THE IMPACT OF CARDIORESPIRATORY FITNESS ON PERIPHERAL VASCULAR FUNCTION IN OLDER ADULTS

by

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ABSTRACT

Optimal regulation of peripheral vascular function ensures adequate blood flow to tissues and the maintenance of vascular homeostasis. This is achieved via endothelium-derived vasodilator and vasoconstrictor chemicals, as well as sympathetic neural inputs. A higher aerobic fitness confers better cardiovascular health. Vascular function and aerobic fitness generally worsen with age. The purpose of this thesis was to investigate the impact of aerobic fitness on peripheral vascular function in older adults.

Relative peak oxygen consumption ($\dot{V}O_2$peak, indirect calorimetry) using a maximal cycle ergometer protocol was assessed in all four studies. Low-flow-mediated constriction (L-FMC) and flow-mediated dilation (FMD) represent endothelial-dependent vasoconstrictor and vasodilator function, respectively. For Studies 1-3, L-FMC and FMD were measured using high-resolution ultrasound and quantified as the percent change in baseline diameter during distal cuff occlusion and the subsequent reactive hyperemia, respectively.

I demonstrated that higher aerobic fitness was associated with larger L-FMC (more negative) and FMD (more positive) responses in the brachial artery of older adults (Study 1). However, the brachial artery is not typically the site of peripheral vascular disease development. I then documented that brachial artery endothelial function was not correlated to that in the popliteal artery. However, aerobic fitness was still associated with better endothelial function in the popliteal artery (Study 2). Building off these cross-sectional studies, a 6-week training study was conducted in which I observed that high-intensity-interval-training (HIIT) and moderate-intensity-continuous-training (MICT) similarly increased brachial FMD, but only HIIT improved brachial L-FMC. Both HIIT and MICT similarly enhanced popliteal FMD and L-FMC responses. Whole-body resistance training did not impact endothelial function in either artery (Study 3).

Vascular function is also influenced by sympathetic activity directed towards resistance vessels within skeletal muscle beds. Peroneal muscle sympathetic nerve activity (MSNA; via microneurography) and arterial pressure (finger photoplethysmography) were recorded during ≥10-min of rest in healthy older adults. Lower aerobic fitness was associated with lower MSNA burst incidence and higher neurohemodynamic transduction, along with greater blood pressure variability in older adults (Study 4).

The studies conducted provide support for the favorable impact of maintaining or increasing aerobic fitness in healthy older adults on peripheral vascular function.

Word Count: 349

LIST OF ABBREVIATIONS USED

5'GMP	5-guanosine monophosphate
AA	arachidonic acid
ACh	acetylcholine
ADMA	asymmetric dimethylarginine
ANCOVA	analysis of covariance
ANOVA	analysis of variance
ATP	adenosine triphosphate
B-mode	brightness mode
BK	bradykinin
$BKCa^{2+}$	big calcium-activated potassium channel
Ca^{2+}	calcium
Ca^{2+}-ATP Pump	calcium-adenosine triphosphatase pump
cAMP	cyclic adenosine monophosphate
cGMP	cyclic guanosine monophosphate
CO_2	carbon dioxide
COX1	cyclooxygenase 1
COX2	cyclooxygenase 2
CVLM	caudal ventrolateral medulla
CYP2C9	cytochrome P450-2C9
DBP	diastolic blood pressure
ECG	electrocardiogram
EDHF	endothelial-derived hyperpolarizing factors
EET	epoxyeicosatrienoic acids
eNOS	endothelial nitric oxide synthase
ER	endoplasmic reticulum
ET_A	endothelin-receptor A
ET_B	endothelin-receptor B
FMD	flow-mediated dilation

Gs	Stimulatory G-protein receptor
GTP	guanosine triphosphate
HIIT	high-intensity interval training
HR	heart rate
$IKCa^{2+}$	intermediate calcium-activated potassium channels
IML	intermediolateral nucleus
IP_3	inositol triphosphate
K^+	potassium
L-FMC	low-flow-mediated constriction
L-NMMA	N^Gmonomethyl-L-arginine
MAP	mean arterial pressure
MICT	moderate-intensity continuous training
MLC	myosin light-chain
MLCK	myosin light-chain kinase
MSNA	muscle sympathetic nerve activity
NADPH	nicotinamide adenine dinucleotide phosphate
NMD	nitroglycerin-mediated dilation
NO	nitric oxide
NO_2-	nitrite
NO_3-	nitrate
NTS	nucleus of the solitary tract
O_2^-	superoxide
$OONO^-$	peroxynitrite
PDE-5	phosphodiesterase-type 5
P_i	inorganic phosphate
PIP_2	phosphatidylinositol biphosphate
PL	cytosolic phospholipase A_2
PPO	peak aerobic power output
$\dot{Q}$	cardiac output
RBCv	red blood cell velocity

RNA	ribonucleic acid
ROS	reactive oxygen species
RT	resistance training
RVLM	rostral ventrolateral medulla
SBP	systolic blood pressure
$SKCa^{2+}$	small calcium-activated potassium channels
SNO	S-nitrosothiols
SR	shear rate
SR_{AUC}	shear rate area under the curve
SV	stroke volume
TVC	total vascular conductance
$\dot{V}O_2$	volume rate of oxygen consumption
$\dot{V}O_2max$	maximum volume rate of oxygen consumption
$\dot{V}O_2peak$	peak volume rate of oxygen consumption
VSM	vascular smooth muscle

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CHAPTER 1: INTRODUCTION

In developed countries, heart disease and stroke are among the leading causes of death (315). In Canada, approximately 1.6 million adults are living with heart disease or have experienced a stroke (219). These high rates of cardiovascular disease contribute to an over-burdened health care system with costs exceeding $23 billion dollars per year in Canada (220). Older adults are at a greater risk of experiencing adverse cardiovascular events due to unfavorable age-related declines in the structure and function of their vascular system (244). Specifically, with increasing age comes an elevated risk of atherosclerosis due to alterations in arterial structural modelling, stiffness and a decline in vascular endothelial (i.e., innermost lining of blood vessels) function (23). Arterial endothelial dysfunction represents an early sign of atherosclerotic development and is characterized by decreased vasodilation (e.g., vessel relaxation) in response to physical or chemical stimuli (244). As well, ageing is accompanied by chronic and excessive sympathetic nervous system activity directed towards the arterioles, especially those located within the skeletal muscle circulation (133). A drastic increase in sympathetic outflow may also be implicated in the development of cardio(vascular) diseases including, but not limited to, arterial hypertension and peripheral vascular diseases (103).

The non-invasive, ultrasound-based flow-mediated dilation (FMD) assessment in conduit arteries provides clinically relevant information regarding endothelial health. Specifically, FMD responses reflect the ability of the endothelium to produce and release the potent vasodilator nitric oxide (NO) following a reactive hyperemia (i.e., increased blood flow) induced by a prior period of distal ischemia (94, 282). Most FMD studies have been performed in the brachial artery, which provides an indication of coronary

artery endothelial health (26) and is predictive of future cardiovascular events (319). However, lower limb arteries such as the popliteal artery are relatively more susceptible to the development of atherosclerosis and peripheral vascular disease (58). Heterogenous FMD responses have been observed between the brachial and popliteal arteries (284), which may be due to the fact that the popliteal artery is exposed to greater gravity-induced hydrostatic pressures (181), has a larger baseline lumen diameter (284) and is exposed to larger oscillations in local shear stress (i.e., frictional forces of blood flow acting on the endothelial cells) during bouts of physical and sedentary activities (182, 294) than the brachial artery. Specifically, lower limb arteries are subjected to more frequent and robust reductions in blood flow during sedentary activities with declines observed in as little as 10 min of sitting (303). Conversely, traditional, lower-limb modes of aerobic exercise (e.g., walking, running, cycling) induce larger hyperemic responses in lower limb arteries (286). Compared to younger adults, the FMD response is attenuated in older adults in both upper- and lower-limb vascular beds (19, 213). The impact of interventions to modify popliteal artery endothelial function in older adults is unclear.

In addition to providing a clinically relevant measure of endothelial-dependent vasodilatory function, the decline in lumen diameter observed during the distal ischemic period of the FMD test, termed low-flow-mediated constriction (L-FMC), provides an index of endothelial-dependent *vasoconstrictor* function (57, 122). Upper-limb L-FMC provides information regarding how reduced shear stress influences vascular tone (i.e., the degree of constriction at rest relative to the maximally dilated state) (90) and is blunted in healthy older adults and patients with coronary artery disease (92). It is unclear if the popliteal artery exhibits an L-FMC response or if such responses are systemic in

nature in older adults. The L-FMC response is unaffected by inhibition of NO production (94). However, upper-limb L-FMC responses have been attenuated following the inhibition of other endothelial-derived vasodilatory factors, including endothelial-derived hyperpolarizing factors (EDHF) and prostaglandins (90), as well as via blockade of endothelin-1A receptors (264). Our understanding of the mechanisms and clinical relevance of endothelial sensitivity to reductions in local blood flow is relatively limited. However, examining the vasomotor response to changes in blood flow and shear stress (increase versus decrease) are comparable to the FMD outcome measure.

The cardiovascular benefits of regular physical activity and exercise are well established in older adults (245). Cardiorespiratory fitness (or aerobic fitness) represents the ability to deliver and utilize oxygen during a maximal exercise test. In older adults, a dose-response relationship exists in that risk-reductions in morbidity and mortality are achieved (~60%) through increases in cardiorespiratory fitness (215). Most studies indicate that higher cardiorespiratory fitness is associated with better brachial FMD responses in older populations (67, 313). The relationship between aerobic fitness and L-FMC is less clear. A cross-sectional study by Bell et al. (13) demonstrated that younger males with higher estimated peak oxygen consumption ($\dot{V}O_2$peak) exhibited greater brachial artery L-FMC responses than their less fit peers. Whether this association holds true in older adults, who exhibit unfavorable alterations in vascular structure and function, or with lower-limb arteries that accrue local adaptations to traditional forms of aerobic exercise is unknown.

Although important information may be gained from cross-sectional studies, interventional studies are a superior study design to determine cause (lifestyle factors)

and effect (vascular function). It is well established that aerobic exercise training augments vasodilator function in older adults with chronic disease (e.g., hypertension, heart failure, type 2 diabetes), with high-intensity interval training (HIIT) often eliciting superior improvements in brachial FMD than moderate-intensity continuous training (MICT) (5, 227). Compared to MICT, these augmented HIIT-induced FMD responses may result from correspondingly higher increases in shear stress and/or a reduction in oxidative stress (oxidative stress converts available NO to toxic peroxynitritate [ONOO-]), thereby increasing NO bioavailability [as reviewed in (227)]. Also, resistance exercise elicits larger post-contraction blood flow responses than aerobic exercise, but this augmented blood flow response is transient and under high-pressure in comparison to the sustained lower-pressure, sustained flow observed during aerobic exercise (65, 82, 286). Despite these differences in exercise-induced shear stress profiles, resistance training (RT) appears to be an effective stimulus for increasing brachial FMD, albeit less than aerobic training in older adults with chronic disease [as reviewed in (5)]. It is unclear if aerobic or RT improves brachial FMD in healthy older adults, improves endothelial function in arteries responsible for supplying blood flow to the active limbs during traditional modes of aerobic exercise (e.g., popliteal), or if HIIT produces greater improvements in FMD than MICT or RT in these arteries. For endothelial-dependent *vasoconstrictor* function, interventional studies have observed augmented brachial L-FMC (i.e., more constriction) responses following short-term (i.e., 6-8 weeks) continuous aerobic exercise training in young healthy (224) and obese adults (240). Whether exercise training also influences L-FMC in either the brachial or popliteal artery of older adults is unclear.

Both sympathetic neural activity and endothelial-derived vasoactive chemicals act together to dictate peripheral vascular function. The sympathetic nervous system represents a fundamental homeostatic system that involves reflexive regulation of arterial blood pressure and the distribution of blood flow. Beat-by-beat homeostatic control of skeletal muscle blood flow, systemic vascular resistance and arterial blood pressure are primarily achieved via the regulation muscle sympathetic nerve activity (MSNA) and subsequent vascular smooth muscle contraction (i.e., vasoconstriction) primarily through the release of norepinephrine (103). Ageing may be characterized by autonomic nervous system dysregulation that results in chronic sympathetic activation, (61, 133) which may have deleterious consequences on overall cardiovascular health (103). Even in the absence of disease, MSNA burst frequency increases with age at a rate of ~0.5 to 1 bursts/min each year (71, 133). In addition, there is some evidence supporting that NO, the primary mediator in endothelial-dependent vasodilator function (94), exerts a tonic inhibitory influence on the regulation of sympathetic outflow directed towards the peripheral vasculature (206, 324). It is unclear whether higher aerobic fitness is associated with lower, similar or greater resting MSNA levels in older adults, as well as the impact of endothelial function on these outcomes.

The transduction of MSNA into a vascular or hemodynamic outcome has gained the attention of numerous researchers as it provides useful information regarding sympathetic regulation of the vasculature. Although not in an older population, aerobic fitness influences reflex-mediated and spontaneous neurovascular or neurohemodynamic transduction in middle-aged (171) and younger adults (174). In middle-aged males, lower-body negative pressure-induced increases in forearm vascular resistance were

positively related to MSNA burst frequency in less aerobically fit ($\dot{V}O_2$peak ~26 ml/kg/min), but unrelated amongst a higher-fit group ($\dot{V}O_2$peak ~43 ml/kg/min) (190). In addition, we demonstrated that aerobic fitness was inversely associated with spontaneous sympathetic neurohemodynamic transduction in young males (200). Whether a similar relationship exists in older adults is unclear. In comparison to young adults, healthy older adults may exhibit decreased α-adrenergic sensitivity (35) and less norepinephrine release per burst (60). Research in animal models demonstrated that α-adrenergic sensitivity or density may be attenuated with greater aerobic fitness, with both young and older male Fischer rats demonstrating reduced α-receptor-mediated vasoconstriction following an exercise training program (15 m/min at 15% incline, 60 min/day, 5 days/week for 10-12 weeks) (64). Whether aerobic fitness influences sympathetic transduction in older adults, of who typically present with a higher resting MSNA burst frequency (176, 183) and lower NO bioavailability (245) is unknown.

The overarching purpose of the proposed thesis was to examine the influence of cardiorespiratory fitness on peripheral vascular function in older adults, with an emphasis on studying the impact of aerobic fitness on endothelial function and the cardiovascular responses to sympathetic activity in older adults. The working hypotheses for this thesis proposal included:

Study 1: Aerobic fitness level was positively associated with brachial artery FMD, but inversely related to brachial artery L-FMC in older adults.

Study 2: Divergent L-FMC responses existed in the brachial and popliteal artery. Aerobic fitness was inversely related to popliteal artery L-FMC.

Study 3: HIIT would elicit superior improvements in both brachial and popliteal endothelial function versus MICT. In addition, both aerobic protocols would produce greater endothelial-dependent vasodilator responses than RT. It was unclear whether endothelial-dependent vasoconstrictor function in either artery would be altered with any type of exercise training.

Study 4: Aerobic fitness would be inversely associated with spontaneous sympathetic transduction. I also explored the association between aerobic fitness with traditional MSNA burst characteristics (e.g., burst frequency) and if these associations were influenced by popliteal macrovascular (flow-mediated dilation) or microvascular (reactive hyperemia) endothelial function.

CHAPTER 2: LITERATURE REVIEW

2.1 Overview of Arterial Structure and Function

2.1.1 Peripheral Arteries

The cardiovascular system is comprised of a pump and a vast network of vessels responsible for the transport of blood throughout the body. The left atria of the heart receives oxygenated blood from the pulmonary system, which is pumped from the left ventricle throughout the body via larger arteries (260). These conduit arteries branch into smaller feed arteries, arterioles and capillaries to exchange gases and nutrients to the organs (261). Once capillary exchange is complete, deoxygenated blood is transported back to the right atria through the venules and subsequently the veins, where it is then pumped from the right ventricle back to the lungs for re-oxygenation (260).

Arteries and arterioles are comprised of three distinct layers (262). The outer layer, referred to as the *tunica adventitia (*or *externa)*, is composed of large amounts of collagen that protect and reinforce the blood vessel. The *tunica adventitia* contains sympathetic nerve endings, as well as the vascular blood supply (i.e., the *vaso vasorum*). The middle layer (*tunica media*) is composed mostly of vascular smooth muscle (VSM) cells embedded in a matrix of collagen, elastin and glycoproteins, which are responsible for the maintenance of arterial lumen diameter and vascular tone (262). The innermost layer (*tunica intima*) consists of a single layer of endothelial cells, which serves as a selectively permeable membrane between the blood and underlying tissues (262).

The arterial system may be characterized along a spectrum from elastic to muscular vessels. The larger (>10 mm vs 0.1-10 mm) conduit arteries are closer to the heart, more distensible with a thicker *tunica adventitial* layer but contains a lower relative

amount of VSM than the downstream arterioles (58, 128). Despite both conduit arteries (macrovascular) and arterioles (microvascular) being responsible for distributing blood flow away from the heart and being comprised of the same three layers, these vessels exhibit distinct structural and functional differences (159). Specifically, an average arteriole is ~30 μm with a thickness that is much smaller in comparison to conduit or feed arteries (159). Arterioles may be referred to as resistance vessels due to their major contribution in regulating blood flow into the capillaries by VSM relaxation (vasodilation) or contraction (vasoconstriction) that alters vascular resistance and arterial blood pressure (43).

2.1.2. Vascular Structure and Function with Ageing

Unfavorable age-related structural and functional vascular adaptations represent a major contributing factor towards the increased risk of developing cardiovascular disease and experiencing an adverse cardio(vascular) event in older adults (283). Structurally, age is associated with a progressive increase in arterial lumen diameter, at a rate of ~0.5% per year (238), as well as a progressive thickening of the intima-media layer (145). The greater diameter with age may be a compensatory response to plaque formation and/or thickening of the arterial wall (145), although this increase occurs even in the absence of plaque formation (95). This chronic dilation may be also be influenced by a loss of elastic fibres, with aging resulting in a decrease in elastin content, elongation of elastin (i.e., less elastic recoil), and a shift towards relying on stiffer collagen content (283). Both larger and smaller sized vessels become stiffer with age (18). An increase in local pressure with age (18), as well as advanced glycation end-products (glycotoxin or highly oxidant

compounds) can bind to collagen in the arterial wall, cause a loss of normal elasticity, strength, and flexibility (327).

Functionally, the vasculature of older adults demonstrates an impaired ability to dilate in response to chemical or physical stimuli (257). This is due to a decreased bioavailability of endothelial-derived NO (244), as well as the sensitivity of VSM cells to NO (192). Endothelial production of the potent vasoconstrictor endothelin-1 increases with age and causes vasoconstriction through endothelin-A receptors (63). Endothelin-1 levels are inversely related to endothelial-dependent dilation, and may be inhibited by NO, which inhibits endothelin-converting enzyme (63). Impaired vascular function with age is also influenced by an age-related increase in inflammation and oxidative stress that decreases NO bioavailability (222, 248). Oxidative stress, and specifically reactive oxygen species (e.g., O_2^-) may impair NO-mediated dilation at both the endothelial (i.e., convert NO to peroxynitrite) and VSM (i.e., source of NADPH oxidases which is a major producer of superoxide dismutase [ROS]) cell layers (246, 281).

Vascular structure and function interact with each other, in that a larger arterial diameter is also associated with a smaller endothelial-dependent dilatory response (287). Poiseuille's Law states that shear stress is equal to (constant × viscosity × blood flow) ÷ ($\pi \times \text{radius}^3$) (214). As demonstrated by the artery size being the cubic term in this equation, a lumen diameter is the primary variable in dictating shear stress, with a smaller lumen diameter promoting higher shear stress. Smaller diameter arteries may also possess more smooth muscle cells relative to the elastic laminae, as well as a larger wall-to-lumen ratio, promoting vasodilator responsiveness (292). Altogether, a number of factors contribute to the adverse structural and functional vascular adaptations that occur with

ageing. These factors may influence either of the individual aspects of, and/or interactions between vascular structure and vascular function.

2.1.3. Vascular Smooth Muscle Control Mechanisms

The proper maintenance of systemic pressure is continuously regulated on a beat-by-beat basis by local- and extrinsically-based vasodilation or vasoconstriction of arteries and arterioles. Locally, vascular tone is regulated by endothelial-independent factors such as transmural pressure (i.e., pressure inside vessel – pressure outside vessel) via myogenic regulation (e.g., if the pressure inside the vessel increases, the vessel responds with a vasoconstrictor response) (155). In addition to local endothelial-independent mechanisms, the endothelium may release chemical mediators that cause vasodilation (i.e., NO, prostaglandins, and EDHFs) or vasoconstriction (i.e., endothelin-1) (261). A more detailed description of each of these chemical vasomotor pathways are described in their own section below. The endothelium regulates vascular tone and mediates the release of anticlotting and pro-clotting factors (41), defends against pathogens (262), controls leukocyte movement in response to inflammation, and may initiate the formation of new blood vessels (i.e., angiogenesis) (23, 114).

Extrinsically, vascular tone can be influenced by the medullary cardiovascular control centres (via sympathetic neural activity), hormones and the renin-angiotensin-aldosterone system (261). The medullary cardiovascular control center primarily elicits peripheral vasoconstriction through stimulation of sympathetic outflow that promotes VSM contraction via the release of norepinephrine, among other neurotransmitters (e.g., neuropeptide-Y, adenosine triphosphate), at sympathetic nerve endings that bind to α_1- and α_2-adrenergic receptors to cause VSM contraction (60). The amount of muscle

sympathetic nerve activity (MSNA), and associated release of norepeinpehrine, are altered in response to feedback from receptors in the body, such as baroreceptors (i.e., pressure-sensing receptors) (134), as well as peripheral (carotid and aortic bodies), muscle (metaboreceptors), and central (medulla oblongata of brainstem) chemoreceptors (i.e., detect changes in blood gases and pH) (256).

The renin-angiotensin-aldosterone system regulates arterial blood pressure on a long-term basis via the release of hormones. To elevate blood pressure, this system relies on retaining or increasing blood volume via sodium and water reabsorption (179). Kidneys secrete the enzyme renin, which converts angiotensinogen (synthesized in the liver) to angiotensin I (217). Angiotensin I is converted to angiotensin II via angiotensin converting enzyme (primarily located on the luminal side of the vascular endothelium). Angiotensin II is a potent vasoconstrictor, thereby increasing vascular resistance and thus arterial blood pressure. In addition, angiotensin II mediates the release of aldosterone, which also increases arterial pressure by stimulating fluid retention and increasing blood volume, and hence cardiac output (165). Compared to young adults, a larger decrease in leg blood flow was observed in older adults in response to an intra-arterial infusion of angiotensin II, indicating that older adults have a hypersensitivity to angiotensin II at rest (317).

2.1.4. Vascular Smooth Muscle Contraction

VSM cells are spindle-shaped excitable cells that are interconnected in an overlapping pattern via gap junctions. VSM cell contraction is achieved via the contractile proteins myosin and actin. Actin is anchored to the cell by dense bodies and dense bands, with the latter being connected via intermediate filaments to form a

cytoskeleton framework across the VSM. Myosin filaments are woven between the actin filaments (27).

The contractile state of VSM cells is achieved through alterations in intracellular calcium (Ca^{2+}) and via depolarization the VSM cell membrane (via L-type calcium channels), with increases or reductions in Ca^{2+} causing a contraction or relaxation, respectively (27). As depicted in the figure below (Figure 2.1.4), an influx of Ca^{2+} ions in the VSM cell binds to a calcium-modulated protein (calmodulin), activating the enzyme, myosin light-chain kinase (MLCK). MLCK phosphorylates the light chain on the myosin head that permits the myosin head to attach with actin, causing contraction of the VSM (i.e., vasoconstriction) (310). VSM relaxation occurs when the phosphorylation of myosin light-chain is reduced. This is achieved via reduced intracellular calcium (via less released from sarcoplasmic reticulum or reduced entry into VSM cell), inhibition of MLCK by increased cyclic adenosine monophosphate (cAMP), and myosin light-chain phosphatase. As such, when intracellular VSM Ca^{2+} concentrations are reduced and MLCK are inhibited, there is less stimuli promoting contraction. Myosin light-chain phosphatase removes the phosphate group from the myosin light chain causing the actin and myosin filaments to separate, resulting in VSM relaxation or vasodilation (262).

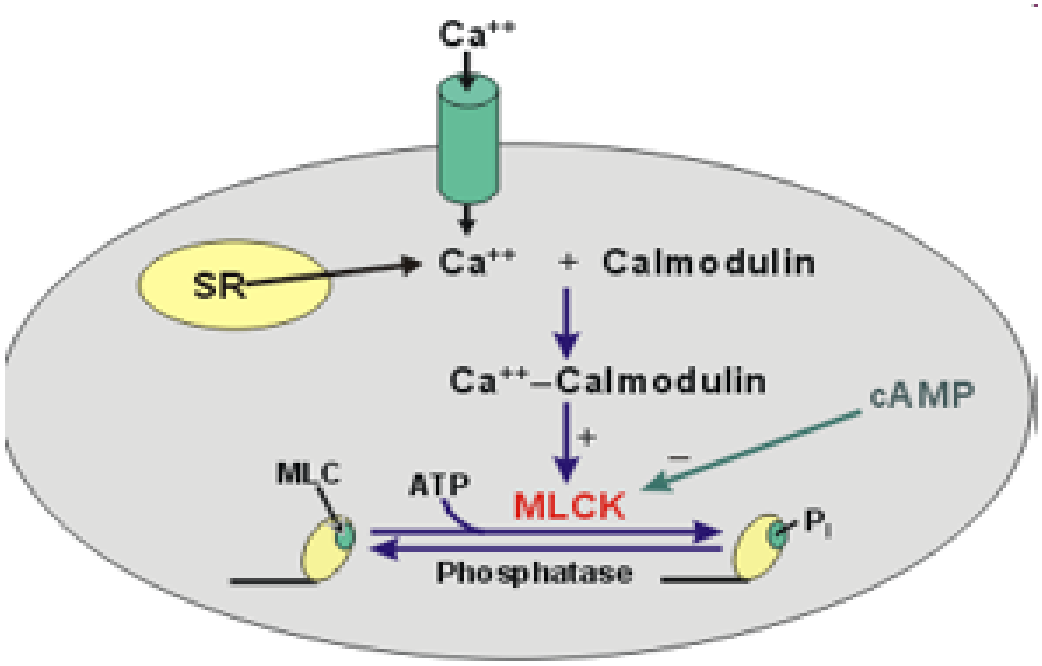

Figure 2.1.4. Contraction of the VSM with MLCK. An influx of Ca++ into the VSM cell, through Ca++ channels or from the SR, binds with calmodulin and activates MLCK. MLCK facilitates the binding of actin to myosin heads resulting in contraction of the VSM. Ca++ reuptake into the SR reverses this process, and allows the VSM to relax. In response to compounds (e.g., epinephrine, adenosine, and prostacyclin), stimulatory G-protein (Gs-protein) increases the second messenger cAMP. cAMP is synthesized from ATP by adenylate cyclase and inhibits MLCK, which reduces the phosphorylation of MLC and causes VSM relaxation. SR, sarcoplasmic reticulum; Ca++, calcium; MLC, myosin light chain; ATP, adenosine triphosphate; MLCK, myosin light-chain kinase; cAMP, cyclic adenosine monophosphate; P_i, inorganic phosphate.

2.2 Ultrasound-Based Measures of Peripheral Vascular Function

Our understanding of the structure and function of the peripheral vasculature has been greatly enhanced through advancements in ultrasound technology to provide non-invasive assessments of the arterial tree (119). Duplex ultrasound provides the ability to simultaneously record artery lumen diameter and blood flow velocity via brightness (B-mode) and pulsed-wave Doppler modes, respectively (328). To achieve this, a hand-held linear array transducer (i.e., probe) containing two arrays of piezoelectric crystals is placed on the surface of the skin to image the artery of interest. The piezoelectric crystals transmit pulsed signals to a specified depth with an emitting frequency (8-13 MHz for B-mode and 5 MHz for pulsed-wave Doppler) that are reflected back to the transducer allowing for artery image and red blood cell velocity (RBCv), respectively (328).

The structure of interest is optimally imaged when the probe is positioned perpendicular, such that the signals are bisecting the vessel at an insonation angle of 90°. However, due to the Doppler frequency shift (f_d) equation: $f_d = 2 \times \text{Transmitted}_{Freq}$ (velocity of moving reflector ÷ velocity of sound in medium) × cosine (degrees), a cosine of 90° equals zero and therefore is not possible. It has been demonstrated that insonation angles >60° introduce a large degree of error in determining RBCv as cosine (degree) decreases markedly as the angle increases beyond 60° (101). Therefore, ultrasound guidelines indicate that RBCv should be determined using an insonation angle ≤60° (282, 285). Since imaging on an angle would be particularly challenging, the machine integrates a ‘steer’ angle, whereby transmitting and received signals are steered by 30°, resulting in a 60° angle being achieved.

As shown in Figure 2.2 below, B-mode imaging creates a two-dimensional image that allows the sonographer to view the anterior and posterior walls of the artery, and as such, the lumen diameter (i.e., dark band between the 2 cyan dotted lines). The 30° steer angle is indicated as the two yellow lines right adjacent to the cyan lines. These yellow lines indicate whether the pulse-wave doppler signal is being sampled from. This recording is then played through semi-automated edge-detection software (Cardiovascular Suite, Quipu), which is an objective and valid method of determining arterial diameter in a variety of populations (74, 86) and will be implemented in the proposed studies.

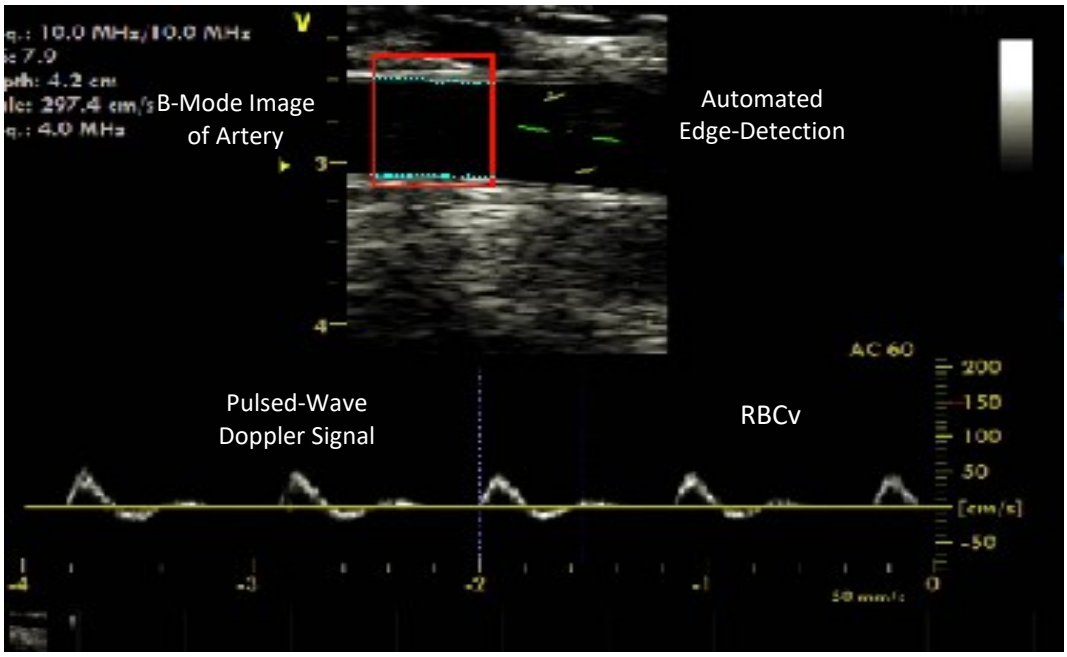

Figure 2.2. Example of the information observed from high-resolution duplex ultrasound imaging using both B-mode imaging and pulsed-wave Doppler signals. B-mode, brightness mode; RBCv, red blood cell velocity.

2.2.1 Flow-Mediated Dilation

Vascular endothelial dysfunction may be characterized by an attenuated vasodilatory response to physical or chemical stimuli, largely mediated by a reduced production of the antiatherogenic chemical NO (244). The FMD technique, first proposed by Celermajer and colleagues in 1992 (33), is a non-invasive assessment of conduit artery endothelial-dependent vasodilatory function (96). The FMD technique is primarily conducted in the brachial artery, with brachial FMD being predictive of future cardiovascular events (124) and strongly associated with coronary artery function (26). Blunted FMD responses are an early marker of atherosclerosis and progression towards the development of vascular diseases (55).

The FMD test uses duplex ultrasound and initially consists of a baseline imaging period followed by a period of ischemia, induced via a pressure-cuff inflated to a supra-systolic pressure located distal to the artery under investigation (e.g., the forearm for brachial artery investigations) (285). Following 5-minutes of ischemia, the cuff is rapidly deflated eliciting a reactive hyperemia and associated shear stress (or frictional forces against the endothelial cells) that results in vasodilation (288). The maximal diameter recorded is then typically expressed as a percentage above baseline (i.e., relative FMD) (282). Additionally, FMD may be normalized to the shear rate area under the curve (SR_{AUC}) between the time of distal cuff deflation until peak dilation occurs to account for inter-individual variations in the shear stress stimulus (209, 285). As well, the baseline diameter may influence the FMD response, with smaller diameters possessing a greater capacity to increase than larger diameters. Therefore, FMD responses may also be

allometrically-scaled to baseline diameter for between-group or between-artery (within same participant) comparisons when differences in artery size are observed (7).

2.2.1.1 Flow-Mediated Dilation Mechanistic Pathways

Most, but not all, studies have demonstrated that FMD responses in the brachial, radial and superficial femoral arteries are largely attenuated (~50%) in response to arterial infusion of the NO synthase inhibitor N^{G}monomethyl-L-arginine (L-NMMA), as reviewed in (94). Accordingly, the FMD test primarily reflects NO bioavailability. Of note, the production of other endothelial-released factors such as prostaglandins and EDHFs increases, while endothelin-1 production decreases, in response to augmented local shear stress (14, 204, 290).

Endothelial production of NO is achieved via nitric oxide synthase (eNOS) as illustrated in Figure 2.2.1.1. With a rise in blood flow there is a corresponding increase in the frictional forces of the red blood cells against the endothelium lining (i.e., shear stress) (30). The anterograde (i.e., forward moving) shear stress is detected by mechanoreceptors on the endothelium. Although unclear, shear-stress mechano-transduction may involve G-protein-coupled receptors, junctional proteins, primary cilia, and the glycocalyx (gel-like physical barrier that surrounds the cell) (3) Certain chemicals such as acetylcholine, bradykinin, histamine and thrombin act as agonists for NO production. Of note, both prostaglandins and EDHF are activated when agonists (i.e., acetylcholine, bradykinin, substance P) bind to their respective receptors, contributing to VSM relaxation. Increased shear stress activated calcium signalling in endothelial cells elevates endothelial cell cytosolic calcium (via direct mechanical gating of calcium permeable membrane channels), activating eNOS and thus, NO (85). Secondarily, the

endoplasmic reticulum releases Ca^{2+}. Together, this results in an increased calcium ion concentrations in the endothelial cell (310). Ca^{2+} binds with calmodulin to form a Ca^{2+}-calmodulin complex and subsequently activating eNOS, which mediates the conversion of L-arginine to NO and L-citrulline (54, 78, 109).

Nitric oxide then rapidly diffuses into the surrounding VSM cells where it stimulates soluble guanylate cyclase and increases cyclic-guanosine monophosphate (cGMP) concentrations. This leads to reductions in intracellular Ca^{2+} levels resulting in VSM relaxation (262), as described in more detail in the *Vascular Smooth Muscle Contraction* section above.

In addition to calcium-dependent pathways of activating eNOS, calcium-independent mechanisms exist in response to increases in shear stress and have been proposed as the predominant mechanism for shear stress-induced NO production, as reviewed in (326). Specifically, shear stress leads to phosphorylation of eNOS primarily via a protein kinase A-dependent manner and secondarily by protein kinase B at Ser1179 and Ser635 sites on eNOS (326).

As well, the production of NO is not exclusively dependent upon eNOS. Specifically, there is some evidence that NO may be released non-enzymatically by *S*-nitrosothiols (SNO) or from nitrate (NO_3-) or nitrite (NO_2-) (259). Endogenous and exogenous forms of NO may react with thiols (sulfur analogues of alcohols) in proteins, such as albumin, to form SNO (259). Circulating SNO may provide a reservoir of NO that may be used in states of NO deficiency. With age, the enzyme required to convert SNO to NO (i.e., S-nitrosoglutathione reductase) decreases (148).

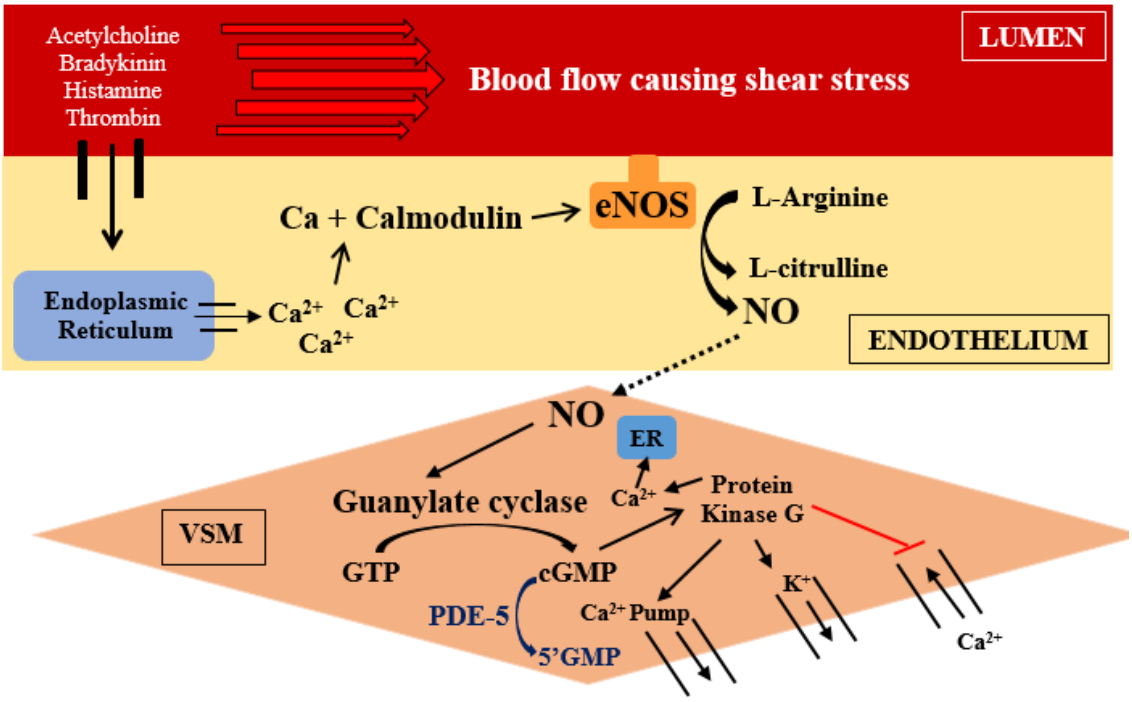

Figure 2.2.1.1. NO production and mechanism of action. Anterograde shear stress is detected by cilia-like mechanoreceptors on the endothelium. Acetylcholine, bradykinin, histamine and thrombin act as agonists for NO production. When these chemicals bind to specific receptor-operated channels, the endoplasmic reticulum releases Ca2+. Increased shear stress activated calcium signalling in endothelial cells elevates endothelial cell cytosolic calcium. Ca2+ binds with calmodulin to form a Ca2+-calmodulin complex and subsequently activating eNOS, which mediates the conversion of L-arginine to NO and L-citrulline. NO then rapidly diffuses into the surrounding VSM cells where it stimulates soluble guanylate cyclase and increases cyclic-guanosine monophosphate (cGMP) concentrations. This leads to reductions in intracellular Ca2+ levels. VSM relaxation occurs when the phosphorylation of myosin light-chain is reduced. This is achieved via reduced intracellular calium (via less released from sarcoplasmic reticulum or reduced entry into VSM cell), inhibition of MLCK by increased cyclic adenosine monophosphate (cAMP), and myosin light-chain phosphatase. Less MLCK activation and greater myosin light-chain phosphatase, which removes the phosphate group from the myosin light chain causing the actin and myosin filaments to separate, resultd in VSM relaxation or vasodilation Red line indicates the closing of L-type calcium channels (and thus calcium entry) via protein kinase G, which contributes to VSM relaxation. Blue reaction represents the degradation of cGMP to 5'GMP via PDE-5, which reduces the vasodilatory effects of cGMP. Ca2+, calcium; eNOS, endothelial nitric oxide synthase; NO, nitric oxide; VSM, vascular smooth muscle; GTP, guanosine triphosphate; cGMP, cyclic-guanosine monophosphate; ER, endoplasmic reticulum; K+, potassium; PDE-5, phosphodiesterase-type 5; 5'GMP, 5-guanosine monophosphate; Ca2+Pump, calcium-adenosine triphosphatase pump.

Similar to SNO, as evident by their chemical formulae, nitrite and nitrate may act as a reservoir of NO, with a number of different enzymes (hemoglobin, myoglobin, xanthine oxidoreductase, mitochondrial cytochrome oxidase, aldehyde dehydrogenase 2, cytochrome P450 reductase, and cytochrome P450) catalyzing the reduction of nitrite/nitrate into NO in certain conditions (37). Most of these enzymes act to convert nitrate/nitrite to NO in periods of hypoxic or anoxic conditions (e.g., cuff-induced distal ischemia), with the exception of cytochrome P450-based mechanisms, which may convert nitrate to NO as a compensatory mechanism during endothelial dysfunction (152, 326).

2.2.1.2 Flow-Mediated Dilation in Older Adults

Increasing age is accompanied by structural and functional vascular adaptations (283). Structural modifications include greater arterial wall thickness, an increased wall-to-lumen ratio and less arterial elasticity (95, 300). Accompanying the age-associated structural changes are chemical changes that attenuate FMD (283). Impaired endothelial function creates a pro-atherosclerotic vascular environment (108). Asymmetric dimethylarginine (ADMA) is formed via the proteolysis (i.e., breakdown of proteins) of L-arginine, is metabolized by a class of enzymes 'dimethylarginine dimethylaminohydrolases' (DDAHs), and is an endogenous inhibitor of eNOS (147). At the endothelium, older adults have higher plasma ADMA concentrations, attenuates the formation of NO (273). Such observations are potentially due to the age-related increase in oxidative stress and/or low-density lipoproteins, which result in diminished DDAH activity, and therefore a higher plasma ADMA (147).

NO-induced vasodilation may also be inhibited by the overproduction of reactive oxygen species (ROS), also referred to as oxidative stress, that overwhelm the antioxidant defense systems (79). When ROS are produced in excess, superoxide (O_2^-) rapidly pairs with NO to form the highly reactive and highly toxic peroxynitrite ($OONO^-$), reducing the bioavailability of NO (11). In general, older adults have exaggerated oxidative stress, which is linked with cardiovascular disease and vascular dysfunction (12, 244). Additionally, older adults have exaggerated levels of endothlin-1 and sympathetic nerve activity, which promote vasoconstriction and may counteract NO-mediated vasodilation (63, 183, 211).

Compared to their younger counterparts, older adults exhibit lower relative FMD responses in both upper- and lower-limb vascular beds (213). Furthermore, older adults have an impaired reactive hyperemic response (i.e., SR_{AUC}), which is the stimuli for shear-stress mediated vasodilation (19, 300). Of relevance, FMD normalized to SR_{AUC} is still lower in older adults compared to younger adults (19, 213). This indicates that some of the negative vascular effects of ageing are not solely related to a reduced reactive hyperemia stimulus, but due to attenuated endothelial-dependent or -independent vasodilatory mechanisms.

2.2.1.3 Nitroglycerin-Mediated Dilation in Older Adults

In addition to shear-stress-mediated, endothelial-dependent NO, vascular health and vasodilatory function are also influenced by VSM function (262). Endothelial-independent dilation (i.e., VSM sensitivity to NO) may be assessed following the sublingual administration of nitroglycerin (39). Nitroglycerin is an NO donor, which activates soluble guanylate cyclase leading to vasodilation (as described above)

independent of endothelium-derived NO (263). NMD is assessed using the same equipment as the FMD test. However, instead of a cuff-induced ischemia and resultant reactive hyperemia, a sublingual dose of nitroglycerin is administered and the artery of interest is imaged for 10-minutes (285). NMD is also represented as a percent change in lumen diameter from baseline to peak dilation. The combination of FMD and NMD permits the distinction of whether changes in FMD are attributed to corresponding alterations at the endothelium versus the VSM (202).

VSM reactivity to nitroglycerin declines with age in both the upper- and lower-limb conduit arteries and resistance vessels (174). Specifically, Parker and colleagues (213) demonstrated that NMD was ~50% lower in older adults compared to young adults in both the brachial and popliteal arteries. Evidence from animal models suggest VSM sensitivity to NO may be decreased with age due to a decrease in soluble guanylyl cyclase activity, which produces cGMP and triggers VSM relaxation (see Figure 2.2.1.1) (38, 140). Aging is also associated with increased inflammation and oxidative stress, which may reduce the bioconversion of nitroglycerin to NO within VSM cells (242) and partly explain the decreased NMD in aged persons (62).

2.2.2 Low-Flow-Mediated Constriction

While the FMD outcome measure has been well-established in the literature, an underappreciated aspect of the response profile during the FMD protocol is the vasoreactivity that occurs during the distal cuff-inflation period that results in reduced blood flow through the artery under investigation (91). The frequently observed endothelial-dependent vasoconstrictor response, termed low-flow-mediated constriction (L-FMC), provides information regarding the impact of reduced shear stress on vascular

tone (90). L-FMC is calculated as a percent decrease in lumen diameter between baseline and the nadir diameter observed during the final 30s of the distal ischemia period. Importantly, FMD guidelines recommend imaging the artery throughout the cuff inflation period (285). A larger L-FMC response (i.e., more negative) is healthy and indicates a greater sensitivity of the vascular endothelium to reductions in shear stress (122, 188).

In the radial artery, Dawson et al. (57) demonstrated that endothelial denudation via transradial catheterization ameliorated L-FMC responses, indicating that the L-FMC response is endothelial-dependent. However, unlike FMD, the infusion of L-NMMA to block NO production did not alter L-FMC (90). There is some evidence that L-FMC responses may be mediated through the inhibition of other vasodilatory signaling (i.e., EDHFs and prostaglandins) (57, 90), as well as enhanced vasoconstrictor signaling via endothelin-1 (264), as described in each section below.

Measuring L-FMC is appealing and convenient for researchers as it is derived by the decrease in lumen diameter during the ischemic portion of the FMD assessment (122). In addition, it is unclear whether a larger upper-limb artery L-FMC is associated with a smaller FMD (126), larger FMD (93, 267), or has no relationship with FMD (91, 92, 267). In the popliteal artery, L-FMC responses were negatively associated with FMD (196), suggesting that larger L-FMC (more negative) is a healthy endothelial response. The clinical relevance or predictive capabilities of L-FMC is much less established than FMD. However, the rationale of this metric is similar to FMD in that the vasomotor responses to changes in local shear stress (i.e., influx vs reduction) are of interest (115).

2.2.2.1 Contribution of EDHFs to L-FMC

Endothelial-derived hyperpolarizing factors (EDHFs) are vasoactive substances [epoxyeicosatrienoic acid (EET), hydrogen peroxide and potassium ions (K^+)] (207). EETs are the primary EDHF and largely involved in endothelial-derived hyperpolarization that results in an activation of transmembrane K^+ channels on VSMs and blunts subsequent VSM contraction. EDHF synthesis may be inhibited via the oral administration of 150 mg Fluconazole, which blocks the cytochrome P450-2C9 (CYP2C9) epoxygenase pathway and has been shown to blunt L-FMC responses in the radial and popliteal arteries (90, 218).

