions in an endurance-trained group of males. However, closer inspection of their individual-level data highlights that 2 participants exhibited uncharacteristically low pre-sitting FMD and an unexpected improvement in FMD response after (increased relative FMD by ~4%) compared to the rest of the group that observed post-sitting decreases (32). This inter-individual variation may have contributed to the lack of sitting-induced declines in popliteal FMD at the group level. In addition, the endurance-trained group in the Morishima et al. (32) study had a much higher relative $\dot{V}O_2$peak compared with the HIIT participants at Follow-up in the current project (60.8 ± 3.6 versus 39.5 ± 6.1 ml/kg/min). As such, it is plausible that the magnitude of the increase in aerobic fitness from the 12-week HIIT protocol was

insufficient to mitigate the negative effects of sitting on endothelial-dependent vasodilatory function.

As this was the first known investigation exploring the impact of prolonged sitting on lower-limb VSMCs function (19), there is a paucity of evidence to draw from regarding NMD responses to prolonged sitting (or the impact of aerobic fitness on sitting-induced changes in NMD). Our lab (113) and others (169, 170) have reported that aerobic fitness is not associated with NMD in either younger or older adults. Furthermore, as mentioned in section **5.1.2**, it has been suggested that exercise training induces vascular structural remodeling that may influence the peak vasodilator response to the administration of NO donors (e.g., nitroglycerin) (171). As such, it is plausible that decreased NMD responses following 3-h of sitting are only reflective of acute functional alterations in VSMCs that are not protected by exercise training-induced structural adaptations (e.g., decreased arterial wall thickness, reduced arterial stiffness).

Popliteal artery reactive hyperemic responses to prolonged sitting were not altered by 12-weeks of HIIT. This indicates that HIIT-mediated increases in aerobic fitness had no impact on sitting-induced impairments in resistance vessel function. This finding is consistent with Garten et al. (34), who observed a similar attenuation in common femoral artery hyperemic responses to a repetitive passive leg movement test following 3-h of sitting between aerobically-trained versus untrained adults. Of relevance, there was a similar population of younger, healthy, and highly physically active males and females included in both the present study, as well as by Garten et al. (34).

Despite the consistent sitting-induced declines in reactive hyperemia within the HIIT group, some intra-individual variation was observed within the Control group. This

is highlighted by the consistent reduction in Baseline peak RBCv responses following sitting, but less consistent responses at Follow-up (Figure 4.3 A). The declines in all reactive hyperemic outcomes were not statistically significant at Follow-up. These results are indicative of some inconsistent resistance vessel responses to sitting on an individual level. For example, reactive hyperemia was decreased following sitting at baseline but increased after sitting at follow-up in some participants. As above-mentioned, a few studies have observed that prolonged sitting attenuates lower-limb reactive hyperemia responses (15, 16, 74, 111, 172). However, conflicting results have also been reported in other studies (14, 32, 104, 105, 173). Collectively, the data from this study and the conflicting evidence from previous studies highlight both intra- and inter-individual differences in lower-limb resistance vessel responses to sitting. Future studies should explore the potential factors contributing to the intra- and inter-individual variance. Additionally, there is still no consensus on the methodological approach for quantifying reactive hyperemia (8). As presented in this study, reactive hyperemia was quantified using peak RBCv, $RBCv_{AUC}$, peak PBF, and PBF_{AUC}. Others have also reported average hyperemic velocity and flow, or sometimes SR_{AUC} in prolonged sitting studies as metrics of reactive hyperemia (8). Consequently, sometimes making direct comparisons with other studies could be challenging. Future research is necessary to determine the most accurate, reliable, and valid approach of quantifying reactive hyperemia and to establish a consensus on which metric(s) of reactive hyperemia should be adopted in future studies.

5.5 Strengths, Limitations, and Future Directions

The present study provided the first longitudinal investigation into the effects of aerobic fitness on lower-limb vascular responses to sitting. As mentioned previously, all

previous studies examining the impact of aerobic fitness on sitting-induced vascular dysfunction were cross-sectional in nature and unable to establish a cause-and-effect relationship (32–34). Furthermore, given that approximately 75% of participants included in published prolonged sitting studies are male (2), with more than half of reports that exclusively assessed males (2), the inclusion of both sexes in the current study enhances the generalizability of the findings to a younger, healthy adults population. Despite this, the current project was underpowered to detect sex differences. Future studies should explore whether sex differences exist in vascular response to HIIT and/or sitting-induced responses post-training.

