Sex Differences Exist in Popliteal, but not Brachial Artery Flow-Mediated Dilation in Physically Active Young Adults

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Abstract

Conflicting reports regarding sex differences in flow-mediated dilation (FMD) may be due to not considering smaller arterial diameters in females, between-group differences in aerobic fitness and/or habitual physical activity (PA) and sedentary behaviours. It was hypothesized that males would exhibit greater brachial (BA-FMD) and popliteal artery FMD (POP-FMD) when scaled for baseline diameter. FMD, aerobic fitness, objectively measured PA and sedentary time (ST), were measured in 13 males (23±3 years) and 13 females (24±2 years). Both groups had similar moderate-vigorous PA (p=0.25) and ST (p=0.08) but males had greater aerobic fitness (p<0.001). Females engaged in more light PA (p=0.03), which was positively correlated to POP-FMD in the pooled sample (r=0.43, p=0.04). When allometrically scaled, BA-FMD was similar between sexes (p=0.75) but POP-FMD was still greater in females (p=0.03). Covarying for light PA did not influence the sex difference in POP-FMD (p=0.008). Sex differences existed in lower-, but not upper-limb FMD.

List of Abbreviations Used

ANOVA – Analysis of variance

ANCOVA – Analysis of covariance

BA – Brachial artery

Ca^{2+} – Calcium

cGMP – Cyclic guanosine-3' 5-monophosphate

CVD – Cardiovascular disease

DBP – Diastolic blood pressure

ECG – Electrocardiogram

eNOS – Endothelial nitric oxide synthase

ET-1 – Endothelin-1

FMD – Flow-mediated dilation

GTP – Guanosine triphosphate

LPA – Light-intensity physical activity

MAP – Mean arterial pressure

MLCK – Myosin light chain kinase

MLCP – Myosin light chain phosphatase

MVPA – Moderate-vigorous physical activity

NO – Nitric oxide

NMD – Nitroglycerin-mediated dilation

POP – Popliteal artery

RBCV – Red blood cell velocity

sGC – Soluble guanylate cyclase

SBP –Systolic blood pressure

SR – Sarcoplasmic reticulum

SR_{AUC} – Shear rate area under the curve

VO_2peak – Peak oxygen consumption

VSM – Vascular smooth muscle

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Cheers!

Jarrett Alexander Johns

1 Introduction

Reduced aerobic fitness and habitual physical activity (PA) along with elevated sedentary time are associated with an increased risk of developing cardiovascular disease (CVD) (Shiroma and Lee 2010). Sex differences exist in the incidence of CVD whereby pre-menopausal females appear to be more protected from CVD until menopause (Bots et al. 2017). Dysfunction of the endothelium (the innermost lining of an artery) is characterized by the reduced ability to produce the potent vasodilator, nitric oxide (NO) and precedes the development of CVD (Sitia et al. 2010). Arterial endothelial function can be quantified via the flow-mediated dilation test (FMD) and serves as an index of NO bioavailability (Joannides et al. 1995; Kooijman et al. 2008; Green et al. 2014). Notably, FMD is clinically relevant as meta-analyses estimate that a 1% increase in brachial artery (BA) FMD is associated with an 8-13% reduced risk of developing CVD (Inaba et al. 2010; Matsuzawa et al. 2015). Seminal work suggested that sex differences in endothelial dysfunction may follow a similar trajectory to CVD incidence (Celermajer et al. 1994), but it is unclear whether sex differences in FMD are related to inter-individual variations in aerobic fitness, habitual PA and/or sedentary time.

Conflicting results from previous studies investigating sex differences in FMD exist (Celermajer et al. 1994; Hashimoto et al. 1995; Juonala et al. 2008; Nishiyama et al. 2008; Harris et al. 2012; Yao et al. 2014; Shenouda et al. 2018; Holder et al. 2019; Tremblay et al. 2019). Specifically, some (Celermajer et al. 1994; Juonala et al. 2008; Nishiyama et al. 2008; Yao et al. 2014; Holder et al. 2019) but not all (Hashimoto et al. 1995; Harris et al. 2012; Shenouda et al. 2018; Tremblay et al. 2019) demonstrated that when expressed as a relative increase in arterial diameter (from baseline), pre-menopausal females exhibited greater BA-FMD compared to age-matched males. However, these studies did not account for possible group differences in

objectively measured aerobic fitness, habitual PA or sedentary time. Furthermore, it is well established that an inverse relationship exists between baseline arterial diameter and FMD (Thijssen et al. 2008b, 2008a). Only two studies (Juonala et al. 2008; Shenouda et al. 2018) have statistically controlled for the larger baseline diameters in males compared to females. Interestingly, males exhibited a greater BA-FMD response when baseline diameter was accounted for (Juonala et al. 2008; Shenouda et al. 2018). It is unclear whether these differences exist in lower-limb arteries.

Historically, FMD has primarily been performed in the BA due to its strong relationship with coronary artery function (Broxterman et al. 2019). However, the popliteal artery (POP) also has clinical relevance. In contrast to the BA, the POP is highly susceptible to the development of atherosclerosis (Debasso et al. 2004; Aboyans et al. 2011). Functionally, the POP is directly exposed to large fluctuations in blood flow during periods of PA (i.e., increased flow) and sedentary time (i.e., reduced flow), which influences endothelial function (Boyle et al. 2013; Padilla and Fadel 2017; Teixeira et al. 2017). Furthermore, it has been demonstrated that 5 days of reduced daily step counts caused marked reductions in POP-, but not BA-FMD (Boyle et al. 2013), highlighting the importance of measuring both upper- and lower-limb endothelial function.

Only one study has compared BA- versus POP-FMD between males and females, with females exhibiting greater relative BA-, but similar POP-FMD compared to males (Nishiyama et al. 2008). Similarly, two recent studies also failed to observe sex differences in the relative POP-FMD response (Vranish et al. 2017; O'Brien et al. 2019). However, none of these studies considered known sex differences in baseline diameter or assessed aerobic fitness. Only O'Brien et al. (2019) considered objectively measured moderate-vigorous PA and sedentary time,

although no differences existed between males and females. Currently, a paucity of research exists comparing POP-FMD in males and females that includes measures of aerobic fitness, habitual PA and sedentary time, all of which are important when considering the involvement of the lower-limb vasculature during exercise and (in)activity.

To date, no study has measured both upper- and lower-limb FMD responses concomitantly in males and females while considering the CVD risk factors indicated above. As such, the purpose of this study was to investigate sex differences in BA- and POP-FMD in young, healthy adults with objectively measured aerobic fitness, habitual PA and sedentary time. It was hypothesized that after the consideration of baseline diameter, males would exhibit greater BA- and POP-FMD responses. Considering that the POP experiences greater fluctuations in blood flow during free-living conditions, the secondary aims included determining whether aerobic fitness, habitual PA and sedentary time were related to POP-FMD in the pooled sample.

2 Review of the Literature

2.1 Peripheral Arteries

Often referred to as muscular arteries because of the large relative proportion of vascular smooth muscle (VSM), peripheral conduit arteries are imperative for effective blood flow distribution (Smith and Fernhall 2010a). Both conduit arteries and downstream arterioles are composed of 3 distinct layers, the *tunica adventitia*, *tunica media* and *tunica intima* as depicted below in Figure 2-1. The *tunica adventitia* is the outermost layer and contains the blood supply for the artery (i.e., the *vaso vasorum*), as well as post-ganglionic sympathetic nerve endings. The middle layer (i.e., *tunica media*) is largely composed of vascular smooth muscle embedded within a matrix of collagen, elastin and glycoproteins. This layer is primarily responsible for the maintenance of lumen diameter and vascular tone (Smith and Fernhall 2011). The innermost layer (i.e., *tunica intima*) consists of a single layer of endothelial cells, an important site for the production of potent vasoactive chemicals such as NO and the vasoconstricting agent endothelin-1 (ET-1) (Sandoo et al. 2010). Regulation of vascular tone, via changes in arteriolar diameter, heavily influences the distribution of cardiac output throughout the systemic circulation.

Local vascular control is achieved via an intricate balance between the production and release of endothelial-derived relaxing (i.e., NO) versus constricting (i.e., ET-1) chemicals (Smith and Fernhall 2011). Transmural pressure (i.e., pressure inside vessel – pressure outside vessel) also contributes to local blood flow via myogenic autoregulation. Myogenic autoregulation occurs independent of the endothelium and is defined as the resultant contraction or relaxation of the VSM in response to an increase or decrease in arteriolar perfusion pressure, respectively. Myogenic autoregulation is believed to serve a protective function by ensuring

relatively constant perfusion pressure and blood flow delivery to the distal capillary beds (Smith and Fernhall 2010b).

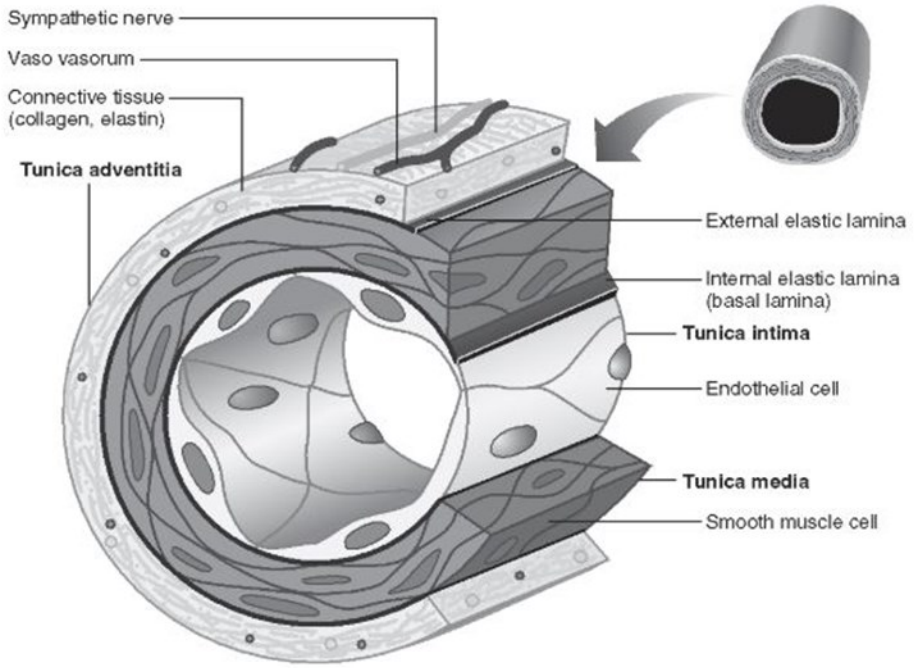

Figure 2-1: *A transverse section highlighting the 3 layers of a conduit artery or arteriole. The innermost layer, the tunica intima is comprised of the endothelium that produce vasoactive chemicals. The tunica media is predominantly composed of vascular smooth muscle cells that contract and relax to regulate blood flow (conduit arteries) and maintain mean arterial pressure (arterioles).*

As shown in Figure 2-2, the orientation of the VSM is unlike skeletal muscle whereby actin and myosin contractile proteins are not organized in parallel within a sarcomere (Smith and Fernhall 2011). Instead, contractile proteins actin and myosin travel transversely towards the long axis of the cell via a cytoskeleton comprised of dense bodies and bands. Actin is anchored to dense bands at the surface of the cell, which are connected to dense bodies located within the interior of the cell (Smith and Fernhall 2011).