Acetylcholine, bradykinin, and shear stress result in increased endothelial intracellular Ca^{2+} concentrations. Ca^{2+} activates cytosolic phospholipase A_2, which releases arachidonic acid. CYP2C9 epoxygenase adds an oxygen to arachidonic acid to create EET, which diffuses into the surrounding VSM and stimulates big calcium-activated K^+ channels (BK_{Ca2+}) (28). The expression of BK_{Ca2+} channels may be variable depending on the vessel bed and the species (111). Activated BK_{Ca2+} channels result in an efflux of K^+, lowering the membrane potential of the VSM cell (180). Low intracellular K^+ hyperpolarizes VSM cells and results in the closure of voltage-dependent Ca^{2+} channels and a decrease in intracellular Ca^{2+} (207). VSM cell contraction and relaxation in response to changes in intracellular Ca^{2+} concentration is described above.

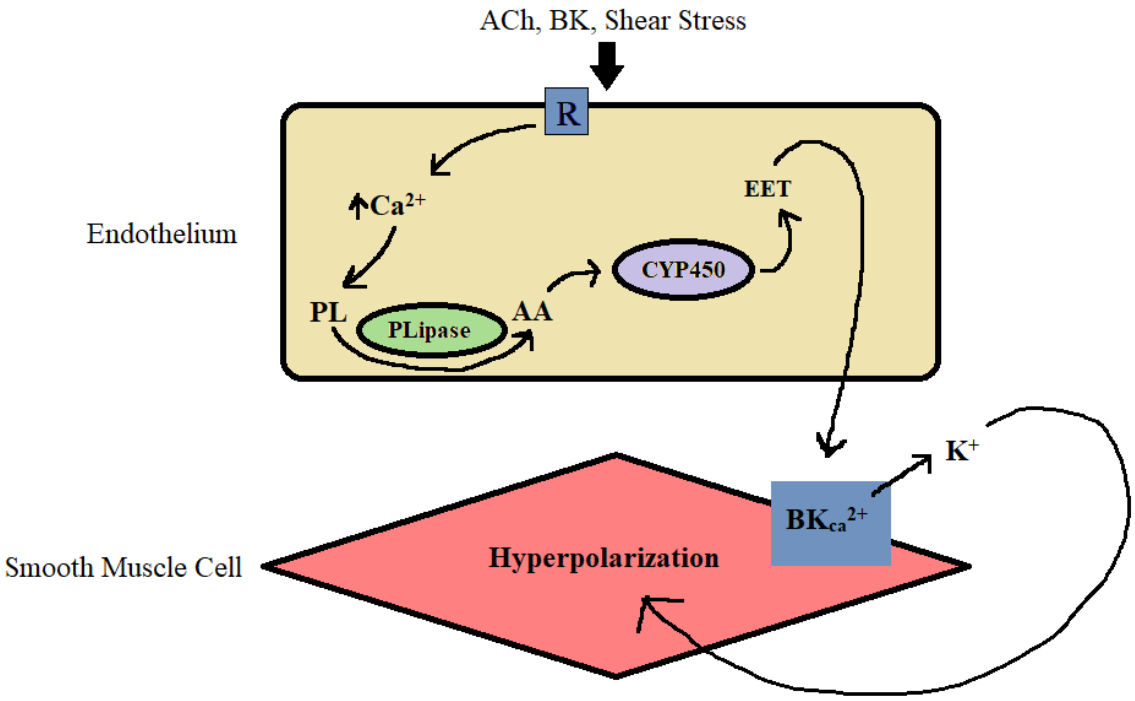

Figure 2.2.2.1. Schematic of the pathway that creates epoxyeicosatrienoic acid (EET) and initates hyperpolarization of vascular smooth muscle cells through the activation of big calcium activated potassium channels (BKca2+). The pathway is initiated by a stimulus such as acetylechonline (ACh), bradykinin (BK), or shear stress. These stimuli open calcium (Ca2+) channels within the endothelial cell membrane (depicted by R in the figure). The opening of Ca2+ channels results in an increase of intracellular calcium within the endothelial cells, which activates cytosolic phospholipase A2 (PL). PL releases arachidonic acid (AA). Cytochrome P450-2C9 (CYP450) adds an oxygen molecule to AA to convert it into EET. EET diffuses out of the endothelial cells to activate BKca2+. The activation of these channels leads to potassium (K+) efflux from the smooth muscle cells, which results in hyperpolarization and vasodilation. Adapted from Busse et al. (28).

In addition to BK_{Ca2+} channels, endothelial bound small (SK_{Ca2+}) and intermediate (IK_{Ca2+}) calcium-activated potassium channels also cause VSM hyperpolarization (329). These channels are also activated in response to increases in endothelial intracellular calcium, leading to K^+ efflux from endothelial cells. EET stimulates SK_{Ca2+} and IK_{Ca2+} channels within the endothelial cells, increasing the probability of these channels opening and K^+ being released (28). K^+ accumulates in the space between the endothelial and

VSM cells (myo-endothelial space), activating inwardly rectifying K^+ channels and increasing the amount of K^+ being pumped from the endothelial cell to the myo-endothelial space, potentiating VSM cell hyperpolarization (207).

Similar to EET, a cytochrome P450 pathway (cytochrome P450 4A3 hydrolase) and arachidonic acid may create hydroxyeicosatetraenoic acid, which is a potent vasoconstrictor (207). Fluconazole is primarily a CYP epoxygenase inhibitor, but also may inhibit other CTY isoforms (e.g., cytochrome P450 4A3 hydrolase) (207). The specific mechanisms regarding how EDHFs are involved in the L-FMC response are not fully understood. However, these factors likely involve these CYP pathways and potentially a disruption of competing CYP isoforms (CYP2C9 vs CYP4A3) that cause vasodilation versus vasoconstriction.

2.2.2.2 Contribution of Prostaglandins to L-FMC

The endothelium also releases prostaglandins, which may be a critical modulator of vascular tone (320). Unlike EDHFs, whether prostaglandins influence the L-FMC response is less clear. Specifically, a 500 mg oral administration of aspirin, an inhibitor of cyclooxygenase products (e.g., prostaglandins) impaired the L-FMC response in the radial (90), but not the brachial or popliteal arteries (218). Furthermore, a separate study that administered 1200 mg of ibuprofen (prostaglandin inhibitor) did not alter L-FMC responses in the brachial artery (32). Of note, the proposed studies will investigate vascular function in the brachial and popliteal arteries, but not the radial artery.

Similar to EDHFs, arachidonic acid is the most common precursor of prostaglandins, and is released from the cell membrane phospholipids, primarily by phospholipase A_2 (171). Arachidonic acid can be metabolized by several enzymatic

systems including prostaglandin synthases, lipoxygenases, and cytochrome P450. Prostaglandins and thromboxane A_2 are formed when arachidonic acid is metabolized by prostaglandin G/H synthase or cyclooxygenase (COX1 and COX2 isoforms) (76). COX1 and COX2 isoforms are the common substrates for a series of different prostaglandin isoforms that have tissue specific locations and actions (231). While PGE_2, PGI_2 and PGF_{2a} may act on VSM cells, PGI_2 also acts on the endothelium. From arachidonic acid, COX-2 may produce PGI_2 in response to increases in local shear stress (143). PGI_2 acts on VSM by binding to PGI receptors, and subsequently activating adenylyl cyclase via a stimulatory G-protein (G_s) that increases cyclic adenosine monophosphate (cAMP) (231) and causes VSM relaxation, as presented above (Figure 2.1.4).

In addition to being a potent vasodilator, PGI_2 is also an inhibitor of platelet aggregation, leukocyte adhesion and VSM cell proliferation (132, 187). Despite these vascular effects, it is unclear whether L-FMC is mediated via the inhibition of prostaglandins-vasodilatory pathway, or if it is dependent upon the artery of interest (radial vs brachial or popliteal).

2.2.2.3 Contribution of Endothelin-1 to L-FMC

In addition to local vasodilators (NO, EDHFs, prostaglandins), the endothelium also regulates local vascular tone by releasing the potent vasoconstrictor, endothelin-1. NO and endothelin-1 are primary regulators of basal vascular tone, antagonize the effects of each other, and are both released in response to shear stress (22, 63).

Endothelin-1, is a short (21-amino acid) peptide released continuously by endothelial cells, but also by VSM cells (20). In response to increases in laminar shear stress, preproendothelin-1 messenger RNA is transiently upregulated in a dose-dependent

manner. Preproendothelin-1 (212 amino acids) undergoes proteolytic cleavage (i.e., breakdown of proteins) to form big-ET-1 (39 amino acids) (52). At the endothelial cell membrane, endothelin converting enzyme converts big-ET-1 to endothelin-1 in the myoendothelial space. The biological effects of endothelin-1 are mediated through the activation of ET_A and ET_B receptor subtypes, with ET_B assisting in the clearance of endothelin-1 and stimulating eNOS enzyme activity and NO formation (235). ET_B receptors are located on both the endothelium and VSM. Conversely, binding to the more dominant ET_A receptor on VSM stimulates the formation of inositol triphosphate (IP_3) from phosphatidylinositol biphosphate (PIP_2) by phospholipase C via a Gq-protein (22, 52). Increased IP_3 within the VSM stimulates Ca^{2+} release from the sarcoplasmic reticulum causing vasoconstriction.

In the radial artery, the contribution of endothelin-1 to the L-FMC response has been investigated by conducting the occlusion-hyperemia test following the intra-arterial infusion of BQ-123 or saline (264). BQ-123 is a selective endothelin receptor antagonist (subtype: ET_A). In this sample of healthy young adults, ET_A inhibition attenuated radial L-FMC (-6.8% to -2.7%), but did not influence the FMD response, indicating that the L-FMC response is largely endothelin-1 mediated (264).

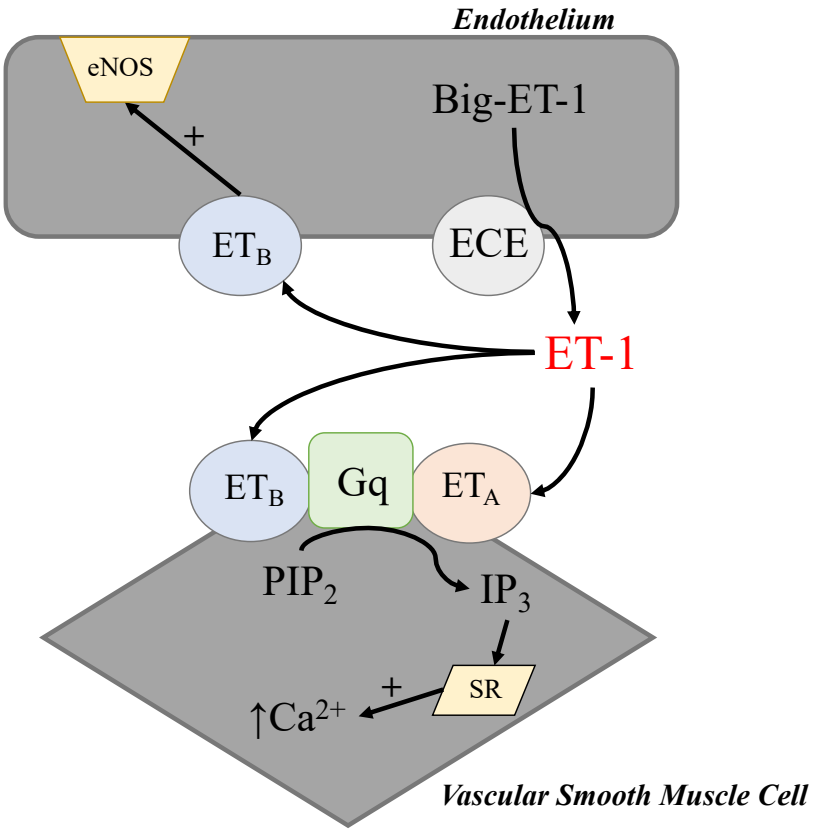

Figure 2.2.2.3. Schematic of the pathway by which endothelin-1 (ET-1) causes vascular smooth muscle cell contraction. ECE, endothelin converting enzyme; ETB, endothelin-B receptor; ETA, endothelin-A receptor; eNOS, endothelial nitric oxide synthase; Gq, phospholipase C coupled Gq-protein; PIP_2, phosphatidylinositol biphosphate; IP_3, inositol triphosphate; SR, sarcoplasmic reticulum; calcium, Ca2+.

2.2.3 Low-Flow-Mediated Constriction in Older Adults

As previously indicated, aging is associated with several unfavorable vascular adaptations that perpetuate endothelial dysfunction, as characterized by attenuated endothelial-dependent and endothelial-independent dilation. While endothelial-dependent vasoconstrictor function, is much less studied than FMD, the response is generally blunted with advanced age (93). Importantly, a greater L-FMC (more constriction) is a healthier response, with radial L-FMC being attenuated in smokers, individuals with hypertension, and coronary artery disease patients (92). In a large sample of 584 patients

(67±11 years), age was an independent predictor of radial L-FMC (β=0.04) in multivariate analysis that considered sex, smoking, coronary artery disease and diabetes (93). This attenuated L-FMC response with age occurs despite ageing being associated with a greater baseline lumen diameter. A larger conduit artery diameter may have a greater capacity to constrict, with baseline diameter being shown to be inversely associated with the magnitude of L-FMC in the brachial and radial arteries of young adults (247).

L-FMC responses may be mediated via prostaglandins (90), EDHFs (90, 218) and/or endothelin-1 (264). While the effects of ageing on L-FMC and the associated mechanisms are poorly understood, it could involve any single or a combination of these endothelial-derived factors. With ageing, COX-derived eicosanoids are decreased, lowering the production of prostaglandins (223). In addition, older adults exhibit higher thromboxane A_2 synthase activity resulting in higher levels of arachidonic acid-derived thromboxane (vasoconstrictor), and a reduced PGI_2/thromboxane ratio (223). In regards to EDHFs, animal models have demonstrated that aging decreases the expression of small, intermediate and big calcium-activated potassium channels, which are responsible for the reduced vasomotor actions of EDHFs with age (69). While prostaglandins and EDHFs are reduced with age, endothelin-1 plasma levels are increased with an associated greater reliance on endothelin-1 to maintain vascular tone (291, 301).

It may not be immediately clear why a reduction in local vasodilators and increase in vasoconstrictors would result in an attenuated L-FMC response (i.e., less constriction to reduction in blood flow). As initially postulated by Gori et al. (90), lower levels of prostaglandins and EDHFs, and higher levels of endothelin-1 at baseline (pre-occlusion)

could influence the amount that these vasodilators could be further inhibited, and/or endothelin-1 increased, in response to the low blood flow stimulus. This would ultimately result in an attenuated sensitivity to reductions in local blood flow.

2.2.4. Between-Limb Arterial Heterogeneity in Endothelial Function

Most studies incorporating FMD have been primarily performed in the brachial artery (285), which can provide predictive information regarding the incidence of future cardiovascular events (124) and are strongly related to coronary artery function (26). Known heterogeneous responses exist between the brachial and lower-limb vessels, including the popliteal artery (292). Such disparities in responses may be due to artery diameter being inversely related to FMD, with larger arteries dilating less than smaller arteries (e.g., brachial or radial) (284). Specifically, Thijssen et al. (284) compared FMD in the brachial, radial, common femoral, superficial femoral and popliteal arteries in young adults and observed that resting arterial diameter was inversely related to the peak FMD responses.

Lower limb arteries are more susceptible to the development of atherosclerosis and peripheral vascular disease (58). Despite this, there is evidence that FMD responses in the radial, brachial and superficial femoral artery are NO-mediated, as reviewed in (94). The limb-specific differences in FMD may be attributed to the increased blood pressure in the lower limb due to greater gravity-induced hydrostatic pressures (181). Specifically, vessels exposed to elevated blood pressure have been reported to have a decreased sensitivity (146, 154) and maximal responsiveness (84) to a variety of nitrovasodilators. With that, the lower-limb arteries supply a greater muscle mass and experience sustained increases in blood flow and blood pressure during locomotion (318).

Conversely, the popliteal artery experiences a greater reduction in local blood flow than upper-limb vessels during sedentary postures due its location along the popliteal fossa (back of the knee) that creates an 'arterial kink' during knee-bent sitting (307). While some evidence exists supporting that divergent FMD responses exist between the brachial and popliteal arteries (186, 292), no study has examined L-FMC responses to determine if brachial endothelial-dependent vasoconstrictor function provides systemic or local information. Importantly, most mechanistic insights into the L-FMC response have been conducted in the radial or brachial arteries (32, 90, 264). Of relevance, the recent study by Petterson et al. (218) in young, healthy adults demonstrated that L-FMC responses in the brachial and popliteal artery are both mediated by EDHFs, but not prostaglandins. Our current understanding regarding limb-specific endothelial function is primarily limited to young adults, with little known about the vasculature of older adults who may be more susceptible to popliteal aneurysm development (58). Although the brachial artery has predictive insight (124), muscular arteries like the brachial artery rarely are the site of atherosclerosis (58). Therefore, future studies investigating clinically relevant arteries such as the popliteal artery that exhibit elastic artery properties (58, 128) are warranted.

2.3 Impact of Aerobic Fitness on Cardiovascular Health in Older Adults

The cardiovascular benefits of regular physical activity and exercise are well established in older adults (245). Cardiorespiratory fitness represents the ability to deliver and utilize oxygen during a maximal exercise test and is quantified as the maximum volume rate of oxygen consumption ($\dot{V}O_2max$). Although influenced by physical activity level, cardiorespiratory fitness is also largely influenced by non-activity related factors such as body composition, sex, and genetics (47, 325).

In older adults, a dose-response relationship exists in that risk-reductions in morbidity and mortality are achieved (~60%) through increases in cardiorespiratory (or aerobic) fitness (215). Conversely, lower aerobic fitness is highly predictive of cardiovascular morbidity and overall mortality (150). Cardiorespiratory fitness declines with age, with a more rapid decline in $\dot{V}O_2$max after the age of 45 years in males and females (127). Despite these robust effects of aging, behaviour change (29) and aerobic exercise training interventions (166) have demonstrated that cardiorespiratory fitness levels are modifiable; attenuating or even reversing the age-associated decline in these physical health measures.

2.3.1 Association Between Aerobic Fitness and Endothelial Function

As reviewed in Montero et al. (173), most studies (>50% of the 29 reviewed) demonstrated a positive association between endothelial vasodilator function and aerobic fitness, which was not different between those that included primarily younger versus older adults. Importantly, studies were conducted almost exclusively in upper-limb arteries. Aerobic fitness was determined via cardiac output and muscle tissue oxygen uptake, and therefore was strongly related to exercise-induced vasodilation in vascular beds related to active muscle. However, little research has been conducted evaluating the relationship between aerobic fitness with shear-stress induced vasodilator function in lower-limb vessels.

Several mechanisms likely underlie the association between higher aerobic fitness and augmented FMD responses. Aerobic exercise has been shown to augment antioxidant systems, upregulate the expression of eNOS and lower plasma levels of endothelin-1 (63, 175). With ageing, a pro-oxidant vascular environment exists whereby ROS are

overproduced and overwhelm the antioxidant defense systems (222). As indicated above, the major ROS superoxide (245) pairs with NO to reduce its bioavailability and forms the highly toxic, peroxynitrite. The formation of peroxynitrite results in less NO diffusing into the VSM and therefore less VSM relaxation (120). Older adults with higher aerobic fitness have a more effective antioxidant system that consists of antioxidant enzymes (e.g., superoxide dismutase) to neutralize the pro-oxidative effects of superoxide, and therefore increase the amount of available NO (254).

The relationship between aerobic fitness and endothelial-dependent vasoconstrictor function (i.e., L-FMC responses) is relatively understudied in comparison to endothelial-dependent vasodilatory function. A cross-sectional study by Bell et al. (13) demonstrated that young males with higher estimated peak oxygen consumption ($\dot{V}O_2$peak) exhibited greater brachial artery L-FMC responses than young males with lower aerobic fitness. Whether this relationship holds true in older adult populations who experience structural and functional changes to the vascular endothelium (283) that cause impairments in brachial artery vasoconstrictor and vasodilator responsiveness is unknown.

2.4 Impact of Exercise Training on Endothelial Function in Older Adults

One of the most important molecular benefits of regular physical activity and exercise is an enhanced NO bioavailability (241). Regular exercise enhances the synthesis and release of NO and attenuates the degradation of NO by free-radicals (87, 142). In general, a 10% increase in aerobic fitness results in an absolute 1% improvement in relative brachial artery FMD (5). As identified in a recent meta-analysis (5), aerobic training ≥4 weeks typically increases the relative brachial FMD (%) of older adults by

2.9%. A 1% increase in relative brachial FMD may confer a 13% risk reduction in cardiovascular events (124) and is indicative of better coronary artery function (26).

The greater endothelial-dependent dilation in exercise-trained older adults is largely due to enhanced NO bioavailability (275), which may be attributed to an increase in eNOS gene and protein expressions (266). In addition, other endothelial-derived vasodilators such as prostaglandins (320) and EDHFs (169) may also be increased following aerobic exercise training, as evident by research in animal models (169, 320). In older adults, aerobic exercise training decreased the production of ROS and expression of oxidant producing enzymes [i.e., NAD(P)H oxidases] (1). Seals and colleagues (243) hypothesized that the beneficial effects of exercise training on endothelial vasodilator function are primarily due to reduced oxidative stress and secondarily to greater NO-production. As well, aerobic exercise training abolishes the tonic ET_A-receptor-mediated vasoconstriction in older adults (301), which may or may not contribute to the augmented endothelial-dependent dilation in this population. In summary, exercise training interventions produce favorable modifications to the peripheral vasculature that may attenuate the detrimental physical and chemical age-associated alterations that promote endothelial dysfunction (245).

Interventional studies have demonstrated that short-term (i.e., 6-8 weeks) continuous aerobic exercise augments brachial artery L-FMC in young healthy adults (224) and young obese adults (240). Such observations could be due to favorable changes in the prostaglandin, EDHF and/or endothelin-1 pathways highlighted above. The effects of exercise training on L-FMC in older adults has yet to be studied.

2.4.1. Aerobic Exercise Training and Endothelial Function in Older Adults

It is well established that aerobic exercise training augments vasodilator function in older adults with chronic disease (e.g., hypertension, heart failure, type 2 diabetes), with high-intensity interval training (HIIT) eliciting superior improvements in brachial FMD than moderate-intensity continuous training (MICT) (5, 227). The superior effects of HIIT have been attributed to the larger, sustained blood flow responses, which may enhance endothelial sensitivity to shear stress and/or a reduction in oxidative stress that decreases NO bioavailability [as reviewed in (227)]. With that, the increase in the shear-stress stimulus (or SR_{AUC}) of an FMD test following HIIT and MICT are relatively similar (227), suggesting there are other mechanisms beyond a greater shear stimulus that are responsible for the enhanced HIIT-induced vascular function.

Most previous reports that have compared the effects of HIIT versus MICT on vasodilator function have been conducted solely in the brachial artery of older adults with chronic disease, as reviewed in (227). To date, the only study to directly evaluate the impact of aerobic exercise intensity in healthy older adults observed no improvement in brachial FMD after 2 weeks of HIIT [10×1-min at 100% peak aerobic power output (PPO)] or MICT (40-min at 65% PPO) in post-menopausal women (139). As such, 2-weeks (6 total exercise sessions) may not provide a sufficient stimulus to elicit favorable FMD changes in this population. Rakobowchuk and colleagues (226) demonstrated that 6-weeks of sprint interval training [i.e., 4-6 repeated 30-second Wingates (0.075 kg/kg body mass) separated by 4.5 minutes of active recovery at 30 watts] and MICT (40-60 minutes at 65% of VO_2peak) similarly improved popliteal artery FMD in young adults. They failed to replicate these findings in a more recent study (249) that used a less

intense sprint protocol (i.e., 3 repeated, 20 second Wingates) and a similar MICT protocol (i.e., 45 minutes at 70% HRpeak). Whether or not older adults exhibit more favorable vascular adaptations to HIIT versus MICT has yet to be studied.

It has been postulated that a theoretical exercise intensity threshold may exist by which NO bioavailability may be jeopardized via high-intensity exercise-induced increases in ROS and a reduction in circulating antioxidants (15, 53). However, these potential detrimental effects may be avoided by limiting the time spent engaging in high-intensity exercise by shortening the high-intensity intervals or introducing more frequent recovery periods between intervals, but specific recommendations are not provided (227). In addition, HIIT protocols incorporating shorter intervals (e.g., 15 seconds) at a higher intensity (e.g., 100% PPO) with passive recovery have been shown to be most optimal for time spent exercising at 80% $\dot{V}O_2$max (i.e., larger stimulus to improve aerobic fitness), a lower perceived exertion level and participant comfort among older adults in comparison to other HIIT protocols (98, 99).

Altogether, it is unclear if aerobic exercise improves brachial FMD in healthy older adults, improves endothelial function in arteries responsible for supplying blood flow to the active limbs during traditional modes of aerobic exercise (e.g., popliteal artery), or if HIIT produces greater improvements in FMD than MICT in these arteries.

2.4.2. Resistance Exercise Training and Endothelial Function in Older Adults

National recommendations indicate that older adults engage in resistance training (RT) a minimum of 2 days per week (42). Compared to aerobic exercise, resistance exercise elicits larger post-contraction blood flow responses, but this augmented blood flow response is transient and under high-pressure in comparison to the sustained low-

pressure flow observed during aerobic exercise (65, 82, 286). Certainly, the blood flow responses are dependent upon the intensity and duration of the resistance training. If blood flow-induced shear stress increases too much, over perfusion could occur resulting in tissue damage (280). As well, it has been speculated that the RT-induced blood flow response is too brief to result in arterial function adaptations (225). Despite these differences in exercise-induced shear stress profiles, RT appears to be an effective stimulus for increasing brachial FMD, albeit less than aerobic training in older adults with chronic disease [as reviewed in (5)]. To date, the influence of resistance training-induced adaptations on lower-limb endothelial-dependent vasodilatory or vasoconstrictor responses remains uncertain in healthy older adults.

2.5 Sympathetic Neural Control of Vascular Smooth Muscle

The sympathetic nervous system represents a fundamental homeostatic system that involves reflexive regulation of arterial blood pressure and distribution of blood flow. The regulation of autonomic outflow directed towards the heart and vasculature is dependent upon peripheral reflex inhibitory and excitatory input, descending neural outputs, central modulators, and circulating factors (77, 250). Peripheral afferent inputs (arterial baroreceptors, cardiopulmonary baroreceptors, peripheral chemoreceptors, respiratory muscle afferents, and skeletal muscle afferents) terminate on the nucleus of the solitary tract (NTS) in the medullary oblongata (77). The NTS projects either directly to the rostral ventrolateral medulla (RVLM) or via the caudal ventrolateral medulla (CVLM). The RVLM directly excites sympathetic preganglionic neurons in the intermediolateral nucleus (IML) of the spinal cord, dictating sympathetic activity. In additional, descending neural inputs from higher brains structures (see Figure 2.5) and the

paraventricular hypothalamic nucleus, as well as central modulators (nitric oxide, angiotensin II, reactive oxygen species), provide direct and/or indirect excitatory drive to the RVLM or IML (77). Circulating angiotensin II can also increase neuronal activation of circumventricular organs (i.e., specialized brain structures characterized by a lack of a normal blood brain barrier) (151). These organs may provide input to the cardiovascular control centres in the medulla oblongata and to the paraventricular hypothalamic nucleus (255). Altogether, the integration of these multiple factors dictate the magnitude of sympathetic outflow to the heart and the vasculature.

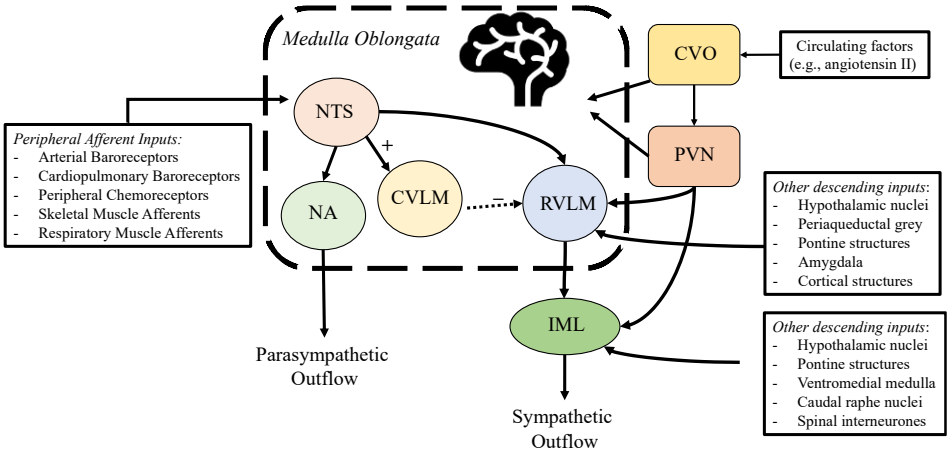

Figure 2.5. Depiction of the factors that contribute to the central regulation of autonomic outflow. The central integration of autonomic outflow occurs in the medulla oblongata from descending neural inputs and peripheral afferent inputs. Peripheral afferents terminate on the nucleus of the solitary tract (NTS) and project to the rostral ventrolateral medulla (RVLM), either directly or through an inhibitory signal from the caudal ventrolateral medulla (CVLM). The NTS also projects to the nucleus ambiguous (NA) to provide excitatory drive to determine parasympathetic outflow (heart only). The RVLM provides excitatory drive to the sympathetic preganglionic neurons on the intermedolateral column (IML) to determine sympathetic outflow. Descending neural inputs from higher brain structures and the paraventricular hypothalamic nucleus (PVN) also provide inhibatory/excitatory drive to the RVLM and/or IML. The circumventricular organs (CVO) provide additional means by which circulating factors can influence autonomic outflow and PVN activity. Separate descending outputs from the RVLM regulate outflow to the heart and vasculature, with further separation to different vascular beds in order for a differentiated control of sympathetic outflow. Adapted from Fisher et al., (77).

As described by Shoemaker et al. (250), the neural control of vascular function represents a highly integrated and complex system that includes: 1) recruitable efferent nerve traffic, 2) neurotransmitters and their patterns of release into, and removal from the neurovascular junctional cleft, 3) postjunctional receptor densities, and 4) post-receptor smooth muscle contractile properties. Post-ganglionic sympathetic nerves are located at the adventitia-media border of most arteries, arterioles, and veins throughout the body (293). Sympathetic nerves primarily produce artery and arteriole VSM contraction through the release of norepinephrine at nerve terminals that binds to α_1- or α_2-adrenergic receptors located on VSM cells (36). The binding of norepinephrine to α_1-adrenergic receptors stimulates phospholipase C through a Gq-protein mechanism, that leads to the formation of inositol triphosphate and diacylglycerol causing increased intracellular Ca^{2+} concentration and protein kinase C activation, respectively (316). In response to the binding of norepinephrine on VSM α_2-adrenergic receptors, an inhibitory G-protein inhibits adenylate cyclase, decreases cAMP concentrations resulting in less MLCK inhibition (i.e., vasoconstriction). In addition to norepinephrine, vascular sympathetic nerves may also release neuropeptide Y and adenosine triphosphate, which can be released as co-transmitters and produce vasoconstriction via the activation of Y_1 receptors or purinergic P_2X receptors, respectively (293). Neuropeptide Y may also potentiate the vasoconstrictor effects of norepinephrine and adenosine triphosphate (208). The density or distribution of these receptors vary across the vascular tree. Arteries and larger arterioles primarily consist of α_1-adrenergic receptors with a relatively lower density of α_2-adrenergic receptors (203). As arterioles become smaller (i.e., pre-capillary arterioles), the proportion of α_2-adrenergic receptors, Y_1 receptors and purinergic P_2X

receptors increase, whereas the proportion of α_1-adrenergic receptors decrease (250). Accordingly, the mechanism by which vasoconstriction is achieved may vary across the vascular tree, with increases in vascular resistance primarily achieved by sympathetic modulation of arterioles (microvasculature) rather than the larger, conduit arteries (80). In a canine model, ageing elevates P_2X receptor-mediated vasoconstriction, but reduces neuropeptide Y-mediated vasoconstriction (59). In comparison to young adults, healthy older adults may exhibit decreased α-adrenergic sensitivity (35) and less norepinephrine release (60).

2.5.1 Microneurography

Direct neurophysiological recordings of postganglionic sympathetic nerves have been reported in rabbits and cats as early as the 1930s (2, 25). However, a major breakthrough occurred in the mid-1960s when Hagbarth and Vallbo (100) performed the first microneurographic method for recording postganglionic sympathetic nerve recordings in conscious humans. Since then, the instruments and processes used have drastically improved, as described in more detail elsewhere (31, 252). The microneurography technique consists of inserting a tungsten microelectrode percutaneously into a nerve fascicle, often a superficial nerve (e.g., peroneal nerve, ulnar nerve, etc.). A non-insulated reference microelectrode is then positioned 2-3 cm subcutaneously from the active recording site. The active and reference electrodes are connected to a pre-amplifier. The active electrode is physically manipulated into the nerve fascicle. The raw nerve signal is then amplified (~75,000×), bandpass filtered (500-2000Hz), full-wave rectified, and integrated (0.1 second time constant) to obtain a mean voltage neurogram (252). From the mean neurogram, characteristic ‘bursts’ of activity

can be detected and analyzed. As microneurography has the capacity to measure sympathetic nerve activity directed towards the skeletal muscle circulation (MSNA), as well as skin sympathetic nerve activity, MSNA recordings are inspected for the presence of cardiac synchronicity (maximum of 1 MSNA burst per cardiac cycle), responsiveness to a sympathoexcitatory maneuver (e.g., breath-hold), and lack of response to arousal or skin stimulation (31, 252). Of note, MSNA and skin sympathetic activity also differ in shape and width (105).

2.5.1.1 Quantifying Muscle Sympathetic Nerve Activity

Multi-unit MSNA is most commonly quantified in terms of burst frequency (bursts per minute) (252). However, since MSNA is pulse synchronous, it is dependent upon resting heart rate, which highlights the need for standardizing activity by calculating a burst incidence (bursts per 100 heart beats) (274). Additionally, because the size of the burst influences the subsequent vasoconstrictor response with a larger burst eliciting greater vasoconstrictor and pressor responses (73), average burst height (or amplitude) and/or burst area are also typically calculated. Although MSNA burst frequency/incidence is reproducible across weeks, months and years (71, 136, 314), the absolute size of the burst is dependent upon the position of the recording microelectrode relative to the sympathetic axons, which cannot be exactly replicated over repeated recordings (156). Accordingly, within- or between-subject comparisons of burst height/area are normalized to the tallest/largest burst within that recording period (typically 5-20 mins) and expressed as a percentage, where the largest burst is normalized to 100% (156). Lastly, total MSNA may be calculated as the product of mean burst frequency and normalized relative burst size (height or area) (136, 251).

2.5.2 Impact of Ageing on Muscle Sympathetic Nerve Activity

Ageing may lead to autonomic nervous system dysregulation, in part characterized by chronic sympathetic activation, which may have deleterious consequences on overall health (36). Even in the absence of disease, MSNA increases with age, with reports suggesting an increase in burst frequency of ~0.5 to 1 burst/min each year (71, 133). Recently, Keir et al. (133) presented the largest cross-sectional evaluation of the influence of age and sex on MSNA in 658 healthy normotensive adults. They observed a progressive increase in sympathetic activity from 20 years (~25 burst/min) to 80 years (~35-40 bursts/min) in males. However, in females, MSNA tended to decrease from age 20 (20 bursts/min) to age ~30 years (~12 bursts/min) followed by a steeper increase in MSNA to 80 years (40-45 bursts/min) (133). Similar findings were observed when burst incidence was used (i.e., bursts per 100 heartbeats). These findings corroborate previous reports that demonstrate lower MSNA in young females versus young males (104), as well as a steeper increase in MSNA with age among females versus males (163, 177). It is unclear why Keir et al. (133) observed a reduction in MSNA from 20 to 30 years in females. Their findings do support that the age-related changes in MSNA vary between the sexes with the hormonal changes such as lower estrogen accompanying menopause ameliorating sex differences in resting MSNA between older males and females. Among both sexes, this age-associated increase in tonic MSNA may be largely implicated in the risk of developing hypertension among older adults (35).

The sympathetic regulation of the vasculature has been well studied using three distinct reflexes that modify MSNA to regulate vasomotor tone, including the arterial

baroreflex, chemoreflex and the skeletal muscle metaboreflex. The impact of ageing on these neurovascular reflexes are described below.

2.5.2.1 Ageing and the Arterial Baroreflex

The arterial baroreflex regulates arterial blood pressure around a set-point in a negative feedback manner by modulating MSNA in responses to ramps in blood pressure (125). Mechanical distensions (via fluctuations in transmural pressure) of the aortic arch and carotid sinus are detected by baroreceptors within these arteries. An increase in vessel distension increases afferent baroreceptor firing to the NTS via the glossopharyngeal (cranial nerve IX) and vagus nerves (cranial nerve X) from the carotid and aortic baroreceptors, respectively (50). Increased baroreceptor firing to the NTS increases parasympathetic outflow to the heart and inhibits sympathetic outflow to the heart and vasculature (34). Attenuated sympathetic outflow directed towards arterioles reduces the tonic vasoconstrictor stimuli, promoting vasodilation and a decrease in total peripheral resistance (103). The opposite set of responses occur following reduced mechanical distension of these barosensory arteries to increase arteriole vasoconstriction and thus arterial pressure back to the arterial baroreflex set-point. The arterial baroreflex acts on a beat-by-beat basis and modulates MSNA depending on the within-beat differences between systolic and diastolic blood pressure. This response is pulse synchronous (i.e., maximum of 1 burst per cardiac cycle). The burst occur during diastole as this is the point in the cardia cycle in which there is the least amount of stretch/pressure within the baroceptor-containing vessels, causing less baroreceptor afferent activation, less NTS input, less caudal ventrolateral medulla activation, and

therefore the least amount of RVLM inhibition (see Figure 2.5), and thus the greatest likelihood of a burst occurring.

In addition to the arterial baroreflex, there is a cardiopulmonary baroreflex with baroreceptors located in the chambers of the right heart, the great veins (superior and inferior vena cavae), as well as the pulmonary veins. In the heart, these low-pressure receptors sense changes in right atria and right ventricle filling pressures and are involved with the regulation of blood volume (137, 234).

Mean arterial pressure (MAP) is determined as a produce of 1/3systolic + 2/3 diastolic blood pressure. The MAP that the arterial baroreflex attempts to achieve is referred to as the 'operating pressure'. The corresponding MSNA burst incidence (i.e., probability of a burst or bursts/100 heartbeats) that is required to maintain that operating pressure is referred to as the 'operating point' (305). The operating point and operating pressure collectively represent the baroreflex 'set-point' mentioned above. Diastolic pressure is used in the example below as bursts occur during diastole (lowest amount of pressure in barosensory containing vessels) and is the accepted pressure metric for sympathetic baroreflex sensitivity (117). Sympathetic baroreflex gain represents the responsiveness of MSNA to changes in diastolic blood pressure (i.e., slope in bursts/100heartbeats/mmHg units) (278). A greater slope value is indicative of a greater reflex gain, better ability to buffer beat-beat changes in arterial pressure and achieve the operating pressure. Depending on the physiological condition (e.g., exercising), the arterial baroreflex may be 'reset' to attempt to maintain a different operating pressure (e.g., higher arterial pressure during exercise) or operating point to continue to effectively

regulate arterial pressure on a beat-beat basis necessary for that physiological condition (51, 229, 305).

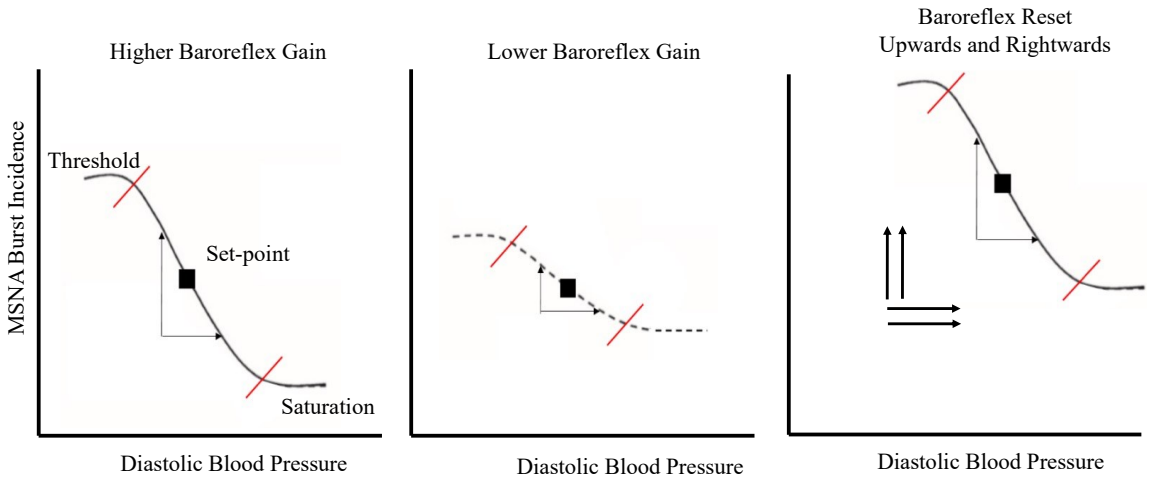

Figure 2.5.2.1. Depiction of sympathetic arterial baroreflex function. The left panel presents the sympathetic baroreflex set-point. Sympathetic baroreflex sensitivity is quantified by plotting the stimulus (diastolic blood pressure) – response (MSNA) curve across a range of blood pressures. This relationship is often sigmodal, with the threshold and saturations regions not consistently observed in humans. The slope of the line between these points provides an index of sympathetic baroreflex sensitivity. The middle panel demonstrates a reduced sensitivity, in which the flatter slope is associated with a smaller change in MSNA for a given change in pressure. The right panel depicts baroreflex resetting, with the example showing an upward resetting to a higher MSNA burst incidence and higher diastolic blood pressure, as is observed with ageing.

With age, barosensory arteries become stiffer (118, 121), which is associated with attenuated distension of the arterial wall and less baroreceptor firing. This results in reduced inhibition of sympathetic outflow directed towards skeletal muscle arterioles (i.e., greater MSNA). However, cross-sectionally, most studies demonstrate a preserved baroreflex gain in older adults in comparison to young adults, as reviewed in (170). Specifically, the responsiveness of the arterial baroreflex to alter MSNA in response to changes in arterial pressure (i.e., bursts/100heartbeats/mmHg) is often (68, 160, 161), but not always (162) similar between young and older adults. Despite similar baroreflex gain

values between age groups, MSNA and diastolic blood pressure are typically higher in older adults. Accordingly, the vascular sympathetic baroreflex is reset upwards (higher MSNA) and rightwards (higher diastolic blood pressure) to regulate arterial pressure on a beat-beat basis around a higher set-point (81).

2.5.3. Relationship Between Muscle Sympathetic Nerve Activity and Endothelial Function

In addition to sympathetic-mediated vasoconstriction, endothelial-derived vasodilatory substances such as NO (326), prostaglandins (90), and EDHFs (90) largely contribute to the maintenance of vascular tone. As indicated above, NO is the most potent vasodilator and is released in response to chemical (e.g., acetylcholine) or physical stimuli (e.g., blood flow against endothelial lining) (78), which all decline with age (245). Brachial artery FMD has been shown to be inversely correlated to resting MSNA in healthy older males, but not in younger males (289). Brachial artery FMD is attenuated in response to a sympathoexcitatory maneuver (cold pressor test; 5 min with hand in 4°C water) in older males, but not in younger males (289). In contrast, Hijmering et al. (110) observed attenuated brachial FMD responses in young adults following mild lower-body negative pressure stimulation (-20 mmHg), which is associated with cardiopulmonary and arterial baroreflex-induced increases in MSNA. Owlya et al. (206) demonstrated that pharmacological infusions of a high dosage of the eNOS inhibitor N^G-monomethyl-l-arginine (500 μg/kg/min for 15 min; L-NMMA) decreased MSNA burst frequency from 22 bursts/min to 7 bursts/min). Conversely, a lower dosage of L-NMMA did not impact MSNA burst frequency, likely due to a baroreflex-mediated sympathoinhibition during the high-dose L-NMMA, as the pharmacological inhibition increased mean arterial

pressure from 80 mmHg to 90 mmHg. In addition, Charkoudian et al. (35) observed that participants with higher resting MSNA had larger increases in blood pressure to pharmacological infusions of L-NMMA than those with lower resting MSNA, without a change in MSNA burst frequency. Altogether conflicting reports exist as to whether neural activity directed toward skeletal muscle arterioles impacts endothelial-mediated dilation, or *vice versa*.

Whether endothelial-dependent vasoconstrictor function (i.e., L-FMC) is associated with MSNA activity is unclear. However, L-FMC is mediated by factors also known to contribute to vascular tone (EDHFs, prostaglandins, endothelin-1). Altogether, there is some evidence supporting that NO, the primary mediator in endothelial-dependent vasodilator function (94), exerts a tonic inhibitory influence on the regulation of sympathetic outflow directed towards the peripheral vasculature. However, pharmacological studies inhibiting NO production challenge the notion that lower NO bioavailability (via L-NMMA) lead to exaggerated sympathetic drive, but instead highlight the intricacies of this relationship to maintain vascular tone.

2.5.4 Sympathetic Transduction

Beat-by-beat homeostatic control of blood flow, systemic vascular resistance and arterial blood pressure is achieved via the regulation MSNA and subsequent vascular smooth muscle contraction (i.e., vasoconstriction) (103). The transduction of sympathetic nerve activity into a hemodynamic or peripheral vascular response has attracted the attention of numerous researchers (24, 46, 72, 73, 189, 190, 233, 269, 302). The transduction of MSNA into a cardio(vascular) outcome has been analyzed using the ratio of time-averaged vascular resistance to MSNA burst frequency during supine rest (106).

In addition, isometric handgrip (276) and pharmacological infusions of vasodilatory agents (56) have been used to evoke robust increases in MSNA to quantify baroreflex-mediated sympathetic transduction responses. For these sympathoexcitatory manoeuvres, the ratio of total increases in vascular resistance to MSNA, or plotting the rise in vascular resistance against MSNA in 30-s bins, have been used to quantify neurovascular transduction (56, 106, 276).

There has been recent interest in examining spontaneous sympathetic transduction by characterizing the pressor and vascular responses to individual MSNA bursts while participants are in a rested state. Examining pressor responses to isolated and clusters of MSNA bursts on a beat-by-beat basis provides novel information regarding sympathetic neural control of arterial blood pressure and vascular resistance. Initially introduced by Wallin and Nerhed (306), and later refined by Vianna et al. (302), the spontaneous transduction method takes advantage of a signal-averaging approach. Specifically, it involves tracking the pressor and/or vascular responses following heartbeats associated with, versus absent of, MSNA bursts. Peak hemodynamic responses (e.g., a rise in mean arterial pressure) occurs ~5-9 cardiac cycles following each burst and is mediated via α-adrenergic receptor-related mechanisms (72). Statistical comparisons can then be made between the hemodynamic responses from isolated bursts (i.e., those surrounded by heartbeats without bursts), between sequences of successive bursts (i.e., 2 or more consecutive heartbeats with bursts) versus heartbeats not associated with a burst. Exaggerated pressor and vasoconstrictor responses to a burst of MSNA may be a major contributor in the development of hypertension (304).