A strength of this study is that habitual activity was objectively measured before and after the 13-week period, given that changes in habitual activity may influence vascular function (13). Habitual physical and sedentary activity patterns remained similar in both groups at baseline and follow-up and therefore, minimizing their potential impact on vascular function measures. Most participants recruited for this study were healthy, young college students. Consistent with previous studies (174, 175), this cohort accumulated an average of ~9 hours per day of sedentary time while maintaining high levels of physical activity. Despite this, a limitation of this study as well as previous studies is the inability to distinguish the time spent in different sedentary postures (i.e., sitting, lying, reclining) (176). Of note, Walsh et al. (163) has shown that a 3-h of lying down attenuated popliteal FMD in the leg bent at 90-degree at the hip and knee but not in the straight leg. This finding indicates: 1) lying posture might not be as detrimental to vascular function as sitting posture, at least when the legs are straight; and 2) hip- and/or knee-flexion angles might play a vital role in vascular dysfunction induced by sedentary

activities. In summary, although no difference in the total time spent in daily sedentary activities was observed between baseline and follow-up, we cannot rule out the possibility of changes in habitual sedentary postures and/or hip-/knee-flexion angles.

Similar to other acute laboratory-based sitting studies, the current project examined lower-limb vascular responses to a 3-h sitting bout (3). However, a recent report from our lab has indicated that young, healthy adults rarely engage in continuous sedentary bouts longer than 2-h (177). As such, utilizing a 3-h sitting bout may have low ecological validity in this population. However, the rationale for choosing a 3-h sitting protocol in the current study was partially related to the inclusion of the NMD assessments, to ensure that pre-sitting nitroglycerin administration would not confound the prolonged sitting-induced vascular impairments. And more importantly, the most common 3-h sitting protocol allowed me to make direct comparisons against other studies.

The clinical relevance of acute impairment of vascular function is still unclear. Most studies have considered the reduction in FMD and reactive hyperemia as 'vascular dysfunction' (2). Nevertheless, an alternative explanation for the reduction in vascular function is that the attenuation in vasodilatory responses following an acute bout of sitting is indicative of a normal, efficient hemodynamic system that adjusts to reduced local blood flow (i.e., a sensitive endothelium responding accordingly to a reduction in blood flow/shear stress). Of relevance, a meta-analysis has indicated that acute sitting only attenuates lower-limb FMD in healthy adults who have normal pre-sitting vascular function, but not in older adults, people with chronic disease risk factors, or overt clinical conditions (3). Future studies should investigate whether the acute effects of sitting on

vascular function are predictive of long-term adaptation to habitual prolonged sitting. For example, Dawson et al. (178) found a significant correlation between acute changes in FMD following a single exercise session and changes in FMD after 2-week of exercise training. Perhaps similar models could be applied in future studies on prolonged sitting to examine if a similar pattern occurs in sitting-induced vascular dysfunction. This will help to better understand the stimulus contributing to vascular maladaptation following habitual prolonged sedentary bouts.

5.6 Conclusion

This study demonstrated that a 12-week HIIT protocol improved aerobic fitness in a group of healthy and physically active younger adults. Despite the increases in aerobic fitness, popliteal artery endothelial function, VSMCs sensitivity to exogenous NO, and resistance vessel function were not altered by training, which may be explained by training-induced vascular structural adaptations (e.g., reduced artery wall thickness and wall-to-lumen ratio). In addition, prolonged sitting still attenuated popliteal vascular function after 12-weeks of HIIT. These results indicate that an improvement in aerobic fitness following short-term aerobic training does not provide protection against the negative effects of sitting on lower-limb vascular health.

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APPENDIX A. GET ACTIVE QUESTIONNAIRE

CSEP | SCPE
THE GOLD STANDARD IN EXERCISE SCIENCE AND PERSONAL TRAINING

Get Active Questionnaire

CANADIAN SOCIETY FOR EXERCISE PHYSIOLOGY –
PHYSICAL ACTIVITY TRAINING FOR HEALTH (CSEP-PATH®)

Physical activity improves your physical and mental health. Even small amounts of physical activity are good, and more is better.

For almost everyone, the benefits of physical activity far outweigh any risks. For some individuals, specific advice from a Qualified Exercise Professional (QEP – has post-secondary education in exercise sciences and an advanced certification in the area – see csep.ca/certifications) or health care provider is advisable. **This questionnaire is intended for all ages – to help move you along the path to becoming more physically active.**

☐ I am completing this questionnaire for myself.