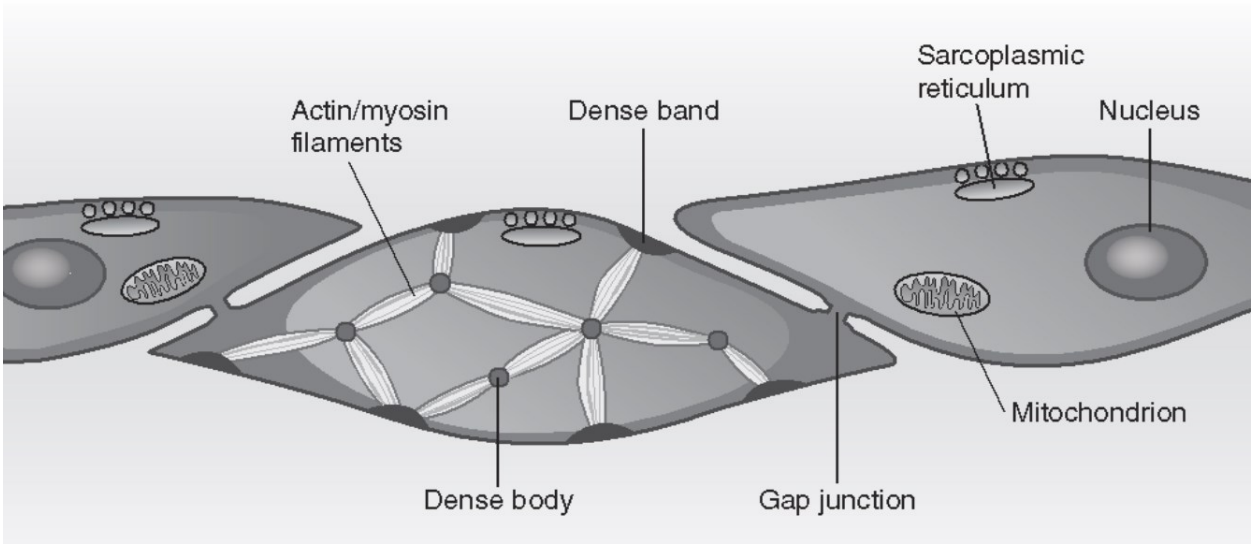

Figure 2-2: *Neighbouring vascular smooth muscle cells (VSMs) are connected via gap junctions to allow for inter-cellular communication. Unlike skeletal muscle, VSMs are comprised of long actin-myosin complexes that are connected via dense bodies and bands. Sarcoplasmic reticulum are the main storage sites for calcium ions, which are necessary for contraction of the VSMs.*

This is particularly important for the increase and decrease in lumen diameter during a relaxed versus contracted state, respectively (See Figure 2-3). When the actin-myosin filaments shorten, the dense bodies are brought closer to the surface-bound dense bands, causing the cell to 'puff up'. The length of a relaxed VSM is able to shorten to more than half its size (Smith and Fernhall 2011), which results in a reduced lumen diameter. The opposite occurs when the actin-myosin filaments lengthen, such that the VSM is now in a 'flattened' state, causing a resultant increase in lumen diameter.

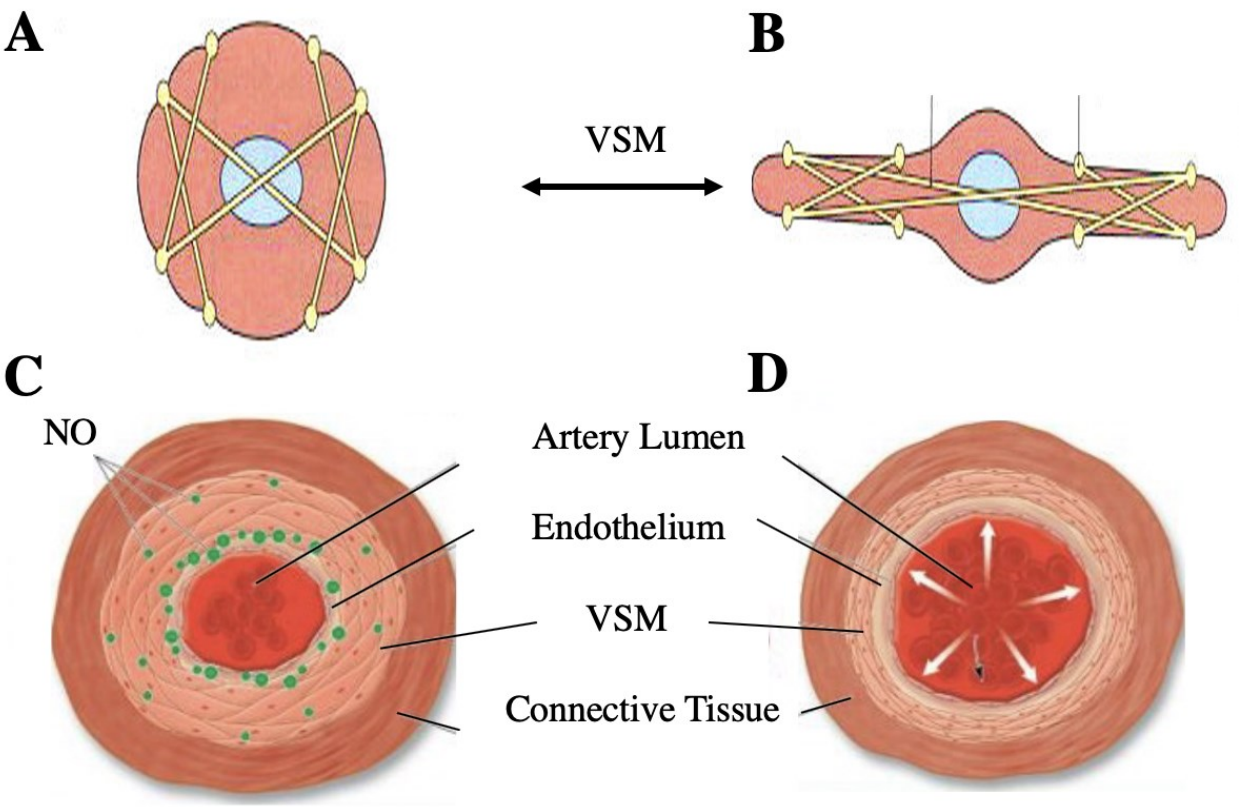

Figure 2-3: *An illustration of how the vascular smooth muscle (VSM) appears depending on the degree of shortening of the actin-myosin complexes (contractile filament). Located in panel A, the VSM is in a contracted state due to the shortening of the actin-myosin complexes and appears 'puffed up'. In panel B, the VSM is in a relaxed state due to lengthening of the actin-myosin complexes. As shown in a cross-sectional view in panel C, when in a contracted state, the VSM is a reduced lumen diameter. However, when the VSM is exposed to the potent vasodilator, nitric oxide (NO; panel C), the resultant relaxation of the VSM causes the lumen diameter to rapidly increase (panel D).*

2.2 The Arterial Endothelium

The vascular endothelium is a single cell layer that serves as the interface between the luminal blood and the underlying vascular smooth muscle cells. Physiologically, the endothelium serves to regulate vascular tone by controlling the release of endothelial-derived vasodilators and vasoconstrictors (Moyna and Thompson 2004). Pathological increases in vascular tone result from impaired vascular homeostasis due to an imbalance between the production and release of

endothelial-derived vasoconstricting versus vasodilating chemicals, leading to CVD complications (Donato et al. 2009).

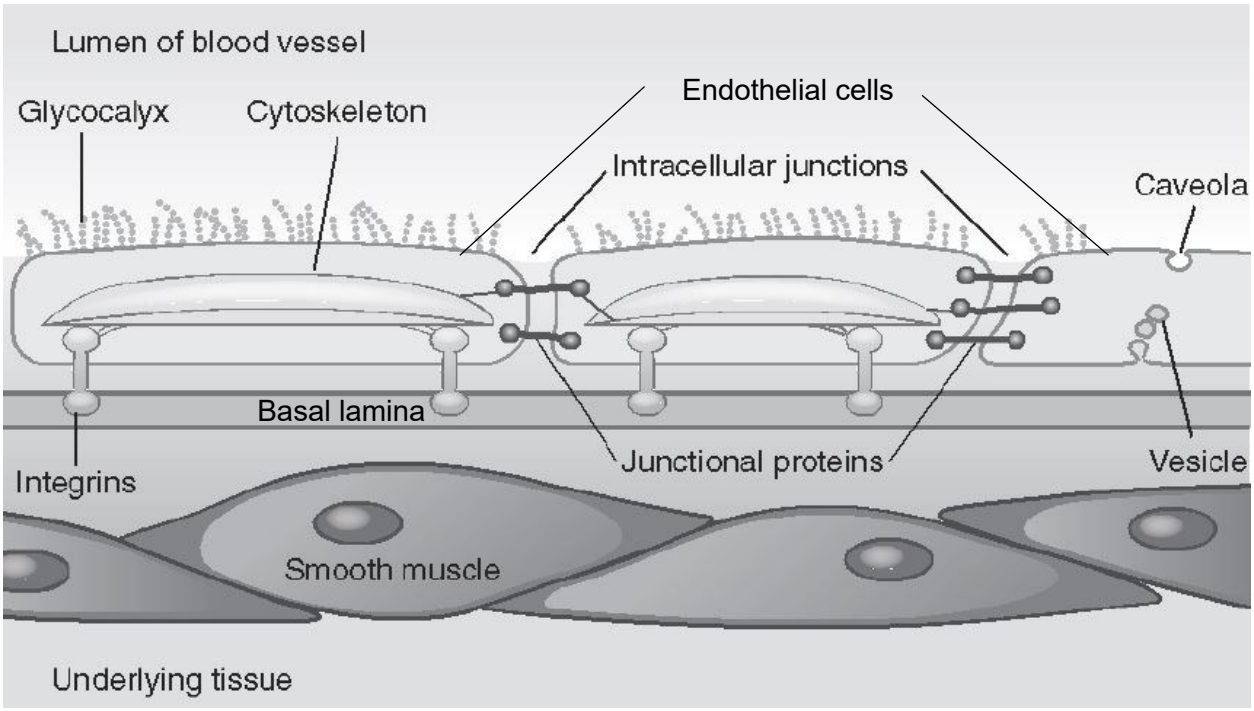

Figure 2-4: *Neighbouring vascular endothelial cells adjoined by junctional proteins. Negatively charged glycocalyx line the endothelial cells and act as protective barriers against large molecules. Integrins act as anchors/receptors to the basement membrane and to detect changes in shear stress. The caveola-vesicle facilitate transportation of larger molecules in/out of the cell. The basal lamina separates the endothelial cells from underlying tissue (i.e., vascular smooth muscle). (Smith and Fernhall 2011).*

In addition to regulating vascular tone, other roles of the endothelium include protection of the underlying vascular smooth muscle cells via the negatively charged glycocalyx, as well as junctional proteins. The junctional proteins permit the passage of water and small molecules into the cell while larger molecules (i.e., albumin) rely on caveola-vesicle transport, as depicted in Figure 2-4 (Smith and Fernhall 2011). The endothelium also regulates the aggregation of platelets and accumulation of other adhering molecules. The luminal surface of the endothelium is non-thrombogenic and anticoagulant, which enables smooth blood flow within an artery.

However, during vascular injury, pro-thrombogenic macromolecules located in the basal lamina are released to initiate cell aggregation, a pro-inflammatory response (Félétou 2011). Altogether, a functioning endothelium is vital for regulation of lumen diameter and blood flow during periods of exercise and (in)activity.

The primary mechanism responsible for endothelial NO production is laminar shear stress, which is detected by mechanoreceptors within (i.e., integrins) and along the luminal surface of the cell (i.e., glycocalyx), as shown in Figure 2-4. This stimulus initiates NO production within the endothelial cell by increasing intracellular calcium (Ca^{2+}) concentrations to subsequently activate endothelial nitric oxide synthase (eNOS), an important enzyme responsible for facilitating the conversion of the amino acid L-arginine to NO and L-citrulline, see Figure 2-5 (Davies 1995; Harrison et al. 2006). Diffusion of NO into the underlying VSM cells activates guanylate cyclase, increasing concentrations of the second messenger cyclic guanosine monophosphate (cGMP) that facilitates the efflux of Ca^{2+}. This reduced intracellular concentration of Ca^{2+} increases activity of the enzyme myosin light-chain phosphatase, which removes the phosphate group from the myosin light chain resulting in the detachment of myosin from actin (i.e., reduced cross-bridge formation) and causes VSM relaxation (Chen et al. 2000; Harrison et al. 2006; Sandoo et al. 2010; Chatterjee 2018). Although NO is the main vasodilator produced in response to increases in shear stress, the endothelium also produces other vasodilators such as prostacyclin and endothelium-derived hyperpolarizing factor. These vasodilators become activated when other blood borne chemicals (e.g. acetylcholine, bradykinin, substance P, ADP or ATP) bind with receptors on the endothelial cell surface, which further contribute to VSM relaxation. For an overall depiction of these mechanisms, refer to Figure 2-5.