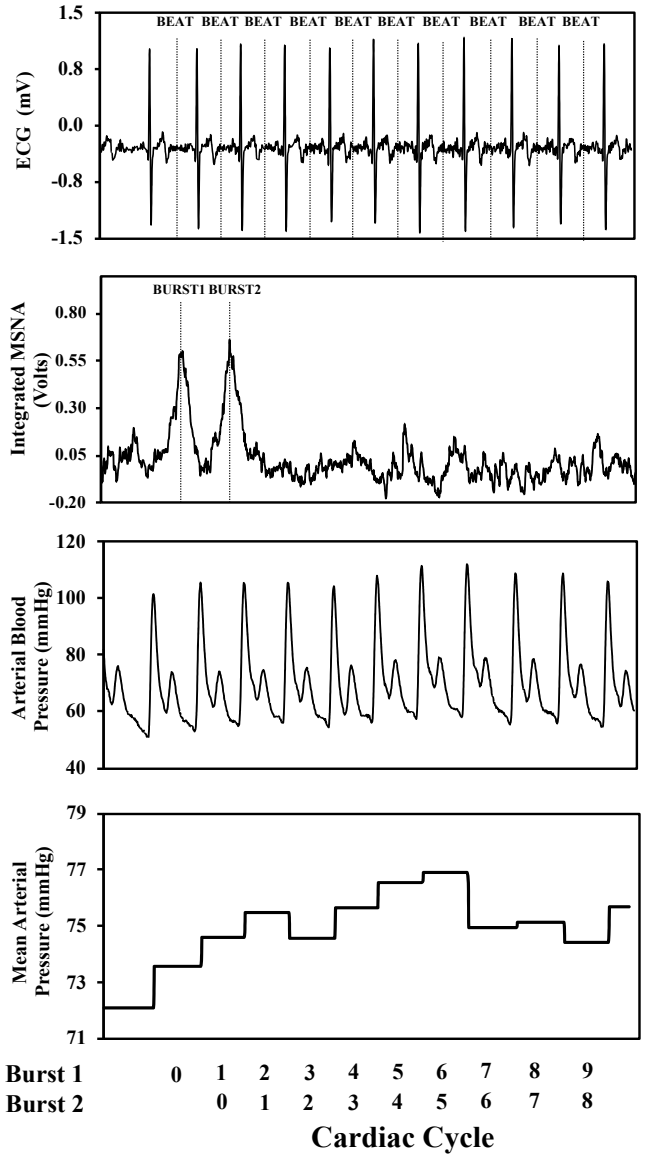

Figure 2.5.4. Example muscle sympathetic nerve activity (MSNA) file with the electrocardiogram (ECG; top panel), integrated MSNA (second panel), beat-by-beat arterial pressure (Portapres; third panel), and the derived mean arterial pressure (MAP; bottom panel) are presented. The 'ECG' and 'Portapres' channels were time-aligned to each other. A analysis macro input BEAT and BURST comments to denote each cardiac cycle and eacch burst of MSNA. The 'Integrated MSNA' channel was aligned with the "BEAT" comments. MAP was determined for each cardiac cycle. The changes in MAP are tracked for each burst over 12 cardiac cycles, with the cardiac cycle associated with the greatest peak average increase in MAP across all bursts representing spontaneous sympathetic transduction. NOTE: the 2 bursts are numerically labelled in this example ('BURST1' and BURST2') to help distinguish the corresponding 'cardiac cycle 0' values for each burst.

Of note, a second method to analyzing spontaneous sympathetic transduction was introduced by Briant et al. (24), which equates a vascular outcome (diastolic blood pressure) to the relative normalized burst area or height (percentage of largest burst) that occurs within a fixed 6-8 cardiac cycles window before each diastolic blood pressure value. In cases where there are multiple bursts within the 6-8 cycle 'window', the individual burst heights/areas are summed. Normalized MSNA burst activity is then ordered into 1% bins (1%•s bins for burst area) and plotted against the corresponding diastolic pressure via linear regression weighted for the number of bursts in each bin. The slope of this regression is used to represent sympathetic transduction. This method is largely reliant on the size of the burst, which proportionately influences the magnitude of the pressor and vascular response (72, 269, 304). However, it does not consider the pressure responses to non-bursts, which may yield important information regarding blood pressure regulation during sympathetic quiescence and ensures that pressor responses do not occur following non-bursts (i.e., internal control). As well, this method assumes that the burst activity 6-8 cardiac cycles prior is responsible for the vascular outcome (diastolic blood pressure). However, it should be noted that there is high inter-individual variability with the peak response occurring often 5-9 cardiac cycles following a burst, but sometimes even earlier or later (4-11 heartbeats) (73, 233, 269). Therefore, this fixed 6-8 cardiac cycle window assumption may underestimate participants' peak spontaneous transduction. This method has been implemented to investigate the impact of untreated hypertension on sympathetic transduction (141). The signal-averaged transduction method described in Vianna et al. (302) has been frequently implemented to provide important information regarding sympathetic regulation of the vasculature between sexes

(46, 114), aerobic fitness levels (200), sodium intake levels (9), and races (304), as well as with pregnancy (269), type 2 diabetes (322), statin therapy (322), and aging (302).

2.5.4.1 Reflex-Mediated Sympathetic Transduction in Older Adults

The transduction of MSNA into vascular outcomes has been studied using sympathoexcitatory stimuli (e.g., handgrip exercise, lower-body negative pressure, infusion of α-adrenergic agonists) between younger and older adults (56, 106, 276). Using a progressive lower-body negative pressure protocol (-5 to -40 mmHg in 5 mmHg increments for 5 min per increment), Davy et al. (56) observed a ~65% lower ratio of forearm vascular resistance to MSNA (Δforearm-resistance/ΔMSNA) in healthy older males versus younger males. Similarly, by analyzing the forearm blood flow responses to separate intra-arterial infusions of norepinephrine and tyramine (evokes endogenous norepinephrine release), Hart et al. (106) observed a positive relationship between the Δforearm blood flow and MSNA burst incidence in young males (r=0.82), but no relationship was observed in older males (r=0.19). Furthermore, Tan et al. (276) introduced an autoregressive model to estimate sympathetic transduction during isometric handgrip exercise to fatigue (35% of maximal voluntary handgrip force) using beat-by-beat arterial pressure, MSNA, and popliteal blood flow. Using this method, older males exhibited a 3-fold lower sympathetic neurovascular transduction values than younger males, despite similar handgrip time to fatigue and sympathetic responses to handgrip (i.e., ΔMSNA) (276). Altogether the results of these studies suggest that the reflex-mediated increases in limb vascular flow are lower in older males versus young males due to attenuated vasoconstrictor responsiveness to sympathetic stimulation. A recent study by Young et al. (321) highlights the fundamental differences between sympathetic

transduction assessed in response to stress versus spontaneous rest, and proposes that information derived from one of these assessments may not be indicative of the other.

2.5.4.2 Spontaneous Sympathetic Transduction in Older Adults

Studies implementing spontaneous sympathetic transduction methods have considered the influence of healthy aging. These age-associated changes in spontaneous sympathetic transduction may be dependent upon the method of analysis (24, 302). Briant et al. (24) observed that females exhibit an age-related *increase* in neurohemodynamic transduction using their regression-slope based analysis. However, the beat-by-beat tracking method of Vianna et al. (302) observed *attenuated* pressor responses in older females (average: ΔMAP: 1.2 mmHg) versus young females (ΔMAP: 2.6 mmHg). It is possible that the method utilized (Briant: relying on burst area/height or 2 cardiac cycle window; Vianna: tracking each burst without a window) explains these divergent findings, with no study to date directly comparing these two methods of analysis. Of note, both studies were conducted in females free of chronic disease with relatively similar resting blood pressure (MAP: 90-100 mmHg) and MSNA burst frequency in young females (15-20 bursts/min). However, MSNA burst frequency was higher among older females in Briant et al. (24) (~38 versus ~28 bursts/min). Resting MSNA burst frequency has been shown to be inversely related with beat-by-beat sympathetic transduction in studies conducted in young adults only (113, 233). However, it is unlikely that the between-study differences in resting MSNA burst frequency fully explain the 2.0-2.5-fold greater sympathetic transduction in older versus young females between the two methods. Certainly, studies using α-adrenergic blockades incorporating both analytical methods are needed to truly elucidate the influence of aging on the sympathetic

regulation of vascular resistance in females. In young adults, the vascular responses to bursts of sympathetic activity using the Vianna method were abolished following the administration of α-adrenergic receptor blockades (phentolamine) with or without additional co-infusion of angiotensin II to control for the elevation in resting blood flow from phentolamine alone (72).

Unlike young females, who demonstrate greater β_2-adrenergic-mediated dilation in response to norepinephrine, young and older males exhibit clear α-adrenergic-mediated vasoconstriction (24, 106). Older males demonstrate a decrease in the magnitude of sympathetic transduction versus young males, regardless of the analytical method utilized (24, 302). Generally, a greater magnitude of neurohemodynamic transduction, as observed with higher sodium intake (vs moderate sodium) (9), in African American young males (vs Caucasian males) (304), or in patients with type 2 diabetes (vs. patients free of disease) (322), has been proposed as a contributing mechanism for greater blood pressure dysregulation and responsible for the increased prevalence of hypertension in these populations. Specifically, the greater spontaneous MSNA-induced surges of arterial blood pressure may result in end-organ damage that contributes to the accelerated development of hypertension. Therefore, it may be counter-intuitive that older males demonstrate a lower sympathetic transduction than young male. This observation may be due to decreased α-adrenergic sensitivity in older males (35) or less norepinephrine release per burst (60). Tan et al. (276) suggest that the blunted vascular response to bursts of MSNA with aging may contribute to the greater resting MSNA in males required to support the maintenance of arterial pressure (e.g., higher burst frequency but lower vascular response per burst). Alternatively, Briant et al. (24) propose

that sympathetic transduction may be less important for males and that alternative mechanisms must be involved in the age-related increase in blood pressure among males.

Altogether, the contribution of sympathetic transduction to the development of hypertension is unclear, particularly since studies have been conducted mostly in younger adults who are less susceptible to blood pressure dysregulation than older adults. However, there is evidence that these responses may be influenced by sex (46, 114) and clearly impacted by diet (9), race (304), pregnancy (269), type 2 diabetes (322), pharmacological interventions (i.e., statins) (322), and healthy aging (24, 302). Studies examining pressor responses to bursts of MSNA on a beat-by-beat basis in an older adult population are warranted given that this analysis provides high-resolution information beyond simply MSNA burst frequency regarding the sympathetic control of arterial blood pressure and vascular tone.

2.6 Impact of Aerobic Fitness on Sympathetic Nerve Activity in Older Adults

Regardless of sex, aging is associated with higher basal MSNA (133). Interestingly, Ng et al. (184) observed that healthy older males and older females who were more aerobically fit ($\dot{V}O_2$max ~40-45 ml/kg/min) and physically active exhibited greater MSNA burst frequency and burst incidence than their less fit/active counterparts (~30-35 ml/kg/min). Conversely, Studinger et al. (271) observed lower resting MSNA burst frequency (but not burst incidence) in Master's athletes (~49 ml/kg/min) versus less aerobically fit age-matched controls (~30-35 ml/kg/min). Although not immediately clear, these conflicting findings may be due to the sex distributions, with the Master's athletes in Studinger et al. (271) being males only, whereas Ng et al. (184) included a combination of fit males and females. In support of this, the between-group differences in

Ng et al. (184) were driven primarily by female participants (trained females: ~48 bursts/min; untrained females: ~25 bursts/min), with similar MSNA burst frequencies between trained and untrained males. In contrast, Baker et al. (10) observed a positive relationship between aerobic fitness and both resting MSNA burst frequency and the change in arterial pressure following ganglionic blockade (infusion of trimethaphan) in young females, that was not replicated in older females suggesting that aerobic fitness may have a lesser role in the autonomic regulation of arterial pressure in older females. However, this is certainly an area worthy of further study, as highlighted in a recent review on the topic (138).

In middle-aged adults (52-59 years), more and less aerobically fit males exhibited similar resting MSNA burst frequency, arterial pressure, and sympathetic baroreflex sensitivity (305). However, the more aerobically fit group had a higher burst incidence (i.e., bursts/100 heartbeat or probability of a burst occurring) than the low fit group (305) due to a lower resting heart rate (43 versus 56 beats/min). Accordingly, higher aerobic fitness was associated with a neural remodelling of the sympathetic baroreflex arc, whereby the arterial baroreflex was shifted upwards (i.e., higher operating point) but not shifted to the right or left (i.e., same arterial pressure). In older adults with cardiovascular disease who present with even higher MSNA burst frequency/incidence, exercise training may lower resting MSNA (189). Specifically, Sales et al. (236) recently demonstrated that 12-weeks (3 sessions per week) of MICT (heart rate between anaerobic threshold and respiratory compensation point) and a progressive HIIT protocol (isocaloric: 200 kcal/session) lowered resting MSNA incidence by 8 bursts/100 heartbeats and 19 bursts/100 heartbeats, respectively in heart failure patients with reduced ejection fraction.

Interestingly, the changes in MSNA (ΔMSNA) were inversely correlated to the changes in brachial artery FMD (ΔFMD; index of NO) (236), suggesting that increased NO bioavailability was associated with a reduction in sympathetic nerve activity-induced peripheral vasoconstriction in patients with heart failure. The influence of aerobic fitness or aerobic exercise training on the interactions between endothelial function and MSNA has yet to be investigated in healthy older adults.

Although not in an older population, aerobic fitness influences reflex-mediated and spontaneous transduction in middle-aged and younger adults. In middle-aged males (~50-55 years), lower-body negative pressure-induced increases in forearm vascular resistance were positively related to MSNA burst frequency in less aerobically fit (~26 ml/kg/min), but these were unrelated amongst higher-fit middle-aged males (~43 ml/kg/min) (190). Importantly, resting MSNA burst frequency was similar between the lower- and higher fit middle-aged males (190). Recent results from our laboratory demonstrated that aerobic fitness was inversely associated with spontaneous sympathetic transduction in young males (200), whereby males with higher aerobic fitness exhibited an attenuated pressor response to a burst of MSNA than males with lower aerobic fitness, despite similar resting MSNA burst frequencies. Conversely, in a separate study (130), young males of higher (~57 ml/kg/min) and lower (~45 ml/kg/min) aerobic fitness demonstrated similar reductions in arterial pressure between these groups following ganglionic blockade (infusion of trimethaphan), indicating that aerobic fitness level did not influence the autonomic support of arterial pressure in this sample (130). Of note, an ~16-week exercise intervention (50-70% heart rate reserve for 25-40 mins/session, 3-4 sessions/week) in pregnant females maintained sympathetic transduction and MSNA

burst frequency, whereas the non-exercise pregnant female group increased MSNA burst frequency but exhibited a reduced neurohemodynamic transduction (258). Altogether, our understanding of the transduction of bursts of MSNA into a vascular outcome has been primarily limited to younger populations, with some conflicting reports as to whether this is impacted by aerobic fitness level.

In comparison to young adults, healthy older adults may exhibit decreased α-adrenergic sensitivity (35) and less norepinephrine release per burst (60). Research in animal models demonstrated that α-adrenergic sensitivity or density may be attenuated with greater aerobic fitness, with both young and older male Fischer rats demonstrating reduced α-receptor-mediated vasoconstriction following an exercise training program (15 m/min at 15% incline, 60 min/day, 5 days/week for 10-12 weeks) (64). Whether aerobic fitness influences sympathetic transduction in older adults, of who present with a higher MSNA burst frequency (176, 183) and lower NO bioavailability (245) than young adults is unknown. Uncovering the relationship between aerobic fitness and the regulation of arterial blood pressure through the sympathetic nervous system may provide insight into a potential mechanism regarding the cardiovascular benefits of higher aerobic fitness in older adults.

2.7 Overall Thesis Rationale, Objectives and Hypotheses

The following studies are proposed to determine the impact of cardiorespiratory fitness and aerobic exercise training on peripheral vascular function in older adults. The below studies and forthcoming chapters are based on the corresponding articles below each section. Some modifications exist between the journal version of the manuscript and the following chapters. A large, noteworthy change has been the the removal of vasoactive range (sum of FMD and L-FMC) from Study 1 and Study 2 in the present thesis. Vasoactive range is proposed as a 'barometer' of vascular health. Within-group changes or between-group differences in vasoactive range alone are unable to differentiate whether such discrepancies are attributed to greater vasodilator or vasoconstrictor function. This is particularly important because different mechanisms are responsible for FMD (i.e., NO) and L-FMC (i.e., endothelin-1, prostaglandins, endothelial-derived hyperpolarizing factor). Given that the magnitude of relative FMD is often greater than relative L-FMC values, vasoactive range is more dependent on FMD than L-FMC. Finally, neither FMD nor L-FMC represent the maximum vasodilator and vasoconstrictor responses, respectively. Therefore, an individuals' actual vasoactive range cannot be truly determined using the FMD technique and therefore have been removed.

An additional change has been the inclusion of ultrasound-derived measures of microvascular function into Study 4, which primarily relies on microneurography determined sympathetic nervous system. The ultrasound-based results were not presented in the published manuscript but are presented in this thesis.

Study 1: Aerobic fitness may be directly related to favorable brachial artery vasodilatory and vasoconstrictor endothelial function in young adults. We aim to determine if such relationships exist in older adults who experience age-related declines in both aerobic fitness and brachial artery endothelial function. I tested the hypothesis that aerobic fitness level was positively associated with brachial artery FMD, but inversely related to brachial artery L-FMC in older adults.

O'Brien, Myles W., Said Mekary, Susan A. Robinson, Jarrett A. Johns, and Derek S. Kimmerly. 2019. The relationship between aerobic fitness and low-flow-mediated constriction in older adults. *European Journal of Applied Physiology* 119(2): 351-359.

Study 2: Endothelial function is typically determined in the brachial artery, but lower-limb arteries such as the popliteal artery are a common site for plaque/aneurysm development and directly exposed to robust increases and decreases in local blood flow during aerobic exercise and sedentary activities, respectively. I aimed to determine if the popliteal artery exhibited an L-FMC response, how popliteal endothelial function compared to brachial artery function, and the association between popliteal endothelial function with aerobic fitness. I tested the hypothesis that L-FMC responses were unique to the vascular bed and that aerobic fitness was inversely related to popliteal artery L-FMC.

O'Brien, Myles W., Jarrett A. Johns, Susan A. Robinson, Said Mekary, and Derek S. Kimmerly. 2019. Relationship between brachial and popliteal artery low-flow-mediated constriction in older adults: impact of aerobic fitness on vascular endothelial function. *Journal of Applied Physiology* 127(1): 134-142.

Study 3: Aerobic and resistance exercise training interventions have been utilized to improve brachial artery FMD in older adults with chronic conditions, with HIIT documented to elicit greater improvements than MICT. The influence of these training regimes on lower-limb endothelial function, L-FMC in either artery, or brachial FMD in otherwise healthy older adults was unknown. I tested the hypothesis that HIIT would elicit superior improvements in both brachial and popliteal function versus MICT, and that these aerobic protocols would result it greater endothelial-dependent vasodilator responses than RT. It was unclear whether endothelial-dependent vasoconstrictor function in either artery would be altered with exercise training in this population.

O'Brien, Myles W., Jarrett A. Johns, Susan A. Robinson, Amanda Bungay, Said Mekary, and Derek S. Kimmerly. 2020. Impact of High-Intensity Interval Training, Moderate-Intensity Continuous Training, and Resistance Training on Endothelial Function in Older Adults. *Medicine and Science in Sports and Exercise* 52(5): 1057-1067.

Study 4: Optimal arterial function requires a balance between endothelial-derived vasodilators and sympathetic neural vasoconstrictor activity. Healthy ageing is associated with an increase in MSNA but a decrease in both endothelial function and aerobic fitness. Aerobic fitness is inversely related to the pressor responses to a burst of MSNA in the peroneal nerve of young males. I will tested the hypothesis that aerobic fitness would be inversely associated with sympathetic transduction and explored the association with traditional MSNA burst characteristics (e.g., burst frequency). I determined if these

expected relationships were influenced by popliteal macrovascular (flow-mediated dilation) or microvascular (reactive hyperemia) endothelial function.

O'Brien, Myles W., Diane J. Ramsay, Carley D. O'Neill, Jennifer L. Petterson, Shilpa Dogra, Said Mekary, and Derek S. Kimmerly. 2021. Aerobic fitness is inversely associated with neurohemodynamic transduction and blood pressure variability in older adults. *GeroScience* 43(6): 2737-2748.

CHAPTER 3

STUDY 1: THE RELATIONSHIP BETWEEN AEROBIC FITNESS AND BRACHIAL LOW-FLOW-MEDIATED CONSTRICTION IN OLDER ADULTS

Chapter 3: Study 1: ABSTRACT

In young adults, aerobic fitness is directly related to favorable brachial endothelial-dependent vasodilatory (i.e., flow-mediated dilation; FMD) and vasoconstrictor (i.e., low-flow mediated constriction; L-FMC) responses. Furthermore, aerobically fit older adults have larger brachial FMD responses than their less fit peers. However, the relationship between aerobic fitness and brachial L-FMC is unknown in older adults. I hypothesized that more aerobically fit older adults would exhibit a greater brachial FMD and L-FMC responses than their less fit counterparts. Forty-seven healthy older adults (67 ± 5 years) were divided into less (LF; *n*=27, 18.3 ± 3.2 ml/kg/min) and more aerobically fit (MF; *n*=20, 29.1 ± 5.8 ml/kg/min; $P < 0.001$) groups based on maximal cycling-based peak oxygen consumption ($\dot{V}O_2$peak, via indirect calorimetry). Brachial FMD and L-FMC were assessed via high-resolution duplex ultrasonography. A larger brachial L-FMC was observed in the MF versus LF groups (–1.2 ± 0.9 versus –0.5 ± 0.6%; $P = 0.01$). Furthermore, the MF group had an enhanced relative FMD response (5.6 ± 1.5 vs. 3.9 ± 1.2%; $P < 0.001$). In the pooled sample, there was a negative correlation ($r = –0.52$; $P < 0.001$) between $\dot{V}O_2$peak (22.9 ± 7.0 ml/kg/min) and L-FMC (–0.8 ± 0.8%). In an older population, greater aerobic fitness was associated with a more favorable endothelial-dependent vasoconstrictor response to reduced blood flow. Interventional or longitudinal aerobic exercise training studies are warranted in this population to determine the impact of training-induced increases in $\dot{V}O_2$peak on L-FMC.

Chapter 3: Introduction

Aging is associated with chronic low-grade inflammation, increased oxidative stress and an impaired ability to produce endothelial-derived vasodilatory autacoids such as nitric oxide (299). Collectively, these factors lead to vascular endothelial dysfunction, the development of atherosclerosis and a greater cardiovascular disease risk (89). The flow-mediated dilation (FMD) technique provides a clinical assessment of conduit artery endothelial-dependent vasodilatory function (96) and is predictive of future cardiovascular events (124). This non-invasive, ultrasound-based procedure measures conduit arterial diameter increases in response to a reactive hyperemia (i.e., increased shear stress) induced by a prior period of distal ischemia (285). However, the frequently observed endothelial-dependent vasoconstrictor response during the ischemic period, termed low-flow-mediated constriction (L-FMC), is relatively understudied. L-FMC has been shown to be partly mediated through the endothelium (57), via the inhibition of vasodilatory signaling via endothelial-derived hyperpolarizing factors and prostaglandins (90), as well as enhanced vasoconstrictor signaling via endothelin-1A receptors (264). Endothelin-1 levels are typically lower in older adults who are more aerobically fit (191) and aerobic exercise training improves vasodilatory signaling of endothelial-derived hyperpolarizing factors (169) and prostaglandins (265) in aged rodent models. L-FMC may provide additional information regarding the role of reduced shear stress on resting diameter and vascular tone (90), is attenuated in individuals with risk factors for coronary artery disease (102), and improves the sensitivity and specificity for detecting patients with cardiovascular disease when complemented with FMD (91).

Aerobic fitness is associated with more favorable brachial artery FMD responses in older populations (67). There is a paucity of research investigating the effects of aerobic fitness on the L-FMC response despite current guidelines recommending that lumen diameter be measured throughout the ischemic (low-flow) portion of the FMD test (285), suggesting that some researchers may be overlooking, or unaware of, this important measure of vasoconstrictor function. Interventional studies have demonstrated that short-term (i.e., 6-8 weeks) continuous aerobic exercise augments brachial artery L-FMC in young healthy adults (224) and young obese adults (240). Recently, a cross-sectional study demonstrated that higher estimated peak oxygen consumption ($\dot{V}O_2peak$) was associated with greater brachial L-FMC in young men (13). It is unknown whether this relationship also exists in older adults.

A limitation in the current state of knowledge regarding the influence of aerobic fitness on endothelial-dependent vasoconstrictor function is that it has been conducted exclusively in young adult populations (13, 224, 240). Older adults experience structural and functional changes to the vascular endothelium that decreases brachial artery vasoconstrictor and vasodilator responsiveness, increasing their risk of cardiovascular events (19, 188). Considering the potential clinical importance of L-FMC, there is an inherent need to investigate the relationship between aerobic fitness and upper-limb vasoconstrictor function in a population that experiences age-related declines in endothelial function, which may lead to the development of peripheral vascular disease. Herein, the purpose of this study was to test the hypothesis that older adults who are more aerobically fit will exhibit greater brachial artery FMD and L-FMC responses than their less aerobically fit peers.

Chapter 3: Methods

Participants:

Forty-seven older adults were recruited from the Active Aging program at Acadia University (Table 1). $\dot{V}O_2$peak was assessed via indirect calorimetry during a progressive cycle ergometer protocol as described in more detail below. Participants were divided into more aerobically fit (MF; $n = 20$) or less aerobically fit (LF; $n = 27$) groups. LF was defined as a $\dot{V}O_2$peak that equated to "poor" according to the Canadian Society for Exercise Physiology age, and sex-specific aerobic fitness classifications (49). Specifically, poor was defined as <23.5 ml/kg/min for both males and females between the ages of 60 to 69 years. MF was defined as a $\dot{V}O_2$peak that equated to "fair" or greater (i.e., > 23.5 ml/kg/min). The breakdown of the specific MF group classifications were as follows: fair ($n = 13$), good ($n = 3$), very good ($n = 3$) and excellent ($n = 1$). Participants were cleared for moderate-vigorous physical activity using the Physical Activity Readiness-Questionnaire plus (308). Twenty-one individuals from a previously published report in my lab that investigated the relationship between physical activity and FMD were included in the present study (202). The protocols and procedures conformed to the Declaration of Helsinki and were approved by the Dalhousie University Health Sciences and Acadia University Research Ethics Boards. Participants were informed of the methods and study design verbally and in writing before providing written informed consent.

Participants had no physical limitations to exercise and a resting blood pressure < 140/90 mmHg. Five participants in the LF group were on Synthroid® for hypothyroidism. Four participants in the LF group were on blood pressure medications.

Specifically, participants were prescribed Teveten® (angiotensin-receptor blocker; n=1), Adalat (calcium channel blocker; n=1); Diuril® (diuretic; n=1) and Coversyl® Plus (angiotensin converting enzyme inhibitor + diuretic; n=1). One person in the LF group was asthmatic. Participants were requested to continue taking all prescribed medications throughout the duration of the study.

Experimental Design:

All 47 participants underwent 2 separate laboratory visits. Visit 1 involved measurements of height and body mass, which were followed by a graded, maximal cycling exercise test to determine $\dot{V}O_2peak$ (see below for details). Visit 2 was dedicated to the assessments of brachial artery vascular function and conducted either before, or between 48 hours and one-week following, the graded exercise test. To minimize confounding influences on endothelial-dependent dilation, vascular assessments were performed 6 hours post-prandial, and participants avoided strenuous physical activity, as well as the consumption of products known to acutely influence FMD responses (e.g., caffeine, chocolate, citrus fruits, saturated fats, folic acid supplements, antioxidant and multivitamin supplements) for 24 hours, consistent with FMD guidelines (285). All study visits were performed in a thermoneutral environment (21°C). To control for diurnal variations in blood pressure and vascular function, Visit 2 was performed at the same time of day for the MF and LF groups (129).

Experimental Protocol:

Anthropometrics and Peak Aerobic Fitness:

Height and weight were measured using a calibrated stadiometer (Health-O-Meter, McCook Il, USA) to the nearest 0.5 cm and 0.1 kg, respectively. Body mass index

(BMI) was calculated as weight/height^2.An incremental and maximal exercise test on a cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands) was administered to determine $\dot{V}O_2$peak via a mixing chamber-based commercial metabolic system (TrueOne 2400®, Parvomedics Inc., Sandy, UT). Following a 5-min warm-up period of light-intensity cycling (30-50W), the workload was initially set at 1 watt per kilogram of body weight and gradually increased by 15 watts every minute until voluntary exhaustion. Strong verbal encouragement was provided throughout the test. Upon completion of the test, the workload was immediately reduced to the warm-up level for a 5-min cool-down period. Relative $\dot{V}O_2$ data were averaged over 15-second intervals for the duration of the graded exercise protocol. $\dot{V}O_2$peak was considered as the greatest 30-second average. All participants achieved ≥2 of the following $\dot{V}O_2$peak/$\dot{V}O_2$max criteria: 1) a plateau in relative $\dot{V}O_2$ (<2.1 ml/min/kg) despite an increase in workload, 2) a respiratory exchange ratio ≥1.10, or 3) a rating of perceived exertion ≥18. Given that not all participants exhibited a plateau in relative $\dot{V}O_2$ (primary criteria for $\dot{V}O_2$max), the term $\dot{V}O_2$peak was used throughout.

Systemic Hemodynamics:

Heart rate (HR) was determined via cardiac intervals obtained from lead II of a bipolar electrocardiography configuration. Beat-by-beat systolic (SBP) and diastolic (DBP) blood pressures were measured using finger photoplethysmography (Portapres®; Finapres Medical Systems, Amsterdam, Netherlands). Brachial measurements of SBP and DBP were also recorded by an automated patient vital signs monitor (Carescape v100®, General Electric Healthcare) and used to perform a 'physiological calibration' of the Portapres® waveform. SBP and DBP were determined from the Portapres® waveform

as the maximum and minimum waveform values, respectively. These pressures were then used to calculate mean arterial pressure (MAP) using the equation ⅓ SBP + ⅔ DBP. All data were sampled continuously at 400 Hz using a PowerLab (PL3508 PowerLab 8/53, ADInstruments, Sydney, Australia) data acquisition system with the exception of the electrocardiography waveform, which was sampled at 1000 Hz. Recordings were displayed in real-time and analyzed offline using LabChart software (Version 8, ADInstruments, Sydney, Australia).

Vascular Measures:

As described in O'Brien et al. (202) the right brachial artery was imaged 3-5 cm proximal to the antecubital fossa with participants in the supine position. A pressure cuff attached to a rapid cuff inflation system (E20 and AG101, Hokanson®, Bellevue, WA) was positioned around the largest circumference of the forearm (~3 cm distal to the antecubital fossa). All images were obtained using a 12-MHz multi-frequency linear array probe attached to a high-resolution ultrasound system (Vivid i, General Electric Healthcare). Simultaneous blood velocity signals were recorded in duplex mode at a pulsed frequency of 5-MHz and corrected with an insonation angle of 60° that remained constant throughout the study. The sample volume was adjusted for each participant such that the anterior to the posterior intima were included, as recommended in published guidelines (285). Artery lumen diameter and blood flow velocity were measured for a minimum of 2 minutes before inflation of the pneumatic cuff. The pressure cuff was then rapidly inflated to 250 mmHg for 5 minutes. Continuous arterial lumen diameter and blood flow velocity recordings were collected throughout the cuff inflation period. Upon

release of cuff pressure, lumen diameter and velocity recordings continued for an additional 5 minutes.

All vascular measurements were blindly analyzed by MWO who has demonstrated an intra-tester analyzing reproducibility of 2.2%, 2.7%, 3.8% and 4.2% for baseline diameter, nadir diameter, L-FMC% and FMD%, respectively. Specifically, MWO blindly analyzed the same ultrasound recording for 20 participants on two different occasions and the variation was calculated for each recording as [(difference between 2 measurements ÷ average value) × 100%]. This intra-tester reliability is consistent with other studies using automatic edge-detection software (228).

Video signals from the ultrasound were exported to a laptop via a video graphics array converter (Epiphan Systems Inc., VGA 2 USB, Ottawa) for offline analysis. Analysis of artery diameter, blood flow velocity and shear rate (i.e., frictional force of blood flow on the endothelium) were performed using automated commercial edge-detection and wall-tracking software (FMD Studio, Cardiovascular Suite, Quipu, Pisa, Italy). This software was used to measure baseline diameter, nadir diameter and peak diameter. All vascular measurements were blindly analyzed by MWO who has demonstrated an intra-tester coefficients of variation (CV) of 2.2%, 2.7%, 3.8% and 4.2% for baseline diameter, nadir diameter, L-FMC% and FMD%, respectively. Blood flow was calculated as mean blood flow velocity × 60 × π × lumen radius^2.

Absolute FMD (in mm) was calculated as: post-cuff deflation peak diameter – baseline diameter. Relative FMD was calculated using the equation: FMD (%) = [(post-cuff deflation peak diameter – baseline diameter) ÷ baseline diameter × 100%]. Shear rate (SR, s^{-1}) was defined as [8 × Mean blood velocity (cm/s)] / diameter (cm). Subsequently,

the SR area under the curve (SR_{AUC}) was calculated between the start of cuff deflation to the time that peak dilation occurred. Time to peak diameter was recorded. The correlation between the SR_{AUC} and the FMD response was $r = 0.43$ ($P = 0.003$), but the 95% confidence intervals of the y-intercept did not encompass zero (1.0-3.9) in the pooled sample. As such, FMD data were not normalized to SR_{AUC} (209). As described by Atkinson and Batterham (7), allometric scaling has been recommended to account for differences in arterial diameter. However, the relationship between the natural log of peak FMD diameter and resting diameter across groups yielded an unstandardized β-coefficient that did not deviate from 1 and had an upper 95% confidence interval that was >1, suggesting allometric scaling of FMD to be unnecessary in the this present study. Specifically, the β-coefficient ± standard error (95% confidence interval) for the FMD measurement was 1.03 ± 0.01 (1.00 - 1.05). However, allometric criteria were met for L-FMC with a regression value of 0.98 ± 0.01 (0.97 – 0.999). Allometrically scaled L-FMC data were analyzed using an analysis of covariance model with the natural log of the difference [ln(nadir diameter) – ln(baseline diameter)] as the dependent variable, group (MF, LF) as the fixed factor, and ln(baseline diameter) as the covariate. Significant findings were followed with pairwise comparisons using Bonferroni post-hoc testing. For each group, the allometrically-scaled brachial L-FMC responses were back transformed and presented as a percentage reduction in diameter from baseline.

L-FMC was represented in absolute (mm) and relative (%) terms using the nadir diameter obtained during the final 30 seconds of the 5-minute distal occlusion period. Relative L-FMC was calculated using the equation: L-FMC (%) = [(baseline diameter – nadir diameter) ÷ baseline diameter ×100%] (13, 224).

Statistical Analyses:

All data were assessed for normality using a Shapiro-Wilk test and found to be normally distributed. Descriptive variables are presented as means ± standard deviations. Independent-samples t-tests compared baseline characteristics, resting hemodynamics and vascular measures between the LF and MF groups. Effect sizes (ES) were calculated for the vascular measurements between the LF and MF groups (i.e., $MF_{mean} - LF_{mean} \div$ pooled sample standard deviation). Small, medium and large effect sizes were defined as 0.2, 0.5, 0.8, respectively (45). Pearson product moment correlational analyses were performed between FMD(%), L-FMC (%), and aerobic fitness ($\dot{V}O_{2peak}$) in the pooled sample. Correlational analyses were computed to determine the association between baseline diameter versus FMD(%) and L-FMC (%).

Some participants in the LF group were taking anti-hypertensive medication (n = 4), which may have influenced their vascular function. However, the removal of these individuals from the analyses did not change the strength of the correlations between aerobic fitness and our vascular measures, nor the magnitude of the differences between the MF and LF groups.

All statistics were completed in SPSS Version 23.0 (IBM, NY) statistical program. Based on previous L-FMC data in young adults (Cohen's d = 1.06) (13), it was estimated that a minimum of 16 participants in each group were required to achieve sufficient statistical power using a two-tailed alpha = 0.05 and assuming 80% power. Statistical significance was accepted as $P < 0.05$. All data are presented as means ± standard deviations (SD).

Chapter 3: Results

Participant characteristics and hemodynamics are summarized in Table 3.1. Age, body mass index, and resting HR were all similar between the MF and LF groups ($P >$ 0.11). Compared to the LF group, the MF group had a lower resting SBP, DBP, and MAP (all $P < 0.05$). As designed, the MF group had a greater $\dot{V}O_2$peak than the LF group ($P <$ 0.001). More individuals in the MF group attained the criterion for a $\dot{V}O_2$max (60%; $n =$ 12) than in the LF group (41%; $n = 11$).

The between-group comparisons of vascular measures are presented in Table 3.2. Baseline, nadir and peak diameters were all larger in the MF group (all, $P < 0.05$). There were no differences in resting blood flow velocity or resting shear rate between the groups ($P > 0.17$), but resting brachial artery blood flow was greater in the MF group (P = 0.04). The MF older adults had greater FMD (5.7 ± 1.5% vs. 3.9 ± 0.6, $P < 0.001$; ES = 1.29; Figure 3.1A) and L-FMC responses (–1.2 ± 0.9% vs. –0.5 ± 0.6%, $P = 0.01$; ES = –0.83; Figure 3.1B) than the LF group. The larger L-FMC was not attributed to the larger baseline diameter in the MF groups as the allometrically scaled L-FMC was also greater in the MF versus LF group (–1.1 ± 0.8 vs. –0.6 ± 0.8%, $P = 0.03$; ES = –0.63).

The correlations in the pooled sample between brachial FMD and L-FMC versus $\dot{V}O_2$peak are presented in Figure 3.2. Aerobic fitness was positively correlated to relative FMD ($r = 0.59$, $P < 0.001$; Figure 3.2A) and negatively associated with relative L-FMC ($r = –0.52$, $P < 0.001$; Figure 3.2B).

Table 3.1. Study 1 participant descriptive characteristics.

	Less Aerobically Fit (n = 27)	More Aerobically Fit (n = 20)	Entire Sample (n = 47)
Age (years)	68 ± 5	66 ± 4	67 ± 5
Sex (Male, Female)	8♂, 19♀	9♂, 11♀	17♂, 30♀
Body Mass Index (kg/m^2)	27.5 ± 4.6	25.2 ± 3.2	26.5 ± 4.2
Resting Heart Rate (beats/minute)	70 ± 10	66 ± 10	68 ± 10
Systolic Blood Pressure (mmHg)	129 ± 10	120 ± 9*	125 ± 11
Diastolic Blood pressure (mmHg)	74 ± 9	66 ± 8*	71 ± 9
Mean Arterial Pressure (mmHg)	93 ± 7	84 ± 7*	89 ± 8
Maximum Heart Rate (beats/minute)	148 ± 9	153 ± 10	150 ± 10
Peak RER ($\dot{V}CO_2/\dot{V}O_2$)	1.19 ± 0.11	1.23 ± 0.08	1.21 ± 0.10
Aerobic Fitness (ml/kg/min)	18.3 ± 3.2	29.1 ± 5.8*	22.9 ± 7.0

Data are presented as means ± standard deviations. RER, respiratory exchange ratio; $\dot{V}CO_2$, peak volume of expired carbon dioxide; $\dot{V}O_2$, peak volume of oxygen consumed. *, $P < 0.05$ versus Less Aerobically Fit.

Table 3.2. Comparison of brachial artery parameters across the pooled sample, more aerobically fit and less aerobically fit.

	Less Aerobically Fit (n = 27)	More Aerobically Fit (n = 20)	Effect Size	Entire Sample (n = 47)
Resting				
Resting Diameter (mm)	3.76 ± 0.52	4.21 ± 0.76*	0.70	3.95 ± 0.66
Blood Flow Velocity (cm/s)	11.8 ± 4.3	12.8 ± 4.6	0.23	12.2 ± 4.4
Blood Flow (ml/min)	80 ± 37	104 ± 40*	0.63	90 ± 37
Resting Shear Rate (s^{-1})	127 ± 50	128 ± 58	0.02	128 ± 53
Flow-Mediated Dilation				
Peak Diameter (mm)	3.91 ± 0.56	4.45 ± 0.80*	0.79	4.14 ± 0.72
Absolute FMD (mm)	0.15 ± 0.06	0.24 ± 0.08*	1.33	0.19 ± 0.08
SR_{AUC}	11516 ± 3078	13255 ± 4485	0.45	12256 ± 3797
Time to Peak Diameter (s)	64 ± 16	67 ± 23	0.14	65 ± 19
Low-Flow-Mediated Constriction				
Nadir Diameter (mm)	3.74 ± 0.51	4.16 ± 0.73*	0.67	3.92 ± 0.64
Absolute L-FMC (mm)	–0.02 ± 0.03	–0.05 ± 0.05*	–0.86	–0.03 ± 0.04

Data are presented as means ± standard deviations or as effect sizes. FMD, flow-mediated dilation; SR_{AUC}, shear rate area under the curve to peak dilation; L-FMC, low-flow-mediated constriction. *, $P < 0.05$ versus Less Aerobically Fit.

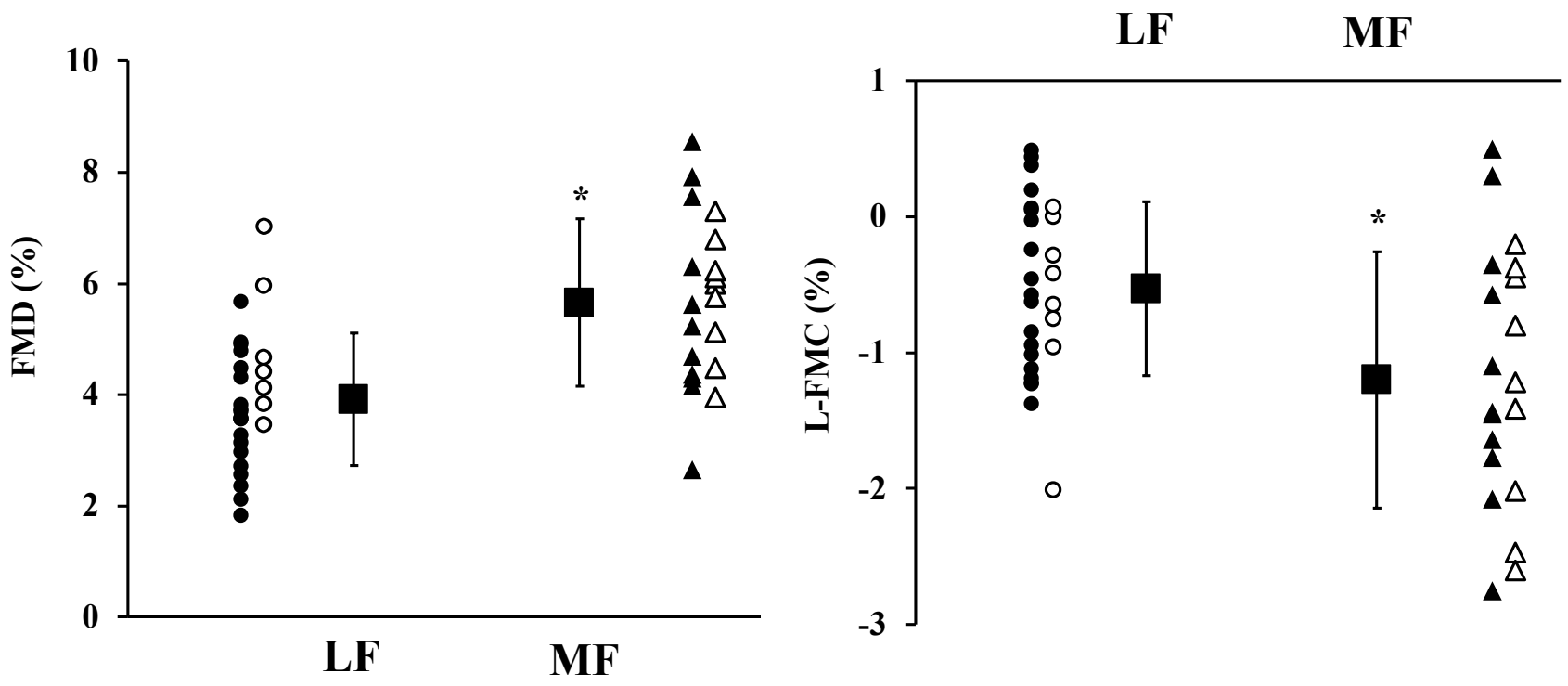

Figure 3.1. Comparison of brachial artery relative flow-mediated dilation [FMD, %; left panel] and low-flow-mediated constriction [L-FMC, %; right panel] between less aerobically fit [LF, white bars] and more aerobically fit [MF, black bars] older adults. Males and females are denoted by the white and black symbols, respectively. Data are presented as means ± standard deviations. *, P < 0.05 versus the lower aerobic fitness group.

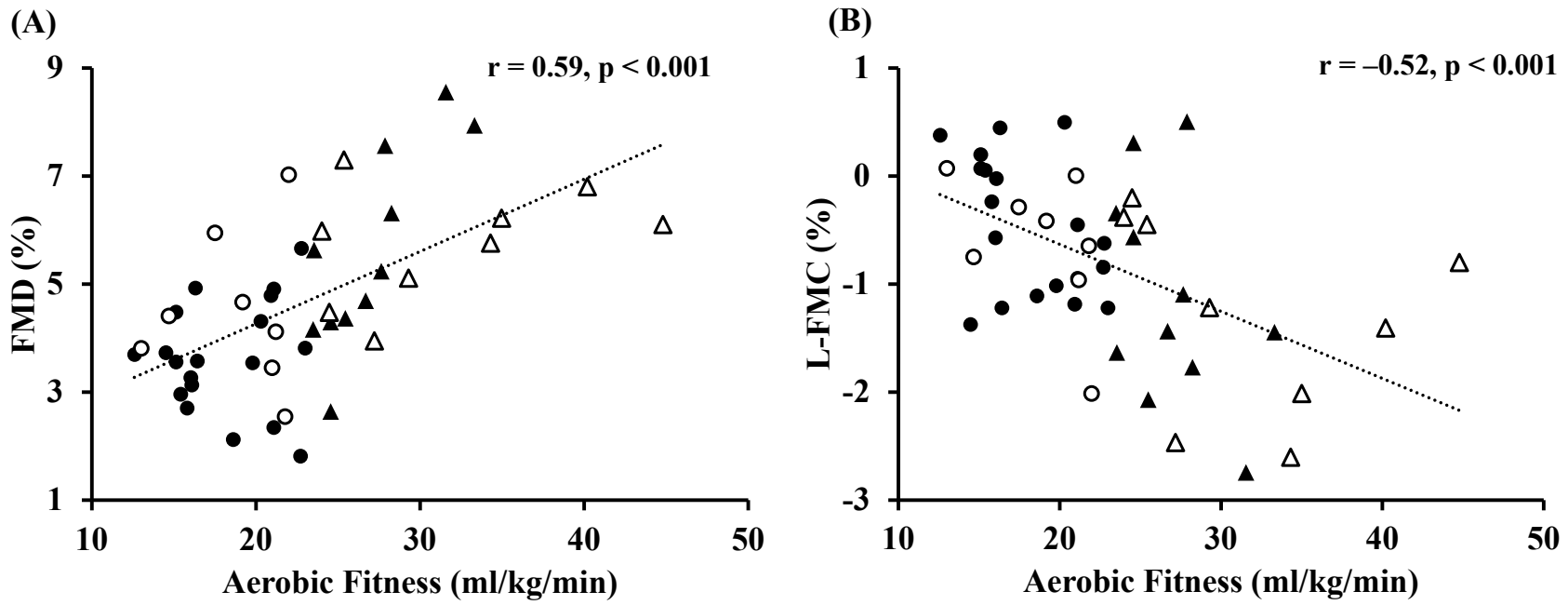

Figure 3.2. Pooled sample correlations between aerobic fitness ($\dot{V}O_2$peak) with (A) brachial artery relative flow-mediated dilation (FMD, %) and (B) relative low-flow-mediated constriction (L-FMC, %). Males and females are denoted by the white and black symbols, respectively. Less aerobically fit participants are denoted as circles and more aerobically fit participants as triangles.

Chapter 3: Discussion

The purpose of this study was to assess the relationship between aerobic fitness and the vasoconstrictor response to low-flow in older adults. Consistent with my hypothesis, aerobic fitness was negatively associated with L-FMC (Figure 3.2B). The vascular endothelium of more aerobically fit older adults appears to be more sensitive to both reductions and increases of shear stress than the vasculature of older adults who are less aerobically fit. This represents the first evidence to highlight that greater aerobic fitness has a beneficial effect on both vasodilatory and vasoconstrictor function in an older population.