☐ I am completing this questionnaire for my child/dependent as parent/guardian.

PREPARE TO BECOME MORE ACTIVE

The following questions will help to ensure that you have a safe physical activity experience. Please answer **YES** or **NO** to each question before you become more physically active. If you are unsure about any question, answer **YES**.

YES	NO	
		1 Have you experienced ANY of the following (A to F) **within the past six months**?
○	○	**A** A diagnosis of/treatment for heart disease or stroke, or pain/discomfort/pressure in your chest during activities of daily living or during physical activity?
○	○	**B** A diagnosis of/treatment for high blood pressure (BP), or a resting BP of 160/90 mmHg or higher?
○	○	**C** Dizziness or lightheadedness during physical activity?
○	○	**D** Shortness of breath at rest?
○	○	**E** Loss of consciousness/fainting for any reason?
○	○	**F** Concussion?
○	○	2 Do you currently have pain or swelling in any part of your body (such as from an injury, acute flare-up of arthritis, or back pain) that affects your ability to be physically active?
○	○	3 Has a health care provider told you that you should avoid or modify certain types of physical activity?
○	○	4 Do you have any other medical or physical condition (such as diabetes, cancer, osteoporosis, asthma, spinal cord injury) that may affect your ability to be physically active?

➤ **NO** to all questions: go to Page 2 – ASSESS YOUR CURRENT PHYSICAL ACTIVITY ➤

YES to any question: go to Reference Document – ADVICE ON WHAT TO DO IF YOU HAVE A YES RESPONSE ➤➤

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Get Active Questionnaire

ASSESS YOUR CURRENT PHYSICAL ACTIVITY

Answer the following questions to assess how active you are now.

1 During a typical week, on how many days do you do moderate- to vigorous-intensity aerobic physical activity (such as brisk walking, cycling or jogging)? ____ DAYS/ WEEK

2 On days that you do at least moderate-intensity aerobic physical activity (e.g., brisk walking), for how many minutes do you do this activity? ____ MINUTES/ DAY

For adults, please multiply your average number of days/week by the average number of minutes/day: ____ MINUTES/ WEEK

Canadian Physical Activity Guidelines recommend that adults accumulate at least 150 minutes of moderate- to vigorous-intensity physical activity per week. For children and youth, at least 60 minutes daily is recommended. Strengthening muscles and bones at least two times per week for adults, and three times per week for children and youth, is also recommended (see csep.ca/guidelines).

GENERAL ADVICE FOR BECOMING MORE ACTIVE

Increase your physical activity gradually so that you have a positive experience. Build physical activities that you enjoy into your day (e.g., take a walk with a friend, ride your bike to school or work) and reduce your sedentary behaviour (e.g., prolonged sitting).

If you want to do **vigorous-intensity physical activity** (i.e., physical activity at an intensity that makes it hard to carry on a conversation), and you do not meet minimum physical activity recommendations noted above, consult a Qualified Exercise Professional (QEP) beforehand. This can help ensure that your physical activity is safe and suitable for your circumstances.

Physical activity is also an important part of a healthy pregnancy.

Delay becoming more active if you are not feeling well because of a temporary illness.

DECLARATION

To the best of my knowledge, all of the information I have supplied on this questionnaire is correct.
If my health changes, I will complete this questionnaire again.

I answered NO to all questions on Page 1

Sign and date the Declaration below

I answered YES to any question on Page 1

Check the box below that applies to you:

- ☐ I have consulted a health care provider or Qualified Exercise Professional (QEP) who has recommended that I become more physically active.
- ☐ I am comfortable with becoming more physically active on my own without consulting a health care provider or QEP.

Name (+ Name of Parent/Guardian if applicable) [Please print] | Signature (or Signature of Parent/Guardian if applicable) | Date of Birth

Date | Email (optional) | Telephone (optional)

With planning and support you can enjoy the benefits of becoming more physically active. A QEP can help.

☐ Check this box if you would like to consult a QEP about becoming more physically active.
(This completed questionnaire will help the QEP get to know you and understand your needs.)

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Get Active Questionnaire – Reference Document

ADVICE ON WHAT TO DO IF YOU HAVE A **YES** RESPONSE

Use this reference document if you answered YES to any question and you have not consulted a health care provider or Qualified Exercise Professional (QEP) about becoming more physically active.