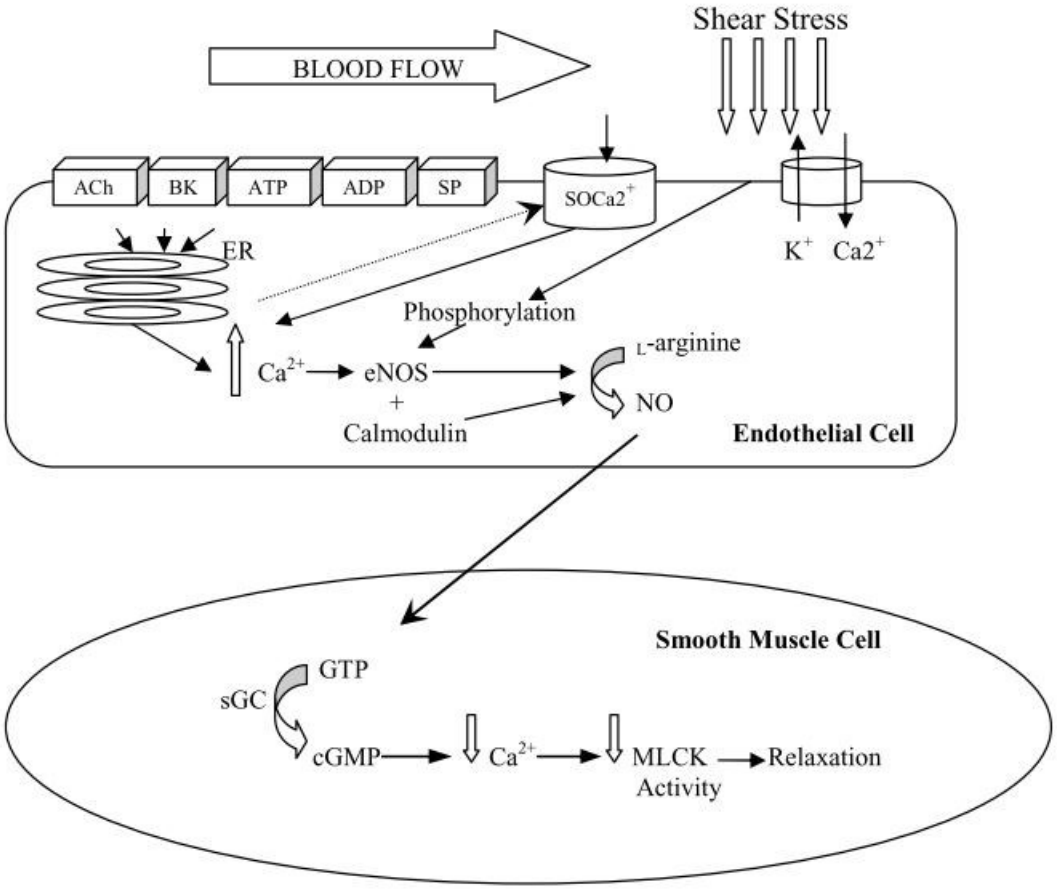

Figure 2-5: *Endothelial nitric oxide (NO) production pathways. An increase in blood flow (shear stress) results in the influx of calcium (Ca^{2+}) into the endothelium via K+/Ca^{2+}channels, release from the endoplasmic reticulum (ER), and from extracellular stores ($SOCa^{2+}$). This initiates endothelial nitric oxide synthase (eNOS) phosphorylation and subsequent conversion of the amino acid L-Arginine into NO. Increases in Ca^{2+}can also be achieved via NO agonists such as acetylcholine (ACh), bradykinin (BK), adenosine tri- and di-phosphate (ATP; ADP), and substance P (SP), all of which activate eNOS. Once synthesized, NO diffuses into the vascular smooth muscle (VSM) where it binds to soluble guanylyl cyclase (sGC), facilitating the conversion of guanosine tri-phosphate (GTP) to cyclic guanosine-3' 5-monophosphate (cGMP). cGMP initiates the efflux of Ca^{2+} within the VSM, reducing the activity of myosin light chain kinase (MLCK) and activating the myosin light chain phosphatase (not pictured), which causes the detachment of the actin-myosin complex, causing relaxation* (Sandoo et al. 2010).

When the VSM is not exposed to NO or other vasodilatory substances, pre-existing cGMP concentrations are relatively low, consequently increasing intracellular Ca^{2+} concentration. The heightened levels of Ca^{2+} reacts with a calcium-modulating protein

(Calmodulin) to activate myosin light chain kinase (MLCK), which phosphorylates the myosin head to cause re-attachment of the myosin head to the actin filaments to produce contraction of the VSM (Smith and Fernhall 2010b). A diagram of this interaction is outlined in Figure 2-6.

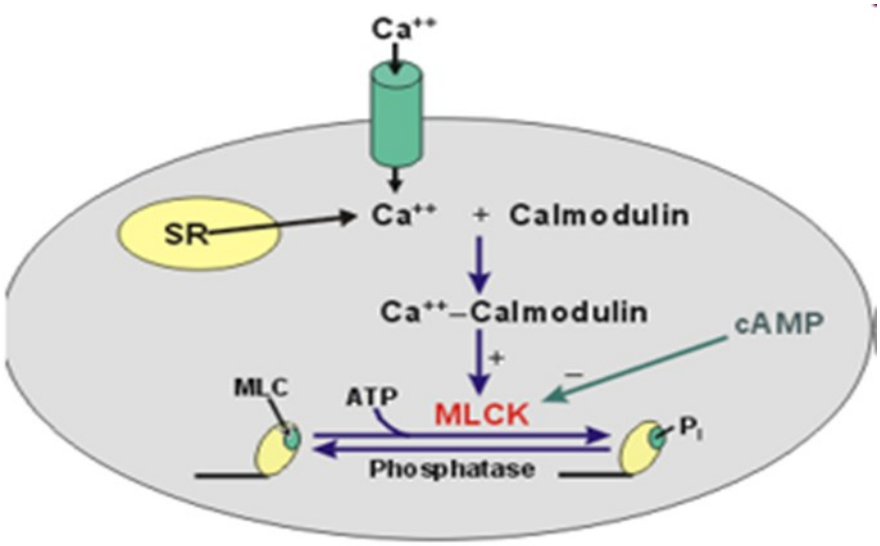

Figure 2-6: *Contraction of the vascular smooth muscle (VSM) via myosin light-chain kinase (MLCK). When calcium (Ca^{++}) concentrations increase in the VSM cell through Ca^{++} channels or from the sarcoplasmic reticulum (SR), Ca^{++} binds with Calmodulin and activates myosin light chain kinase (MLCK), the enzyme responsible for facilitating the binding of actin filaments to myosin heads resulting in contraction of the VSM. Conversely, when Ca^{++} concentrations decrease, myosin light chain (MLC) phosphatase activity results in the detachment of the actin-myosin complex causing VSM relaxation. Cyclic adenosine monophosphate (cAMP), formed via prostaglandin pathways (not pictured) can also inhibit the activity of MLCK, causing further relaxation. ATP, adenosine triphosphate; P_i, inorganic phosphate*

The bioavailability of NO also depends on the amount of oxidative stress within the endothelial cells. Endothelial dysfunction, (i.e., reduced NO bioavailability) has been linked to increased oxidative stress (Taddei et al. 2001) and diminishes the production of NO. Specifically, oxidative stress is associated with high levels of superoxide radicals (O_2^-). When high concentrations of O_2^- are present within the cell, NO interacts with O_2^- and produces peroxynitrate ($ONOO^-$), which prevents NO from activating VSM relaxation (Smith and Fernhall

2011), as depicted in Figure 2-7. Encouragingly, engaging in regular physical activity reduces oxidative stress and preserves endothelial function (Taddei et al. 2000; Seals et al. 2009)

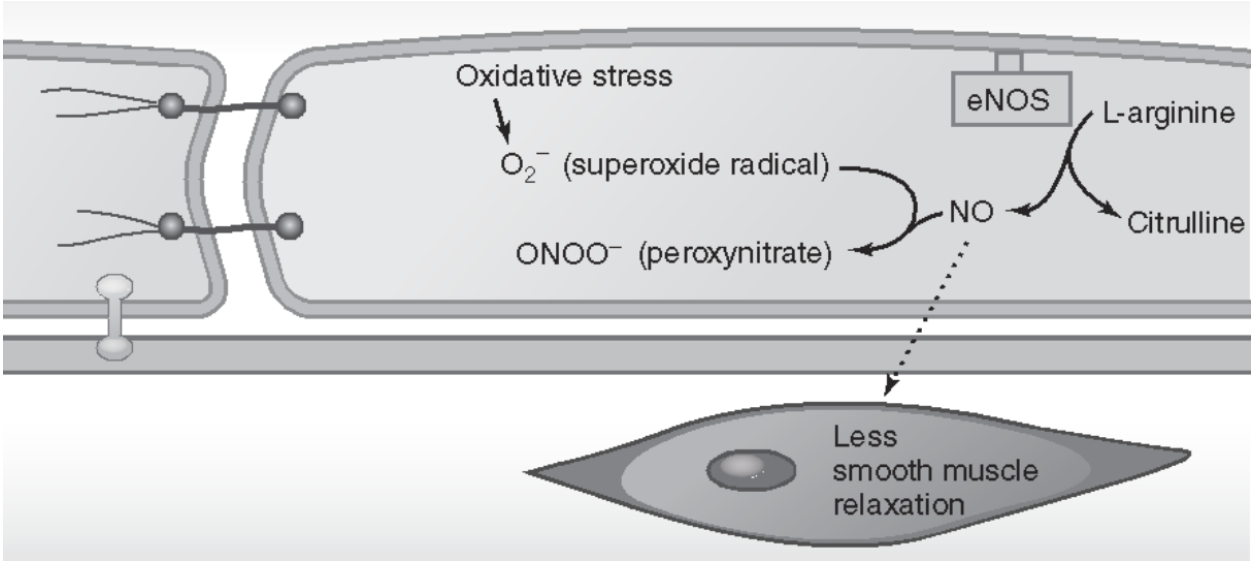

Figure 2-7: *Nitric oxide (NO) acts to initiate vascular smooth muscle (VSM) relaxation via activation of the enzyme endothelial nitric oxide synthase (eNOS). When high levels of superoxide radicals (O_2^-) are present within the cell as a result from increased oxidative stress, it interacts with NO and produces peroxynitrate ($ONOO^-$). This prevents the NO-mediated VSM relaxation* (Smith and Fernhall 2011).

2.3 Assessment of Peripheral Arterial Endothelial Function

2.3.1 Flow-Mediated Dilation Test

Atherosclerosis is preceded by endothelial dysfunction (Sitia et al. 2010). Assessment of conduit artery endothelial function can be performed via the ultrasound-based, flow-mediated dilation (FMD) test, originally utilized by Celermajer and colleagues (Celermajer et al. 1992). The FMD test is highly reproducible when performed according to published guidelines (Greyling et al. 2016; Thijssen et al. 2019) and serves as an index of NO bioavailability (Joannides et al. 1995; Kooijman et al. 2008; Green et al. 2014). The FMD test requires duplex ultrasonography, which involves simultaneous recording of artery lumen diameter and red blood

cell velocity (RBCV) via brightness-mode (B-mode) and pulsed-wave Doppler, respectively (Thijssen et al. 2011a), as depicted in Figure 2-8.

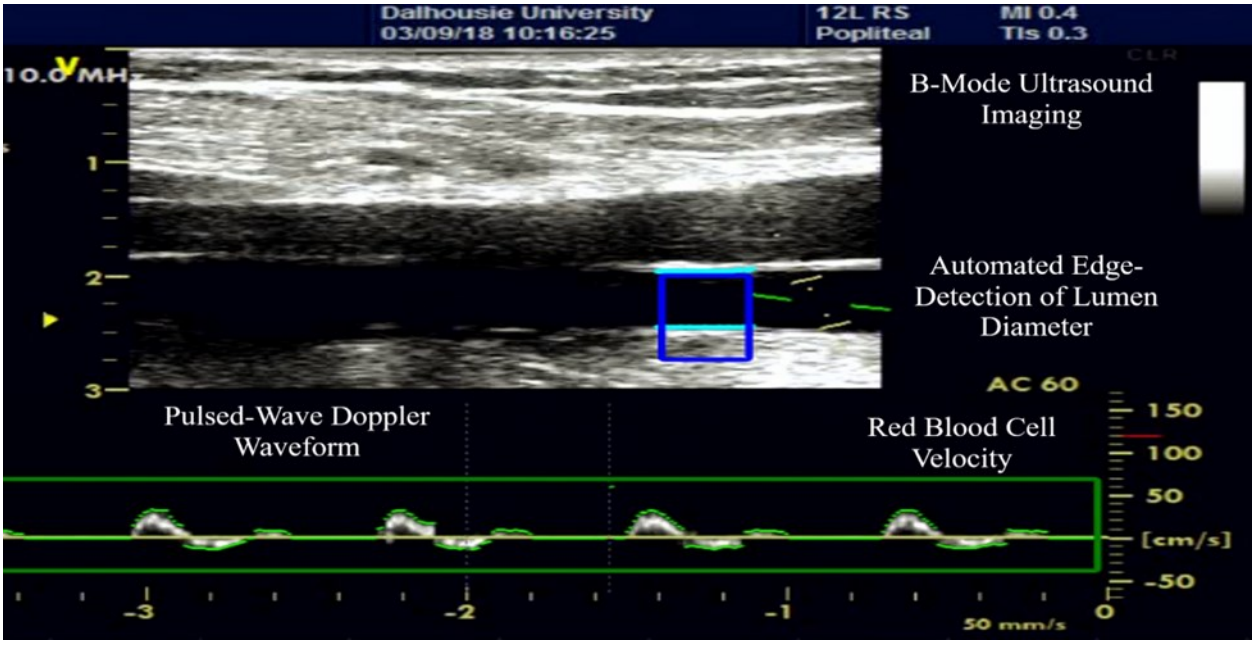

Figure 2-8: *A representative example of high-resolution duplex ultrasound imaging using Brightness-mode imaging and pulsed-wave Doppler waveforms. The dark blue box indicates the region of interest where the light blue horizontal lines represent the automated edge-detection of lumen diameter. The green and yellow lines within the artery are the angle of correction, and sample volume, respectively, where red blood cell velocity (RBCV) is recorded. Detection of RBCV occurs via automated tracking of the pulsed-wave Doppler waveform (within green rectangle).*