My results are consistent with the current literature in that aerobic fitness has been previously associated with augmented vasodilator function (i.e., FMD) in young and older populations (172, 202). However, the relationship between aerobic fitness and L-FMC was less clear. Specifically, the only other cross-sectional study demonstrated a moderate negative correlation ($r = –0.50$) between estimated $\dot{V}O_2$peak and L-FMC in young males (13), which is reflective of the present study that used a maximal aerobic protocol and was comprised of older males and females ($r = –0.52$; Figure 2B). Together, these results lend evidence that the L-FMC frequently observed during the distal ischemic period of the traditional FMD test is moderately dependent upon aerobic fitness with more aerobically fit older adults exhibiting greater vasoconstriction in response to a low-flow/shear stress stimulus. The brachial L-FMC response remained larger in the MF group in comparison to the LF group when allometrically scaled, suggesting the more favorable vasoconstrictor response is not simply due to the MF having a greater baseline diameter. This observation supports the notion that aerobic fitness is likely more

responsible for the greater vasoconstrictor sensitivity in response to low blood flow than artery size in this present study.

The mechanisms responsible for the enhanced L-FMC in aerobically-trained individuals is unclear, but may be due to a greater sensitivity of the vascular endothelium to reductions in shear stress (224). It is likely that older adults who are more aerobically fit have improved endothelium sensitivity to both reductions and influxes of shear stress in older adults, as evident by the greater L-FMC and FMD (Figure 3.1A-B). However, future studies that include markers of endothelial-derived vasodilators (i.e., endothelial-derived hyperpolarizing factor and prostaglandins) and vasoconstrictors (i.e., endothelin-1) are warranted.

As mentioned by Humphreys et al. (122), L-FMC is an appealing and convenient measure considering FMD guidelines recommend imaging the artery throughout the cuff inflation period (116). My study adds to the available evidence by associating aerobic fitness with the 'constrictor-side' of the FMD test and suggesting it, as well as FMD, is greater in older adults who are more aerobically fit. Altogether, accumulating evidence suggests that incorporating a measure of vasoconstrictor capacity may be as important as assessing vasodilator function as an indication of peripheral vascular health in a variety of populations.

As with all cross-sectional studies, I was unable to provide direct evidence between exercise training-induced increases in aerobic fitness with changes in L-FMC. However, the present study was the first to investigate the role of aerobic fitness on L-FMC in an older adult population. As such, interventional or longitudinal studies are needed in this population similar to what has been conducted in younger populations

(224, 240). Also, it is currently unclear if brachial artery L-FMC differs between older males and females. No differences in relative L-FMC were observed between males and females in the MF ($P > 0.13$; 9♂, 11♀) or LF groups ($P > 0.60$; 8♂, 19♀). However, the present study was not designed or powered to test if sex influences the magnitude of vasoconstriction in response to low-flow. Future studies are needed to determine if sex-differences exist in L-FMC in both younger and older adults. Future research should investigate if the relationship between aerobic fitness with vasodilator and vasoconstrictor function remains valid for older individuals of "excellent" aerobic fitness status, such as in Master's athletes. Of relevance, our sample is representative of a typical older Canadian with an average $\dot{V}O_2$peak of ~25 ml/kg/min (268), increasing the generalizability of our results.

In an older population, a greater aerobic fitness was associated with a more favorable vasoconstrictor response to low-flow, which may represent an independent marker of cardiovascular health. Future studies are needed to determine the clinical relevance of L-FMC as a measure of vascular health and the causal relationships associated with aerobic fitness and vasoconstrictor capacity.

LINKING STUDY 1 & STUDY 2

Study 1, entitled, "The Relationship Between Aerobic Fitness and Low-Flow Mediated Constriction in Older Adults" evaluated the association between aerobic fitness level and L-FMC in the brachial artery of older adults. Consistent with my hypothesis, more aerobically fit participants exhibited a larger L-FMC response than those who were less aerobically fit. While this study provided evidence for the association between aerobic fitness and better vascular endothelial health, it was specific to the brachial artery. Unlike upper-limb arteries, lower-limb vessels (e.g., the popliteal artery) are more susceptible to peripheral vascular disease (58). In addition, the lower limb vasculature is responsible for supplying the active limbs involved in common forms of aerobic exercise (e.g., walking, cycling, etc.).

Based on the outcomes of Study 1, Study 2 sought to answer the questions:

1) Do the brachial and popliteal arteries exhibit similar endothelial-dependent responses (FMD and L-FMC)?

2) Is the popliteal L-FMC response also inversely related to aerobic fitness?

CHAPTER 4

STUDY 2: RELATIONSHIP BETWEEN BRACHIAL AND POPLITEAL ARTERY LOW-FLOW-MEDIATED CONSTRICTION IN OLDER ADULTS: IMPACT OF AEROBIC FITNESS ON VASCULAR ENDOTHELIAL FUNCTION

Chapter 4: ABSTRACT

I previously observed that brachial artery (BA) low-flow-mediated constriction (L-FMC) was inversely related to aerobic fitness (i.e., $\dot{V}O_2$peak) in older adults. However, it was unclear if an L-FMC response can be elicited in the popliteal artery (POP) or if a similar inverse relationship exists with aerobic fitness exists. Considering that the POP experiences larger shear stress fluctuations during sedentary behaviors and traditional lower-limb modes of aerobic exercise, I tested the hypotheses that: 1) heterogeneous L-FMC responses exist between the BA versus POP of older adults, and 2) that aerobic fitness was inversely related to POP L-FMC. L-FMC was assessed in 47 healthy older adults (30♀, 67 ± 5 yr) using duplex ultrasonography and quantified as the percent decrease in diameter (from baseline) during the last 30 seconds of a 5-minute distal cuff occlusion period. When allometrically scaled to baseline diameter, the BA exhibited a greater L-FMC response than the POP (–1.3±1.6% vs. –0.4±1.6%; P=0.03). Furthermore, L-FMC responses in the BA and POP were not correlated (r=0.22; P=0.14). $\dot{V}O_2$peak was strongly correlated to POP L-FMC (r=–0.73; P<0.001). The heterogeneous BA versus POP L-FMC data indicate that upper-limb L-FMC responses do not represent a systemic measure of endothelial-dependent vasoconstrictor capacity in older adults. The strong association between $\dot{V}O_2$peak and POP L-FMC suggests that localized shear stress patterns, perhaps induced by lower-limb dominant modes of aerobic exercise, may result in greater vasoconstrictor responsiveness in healthy older adults.

Chapter 4: Introduction

Older adults are at a greater risk of experiencing a cardiovascular event due to unfavorable age-related changes to their cardiovascular system (299). Combined with a sedentary/inactive lifestyle, ageing leads to alterations in arterial structural modelling, increased stiffness and a decline in endothelial function that elevates the risk of atherosclerosis (23). Vascular endothelial dysfunction may be characterized by an attenuated vasodilatory response to physical or chemical stimuli mediated by a reduction in the production of the antiatherogenic chemical nitric oxide (NO) (244). Flow-mediated dilation (FMD) provides a clinically relevent assessment of conduit artery endothelial-dependent vasodilatory function (97). The FMD test provides an index of endothelial-derived NO bioavailability via the increase in conduit arterial diameter in response to a reactive hyperemia (i.e., increased shear stress) induced by a prior period of distal ischemia (285). Blunted FMD responses are an early marker of atherosclerosis and progression towards the development of vascular diseases (55).

An often overlooked aspect of the vascular profile during the traditional FMD protocol is the endothelial-dependent vasoconstrictor response that occurs during the distal cuff-induced ischemia, termed low-flow-mediated constriction (L-FMC). L-FMC provides additional information regarding the impact of reduced shear stress on vascular tone (90) and is impaired in individuals with risk factors for coronary artery disease (102). Most FMD studies have been performed in the brachial artery (BA), which provides an indication of coronary artery endothelial health and is predictive of future cardiovascular events (124). However, lower-limb arteries such as the popliteal (POP) are more susceptible to the development of atherosclerosis and peripheral vascular disease

(58). Furthermore, known heterogenous endothelial-dependent dilatory responses have been observed between the BA and POP (213, 292). The limb-specific differences in FMD may be attributed to the greater gravity-induced hydrostatic pressures (181), larger baseline arterial diameter (284) and/or exposure to larger oscillations in local shear stress patterns during bouts of physical activity and sedentary behaviours in the POP (182, 294). Specifically, lower-limb vessels are subjected to more frequent and robust reductions in blood flow during sedentary activities than upper-limb arteries, with declines observed in as little as 10 minutes of sitting (303). Conversely, traditional modes of aerobic exercise (walking, running, cycling, etc.) induce larger hyperemic responses in lower-limb arteries (286). L-FMC has been predominantly studied in the radial (57, 90) and BA (13, 102). As such, it is unclear if upper-limb L-FMC responses are reflective of lower-limb endothelial-dependent vasoconstrictor function.

Considering that the POP experiences larger shear stress fluctuations during sedentary behaviors and traditional lower limb modes of aerobic exercise, the primary purpose of the present study was to test the hypothesis that older adults will exhibit heterogeneous endothelial-dependent vasoconstrictor responses between the BA and POP. Additionally, I previously demonstrated that higher cardiorespiratory fitness was associated with greater L-FMC responses in the BA in a sample of healthy older adults (195). Therefore, the secondary objective of this study was to test the hypothesis that a stronger relationship exsits between aerobic fitness and L-FMC in the POP.

Chapter 4: Methods:

Participants

Forty-seven older adults (30 ♀) were recruited from the Active Aging program at Acadia University (Table 1). Participants were cleared for moderate-vigorous physical activity using the Physical Activity Readiness-Questionnaire plus (308). The protocols and procedures conformed to the Declaration of Helsinki and were approved by the Dalhousie University Health Sciences and Acadia University Research Ethics Boards. Participants were informed of the methods and study design verbally and in writing before providing written informed consent.

Participants had no physical limitations to exercise, did not use tobacco products and were normotensive (i.e., resting blood pressure < 140/90 mmHg). Five participants were on Synthroid® for hypothyroidism. Four participants were prescribed medications to treat high blood pressure. Specifically, some participants were taking Teveten® (angiotensin-receptor blocker; $n = 1$), Adalat® (calcium channel blocker; $n = 1$); Diuril® (diuretic; $n = 1$) and Coversyl Plus® (angiotensin converting enzyme inhibitor + diuretic; $n = 1$). One person was asthmatic. Participants were requested to continue taking all prescribed medications throughout the duration of the study. All female participants were postmenopausal and were not undergoing any form of hormone replacement therapy. All 47 individuals from my previously published study investigating the relationship between aerobic fitness and BA L-FMC (195) were included in the present study.

Experimental Design

Participants reported to the laboratory twice. The first visit involved completion of the informed consent process and Physical Activity Readiness Questionnaire plus,

measurements of height and body mass, followed by a graded, maximal cycling exercise test to determine peak oxygen consumption ($\dot{V}O_2$peak, see below for details). The second visit was dedicated to the assessment of vascular function and conducted between 48 hours and one week following the graded exercise test. All vascular measurements were performed under standardized conditions. Specifically, assessments were performed 6 hours post-prandial, and participants avoided strenuous physical activity, as well as the consumption of products known to acutely influence vascular endothelial function (e.g., caffeine, chocolate, citrus fruits, saturated fats, folic acid supplements, antioxidant and multivitamin supplements) for 24 hours, consistent with FMD guidelines (285). Upon arriving to the laboratory, participants rested for ~20 minutes prior to the vascular assessments. Vascular function was assessed first in the BA (12 minutes), followed by at least 10 minutes of rest before the POP measures (12 minutes). All study visits were performed in a thermoneutral environment (21°C).

Experimental Procedures

Anthropometrics and Peak Aerobic Fitness. Height and weight were measured using a calibrated stadiometer (Health-O-Meter, McCook Il, USA) to the nearest 0.5 cm and 0.1 kg, respectively. An incremental and maximal exercise test on a cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands) was administered to determine maximal oxygen uptake ($\dot{V}O_2$max) via a mixing chamber-based commercial metabolic system (TrueOne 2400®, Parvomedics Inc., Sandy, UT). Following a 5-minute warm-up period of light-intensity cycling (30-50W), the power output was set to a pre-determined workload (1W/kg body mass) that progressively increased by 15W/min until voluntary exhaustion. Strong verbal encouragement was provided throughout the test. Upon

completion of the test, the workload was immediately reduced to the warm-up level for a 5 min cool-down period. Relative $\dot{V}O_2$ data were averaged over 15 second intervals for the duration of the graded exercise protocol. Maximum or peak $\dot{V}O_2$ were considered as the greatest 30 second averaged $\dot{V}O_2$. All participants achieved ≥2 of the following $\dot{V}O_2$peak/$\dot{V}O_2$max criteria: 1) a plateau in relative $\dot{V}O_2$ (<2.1 ml/min/kg) despite an increase in workload, 2) a respiratory exchange ratio ≥1.10, or 3) a rating of perceived exertion ≥18. Given that not all participants exhibited a plateau in relative $\dot{V}O_2$ (primary criteria for $\dot{V}O_2$max), the term $\dot{V}O_2$peak was used throughout.

Systemic Hemodynamics. Heart rate (HR) was determined via cardiac intervals obtained from lead II of a standard bipolar limb lead electrocardiogram. Beat-by-beat systolic (SBP) and diastolic (DBP) blood pressures were measured using finger photoplethysmography (Portapres®; Finapres Medical Systems, Amsterdam, Netherlands). Left brachial artery measurements of SBP and DBP were also recorded by an automated patient vital signs monitor (Carescape v100®, General Electric Healthcare) and used to perform a 'physiological calibration' of the Portapres® waveform. All data were sampled continuously at 200 Hz using a PowerLab (PL3508 PowerLab 8/53, ADInstruments, Sydney, Australia) data acquisition system with the exception of the electrocardiogram waveform, which was sampled at 1000 Hz. Recordings were displayed in real-time and analyzed offline using LabChart software (ADInstruments, Sydney, Australia). SBP and DBP were determined from the Portapres® waveform as the maximum and minimum waveform values, respectively (66). These pressures were then used to calculate mean arterial pressure (MAP) using the equation ⅓ SBP + ⅔ DBP.

Hemodynamic data were averaged over at least 5 min of beat-by-beat data recoded immediately prior to the POP-FMD protocol.

Vascular Measures. As described in O'Brien et al. (202), the right BA and left POP were imaged with the participants in the supine and prone positions, respectively. The BA was imaged 3-5 cm proximal to the antecubital fossa and the POP was imaged proximal to the bifurcation at or slightly above the popliteal fossa. A pressure cuff attached to a rapid inflation system (E20 and AG101, Hokanson®, Bellevue, WA) was positioned around the largest circumference of the forearm (BA; ~3 cm distal to the antecubital fossa) or lower leg (POP; ~10 cm distal to the popliteal fossa). All images were obtained using a 12-MHz multi-frequency linear array probe attached to a high-resolution ultrasound system (Vivid i, General Electric Healthcare). Blood velocity signals were recorded in duplex mode at a pulsed frequency of 5-MHz and corrected with an insonation angle of 60° that remained constant throughout the study. The sample volume was adjusted for each participant such that the anterior to the posterior intima were included, as recommended in published guidelines (285). Artery lumen diameter and blood velocity were measured for a minimum of 2 minutes prior to inflation of the pneumatic cuff. The pressure cuff was then rapidly inflated to 250 mmHg for 5 minutes. Continuous arterial lumen diameter and blood velocity recordings were collected throughout the cuff inflation period. Upon release of cuff pressure, lumen diameter and blood velocity recordings continued for an additional 5 minutes.

Video signals from the ultrasound were exported to a laptop via a video graphics array converter (Epiphan Systems Inc., VGA 2 USB, Ottawa) for offline analysis. Analysis of artery diameter, blood velocity and shear rate (i.e., frictional force of blood

flow on the endothelium) were performed using automated commercial edge-detection and wall-tracking software combined with simultaneous Doppler waveform envelope analysis (FMD Studio, Cardiovascular Suite, Quipu, Pisa, Italy). This software was used to measure baseline diameter, nadir diameter (i.e., L-FMC) and peak diameter (i.e., FMD). All vascular measurements were blindly analyzed by MWO who has demonstrated an intra-tester analyzing reproducibility of 2.2%, 2.7%, 3.8% and 4.2% for baseline diameter, nadir diameter, L-FMC% and FMD%, respectively. Specifically, MWO blindly analyzed the same ultrasound recording for 20 participants on two different occasions and the variation was calculated for each recording as [(difference between 2 measurements ÷ average value) × 100%]. This intra-tester reliability is consistent with other studies using automatic edge-detection software (228). Blood flow (ml/min) was calculated as mean blood velocity (cm/s) × π × lumen radius $(cm)^2$ × 60 (s/min).

Absolute FMD was calculated as the difference (in mm) between the post-cuff deflation peak and baseline diameters. Relative FMD was calculated using the equation: FMD (%) = [(post-cuff deflation peak diameter – baseline diameter) ÷ baseline diameter × 100%]. Shear rate (SR, s^{-1}) was defined as [8 × mean blood velocity (cm/s)] / diameter (cm). Subsequently, the SR area under the curve (SR_{AUC}) was calculated between the start of cuff deflation to the time that peak dilation occurred. Time to peak dilation was recorded. Significant correlations between the SR_{AUC} and FMD responses were observed for both the BA ($r = 0.43$, $P = 0.003$) and the POP ($r = 0.47$, $P = 0.001$). However, the 95% of the y-intercept did not encompass 0 for the BA (1.0-3.9) but did for the POP (-0.5-2.4). As such, FMD data were not normalized to SR_{AUC} (209).

L-FMC was represented in absolute (mm; nadir diameter – baseline diameter) and relative (%) terms using the nadir diameter obtained during the final 30 seconds of the 5-minute distal cuff occlusion period. Relative L-FMC was calculated using the equation: L-FMC (%) = [(nadir diameter – baseline diameter) ÷ baseline diameter ×100%].

Statistical Analysis

The following procedures were used to help address my primary hypothesis. All data were assessed for normality using a Shapiro-Wilk test and found to be normally distributed. Consistent with the analysis approach of Thijssen et al. (292), paired t-tests were used to compare FMD and L-FMC responses between the BA and POP. Furthermore, Pearson product moment correlational analyses were performed to determine the relationships of FMD and L-FMC between the BA and POP in both relative (%) and absolute (mm) terms. To determine if baseline artery size was a determinant of the magnitude of the vasodilatory and vasoconstriction response, correlational analyses compared the association between baseline diameter and vascular responses in the BA and POP separately, as well as with the combined BA and POP sample (n = 94). Effect sizes (ES) were calculated for the vascular measurements between the POP and BA ($POP_{mean} – BA_{mean}$ ÷ pooled standard deviation) and interpreted as small (ES = 0.2), medium (ES = 0.5) and large (ES = 0.8) (45).

As described by Atkinson and Batterham (7), allometric scaling has been recommended to account for differences in baseline arterial diameter. Allometric scaling is recommended if the relationship between the natural log of peak FMD diameter (or nadir diameter for L-FMC) and resting diameter yield an unstandardized β-coefficient that deviates from 1 and/or have upper 95% confidence intervals <1. These assumptions

were met for L-FMC measurements in the BA and POP with β ± standard error (95% confidence intervals; lower-upper) of 0.984 ± 0.007 (0.97-1.00) and 0.979 ± 0.010 (0.96-1.00), respectively. However, the allometric assumptions were not met for BA-FMD [1.025 ± 0.013 (1.00-1.05)] and POP-FMD [1.028 ± 0.014 (1.00-1.06)], suggesting allometric scaling to be unnecessary in this study. Allometrically scaled L-FMC were examined using an analysis of covariance (ANCOVA) model with the natural log of the difference [ln(nadir diameter) – ln(baseline diameter)] as the dependent variable and ln(baseline diameter) as the covariate. Artery (BA/POP) was used as a fixed factor. Statistically significant ANCOVAs were followed up with pairwise comparisons using Fisher's least squares difference *post hoc* testing (8). For each instance, the allometrically-scaled L-FMC responses were back transformed and presented as a percent change from the baseline diameter, as described in more detail by Atkinson and Batterham (8).

To help address my secondary hypothesis, Pearson product moment correlational analyses were performed between POP-FMD and POP L-FMC with $\dot{V}O_2$peak. Pearson correlations were interpreted as follows: <0.5 (weak), 0.5-0.7 (moderate) and >0.7 (strong) (112).

All statistics were completed in SPSS Version 23.0 (IBM, NY). Some participants were taking anti-hypertensive medication (n = 4), which may have influenced their vascular function. Removing these individuals from analyses did not change the strength of the correlations between aerobic fitness and POP L-FMC (r = -0.73, P < 0.001) nor the comparisons of L-FMC between the BA and POP (r = 0.20, P = 0.20; allometrically-

scaled L-FMC: -1.4 ± 1.6% vs. -0.4 ± 1.6%, $P = 0.03$). Statistical significance was accepted as $P < 0.05$. All data are presented as means ± standard deviations (SD).

Chapter 4: Results

Participant characteristics and hemodynamics are summarized in Table 4.1. According to the Canadian Society for Exercise Physiology age, and sex-specific aerobic fitness classifications (49), participants were classified as poor (n = 27), fair (n = 13), good (n = 3), very good (n = 3) or excellent (n = 1).

Brachial versus Popliteal Comparisons

At baseline, the POP had a larger diameter than the BA, but lower mean blood velocity and shear rate (Table 4.2). Resting blood flow was similar between the arteries (Table 4.2). Compared to the POP, the BA elicited a greater SR_{AUC} stimulus with shorter time to peak dilation (Table 4.2). Although a smaller absolute FMD was observed in the BA (Table 4.2) the BA exhibited a larger relative FMD (4.7 ± 1.6% vs. 3.3 ± 2.0%, P < 0.001; ES = -0.7; Fig. 4.1A). Furthermore, the absolute L-FMC was smaller in the BA (Table 4.2) but the two arteries had similar relative L-FMC responses (–0.8 ± 0.8% vs. –0.9 ± 1.4%; P = 0.72; ES = -0.1). When adjusted for baseline diameters, the allometrically-scaled relative L-FMC response was greater in the BA (–1.3 ± 1.6% vs. –0.4 ± 1.6%, $P = 0.03$; ES = 0.6; Fig. 4.1B).

A positive moderate-strength correlation was observed between the BA and POP for relative FMD (Fig. 4.2A). A similar correlation was observed when absolute values were used for FMD (r = 0.59, $P < 0.001$). However, no correlation was observed between constrictor responses in the BA and POP when L-FMC was represented as either a relative (Fig. 4.2B) or absolute change from baseline diameter (r = 0.24, $P = 0.10$).

Table 4.1. Study 2 participant demographics and characteristics.

Variable	Sample (n=47)
Age, yrs	67 ± 5 (56-83)
Body Mass Index, kg/m^2	26.5 ± 4.2 (19-37)
Resting heart rate, beats/minute	68 ± 10 (50-90)
Systolic blood pressure, mmHg	125 ± 11 (102-139)
Diastolic blood pressure, mmHg	71 ± 9 (55-85)
Mean arterial pressure, mmHg	89 ± 8 (70-99)
Peak heart rate, beats/minute	150 ± 10 (133-166)
Peak RER ($\dot{V}CO_2/\dot{V}O_2$)	1.21 ± 0.10 (1.07-1.39)
Aerobic Fitness, ml/kg/min	22.9 ± 7.0 (12.6-44.8)

Data are presented as means ± SD (range). RER, respiratory exchange ratio; $\dot{V}CO_2$, volume of carbon dioxide produced; $\dot{V}O_2$, volume of oxygen consumed.

Table 4.2. Comparison of brachial and popliteal parameters.

	BA	POP	ES	*P*–Value
Resting				
Resting diameter, mm	3.95 ± 0.66	6.59 ± 1.38	2.4	<0.001
Mean blood velocity, cm/s	12.2 ± 4.4	4.1 ± 2.0	-2.4	<0.001
Blood flow, ml/min	90 ± 37	88 ± 48	-0.1	0.74
Resting shear rate, s^{-1}	128 ± 53	26 ± 15	-2.6	<0.001
Flow-Mediated Dilation				
Peak diameter, mm	4.14 ± 0.72	6.82 ± 1.46	2.3	<0.001
Absolute FMD, mm	0.19 ± 0.08	0.23 ± 0.16	0.3	0.048
SR_{AUC}	12256 ± 3797	6347 ± 2579	-1.8	<0.001
Peak FMD:SR_{AUC} Ratio, a.u.	0.17 ± 0.09	0.37 ± 0.21	1.3	<0.001
Time to peak diameter, s	65 ± 19	105 ± 32	1.5	<0.001
Low-Flow-Mediated Constriction				
Nadir diameter, mm	3.92 ± 0.64	6.53 ± 1.35	2.5	<0.001
Absolute L-FMC, mm	–0.03 ± 0.04	–0.06 ± 0.09	-0.4	0.03

Data are presented as means ± SD with the corresponding effect sizes (ES). FMD, flow-mediated dilation; SR_{AUC}, shear rate area under the curve to peak dilation; L-FMC, low-flow-mediated constriction; SBP, systolic blood pressure; DBP, diastolic blood pressure. Data were analyzed using paired t-tests.

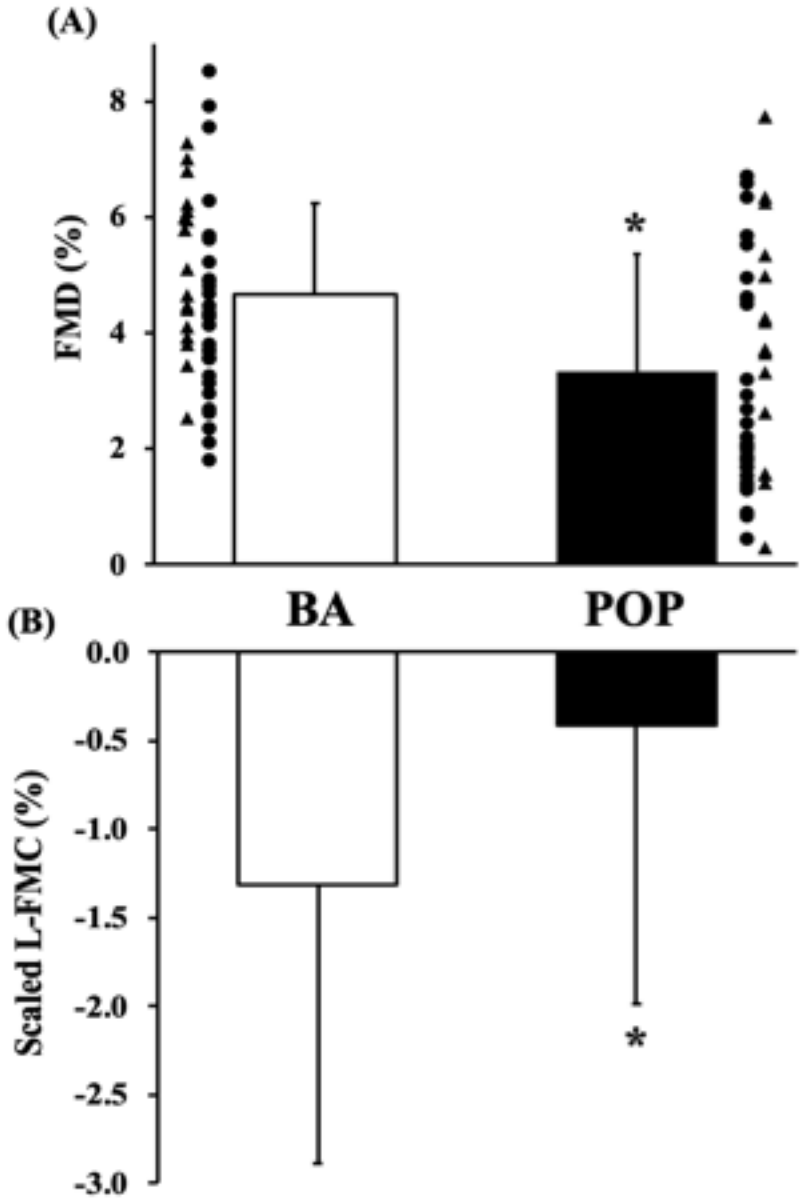

Figure 4.1. Comparison of (A) relative flow-mediated dilation [FMD, %] and (B) allometrically-scaled low-flow-mediated constriction [L-FMC, %] between the brachial artery [BA, white bars] and popliteal artery [POP, black bars]. Data are presented as means ± standard deviations. Individual data are presented as circles and triangles for females and males, respectively. Individual data are not dispayed for scaled L-FMC as group means only are provided following allometric scaling procedures. Data were analyzed using paired t-tests. N = 47, 30 women. *, P < 0.05 vs. brachial artery.

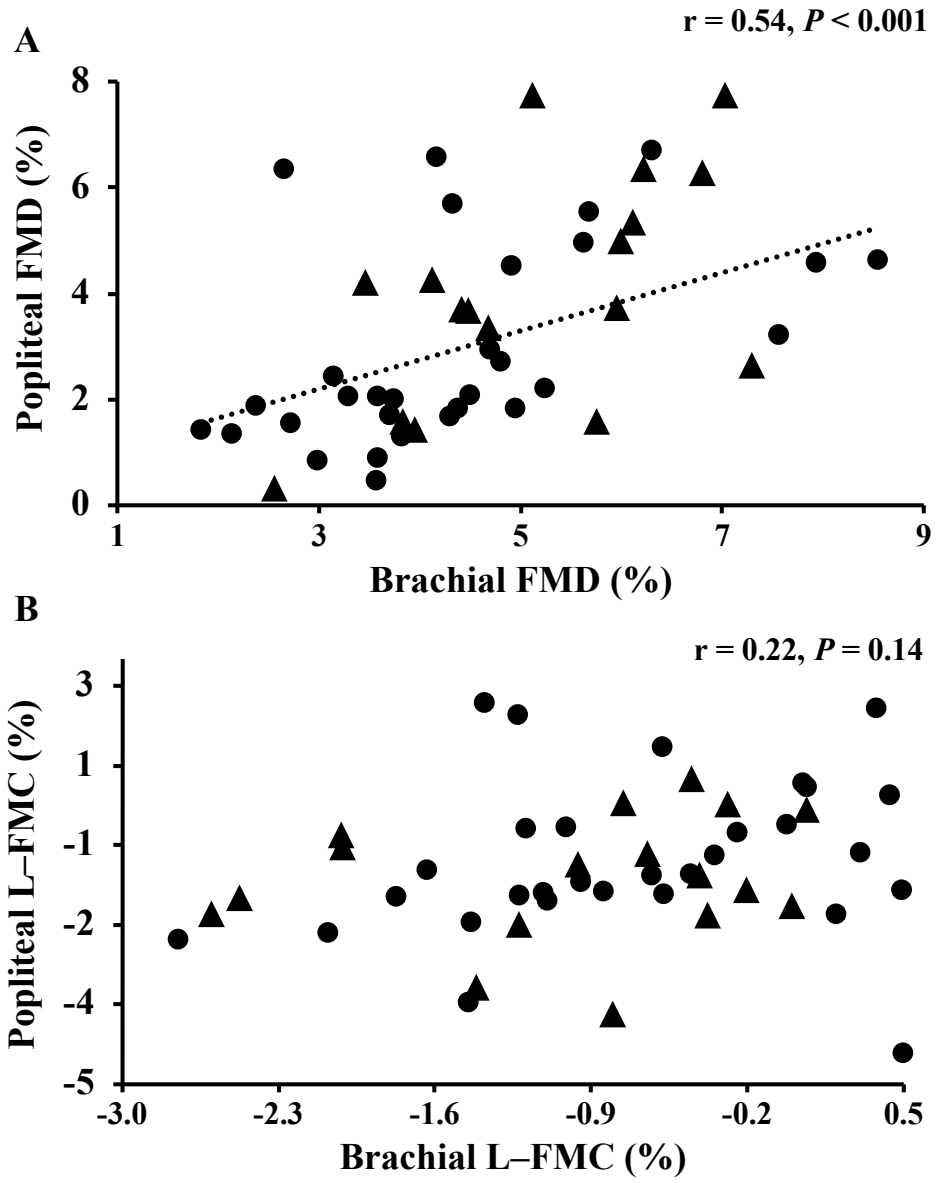

Figure 4.2. Pearson correlations between the brachial artery and popliteal artery for (A) relative flow-mediated dilation (FMD, %) or (B) relative low-flow-mediated constriction (L-FMC, %). Individual data are presented as circles and triangles for females and males, respectively. N = 47, 30 females.

Relationship Between Baseline Diameter and Endothelial Vascular Function

When BA and POP data were combined, weak correlations were observed between baseline diameter and both relative FMD ($r = 0.41$, $P < 0.001$) and relative L-FMC ($r = –0.41$, $P < 0.001$). Moderate correlations were observed between resting BA diameter with BA-FMD ($r = 0.63$, $P < 0.001$) and with BA L-FMC ($r = –0.54$, $P < 0.001$). However, weak correlations were observed between resting POP diameter with POP-FMD ($r = 0.42$, $P = 0.003$) and POP L-FMC ($r = –0.38$, $P = 0.01$).

Relationships Between Endothelial Vascular Function and Aerobic Fitness

The correlations between $\dot{V}O_2$peak with FMD and L-FMC in the POP are presented in Fig. 4.3. Aerobic fitness was positively correlated to relative POP-FMD (Fig. 4.3A) and negatively associated with relative POP L-FMC (Fig. 4.3B). Similar correlations were observed when absolute changes were used for POP-FMD ($r = 0.49$; $P < 0.001$) or POP L-FMC ($r = –0.76$; $P < 0.001$)

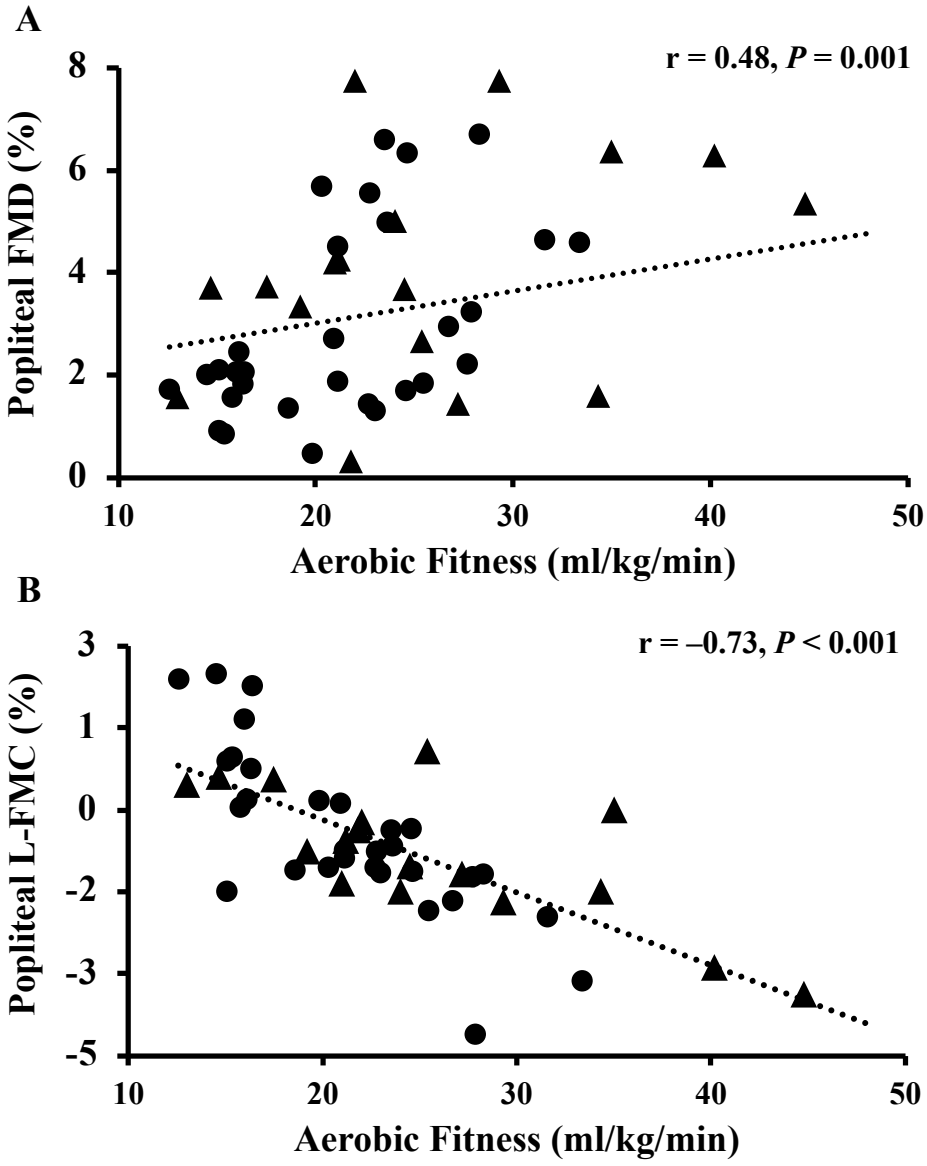

Figure 4.3. Pearson correlations between aerobic fitness ($\dot{V}O_2$peak) with (A) popliteal artery relative flow-mediated dilation (FMD, %) or (B) relative low-flow-mediated constriction (L-FMC, %). Individual data are presented as circles and triangles for females and males, respectively. N = 47, 30 females.

Chapter 4: Discussion

The primary purpose of this study was to assess the relationship between endothelial-dependent vasoconstrictor responses to low-flow in the brachial and popliteal arteries of older adults. Consistent with my hypothesis, L-FMC responses in the BA and POP were not correlated (Fig. 4.2B) and when adjusted for differences in baseline diameter, relative L-FMC was larger in the BA than the POP (Fig. 4.1B). Additionally, aerobic fitness was negatively associated with POP L-FMC (Fig. 4.3B). Similar to my previous observation in the BA of this sample (195), the vascular endothelium of the POP appears to be more sensitive to both reductions (i.e., L-FMC) and increases (i.e., FMD) of local shear stress in more aerobically fit older adults. This study represents the first evidence that the BA and POP exhibit differential vasoconstrictor responses and documents the relationship between aerobic fitness and POP endothelial-dependent vasodilatory and vasoconstrictor function in an older population.

Consistent with previous literature in older adults (213), I observed a larger relative FMD response in the BA versus the POP. Conversely, the FMD response was greater in the POP than the BA when presented as an absolute change in diameter. This between-artery discrepancy in absolute versus relative FMD was primarily due to differences in baseline diameter. In young adults, within-individual differences in artery size influence the corresponding functional vasodilatory responses (284). My results corroborate these findings in older persons (see Fig. 4.1A & Table 4.2) and extend these observations to endothelial-dependent vasoconstrictor function in this population (see Fig. 4.1B). Specifically, baseline diameter was correlated to the corresponding relative FMD ($r = 0.4$) and relative L-FMC ($r = –0.4$) responses. Previous findings in young

healthy people have demonstrated no relationship between relative FMD in the BA and POP arteries (r = 0.1; *P* = 0.8) (292), whereas the current FMD responses in the BA and POP of older adults were moderately correlated (*r* = 0.5; Fig. 4.2A). Of relevance, the BA-FMD, BA L-FMC and POP-FMD data from my sample of older adults are consistent with those reported by Siasos et al. (253) (BA-FMD; 5.3 ± 2.9%), Harrison et al. (102) (BA L-FMC; –1.8 ± 0.8%), and Angerer et al. (4) (POP-FMD; 2.9 ± 3.6%).

L-FMC has been primarily studied in upper-limb arteries (e.g., radial and brachial), provides additional information regarding the role of reduced shear stress on resting diameter and vascular tone (90) and is impaired in individuals with risk factors for coronary artery disease (102). Adding to the current literature, my findings demonstrate that heterogenous L-FMC responses were exhibited in the BA and POP of older adults. Specifically, a smaller absolute L-FMC response was observed in the BA than the POP. However, the POP has a larger lumen diameter than the BA (6.6 ± 1.4mm vs 4.0 ± 0.7 mm; Table 4.2), which demonstrated the need to account for baseline diameter via allometric scaling. Herein, a larger allometrically-scaled relative L-FMC was observed in the BA (see Fig. 4.1B). No correlation was observed for L-FMC between the BA and POP. As such, the influence of interventions (i.e., exercise training) and the prognostic value of L-FMC should be determined in both the upper- and lower limb vascular beds. While speculative, the disparity between L-FMC responses in the BA and POP are likely attributed to limb-specific differences in local hemodynamic forces. This differential L-FMC response may also be influenced by variations in endothelial expression of anti-atherogenic (e.g., eNOS, SOD-1, SOD-2, etc.) proteins, which have been shown to be different between the brachial and femoral arteries in porcine models (210). Of relevance,

the brachial and femoral arteries of pigs are similar in size, endothelium and smooth muscle content to humans (297). Future mechanistic research investigating gene expression between upper- and lower-limb arteries is warranted to determine if the observed heterogeneous responses are inherently predetermined between vascular beds.

Consistent with previous literature (172, 202), higher aerobic fitness was positively associated with augmented POP vasodilator function. However, I observed a stronger correlation between $\dot{V}O_2$peak and POP L-FMC ($r = –0.7$) than previous cross-sectional studies comparing aerobic fitness and BA L-FMC. Specifically, $\dot{V}O_2$peak was negatively correlated to BA L-FMC in young males [$r = –0.5$; (13)] and to BA L-FMC from the sample of older adults in the present study [$r = –0.5$; (195)]. The stronger correlation between cardiorespiratory fitness with the lower-limb vasculature is likely due to the larger influxes and reductions in shear stress associated with locomotion (or lack thereof) that do not occur to the same magnitude in the BA (286). Radial artery L-FMC is mediated via the inhibition of non-NO vasodilators, endothelial-derived hyperpolarizing factors and prostaglandins (90), as well as enhanced vasoconstrictor signaling via endothelin-1 (264). It is unknown whether the same mechanisms that govern L-FMC in the radial artery are responsible for the vasoconstrictor response to low-flow in the BA and POP.

Measuring L-FMC is appealing and convenient for researchers as it is derived by the change in lumen diameter during the ischemic portion of the commonly used FMD protocol (122). As previously mentioned, L-FMC and FMD provide distinctly different information but when combined provide additive and complementary insight into vascular endothelial function (91). Accumulating evidence demonstrates the importance

of measuring vasoconstrictor capacity as an indication of peripheral vascular health (102, 122). Of relevance, Weissgerber et al. (312) failed to observe an L-FMC response in the BA of females who were inactive and pregnant, inactive and not pregnant, or active and not pregnant. However, they did observe a BA L-FMC response in their active, pregnant group. Although they did incorporate measures of physical activity (i.e., 3 day physical activity record) and physical fitness (i.e., exercise workload to achieve a rating of perceived exertion of 13 on a scale of 6-20), an assessment of maximal aerobic fitness was not completed. Based on our inverse correlation between aerobic fitness and L-FMC in the brachial (195) and popliteal (Fig. 4.3B) arteries, it may be possible that less fit participants were recruited in their study, which may have impacted the magnitude of the L-FMC response. Altogether, higher aerobic fitness was associated with greater FMD and L-FMC responses. These findings support the overarching hypothesis that increased aerobic fitness is associated with both a greater sensitivity to low-flow, as well as large increases of shear stress (i.e., reactive hyperemia). There is a need to determine the clinical relevance and prognostic value of L-FMC and changes in L-FMC, as is established for the traditional FMD test (124).

The findings of this study are limited to an older adult population. As such, it is unclear if heterogenous responses exist between the upper- and lower-limb conduit arteries of young adults or clinical populations with vascular disorders. My sample was comprised of individuals with lower aerobic fitness (n = 27/47 rated as 'Poor'). However, the average $\dot{V}O_2$peak (23 ± 7 ml/kg/min) of my participants is reflective of national averages for older males and females (21-29 ml/kg/min) (131, 268). The time of day when the vascular assessments were conducted varied between participants (i.e., 8am to

5pm). Whether BA-FMD is influenced by diurnal variation in healthy, younger populations is controversial with some (129, 205, 232), but not all (135, 279) studies observing a blunted BA-FMD in the morning hours. It is unclear whether FMD or L-FMC in the POP is influenced by time of day, particularly in older populations. Of relevance, no differences (both $P > 0.56$*)* were observed when dichotomizing participants by those tested before noon ($n = 30$) and afternoon ($n = 17$) for L-FMC in the BA (-0.8 ± 0.8% vs. -0.9 ± 0.8%) and POP (-0.9 ± 1.6% vs. -0.9 ± 1.1%). Similarly, no differences (both $P > 0.57$) in FMD responses were observed between those tested in the morning and afternoon for the BA (4.6 ± 1.6% vs. 4.7 ± 1.7%) or POP (3.2 ± 2.1% vs. 3.6 ± 2.0%). Additionally, mechanistic work is warranted to determine if the signaling pathways responsible for radial artery L-FMC responses (e.g., endothelial-derived hyperpolarizing factors, prostaglandins and endothelin-1) are similar in the BA and POP of older adults. The demonstrated correlation between aerobic fitness and POP L-FMC warrants interventional inquiry to provide direct evidence between exercise training-induced changes in aerobic fitness and L-FMC in older populations, as has been done in younger populations (224, 240). Also, it is unclear if the magnitude of, and relationship between BA and POP L-FMC responses differs between older males and females, which the present study was not designed, nor powered to detect. Previous studies have observed that higher aerobic fitness was not associated with a greater BA-FMD in postmenopausal females (239), which contrasts with our previous observations in the BA (195) and current observations in the POP (Fig. 4.3A) in a relatively large sample of females (17♂ vs. 30♀). With that, I have previously demonstrated that weekly moderate-vigorous physical activity has a greater association with BA-FMD and POP-FMD than

cardiorespiratory fitness in older adults (202), suggesting that divergent observations between studies may be attributed to differences in intensity-related physical activity. Certainly, future research is needed to elucidate the influences of lifestyle behaviours on endothelial function in older males and females.

The brachial and popliteal arteries exhibit differential vasoconstrictor responses to low-flow in older adults, demonstrating that BA L-FMC does not represent a systemic measure of vasoconstrictor capacity. Higher aerobic fitness was associated with a greater sensitivity to low-flow and to a reactive hyperemia in the popliteal arteries of older persons. Given that lower-limb arteries are more susceptible to peripheral vascular disease, future research is needed to determine the clinical relevance and prognostic value of POP L-FMC.

LINKING STUDIES 1 & 2 WITH STUDY 3

Studies 1 and 2 together demonstrated that aerobic fitness was positively associated with FMD and negatively associated with L-FMC in both the brachial and popliteal arteries. Despite these relationships observed in both the upper- and lower-limb arteries, there was no correlation for L-FMC responses between the vascular beds. As well, relative FMD and allometrically-scaled L-FMC responses were smaller in the popliteal artery. A limitation of studies 1 and 2 was the cross-sectional design, with an inability to establish causality. Accordingly, an intervention design investigating the impact of physical exercise on endothelial function was warranted. Within exercise training interventions, the type of training may have an impact on endothelial function. Some evidence supports that brachial FMD is augmented to a greater extent following high-intensity interval training than moderate-intensity continuous training, which both may provoke larger improvements in FMD than resistance training. Elaborating upon my cross-sectional studies, I conducted an intervention study that examined the impact of exercise training on FMD and L-FMC.

CHAPTER 5:

STUDY 3: IMPACT OF HIGH-INTENSITY INTERVAL TRAINING, MODERATE-INTENSITY CONTINUOUS TRAINING, AND RESISTANCE TRAINING ON ENDOTHELIAL FUNCTION IN OLDER ADULTS

Chapter 5: ABSTRACT

It is unclear if high-intensity-interval-training (HIIT) elicits superior improvements in brachial artery (BA) flow-mediated dilation (FMD) responses than moderate-intensity-continuous-training (MICT) or resistance training (RT) in healthy older adults. Whether HIIT enhances lower-limb FMD responses and/or augments low-flow-mediated constriction (L-FMC) responses more than MICT or RT in either upper-limb or lower-limb arteries is also unknown. I tested the hypothesis that HIIT would improve BA and popliteal artery (POP) FMD and L-FMC responses more than MICT or RT in healthy older adults. Thirty-eight older adults (age: 67±6 yrs) performed 6-weeks of either HIIT [2×20-minute bouts alternating between 15s intervals at 100% of peak aerobic power output (PPO) and passive recovery (0% PPO); *n*=12], MICT (34-minute at 60% PPO; *n*=12), or whole-body RT (8 exercises, 2×10 repetitions; *n*=14). L-FMC and FMD were measured before and after training using high-resolution ultrasound and quantified as the percent change in baseline diameter during distal cuff occlusion and the ensuant reactive hyperemia, respectively. Resting BA blood flow ($P<0.003$) was greater following HIIT only. HIIT and MICT similarly increased BA-FMD (pre-post: both, $P<0.001$), but only HIIT improved BA L-FMC ($P<0.001$). Both HIIT and MICT similarly enhanced POP FMD and L-FMC responses (both, $P<0.045$). RT did not impact FMD or L-FMC responses in either artery (all, $P>0.20$). HIIT and MICT, but not RT, similarly improved

lower-limb vasodilator and vasoconstrictor endothelial function in older adults. While HIIT and MICT enhanced BA vasodilator function, only HIIT improved resting blood flow and endothelial sensitivity to low-flow in the BA. In the short-term, HIIT may be most effective at improving peripheral vascular endothelial function in older adults.