1 Have you experienced ANY of the following (A to F) within the past six months?	
A A diagnosis of/treatment for heart disease or stroke, or pain/discomfort/pressure in your chest during activities of daily living or during physical activity? ☐ YES	Physical activity is likely to be beneficial. If you have been treated for heart disease but have not completed a cardiac rehabilitation program within the past 6 months, consult a doctor – a supervised cardiac rehabilitation program is strongly recommended. If you are resuming physical activity after more than 6 months of inactivity, begin slowly with light- to moderate-intensity physical activity. If you have pain/discomfort/pressure in your chest and it is new for you, talk to a doctor. Describe the symptom and what activities bring it on.
B A diagnosis of/treatment for high blood pressure (BP), or a resting BP of 160/90 mmHg or higher? ☐ YES	Physical activity is likely to be beneficial if you have been diagnosed and treated for high blood pressure (BP). If you are unsure of your resting BP, consult a health care provider or a Qualified Exercise Professional (QEP) to have it measured. If you are taking BP medication and your BP is under good control, regular physical activity is recommended as it may help to lower your BP. Your doctor should be aware of your physical activity level so your medication needs can be monitored. If your BP is 160/90 or higher, you should receive medical clearance and consult a QEP about safe and appropriate physical activity.
C Dizziness or lightheadedness during physical activity ☐ YES	There are several possible reasons for feeling this way and many are not worrisome. Before becoming more active, consult a health care provider to identify reasons and minimize risk. Until then, refrain from increasing the intensity of your physical activity.
D Shortness of breath at rest ☐ YES	If you have asthma and this is relieved with medication, light to moderate physical activity is safe. If your shortness of breath is not relieved with medication, consult a doctor.
E Loss of consciousness/fainting for any reason ☐ YES	Before becoming more active, consult a doctor to identify reasons and minimize risk. Once you are medically cleared, consult a Qualified Exercise Professional (QEP) about types of physical activity suitable for your condition.
F Concussion ☐ YES	A concussion is an injury to the brain that requires time to recover. Increasing physical activity while still experiencing symptoms may worsen your symptoms, lengthen your recovery, and increase your risk for another concussion. A health care provider will let you know when you can start becoming more physically active, and a Qualified Exercise Professional (QEP) can help get you started.

After reading the ADVICE for your YES response, go to Page 2 of the *Get Active Questionnaire* – ASSESS YOUR CURRENT PHYSICAL ACTIVITY

CSEP | SCPE
THE GOLD STANDARD IN EXERCISE SCIENCE AND PERSONAL TRAINING

Get Active Questionnaire – Reference Document

ADVICE ON WHAT TO DO IF YOU HAVE A **YES** RESPONSE

Use this reference document if you answered YES to any question and you have not consulted a health care provider or Qualified Exercise Professional (QEP) about becoming more physically active.

2 Do you currently have pain or swelling in any part of your body (such as from an injury, acute flare-up of arthritis, or back pain) that affects your ability to be physically active? ☐ YES

If this swelling or pain is new, consult a health care provider. Otherwise, keep joints healthy and reduce pain by moving your joints slowly and gently through the entire pain-free range of motion. If you have hip, knee or ankle pain, choose low-impact activities such as swimming or cycling. As the pain subsides, gradually resume your normal physical activities starting at a level lower than before the flare-up. Consult a Qualified Exercise Professional (QEP) in follow-up to help you become more active and prevent or minimize future pain.

3 Has a health care provider told you that you should avoid or modify certain types of physical activity? ☐ YES

Listen to the advice of your health care provider. A Qualified Exercise Professional (QEP) will ask you about any considerations and provide specific advice for physical activity that is safe and that takes your lifestyle and health care provider's advice into account.

4 Do you have any other medical or physical condition (such as diabetes, cancer, osteoporosis, asthma, spinal cord injury) that may affect your ability to be physically active? ☐ YES

Some people may worry if they have a medical or physical condition that physical activity might be unsafe. In fact, regular physical activity can help to manage and improve many conditions. Physical activity can also reduce the risk of complications. A Qualified Exercise Professional (QEP) can help with specific advice for physical activity that is safe and that takes your medical history and lifestyle into account.

After reading the ADVICE for your YES response, go to Page 2 of the *Get Active Questionnaire* – ASSESS YOUR CURRENT PHYSICAL ACTIVITY

WANT ADDITIONAL INFORMATION ON BECOMING MORE PHYSICALLY ACTIVE?