To perform duplex ultrasonography, a multi-frequency linear array transducer containing two arrays of piezoelectric crystals is placed on the surface of the skin above 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 RBCV, respectively. The brachial and popliteal arteries often run parallel to the skin. An optimal image is achieved when the probe is perpendicular to the artery of interest such that the signals are bisecting the vessel at an insonation angle of 90º (Harris et al. 2010). Unfortunately, the measurement of RBCV achieves

an optimal signal at 0º as per the Doppler equation (fD = [(2 fo•v) / c] × cos θ). Specifically, the signal is strongest when the cosine θ = 0 (i.e., 1). Therefore, to ensure that both a quality image is maintained and that valid RBCV signals are achieved, an insonation angle of ≤60º is recommended (Thijssen et al. 2019). Previous work has demonstrated that increasing the insonation angle ≥60º introduces a large degree of error, as cosine θ decreases markedly with larger angles (Harris et al. 2010). It was demonstrated that RBCV values were not different when insonation angles of 40º, 50º and 60º were implemented, but were significantly larger at 70º and 80º (Harris et al. 2010) (See Table 2-1). However, obtaining a 60º via manual movements of the probe is difficult for a sonographer to achieve. Therefore, a 'steer' angle function in the ultrasound machine is utilized whereby transmitting and received signals are 'steered'. With the ultrasound signal 'steered' by 30º, the recommended 60º angle can be achieved. For a depiction, see Figure 2-9.

Table 2-1: *An example of how red blood cell velocity (RBCV) changes depending on the insonation angle selected by the sonographer. Published guidelines recommend an angle of ≤60º* (Thijssen et al. 2019)*. Table obtained from (Harris et al. 2010).*

Evidence of alterations in blood velocity and blood flow as a consequence of insonation angle

				Theoretical		
Insonation Angle	**Velocity (cm/s)**	**Blood Flow (ml/min)**	**% Δ from 60°**	**FMD (%)**	**Shear (s^{-1})**	**FMD/Shear**
40°	3.52±0.46	24.8±8.3	−49%	7.0	33.52	0.21
50°	4.24±0.57	29.5±9.9	−25%	7.0	40.38	0.17
60°	5.27±0.50	36.7±11.1	0%	7.0	50.19	0.14
70°	7.97±1.07*	55.5±18.7	51%	7.0	75.90	0.09
80°	15.69±2.12*	109.4±36.8*	197%	7.0	149.43	0.05

* Significant ($p < 0.05$) from 60°. Values are mean ±SD.

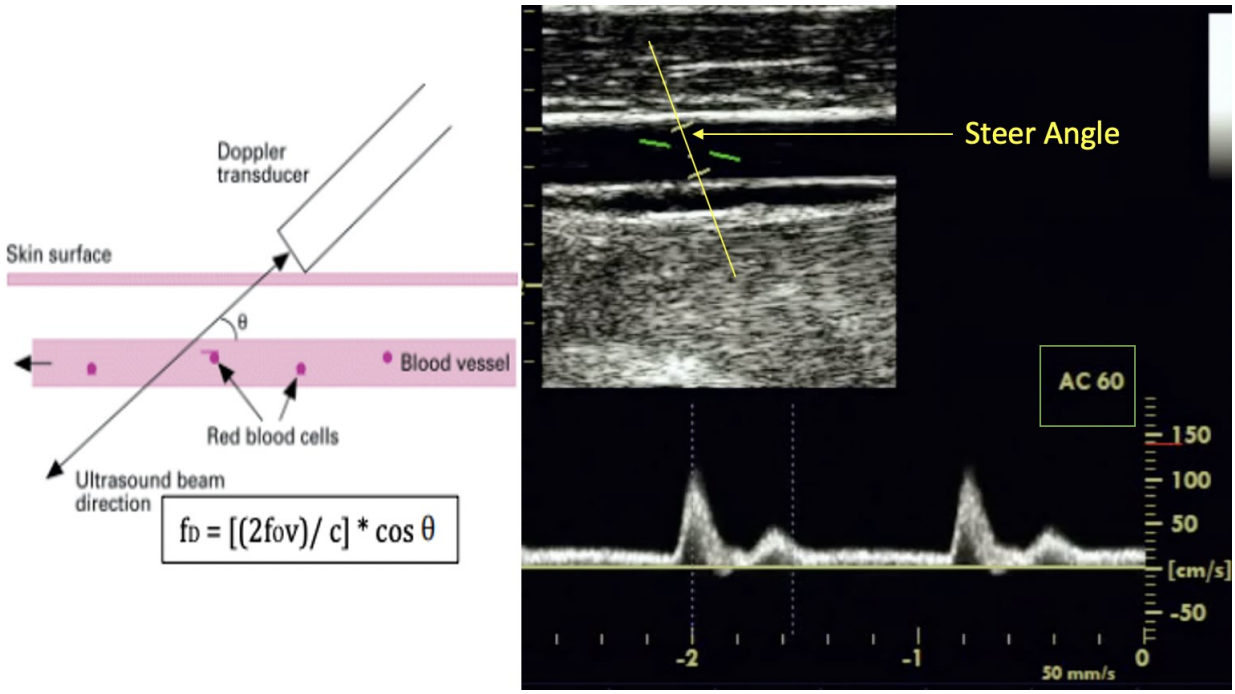

Figure 2-9: *An insonation angle of 60° permits an optimal Doppler signal in a blood vessel parallel to the probe (i.e., perpendicular, 90° to the vessel). In the Doppler equation {[(2 • fo • V) ÷ C] • cos θ}, a cosine of 90° = 0. This results in an unreliable Doppler signal. Blood cell velocity (RBCV) differs greatly at angles 70-80°* (Harris et al. 2010)*, therefore the angle is corrected to 60°* (Thijssen et al. 2019)*. This is technically challenging for the sonographer, so a steering angle of 30° (yellow line) is implemented to ensure the probe can be held approximately parallel to the vessel while still achieving the insonation angle of 60°. °, degrees; cos, cosine; fo, frequency of transmitted beam; v, velocity of red blood cells; c, speed of sound in tissue; US, ultrasound probe.*

The FMD test is 12 minutes in duration and begins with 2 minutes of baseline imaging to obtain resting RBCV and lumen diameter. This is followed by 5 minutes of distal ischemia induced by inflating a pneumatic pressure-cuff secured to the forearm (BA-FMD) or calf (POP-FMD) to supra-systolic pressures (250 mmHg). The ultrasonographer continues to measure lumen diameter and RBCV throughout this ischemic period. Following the ischemic period, the pressure cuff is instantly deflated, which elicits hyperemia (i.e., increase in blood flow and shear stress) causing NO release from the endothelial cells. The corresponding vasodilation occurs

while the operator continually measures artery lumen diameter and RBCV for an additional 5 minutes post-cuff deflation.

Flow-mediated dilation is often quantified as the maximum increase in diameter (compared to baseline) and expressed in both absolute (i.e., mm) and relative (%) terms. To minimize inter-individual variation in shear stress profiles, it has been recommended that FMD be normalized to the shear rate area under the curve (SR_{AUC}) (Pyke and Tschakovsky 2007; Padilla et al. 2008) if it correlates with FMD (Atkinson et al. 2009), as shown in Figure 2-10.

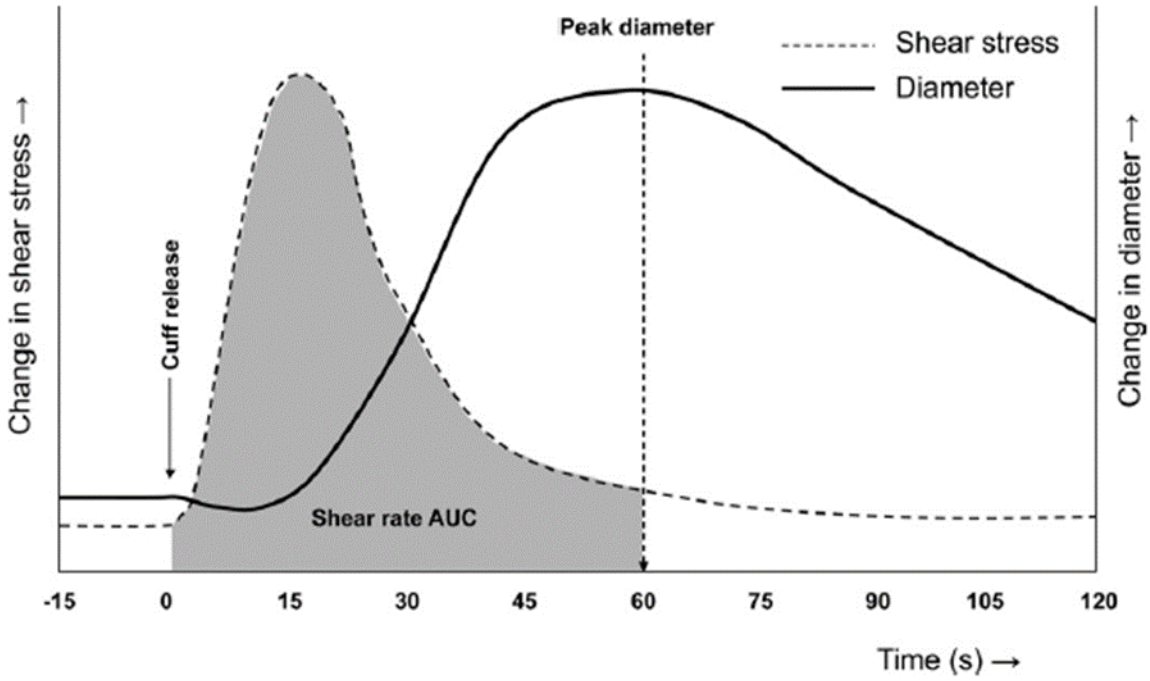

Figure 2-10: *Time course of the FMD response. At the group-level, it has been recommended that the FMD response be normalized to the shear rate area under the curve (SR_{AUC}) stimulus (i.e., the time between distal cuff deflation to when peak dilation occurred) if SR_{AUC} correlates with FMD.*

Recent studies have also suggested statistically correcting for inter-individual differences in baseline lumen diameter (Thijssen et al. 2008b) via allometric scaling (Atkinson and Batterham 2013). Specifically, the calculation of relative FMD (% baseline) is mathematically biased whereby smaller arteries exhibit greater relative increases (from baseline) compared to

larger arteries for a given absolute increase in diameter. This challenge may be overcome by the method introduced by Atkinson and Batterham (2013) when the slope of the relationship between baseline and peak diameters differ from 1 (Atkinson 2013; Atkinson et al. 2013). This strategy is employed by performing an analysis of covariance using the absolute change in the logarithmically transformed peak and baseline diameters as the dependent variable and logarithmically transformed baseline diameter as the covariate. Calculated estimated means from the model are covariate-adjusted, back transformed (i.e., power of 10), and converted into a percentage by subtracting '1' and multiplying by 100. However, pitfalls to this approach exist as individual data points cannot be calculated (Thijssen et al. 2019) and the current difficulty associated with comparing results with previous published work that have not employed this approach. For the present study, regression analysis will be performed to confirm whether the data meets criteria for allometric scaling (Atkinson and Batterham 2013) and normalization of FMD to SR_{AUC} (Pyke and Tschakovsky 2007).