Chapter 5: Introduction

Older adults are at an elevated risk for cardiovascular disease due to the development of atherosclerosis, which is initially characterized by an impaired endothelial-dependent dilation in response to physical or chemical stimuli (55). The non-invasive, flow-mediated dilation (FMD) assessment provides clinically relevant information regarding the ability of the endothelium to produce and release nitric oxide (NO) following a reactive hyperemia elicited by a prior period of distal ischemia. Most FMD studies have been performed in the brachial artery (BA), which provides an indication of coronary artery endothelial health (26) and is predictive of future cardiovascular events (319). However, lower-limb arteries such as the popliteal artery (POP) are more susceptible to the development of atherosclerosis and peripheral vascular disease than upper-limb arteries (58). In addition to providing a clinically relevant measure of endothelial vasodilator function, the decline in conduit artery diameter observed during the distal ischemic period of the FMD test, termed low-flow-mediated constriction (L-FMC), provides an index of endothelial-dependent vasoconstrictor function. L-FMC provides information regarding how reduced shear stress influences vascular tone (90) and is blunted in older adults and patients with coronary artery disease (92). I previously reported that the POP exhibits smaller L-FMC responses than the BA in older adults (194), which highlights the importance of determining whether exercise training impacts vascular endothelial function differently between upper- and lower-limb arteries.

It is well established that aerobic exercise training augments vasodilator function in older adults with chronic disease (e.g., hypertension, heart failure, type 2 diabetes),

with high-intensity interval training (HIIT) eliciting superior improvements in BA-FMD than moderate-intensity continuous training (MICT) (5, 227). The superior effects of HIIT have been attributed to larger and more sustained blood flow responses and/or greater reductions in oxidative stress, which together may enhance endothelial cell sensitivity to shear stress and increase NO bioavailability [as reviewed in (227)]. Most previous reports that have compared the effects of HIIT and MICT on vasodilator function have been conducted solely in the BA of older adults with chronic disease and of a longer duration (i.e., >10 weeks) [as reviewed in (227)]. To date, the only study to directly evaluate the impact of aerobic exercise training intensity in healthy older adults observed no improvement in BA-FMD after 2 weeks of HIIT [10×1-minute at 100% peak power output (PPO)] or MICT (40-minutes at 65% PPO) in post-menopausal females (139). As such, 2-weeks (6 total exercise sessions) may not provide a sufficient stimulus to elicit favorable BA-FMD changes in this population. It is unclear if 6-weeks of aerobic training enhances BA-FMD in healthy older adults, improves endothelial function in arteries responsible for supplying blood flow to the active limbs during traditional modes of aerobic exercise (e.g., POP), or if HIIT produces greater improvements in FMD than MICT in these arteries. Cross-sectionally, I have demonstrated that cardiorespiratory fitness is moderately related to BA L-FMC (195) and strongly related to POP L-FMC (194) in older adults. Whether or not short-term aerobic exercise training interventions enhances L-FMC in either artery in older adults is unclear, and if HIIT augments the endothelial-dependent vasoconstrictor response more than MICT is also unknown in this population.

It is recommended that older adults engage in resistance training (RT) a minimum of 2 days per week (42). Resistance exercise elicits larger post-contraction blood flow responses than aerobic exercise, but this augmented blood flow response is transient and under high-pressure in comparison to the sustained low-pressure flow observed during aerobic exercise (65, 82, 286). Despite these differences in exercise-induced shear stress profiles, RT appears to be an effective stimulus for increasing BA-FMD, albeit less than aerobic training in older adults with chronic disease [as reviewed in (5)]. To date, the influence of short-term RT-induced adaptations on lower-limb endothelial-dependent vasodilatory or vasoconstrictor responses remains uncertain in healthy older adults.

I investigated the effects of short-term (6-weeks) HIIT, MICT and RT on upper- and lower-limb endothelial function in older adults. I hypothesized that HIIT would elicit superior improvements in BA-FMD and POP-FMD than MICT, and that these aerobic protocols would elicit greater endothelial-dependent vasodilator responses than RT. Based on my previous observations that greater aerobic fitness was associated with larger L-FMC responses in both the BA and POP (194, 195), I anticipated that 6-weeks of HIIT or MICT, but not RT, would augment endothelial-dependent vasoconstrictor function in this population.

Chapter 5: Methods

Participants. Thirty-eight older adults (23 females; age 56-83 years) were recruited from the Active Aging program at Acadia University (Table 5.1). Participants had no physical limitations to exercise and a resting blood pressure <140/90 mmHg. Three participants were on Synthroid for hypothyroidism (n = 1 in each group). Four participants were prescribed medications to treat high blood pressure. Specifically,

participants were taking Teveten® (angiotensin-receptor blocker; *n* = 1; HIIT), Adalat® (calcium channel blocker; *n* = 1; RT); Diuril® (diuretic; *n* = 1; HIIT) and Coversyl Plus® (angiotensin converting enzyme inhibitor + diuretic; *n* = 1; RT). One person was asthmatic (RT). During the study, participants were requested to continue taking all prescribed medications. Participants were informed of the methods and study design verbally and in writing before providing written informed consent. Participants were randomized to HIIT (*n* = 12; 7 females), MICT (*n* = 12; 8 females), or RT (*n* = 14; 8 females) after the pre-training determination of aerobic fitness and initial vascular testing day. The pre-training vascular endothelial data have previously been presented in a cross-sectional study investigating L-FMC responses between the BA and POP (194). All protocols and procedures conformed to the Declaration of Helsinki and were approved by the Dalhousie University Health Sciences and Acadia University Research Ethics Boards.

Experimental Design. Participants underwent four separate laboratory visits in total. Days 1 and 2 were conducted pre-training while days 3 and 4 were completed following training. Days 1 and 3 involved measurements of height and body mass (body mass index: weight/height^2), which were followed by a graded, maximal cycling exercise test to determine aerobic fitness ($\dot{V}O_2$peak). PPO was also recorded and used to establish exercise intensities for the two aerobic training protocols. Days 2 and 4 were dedicated to the assessments of BA and POP vascular function and conducted either prior to, or a minimum of 48-hours (or maximum one-week) following the graded exercise tests (see below for details). To minimize known confounding influences on endothelial-dependent dilation, vascular assessments were performed 6-hours post-prandial while participants avoided strenuous physical activity, as well as the consumption of products known to

acutely influence endothelial responses (e.g., caffeine, chocolate, citrus fruits, saturated fats, folic acid supplements, antioxidant and multivitamin supplements) for 24-hours, consistent with FMD guidelines (282). Upon arriving to the laboratory, participants rested in the supine position for a minimum of 20-minutes prior to the vascular assessments. Vascular function was assessed first in the BA, followed by at least 10-minutes of rest before the POP measures. All study visits were performed in a thermoneutral environment (21°C). Days 2 and 4 were performed at the same time of day within each participant to control for diurnal variations in blood pressure and vascular function.

Anthropometrics and peak aerobic fitness. Height and weight were measured using a calibrated stadiometer (Health-O-Meter, McCook Il, USA) to the nearest 0.5-cm and 0.1-kg, respectively. An incremental and maximal exercise test on a cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands) was administered to determine $\dot{V}O_2$peak via a mixing chamber-based commercial metabolic system (TrueOne 2400®, Parvomedics Inc., Sandy, UT). Following a 5-minute warm-up period of light-intensity cycling (30-50W), the workload was set at 1 watt/kg body mass and gradually increased by 15 watts/min until voluntary exhaustion. Strong verbal encouragement was provided throughout the test. Upon completion of the test, the workload was immediately reduced to the warm-up level for a 5-minute cool-down period. $\dot{V}O_2$ data were averaged over 15-second intervals for the duration of the graded exercise protocol. Maximum or peak $\dot{V}O_2$ were considered as the greatest 30-second averaged $\dot{V}O_2$. All participants achieved ≥2 of the following $\dot{V}O_2$peak/$\dot{V}O_2$max criteria: 1) a plateau in relative $\dot{V}O_2$ (<2.1 ml/min/kg) despite an increase in workload, 2) a respiratory exchange ratio ≥1.10, or 3) a rating of

perceived exertion ≥18. Given that not all participants exhibited a plateau in relative $\dot{V}O_2$ (primary criteria for $\dot{V}O_2$max), the term $\dot{V}O_2$peak was used throughout. The workload of the last completed stage was considered as the PPO, measured in watts.

Training protocols. For the three training protocols, all sessions were supervised and conducted 3 days per week (Mondays, Wednesdays and Fridays) for 6-weeks. Six weeks was implemented for logistical reasons to accomplish all testing and training during the summer months. However of note, 6-weeks (3x/week) sprint-interval and MICT augmented aerobic fitness by ~12% in younger adults (88). Given that older adults generally have lower pre-training aerobic fitness levels than younger adults, there is greater room to increase, supporting 6-weeks as a reasonable length to augment aerobic fitness. As outlined in Figure 5.1, all participants completed 18 supervised sessions with no participants dropping out of the study. Warm-up and cool-down periods consisted of 5-minutes at 25% PPO for both the HIIT and MICT protocols. The HIIT protocol was based on a previous study that compared the time to exhaustion, safety, participant preference, and time spent near $\dot{V}O_2$max in older adults (98). Specifically, the HIIT protocol implemented in the present study resulted in the longest time to exhaustion and a similar amount of time spent above 80% and 90% $\dot{V}O_2$max versus 3 other HIIT protocols varying in work:rest ratios and recovery intensity (active vs. passive) (98). Furthermore, previous research has demonstrated that this HIIT protocol elicits greater mean $\dot{V}O_2$ responses than an isocaloric bout of moderate-intensity continuous exercise performed at 70% PPO (99). For the first 2-weeks, the HIIT protocol consisted of forty, 15-second intervals at 100% PPO interspersed with 15-seconds of passive recovery. Following a 5-minute passive recovery period, a second set of 40 intervals was completed (i.e., 40-

minutes total time). To adjust for anticipated improvements in aerobic fitness and exercise tolerance, the duration of the HIIT protocol was increased to 45-minutes (2 × 22.5-minutes; 2 sets of 45 intervals) for the remaining 4-weeks and workload increased by 15 watts for the final 2-weeks. Training volume was decreased by 10-minutes (from 2 sets of 45 intervals to 2 sets of 35 intervals) for the final 2 training sessions to ensure appropriate recovery prior to the post-training assessment of aerobic fitness.

The MICT protocol was based on the American College of Sports Medicine physical activity guidelines that recommend at least 30-minutes of daily moderate aerobic physical activity (83). Continuous cycling at 60% PPO for ~34-minutes was initially prescribed. This duration was adjusted to ensure that the MICT protocol was isoenergetic to the HIIT protocol based on the assumption that mechanical efficiency, aerobic fitness and PPO were similar between groups; in that 20-minutes at 100% PPO expends the same energy as 34-minutes at 60% PPO. To support this assumption, the HIIT and MICT groups demonstrated similar pre-training $\dot{V}O_2$peak and PPO (both, P=1.00, Table 5.1). To accommodate the matched increase in energy expenditure, total exercise time was prolonged to 39-minutes for the remaining 4-weeks and power output increased by 15W for the final 2-weeks. For the final 2 MICT sessions, participants decreased their cycling time from 39 to 30-minutes in preparation for the post-training determination of maximal aerobic fitness.

Each RT session began and ended with 3-minutes of light cycling at 25% PPO. Thereafter, participants completed a total of 8 strength exercises, alternating between muscle groups. The order of exercises was not strictly controlled. The exercises were primarily isokinetic machine-based and included leg press, bench press, hamstrings curl,

shoulder press and leg extensions. Cable-exercises included seated row and latissimus pull-down, and bird-dogs (i.e., a core exercise that involves kneeling on the floor and simultaneously extending the hip while flexing the contralateral shoulder). Each participant performed 2×10 repetitions at 70% of perceived one repetition maximum (1RM) for the first 2-weeks. Both sets of each exercise were performed before starting the next exercise with 30-60 seconds of rest between sets. There were ~1-2 minutes between exercises. Upper- and lower-body exercises were alternated. Participants were instructed to increase the number of repetitions to 12 *ad libitum*. Once participants were able to perform 2×12 repetitions, the resistance was proportionally increased to a weight that equated to 10 repetitions of their new estimated 70% of 1RM with the assistance of the supervising Canadian Society for Exercise Physiology Clinical Exercise Physiologist.

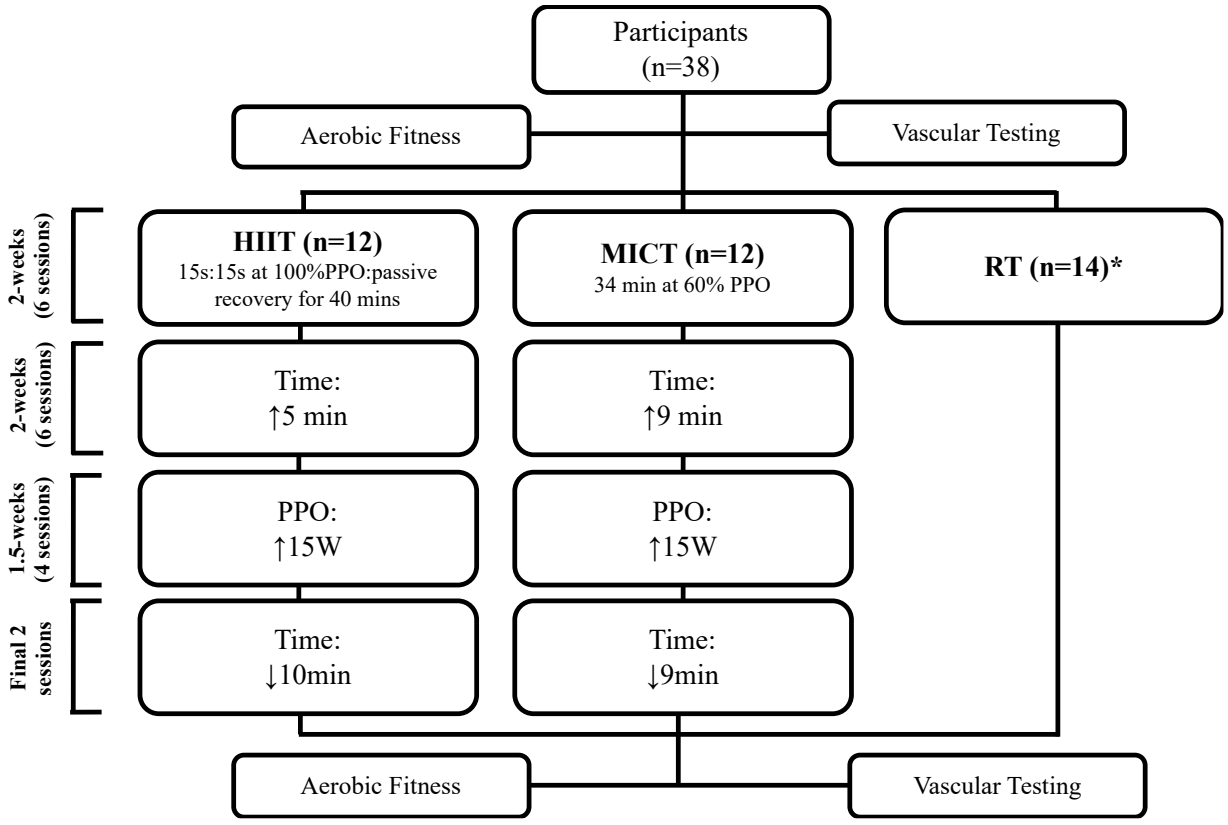

Figure 5.1. Schematic of the experimental design and each of the high-intensity interval training (HIIT), moderate-intensity continuous training (MICT) and whole-body resistance training (RT) protocols. PPO, peak aerobic power output (measured in watts). *The RT protocol consisted of 8 exercises targeting all major muscle groups for 2 sets of 10 repetitions.

Systemic Hemodynamics. Heart rate (HR) was determined via cardiac intervals obtained from lead II of a standard bipolar limb lead electrocardiogram. Beat-by-beat systolic (SBP) and diastolic (DBP) blood pressures were measured using finger photoplethysmography (Portapres®; Finapres Medical Systems, Amsterdam, Netherlands). The finger used for recording blood pressure was maintained at heart level throughout the protocol and minor deviations in height between the heart and finger were corrected using the Portapres® height correction unit. Left brachial artery measurements of SBP and DBP were also recorded by an automated patient vital signs monitor

(Carescape v100®, General Electric Healthcare) and used to perform a 'physiological calibration' of the Portapres® waveform. All data were sampled continuously at 200 Hz using a PowerLab (PL3508 PowerLab 8/53, ADInstruments, Sydney, Australia) data acquisition system with the exception of the electrocardiogram waveform, which was sampled at 1000 Hz. Recordings were displayed in real-time and analyzed offline using LabChart software (Version 8, ADInstruments, Sydney, Australia).

Relative SBP and DBP were determined from the Portapres® waveform as the maximum and minimum within beat values, respectively. These pressures were then used to calculate mean arterial pressure (MAP) using the equation ⅓SBP + ⅔DBP. In addition, stroke volume (SV) was derived from the raw finger blood pressure waveforms using the ModelFlow® method incorporated into the non-invasive cardiac output add-on for LabChart® (ADInstruments, Sydney, Australia). Cardiac output (Q) was calculated as the product of HR and SV and total vascular conductance (TVC) as Q÷MAP. Portapres® data (SV, Q and TVC) were not recorded from two participants in the MICT group (1♂, 1♀) due to equipment malfunctions. Beat-by-beat hemodynamic data were averaged over at least 5-minutes of supine rest 10-minutes after the BA-FMD and immediately prior to the POP-FMD protocol.

Vascular Measures. As described in O'Brien et al. (202), the right BA and left POP were imaged with the participants in the supine and prone positions, respectively. The BA was imaged 3-5 cm proximal to the antecubital fossa and the POP was imaged proximal to the bifurcation at or slightly above the popliteal fossa. A pressure cuff attached to a rapid inflation system (E20 and AG101, Hokanson®, Bellevue, WA) was positioned around the largest circumference of the forearm (BA; ~3 cm distal to the

antecubital fossa) or lower leg (POP; ~10 cm distal to the popliteal fossa). All images were obtained using a 12-MHz multi-frequency linear array probe attached to a high-resolution ultrasound system (Vivid i, General Electric Healthcare). Blood velocity signals were recorded in duplex mode at a pulsed frequency of 5-MHz and corrected with an insonation angle of 60° that remained constant throughout the study. The sample volume was adjusted for each participant such that the anterior to the posterior intima were included, as recommended in published guidelines (282). Artery lumen diameter and blood velocity were measured for a minimum of 2-minutes prior to inflation of the pneumatic cuff. The pressure cuff was then rapidly inflated to 250 mmHg for 5-minutes. Continuous arterial lumen diameter and blood velocity recordings were collected throughout the cuff inflation period. Upon release of cuff pressure, lumen diameter and blood velocity recordings continued for an additional 5-minutes.

Following at least 10-minutes of rest after the FMD test, the POP was imaged for 1-minute before and 10-minutes following a sublingual administration of nitroglycerin spray (0.4 mg). This nitroglycerin test provides a measure of endothelial-independent vasodilation (39). POP nitroglycerin-mediated vasodilation was calculated as a percentage increase from baseline to the peak lumen diameter obtained during the 10-minute period following sublingual administration of nitroglycerin. POP nitroglycerin-mediated vasodilation was not conducted in two participants (total participants: HIIT: n=11/12 and MICT: n=11/12).

Video signals from the ultrasound were exported to a laptop via a video graphics array converter (Epiphan Systems Inc., VGA 2 USB, Ottawa) for offline analysis.

Analysis of artery diameter, blood velocity and shear rate (i.e., frictional force of blood flow on the endothelium) were performed using automated commercial edge-detection and wall-tracking software combined with simultaneous Doppler waveform envelope analysis (FMD Studio, Cardiovascular Suite, Quipu, Pisa, Italy). This software was used to measure baseline diameter, nadir diameter (i.e., L-FMC) and peak diameter (i.e., FMD). All vascular measurements were blindly analyzed by a single investigator, who has demonstrated coefficients of variation of 2.2%, 2.7%, 3.8%, and 4.2% for baseline diameter, nadir diameter, L-FMC% and FMD%, respectively.

Absolute FMD was calculated as the difference (in mm) between the post-cuff deflation peak and baseline diameters. Relative FMD was calculated using the equation: FMD (%) = [(post-cuff deflation peak diameter – baseline diameter) ÷ baseline diameter × 100%]. Blood flow (ml/min) was calculated as mean blood velocity (cm/s) × π × lumen radius $(cm)^2$ × 60 (s/min). Popliteal and brachial vascular conductance were calculated by dividing POP and BA blood flow by MAP, respectively. All blood flow and MAP data from the corresponding FMD-baseline periods were used for these calculations. Shear rate (SR, s^{-1}) was defined as [8 × mean blood velocity (cm/s)] / diameter (cm). Subsequently, the SR area under the curve (SR_{AUC}) was calculated between the start of cuff deflation to the time that peak dilation occurred. Whether or not, and how, FMD responses should be normalized to SR_{AUC} is unclear (6, 282). Our data did not meet the statistical assumptions required to normalize the FMD responses to SR_{AUC} (6, 296) in either the BA or POP [see Appendices, which contains scatterplots demonstrating the relationship between brachial (Appendix C) and popliteal (Appendix D) shear rate area

under the curve versus FMD]). However, SR_{AUC} responses and the relationships between FMD and SR_{AUC} are presented for both arteries, as per recommendations (6, 282).

L-FMC was represented in absolute (mm) and relative (%) terms using the nadir diameter obtained during the final 30-seconds of the 5-minute distal cuff occlusion period. Relative L-FMC was calculated using the equation: L-FMC (%) = [(baseline diameter – nadir diameter) ÷ baseline diameter ×100%].

As described by Atkinson and Batterham (7), allometric scaling of FMD has been recommended to account for differences in baseline arterial diameter. Although based on FMD analysis, we previously demonstrate that baseline diameter is correlated to the L-FMC responses in older adults (194). Allometric scaling is recommended if the relationship between the natural log of peak FMD diameter (or nadir diameter for L-FMC) and natural log of resting diameter yield an unstandardized β-coefficient that deviates from 1 and/or have an upper 95% confidence interval <1. These assumptions were met for L-FMC measurements in the BA and POP with β ± standard error (95% confidence intervals) of 0.979 ± 0.01 (0.97-0.99) and 0.989 ± 0.01 (0.98-1.00), respectively. However, the allometric assumptions were not met for BA-FMD [1.036 ± 0.01 (1.01-1.06)] and POP-FMD [1.008 ± 0.01 (0.99-1.03)], suggesting allometric scaling of FMD to be unnecessary in this study. These assumptions remained when checked for each group individually. Allometrically-scaled L-FMC were examined using an analysis of covariance (ANCOVA) model with the natural log of the difference [ln(nadir diameter) – ln(baseline diameter)] as the dependent variable and ln(baseline diameter) as the covariate. Group (HIIT, MICT, RT) and Time (pre-training, post-training) were used as a fixed-factors. Statistically significant ANCOVAs were followed up with pairwise

comparisons using Fisher's least squares difference *post hoc* testing (7). For each instance, overall group (not individual) allometrically-scaled arterial diameter changes were back transformed and presented as a percent change from the baseline diameter, as described in more detail previously (7). Separate ANCOVA models were conducted for each artery.

Statistical Analysis. All data were assessed for normality using a Shapiro-Wilk test, and non-normalized data were appropriately transformed (e.g., log transformation) prior to statistical analysis (body mass index, resting SV, resting CO, resting TVC, resting SBP, peak HR, absolute BA FMD, BA blood flow, BA resting SR, POP resting blood flow, POP resting SR). Participant descriptive characteristics were compared using one-way analysis of variance (ANOVA). The effects of exercise training on resting hemodynamic and vascular measurements were compared using a between-subjects (Group × Time) repeated measures ANOVA. The variance of differences was assessed using Mauchly's test of sphericity and when violated, the Greenhouse-Geisser correction to the degrees of freedom was applied. Bonferroni post-hoc testing was conducted on statistically significant ANOVAs. Based on the previously observed relationship between aerobic fitness and L-FMC (194, 195), correlational analyses were conducted to determine if the change in aerobic fitness ($\Delta\dot{V}O_2peak$) was related to the change (ΔL-FMC) in either artery. Some participants were taking anti-hypertensive medication (n = 4), which may have influenced their vascular function. Removing these individuals from analyses did not alter the significant findings determined from the full sample (see below).

Effect sizes (ES) were calculated post- to pre-training within each training group via Cohen's *d* (post-training mean – pre-training mean) ÷ pooled sample mean. Cohen's *d* effect sizes were interpreted as small (ES = 0.2 or -0.2), medium (ES = 0.5 or -0.5) and large (ES = 0.8 or -0.8) (45).

All statistics were completed in SPSS Version 25.0 (IBM, NY). Statistical significance was accepted as $P < 0.05$. All data are presented as means ± standard deviations (SD).

Chapter 5: Study 3: Results

Participant characteristics, systemic hemodynamics and aerobic fitness were similar between the groups at baseline (all, P>0.29) and are summarized in Table 5.1. No pre-post differences were observed for body mass index (RT: P=0.09), SV (HIIT: P=0.08), CO (P=0.44), and TVC (HIIT: P=0.06). Resting HR decreased (P<0.04) in the MICT group only. The HIIT group had lower post-training resting SBP, DBP, and MAP (all, P<0.03), but MICT only decreased DBP (P=0.02). RT did not change any systemic resting hemodynamic measurements (MAP: P=0.06; rest of variables, P>0.11). No pre-post differences were observed for peak respiratory exchange ratio (HIIT: P=0.08) or peak HR (P=0.11). Compared to pre-training, all groups had a greater $\dot{V}O_2$peak and PPO post-training (both, P<0.04).

Brachial Artery Hemodynamics: BA lumen diameter was larger following RT (P=0.02), but not HIIT or MICT (both, *P*>0.79; see Table 2). The increase in resting BA diameter lead to a lower resting BA-SR in the RT group (*P*=0.048). Resting BA blood flow velocity and blood flow were increased following HIIT only (both, *P*<0.003), which, resulted in a higher BA-SR (*P*=0.01). The increases in BA blood flow and

corresponding decreases in MAP following HIIT resulted in a larger resting BA vascular conductance in this group ($P<0.001$). MICT did not change any resting BA variables (all, $P>0.38$).

Table 5.1. Study 3 participant descriptive characteristics, resting hemodynamics and aerobic fitness.

	HIIT		MICT		RT	
	Pre-Training	Post-Training (ES)	Pre-Training	Post-Training (ES)	Pre-Training	Post-Training (ES)
Age (years)	68 ± 5		68 ± 6		66 ± 7	
Sex (Male, Female)	5♂, 7♀		4♂, 8♀		6♂, 8♀	
Body Mass Index (kg/m^2)	25.9 ± 3.1	25.8 ± 3.0 (-0.03)	25.2 ± 3.6	26.0 ± 3.1 (-0.01)	27.2 ± 5.1	27.0 ± 5.1 (-0.05)
Heart Rate (beats/min)	70 ± 11	68 ± 10 (-0.21)	68 ± 11	65 ± 10* (-0.32)	68 ± 8	66 ± 10 (-0.21)
Stroke Volume (ml/beat)[a]	67 ± 27	73 ± 21 (0.24)	71 ± 25	73 ± 23 (0.10)	69 ± 25	74 ± 22 (0.21)
Cardiac Output (L/min)[a]	4.7 ± 2.1	4.9 ± 1.4 (0.12)	5.0 ± 2.0	5.0 ± 1.9 (-0.002)	4.7 ± 1.8	4.8 ± 1.4 (0.09)
Systolic Blood Pressure (mmHg)	126 ± 12	120 ± 10* (-0.60)	121 ± 12	121 ± 10 (-0.02)	128 ± 9	125 ± 11 (-0.35)
Diastolic Blood Pressure (mmHg)	74 ± 9	68 ± 9* (-0.63)	71 ± 9	66 ± 7* (-0.62)	70 ± 11	67 ± 9 (-0.29)
Mean Arterial Pressure (mmHg)	91 ± 8	84 ± 8* (-0.75)	87 ± 9	83 ± 8 (-0.44)	89 ± 9	85 ± 9 (-0.39)
Total Vascular Conductance (ml/min/mmHg)[a]	53 ± 24	59 ± 17 (0.31)	60 ± 28	62 ± 29 (0.09)	54 ± 23	57 ± 18 (0.16)
Aerobic Fitness (ml/kg/min)	23 ± 7	28 ± 7* (0.86)	23 ± 4	29 ± 8* (0.69)	23 ± 7	26 ± 10* (0.25)
Peak Aerobic Power Output (W)	145 ± 39	170 ± 50* (0.69)	150 ± 36	179 ± 43* (0.55)	135 ± 60	144 ± 60* (0.15)
Peak Heart Rate (beats/min)	156 ± 7	153 ± 16 (-0.21)	149 ± 13	151 ± 19 (0.12)	150 ± 9	148 ± 20 (-0.08)

Peak RER ($\dot{V}CO_2/\dot{V}O_2$)	1.22 ± 0.12	1.18 ± 0.12 (-0.31)	1.23 ± 0.06	1.25 ± 0.08 (0.29)	1.16 ± 0.12	1.17 ± 0.12 (0.12)

Data are presented as means ± standard deviations. HIIT, high-intensity interval training; MICT, moderate-intensity continuous training; RT, resistance training. *, $P < 0.05$ versus pre-training within same training group. [a]n = 10 for MICT group. Between-subject repeated measures (Group × Time) ANOVA with Bonferroni *post-hoc* testing compared pre- versus post-training data within each group. Effect sizes (ES) were calculated post- to pre-training within each group via Cohen's *d*.

Table 5.2. Comparison of Brachial artery parameters across the HIIT, MICT and RT protocols.

	HIIT		MICT		RT	
	Pre-Training	Post-Training (ES)	Pre-Training	Post-Training (ES)	Pre-Training	Post-Training (ES)
Resting						
Resting diameter, mm	4.28 ± 0.74	4.28 ± 0.72 (-0.01)	3.91 ± 0.51	3.91 ± 0.51 (-0.01)	3.69 ± 0.63	3.74 ± 0.64* (0.07)
Mean blood velocity, cm/s	11.8 ± 3.7	14.2 ± 4.2* (0.59)	12.4 ± 5.0	12.6 ± 4.2 (0.04)	13.3 ± 5.1	12.0 ± 3.8 (-0.28)
Blood flow, ml/min	102 ± 43	120 ± 42* (0.43)	90 ± 38	93 ± 41 (0.06)	83 ± 36	78 ± 28 (-0.17)
Resting shear rate, s^{-1}	113 ± 39	137 ± 47* (0.55)	130 ± 61	131 ± 48 (0.01)	150 ± 62	134 ± 50* (-0.28)
Vascular conductance, ml/min/mmHg[a]	1.1 ± 0.5	1.5 ± 0.5* (0.57)	1.1 ± 0.5	1.1 ± 0.6 (0.11)	1.0 ± 0.4	0.9 ± 0.3 (.11)
Flow-Mediated Dilation						
Peak diameter, mm	4.49 ± 0.80	4.58 ± 0.79* (0.11)	4.10 ± 0.56	4.18 ± 0.57* (0.16)	3.87 ± 0.69	3.93 ± 0.70* (0.09)
Absolute FMD, mm	0.21 ± 0.09	0.30 ± 0.09* (0.86)	0.19 ± 0.08	0.28 ± 0.09* (0.92)	0.18 ± 0.08	0.20 ± 0.08 (0.17)
SR_{AUC}, a.u.	11589 ± 3998	15334 ± 6124* (0.69)	12880 ± 4988	14641 ± 4201 (0.38)	12056 ± 2683	14941 ± 4961* (0.69)
Time-to-peak dilation, s	54 ± 13	55 ± 12 (0.07)	66 ± 21	68 ± 18 (0.08)	63 ± 14	65 ± 11 (0.19)
Low-Flow-Mediated Constriction						
Nadir diameter, mm	4.24 ± 0.70	4.20 ± 0.66 (-0.06)	3.87 ± 0.49	3.86 ± 0.49 (-0.03)	3.66 ± 0.63	3.70 ± 0.64* (0.07)

Absolute L-FMC, mm	–0.04 ± 0.06	–0.08 ± 0.07* (-0.54)	–0.04 ± 0.04	–0.05 ± 0.04 (-0.26)	–0.03 ± 0.02	–0.03 ± 0.03 (-0.10)

Data are presented as means ± standard deviations. HIIT, high-intensity interval training; MICT, moderate-intensity continuous training; RT, resistance training; FMD, flow-mediated dilation; SR_{AUC}, shear rate area under the curve to peak dilation. *, $P < 0.05$ to pre-training within training group. [a]n = 10 for MICT group. Between-subject repeated measures (training group × time) ANOVA with Bonferroni *post-hoc* testing. Effect size (ES) were calculated post- to pre-training within each group via Cohen's *d*.

Brachial Artery Vasoreactivity: As shown in Table 5.2, BA peak diameter was greater following training in all three groups (all, $P<0.03$). BA-SR_{AUC} was larger following HIIT and RT (both, $P<0.01$), but not MICT ($P=0.12$). Training did not change the time-to-peak diameter in any group (all, $P>0.37$). Relative BA-FMD was enhanced following HIIT (4.8±1.8% to 6.7±1.3%; $P<0.001$; ES=1.08) and MICT (4.7±1.9% to 6.8±1.7%; $P<0.001$; ES=1.01), but not RT (4.7±1.4% to 5.0 ± 1.4%; $P=0.52$; ES=0.15; Figure 5.4A).

BA L-FMC was greater following HIIT (-0.9±1.7% to -1.7±1.2%; $P<0.001$; ES=-0.67), but not MICT (-0.9±1.0% to -1.2±0.9%; $P=0.14$; ES=-0.32) or RT (-0.8±0.6% to -0.9±0.7%; $P=0.68$; ES=-0.11; Figure 5.4B). This observation was unchanged after allometric scaling to baseline diameter (HIIT: P=0.03; MICT & RT: $P>0.42$; Figure 5.4C). The change in BA L-FMC was moderately correlated with the change in aerobic fitness (r = -0.51, $P=0.001$; $\Delta\dot{V}O_2$peak vs. ΔBA L-FMC; see Figure 5.5).

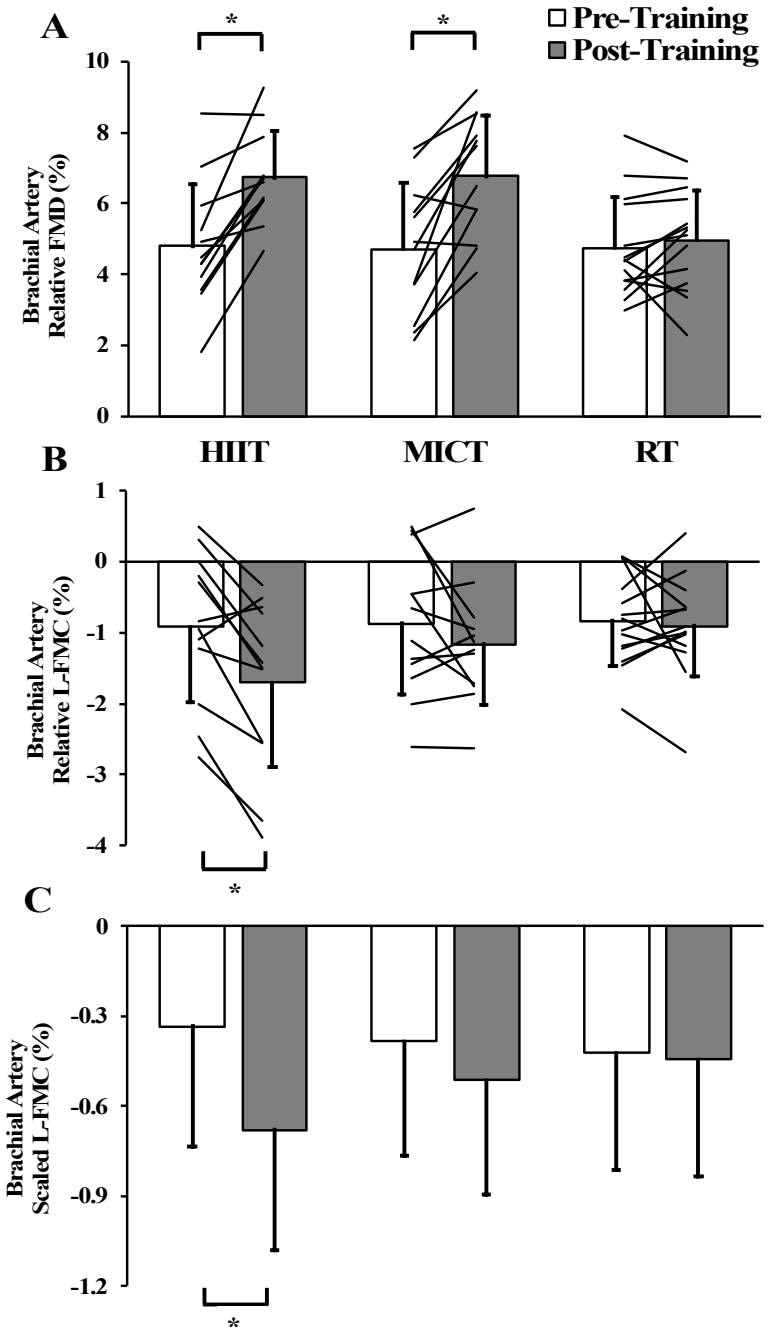

Figure 5.4. Comparison of brachial artery relative flow-mediated dilation (FMD, %; *A*), relative low-flow-mediated constriction (L-FMC, %; *B*), and allometrically-scaled L-FMC (%; *C*) from pre- to post-training following high-intensity interval training (HIIT), moderate-intensity continuous training (MICT) and whole-body resistance training (RT). Data are presented as means ± SD. Individual data are presented in panels *A* and *B*. *, P < 0.05 versus pre-training within same training group. Data were analyzed via a between-subjects repeated measures (training Group × Time) ANOVA with Bonferroni *post-hoc* testing. HIIT: *n*=12; MICT: *n*=12; RT: *n*=14.

Popliteal Artery Hemodynamics: As shown in Table 5.3, POP lumen diameter was larger after RT (P=0.02), but not following HIIT or MICT (both, P>0.62). Mean POP blood flow velocity (MICT: P=0.08), blood flow, resting SR (MICT: P=0.08), and POP vascular conductance were unchanged following any of the three-training protocol (all, P>0.10 unless specified).

Popliteal Artery Vasoreactivity: A greater POP peak diameter in response to reactive hyperemia was observed after MICT and RT (both, P<0.02), but not HIIT (P=0.052) (Table 5.3). All groups had a post-training increase in the SR_{AUC} (all, P<0.001) with no changes in the time-to-peak diameter (P>0.52). POP-FMD was enhanced following HIIT (3.6±1.9% to 4.9±1.5%; P<0.001; ES=0.72) and MICT (2.6±1.7% to 4.0± 1.9%; P<0.001; ES=0.71), but not RT (3.1±1.9% to 3.4±1.8%; P=0.20; ES=0.16; see Figure 5.6A).

Similarly, POP L-FMC was increased after HIIT (-0.9±1.1% to -1.8±0.9%; P=0.01; ES=-0.82) and MICT (-0.8±1.6 to -1.5±0.7%; P=0.045; ES=-0.56) but was unchanged following RT (-1.0±1.7% to -1.0±1.1%; P=1.00; ES=0.0001; Figure 5.6B). However, allometrically-scaled POP L-FMC was not statistically greater in the MICT group (-0.4±0.5% to -0.7±0.6%; P=0.16). In contrast, the greater L-FMC response following HIIT remained significant after scaling for baseline diameter (-0.4±0.6% to -0.8±0.6; P=0.047; Figure 5.6C). The change in POP L-FMC was not correlated to the change in aerobic fitness (r = -0.27, P=0.10; $\Delta\dot{V}O_2$peak vs. ΔPOP L-FMC; see Figure 5.7).

POP nitroglycerin-mediated vasodilation was unchanged following any of the training protocols (all, P>0.33).

Table 5.3. Comparison of Popliteal artery parameters across the HIIT, MICT and RT protocols

	HIIT		MICT		RT	
	Pre-Training	Post-Training (ES)	Pre-Training	Post-Training (ES)	Pre-Training	Post-Training (ES)
Resting						
Resting diameter, mm	6.35 ± 1.12	6.35 ± 1.13 (-0.001)	6.65 ± 2.04	6.63 ± 2.02 (-0.01)	6.93 ± 0.86	7.00 ± 0.83* (0.08)
Mean blood velocity, cm/s	5.3 ± 1.6	5.5 ± 1.8 (0.16)	3.7 ± 2.0	4.2 ± 1.8 (0.28)	4.0 ± 2.3	4.1 ± 2.1 (0.05)
Blood flow, ml/min	111 ± 54	110 ± 56 (-0.03)	75 ± 38	83 ± 37 (0.20)	96 ± 55	95 ± 47 (-0.02)
Resting shear rate, s^{-1}	35 ± 14	36 ± 15 (0.11)	25 ± 17	28 ± 16 (0.21)	24 ± 15	24 ± 14 (0.03)
Vascular conductance, ml/min/mmHg[a]	1.2 ± 0.5	1.3 ± 0.7 (0.15)	0.9 ± 0.5	1.0 ± 0.5 (0.31)	1.1 ± 0.7	1.1 ± 0.6 (0.04)
Flow-Mediated Dilation						
Peak diameter, mm	6.59 ± 1.23	6.67 ± 1.23 (0.06)	6.81 ± 2.04	6.88 ± 2.01* (0.03)	7.16 ± 0.98	7.25 ± 0.95* (0.10)
Absolute FMD, mm	0.24 ± 0.16	0.32 ± 0.13* (0.53)	0.17 ± 0.11	0.25 ± 0.12* (0.69)	0.22 ± 0.15	0.24 ± 0.15 (0.15)
SR_{AUC}, a.u.	6685 ± 2317	8208 ± 2812* (0.58)	5308 ± 2404	7066 ± 2805* (0.65)	5485 ± 2030	7429 ± 2485* (0.80)
Time-to-peak dilation, s	110 ± 32	111 ± 30 (0.03)	111 ± 20	109 ± 21 (-0.12)	89 ± 32	88 ± 29 (-0.04)
Low-Flow-Mediated Constriction						
Nadir diameter, mm	6.29 ± 1.10	6.24 ± 1.13 (-0.05)	6.60 ± 2.03	6.53 ± 2.00 (-0.03)	6.86 ± 0.76	6.93 ± 0.77* (0.10)
Absolute L-FMC, mm	–0.06 ± 0.06	–0.11 ± 0.06* (-0.78)	–0.05 ± 0.10	–0.10 ± 0.05* (-0.59)	–0.08 ± 0.13	–0.07 ± 0.09 (0.04)

Nitroglycerin-Mediated Dilation						
Relative NMD, %[b]	5.89 ± 2.22	6.04 ± 1.88 (0.07)	5.66 ± 2.12	6.00 ± 1.95 (0.17)	5.43 ± 2.30	5.42 ± 2.16 (0.001)
Absolute NMD, mm[b]	0.38 ± 0.19	0.39 ± 0.17 (0.06)	0.35 ± 0.11	0.36 ± 0.10 (0.16)	0.39 ± 0.19	0.39 ± 0.19 (0.001)

Data are presented as means ± standard deviations. HIIT, high-intensity interval training; MICT, moderate-intensity continuous training; RT, resistance training; FMD, flow-mediated dilation; SR_{AUC}, shear rate area under the curve to peak dilation; NMD, nitroglycerin-mediated dilation. *, $P < 0.05$ to pre-training within training group. [a]n = 11 for MICT group; [b]*n*=11 for MICT and HIIT groups. Between-subject repeated measures (Group × Time) ANOVA with Bonferroni *post-hoc* testing. Effect sizes (ES) were calculated post- to pre-training within each group via Cohen's *d*.

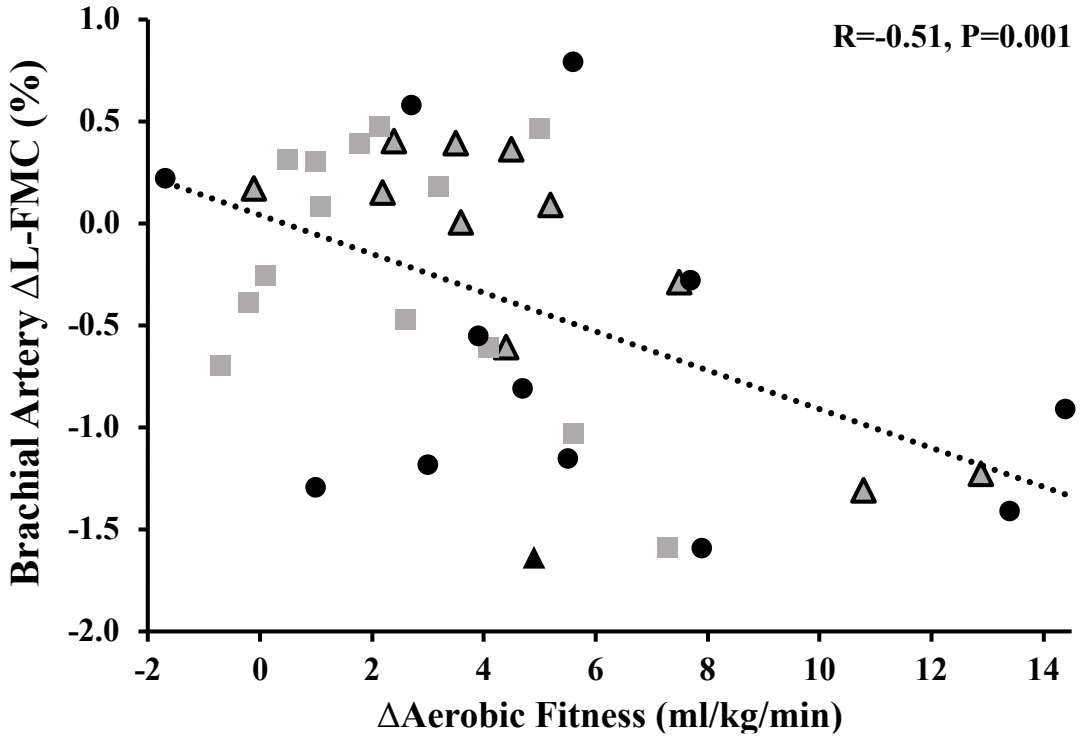

Figure 5.5. Scatterplot showing the relationship between the change in aerobic fitness (peak oxygen consumption) with the change in brachial artery relative low-flow-mediated constriction (L-FMC) in the pooled sample. Data are presented for high-intensity interval training (circles), moderate-intensity continuous training (triangles) and resistance training groups (squares). The unstandardized β ± standard errors (95% confidence intervals) was -0.10±0.03, (-0.15 to -0.04)

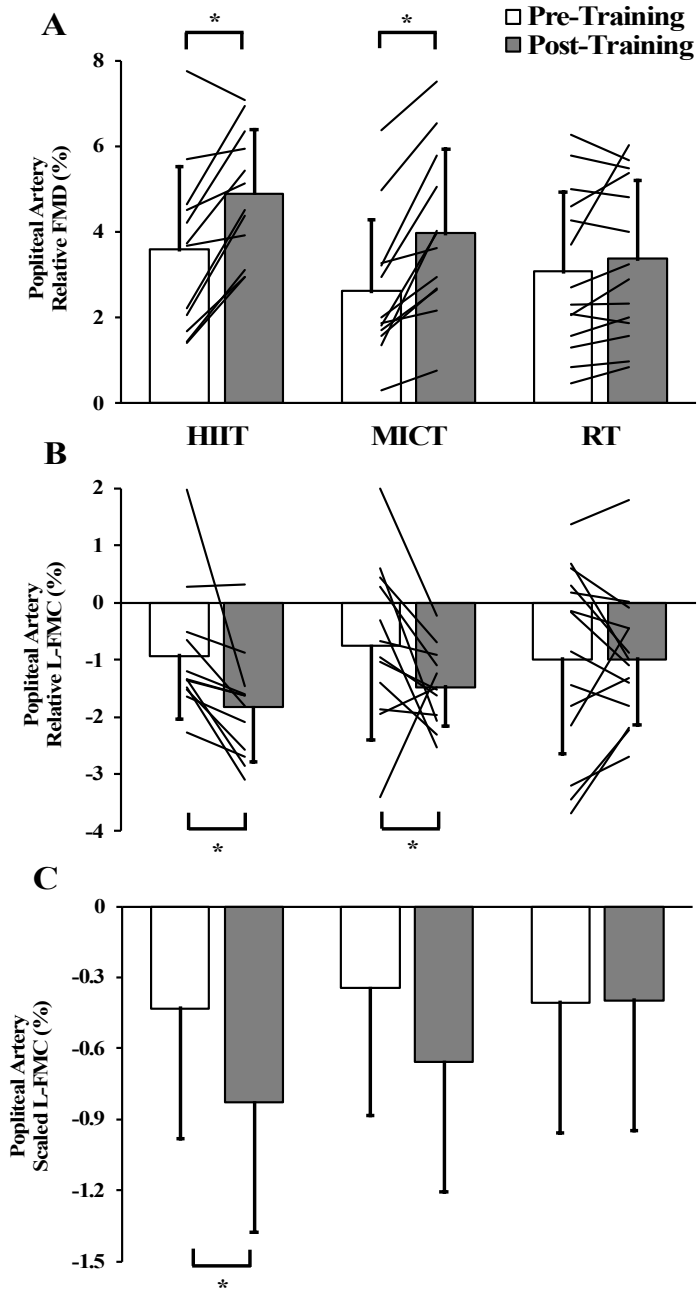

Figure 5.6. Comparison of popliteal artery relative flow-mediated dilation (FMD, %; A), relative low-flow-mediated constriction (L-FMC, %; B), and allometrically-scaled L-FMC (%; C) from pre- to post-training following high-intensity interval training (HIIT), moderate-intensity continuous training (MICT) and whole-body resistance training (RT). Data are presented as means ± SD. Individual data are presented in panels A and B. *, P < 0.05 to pre-training within same training group. Data were analyzed via a between-subjects repeated measures (Group × Time) ANOVA with Bonferroni *post-hoc* testing. HIIT: n=12; MICT: n=12; RT: n=14.