- **csep.ca/certifications**
  CSEP Certified members can help you with your physical activity goals.
- **csep.ca/guidelines**
  Canadian Physical Activity Guidelines for all ages.

APPENDIX B. HEALTH HISTORY QUESTIONNAIRE

Age: _____ years

PARTICIPANT I.D. (Completed by Research Team): _____

Determination of body mass index (BMI):

i. What is your approximate weight in kilograms? _____
To convert from pounds to kilograms, multiply by 0.454

ii. What is your approximate height in meters? _____
To convert from inches to meters, multiple by 0.0254

iii. To calculate BMI, please click this link:
http://www.nhlbi.nih.gov/health/educational/lose_wt/BMI/bmicalc.htm

Calculated BMI: _____kg/m^2

The following questions will determine your eligibility for the study. If you answer 'Yes to any question you will not be able to participate in the study.

1. Was your calculated body mass index above greater than 30 kg/m^2? ☐YES ☐ NO
2. Are you younger than 18 years old? ☐YES ☐ NO
3. Have you smoked or consumed any nicotine/marijuana-containing products daily within the past 6 months? ☐YES ☐ NO
4. Are you allergic to Tegaderm™ (3M) medical adhesive dressing? ☐YES ☐ NO
5. Have you been prescribed medications for high blood pressure? ☐YES ☐ NO
6. Do you have a cardiovascular, neural (e.g., Raynaud's disease), respiratory or metabolic disorder (e.g., diabetes)? ☐YES ☐ NO
7. Do you have a history of fainting or dizziness during sitting or standing? ☐YES ☐ NO

For females only:

7. Are you pregnant, breastfeeding or intending to become pregnant in the next 3 months? ☐YES ☐ NO
8. Are you currently on, or planning on starting, hormone replacement therapy? ☐YES ☐ NO
9. If you are 55 years or older: Have you *had* a menstrual period in the last 12 months? ☐ not applicable ☐YES ☐ NO
10. If you are younger than 55 years: Have you been *without* a menstrual period for the last 12 months? ☐ not applicable ☐YES ☐ NO

APPENDIX C. CONSENT FORM

CONSENT FOR STUDY PARTICIPATION

Project Title: The impact of a 12-week high-intensity interval training program on prolonged-sitting induced reductions to popliteal artery health

I _____ have read the explanation about this study. I have been given the opportunity to discuss it and my questions have been answered to my satisfaction. I agree to take part in this study. However, I realize that my participation is voluntary and that I am free to withdraw from the study while it is ongoing, and up to one month following testing. I am aware that others participating in this study will be aware of my identity if I am assigned to the exercise intervention group.

_____	_____
Participant's Signature	*DATE*
_____	_____
Print Name of Participant	*DATE*
_____	_____
Signature of Witness	*DATE*

I confirm that I have explained the nature and purpose of the study to the participant names above and have answered all questions. In my judgment the participant is voluntarily and knowingly giving informed consent.

_____	_____	_____
Name of Person Obtaining Consent	Signature	Relationship to Participant

Please contact me at (please list a phone number, e-mail address, or mailing address):

_____

_____

_____

APPENDIX D. ETHICS APPROVAL

DALHOUSIE UNIVERSITY
Research Services

Health Sciences Research Ethics Board
Letter of Approval

May 31, 2021

Derek Kimmerly
Health\School of Health and Human Performance

Dear Derek,

REB #: 2021-5645
Project Title: The impact of a 12-week high-intensity interval training program on prolonged-sitting induced reductions to popliteal artery health