2.3.2 Sex Differences in Flow-Mediated Dilation Responses

2.3.2.1 Female Hormones

Previous investigations have reported enhanced relative BA-FMD in pre-menopausal females compared to males (Juonala et al. 2008; Nishiyama et al. 2008; Yao et al. 2014; Holder et al. 2019). However, this has not been consistently corroborated (Hashimoto et al. 1995; Harris et al. 2012; Shenouda et al. 2018; Tremblay et al. 2019) (See Figure 2-11). In the limited body of literature investigating sex differences in POP-FMD, previous studies have demonstrated similar responses between young males and females (Nishiyama et al. 2008; Vranish et al. 2017; O'Brien et al. 2019). The majority of studies that have reported greater FMD responses in pre-menopausal females have proposed that 17-ß Estradiol (E2), the most common form of estrogen

found within the body (Chakrabarti et al. 2014) may play an important role (Yao et al. 2014; Green et al. 2016; Vranish et al. 2017). Indeed, seminal work has demonstrated that the differential pattern of age-related declines in BA-FMD between males and females are E2-mediated. Specifically, Celermajer et al., (1994) demonstrated that males exhibited reductions in FMD in their early 40's, whereas females experience this reduction in their 50's, coinciding with menopause (Celermajer et al. 1994). Menopause is classically characterized as a period of time where E2 production halts (Moreau 2019). The benefits of E2 on the endothelium in both animal and human studies have been supported in multiple reviews elucidating the E2 mechanisms that influence the vasculature (Mendelson and Karas 1999; White 2002; Chakrabarti et al. 2014; Green et al. 2016; Usselman et al. 2016; Stanhewicz et al. 2018). 17-ß Estradiol promotes an antiatherogenic environment within the endothelium via various mechanisms involving ligand bound α and ß estrogen receptors located inside the cell, and on the membrane of the endothelial cells (Mendelsohn 2002). 17-ß Estradiol binding to these receptors increases NO bioavailability, reduces ET-1 concentrations, and augments antioxidant and anti-inflammatory ability (Mendelson and Karas 1999; White 2002; Chakrabarti et al. 2014). As FMD is highly NO-mediated (Green et al. 2014), particular relevance surrounds the apparent enhanced ability of E2 to increase NO bioavailability. Through genomic and non-genomic pathways, E2 increases NO bioavailability primarily via phosphorylation of eNOS and enhanced expression of eNOS proteins (White 2002; Chakrabarti et al. 2014). Despite the well-developed base of literature surrounding the beneficial effects of E2 on NO bioavailability, a recent report suggests that E2 may not affect BA-FMD in pre-menopausal females (Shenouda et al. 2018). In contrast to seminal work in the field (Hashimoto et al. 1995), Shenouda et al., (2018) demonstrated no differences in BA-FMD across the early-follicular phase (i.e., nadir concentrations of E2) and

late-follicular/mid-luteal phases of the menstrual cycle (i.e., peak concentrations of E2) (Shenouda et al. 2018). Regardless, current FMD guidelines recommend that females be tested in the early-follicular phase of the menstrual cycle to minimize the potential effects of E2 on the endothelium (Thijssen et al. 2019). These recommendations have been followed in previous studies investigating sex differences in FMD, suggesting other factors (i.e., structural differences) may be involved.

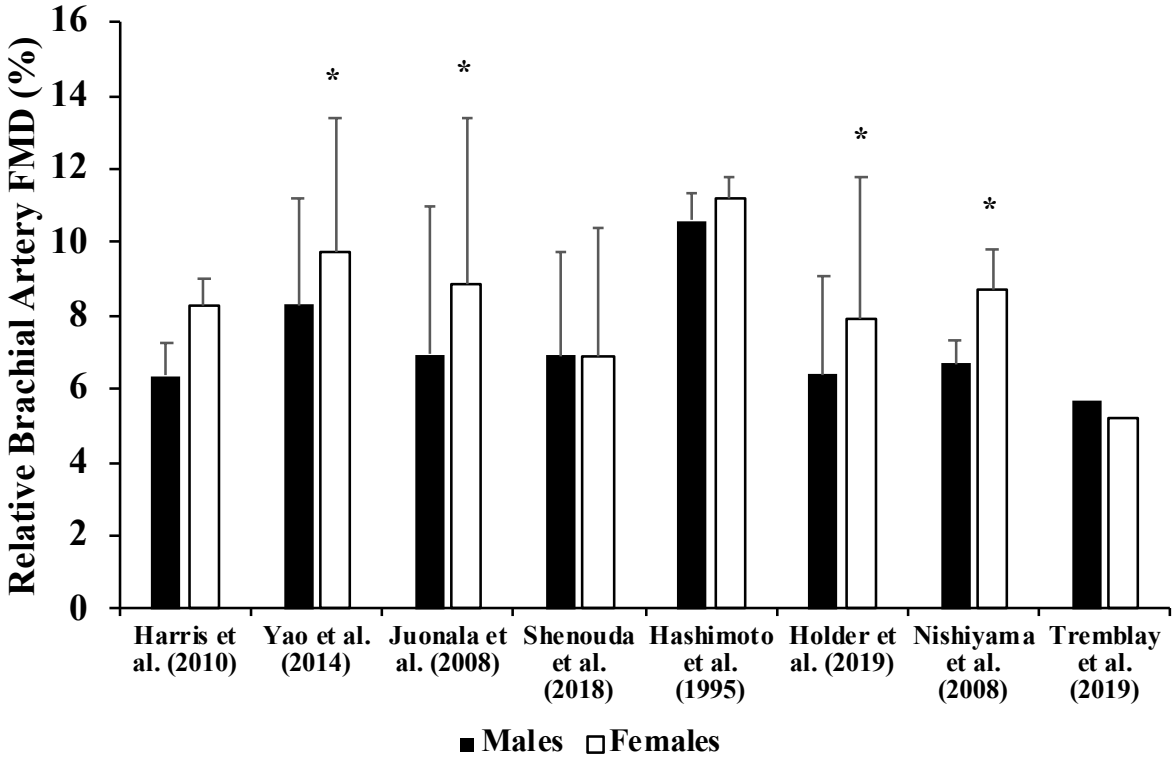

Figure 2-11: *Comparison of previous reports that have investigated brachial artery relative flow-mediated dilation. Figure is adapted from data reported within the respective published articles. Tremblay et al. (2019) did not provide standard deviation values. FMD, flow-mediated dilation.*

2.3.2.2 Differences in Arterial Structure

It is well established that females have smaller arteries than age-matched males, (Juonala et al. 2008; Nishiyama et al. 2008; Harris et al. 2012; Shenouda et al. 2018; Holder et al. 2019)

which may inflate FMD responses via mathematical bias (see allometric scaling discussed above). In two studies that statistically controlled for sex differences in baseline diameter, males demonstrated enhanced BA-FMD (Juonala et al. 2008; Shenouda et al. 2018). Juonala and colleagues (2008) measured BA-FMD in 1018 males and 1247 pre-menopausal females as part of the Cardiovascular Risk in Young Finns Study spanning from 1980 to 2001. With ages ranging from 24 – 39 years in both groups, females exhibited greater relative BA-FMD compared to males, but this outcome was reversed when the smaller baseline diameter in females versus males was corrected for. Juonala et al. (2008) also compared BA-FMD in 224 pairs of males and females with equivalent baseline diameters and found that males exhibited a greater BA-FMD response compared to females. A similar outcome was reported recently where allometrically scaled BA-FMD responses were greater in young males compared to both naturally menstruating young females and young females using oral contraceptives (Shenouda et al. 2018). Furthermore, the results from Shenouda et al. (2018) suggested that BA-FMD was unaffected in young females who were exposed to high levels of endogenous (naturally menstruating) or exogenous (oral contraceptive users) E2. In addition to smaller diameters, one study suggested that females have a greater wall thickness to lumen diameter ratio compared to age-matched males (Green et al. 2010). The 'Folkow Effect', a term coined by Thijssen et al., (2011), is related to findings from a study indicating that arteries with a greater wall-lumen ratio exert a hyper-responsiveness to vasoactive stimuli (Folkow 1978). Specifically, a larger VSM relative to lumen diameter will have a greater influence on the change in diameter during phases of contraction and relaxation. Smaller arteries such as the BA versus POP (or females versus males) typically have greater VSM content relative to their respective lumen diameter size (Green et al. 2010; Thijssen et al. 2011b). Importantly, the VSM thickness to lumen diameter

ratio is positively correlated with FMD responses even after correcting for the SR_{AUC} stimulus (Thijssen et al. 2011b). Though wall thickness is not being measured in the present study, potential sex differences related to wall-lumen ratio should be considered, given its strong, inverse relationship with baseline diameter (Thijssen et al. 2011b).

2.3.3 Between-Limb Arterial Heterogeneity

The BA is the most common artery to measure FMD (Yan et al. 2005; Soga et al. 2008; Sitia et al. 2010) due to its ability to predict future CVD events (Inaba et al. 2010; Green et al. 2011; Matsuzawa et al. 2015) and strong correlation with coronary artery function (Broxterman et al. 2019), despite being a relatively atherosclerosis-resistant artery (Thijssen et al. 2012). Lower-limb arteries such as the POP are less studied, but may be equally important due to an increased susceptibility for atherosclerotic development (Debasso et al. 2004; Aboyans et al. 2011). There are important limb differences in the FMD response between the POP and the BA (Thijssen et al. 2008b). The FMD response is highly and negatively correlated with baseline diameter, with larger arteries such as the POP eliciting an attenuated ability to dilate in response to a reactive hyperaemia despite similar shear rates compared to the smaller BA diameter (Thijssen et al. 2008b). These differences have been related to lower-limb arteries having greater gravity-induced hydrostatic pressure during upright postures such as standing and walking (Nishiyama et al. 2007). The POP also has a smaller wall-to-lumen ratio (Green et al. 2010; Thijssen et al. 2011b) , thus lessening the vasodilatory responses as per the “Folkow Effect”. The POP also experiences functional increases in blood flow due to repetitive contractions during locomotion, which may make the lower-limbs conditioned to shear stress such that they have a lower dilatory response to the hyperemic stimulus induced by the FMD test (Proctor and Newcomer 2006). As such, it can be expected that BA-FMD may elicit greater responses than

the POP-FMD in the present study (Thijssen et al. 2008b). To our knowledge, only one study has examined sex differences in FMD within the POP and BA simultaneously (Nishiyama et al. 2008). Specifically, relative BA-FMD was greater in young, pre-menopausal females versus males. However, they did not observe sex differences in relative POP-FMD (Nishiyama et al. 2008).

2.3.4 Nitroglycerin-Mediated Dilation

Endothelial-independent vasodilation is reflective of the capacity of the VSM to dilate in response to an extrinsic vasodilator and gives an overall indicator of VSM sensitivity (Smith and Fernhall 2011). Nitroglycerin acts as an NO donor and is typically administered sublingually (i.e., under the tongue) (Chen et al. 2002). Nitroglycerin then directly diffuses into the VSM, which facilitates the activation of soluble guanylate cyclase, increasing cGMP, decreases intracellular Ca^{2+} concentration and results in vasodilation (Kleschyov et al. 2003). This nitroglycerin-mediated dilation test will provide an indication as to whether or not potential sex differences in endothelial-dependent vasodilation are due to NO bioavailability *per* se or the ability of the VSM to relax in response to an increased production of NO.

2.3.4.1 Sex Differences in Nitroglycerin-Mediated Dilation (NMD)

Of the limited studies investigating sex differences in BA-NMD, similar responses have been observed between young males and females (Hashimoto et al. 1995; Black et al. 2009; Shenouda et al. 2018). This is somewhat surprising considering the results from Hashimoto et al., (1995) and Black et al., (2009) failed to consider baseline diameter. In addition to FMD, BA-NMD has been shown to be inversely related to baseline diameter in young adults, which was also true in POP-NMD (Thijssen et al. 2008b). In the only study controlling for differences in baseline diameter between males and females, Shenouda et al., (2018) demonstrated similar BA-

NMD in males and females using oral contraceptives, but was lower in naturally menstruating females compared to both groups (Shenouda et al. 2018). For the present study, attention to potential differences in POP-NMD responses between females who use oral contraceptives versus naturally menstruating females may be necessary.

2.4 Physical Activity and Sedentary Behaviour Monitoring

Achieving the Canadian aerobic moderate-vigorous physical activity (MVPA) guidelines can reduce the risk of premature death, CVD and many others conditions (Tremblay et al. 2011). Conversely, physical inactivity acutely impairs POP-FMD responses (Padilla and Fadel 2017) and is associated with an increased risk for premature death and CVD (Warburton et al. 2010). With this information, scientists have been using activity monitoring both subjectively (Rakobowchuk et al. 2008; Shenouda et al. 2018) and objectively (Hopkins et al. 2011; O'Brien et al. 2018b) to determine physical activity patterns and how they influence endothelial function. However, caution should be used when interpreting studies that solely use subjectively measured physical activity levels as the likelihood for error is higher than objectively-measured devices (Ahn et al. 2015; Urda et al. 2017).