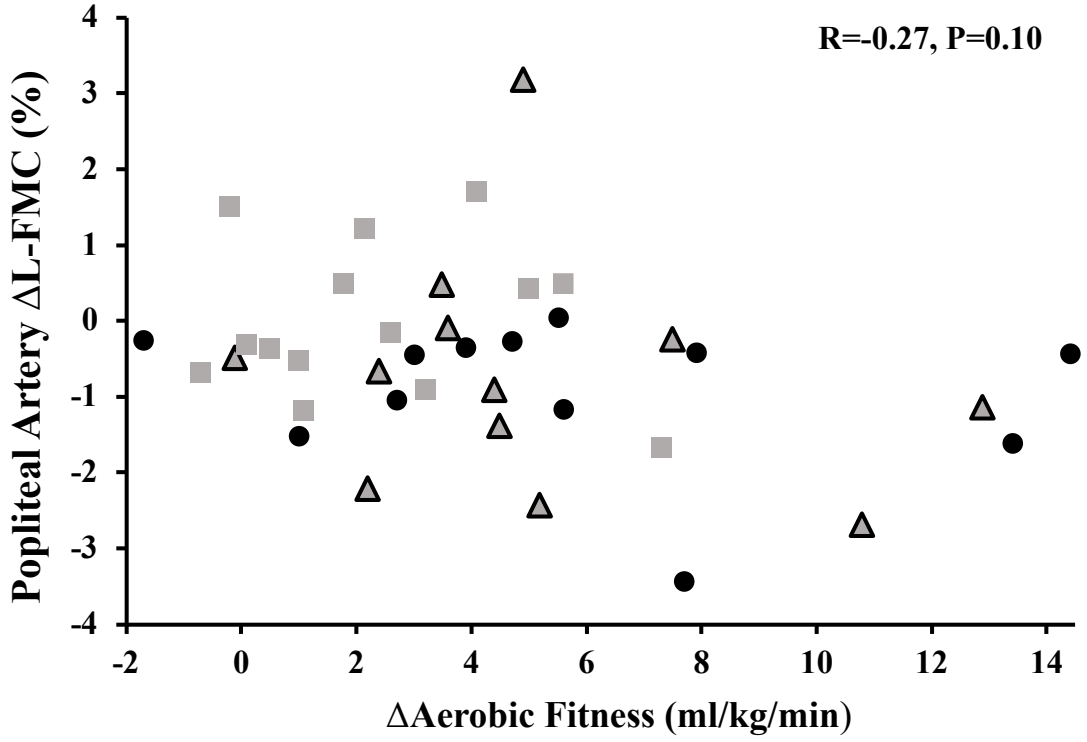

Figure 5.7. Scatterplot showing the relationship between the change in aerobic fitness (peak oxygen consumption) with the change in popliteal artery L-FMC in the pooled sample. Data are presented for high-intensity interval training (circles), moderate-intensity continuous training (triangles) and resistance training groups (squares). The unstandardized β ± standard errors (95% confidence intervals) was -0.09±0.05, (-0.19 to 0.02).

Chapter 5: Discussion

The purpose of this study was to compare endothelial-dependent vasodilator and vasoconstrictor responses in the brachial and popliteal arteries following short-term HIIT, MICT and RT in healthy older adults. Contrasting with my hypothesis, 6-weeks of HIIT and MICT similarly improved FMD responses in the brachial and popliteal arteries. Adding to the current literature, HIIT enhanced endothelial vasoconstrictor responses in both arteries, but MICT only increased L-FMC in the POP (i.e., to active limbs). Whole-

body RT did not improve either measure of vascular endothelial function in the BA nor POP. Favorable increases in resting blood flow and vascular conductance were observed in the BA following HIIT, but not MICT or RT. Similar responses were not identified in the POP. This is the first study to compare the effects of different exercise modalities on both endothelial vasodilator and vasoconstrictor function in healthy older adults. Furthermore, I report the first data to document vascular endothelial responses to training in the POP of older persons, which experiences different shear stress profiles during cycling exercise training than the BA.

These results support the notion that the vasculature may be favourably modified by short-term aerobic exercise training in older adults known to experience the deteriorating effects of advanced aging (67), such as chronic low-grade inflammation and increased oxidative stress (299). Adding to the current body of literature, the short-term HIIT and MICT models used in the present study (but not RT) elicited a sufficient stimulus for modifying both BA-FMD (ΔHIIT: 1.9%; ΔMICT: 2.1%; Figure 5.4) and POP-FMD (ΔHIIT: 1.3%; ΔMICT: 1.4%; Figure 5.6). Similar to observations in younger adults (295), short-term (i.e., 6-8 weeks) aerobic training augmented vasodilator function in two arteries that experience very different shear rate stimuli during cycling exercise in our population of older adults. Future research should aim to uncover the time course of these changes and if longer training periods continue to increase vasodilator function. Of particular importance, a 1% increase in relative BA-FMD equates to an ~10% reduction in the relative risk of a future cardiovascular event and all-cause mortality (319). Furthermore, BA-FMD has been shown to be predictive of coronary artery function; dysfunction of the coronary arteries precedes or is responsible for most cardiovascular

disease (26). In addition, endothelial dysfunction is an early marker of atherosclerosis (55), and the POP is a common site for plaque development (58). Herein, both HIIT and MICT resulted in a clinically significant reduction in cardiovascular disease risk in a relatively short period of exercise training among healthy, older adults.

Of relevance, we did not observe any changes in POP nitroglycerin-mediated vasodilation following any training regimen, suggesting that the improved POP-FMD responses in the HIIT and MICT groups are not attributed to enhanced vascular smooth muscle sensitivity. This finding is consistent with previous literature conducted in the BA, in that a meta-analysis of 26 exercise training studies concluded that BA nitroglycerin-mediated vasodilation was only marginally greater or was unchanged following exercise training (5). Perhaps longer training periods may be needed to combat the diminished POP nitroglycerin-mediated vasodilation associated with advancing age (213). Certainly, the present study was not designed to test the mechanisms responsible for our observed findings, but possible mechanisms behind the improved relative FMD responses is a reduction in oxidative stress (i.e., reactive oxygen species) or resting levels of the potent vasoconstrictor endothelin-1. Aging is associated with exacerbated oxidative stress that promotes the uncoupling of endothelial nitric oxide synthase (62) and endothelin-1 expression (63), which has been shown to be favourably modulated via exercise training (191, 237). Future studies are needed to confirm or refute this hypothesis.

We have previously demonstrated that higher cardiorespiratory fitness is associated with a greater L-FMC response in healthy older adults (194, 195). The present study is the first to determine if an aerobic or resistance exercise training intervention can

augment L-FMC in this population. Interestingly, HIIT augmented endothelial-dependent vasoconstrictor function in the BA and POP, but MICT only enhanced L-FMC in the POP (Figures 5.4 & 5.6). Considering that POP L-FMC was similarly improved following HIIT and MICT, but not after RT, the increased endothelial sensitivity to low-flow in the popliteal artery may be attributed to the increased shear stress in the active limb arteries during aerobic exercise, which translates to more effective signalling of vasodilatory/vasoconstrictor pathways. With that, our results suggest that the higher shear stress during HIIT versus MICT may be needed to elicit favorable adaptations in the vasoconstrictor side of endothelial function in the BA. L-FMC has been shown to be mediated through the endothelium (57), via the inhibition of vasodilatory signaling via endothelial-derived hyperpolarizing factors and prostaglandins (90), as well as enhanced vasoconstrictor signaling via endothelin-1 (264). These results suggest that HIIT may be more effective at promoting the inhibition of these vasodilatory pathways and/or stimulation of the endothelin-1 pathway in response to reductions in shear stress to a greater extent than MICT. The importance of enhancing these pathways and the L-FMC response to overall vascular health and risk of adverse cardiovascular events is currently unknown. Contrary to my previous cross-sectional observations equating cardiorespiratory fitness and vasoconstrictor function (194, 195), aerobic fitness, but not L-FMC, was improved in all three groups. It appears that other factors independent of increased aerobic fitness are responsible for subsequent enhancements to endothelial-dependent vasoconstrictor function. Of importance, these observations differ between arteries, with the relationship between the change in aerobic fitness and change in L-FMC being of moderate-strength in the BA (see Figure 5.5) but non-existent in the POP (see

Figure 5.7). As such, it is plausible that different mechanisms may be responsible for the improvements in L-FMC between the BA and POP.

With advancing age, there is a reduction in BA blood flow (48). Interestingly, HIIT, but not MICT or RT, resulted in a greater resting BA blood flow and shear stress through an increase in blood flow velocity with no corresponding change in resting BA diameter. Higher anterograde and lower retrograde BA blood flow velocity is associated with greater physical function in older persons (48) and a higher resting shear stress may promote anti-atherogenic gene expression (149). This suggests our HIIT protocol may be a more effective training program than MICT or RT alone at combatting the decline in physical function and progression of atherosclerosis in an aged population. However, in the lower-limb vasculature, pre-post outcomes were similar between HIIT and MICT.

Few studies have investigated if RT influences vascular function in older adults (5). Our results show that short-term RT did not alter endothelial-dependent vasodilator or vasoconstrictor function in either artery in this population. It may be possible that a longer duration of RT, or more intense RT, may be needed to elicit these functional adaptations. Interestingly, the whole-body RT did increase resting lumen diameter. Despite not meeting statistical assumptions, conducting allometric scaling to account for these changes in diameter did not result in RT exhibiting an increased FMD. While the mechanisms behind this are unclear, our observations are consistent in both the BA and POP. This warrants future investigations regarding the influence of transient, high-pressure shear stress patterns on local resting diameter in older adults, who are often encouraged to engage in regular RT (42). Of relevance, resting diameter is influenced by numerous competitive vasodilator and constrictor influences, limiting its use as an index

of vascular structure (178). With that, the maximal dilation in response to nitroglycerin, which represents near maximal diameter, has been used as a preferred index of arterial structure (178) and was not influenced by RT in the POP. This suggests that RT likely did not elicit structural changes in this short-term intervention.

Although improvements in BA-FMD were observed in the HIIT and MICT groups, endothelial-independent dilation was not assessed in the BA. However, it is unlikely that 6-weeks is long enough to enhance vascular smooth muscle function in the BA (5). I appreciate that this study was unable to provide information regarding the mechanisms of the observed findings. Future research incorporating blockades of endothelial-dependent vasodilatory and vasoconstrictor pathways pre- and post-training are warranted. With that, I did not observe training-induced changes in POP nitroglycerin-mediated dilation (i.e., endothelial-independent dilation), excluding alterations in vascular smooth cell sensitivity to endothelial-derived nitric oxide as a potential mechanism for the enhanced FMD responses. Sex-differences in the vascular responses to exercise training may exist (212). However, the number of males and females in the exercise groups were similar. Future studies should examine the role that sex has on exercise mode- and aerobic exercise intensity-induced vascular responses in older populations. I acknowledge this study did not include a control group, but the repeated measures design allowed participants to serve as their own controls. As well, randomized controlled studies investigating exercise modalities and endothelial function consistently demonstrate no improvement in the control group, as reviewed in (5), which in conjunction with the high-reproducibility of our ultrasound measures suggest the lack

of a control group to be a minor limitation that does not take away from our primary findings.

The findings of this study demonstrate that 6-weeks of HIIT and MICT were superior to RT at eliciting improvements in endothelial-dependent dilation in the brachial and popliteal arteries of older adults. While HIIT and MICT enhanced endothelial-dependent vasoconstrictor function in the POP, only the HIIT group exhibited a greater endothelial sensitivity to low-flow and baseline blood flow in the BA. HIIT produced clinically meaningful increases in vascular endothelial function in the short-term, which translates to a reduced risk of cardiovascular disease of a greater magnitude than MICT or RT in older adults. Therefore, exercise training programs aimed at improving peripheral vascular function in healthy older adults should consider implementing HIIT.

LINKING STUDIES 1 & 2 & 3 WITH STUDY 4

Studies 1-3 utilized the flow-mediated dilation technique to investigate the impact of aerobic fitness and exercise training intervention on endothelial function. Together, these studies supported that aerobic fitness and aerobic training improved endothelial, and by extension, peripheral vascular health in both the upper- and lower-limb vascular bed. However, proper peripheral vascular function involves a combination of vasodilatory and vasoconstrictor stimuli that act together to regular arterial function. While endothelial function is a major aspect of vascular function, efferent sympathetic neural activity directed towards skeletal muscle resistance vessels (i.e., MSNA) that promotes vasoconstriction also contributes to healthy blood vessel function. To thoroughly examine the impact of aerobic fitness on peripheral vascular function, both endothelial and neural aspects should be considered. Based on our findings observing that higher aerobic fitness is associated with better endothelial-dependent vasodilator and vasoconstrictor function, we sought to examine the association of aerobic fitness with sympathetic nerve activity. Recently, the quantification of sympathetic nerve activity has extended beyond time-averaged resting metrics (e.g. burst frequency and incidence) to also characterizing the vascular or hemodynamic outcome of each burst of MSNA (i.e., sympathetic transduction). Accordingly, the purpose of study 4 was to answer the question: Is aerobic fitness inversely associated with sympathetic neurohemodynamic transduction and blood pressure variability?

CHAPTER 6

STUDY 4: AEROBIC FITNESS IS INVERSELY ASSOCIATED WITH SYMPATHETIC TRANSDUCTION AND BLOOD PRESSURE VARIABILITY IN OLDER ADULTS

Chapter 6: ABSTRACT

Higher aerobic fitness is independently associated with better cardiovascular health in older adults. The transduction of muscle sympathetic nerve activity (MSNA) into mean arterial pressure (MAP) responses provides important insight regarding beat-by-beat neural circulatory control. Blood pressure is regulated on a beat-by-beat basis. Aerobic fitness is negatively associated with peak MAP responses to spontaneous MSNA in young males. Whether this relationship exists in older adults is known. I tested the hypothesis that aerobic fitness was inversely related to sympathetic transduction and blood pressure variability (BPV) in older adults. Relative peak oxygen consumption ($\dot{V}O_2$peak, indirect calorimetry) was assessed in 22 older adults (9 females, 65±5 years, 36.3±11.5 ml/kg/min). Common peroneal MSNA (microneurography) and arterial pressure (finger photoplethysmography) were continuously recorded during ≥10-minutes of rest. BPV was assessed using the average real variability index. MAP was tracked for 12 cardiac cycles following heartbeats associated with MSNA bursts (i.e., peak ΔMAP). Peak ΔMAP responses (0.9±0.6 mmHg) were negatively associated (all, $P<0.04$) with resting burst frequency (30±11 bursts/min; R=-0.47) and burst incidence (54±22 bursts/100heartbeats; R=-0.51), but positively associated with BPV (ρ=0.47). $\dot{V}O_2$peak was inversely related to the pressor responses to spontaneous MSNA bursts (R=-0.47, P=0.03) and BPV (ρ=-0.54, P=0.01), positively related to burst incidence (R=0.42, P=0.05), but unrelated to MSNA burst frequency (P=0.20). The $\dot{V}O_2$peak-BPV relationship remained after controlling for burst frequency, peak ΔMAP, age, and sex. Lower $\dot{V}O_2$peak was associated with augmented sympathetic transduction and BPV in older adults. These negative hemodynamic outcomes highlight the importance of higher aerobic fitness with ageing for optimal cardiovascular health.

Chapter 6: Introduction

The cardiovascular benefits of higher levels of aerobic fitness are well-established (164). Older adults exhibit an age-related decline in aerobic fitness (127) and an increased risk of developing hypertension (150). The mechanisms that contribute to the protective cardiovascular effects of being an older adult with a higher level of aerobic fitness are unclear, but may involve the sympathetic nervous system. Optimal beat-by-beat regulation of vascular resistance and arterial blood pressure are achieved via modulation of post-ganglionic sympathetic outflow directed towards the skeletal muscle circulation, termed muscle sympathetic nerve activity (MSNA) (103).

Ageing is characterized by autonomic dysregulation manifested as chronic sympathetic activation (61, 133), which may have deleterious consequences on cardiovascular health (103). Even in the absence of disease, healthy ageing is associated with an increase in MSNA burst frequency at a rate between ~0.5 to 1 bursts per minute each year (71, 133), with older adults exhibiting a greater reliance on sympathetic regulation of arterial pressure compared to younger adults (107). Conflicting evidence exists regarding the impact of greater aerobic fitness on resting sympathetic outflow in older adults, with reports indicating higher (184), lower (271) or no change (10) in MSNA burst frequency. The transduction of spontaneous bursts of MSNA into a pressor response are mediated via α-adrenergic receptor-mediated mechanisms (72), exaggerated pressor responses may (9, 304) or may not (141) be a major contributor in the development of hypertension, and have attracted the attention of numerous researchers (114, 200, 321). This analytical method has been used to provide important information

regarding sympathetic regulation of the vasculature between sexes in young adults (46), aerobic fitness levels (200, 270), sodium intake levels (9), and races (304), as well as with pregnancy (269), type 2 diabetes (322), statin therapy (322), aging (302), and in response to high altitude (16). The postulated negative consequences of an augmented sympathetic transduction may be attributed to greater fluctuations in blood pressure (i.e., larger blood pressure variability), which may result in end-organ damage overtime (233, 309, 311). Tracking transduction on a beat-by-beat basis provides high resolution information regarding the sympathetic neural control of arterial pressure beyond the standard, time-averaged metrics (burst frequency/incidence). In addition to chronic sympathoexcitation (133), older adults exhibit attenuated pressor responses to spontaneous MSNA burst compared to younger adults (302). In young males, we previously demonstrated that aerobic fitness ($\dot{V}O_2$peak) was inversely associated with spontaneous neurohemodynamic transduction (200). It is unknown if the same relationship exists in older adults, who exhibit exaggerated resting sympathetic outflow (133), and whether this contributes to augmented blood pressure variability.

In young adults, the influence of sympathetic baroreflex sensitivity (sBRS) may (113), or may not (200), be inversely related to spontaneous sympathetic transduction. Similarly, resting MSNA characteristics (burst frequency [bursts/min] and burst incidence [bursts/100heartbeats]) have been shown to be inversely related to the magnitude of sympathetic transduction in young adults (113, 233) It is unclear whether sBRS or traditional MSNA characteristics impact sympathetic transduction in older adults, but previous studies indicate that these factors need to be considered.

To provide insight into the impact of aerobic fitness ($\dot{V}O_2$peak) on sympathetic neural regulation of arterial pressure in older adults, the purpose of the present study was to investigate the association between aerobic fitness with sympathetic transduction in older adults. It was hypothesized that higher aerobic fitness would be inversely related to sympathetic transduction of spontaneous bursts of MSNA and to BPV in this population. The association between aerobic fitness and sympathetic transduction with traditional, time-averaged measures of MSNA characteristics were also explored.

Chapter 6: Methods

Participants

Twenty-two (9 females) healthy Caucasian older adults (≥55 years) participated in the study. Participants had no physical limitations to exercise, a body mass index <30 kg/m^2 and resting seated blood pressure (systolic [SBP] / diastolic [DBP]) of <140/<90 mmHg. All females were post-menopausal and not using any form of hormonal support. A power analysis was conducted using G*Power (75) based on the correlation between peak ΔMAP and relative $\dot{V}O_2$peak (R=0.69) from a previous study conducted in our lab in young males (200), which indicated that 11 participants were needed to achieve a power of 80%, assuming a two-tailed α=0.05. Given the discrepancies in the literature as to whether (302) or not (24) sex impacts neurohemodynamic transduction in older adults, data are presented separately for males and females. The protocols and procedures conformed to the Declaration of Helsinki and were approved by the local ethics boards at both Dalhousie and Acadia University. Participants were informed of the methods and study design verbally and in writing before providing consent.

Experimental Design

All participants completed 2 separate laboratory visits. Visit 1 involved a graded, maximal cycling exercise test to determine $\dot{V}O_2$peak. Visit 2 was dedicated to the microneurography session and conducted at least 5-days following the graded exercise test. All microneurography sessions were performed 6-hours post-prandial. Participants avoided strenuous physical activity, as well as the consumption of products known to acutely influence vascular function (e.g., caffeine, chocolate, citrus fruits, saturated fats, folic acid supplements, antioxidant and multivitamin supplements) for 24-hours (282). No participants were taking any prescribed medications known to influence the cardiovascular or nervous systems. All study visits were performed in a thermoneutral environment (~21°C).

Experimental Procedures

Anthropometrics and Aerobic Fitness. Height and weight were measured using a calibrated stadiometer/physician's scale (Health-O-Meter, McCook Il, USA) to the nearest 0.5-cm and 0.1-kg, respectively. An incremental and maximal exercise test on a cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands) was administered to determine $\dot{V}O_2$peak via a mixing chamber-based commercial metabolic system (TrueOne 2400, Parvo Medics Inc., Sandy, UT). Following a 5-minute warm-up period of light-intensity cycling (30-50W), the workload gradually increased by 15-20 watts/min until voluntary exhaustion. The peak aerobic power output achieved at the end of the test was recorded. Ratings of perceived exertion were determined every 2-minutes using the Borg 6-20 scale (21). Respiratory exchange ratio was calculated as the volume rate of carbon dioxide (CO_2) divided by $\dot{V}O_2$. All participants achieved ≥2 of the following $\dot{V}O_2$peak/$\dot{V}O_2$max criteria: 1) a plateau in relative $\dot{V}O_2$ (<2.1 ml/min/kg) despite an

increase in workload, 2) a respiratory exchange ratio ≥1.10, or 3) a rating of perceived exertion ≥18. Given that not all participants exhibited a plateau in relative $\dot{V}O_2$ (primary criteria for $\dot{V}O_2$max), the term $\dot{V}O_2$peak was used throughout. Relative $\dot{V}O_2$ data were averaged over 15-second intervals for the duration of the graded exercise protocol. Peak $\dot{V}O_2$ was considered as the greatest 30-second average.

Systemic Hemodynamics. Heart rate (HR) was determined via cardiac intervals obtained from lead II of a standard bipolar limb lead electrocardiogram. Beat-by-beat SBP and DBP were measured using finger photoplethysmography (Portapres; Finapres Medical Systems, Amsterdam, Netherlands). Left brachial artery measurements of SBP and DBP were also recorded by an automated patient vital signs monitor (Carescape v100, General Electric Healthcare) and used to perform a 'physiological calibration' of the Portapres waveform. Portapres data were sampled continuously at 200 Hz using a PowerLab (PL3508 PowerLab 8/53, ADInstruments, Sydney, Australia) data acquisition system and the electrocardiogram waveform was sampled at 1000 Hz. Recordings were displayed in real-time and analyzed offline using LabChart software (Version 8, ADInstruments, Sydney, Australia). Mean arterial pressure (MAP) was calculated from the beat-by-beat Portapres waveform in LabChart using the equation ⅓ SBP + ⅔ DBP. Beat-by-beat hemodynamic data were averaged over at least 5-minutes during the MSNA recording period (mean: 10.4 ± 0.6-min).

Popliteal Macrovascular and Microvascular Function. The popliteal artery was imaged with the participants in the prone positions, proximal to the bifurcation at or slightly above the popliteal fossa. A pressure cuff attached to a rapid inflation system (E20 and AG101, Hokanson®, Bellevue, WA) was positioned around the largest

circumference of the lower leg (~10 cm distal to the popliteal fossa). All images were obtained using a 12-MHz multi-frequency linear array probe attached to a high-resolution ultrasound system (Vivid i, General Electric Healthcare). Blood velocity signals were recorded in duplex mode at a pulsed frequency of 5-MHz and corrected with an insonation angle of 60° that remained constant throughout the study. The sample volume was adjusted for each participant such that the anterior to the posterior intima were included, as recommended in published guidelines (282). Artery lumen diameter and blood velocity were measured for a minimum of 2-minutes prior to inflation of the pneumatic cuff. The pressure cuff was then rapidly inflated to 250 mmHg for 5-minutes. Continuous arterial lumen diameter and blood velocity recordings were collected throughout the cuff inflation period. Upon release of cuff pressure, lumen diameter and blood velocity recordings continued for an additional 5-minutes.

Cineloops were saved directly onto the ultrasound machine (n=18) or video signals from the ultrasound were exported to a laptop (n=4) via a video graphics array converter (Epiphan Systems Inc., VGA 2 USB, Ottawa) for offline analysis. Analysis of artery diameter, blood velocity and shear rate (i.e., frictional force of blood flow on the endothelium) were performed using automated commercial edge-detection and wall-tracking software combined with simultaneous Doppler waveform envelope analysis (FMD Studio, Cardiovascular Suite, Quipu, Pisa, Italy).

Absolute FMD was calculated as the difference (in mm) between the post-cuff deflation peak and baseline diameters. Relative FMD was calculated using the equation: FMD (%) = [(post-cuff deflation peak diameter – baseline diameter) ÷ baseline diameter × 100%]. Blood flow (ml/min) was calculated as mean blood velocity (cm/s) × π × lumen

radius $(cm)^2 \times 60$ (s/min). Popliteal vascular conductance was calculated by dividing popliteal blood flow by mean arterial pressure (via finger photoplethysmography mentioned above). Shear rate (SR, s^{-1}) was defined as [8 × mean blood velocity (cm/s)] / diameter (cm). Subsequently, the SR area under the curve (SR_{AUC}) was calculated between the start of cuff deflation to the time that peak dilation occurred. Whether or not, and how, FMD responses should be normalized to SR_{AUC} is unclear (6, 282). Our data did not meet the statistical assumptions required to normalize the FMD responses to SR_{AUC} or allometrically scale the responses (6, 296).

Resistance vessel function may also be determined by examining the post-occlusion reactive hyperemia following the release of the ischemic portion of the FMD technique (153). While inter-related with FMD, this reactive hyperemia reflects the dilation of downstream resistance vessels. Importantly, sympathetic nerve activity is primarily directed towards the resistance vessels (i.e., arterioles) to modify arterial pressure (80). While recent guidelines have been produced regarding how to determine resistance vessel function (153), there is not a clear metric for researchers to use. In the absence of a clear metric, it is recommended that data are presented in multiple ways (i.e., absolute blood flow and velocity, peak, and AUC) to be comprehensive and allow for comparisons with other studies. Accordingly, we present peak (always within first 5 seconds following cuff-deflation) and 60s AUC for popliteal blood flow, blood flow velocity, and popliteal vascular conductance (blood flow/MAP). Our vascular conductance is acknowledged to be an estimate given that MAP was based on heart-level derived MAP as opposed to pressure at the level of the leg, although all participants were in the supine/prone positions with no major hydrostatic differences between the leg and

heart. While Limberg et al. (153) highlight that typically 60s or 120s AUC have been used, the use of cineloops to save ultrasound data directly onto the machine required us to use 60s, due to some brief breaks in recording among the older ultrasound files.

Muscle Sympathetic Nerve Activity. Multi-unit post-ganglionic MSNA was recorded using standard microneurographic techniques (105). As previously described (200), the right common peroneal nerve was mapped using transcutaneous electrical stimulation posterior to the fibular head. A 2MΩ unipolar tungsten microelectrode (FHC; Bowdoin, MA), connected to an isolated pre-amplifier, was inserted percutaneously into a nerve fascicle. A non-insulated reference microelectrode was positioned 2-3 cm subcutaneously from the active recording site. Neural signals were amplified (~75,000×), bandpass filtered (500-2000Hz), full-wave rectified, and integrated (0.1 second time constant) to obtain a mean voltage neurogram (662C-4 Nerve Traffic Analysis System, University of Iowa Bioengineering, Iowa City, IA). MSNA recordings were identified by the presence of cardiac synchronicity, responsiveness to an end-expiratory apnea and/or Valsalva's maneuver, as well as a lack of response to arousal or skin stimulation. Once a stable MSNA signal was obtained, participants rested quietly in a darkened room for a minimum of 10-minutes while resting data were collected. The MSNA mean voltage neurogram was sampled at 400 Hz.

Time-averaged MSNA was quantified as burst frequency (bursts per minute) and burst incidence (bursts per 100 heart beats). Absolute burst amplitude (mV) was determined for each burst and normalized as a percentage of the tallest spontaneous burst measured during the recording period. Total MSNA activity was estimated as the product of the mean relative burst amplitude and burst frequency (136). Each mean neurogram

recording was inspected for shifts in the underlying baseline activity, which would indicate potential movement of the active microelectrode. Only stable MSNA recordings were used for subsequent analyses.

Sympathetic Transduction. The signal-average method of determining sympathetic transduction and the open-source program I developed are described in detail elsewhere (197). In brief, beat-by-beat cardiac intervals, arterial blood pressure and the integrated MSNA signal were time-aligned so that bursts could be identified within the cardiac cycle they were generated. The peak of each MSNA burst was identified using a semi-automatic peak detection algorithm within LabChart (Version 8, AD Instruments, Sydney, Australia). A separate macro identified the peak of the R-waves in the electrocardiogram signal. Bursts were confirmed or denied by a trained observer based on pulse synchrony (i.e., alignment with the corresponding peak R-wave), a 3:1 signal-to-inter-burst noise ratio and overall burst morphology. Time-aligned hemodynamic and MSNA data were extracted on a beat-by-beat basis and exported to a Microsoft Excel spreadsheet (Microsoft, Washington, USA). This sheet contained built-in functions that tracked absolute changes in MAP for 12 cardiac cycles following each burst (i.e., starting from cardiac cycle 0). Twelve cardiac cycles were selected to ensure the peak pressor responses were captured. For each participant, the average ΔMAP across each successive cardiac cycle was obtained and the peak response averaged, regardless of the cardiac cycle number that it occurred. This peak ΔMAP was the primary outcome measure used to represent the hemodynamic response to spontaneous bursts of MSNA. The average and nadir MAP responses to cardiac cycles absent of bursts were similarly analyzed as an internal control to ensure peak MAP increases were not due to random noise but truly due

to bursts of MSNA. Fairfax et al. (72) demonstrated that non-bursts or random samples of white noise were associated with an increase or no change in vascular conductance, respectively. In contrast, heartbeats associated with MSNA bursts caused a decrease in vascular conductance, substantiating that the vascular/pressor responses were due to sympathetic vasoconstrictor activity (72). These non-burst nadir MAP responses also provided information regarding non-neural hemodynamic regulation during sympathetic quiescence. All hemodynamic and pressor response analyses were conducted by a single investigator, who was blinded to participant identity at the time of MSNA analysis. Our laboratory has established high inter-observer reliability in analyzing burst frequency (R=0.97, P<0.001; absolute mean error: 1.7 ± 1.7 bursts/min), sympathetic transduction (peak ΔMAP; R=0.96, P<0.001; 0.19 ± 0.25 mmHg) and hemodynamic responses to non-bursts (nadir ΔMAP; R=0.97, P<0.001; 0.20 ± 0.14 mmHg) in 28 participants (n=12 older adults; n=16 younger adults). Although sympathetic transduction using ΔMAP was our primary outcome, the cardiac output (stroke volume estimated via the Modelflow method) and total peripheral resistance (MAP/cardiac output) responses to burst and non-burst heartbeats were also determined and presented in Figure 6.1 for completeness.

Aerobic fitness was unrelated to peak increases in total peripheral resistance (P=0.65), but negatively correlated to the peak rise in cardiac output (R=-0.43, P=0.05) following spontaneous bursts. Relative $\dot{V}O_2$peak was not associated with nadir total peripheral resistance or cardiac output responses following cardiac cycles without bursts (all, P>0.09).

Sympathetic Baroreflex Sensitivity. For each participant, DBP values were assigned to 3 mmHg bins and burst incidence calculated for each bin (bursts per cardiac cycle × 100). The slope (bursts/100heartbeats/mmHg) of the regression between DBP bin (independent variable) and burst incidence (dependent variable), weighted for the number of cardiac cycles in each bin, provided an index of sBRS. Participant sBRS outcomes were excluded if the strength of this relationship was weak (i.e., R-value < -0.50) (113). Data from 2 participants were removed for this reason. Therefore, sBRS data are presented for 20 participants (average: R=0.91 ± 0.10; 8 females).

Blood Pressure Variability. Short-term blood pressure variability was determined for SBP, DBP and MAP using the average real variability index (ARV). This index accounts for the beat-by-beat order of arterial pressure measurements, corrects the limitations of other measures (e.g., standard deviation or coefficient of variation) that only reflect the dispersion around the mean (167), and has demonstrated prognostic value (168). The ARV was determined as the average absolute change between successive beat-by-beat arterial pressure measurements (323), as described in equation 1 below, where N is the number of blood pressure measurements, k is the order of measurements, and X is the outcome (SBP, DBP, or MAP),

$$\text{Equation 1. ARV} = \frac{1}{N-1}\sum_{k=1}^{N-1}|X_{k+1}-X_k|$$

Statistical Analysis.

All data were assessed for normality using a Shapiro-Wilk test, and non-normalized data were analyzed via non-parametric tests (nadir ΔMAP: Spearman's rank-order correlations or Wilcoxon signed-rank tests). The ΔMAP responses across cardiac

cycles between bursts and non-bursts were determined (Burst Presence × Cardiac Cycle) via a repeated measures analysis of variance (RM-ANOVA). The variance of differences was assessed using Mauchly's test of sphericity and when violated, the Greenhouse-Geisser correction to the degrees of freedom was applied. Bonferroni *post-hoc* testing for multiple comparisons was conducted on statistically significant ANOVAs. Correlational analysis (Pearson's or Spearman's) was conducted to examine the relationship between aerobic fitness (i.e., relative $\dot{V}O_2$peak) and the greatest average peak and nadir (non-burst cardiac cycles only) ΔMAP, blood pressure variability (ARV), sBRS, and traditional time-averaged MSNA burst characteristics. The relationship between ultrasound measures of resistance vessel function with aerobic fitness and sympathetic outcomes were investigated. Effect sizes (*R*-value or rho-value [ρ]) are presented. Statistically significant associations between MSNA measurements and aerobic fitness were followed up with exploratory partial correlations simultaneously controlling for age, sex, MSNA burst frequency and/or peak ΔMAP. All statistics were completed in SPSS Version 26.0 (IBM, NY). Statistical significance was accepted as $P<0.05$. All data are presented as means ± standard deviations.

Chapter 6: Results

Participant descriptive, resting hemodynamics, MSNA and hemodynamic transduction characteristics (i.e., cardiac cycles to peak/nadir MAP responses) are presented in Table 6.1. Compared to males, older females had lower aerobic fitness and higher resting heart rate (both, $P<0.02$). All other variables were similar between sexes (all, $P>0.13$).

Table 6.1. Study 4 participant descriptive characteristics, aerobic fitness, resting hemodynamics, and muscle sympathetic nerve activity outcomes.

	Participants (*n*=22)	Males (n=13)	Females (n=9)
Participant Characteristics			
Age (years)	65 ± 5	66 ± 4	63 ± 6
Height (cm)	169 ± 8	173 ± 9	166 ± 5*
Weight (kg)	73 ± 11	78 ± 10	66 ± 7*
Body Mass Index (kg/m^2)	25.4 ± 2.5	26.1 ± 2.2	24.5 ± 2.6
Aerobic Fitness			
$\dot{V}O_2$peak (ml/kg/min)	36.3 ± 11.5	41.5 ± 10.8	28.9 ± 8.3*
Peak Aerobic Power Output (W)	240 ± 58	260 ± 60	211 ± 42*
Peak Heart Rate (beats/min)	159 ± 11	160 ± 12	156 ± 11
Peak RPE (6-20 Borg scale)	18 ± 1	18 ± 1	18 ± 1
Peak RER ($\dot{V}CO_2/\dot{V}O_2$)	1.19 ± 0.08	1.21 ± 0.09	1.17 ± 0.08
Resting Hemodynamics			
Heart Rate (beats/min)	56 ± 5	54 ± 5	59 ± 4*
Systolic Blood Pressure (mmHg)	121 ± 11	120 ± 10	122 ± 12
Diastolic Blood Pressure (mmHg)	67 ± 9	65 ± 8	69 ± 9
Mean Arterial Pressure (mmHg)	85 ± 8	83 ± 7	87 ± 8
Muscle Sympathetic Nerve Activity			
Burst Frequency (bursts/min)	30 ± 11	31 ± 11	28 ± 12
Burst Incidence (bursts/100 heartbeats)	54 ± 22	57 ± 21	49 ± 23
Overall Burst Amplitude (% of maximum)	48 ± 1	48 ± 11	50 ± 9
Total Relative MSNA (% maximum • bursts/min)	1420 ± 641	1433 ± 634	1402 ± 689
Cardiac Cycles to Peak MAP (bursts)	6.5 ± 2.5	7.2 ± 2.8	5.6 ± 1.7
Cardiac Cycles to Nadir MAP (non-bursts)	6.1 ± 2.2	6.4 ± 1.7	5.7 ± 2.8

Data presented as means ± standard deviations. RER, respiratory exchange ratio; RPE, ratings of perceived exertion; $\dot{V}CO_2$, volume rate of carbon dioxide production; $\dot{V}O_2$, volume rate of oxygen consumption; MSNA, muscle sympathetic nerve activity; MAP, mean arterial pressure; RPE, ratings of perceived exertion. Total Relative MSNA was calculated as the product of burst frequency and mean relative burst amplitude. Sex differences identified via independent sample *t*-tests and indicated as an asterisk (P<0.05).

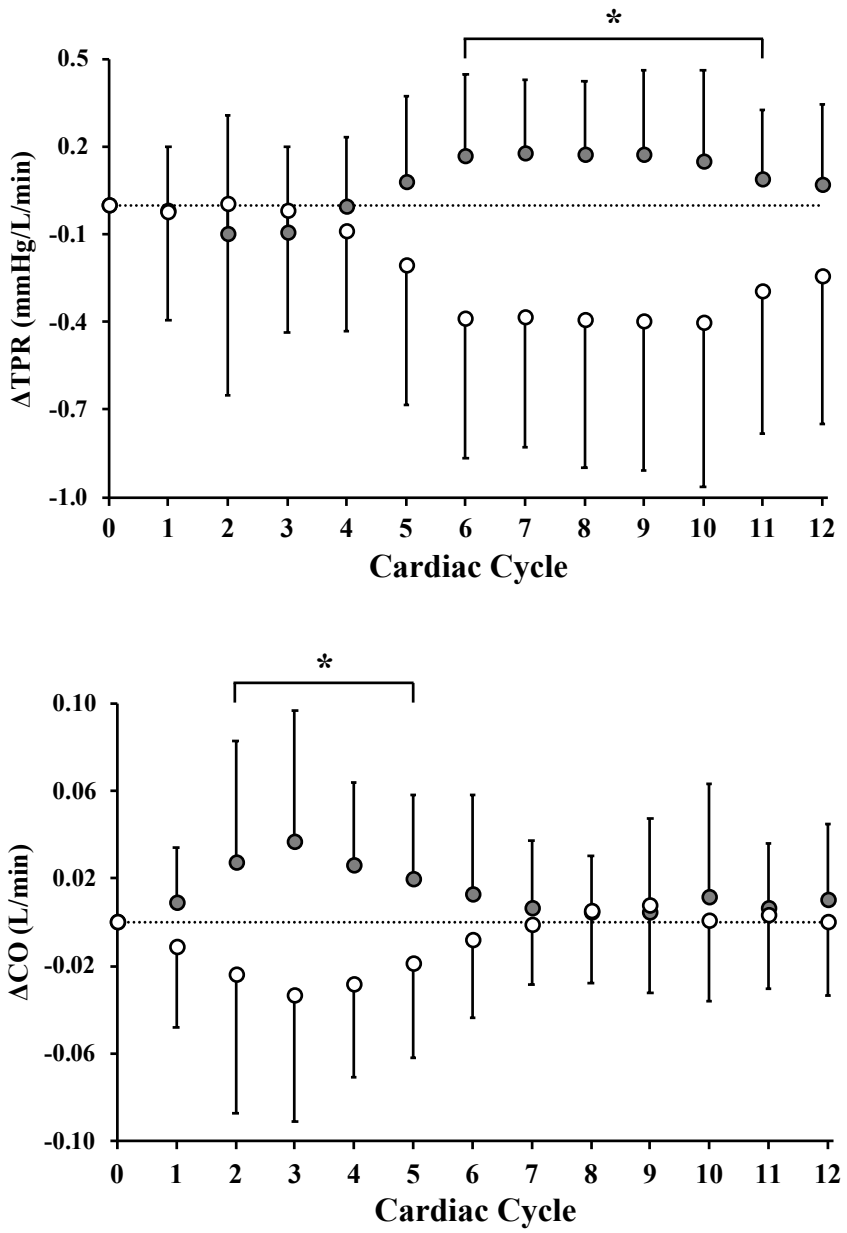

Figure 6.1. Beat-by-beat total peripheral resistance (TPR; top panel) and cardiac output (CO; bottom panel) responses following cardiac cycles associated with (bursts), or absent of (non-bursts), muscle sympathetic nerve activity. Data presented separately for bursts (filled circles) and non-bursts (white circles). The broken black line represents no changes in TPR or CO from cardiac cycle zero. Data presented as means ± standard deviations. Data were analyzed using a Burst Presence (2) × Cardiac cycle (12) repeated measure analysis of variance with Bonferroni post hoc testing. *P<0.05 between bursts and non-bursts.

The average ΔMAP responses to bursts and non-bursts are presented in Figure 6.2. As expected, ΔMAP rises were greater following MSNA bursts compared to non-burst cardiac cycles.

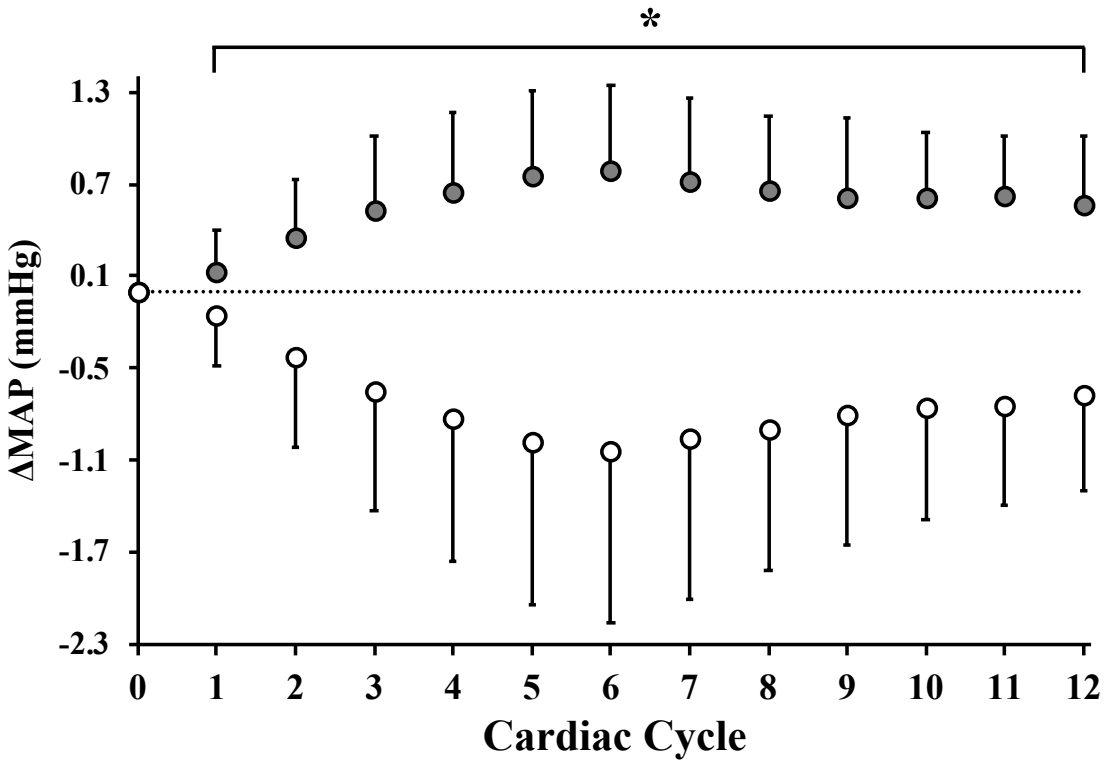

Figure 6.2. Beat-by-beat mean arterial pressure (MAP) responses following cardiac cycles associated with (bursts), or absent of (non-bursts), muscle sympathetic nerve activity. Data presented separately for bursts (filled circles) and non-bursts (open circles). The broken black line represents no change (0 mmHg) in MAP from cardiac cycle zero. Data presented as means ± standard deviations. Data were analyzed using a Burst Presence (2) × Cardiac Cycle (12) repeated measures analysis of variance with Bonferroni post hoc testing. *P<0.05 between bursts and non-bursts at the same cardiac cycle.

The MAP responses to bursts (peak ΔMAP; average: 0.9±0.6 mmHg) and non-bursts (nadir ΔMAP; average: -1.1±1.1 mmHg) were both inversely associated with

MSNA burst frequency (peak: Figure 6.3A; nadir: ρ=-0.61, *P*=0.003) and MSNA burst incidence (peak Figure 6.3B; ρ=-0.57, *P*=0.01), but unrelated to sBRS (peak: Figure 6.3C; ρ=0.29, *P*=0.21; average: -3.8 ± 1.6 bursts/100heartbeats/mmHg).