Effective Date: May 31, 2021
Expiry Date: May 31, 2022

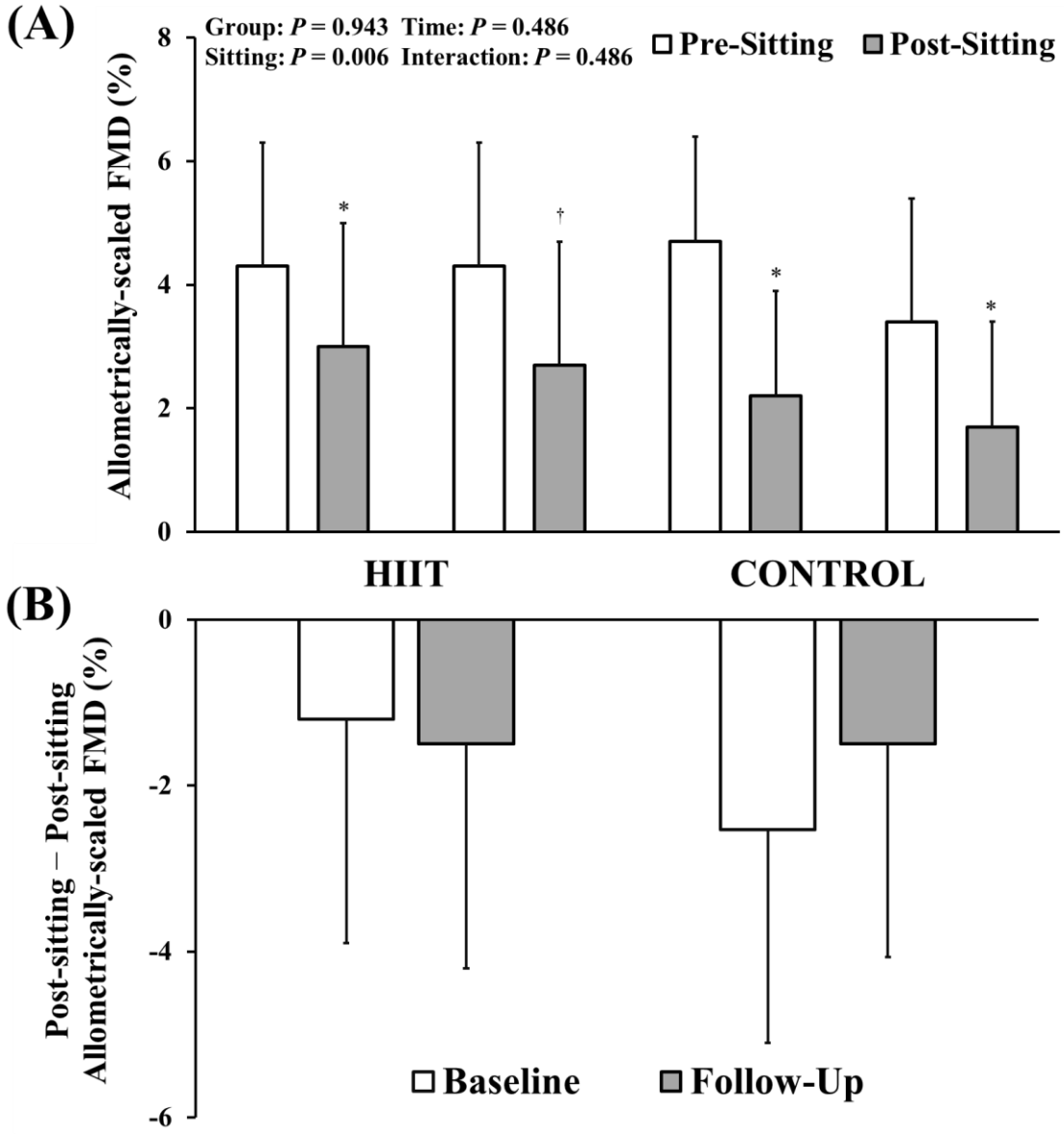
The Health Sciences Research Ethics Board has reviewed your application for research involving humans and found the proposed research to be in accordance with the Tri-Council Policy Statement on *Ethical Conduct for Research Involving Humans*. This approval will be in effect for 12 months as indicated above. This approval is subject to the conditions listed below which constitute your on-going responsibilities with respect to the ethical conduct of this research.

Effective March 16, 2020: Notwithstanding this approval, any research conducted during the COVID-19 public health emergency must comply with federal and provincial public health advice as well as directives from Dalhousie University (and/or other facilities or jurisdictions where the research will occur) regarding preventing the spread of COVID-19.

Sincerely,

Dr. Lori Weeks, Chair

APPENDIX E. SUPPLEMENTAL FIGURE



Supplemental Figure 1. (A) Popliteal allometrically-scaled FMD (%) responses to 3-h of prolonged sitting between Baseline and Follow-up for both HIIT (n=11, 7♀) and Control (n=8, 5♀) groups. Group × Time × Pre-/Post-Sitting interaction effects were determined via a 3-way repeated measures analysis of covariance with Bonferroni *post hoc* pairwise comparisons. Means ± SD data are presented. *, $P < 0.05$ versus Pre-Sitting within the same group. †, $P < 0.05$ versus Control group. (B) Sitting-induced changes (Post-sitting – Pre-sitting) in allometrically-scaled FMD (%).