Sedentary behaviour is classified as activities that involve sitting or lying with an energy expenditure ≤1.5 metabolic equivalents of task (METs) (Dunstan et al. 2012). The present study will utilize the PiezoRxD® and ActivPAL™ to measure levels of physical activity and sedentary time, respectively. The PiezoRxD® uses step rate thresholds to categorize intensity-related physical activity and has been validated in both laboratory- (Saunders et al. 2014) and free living-based settings (O'Brien et al. 2018c). This device is particularly useful when individualizing step-rate thresholds as these can be adjusted depending on the height of an individual (O'Brien et al. 2018d). For example, based on the results by O'Brien et al. (2018d), a

person with a stature of 180 cm would have moderate and vigorous physical activity thresholds of 95 and 125 steps•minute^{-1}, respectively. However, someone who is 170 cm would have thresholds that correspond to 100 and 130 steps•minute^{-1}.

The activPAL^{TM} accelerometer is a thigh-worn device that can determine the amount of time spent engaged in sedentary and standing postures by measuring static acceleration (Edwardson et al. 2017). Built in algorithms enable the device to differentiate sitting/lying versus upright positions. When someone is standing, the device would be vertical and thus document that as time spent upright. When sitting, the device would be horizontal and is considered as time spent sitting or lying. Information regarding transitions between these postures are available and have been validated numerous times when worn on the mid-thigh (Edwardson et al. 2017). Conveniently, the activPAL^{TM} can be worn continuously during water activities (i.e., bathing, swimming) when water-proofed using an adhesive medical dressing, and while sleeping. See Figure 2-12 for a depiction of the two devices.

Figure 2-12: *The activPAL^{TM} and PiezoRx®, placed beside a one dollar coin for size reference. The activPAL^{TM} will be placed anteriorly on the mid-thigh. The PiezoRx® will be worn on the waist or belt in accordance to manufacturer recommendations.*

2.5 Influence of Habitual Behaviours on Peripheral Vasodilator Health

Flow-mediated dilation acts a surrogate measure of NO-bioavailability (Green et al. 2014) and regular physical activity improves NO-bioavailability via increased phosphorylation of eNOS (Nosarev et al. 2015). Investigations into the effects of objectively measured physical activity and sedentary behaviour patterns on FMD is limited. Boyle et al., (2013) measured BA- and POP-FMD in young, healthy adults at baseline and after 5 days of reduced daily step counts (i.e., $\geq$ 10 000 steps•day^{-1} $\rightarrow$ $\leq$ 5 000 steps•day^{-1}) where a decrement in POP-FMD was observed. They observed no difference in BA-FMD between baseline and the 5 day reduced physical activity intervention suggesting that the decrease in regular shear stress accomplished during locomotion is more important in the lower- compared to upper-limb arterial endothelial function (Boyle et al. 2013), as depicted in Figure 2-13. This hypothesis was tested in a follow-up study that repeated the reduced physical activity intervention with the addition of inducing brief periods of increased shear stress to one lower-limb via a heated foot bath 3 times per day, with the contralateral limb acting as an internal control (Teixeira et al. 2017). Limb heating can improve vascular function via shear stress-mediated mechanisms (Romero et al. 2017), driven largely by increases in vasodilation of the skin microcirculation (Edholm et al. 1956). Following 5 days of reduced activity, POP-FMD in the control leg (no heat) was attenuated but the experimental leg experienced no reductions in POP-FMD (Teixeira et al. 2017).

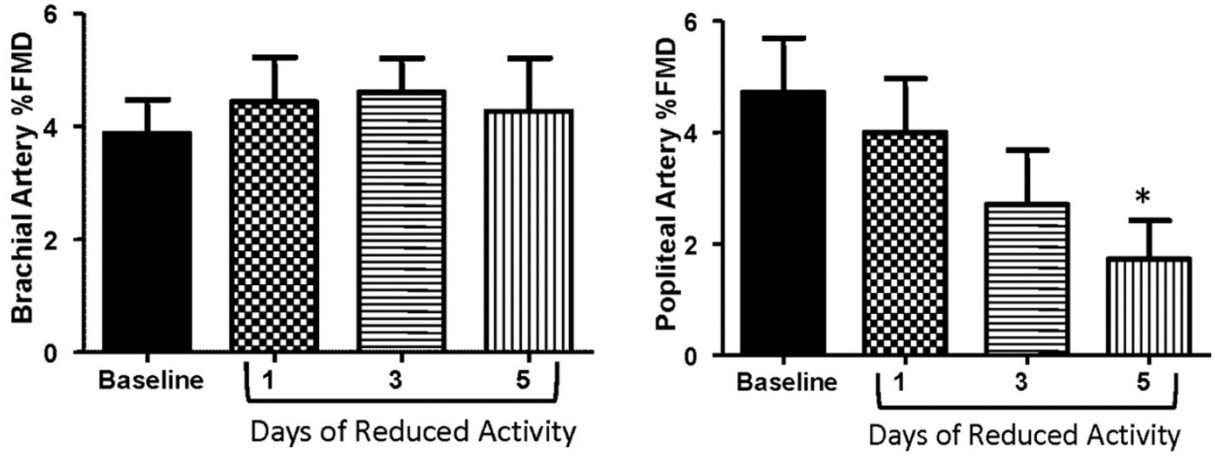

Figure 2-13: *Boyle et al. (2013) recruited healthy young males who typically take approximately 10 000 steps•day^{-1} and reduced their daily step counts to approximately 5 000 steps•day^{-1} while measuring endothelial function via the flow-mediated dilation technique (FMD) throughout the intervention. As indicated above, relative brachial artery (BA-FMD) was unaffected by 5 days of reduced steps count whereas popliteal (POP-FMD) was markedly reduced following the 5th day of reduced daily steps count. This highlights the effect of physical activity on lower-, but not upper-limb FMD (Boyle et al. 2013).*

In children, investigators sought to understand the relationship between the changes in BA-FMD and physical activity levels during seasonal transitions (i.e., Summer – Fall) (Hopkins et al. 2011). They observed a concomitant decrease in BA-FMD along with physical activity levels between Summer and Fall. Further, they also discovered a direct relationship between increases in BA-FMD (%) and daily vigorous-intensity physical activity (VPA), which suggests that higher intensity physical activity levels may have a positive influence on upper-limb vasodilatory health (Hopkins et al. 2011). However, reductions in lower-intensity activities such as stepping failed to influence BA-FMD in adults (Boyle et al. 2013). There is currently a paucity of information regarding the relationship between FMD with objectively measured, habitual PA and sedentary levels in young, healthy adults. This is important as many young adults are students, who are at a higher risk of progressively decreasing their PA levels and

increasing their sedentary time over the course of a 4-year degree (Smetaniuk et al. 2017). In an acute setting, physical inactivity results in reduced POP- (Padilla and Fadel 2017) but not BA-FMD (Thosar et al. 2014) in young adults. There are disparate results from studies investigating sex differences in response to acute sitting bouts with one study demonstrating attenuated decrements in POP-FMD in young females (Vranish et al. 2017) and no differences between males and females in sitting-induced impairments in POP-FMD (O'Brien et al. 2019). To date, no study has investigated the influence of habitual sedentary time on BA- and POP-FMD concomitantly, and if this measure modulates sex differences in FMD.

2.6 Influence of Aerobic Fitness on Peripheral Vasodilatory Health

Reduced aerobic fitness is associated with increased cardiovascular risk (Shiroma and Lee 2010). Since poor BA-FMD serves as an indicator for future cardiovascular events (Green et al. 2011), it is not surprising that many studies have investigated the relationship between aerobic fitness (as measured via peak oxygen consumption during incremental maximal exercise tests) and BA-FMD (Veves et al. 1997; Trigona et al. 2010; Bell et al. 2017; O'Brien et al. 2018a). The well-established, positive relationship between aerobic fitness and BA-FMD is mostly limited to studies investigating older adults and clinical populations (Montero 2014; Montero et al. 2014). A recent study performed in young males corroborated previous findings that BA-FMD was not related to aerobic fitness (Bell et al. 2017). A meta-analysis performed by Montero et al., (2014) found that older master athletes exhibited superior BA-FMD compared to controls. A relationship that was absent in young, athletically trained males versus controls (Montero et al. 2014).

While relationships between aerobic fitness and BA-FMD are not anticipated in the present study in young adults, there is a paucity of research investigating the influence of aerobic fitness

on POP-FMD. The POP is exposed to large increases in blood flow during activities such as cycling and jogging. As such, there may be a greater likelihood that aerobic fitness is related to POP-FMD due to greater exposure to augmented blood flow/shear stress patterns during these commonly utilized lower-limb modes of aerobic exercise training. However, the primary purpose of including an objective measure of aerobic fitness was to assess whether inter-individual differences in aerobic fitness could help explain potential sex differences in FMD.

2.7 Research Question and Hypothesis

Peripheral artery vasodilator health is viewed as an important contributor to overall cardiovascular health and may be different between arterial beds in the upper- versus lower-limb. Females exhibit differential reductions in endothelial health measured via FMD than males, whereby males exhibit reductions in FMD more than a decade earlier than females. Contradicting evidence between studies suggest that pre-menopausal females may exhibit superior BA-FMD, but not POP-FMD compared to age-matched males. These disparities may be due to the lack of studies not controlling for known sex differences in baseline diameter when performing the relative FMD calculation, sex differences due to female hormones (most notably, estrogen), or inter-individual variation in aerobic fitness, habitual PA and sedentary time. As such, the purpose of this study was to investigate sex differences in BA- and POP-FMD in young, healthy adults with objectively measured aerobic fitness, habitual PA and sedentary time. It was hypothesized that after the consideration of baseline diameter, males would exhibit greater BA- and POP-FMD. Considering that the POP experiences greater fluctuations in blood flow, secondary aims included determining whether habitual PA and sedentary time, along with aerobic fitness are related to arterial health in the pooled sample.

3 Methodology

3.1 Participants

Twenty-six young, healthy, non-obese participants (13 females; See Table 4-1) were recruited via advertising posters and word of mouth. Participants provided written consent to participate in the study and were cleared for exercise testing via the Physical Activity Readiness Questionnaire Plus (PAR-Q+) (Warburton et al. 2011). The experimental design and protocols conformed to the Declaration of Helsinki and were approved by the Dalhousie Health Sciences Research Ethics Board (REB: 2018-4518; Appendix A).

3.2 Experimental Design

Participants visited the laboratory twice. Visit 1 included familiarization of the lab and all data collection procedures, anthropometric measurements (i.e., stature and weight), and resting blood pressure. Participants were eligible to participate if they were healthy (i.e., no chronic diseases, BMI <30 $kg \bullet m^{-2}$, resting blood pressure <140/<90 mmHg; Health History Questionnaire; Appendix B) and had no contraindications to exercise as determined by the PAR-Q+ (Appendix C). Females were asked to confirm that they experienced regular menstrual cycles (~28 days) and were not currently pregnant (Health History Questionnaire; Appendix B). If eligible, participants provided signed consent (Appendix D) and a research member instrumented them with the physical activity and sedentary behavior monitors (see below). Participants returned 7 days later for their second visit, which included the assessment of BA and POP arterial health, followed by an assessment of aerobic fitness. To minimize confounding factors on endothelial-dependent function, participants refrained from engaging in MVPA and the consumption of alcohol for 24 hours prior to the second visit. Additionally, participants arrived to the lab ~6-hours postprandial while avoiding the consumption of products known to acutely

influence FMD responses (e.g., caffeine, chocolate, kiwi, saturated fats, citrus fruits, folic acid and multivitamin supplements and antioxidants) at least 12-hours before the session (Thijssen et al. 2019). Females were tested in the early-follicular phase of their natural menstrual cycle (i.e., Days 1-5 following menstruation; n=5) or during the placebo phase of their combination oral contraceptive pill pack (n=5), to minimize the influence of fluctuating hormonal levels on FMD. There were also 3 female participants using intrauterine devices (IUD). All vascular assessments were performed in a thermoneutral (20-22ºC), dimly lit room.