Aerobic fitness was unrelated to burst frequency (Figure 6.4A), average normalized burst amplitude (*R*=-0.06, *P*=0.80), total relative MSNA activity (*R*=0.22, *P*=0.32) and sBRS (*R*=-0.24, *P*=0.30), but positively related to burst incidence (Figure 6.4B). Accordingly, aerobic fitness was negatively associated with resting heart rate (*R*=-0.69, *P*<0.001). Controlling for sex, age and peak ΔMAP via partial correlation attenuated the relationship between burst incidence and aerobic fitness (*R*=0.29, *P*=0.23). $\dot{V}O_2$peak was inversely associated with the pressor responses to spontaneous bursts of MSNA (Figure 6.4C), but not correlated to the nadir responses following cardiac cycles without bursts (ρ=-0.21, *P*=0.36). Controlling for burst frequency, age and sex attenuated the relationship between aerobic fitness and peak neurohemodynamic transduction (*R*=-0.27, *P*=0.26).

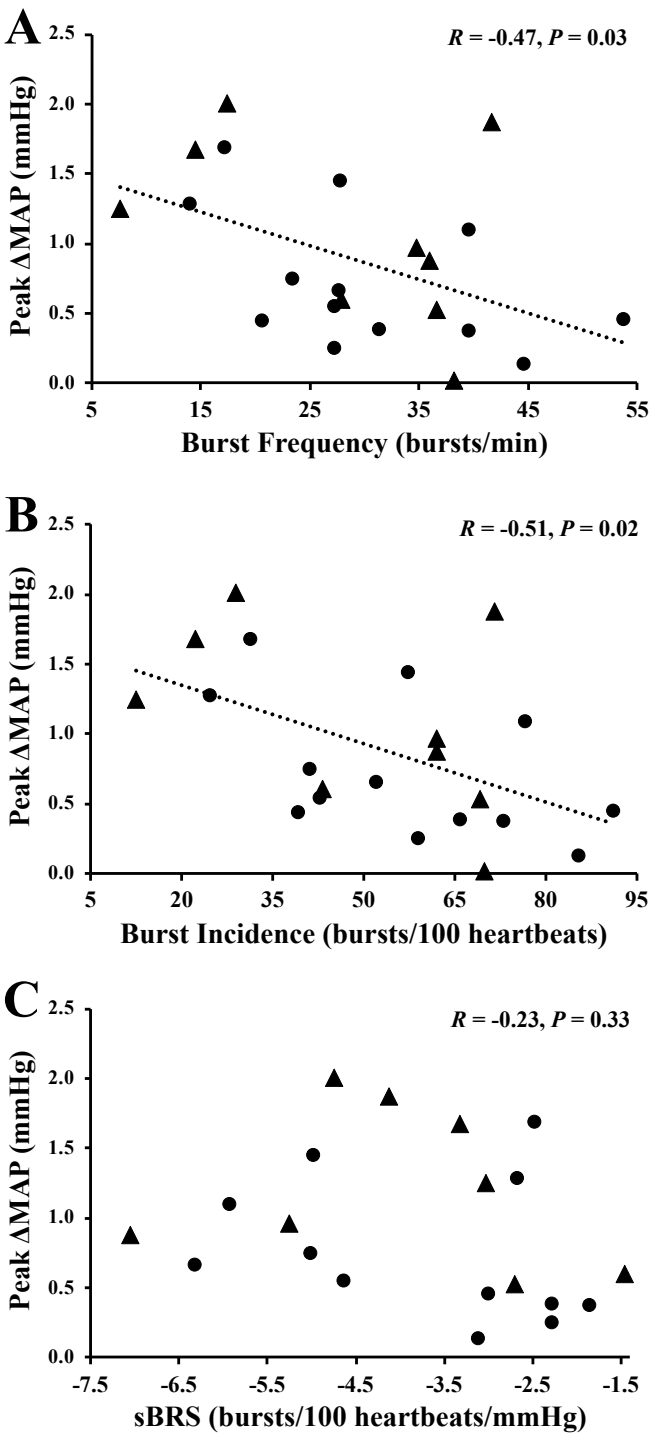

Figure 6.3. The relationship between peak pressor responses (peak ΔMAP) following bursts of muscle sympathetic nerve activity (MSNA) versus MSNA burst frequency (A), MSNA burst incidence (B), and sympathetic baroreflex sensitivity (C). The relationship between sympathetic transduction and these MSNA measures were determined via Pearson correlations (peak ΔMAP). Males and females are presented as circles and triangles, respectively.

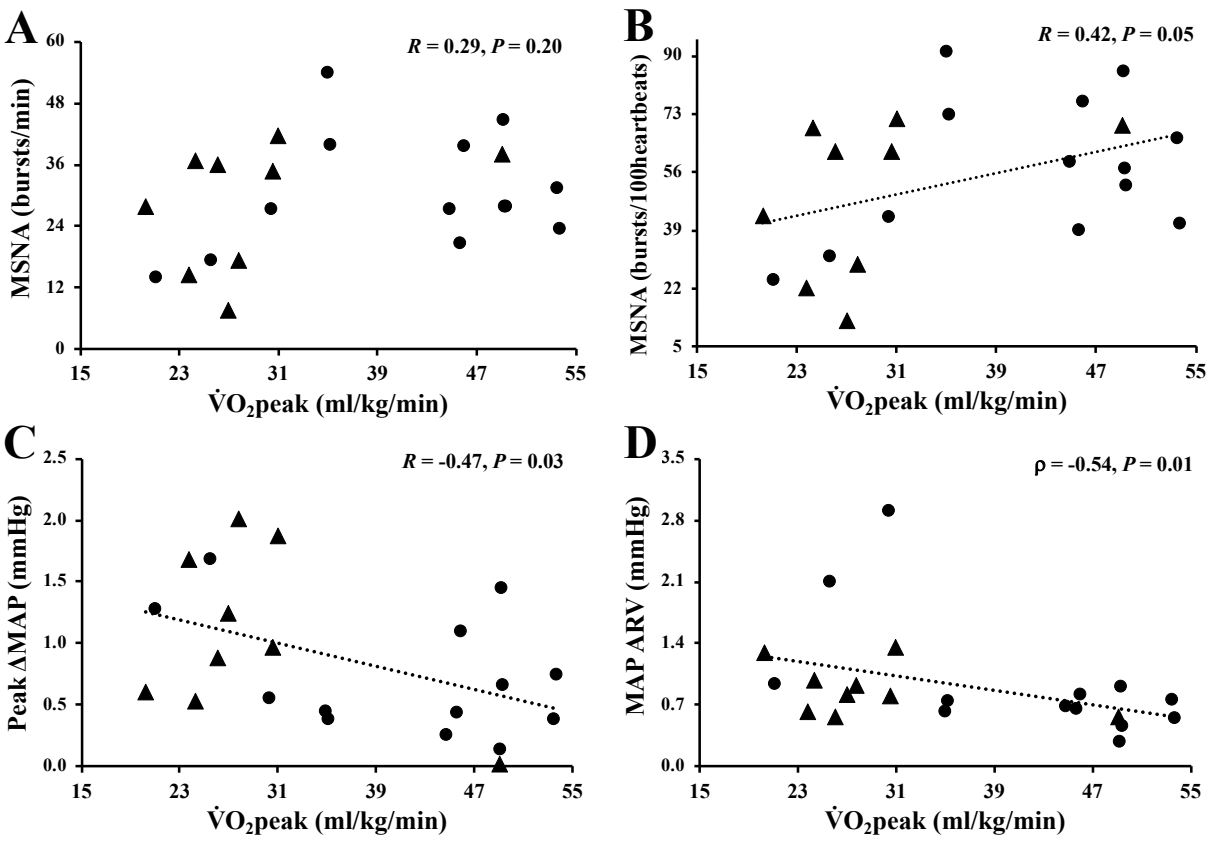

Figure 6.4. The relationship between aerobic fitness (relative V̇O2peak) versus muscle sympathetic nerve activity (MSNA) burst frequency (A), burst incidence (B), the peak pressor responses following bursts of MSNA (C) and mean arterial pressure (MAP) average real variability (ARV) (D). The relationship between aerobic fitness with these measures were determined via Pearson correlations (burst frequency, burst incidence, peak ΔMAP) or Spearman's rank-order correlations (MAP-ARV). Males and females are presented as circles and triangles, respectively.

Both aerobic fitness (negatively) and the peak ΔMAP response (positively), but not the nadir ΔMAP responses were associated with ARV-derived MAP variability. Similar observations were noted when assessed using SBP and DBP variability outcomes (Appendix E). Controlling for MSNA burst frequency, peak transduction, age, and sex did not alter the relationship between aerobic fitness and MAP-ARV (R=0.53, P=0.02).

Controlling for burst frequency, age, and sex lessened the relationship between MAP-ARV and the peak ΔMAP response (R=0.27, P=0.26).

Ultrasound measures, including popliteal hemodynamics, FMD, and resistance vessel function are presented in Table 6.2. Resting popliteal vascular conductance (P=0.04) was higher in males versus females, with a trend for resting popliteal blood flow following the same pattern (P=0.054). No other ultrasound outcome (resting, FMD, or resistance vessel function differed between the sexes (all, P>0.13).

There were no relationships between any index of resistance vessel function with burst frequency, burst incidence, total MSNA, peak ΔMAP, sBRS, or MAP-ARV (all, P>0.24). We were under-powered to observe a relationship between aerobic fitness and blood flow velocity 60s AUC (R=0.41, P=0.06), whereas aerobic fitness was unrelated to the other indices of resistance vessel function (rest, P>0.20).

Table 6.2. Participant ultrasound outcomes, including popliteal hemodynamics, flow-mediated dilation, and resistance vessel function.

	Participants (*n*=22)	Males (*n*=13)	Females (*n*=9)
Resting Measures			
Resting Diameter, mm	6.64 ± 1.17	6.92 ± 0.84	6.23 ± 1.49
Mean blood velocity, cm/s	2.9 ± 0.9	2.9 ± 0.8	2.9 ± 1.0
Blood flow, ml/min	59 ± 21	66 ± 23	49 ± 13
Resting shear rate, /s	38 ± 20	35 ± 12	42 ± 29
Resting PVC, 10^2×ml/min/mmHg	71 ± 25	80 ± 26	57 ± 18*
Flow-Mediated Dilation			
Peak diameter, mm	6.98 ± 1.27	7.30 ± 0.88	6.51 ± 1.62
Time-to-Peak Diameter, s	115 ± 16	112 ± 14	118 ± 19
SR_{AUC}, a.u.	9758 ± 1491	9374 ± 1253	10312 ± 1701
Relative FMD, %	5.0 ± 2.0	5.5 ± 1.6	4.3 ± 2.4
Absolute FMD, mm	0.34 ± 0.15	0.38 ± 0.11	0.28 ± 0.17
Resistance Vessel Function			
Peak blood flow velocity, cm/s	29 ± 11	30 ± 13	28 ± 8
Blood flow velocity 60s AUC, a.u	685 ± 300	725 ± 362	627 ± 183
Peak blood flow, ml/min	638 ± 329	704 ± 368	543 ± 253
Blood flow 60s AUC, a.u	14490 ± 9458	16790 ± 11225	11168 ± 4978
Peak PVC, 10^2×ml/min/mmHg	764 ± 386	843 ± 407	649 ± 344
PVC 60s AUC, a.u	17557 ± 10844	20504 ± 12382	13299 ± 6648

Data presented as means ± standard deviations. PVC, popliteal vascular conductance; FMD, flow-mediated dilation; SR_{AUC}, shear rate area under the curve to peak dilation; AUC, area under the curve. Sex differences identified via independent sample *t*-tests and indicated as an asterisk ($P<0.05$).

Chapter 6: Discussion

The present study characterized the association of aerobic fitness with measures of the sympathetic arm of the autonomic nervous system in older adults. Consistent with my hypothesis, aerobic fitness level was inversely associated with the pressor responses to spontaneous bursts of MSNA. The peak (bursts) and nadir (non-bursts) MAP responses were both negatively associated with traditional measures of resting MSNA (burst frequency and burst incidence). Aerobic fitness was positively associated with

burst incidence, but not burst frequency. A lower aerobic fitness and higher sympathetic transduction were associated with greater blood pressure variability, which may provide longer term insight into the cardiovascular consequences of lower fitness level in older adults.

There is an increased reliance on sympathetic regulation of arterial pressure with ageing (107). Despite this higher sympathoexcitation in older adults, our findings are consistent with previous findings from younger (200) and middle-aged males (190) that better aerobic fitness attenuates the pressor and vascular responses to MSNA. Young et al. (321) have recently highlighted the challenges and limitations associated with comparing sympathetic transduction determined from spontaneous, resting sympathetic recordings (200) versus reflex-mediated increases in MSNA (190). Similar to my previous observations in young males (200), the nadir ΔMAP responses were unaffected by aerobic fitness. However, both the peak and nadir ΔMAP were negatively associated with burst frequency and burst incidence, but unrelated to sympathetic baroreflex sensitivity, in older adults (see Figure 6.3A-C for peak ΔMAP). These observations suggest that the regulation of arterial pressure in older adults with higher resting sympathetic activity may exhibit compensatory lower pressor responses for each burst. Similarly, those with higher resting sympathetic outflow exhibited an augmented decline in MAP following cardiac cycles without bursts, suggestive of a dependence on tonic vasoconstrictor activity to maintain arterial pressure in this population. Given the significant relationships between aerobic fitness with sympathetic transduction (inverse) and ARV (positive), but not burst frequency (Figure 6.4), suggests that older adults with lower aerobic fitness have larger spontaneous MSNA-induced surges in arterial blood

pressure (i.e., greater blood pressure variability; see Figure 6.4D). Of note, neither aerobic fitness nor ARV were related with the nadir ΔMAP response, indicating that the fluctuations in arterial pressure appear to be driven by spontaneous MSNA-induced MAP increases and are not counterbalanced by smaller non-burst decreases in MAP in older adults with lower aerobic fitness. Altogether, these results suggest that lower aerobic fitness may contribute to a greater sympathetic transduction, which was associated with greater arterial pressure variability. Importantly, the inverse association between ARV and aerobic fitness remained after controlling for MSNA burst frequency, peak transduction, sex, and age. The increased ARV and brief periods of larger 'blood pressure fluctuations could contribute to end-organ damage over time, which accelerates the development of hypertension (167, 168) and may be implicated in the development of cardiovascular diseases.

The relationship between aerobic fitness and resting MSNA in healthy older adults is controversial (10, 184, 271). We observed that better aerobic fitness was associated with higher resting MSNA burst incidence, but not MSNA burst frequency (Figure 6.4A-B). Our results are generally consistent with Ng et al. (184), who observed greater MSNA burst incidence among Master's athletes (~40-45 ml/kg/min; ~77 bursts/100heartbeats) versus less aerobically fit controls (30-35 ml/kg/min; ~50 bursts/100heartbeats). However, they also observed a larger burst frequency in their sample of athletes (~31 bursts/min vs ~45 bursts/min). In Studinger et al. (271), the lower resting MSNA burst frequency observed in male Master's athletes (49±8 ml/kg/min; 17±7 bursts/min) versus less aerobically fit, age-matched controls (34±7 ml/kg/min; 22±9 bursts/min) was abolished when normalized for resting heart rate (i.e., similar burst

incidences). Thus, burst incidence may be the most appropriate comparator of resting sympathetic outflow when comparing those with different levels of aerobic fitness due to the well-established bradycardia that accompanies chronic aerobic exercise training (17). Baker et al. (10) recently observed no relationship between $\dot{V}O_2$peak with either MSNA burst frequency or incidence in older females. Differences between Baker et al. (10) and the present study regarding population (13 females only vs. 9 males/13 females), age (average: 58 vs. 65 years), aerobic fitness average and range (29 ml/kg/min [20-45] vs. 36 ml/kg/min [20-54]), or resting MSNA burst incidence (50-90 bursts/100heartbeats vs. 12-90 burst/100heartbeats) may have contributed to these divergent relationships. The wider range of resting MSNA and aerobic fitness levels may have improved my ability to detect a correlational relationship. It is well established that exercise training induces bradycardia (17), which was supported in this study ($\dot{V}O_2$peak and heart rate: R=-0.69, P<0.001) and contributed to the direct relationship between aerobic fitness and MSNA burst incidence.

While the present study highlights the impact of aerobic fitness on neuro-cardiovascular control in older adults, the mechanisms responsible for these observations are unclear. It is possible that aerobic fitness alters norepinephrine kinetics in older adults (i.e., prejunctional release, reuptake and clearance, spillover, etc.) (221). However, neither MSNA burst frequency nor burst amplitude (i.e., indices of norepinephrine release) (70, 185) were related to aerobic fitness. At the receptor level, research in older male Fischer rats observed reduced α-receptor-mediated vasoconstriction following an exercise training program (15 m/min at 15% incline, 60 min/day, 5 days/week for 10-12 weeks) (64). Aerobic exercise training (12-wk, 4-d/wk, at ~70% heart rate reserve) has

been shown to augment popliteal artery resistance vessel function, as assessed using magnetic resonance imaging during a plantar flexion contraction in older adults (123). The present data, albeit cross-sectional, suggests that alterations in resistance vessel function do not explain the impact of aerobic fitness on sympathetic outcomes. Specifically, I did not observe relationships between any measure of resistance vessel function with time-averaged MSNA outcomes, sympathetic transduction, or aerobic fitness. Future research is required to determine the exact mechanisms contributing to the blunted sympathetic transduction with enhanced aerobic fitness. The present study helps direct such research in that local vascular responses may be less impactful on sympathetic transduction in this population.

Using the same signal-averaging approach, Vianna et al. (302) observed sex-differences in the MAP responses to bursts and non-burst cardiac cycles in older adults with similar resting MSNA levels to those included in this study. Specifically, older males (peak ΔMAP: 1.7 mmHg; nadir ΔMAP: -2.0 mmHg) exhibited larger peak MAP responses versus older females (peak ΔMAP: 1.2 mmHg; nadir ΔMAP: -1.0 mmHg). My findings were more reflective of their older females (present study, peak ΔMAP: 0.9 mmHg; nadir ΔMAP: -1.1 mmHg). Based on the observed relationship between aerobic fitness and peak ΔMAP in this study (Figure 6.4C), it is possible that their older males had lower aerobic fitness and thus, exhibited larger pressor responses. For completeness, I presented male and female data separately, but were statistically underpowered to determine sex differences or sex-specific associations between aerobic fitness and sympathetic transduction in samples matched for aerobic fitness.

While this study benefited from a heterogenous sample across a wide range of aerobic fitness levels, it was limited by a cross-sectional design and highlights the need for future investigation using an exercise training intervention. I acknowledge that these findings are specific to healthy older adults who do not exhibit autonomic nervous system dysfunction. Future research investigating the interplay between lifestyle factors and neuro-cardiovascular function in clinical populations is warranted. While an objective measure of maximal aerobic fitness was implemented in the present study, whether or not physical behaviours (e.g., step counts, moderate-vigorous physical activity, sedentary time, etc.) (199, 309) or nutritional factors (e.g., sodium intake) (9) influenced these results is unclear and should be determined in future studies.

In conclusion, the current results demonstrated that aerobic fitness modulates the sympathetic regulation of arterial pressure, with lower aerobic fitness being associated with lower burst incidence and higher sympathetic transduction that corresponded to greater blood pressure variability in older adults. This information may provide some insight into the neuro-cardiovascular benefits of higher aerobic fitness among older adults.

CHAPTER 7: DISCUSSION OF STUDIES 1-4

The objective of this thesis was to examine the associations of aerobic fitness and impact of exercise training on peripheral vascular function in older adults. The projects addressed this overarching objective by considering the endothelial, as well as neural inputs that contribute to regulating arterial function. Special attention was placed on the endothelial function across the upper-body (brachial artery) and lower-limb (popliteal artery) arteries, uncovering the divergent reponses between vascular beds. Altogether, the studies included advance our understanding of aerobic fitness and/or exercise training on endothelial-dependent dilation (FMD), endothelial-dependent vasoconstriction (L-FMC), and sympathetic transduction among healthy older adults.

It is well established that a higher aerobic fitness is associated with a reduced risk of all-cause mortality (157). For example, a retrospective cohort study with a median follow-up ~8 years demonstrated that the consequences of low aerobic fitness (hazard ratio between below vs above average fitness: 1.41) was comparable to traditional cardiovascular risk factors, including coronary artery disease (1.29), smoking (1.41), and type II diabetes mellitus (1.40) (157). Despite the frequently observed cardiovascular health benefits of a higher aerobic fitness, our understanding of *why* being more fit confers cardioprotective benefits among older adults is less clear. To address this concept, the studies conducted within this thesis aimed to better understand the relationship of aerobic fitness with and impact of exercise training on measures of peripheral vascular function. This was achieved by examining the relationship between directly measured aerobic fitness with a thorough ultrasound assessment of endothelial-dependent and independent function in conduit arteries, as well as invasive assessment of

sympathetic neural activity directed towards skeletal muscle resistance vessels. Altogether, the information gained from these studies provide support for, or areas of further inquiry, the cardiovascular benefits of aerobic fitness in older adults.

FMD in the brachial artery has been shown to be reflective of coronary artery function (central measure) (26), mediated via NO (94), and predictive of future cardiovascular events (124). While substantially less is known about FMD responses in the popliteal artery, this conduit artery is responsible for delivering blood to tissues in the lower-limb, which are directly involved in changes in blood flow during traditional forms of aerobic exercise (increases) and sedentary postures (decreases) (230, 307). Unlike the brachial artery, the popliteal artery is more susceptible to arthrosclerosis (128) and is the most common site of peripheral vascular aneurysms (58). While the participants included in the present study have not experienced a cardiovascular event or history of a popliteal aneurysm, understanding peripheral vascular function in healthy older adults may be informative from a preventative perspective. Despite my research demonstrating that endothelial function in the brachial does not reflect that of the popliteal artery of older adults (Study 2) (194), aerobic fitness was associated with larger FMD and L-FMC responses in both arteries (194, 195). As well, FMD increased in both the brachial and popliteal arteries following short-term MICT and HIIT, but unchanged following RT (193). Interestingly, RT also increased aerobic fitness in my group of older adults as well as resting lumen diameter pre-post in both arteries (193). In summary, the impact of- and relationships between aerobic fitness and endothelial function follow the same patterns between the brachial and popliteal arteries (i.e., associated with larger FMD and L-FMC responses), despite such arteries exhibiting unique responses. Such observations indicate

the limb-specific nature of these responses and encourage future investigators to consider endothelial function in lower-limb vessels.

The FMD technique is a frequently implemented test by researchers to assess arterial function (40). Despite researchers be encouraged to continuously track diameter throughout the ischemia portion of the FMD test (282), the prevalence of L-FMC being reported in the literature is substantially less than FMD (198). The inclusion of L-FMC is a noteworthy aspect of this thesis as it has been proposed of providing complementary information to FMD regarding endothelial function (122). While previous research has documented the positive association between aerobic fitness and upper-limb FMD (173), I have extended these observation to endothelial-dependent vasoconstrictor function in both the brachial and popliteal arteries (194, 195).

Similarly, L-FMC responses were divergent across limbs (194), which is consistent with the FMD literature (95, 186). Moreover, aerobic training augmented L-FMC in the popliteal artery, but only HIIT increased L-FMC in the brachial artery (193). Together, this provides insight into potential mechanistic pathways involved with aerobic fitness and L-FMC, which may include some combination of: prostaglandins (90), EDHFs (90, 218), and/or endothelin-1 (264). In addition, non-endothelial-dependent mechanisms may be involved. Another contributor to vessel tone is the transmural pressure (or the pressure gradient across a vessel wall). While an increase in transmural pressure promotes vasoconstriction via myogenic autoregulation primarily in small arteries (<400 μm) or arterioles, the increase in local pressure during cuff inflation may impact conduit artery function. Specifically, the suprasystolic cuff-induced increase in local pressure may promote a myogenically-mediated vasoconstriction response that

contributes, at least in part, to the observed L-FMC response. In rats with heart failure, 8 weeks of aerobic exercise (~50% max speed for 1 hr/day, 5 days/week) impoved the myogenic vasoconstrictor response of their tibial artery (216). As well, the percent of myogenic vasoconstriction was positively correlated to maximal running distance (i.e., more distance was associated with greater constriction). Accordingly, the larger L-FMC responses observed in the more aerobically fit older adults in Studies 1-2 may have been related to a better myogenic autoregulatory response, whereas the less aerobically fit older adults (i.e., "poor" fitness) may have impaired myogenic responses, which result in no change in local diameter or even a slight dilation. Aerobic fitness is inversely related with arterial stiffness in older adults (277). No relationship has been observed between brachial artery L-FMC and arterial stiffness (carotid-femoral pulse wave velocity) in pregnant and non-pregnant females (158). Despite this, stiffer brachial or popliteal arteries among the lower fit older adults in the present study could impair vasoreactivity of the vessel to both reductions and influxes of local blood flow. The stiffness/compliance of these vessels could contribute to the attenuated L-FMC and FMD responses observed among older adults with lower aerobic fitness. The study of mechanisms that may explain the cross-sectional and interventional observations presented in this thesis are warranted.

The present thesis and associated manuscripts advocate for the inclusion of L-FMC among researchers to better understand endothelial function. Importantly, the articles includes in a recent review from our group on the impact of aerobic fitness and movement interventions on L-FMC were primarily conducted in the last 5 years (198), indicating that there is some recent adoption of this measure among researchers. The

studies included in this thesis may contribute to such adoptions and demonstrates that L-FMC is modifiable (e.g., in 6-weeks of training) in older adults (193), and suggests that a larger vasoconstriction to reductions in local flow is a healthy response. While the clinical or prognostic value of L-FMC has yet to be established, the notion of examining vasoreactivity to changes in local blood flow has proven utility among vascular researchers and follows a similar line of thinking as the FMD technique but under unique mechanisms. Establishing the clinical relevance may further propagate this metric among researchers.

In conscious humans, the recording of MSNA has been conducted for ~55 years (100), permitting great insight into the sympathetic regulation of vascular function. It is well-established that time-averaged measures of MSNA (i.e., burst frequency/incidence) increases with age (133). However, the impact of aerobic fitness on MSNA burst characteristic is less clear (138). My final study supports that higher aerobic fitness was associated with a greater burst incidence (bursts per 100 heartbeats) (201), which was grounded in the bradycardia that accompanies older adults with higher versus lower fitness, as aerobic fitness was unrelated to burst frequency (201). The communication between spontaneous bursts of MSNA into a cardio(vascular) outcome, termed sympathetic transduction (321), provides insight the neural control of the vasculature. Interest in this metric among autonomic cardiovascular-focused researchers has increased over the last ~5 years (298). Adding to the current literature, I have demonstrated that aerobic fitness level was inversely related to sympathetic transduction (201), mirroring my previous findings in young males (200). This may indicate a role of aerobic fitness on further attenuating α-adrenergic receptor-mediated vasoconstriction, which typically

accompanies aging (106). Importantly, these changes lead to an augmented blood pressure variability among older adults with lower aerobic fitness (201). This link to blood pressure variability provides clinical context into the sympathetic regulation of arterial pressure and indicates that a potential mechanism by which higher aerobic fitness leads to better cardiovascular health. Cross-sectionally, these MSNA and sympathetic transduction outcomes were unrelated to the ultrasound-derived measures of macrovascular or resistance vessel function. Together, this suggests that counteracting greater vasodilator function with higher aerobic fitness does not explain the attenuated sympathetic transduction in this population, although longitudinal studies are needed to confirm.

Limitations & Future Directions. The transition from a cross-sectional design in studies 1 and 2 to an exercise intervention in study 3 provided greater confidence into the impact of aerobic exercise on FMD and L-FMC outcomes. Based on aerobic fitness being associated with a lower blood pressure variability and sympathetic transduction in study 4, a logical next step that would permit cause-effect conclusions would be to conduct a randomized controlled exercise training intervention with MSNA conducted before and after the intervention in older adults. This would confirm whether it is the exercise training specifically that alters the sympathetic regulation of arterial pressure and not inter-individual factors responsible for such observations.

The ultrasound and microneurographic measures provided insight into endothelial function and sympathetic nervous system factors, respectively. However, the specific endothelial/neural physiologic mechanisms involved were not determined. A future direction that would uncover more specific mechanisms would be to include blockades or

inhibitors of eNOS [e.g., L-NMMA (144)], EDHFs [e.g., fluconazole (218)], prostaglandins [e.g., aspirin (90)], endothelin-1 [e.g., BQ-123 (264)], and/or α-adrenergic receptors [e.g., phentolamine (72)]. This would provide further insight into which endothelial pathways or neural aspects are most impacted by aerobic fitness and/or aerobic exercise training.

As highlighted above, the present thesis studies are specific to healthy older adults. However, given that an effect was observed in healthy older adults substantiates that aerobic fitness or exercise training may have an even greater impact among older adults with chronic disease and therefore worse vascular function at baseline. Nevertheless, the inclusion of participants with cardiovascular conditions would be an important future direction. Of note, the specific magnitude of cardiovascular improvements based on exercise intensity (HIIT vs. MICT) or type (aerobic vs. resistance training) may vary for these less healthy populations. However, the HIIT training protocol was originally developed based on time spent at a higher intensity and ratings of perceived exertion in patients with coronary artery disease (98), lending support for future training interventions using the HIIT protocol implemented herein.

Conclusion. A higher aerobic fitness was associated with greater FMD and L-FMC responses in both the brachial and popliteal arteries, an attenuated sympathetic transduction response to spontaneous bursts of MSNA, and lower blood pressure variability in older adults. A relatively short period of aerobic exercise training sufficiently augmented aerobic fitness, endothelial-dependent vasodilatory, and endothelial-dependent vasoconstrictor function. The studies conducted add to the field by providing evidence for the favorable impact of maintaining or increasing aerobic fitness

in healthy older adults on peripheral vascular function and offer insight into the specific benefits or adaptations from an endothelial and neural perspective.

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APPENDIX A: Copyright Approvals

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APPENDIX B: STUDENT CONTRIBUTION TO MANUSCRIPTS IN THESIS

DALHOUSIE UNIVERSITY

Faculty of Graduate Studies

STUDENT CONTRIBUTION TO MANUSCRIPTS IN THESIS

MUST BE WORD-PROCESSED OR TYPEWRITTEN.

NAME: Myles W. O'Brien	**STUDENT ID #: B00593444**
DEPARTMENT: Faculty of Health	**PROGRAMME: PhD in Health**
PHONE: (902) 301-2523	**E-MAIL: myles.obrien@dal.ca**

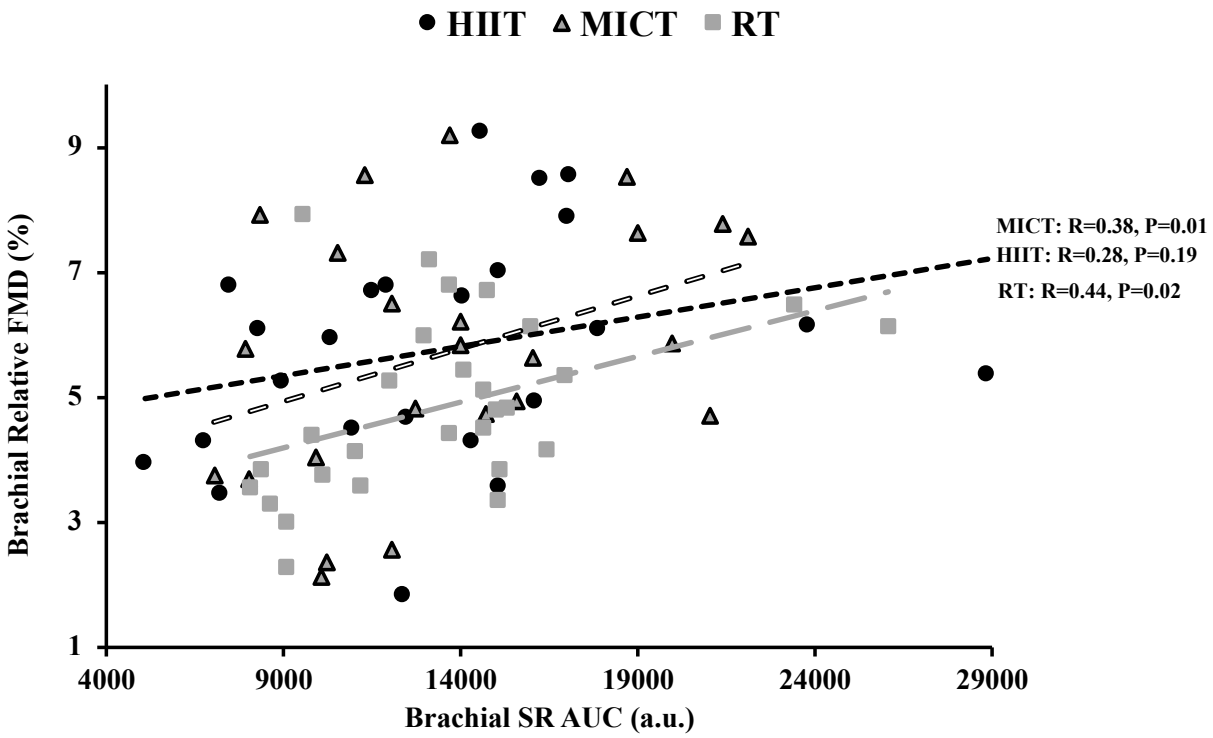
MANUSCRIPT AUTHORS: Myles W.,O'Brien, Said Mekary, Susan A. Robinson, Jarrett A. Johns, and Derek S. Kimmerly.
MANUSCRIPT TITLE: The relationship between aerobic fitness and low-flow-mediated constriction in older adults
JOURNAL: *European Journal of Applied Physiology*
STUDENT CONTRIBUTION: Contribution for authors is included with specific contribution by MWO bolded. The format for contribution is consistent with the journal each article is published in where applicable. The study was designed by **MWO**, SM and DSK; data were collected by SAR, JAJ and **MWO**; data were analyzed by SAR, JAJ and **MWO**; data interpretation and manuscript preparation were undertaken by **MWO** and DSK. All authors approved the final version of the paper.
SUPERVISOR SIGNATURE:

MANUSCRIPT AUTHORS: Myles W. O'Brien, Jarrett A. Johns, Susan A. Robinson, Said Mekary, and Derek S. Kimmerly.
MANUSCRIPT TITLE: Relationship between brachial and popliteal artery low-flow-mediated constriction in older adults: impact of aerobic fitness on vascular endothelial function
JOURNAL: *Journal of Applied Physiology*
STUDENT CONTRIBUTION: M.W.O. and D.S.K. conceived and designed research; **M.W.O.,** J.A.J., S.A.R., and S.M. performed experiments; **M.W.O.** analyzed data; **M.W.O.** and D.S.K. interpreted results of experiments; **M.W.O**. prepared figures; **M.W.O.** drafted manuscript; **M.W.O.,** J.A.J., S.A.R., S.M., and D.S.K. edited and revised manuscript; All authors approved final version of manuscript.
SUPERVISOR SIGNATURE:

MANUSCRIPT AUTHORS: Myles W. O'Brien, Jarrett A. Johns, Susan A. Robinson, Amanda Bungay, Said Mekary, and Derek S. Kimmerly.
MANUSCRIPT TITLE: Impact of High-Intensity Interval Training, Moderate-Intensity Continuous Training, and Resistance Training on Endothelial Function in Older Adults.
JOURNAL: *Medicine and Science in Sports and Exercise*
STUDENT CONTRIBUTION: M.W.O. S.M, and D.S.K. conceived and designed research; **M.W.O.,** J.A.J., S.A.R., A.B, and S.M. performed experiments; **M.W.O.** and S.M analyzed data; **M.W.O.**, S.M., D.S.K. interpreted results of experiments; **M.W.O**. prepared figures; **M.W.O.** drafted manuscript; **M.W.O.,** J.A.J., S.A.R., S.M., A.B., and D.S.K. edited and revised manuscript; All authors approved final version of manuscript.
SUPERVISOR SIGNATURE:

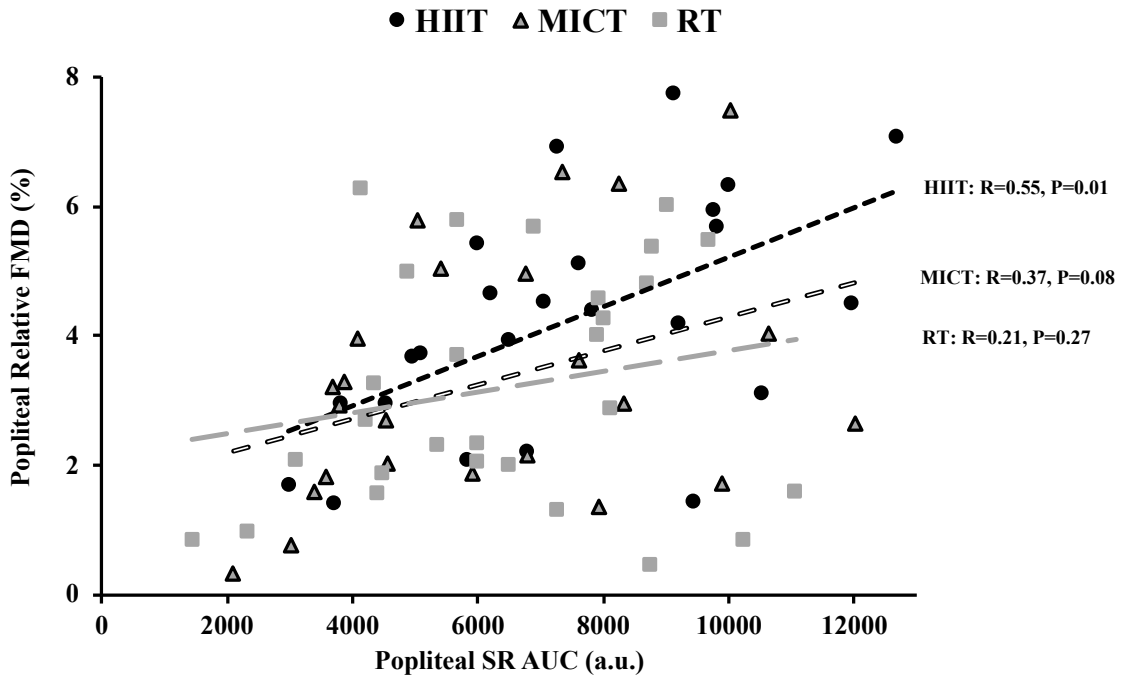
MANUSCRIPT AUTHORS: Myles W. O'Brien, Diane J. Ramsay, Carley D. O'Neill, Jennifer L. Petterson, Shilpa Dogra, Said Mekary, and Derek S. Kimmerly.
MANUSCRIPT TITLE: Aerobic fitness is inversely associated with neurohemodynamic transduction and blood pressure variability in older adults.
JOURNAL: *GeroScience*
STUDENT CONTRIBUTION: M.W.O., D.S.K., S.D., and S.M. conceived and designed research; **M.W.O.,** D.J.R., C.D.O., S.D., S.M., and D.S.K performed experiments; **M.W.O.** and J.L.P. analyzed data; **M.W.O.** and D.S.K. interpreted results of experiments; **M.W.O**. prepared figures; **M.W.O.** drafted manuscript; All authors edited and revised manuscript; All authors approved final version of manuscript.
SUPERVISOR SIGNATURE:

APPENDIX C: SR AUC AND BRACHIAL FMD WITH TRAINING



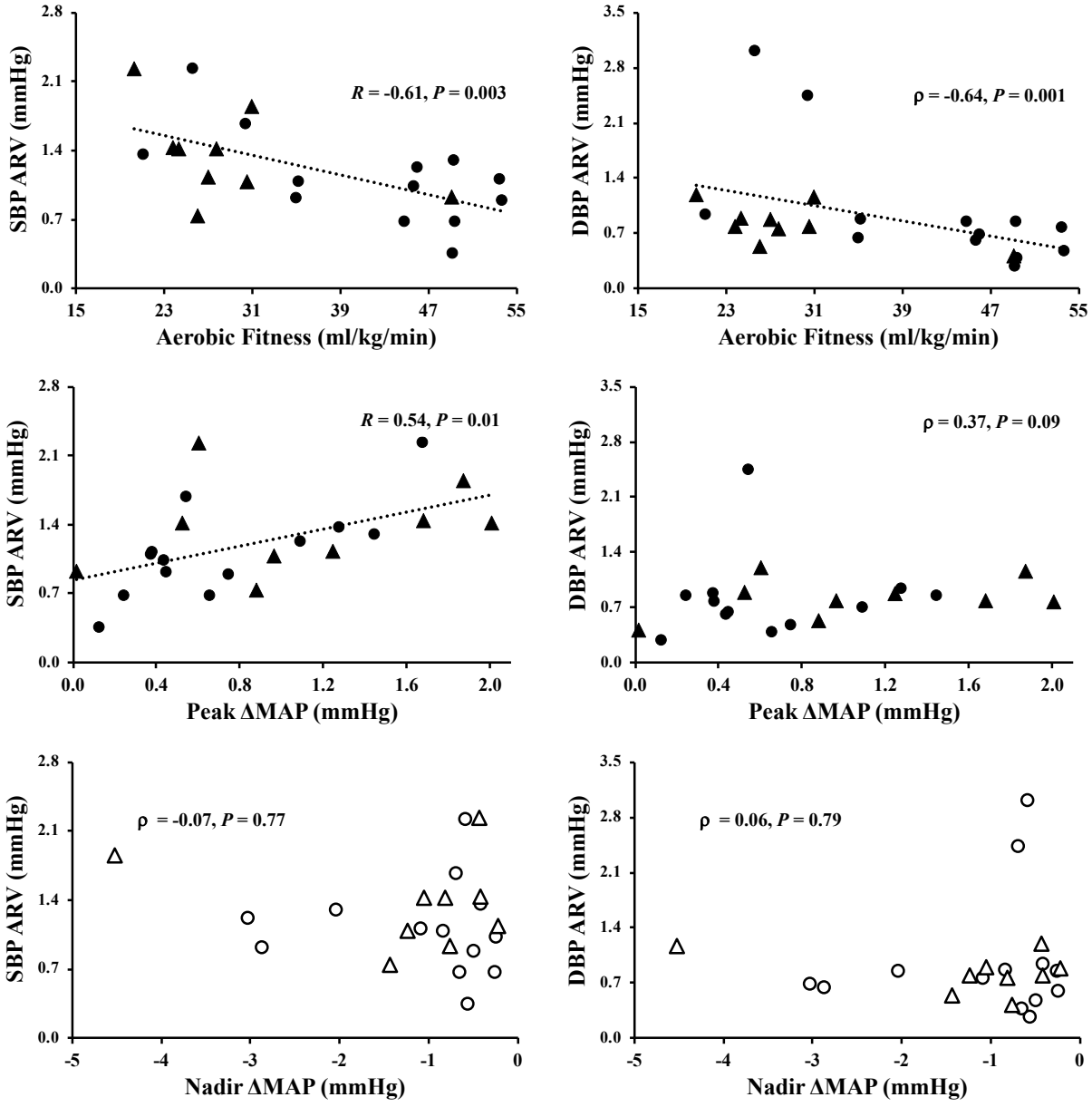
Scatterplot showing the relationship between the shear stress stimulus (shear rate area under the curve; SR AUC) and brachial flow-mediated dilation (FMD) with data from both pre-training and post-training for each of the high-intensity interval training (HIIT; circles; black regression line), moderate-intensity continuous training (MICT: triangles; black and white regression line) and resistance training groups (RT: squares; grey regression line). The slopes (10^4) were 0.93, 1.70 and 1.46 for the HIIT, MICT and RT groups, respectively. The correlation of the pooled sample was r = 0.35 (*P*=0.002) and the y-intercept was greater than zero with β=3.6±0.6, *P*<0.001 (95% CI: 2.4-4.8).

APPENDIX D: SR AUC AND POPLITEAL FMD WITH TRAINING



Scatterplot showing the relationship between the shears stress stimulus (shear rate area under the curve; SR AUC) and popliteal flow-mediated dilation (FMD) with data from both pre-training and post-training for each of the high-intensity interval training (HIIT; circles; black regression line), moderate-intensity continuous training (MICT: triangles; black and white regression line) and resistance training groups (RT: squares; grey regression line). The slopes (10^4) were 3.81, 2.62 and 1.60 for the HIIT, MICT and RT groups, respectively. The correlation of the pooled sample was r = 0.40 (*P*<0.001) and the y-intercept was greater than zero with β=1.6±0.6, *P*=0.004 (95% CI: 0.5-2.7).

APPENDIX E: RELATIONSHIP BETWEEN ARTERIAL PRESSURE AND BPV



The relationship between systolic blood pressure (SBP; left panels) and diastolic blood pressure (DBP; right panels) average real variability (ARV) with aerobic fitness (relative $\dot{V}O_2$peak; top panels), the peak pressor responses following bursts of MSNA (middle panels; peak ΔMAP) and following cardiac cycles absent of bursts (bottom panels; nadir ΔMAP; white circles). Males and females are presented as circles and triangles, respectively. Relationships were determined via Pearson correlations or non-parametric Spearman's rank-order correlations.

APPENDIX F: CURRICULUM VITAE

Myles William O'Brien, CSEP-CEP, Ph.D (Cand.)

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Updated July 13, 2022

EDUCATION HISTORY

Bachelor of Kinesiology (Honours) 2012-2016
Acadia University, Wolfville, Nova Scotia, Canada
Supervisor: Dr. Jonathon Fowles
"Implementation and perceived effectiveness of the Exercise is Medicine Canada exercise prescription resources among health care providers across Canada"

- GPA: 3.7/4.0

Master of Science in Kinesiology 2016-2018
Dalhousie University, Halifax, Nova Scotia, Canada
Supervisor: Dr. Derek Kimmerly
Thesis Committee: Dr. Gail Dechman, Dr. Scott Grandy
"The effects of short-term high-intensity interval, moderate-intensity continuous and resistance training on cardiovascular health in older adults"

- GPA: 4.1/4.3

Doctor of Philosophy in Health 2018-Present
Dalhousie University, Halifax, Nova Scotia, Canada
Supervisors: Dr. Derek Kimmerly & Dr. David Westwood
Thesis Committee: Dr. Olga Theou, Dr. Shilpa Dogra
"The impact of cardiorespiratory fitness on peripheral vascular function in older adults"

- GPA: 4.24/4.3

ACADEMIC INTERESTS

- Influence of Aging and Activity on Peripheral Vascular Function.
- The Independent Health Effects of Aerobic Fitness, Physical Activity, and Sedentary Postures.
- Physical (In)activity and Neural Regulation of Arterial Blood Pressure.
- Development and Validity of Physical Activity Monitors and Metrics.
- Integration of Physical Activity and Exercise in Healthcare by Primary Care Providers and Qualified Exercise Professionals.

PEER-REVIEWED JOURNAL ARTICLES

Published Articles (Total: *n*=60*; n*=43 first-author, *n*=6 senior-author)

1) **O'Brien MW**, Schwartz BD, Petterson JL, Kimmerly DS. (2022). Comparison of Signal-Averaging and Regression Approaches to Analyzing Sympathetic Transduction. *Clinical Autonomic Research. In Press.*

2) Petterson JL, Mekari S, **O'Brien MW**. (2022). An Open-Source Stroop Task Program That Incorporates a Switching Condition to Determine Executive Function. *Software Impacts. In Press*.

3) **O'Brien MW**, Shivgulam ME, Petterson JL, Wu Y, Johns JA, Frayne RJ, Kimmerly DS. (2022). Substituting Stationary Time with Moderate-Intensity Activity May Improve Flow-Mediated Dilation. Journal of Cardiopulmonary Rehabilitation and Prevention. *In Press*.

4) **O'Brien MW**, Mekary S, Kimmerly DS. (2022). Aging, Cardiorespiratory Fitness and Sympathetic Transduction. *Aging.* 14(10): 4189-4190

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6) **O'Brien MW**, Wu Y, Petterson JL, Bray NW, Kimmerly DS. Validity of the ActivPAL to Distinguish Postures: A Systematic Review. *Gait & Posture*. 94: 107-113.