3.3 Experimental Measurements

3.3.1 Anthropometrics and Physical Activity Monitoring

Stature and weight were measured using a stadiometer and physician's scale (Health-O-Meter, McCook IL, USA) to the nearest 0.5 cm and 0.1 kg, respectively. Participants wore a PiezoRxD® medical grade pedometer (StepsCount, Ont., Canada) and an activPAL™ accelerometer (micro 3; PAL Technologies, Glasgow, UK) for one week to assess physical activity and sedentary behaviour patterns, respectively. The PiezoRxD® was secured to the waistband on their right side, in line with the mid-thigh and used to measure daily step counts. The PiezoRxD® uses step-rate thresholds to determine time spent in light-, moderate- and vigorous- intensity physical activity. Individualized step-rate thresholds based on participant stature were selected to accurately identify physical activity intensity classification (O'Brien et al. 2018d). The activPAL™ monitor determines time spent laying/sitting and standing via its static acceleration profile. It was waterproofed by inserting the device in a finger cot then attaching to the mid-line of the right thigh, one third of the way between the hip and knee, distal to the greater trochanter via transparent medical dressing (Tegaderm™, 3M, London, ON., Canada) (Edwardson et al. 2017).

3.3.2 Resting Hemodynamics

Heart rate was determined via cardiac intervals obtained from lead II of a bipolar electrocardiogram (ECG) configuration. Beat-by-beat blood pressure was measured from the left index or middle finger using photoplethysmography (Portapres®, Finapres Medical Systems, Amsterdam, The Netherlands) and physiologically calibrated via brachial artery blood pressure measurements recorded by an automated patient vital signs monitor (Carescape v100®, General Electric Healthcare). The finger used for blood pressure recordings was height corrected to heart level using the Portapres® height correction unit. The ECG and Portapres® recordings were sampled continuously at 1000 Hz and 200 Hz, respectively (PL3508 PowerLab 8/53; ADInstruments, Sydney, Australia). All signals were displayed in real-time and analyzed offline using LabChart software (Version 8.1.16, ADInstruments, Sydney, Australia).

3.3.3 Assessment of BA and POP Endothelial-Dependent and Independent Dilation

Brachial and popliteal artery endothelial-dependent function were assessed via duplex ultrasonography using the FMD test with participants in the supine and prone positions, respectively. Following an initial 15 minute resting period, BA- and POP-FMD were performed in sequence, using a 12-MHz multi-frequency linear array probe attached to a high-resolution ultrasound system (Vivid i, General Electric Healthcare, USA). Red blood cell velocity was recorded simultaneously with arterial lumen diameter at a pulsed frequency of 5-MHz corrected using an insonation angle of 60°. The sample volume was adjusted such that it encompassed the entire lumen but did not exceed the anterior and posterior intima according to published guidelines (Thijssen et al. 2019). The BA was imaged 3-5 cm proximal to the antecubital fossa and the POP was imaged 2-3 cm proximal to the bifurcation in the popliteal fossa. A pneumatic pressure cuff attached to a rapid cuff inflation system (E20 and AG101, Hokanson®, Bellevue,

WA) was placed ~3 cm distal to the antecubital fossa and around the mid-calf (~10 cm distal to the popliteal fossa) for the BA- and POP-FMD protocols, respectively (Thijssen et al. 2019). Resting arterial lumen diameter and RBCV were measured for at least 2 minutes. While continuing to record arterial lumen diameter and RBCV, the pressure cuff was rapidly inflated to 250 mmHg for 5 minutes. Ultrasound recording continued for an additional 5 minutes following cuff deflation. To ensure resting blood flow returned to baseline levels, there was a minimum 10 minute break separating the BA- and POP-FMD tests. Following another 10 minute rest period after the POP-FMD test, popliteal endothelial-independent function was assessed by measuring the peak increase in lumen diameter following a 0.4 mg sublingual administration of nitroglycerin (POP-NMD) compared to a preceding 1 minute baseline measurement (Chen et al. 2002). Lumen diameter and RBCV were continuously measured for 10 minutes following the nitroglycerin administration.

3.3.4 Assessment of Aerobic Fitness

To assess aerobic fitness, participants performed an incremental maximal exercise test on an electronically-braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands). Peak oxygen consumption (VO_2peak) was measured via indirect calorimetry using a commercial mixing chamber-based metabolic system (TrueOne 2400®, Parvomedics Inc. Sandy, UT). Following a 5 minute warm up at 50 W, workload increased by 20 W every minute until participants were unable to continue cycling at a minimum cadence of 40 rpm. Following termination of the test, participants cooled down at a workload of 50 W for a minimum of 5 minutes. No participants achieved a plateau in VO_2 (i.e., an increase in absolute VO_2 ≤150mL/min despite an increase in workload). Therefore, the attainment of VO_2max was based upon meeting 2 of the following criteria (Howley et al. 1995) : a respiratory exchange ratio

(VCO_2:VO_2) of ≥1.10, a maximum HR ≥90% of age-predicted (age-220), and/or a maximum rating of perceived exertion on the Borg 6-20 scale of ≥18 (Borg 1998). Thirteen (100%) and 12 (92%) of the participating males and females met ≥2 of these criteria, respectively.

3.4 Data Analysis

Peak oxygen consumption was presented in relative units ($ml \cdot kg^{-1} \cdot min^{-1}$) and averaged over 15 second epochs. Full days of activity data obtained from the PiezoRxD® (i.e., ≥10 hours of wear time per day) and activPAL™ were analyzed. However, partial days (i.e., days 1 and 7) were not included for analysis. Furthermore, since participants were asked to avoid engaging in MVPA 24 hours before the second visit, this day was never included in analysis of habitual PA, sedentary and standing times. At least 4 days of valid PiezoRxD® data (including 1 weekend day) were considered sufficient for analysis. Activity accumulated below the individualized moderate-intensity step rate threshold was considered light intensity physical activity (LPA), whereas activity above this threshold was considered MVPA. Activity accumulated above the moderate- but below the vigorous-intensity threshold was considered moderate intensity physical activity and any activity above the vigorous-intensity threshold was considered vigorous physical activity (O'Brien et al. 2018d). ActivPAL™ acceleration data were exported from the device and imported into the activPAL™ software (PAL Analysis, version 5.8.5), which produces activity data in 15s epochs. These data were then analyzed using a customized LabVIEW program (LabVIEW 2018; National Instruments, Austin, TX, USA) that summarized daily averages of awake time spent standing and sitting/lying down. Heart rate was determined using inter-beat cardiac intervals from the ECG tracing. Systolic (SBP and diastolic (DBP) blood pressures were determined from the Portapres® waveform as the maximum and minimum values per cardiac cycle, respectively. Mean arterial pressure (MAP) was calculated as:

$$MAP = \frac{1}{3}\ SBP + \frac{2}{3}\ DBP$$

Ultrasound-derived vascular recordings were exported as video signals directly to a laptop via a video graphics array converter (Epiphan Systems Inc., VGA 2 USB, Ottawa, CAN). Continuous analysis of beat-by-beat POP and BA diameter, RBCV and shear rate were performed using an automated commercial edge-detection and wall-tracking software combined with simultaneous Doppler RBCV waveform envelope analysis (FMD Studio, Cardiovascular Suite, Quipu, Pisa, Italy).

Arterial blood flow (BF; ml/min) and shear rate (SR, s^{-1}) were calculated as:

$$BF = [mean\ RBCV\ (cm/s) \times \pi \times lumen\ radius^2\ (cm^2) \times 60\ (s/min)].$$

$$SR: [8 \times RBCV\ (cm/s) / diameter\ (cm)].$$

The constant within the calculation of SR could be 4 or 8 depending on the size of the sample volume when measuring RBCV (Thijssen et al. 2019). For the present study, a constant value of 8 was used as the sample volume encompassed the entire lumen of the artery, whereas a constant value of 4 would have been used if the sample volume encompassed only the middle portion of the lumen (Parker et al. 2009). Subsequently, the SR area under the curve (SR_{AUC}) was calculated between the start of cuff deflation to the time that peak dilation occurred (Pyke and Tschakovsky 2007).

Flow-mediated dilation was quantified in the following forms:

$$Relative\ FMD\ (\%) = [(peak\ diameter - baseline\ diameter) \div baseline\ diameter \times 100\%]$$

$$Absolute\ FMD\ (mm) = (peak\ diameter - baseline\ diameter)$$

It has been recommended to statistically control for individual differences in the magnitude of the SR response following cuff deflation (Pyke and Tschakovsky 2007; Padilla et al. 2008). However, this method is unnecessary if the relationship between SR_{AUC} and relative FMD is weak, or not significant (Atkinson et al. 2009). In our sample, BA- and POP-FMD were not correlated to their respective SR_{AUC} stimuli (BA: $r = 0.31$, $p = 0.15$; POP: $r = 0.33$, $p = 0.11$). As such, relative FMD responses normalized to SR_{AUC} were not reported.

Allometric scaling of FMD responses, to account for known sex differences in baseline diameter, is recommended if the relationship between the natural log of the peak FMD diameter and baseline diameter yield an unstandardized β-coefficient that deviates from 1 and/or has an upper 95% confidence interval (CI) <1 (Atkinson and Batterham 2013). Linear regression revealed that in our sample, the BA- (ß = 0.92, SE = 0.03, CI: 0.87 – 0.97), POP-FMD (ß = 0.94, SE = 0.019, CI: 0.90 – 0.98), as well as the POP-NMD diameters (ß = 0.85, SE = 0.04, CI: 0.77 – 0.94) met these assumptions. The resultant allometrically-scaled FMD responses were calculated using an analysis of covariance (ANCOVA), with the difference between the logarithmically transformed baseline and peak diameters as the dependent variable and the logarithmically transformed baseline diameter as the covariate. This statistical test results in a group estimated mean with a corresponding standard deviation, which gets converted to an individual percentage via:

$$\textit{Allometrically scaled FMD (\%)} = [(e^{EM} - 1) \times 100\%], \textit{ where EM = estimated mean}$$

The pitfall to this approach is that individual data points are no longer achievable following the calculation of an estimated group mean, and thus, are unable to be utilized for further analysis with independent variables (i.e., ANCOVA and correlations).

3.5 Statistical Analysis

To ensure data were normally distributed, Shapiro-Wilk normality tests were conducted. Relative and absolute BA-FMD responses, BA resting blood flow, RBCV and vigorous physical activity were not normally distributed ($p < 0.05$). These non-normal data were consequently logarithmically transformed and confirmed to be normally distributed (all, $p > 0.13$) when analyzed again using the Shapiro-Wilk normality test. Independent t-tests were performed to detect sex differences in descriptive characteristics, habitual physical activity and sedentary behaviours, aerobic fitness, and vascular function. Non-normal data that were logarithmically transformed were also tested via independent t-tests but were back-transformed to absolute values for presentation. Pearson's product moment correlation tests were performed to evaluate relationships between BA and POP endothelial-dependent and independent vasodilation versus weekly physical activity, daily sedentary time and aerobic fitness. Pearson correlations were interpreted as follows: 0.3–0.5 (low), 0.5–0.7 (moderate), 0.7–0.9 (high), and ≥0.9 (very high) (Mukaka 2012). Separate ANCOVA analyses were performed to investigate the influence of LPA, and SBP on POP-FMD as significant relationships existed between these variables in the pooled sample. Additional ANCOVA analyses were performed to identify whether certain independent variables influenced POP-FMD. Specifically, these analyses were performed using VO_2peak, MVPA and sedentary time as covariates, as these were pertinent independent variables for secondary aims of the present study. Data are presented as means ± standard deviation. Significance was accepted at $P < 0.05$. All statistical testing was performed using SPSS V25 (IBM, New York, USA).

4 Results

4.1 Participants

Age, weekly MPA and VPA, daily steps and sedentary time, diastolic and MAP were similar between males and females (all, $p>0.06$; Table 4-1), but females spent more time per week engaged in LPA ($p=0.03$). As shown in Table 4-1, males had a greater body mass index, resting SBP and lower resting heart rates compared to females (all, $p<0.05$). During the maximal incremental cycle ergometry test, males and females had similar maximum heart rate (183 ± 8 vs. 187 ± 8 beats•min^{-1}, $p=0.25$), respiratory exchange ratio (1.21 ± 0.05 vs. 1.24 ± 0.06, $p=0.43$), and ratings of perceived exertion (19 ± 1 vs. 18 ± 1, $p=0.10$). Males had greater relative VO_2peak than females (48.0 ± 7.1 vs. 36.8 ± 6.0 ml•kg^{-1}•min^{-1}, $p=0.001$). However, when the average VO_2peak scores for males and females were compared to the age- and sex-based reference values for cycle ergometry, both were between the 70th (Males: 47.9 ml•kg^{-1}•min^{-1}, Females: 35.6 ml•kg^{-1}•min^{-1}) and 80th percentiles (Males: 51.4 ml•kg^{-1}•min^{-1}, Females: 38.8 ml•kg^{-1}•min^{-1}) (Kaminsky et al. 2017).