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11) **O'Brien MW**, Kimmerly DS. (2022). Is 'Not Different' Enough to Establish Similar Cardiovascular Responses Between Sexes? *American Journal of Physiology - Heart & Circulatory Physiology.* 322(3): H355-H358

12) **O'Brien MW**, Petterson JL, Wu Y, Bray NW, Kimmerly DS. (2022). What is the Impact of Aerobic Fitness and Movement Interventions on Low-Flow-Mediated Vasoconstriction? A Systematic Review of Observational and Intervention Studies. *Vascular Medicine.* 27(2): 193-202.

13) Petterson JL, **O'Brien MW**, Ramsay DJ, Johnston WJ, O'Neill CD, Dogra S, Mekary S, Floras JS, Kimmerly DS. (2022). Sympathetic Neurohemodynamic Transduction is Attenuated in Older Males Independent of Aerobic Fitness. *Clinical Autonomic Research.* 32(1): 73-76.

14) **O'Brien MW**, Johns JA, Frayne RJ, Kimmerly DS. (2022). Comparison of Habitual Stepping Cadence Analysis Methods: Relationship with Step Counts. *Gait & Posture.* 92: 328-332.

15) Shivgulam ME*, **O'Brien MW***, Johns, JA, Petterson JL, Wu Y, Frayne RJ, Kimmerly DS. (2022). Impact of Habitual Sedentary Patterns on Popliteal Artery Endothelial-Dependent Vasodilation in Healthy Adults. *Vascular Medicine.* 27(2): 120-126. *Co-First Authors

16) Petterson JL, McPhee BN, Wu Y, **O'Brien MW**. (2021). Does COVID-19 Influence the Sympathetic Regulation of Blood Pressure? *Journal of Physiology.* 599(22): 4951-4953. **Prize: Top Journal Club Article for 2021 by Journal of Physiology.**

17) **O'Brien MW**, Petterson JL, Johns JA, Mekary S, Kimmerly DS. (2021) A Larger Low-Flow Mediated Constrictor Response is Associated with Augmented Flow-Mediated Dilation in the Popliteal Artery. *Clinical Physiology and Functional Imaging.* 41(6): 497-504.

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19) Liu H, **O'Brien MW**, Johns JA, Kimmerly DS. (2021) Does aerobic fitness impact prolonged sitting-induced popliteal artery endothelial dysfunction. *European Journal of Applied Physiology.* 121(11): 3233-3241.

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21) **O'Brien MW**, Wu Y, Johns JA, Poitras J, Kimmerly DS. (2021). Development and Validation of Accelerometry Count-Based Model of Physical Activity Intensity in Adults. *Medical Engineering and Physics*. 95: 45-50.

22) **O'Brien MW***, Al-Hinnawi A*, Johns JA, Kimmerly DS. (2021) Spontaneous cardiovagal baroreflex sensitivity is unaffected by an acute bout of prolonged sitting: No impact of sex, menstrual or oral contraceptive phases. *Clinical Autonomic Research.* 31(6): 783-786. *Co-first authors.

23) **O'Brien MW**, Shields CA, Dunbar MJ, Crowell SJ, Fowles JR. (2021). The Effects of Previous Educational Training on the Physical Activity Counselling Practices among Dietitians Across Canada. *Canadian Journal of Dietetic Practice and Research.* 83(1): 35-40.

24) **O'Brien MW**, Ramsay DJ, O'Neill CD, Petterson JL, Dogra S, Mekary S, Kimmerly DS. (2021). Aerobic Fitness is Inversely Associated with Neurohemodynamic Transduction and Blood Pressure Variability in Older Adults. *GeroScience*. 43(6): 2737-2748.

25) **O'Brien MW***, Wong MYS*, Sui W, Voss ML, Bray NW, Turnbull CN, Nagpal TS, Fowles JR. Learnings from Exercise is Medicine Canada Workshops as Strategies to Assist Medical Students Promote Physical Activity During and Beyond the COVID-19 Pandemic. *Journal of Medical Education Research*. 1(1): 25-33. *Co-first authors.

26) **O'Brien MW**, Kimmerly DS, Mekary S. 2021. Greater Habitual Moderate-To-Vigorous Physical Activity is Associated with Better Executive Function and Higher Prefrontal Oxygenation in Older Adults. *GeroScience.* 43(6): 2707-2718.

27) Fowles JR, **O'Brien MW**, Comeau KG, Thurston B, Petrie HJ. (2021). Flattened Cola Improves High-Intensity Interval Performance in Competitive Cyclists. *European Journal of Applied Physiology.* 121: 2859-2867.

28) **O'Brien MW**, Al-Hinnawi A, Wu Y, Petterson JL, Shivgulum ME, Johns JA, Frayne RJ, Kimmerly DS. (2021). The Influence of Habitual Breaks in Sedentary Time on Cardiovagal Baroreflex Function. *Applied Physiology, Nutrition and Metabolism.* 46(9): 1143-1146

29) **O'Brien MW**, Bray NW, Kivell MJ, Fowles JR. A Scoping Review of Exercise Referral Schemes Involving Qualified Exercise Professionals in Primary Health Care. (2021). *Applied Physiology, Nutrition and Metabolism.* 46(9): 1007-1018. **Editor's Choice Article for September 2021.**

30) **O'Brien MW,** Ramsay D, Johnston W, Kimmerly DS. (2021). The Association Between Habitual Posture and Intensity-Related Physical Activity with Sympathetic Neurohemodynamic Transduction in Young Males. *Clinical Autonomic Research*. 31(2): 339-341.

31) **O'Brien MW**, Petterson JL, Kimmerly DS. (2021). An Open-Source Program to Analyze Spontaneous Sympathetic Neurohemodynamic Transduction. *Journal of Neurophysiology*. 125(3): 972-976.

32) **O'Brien MW**, Johns JA, Petterson JL, Mekary S, Kimmerly DS. (2021). The Impact of Age and Sex on Popliteal Artery Endothelial-Dependent Vasodilator and Vasoconstrictor Function. *Experimental Gerontology*. 145: 111221.

33) Wu Y, Johns JA, Poitras J, Kimmerly DS, **O'Brien MW**. (2021). Improving the Criterion Validity of The Activpal in Determining Physical Activity Intensity During Laboratory and Free-Living Conditions. *Journal of Sport Science*. 39(7) 826-834.

34) **O'Brien MW**, Wojcik WR, Fowles JR. (2021) Validity and Inter-Instrument Reliability of a Medical Grade Physical Activity Monitor in Older Adults. *Journal for the Measurement of Physical Behaviour.* 4(1): 31-38.

35) Mekari S, Neyedli HF, Fraser S, **O'Brien MW**, Martins R, Evans K, Earle M, Aucoin R, Chiekwe J, Hollohan Q, Kimmerly DS, Dupuy O. (2020). High-Intensity interval training Improves Cognitive Flexibility in Older Adults. *Brain Sciences*. 10(11): 796.

36) Petterson JL, **O'Brien MW**, Johns JA, Chiasson J, Kimmerly DS. Influence of Prostaglandins and Endothelial-Derived Hyperpolarizing Factors on Brachial and Popliteal Endothelial-Dependent Function in Young Adults. *Journal of Applied Physiology.* 130(1): 17-25.

37) **O'Brien MW**, Ramsay D, Johnston W, Kimmerly D.S. Aerobic Fitness and Sympathetic Responses to Spontaneous Muscle Sympathetic Nerve Activity in Young Males. *Clinical Autonomic Research.* 31(2): 253-261.

38) **O'Brien MW,** Johns JA, Al-Hinnawi A, Kimmerly DS. (2020). Popliteal Flow-Mediated Dilatory Responses to A Bout of Prolonged Sitting Between Earlier and Later Phases of Natural Menstrual and Contraceptive Pill Cycles. *Journal of Applied Physiology.* 129(4): 637-645.

39) Johns JA, **O'Brien MW**, Bungay A, Kimmerly DS. (2020). Sex and Light Physical Activity Impact Popliteal, but not Brachial Artery, Flow-Mediated Dilation in Physically Active Young Adults. *Applied Physiology, Nutrition and Metabolism.* 45(12): 1387-1395.

40) Johns JA, Frayne RJ, Goreham JA, Kimmerly DS, **O'Brien MW**. (2020). The Bout Cadence Method Improves the Quantification of Stepping Cadence in Free-Living Conditions. Gait & Posture. 79: 96-101.

41) **O'Brien MW**, Shields CA, Solmundson K, Fowles JR. (2020). Exercise is Medicine Canada Workshop Training Improves Physical Activity Practices of Physicians

Across Canada, Independent of Initial Confidence. Canadian Medical Educational Journal. 11(5): e5-e15.

42) **O'Brien MW**, Johns JA, Fowles JR, Kimmerly DS. (2020). Validity of the activPAL and Height-Adjusted Curvilinear Cadence-METs Equations in Healthy Adults. Measurement in Physical Education and Exercise Science. 24(2): 147-156.

43) **O'Brien MW**, Johns JA, Robinson SA, Bungay A, Mekary S, Kimmerly DS. (2020). Impact of High-Intensity Interval Training, Moderate-Intensity Continuous Training, and Resistance Training on Endothelial Function in Older Adults. *Medicine & Science in Sport & Exercise.* 52(5): 1057-1067.

44) **O'Brien MW**, Johns JA, Dorey TW, Frayne RJ, Fowles JR, Mekary S, Kimmerly DS. (2020). Meeting International Aerobic Physical Activity Guidelines is Associated with Enhanced Cardiovagal Baroreflex Sensitivity in Healthy Older Adults. *Clinical Autonomic Research.* 30: 139-148.

45) **O'Brien MW**, Johns JA, Williams TD, Kimmerly DS. (2019). Sex does not Influence Impairments in Popliteal Endothelial-Dependent Vasodilator or Vasoconstrictor Responses Following Prolonged Sitting. *Journal of Applied Physiology.* 127 (3): 679-687.

46) **O'Brien MW**, Mekary S, Robinson SA, Johns JA, Kimmerly DS. (2019). Relationship Between Brachial and Popliteal Artery Low-Flow-Mediated Constriction in Older Adults: Impact of Aerobic Fitness on Vascular Endothelial Function. *Journal of Applied Physiology.* 127(1): 134-142.

47) **O'Brien MW**, Shields CA, Campbell KL, Crowell S, Fowles JR. (2020). Perceptions and Practices of Providing Physical Activity Counselling and Exercise Prescriptions Among Physiotherapists in Nova Scotia. *Physiotherapy Canada*. 72(3): 230-238.

48) Robinson SA, **O'Brien MW**, Grandy S, Heinze-Milne S, Kimmerly DS. (2019). Short-Term Supplement of Virgin Coconut Oil Improves Endothelial-Dependent Dilation but not Exercise-Mediated Hyperemia in Young Adults. *Nutrition Research*. 67: 17-26.

49) Dorey TW, **O'Brien MW**, Kimmerly DS. (2019). The Influence of Aerobic Fitness on Electrocardiographic and Heart Rate Variability Parameters in Young and Older Adults. *Autonomic Neuroscience*. 217: 66-70.

50) **O'Brien MW**, Mekary S, Robinson SA, Johns JA, Kimmerly DS. (2019). The Relationship Between Aerobic Fitness and Low-Flow-Mediated Constriction in Older Adults. *European Journal of Applied Physiology.* 119(2): 351-359.

51) **O'Brien MW**, Kivell MJ, Wojcik WR, d'Entremont G, Kimmerly DS, Fowles JR. (2018). Influence of Anthropometrics on Step-Rate Thresholds for Moderate and

Vigorous Physical Activity in Older Adults: Scientific Modeling Study. *JMIR Aging*. 1(2): 12363.

52) **O'Brien MW**, Kivell MJ, Wojcik WR, d'Entremont G, Kimmerly DS, Fowles JR. (2018). Step Rate Thresholds Associated with Moderate and Vigorous Physical Activity in Adults. *International Journal of Environmental Research and Public Health*. 15(11): 2454.

53) **O'Brien MW**, Shields CA, Crowell S, Theou O, McGrath P, Fowles JR. (2018). The Effects of Previous Educational Training on Physical Activity Counselling and Exercise Prescription Among Physicians Across Nova Scotia: A Cross-Sectional Study. *Canadian Medical Education Journal*. 9(4): e35-e45.

54) **O'Brien MW**, Wojcik WR, Fowles JR. (2018). Medical-Grade Physical Activity Monitoring for Measuring Step Count and Moderate-to-Vigorous Physical Activity: Validity and Reliability Study. *JMIR mHealth uHealth*. 6(9): e10706.

55) **O'Brien MW**, Robinson S, Frayne R, Mekary S, Fowles JR, Kimmerly DS. (2018). Achieving Canadian Physical Activity Guidelines is Associated with Better Vascular Function Independent of Aerobic Fitness and Sedentary Time in Older Adults. *Applied Physiology, Nutrition and Metabolism.* 43(10):1003-1009.

56) Dorey TD, **O'Brien MW**, Robinson S, Kimmerly DS. (2018). Knee-High Compression Socks Minimize Head-Up Tilt-Induced Cerebral and Cardiovascular Responses Following Exercise. *Scandinavian Journal of Medicine in Science and Sports.* 28(7):1766-1774.

57) Fowles JR, **O'Brien MW**, Solmundson K, Oh PI, Shields CA. (2018). Exercise is Medicine Canada Physical Activity Counselling and Exercise Prescription Training, Improves Counselling, Prescription and Referral Practices Among Physicians Across Canada. *Applied Physiology, Nutrition and Metabolism*. 43(5):535-539.

58) **O'Brien MW**, Wojcik WR, d'Entremont L, Fowles JR. (2018). Validation of the PiezoRx® Step Count and Moderate to Vigorous Physical Activity in Free-Living Conditions in Adults: A Pilot Study. *International Journal of Exercise Science.* 11(7):541-551.

59) Fowles JR, **O'Brien MW**, Wojcik WR, d'Entremont L, Shields CA. (2017). A Pilot Study: Validity and Reliability of the CSEP-PATH PASB-Q and a new Leisure Time Physical Activity Questionnaire to Assess Physical Activity and Sedentary Behaviors. *Applied Physiology, Nutrition and Metabolism*. 42(6):677-680.

60) **O'Brien MW**, Shields CA, Oh PI, Fowles JR. (2017). Health Care Provider Confidence and Exercise Prescription Practices of Exercise is Medicine Canada Workshop Attendees. *Applied Physiology, Nutrition and Metabolism*. 42(4):384-390.

KNOWLEDGE TRANSLATION ARTICLES

- Shivgulam ME, **O'Brien MW**, Johns, JA, Petterson JL, Wu Y, Frayne RJ, Kimmerly DS. Impact of Habitual Sedentary Patterns on Popliteal Artery Endothelial-Dependent Vasodilation in Healthy Adults. 2022. Sedentary Behaviour Research Network Knowledge Translation.

- **O'Brien MW**, Bray NW, Kivell MJ, Fowles JR. A Scoping Review of Exercise Referral Schemes Involving Qualified Exercise Professionals in Primary Health Care. 2021. September Editor's Choice Article for Applied Physiology, Nutrition, and Metabolism. Knowledge translation summary prepared by Editors.

- **O'Brien MW,** Johns JA, Al-Hinnawi A, Kimmerly DS. Popliteal flow-mediated dilatory responses to an acute bout of prolonged sitting between earlier and later phases of natural menstrual and oral contraceptive pill cycles. 2020. Sedentary Behaviour Research Network Knowledge Translation.

- **O'Brien MW**, Kimmerly DS. "Active Voice: What Type of Exercise Training Most Improves Artery Health in Older Adults?" 2020. American College of Sports Medicine: Sports Medicine Bulletin – Active Voice.

- **O'Brien MW**, Robinson SA, Frayne RJ, Mekary S, Fowles JR, Kimmerly DS. "Is Achieving Canadian Physical Activity Guidelines Important For Blood Vessel Health in Older Adults?" 2018. Canadian Society for Exercise Physiology Knowledge Translation.

ACADEMIC CONFERENCE PRESENTATIONS

Published Conference Presentations

1) **O'Brien MW**, Shivgulam ME, Petterson JL, Wu Y, Johns JA, Frayne RJ, Kimmerly DS. (2022). Substituting Stationary Time With Moderate-Intensity Activity May Improve Brachial Flow-Mediated Dilation: An Isotemporal Substitution Approach. *Medicine & Science in Sport & Exercise. In Press.*

2) Petterson JL, **O'Brien MW**, McPhee BN, Johnston WJ, Ramsay DJ, Floras JS, Kimmerly DS. (2022). Lower Neurohemodynamic Transduction In Young Females Versus Males With Similar Aerobic Fitness And Sympathetic Outflow. *Medicine & Science in Sport & Exercise. In Press.*

3) Wu Y, Peddle A, Daley WS, **O'Brien MW**, Frayne RJ. (2022). Criterion Validity of Tri-Monitor ActivPAL Configuration in Determining Knee-Flexion Angles during Sitting in Laboratory Setting. *Medicine & Science in Sport & Exercise. In Press.*

4) Liu H, **O'Brien MW**, Johns JA, Kimmerly DS. (2021). Does aerobic fitness impact prolonged sitting-induced popliteal artery endothelial dysfunction? *Applied Physiology, Nutrition and Metabolism.* 46(10): S63.

5) Pellerine LP, Kimmerly DS, Fowles JR, **O'Brien MW**. (2021). Criterion-validity of the Physical Activity Vital Sign for estimating habitual moderate-to-vigorous physical activity in active younger and older adults. *Applied Physiology, Nutrition and Metabolism.* 46(10): S70.

6) Petterson JL, **O'Brien MW**, Ramsay DJ, Johnston W, O'Neill CD, Dogra S, Mekary S, Floras JS, Kimmerly DS. (2021). Sympathetic neurohemodynamic transduction is attenuated in older males independent of age-related declines in aerobic fitness. *Applied Physiology, Nutrition and Metabolism.* 46(10): S70.

7) Shivgulam ME, **O'Brien MW**, Johns JA, Petterson JL, Wu Y, Frayne RJ, Kimmerly DS. (2021). Impact of habitual sedentary patterns on popliteal artery endothelial-dependent vasodilation in healthy adults. *Applied Physiology, Nutrition and Metabolism.* 46(10): S75.

8) Wu Y, **O'Brien MW**, Johns JA, Petterson JL, Kimmerly DS. (2021). More frequent breaks in sedentary time are associated with better autonomic heart rate control in adults. *Applied Physiology, Nutrition and Metabolism.* 46(10): S81.

9) **O'Brien MW**, Ramsay D, Johnston W, Kimmerly DS. (2020). Higher aerobic fitness attenuates the neurovascular responses to spontaneous bursts of muscle sympathetic nerve activity in males. *Applied Physiology, Nutrition and Metabolism.* 45(11): S313.

10) Petterson JL, **O'Brien MW**, Johns JA, Chiasson J, Kimmerly DS. (2020). Influence of prostaglandins and endothelial-derived hyperpolarizing factors on brachial and

popliteal endothelial-dependent function in young adults. *Applied Physiology, Nutrition and Metabolism.* 45(11): S315.

11) Al-Hinnawi A, **O'Brien MW**, Johns JA, Kimmerly DS. (2020). Cardiovagal baroreflex sensitivity is unaffected by sex, menstrual or oral contraceptive pill phases in response to an acute bout of prolonged sitting. *Applied Physiology, Nutrition and Metabolism.* 45(11): S285.

12) Wu Y, Johns JA, Poitras J, Kimmerly DS, **O'Brien MW**. (2020). Improving the criterion validity of the activPAL in determining physical activity intensity during laboratory and free-living conditions. *Applied Physiology, Nutrition and Metabolism.* 45(11): S324.

13) **O'Brien MW**, Johns JA, Robinson SA, Mekary S, Kimmerly DS. (2019). The effects of short-term aerobic and resistance exercise training on popliteal artery low-flow-mediated constriction in older adults. Canadian Society for Exercise Physiology. *Applied Physiology, Nutrition and Metabolism.* 44(10), s97.

14) Johns JA, Bungay A, **O'Brien MW**, Kimmerly DS. (2019). The influence of sex, aerobic fitness, moderate-vigorous physical activity and sedentary time on brachial and popliteal artery *vasoconstrictor* function in healthy young adults. Canadian Society for Exercise Physiology. *Applied Physiology, Nutrition and Metabolism.* 44(10), s82.

15) Johns JA, Bungay A, **O'Brien MW**, Kimmerly DS. (2019). The influence of sex, aerobic fitness, moderate-vigorous physical activity and sedentary time on brachial and popliteal artery *vasodilator* function in healthy young adults. Canadian Society for Exercise Physiology. *Applied Physiology, Nutrition and Metabolism*. 44(10), s82

16) **O'Brien MW**, Johns JA, Robinson SA, Mekary S, Kimmerly DS. (2019). Comparison of Endothelial-Dependent Vasodilatory and Vasoconstrictor Responses Between Upper- and Lower-Limb Arteries in Older Adults. American College of Sports Medicine. *Medicine & Science in Sport & Exercise.* 51(6), 664.

17) Johns JA, **O'Brien MW**, Bungay A, Mekary S, Kimmerly DS. (2019). The Influence of Habitual Moderate-Vigorous Physical Activity Level Versus Aerobic Fitness on Age-Related Endothelial Function. American College of Sports Medicine. *Medicine & Science in Sport & Exercise*. 51(6), 806.

18) **O'Brien MW**, Shields CA, Crowell S, McGrath P, Fowles JR. (2018). Perspectives on Facilitating and Sustaining Physical Activity and Exercise Promotion in Healthcare Among Diabetes Care Providers in Nova Scotia. Diabetes Canada Conference. *Canadian Journal of Diabetes*. 42(5), s29-s30.

19) Johns JA, **O'Brien MW**, Williams TD, Kimmerly DS. (2018). Influence of Menstrual Phase on Sitting-Induced Vascular Dysfunction. Canadian Society for Exercise Physiology. *Applied Physiology, Nutrition and Metabolism*. 43(10), s67.

20) **O'Brien MW**, Shields CA, Fowles JR. (2018). Exercise is Medicine Canada Training Improves Physical Activity Practices in Physicians Across Canada Independent of Initial Confidence. Canadian Society for Exercise Physiology. *Applied Physiology, Nutrition and Metabolism*. 43(10), s85.

21) **O'Brien MW**, Shields CA, Crowell S, McGrath P, Fowles JR. (2018). Physical Activity Counselling and Exercise Prescription Practices of Physiotherapists in Nova Scotia. American College of Sports Medicine. *Medicine & Science in Sport & Exercise.* 50(5), 361.

22) **O'Brien MW**, Robinson S, Evans K, Mekary S, Kimmerly DS. (2018). Can Six-Weeks of Whole-Body Resistance Training Improve Endothelial Function in Older Adults?. Experimental Biology. *The FASEB Journal*. 32, 855.22.

23) **O'Brien MW**, Robinson S, Mekary S, Kimmerly DS. (2018). The Effects of Short-Term High-Intensity Interval Versus Moderate-Intensity Continuous Training on Vascular Function in Older Adults. Experimental Biology. *The FASEB Journal*. 32, 855.8.

24) Robinson S, **O'Brien MW**, Kimmerly DS. (2018). Short-Term Ingestion of Virgin Coconut Oil Improves Endothelial-Dependent Dilation but not Exercise-Mediated Hyperemia in Young Adults. Experimental Biology. *The FASEB Journal*. 32, 742.8.

25) **O'Brien MW**, Shields CA, Crowell S, Theou O, McGrath P, Fowles JR. (2017). The Effect of Previous Training on Perceptions and Practices of Physical Activity Counselling and Exercise Prescription Among Healthcare Providers Across Nova Scotia. Canadian Society for Exercise Physiology. *Applied Physiology, Nutrition and Metabolism*. 42(10), s91.

26) **O'Brien MW**, Shields CA, Fowles JR. (2017). "Stepping-Up" to the Challenge: Diabetes Care Professionals Promoting Physical Activity. Diabetes Canada Conference. *Canadian Journal of Diabetes*. 41(5), s24.

27) **O'Brien MW**, Shields C, Oh PI, Fowles JR. (2017). Effectiveness of the Exercise is Medicine Canada Training Workshops on Physician Counselling and Prescription Practices. American College of Sports Medicine. *Medicine & Science in Sport & Exercise*. 49(5), 298.

28) **O'Brien MW**, Ramsay D, Mekary S, Dogra S, O'Neill C, Kimmerly DS. (2017). Is There a Relationship Between Resting Sympathetic Nerve Activity and Leg Vascular Conductance in Aerobically-Trained Older Adults?. Experimental Biology. *The FASEB Journal*. 31, 1056.3.

29) **O'Brien MW**, Shields CA, Yungblut S, Oh PI, Fowles JR. (2016). Effectiveness of the Exercise is Medicine Canada Physical Activity Counselling and Exercise

Prescription Resources Among Physicians Across Canada. Canadian Society for Exercise Physiology. *Applied Physiology, Nutrition and Metabolism*. 41(9), s378.

30) **O'Brien MW**, Shields CA, Yungblut S, Fowles JR. (2016). Opposition and Opportunity: Reported Challenges and Changes to Practice Within the Context of Exercise is Medicine Canada Initiative. The Canadian Society for Psychomotor Learning and Sport Psychology. *Journal of Exercise, Movement and Sport*. 48(1), 205.

31) Fowles JR, **O'Brien MW**, Yungblut S, Oh PI, Shields CA. (2016). Implementation of Exercise is Medicine Canada Exercise Prescription Resources Among Health Care Providers Across Canada. Canadian Society for Exercise Physiology. *Applied Physiology, Nutrition and Metabolism*. 41(9), s357.

32) Shields CA, Fowles JR, **O'Brien MW**, Yungblut S, Fortier MS, Oh PI. (2015). Exercise is Medicine Canada: Early But Important Signs of the Effectiveness of this National Initiative. The Canadian Society for Psychomotor Learning and Sport Psychology. *Journal of Exercise, Movement and Sport*. 47(1), 222.

33) **O'Brien MW**, Wojcik WR, D'Entremont L, Mekary S, Fowles JR. (2015). Validation of PiezoRx® Step Count and Moderate to Vigorous Physical Activity Times in Free Living Conditions in Adults. Canadian Society of Exercise Physiology. *Applied Physiology, Nutrition and Metabolism.* 40(9), s48.

34) Wojcik WR, **O'Brien MW**, Fowles JR. (2015). Validity of Step Count and Intensity Related Physical Activity Measures of Several Physical Activity Monitoring Devices. Canadian Society of Exercise Physiology. *Applied Physiology, Nutrition and Metabolism.* 40(9), s69.

35) Fowles JR, **O'Brien MW**, Yungblut S, Oh P, Shields CA. (2015). Exercise is Medicine Canada: Implementation and Perceived Effectiveness of the Exercise is Medicine Canada Workshops Among Health Care Providers. Canadian Society of Exercise Physiology. *Applied Physiology, Nutrition and Metabolism.* 40(9), s23.

Professional Meetings and Other Academic Conferences

1) McPhee B, Petterson JL, **O'Brien MW**, Kimmerly DS. (2022). Impact of Aerobic Fitness and a 12-week HIIT program on Sympathetic Neurovascular Transduction in Young Adults Atlantic Provinces Exercise Science Conference. University of New Brunswick, Fredericton, New Brunswick, Canada.

2) Bustamante C, **O'Brien MW**, Kimmerly DS. (2022). Impact of Aerobic Fitness and a 12-week HIIT Program on Resistance Vessel Function in Young Adults. Atlantic Provinces Exercise Science Conference. University of New Brunswick, Fredericton, New Brunswick, Canada.

3) Pellerine L, Kimmerly DS, Fowles JR, **O'Brien MW**. (2021). Criterion Validation of the Physical Activity Vital Sign Questionnaire Estimation of Habitual Moderate-Vigorous Physical Activity in Younger and Older Adults. Atlantic Provinces Exercise Science Conference. University of New Brunswick, Fredericton, New Brunswick, Canada.

4) Schwartz B, **O'Brien MW**, Peddle A, Frayne RJ. (2021). Validation of activPAL Determined Knee Angles. Atlantic Provinces Exercise Science Conference. University of New Brunswick, Fredericton, New Brunswick, Canada.

5) Petterson JL, **O'Brien MW**, Kimmerly DS. (2021). Impact of Aerobic Fitness on Sympathetic Neurohemodynamic Transduction in Older Males. Atlantic Provinces Exercise Science Conference. University of New Brunswick, Fredericton, New Brunswick, Canada.

6) Wu Y, **O'Brien MW**, Johns JA, Petterson JL, Kimmerly DS. (2021). Relationship Between Habitual Sedentary Patterns and Autonomic Heart Rate Control in Younger Adults. Atlantic Provinces Exercise Science Conference. University of New Brunswick, Fredericton, New Brunswick, Canada.

7) Shivgulam M, **O'Brien MW**, Johns JA, Petterson JL, Kimmerly DS. (2021). Impact of Habitual Sedentary Patterns on Popliteal Artery Endothelial-Dependent Vasodilation in Healthy Adults. Atlantic Provinces Exercise Science Conference. University of New Brunswick, Fredericton, New Brunswick, Canada.

8) Liu H, **O'Brien MW**, Johns JA, Al-Hinnawi A, Kimmerly DS. (2021). Does Higher Aerobic Fitness Impact Prolonged Sitting-Induced Popliteal Artery Endothelial Dysfunction in Younger Healthy Adults? Atlantic Provinces Exercise Science Conference. University of New Brunswick, Fredericton, New Brunswick, Canada.

9) **O'Brien MW**, Shields CA, Crowell S, Fowles JR. (2019). Nova Scotia Healthcare Providers' Perspectives on Promoting and Sustaining Physical Activity and Exercise in Healthcare. Primary Healthcare Research Day, Halifax, Nova Scotia, Canada.

10) Martin L, Allan V, **O'Brien MW**, Kivell M, Bruner M, Shields CA. (2019). I'm Doing the Right Thing for My Child…Aren't I? Parents' Perspectives of Specialized Hockey Programming as a Context for Youth Development. The Hockey Conference, Saint Mary's University, Halifax, Nova Scotia, Canada.

11) Johns JA, **O'Brien MW**, Bungay A, Mekary S, Kimmerly DS. (2019). The Influence of Habitual Moderate-Vigorous Physical Activity Levels Versus Aerobic Fitness on Age-Related Endothelial Function. Atlantic Provinces Exercise Science Conference. Acadia University, Wolfville, Nova Scotia, Canada.

12) Bungay A, Johns JA, **O'Brien MW**, Kimmerly DS. (2019). Sex Differences in Brachial and Popliteal Artery Endothelial Health Independent of Habitual Physical

Activity and Sedentary Time. Atlantic Provinces Exercise Science Conference. Acadia University, Wolfville, Nova Scotia, Canada.

13) Bungay A, Johns JA, **O'Brien MW**, Kimmerly DS. (2019). Sex Differences in Brachial and Popliteal Artery Endothelial Health Independent of Habitual Physical Activity and Sedentary Time. Crossroads Interdisciplinary Health Research Conference, Halifax, Canada.

14) Fowles JR, **O'Brien MW**, Shields CA, Crowell S, McGrath P. (2018). Exercise is Medicine Initiative in Nova Scotia. Nova Scotia Health Authority Annual General Meeting, Halifax, Nova Scotia, Canada.

15) **O'Brien MW**, Shields CA, Crowell S, Theou O, McGrath P, Fowles JR. (2018). Nova Scotia Physicians Perspectives on Promoting and Sustaining Physical Activity and Exercise in Healthcare. Primary Healthcare Research Day, Dartmouth, Nova Scotia, Canada.

16) **O'Brien MW**, Robinson SA, Frayne RJ, Mekary S, Fowles JR, Kimmerly DS. (2018). Achieving Canadian physical activity guidelines is associated with better vascular function independent of aerobic fitness and sedentary time in older adults. Atlantic Provinces Exercise Science Conference. Dalhousie University, Halifax, Nova Scotia, Canada.

17) Williams TD, **O'Brien MW**, Johns JA, Kimmerly DS. (2018). Influence of menstrual phase on sitting-induced vascular dysfunction. Atlantic Provinces Exercise Science Conference. Dalhousie University, Halifax, Nova Scotia, Canada.

18) Johns JA, **O'Brien MW**, Williams TD, Kimmerly DS. (2018). Sex does not influence impairments in popliteal endothelial-dependent vasodilator or vasoconstrictor responses following prolonged sitting. Atlantic Provinces Exercise Science Conference. Dalhousie University, Halifax, Nova Scotia, Canada.

19) MacLellan K, **O'Brien MW**, Johns JA, Kimmerly DS. (2018). The effects of caffeine dosage on the cardiovascular and popliteal blood flow responses to a cycling time to exhaustion trial. Atlantic Provinces Exercise Science Conference. Dalhousie University, Halifax, Nova Scotia, Canada.

20) **O'Brien MW**, Shields CA, Crowelll S, Theou O, McGrath P, Fowles JR. (2017). The Effect of Previous Training on Perceptions and Practices of Physical Activity Counselling and Exercise Prescription Among Nova Scotian Health Care Providers. Primary Healthcare Research Day, Halifax, Nova Scotia, Canada.

21) Dorey TD, **O'Brien MW**, Robinson S, Kimmerly DS. (2017). Knee-High Compression Socks Minimize Head-Up Tilt-Induced Cerebral and Cardiovascular Responses Following Exercise. Primary Healthcare Research Day, Halifax, Nova Scotia, Canada.

22) Theou O, **O'Brien MW**, Shields CA, Crowell S, McGrath P, Fowles JR. (2017). Physical Activity Counselling and Exercise Prescription Practices of Nova Scotian Physicians. Dalhousie Department of Medicine Research Day, Halifax, Nova Scotia, Canada.

23) **O'Brien MW**, Shields CA, Crowell S, McGrath P, Fowles JR. (2017). Practices and Perceptions of Physical Activity Counselling and Exercise Prescription among Nova Scotian Health Care Providers. Atlantic Provinces Exercise Science Conference. Charlottetown, Prince Edward Island, Canada.

24) Robinson S, **O'Brien MW**, Kimmerly DS. (2017). Short-Term Ingestion of Virgin Coconut Oil Improves Endothelial-Dependent Dilation but not Exercise-Mediated Hyperemia in Young Adults. Atlantic Provinces Exercise Science Conference. Charlottetown, Prince Edward Island, Canada.

25) Dorey TD, **O'Brien MW**, Robinson S, Kimmerly DS. (2017). Knee-High Compression Socks Minimize Head-Up Tilt-Induced Cerebral and Cardiovascular Responses Following Exercise. Atlantic Provinces Exercise Science Conference. Charlottetown, Prince Edward Island, Canada.

26) **O'Brien MW**. (2017). *Invited Presentation*: The Exercise is Medicine Canada Initiative. Atlantic Provinces Exercise Science Conference. Charlottetown, Prince Edward Island, Canada.

27) **O'Brien MW**, Fowles JR, Shields CA, Oh PI, Yungblut S. (2016). Implementation and perceived effectiveness of the Exercise is Medicine Canada workshops in primary care providers across Canada. Atlantic Provinces Exercise Scientists and Socio-Culturalists, Antigonish, Nova Scotia, Canada.

28) Wojcik WR, **O'Brien MW**, Fowles JR. (2016). Validation of step count and intensity measures of physical activity in several physical activity monitoring devices. Atlantic Provinces Exercise Scientists and Socio-Culturalists, Antigonish, Nova Scotia, Canada.

29) **O'Brien MW**, Fowles JR, Shields CA, Oh PI, Yungblut S. (2016). Implementation and perceived effectiveness of the Exercise is Medicine Canada workshops in primary care providers across Canada. Oral Presentation. Crossroads Interdisciplinary Health Research Conference, Halifax, Canada.

RESEARCH EMPLOYMENT & CLINICAL EXPERIENCE

Research Coordinator
Geriatric Medicine Research Unit, Halifax, NS — Dec 2021-Present
"Clinical Trial: Breaking Bad Rest"
Dr. Olga Theou

Research Assistant
Nova Scotia Health Authority, Halifax, NS — April 2016-Dec 2018
"Exercise is Medicine Nova Scotia Initiative"
Dr. Jonathon Fowles and Mrs. Sandra Crowell

Exercise is Medicine Canada (EIMC) — June 2015-Dec 2018
"Exercise is Medicine Nova Scotia Initiative"
"Exercise is Medicine Canada National Training Workshop Evaluation"
Dr. Jonathon Fowles

Centre of Lifestyle Studies, Acadia University, Wolfville, NS — May 2015-March 2019
"Exercise is Medicine Canada National Training Workshop Evaluation"
"Validity and Reliability of a Medical-Grade Physical Activity Monitor"
Dr. Jonathon Fowles

"Finding the balance: Unpacking the youth sport experience in an era of specialized sport and recreation opportunities"
Dr. Chris Shields

Research Internship
Mitacs Accelerate — March 2019-June 2019
Canadian Society for Exercise Physiology & Exercise is Medicine Canada
"An Evaluation of Exercise Referral Schemes in Healthcare"

Practicing Clinical Exercise Physiologist — Feb 2016-Aug 2018
Received patient referrals from general and specialist physicians for physical activity counselling, exercise assessments, and exercise prescription.
Wolfville Professional Centre, Wolfville, Nova Scotia.

TEACHING & MENTORING EXPERIENCE

Instructor 2018

- Department of Physiology and Biophysics, Faculty of Medicine, Dalhousie University
- Human Physiology Lab (PHYL 3620). 3 credit hours. Class size (*n*=11). The course was split into traditional lecturing (2-hr per week) and applied laboratory experiments (2-hr per week).
- Overall course evaluation: 4.5/5.0

Instructor 2021

- School of Health and Human Performance, Division of Kinesiology, Dalhousie University
- Kinesiology Honours 1 (KINE 4901). 3 credit hours. Class size (*n*=13).
- Overall course evaluation: 4.9/5.0

Instructor

- School of Human Kinetics, St. Francis Xavier University 2022
- Aging and Exercise (HKIN 357). 3 credit hours. Class size (*n*=42).
- *Created syllabus, all course materials, and evaluations.*
- Overall course evaluation (4.6-4.9/5.0), each criteria was above department and university average (3.9-4.3).

Adjunct Scholar

- School of Health and Human Performance, Dalhousie University Present-2023
- Faculty of Graduate Studies

Module Instructor 2018

- Dalhousie University – Measurement Evaluation and Instrumentation (KINE 5590)
- "Ultrasound Theory and Applications" – 4 in person Lectures.

Teaching Assistant: Dalhousie University

- Fitness Assessment and Program Design (KINE 3414) 2016/2017, 2017/2018, 2018/2019
- Applied Physiological Principles of Performance (KINE 3419) 2016/2017, 2017/2018, 2019/2020
- Exercise Physiology (KINE 2310) 2017/2018, 2018/2019, 2019/2020

Teaching Assistant: Acadia University

- Human Physiology 1 (KINE 2413) 2014/2015
- Human Physiology 2 (KINE 2423) 2014/2015
- Exercise Physiology (KINE 3013) 2016

Guest Lecturer

- Fitness Assessment and Program Design (KINE 3414) Nov. 2018
  - "Exercise Testing and Programming in Older Adults"
  - Dalhousie University
- Physical Activity & Chronic Disease (KINE 4709) Feb. 2021
  - "Physical Behaviours and Vascular Function" Oct. 2021
  - Dalhousie University
- Graduate Seminar (HLTH 6000) April 2021
  - "Funding Your Graduate Studies"
  - Dalhousie University
- Special Topics: Personal Health (KINE 4593) Oct 2021
  - Cardiovascular Disease & Physical Behaviours
  - Acadia University

Master's Thesis Supervisor

Liam Pellerine (MSc Kinesiology) Present

- Co-supervised with Dr. Ryan Frayne

Master's Thesis Independent Study Supervisor

Yanlin Wu (MSc Kinesiology) 2022

- Co-supervised with Dr. Ryan Frayne

Liam Pellerine (MSc Kinesiology) 2022

- Co-supervised with Dr. Ryan Frayne

Beverly Schwartz (MSc Kinesiology) 2022

- Co-supervised with Dr. Ryan Frayne

Honours Thesis Supervisor

Liam Pellerine (BSc Kinesiology Honours). 2021

- *Criterion-Validity of the Physical Activity Vital Sign for Estimating Moderate-to-Vigorous Intensity Physical Activity Levels in Younger and Older Adults.*
- Co-supervised with Dr. Derek Kimmerly.

Honours Thesis Committee (Reader)

Breanna McPhee (BSc Kinesiology Honours). 2022
- *The Impact of High-Intensity Interval Training on Sympathetic Neurovascular Transduction in Young Adults*

Carolina Bustamante (BSc Kinesiology Honours). 2022
- *The Impact of a 12-week High-Intensity Interval Training Program on Resistance Vessel Function in Healthy Adults.*

Haoxuan Liu (BSc Kinesiology Honours). 2021
- *Impact of Aerobic Fitness on Prolonged Sitting-Induced Popliteal Artery Endothelial Dysfunction.*

Madeline Shivgulam (BSc Kinesiology Honours). 2021
- *Impact of Sedentary Time and Patterns on Popliteal Artery Health in Adults.*

Beverley Schwartz (BSc Kinesiology Honours). 2021
- *Validation of activPal Determined Knee-Bent Sitting*

Mentored Students

Jarrett Johns (MSc Kinesiology) 2020
- *Sex Differences Exist in Popliteal, but not Brachial Artery Flow-Mediated Dilation in Physically Active Young Adults.*

Amera Al-Hinnawi (BSc Kinesiology Honours) 2020
- Independent Study: *Cardiovagal baroreflex sensitivity is unaffected by sex in response to an acute bout of prolonged sitting.*
- Independent Study: *The relationship between habitual sedentary time and cardiovagal baroreflex sensitivity.*
- Thesis: *Cardiovagal baroreflex sensitivity is unaffected by sex, menstrual or oral contraceptive pill phases in response to an acute bout of prolonged sitting.*

Justine Poitras (BSc Kinesiology) 2020
- Independent Study: *Criterion validity of the activPAL*

Yanlin Wu (BSc Kinesiology Honours) 2020
- *Criterion validity of the activPAL in determining physical activity intensity during laboratory conditions*

Jennifer Petterson (BSc Kinesiology Honours) 2020
- *Influence of endothelial-derived hyperpolarizing factors on popliteal endothelial-dependent function in young adults*

Jack Chiasson (BSc Kinesiology Honours) 2020
- *Influence of prostaglandins on popliteal endothelial-dependent function in young adults*

William Johnston (BSc Kinesiology Honours) 2020

- *Influence of aerobic fitness on sympathetic neurovascular transduction in response to handgrip exercise.*

Amanda Bungay (BSc Kinesiology Honours) 2019

- *Sex Differences in Popliteal Flow-Mediated Dilation in Young Adults.*

Kelly MacLellan (BSc Kinesiology Honours) 2018

- *The effects of caffeine dosage on the cardiovascular and popliteal blood flow responses to a cycling time to exhaustion trial.*

Tanner Williams (BSc Kinesiology Honours) 2018

- *Sex does not influence impairments in popliteal endothelial-dependent vasodilator or vasoconstrictor responses following prolonged sitting*

Tristan Dorey (BSc Kinesiology Honours) 2017

- *Knee-High Compression Socks Minimize Head-Up Tilt-Induced Cerebral and Cardiovascular Responses Following Exercise*

FUNDING, DISTINCTIONS, & AWARDS

Canadian Institutes of Health Research Post-Doctoral Fellowship 2022-2025
- Rated #2 of all CIHR post-doctoral fellowship applicants.
- *$150 000*

Dalhouise University Post-Doctoral Fellowship Level
University Internal Medicine Research Fund 2022
- *$60 000 for one year, available for renewal for year 2.*

Dalhousie University Ph.D Level
Fredrick Banting and Charles Best CIHR Doctoral Award 2020-2023
- *$105 000*

Killam PreDoctoral Scholarship 2019-2022
- *$90 000*

Nova Scotia Health Research Foundation Scotia Scholar 2019-2022
- *$45 000*

Nova Scotia Graduate Scholarship 2018-2020
- *$30 000*

Heart & Stroke: BrightRed Mission Award 2018
- *$5 000*

CGS Michael Smith Foreign Study Supplement 2020
- *$6 000*
- CIHR award to work with Dr. Danny Green at the University of Western Australia in Perth, Australia. COVID pandemic prevented travel.

Mitacs Accelerate Internship 2019
- *$15 000*
- Internship with CSEP and EIMC

Pictou County Municipality Community Recognition Award 2019
- Awarded for academic achievements and research initiatives in cardiovascular activity by District 7 counsellor David Parker and Warden Robert Parker.

Dalhousie University M.Sc Level
Nova Scotia Health Research Foundation Scotia Scholar 2017
- *$10 000*

Nova Scotia Graduate Scholarship 2016/2017
- *$20 000*

Heart & Stroke: BrightRed Award 2016
- *$5 000*

Acadia University BKinH Level

Acadia Excellence Scholarship 2012-2015
- *$5 000*

Henry and Rena Demone Scholarship 2012-2015
- *$10 000*

Thomas and Mildred Irvine Scholar-Bursary 2016
- *$5 000*

Acadia University Scholar 2012-2016
- GPA of A- or higher, with no grade less than B- in any course.

CCUPEKA Leadership Award 2016
- In recognition of an exceptional graduating student leader who will make a difference in their chosen field.

Eastern Kings Memorial Award of Excellence 2015
- Awarded for contribution on "Exercise – The 5th Vital Sign"

Grant: Research Nova Scotia: New Health Investigator 2022
- Primary: Olga Theou; *$100 000 over 2 years*
- Role: Associate

PROFESSIONAL AFFILIATIONS & SERVICE

Journal Reviewer

Clinical Autonomic Research	2022; *n*=1
Journal of Cardiopulmonary Rehabilitation & Prevention	2022; *n*=1
JMIR Cardio	2022; *n*=1
Experimental Gerontology	2022; *n*=1
Journal for Medical Internet Research	2022; *n*=2
AIMS Public Health	2022; *n*=1
Frontiers Digital Medicine	2022; *n*=1
JMIR Diabetes	2022; *n*=1
Healthy Populations Journal	2021/22; *n*=2
JMIR Mhealth & Uhealth	2021; *n*=1
Scandinavian Journal of Science & Medicine in Sports	2021; *n*=1
European Journal of Sport Science	2021; *n*=1
JMIR Formative Research	2021/22; *n*=2
Scientific Reports	2020; *n*=1
Teaching and Learning in Medicine	2020; *n*=1
Physiotherapy Theory and Practice	2020; *n*=1
NPJ Digital Medicine	2020; *n*=1
Journal of Aging and Physical Activity	2020; *n*=1
BioMed Research International	2019; *n*=1
International Journal of Sport and Exercise Medicine	2019; *n*=1
Applied Physiology, Nutrition and Metabolism	2019; *n*=1

Certifications

Canadian Society for Exercise Physiology – Clinical Exercise Physiologist	2016-Present
Canadian Society for Exercise Physiology – High Performance Specialist	2016-Present
Canadian Society for Exercise Physiology – Certified Personal Trainer	2015-2016

Memberships

Canadian Society for Exercise Physiology (CSEP) – Student Member	2015-Present
American College of Sports Medicine (ACSM) – Student Member	2017-Present
American Physiological Society (APS) – Student Member	2018-Present
Exercise is Medicine Canada (EIMC) – Student Member	2021-Present

Committees & Volunteering

Dalhousie University Hiring Committee – Student Representative

- *Division of Kinesiology – Laboratory Instructor Position* 2020
- *Division of Kinesiology – Laboratory Instructor Position* 2021
- *Division of Kinesiology – Limited Term Instructor Position* 2021
- *Health & Human Performance – Continuing Term Instructor* 2022

- *Health & Human Performance – Limited Term Laboratory Instructor* 2022

Editorial Board Member: Healthy Populations Journal 2021-Present

Exercise is Medicine Canada – Graduate Student Committee 2019-Present

Prospective Physical Activity, Sitting and Sleep (PROPASS) Consortium 2020-Present
- *International consortium with specific task of developing analysis software*

Canadian Society for Exercise Physiology Student Committee 2016
- *Undergraduate Student Representative*

Exercise is Medicine Canada on Campus – Acadia University Campus 2015/2016
- *Student Executive President*

Exercise Science and Training Practicum – CSEP-CEP Candidacy Volunteer 2014-2016
- *Acadia Active Aging, Cardiac Rehabilitation, etc.*