Table 4-1: *Participant descriptive characteristics.*

Variable	Males (n=13)	Females (n=13)	*P*-Values
Age, years	23 ± 3	24 ± 2	0.31
BMI, $kg \cdot m^{-2}$	25.8 ± 2.8	23.0 ± 2.5	0.01
Daily step count, $steps \cdot day^{-1}$	8585 ± 2721	10753 ± 3360	0.06
Sedentary time, $min \cdot day^{-1}$	625 ± 96	544 ± 126	0.08
LPA, $min \cdot week^{-1}$	127 ± 53	182 ± 67	0.03
MPA, $min \cdot week^{-1}$	335 ± 95	424 ± 132	0.06
VPA, $min \cdot week^{-1}$	91 ± 90	79 ± 72	0.54
MVPA, $min \cdot week^{-1}$	430 ± 142	503 ± 174	0.25
HR, $beats \cdot min^{-1}$	59 ± 7	68 ± 8	<0.01
SBP, mmHg	123 ± 7	110 ± 9	<0.01
DBP, mmHg	65 ± 9	67 ± 8	0.67
MAP, mmHg	84 ± 8	81 ± 6	0.23

Data presented as means ± SD. Comparisons made via independent t-tests. BMI, body mass index; VO_2peak, peak oxygen consumption; LPA, light physical activity; MVPA, moderate-vigorous physical activity; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; mmHg, millimetres of mercury; MAP, mean arterial pressure.

4.2 Arterial Measurements

4.2.1 Impact of Habitual Activity and Aerobic Fitness on Arterial Health

In the pooled sample, results from the correlational analyses between weekly LPA, MVPA, daily sedentary time and VO_2peak versus relative BA-FMD, POP-FMD, and POP-NMD are presented in Table 4-2. Weekly LPA was positively correlated with POP-FMD (r=0.43 p=0.04) but no other significant correlations for any of these relationships existed, including when separated by sex. No relationships were observed between weekly LPA, MVPA, sedentary time or VO_2peak (r=0.36, p = 0.07).

Table 4-2: *Relationships between vascular function versus weekly habitual physical activity, daily sedentary time and aerobic fitness in the pooled sample and separated by sex*

Independent Variable	Group	Brachial FMD	Popliteal FMD	Popliteal NMD
LPA, min•week^{-1}	Pooled	r=0.17, p=0.43	***r*=0.43, *p*=0.04**	r=0.30, p=0.16
	Males	r=0.06, p=0.83	r=0.47, p=0.13	r=-0.11, p=0.74
	Females	r=-0.03, p=0.93	r=-0.10, p=0.75	r=0.33, p=0.33
MVPA, min•week^{-1}	Pooled	r=0.05, p=0.80	r=0.11, p=0.60	r=-0.12, p=0.60
	Males	r=-0.34, p=0.26	r=-0.0, p=0.99	r=-0.5, p=0.1
	Females	r=-0.10, p=0.75	r=-0.09, p=0.78	r=-0.02, p=0.96
Sedentary Time, min•day^{-1}	Pooled	r=-0.17, p=0.41	r=-0.25, p=0.24	r=0.0, p=0.98
	Males	r=-0.42, p=0.16	r=-0.13, p=0.69	r=-0.02, p=0.94
	Females	r=-0.09, p=0.78	r=0.13, p=0.69	r=0.18, p=0.60
VO_2peak, ml•kg^{-1}•min^{-1}	Pooled	r=-0.23, p=0.26	r=-0.30, p=0.15	r=-0.15, p=0.49
	Males	r=0.35, p=0.24	r=0.28, p=0.38	r=-0.16, p=0.62
	Females	r=-0.22, p=0.48	r=-0.06, p=0.86	r=0.38, p=0.25

Data obtained from Pearson product moment correlation tests and presented as Pearson product moment coefficients (r) and corresponding significance value (p). FMD, flow-mediated dilation; NMD, nitroglycerin-mediated dilation; LPA, light physical activity; MVPA, moderate-vigorous physical activity, VO_2peak, peak oxygen consumption

4.2.2 Brachial Artery FMD Test

Males had larger baseline and peak diameters, and higher resting blood flow (all, p<0.001; Table 4-3). There were no sex differences in relative BA-FMD (females 7.8 ± 2.7% vs. males, 6.2 ± 1.5%, p=0.09; Figure 4-1), absolute FMD, resting shear rate, mean RBCV, SR_{AUC}

or time to peak dilation (all, $p>0.16$; Table 4-3). As shown in Figure 4-2, when accounting for sex differences in baseline diameter via allometric scaling, there were no differences between males (8.0 ± 2.7%) and females (7.6 ± 2.7%, $p=0.75$).

Table 4-3: *Brachial and popliteal artery measurements during rest and in response to reactive hyperaemia between males and females*

	Brachial Artery		**Popliteal Artery**	
	Males	**Females**	**Males**	**Females**
Resting				
Baseline diameter, cm	0.43 ± 0.04*	0.33 ± 0.04	0.65 ± 0.07*	0.51 ± 0.04
Shear rate, s^{-1}	227 ± 94	235 ± 114	80.1 ± 24.6	80.8 ± 33.8
Red blood cell velocity, $cm•s^{-1}$	13.1 ± 7.2	9.4 ± 4.1	6.4 ± 1.8	5.1 ± 1.9
Blood flow, $ml•min^{-1}$	116 ± 66*	45 ± 23	125 ± 32*	61 ± 25
Post-Deflation				
Peak diameter, cm	0.46 ± 0.04*	0.36 ± 0.04	0.68 ± 0.08*	0.55 ± 0.04
Absolute FMD, cm	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.004
Time to peak diameter, s	64 ± 16	56 ± 14	75 ± 17	77 ± 21
SR_{AUC}	39799 ± 12929	48135 ± 17052	20399 ± 6907	24510 ± 6449

*Data presented as means ± SD. Comparisons made via independent t-tests. FMD, flow-mediated dilation; SR_{AUC}, Shear rate area under the curve until peak dilation. *, $p<0.05$ versus females for the same artery as compared by independent t-tests.*

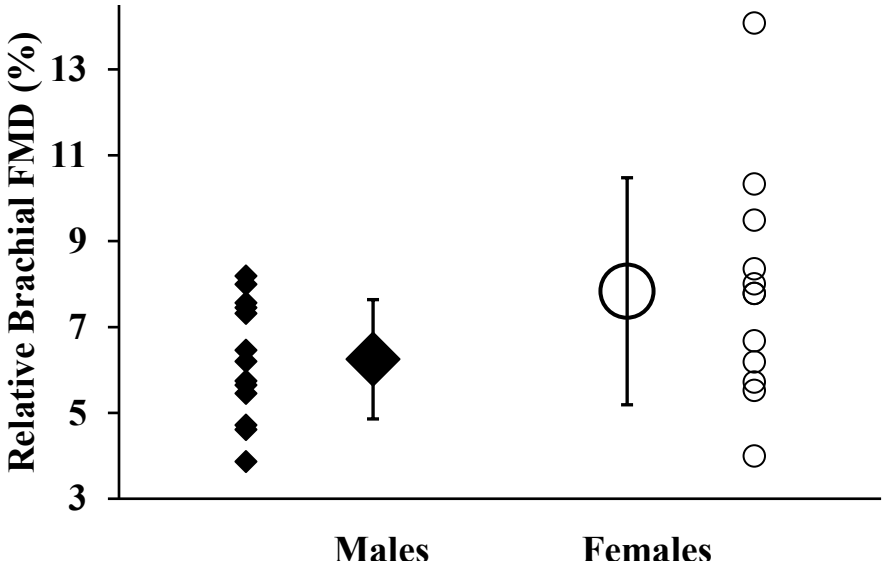

Figure 4-1: *No differences between males and females in relative brachial artery flow-mediated dilation responses (% from baseline) as determined by an independent t-test (p=0.09). Group means ± standard deviations are displayed as the larger symbols and individual data points are depicted as diamonds and circles for males and females, respectively. FMD, flow-mediated dilation.*

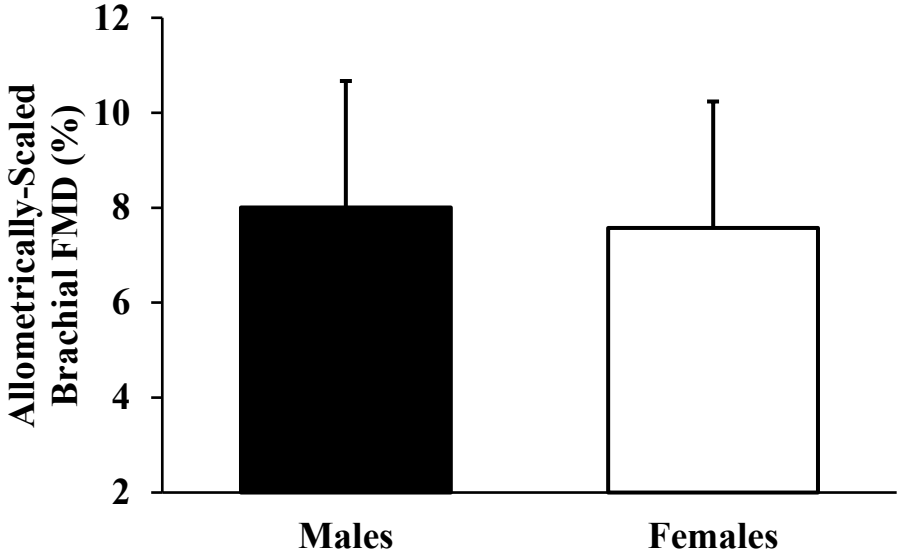

Figure 4-2: *After allometrically scaling for baseline diameter, brachial artery flow-mediated dilation was similar between males and females, as compared by least significant differences post-hoc testing from an analysis of covariance (p=0.75). Data are presented as estimated means ± standard deviation, therefore individual data points are not available. FMD, flow-mediated dilation*

4.2.3 Popliteal Artery FMD Test

Males had larger baseline and peak diameters, and greater resting blood flow (all, $p<0.001$; Table 4-3). Conversely, there were no differences between males and females in resting shear rate, mean RBCV, SR_{AUC}, or time to peak dilation (all, $p>0.15$; Table 4-3). Females exhibited greater relative POP-FMD (6.2 ± 1.0% vs. 4.6 ± 1.4%; $p=0.001$; Figure 4-3) and after allometrically scaling for baseline diameter (6.8 ± 1.7% vs. 4.7 ± 1.7%, $p=0.03$; Figure 4-4) but not when represented in absolute terms ($p=0.61$; Table 4-3). Additionally, the observed sex differences were not altered when including LPA ($p=0.008$; Figure 4-5), SBP, MVPA, sedentary time or VO_2peak (all, $p<0.01$) as covariates.

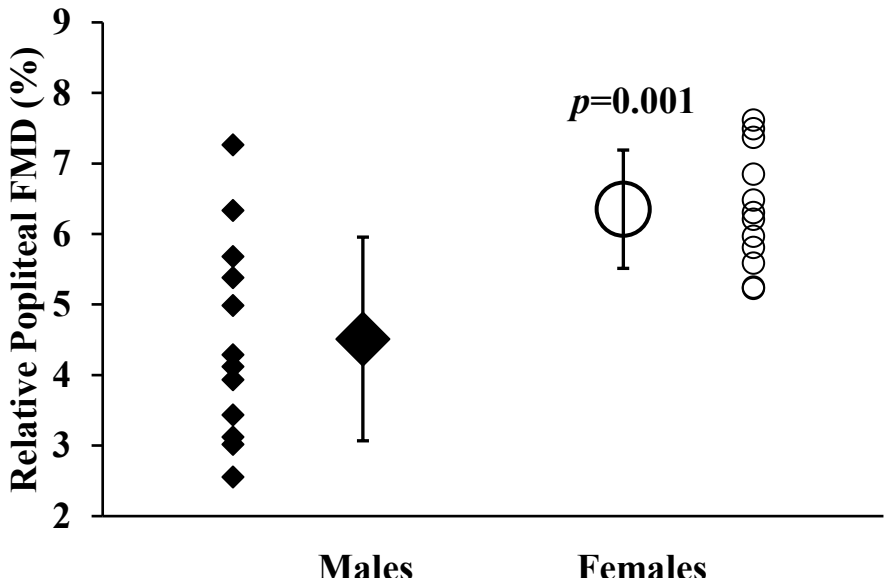

Figure 4-3: *Females exhibited greater popliteal flow-mediated dilation compared to males, via an independent t-test. Group means ± standard deviations are displayed as the larger symbols and individual data points are depicted as diamonds and circles for males and females, respectively. FMD, flow-mediated dilation.*

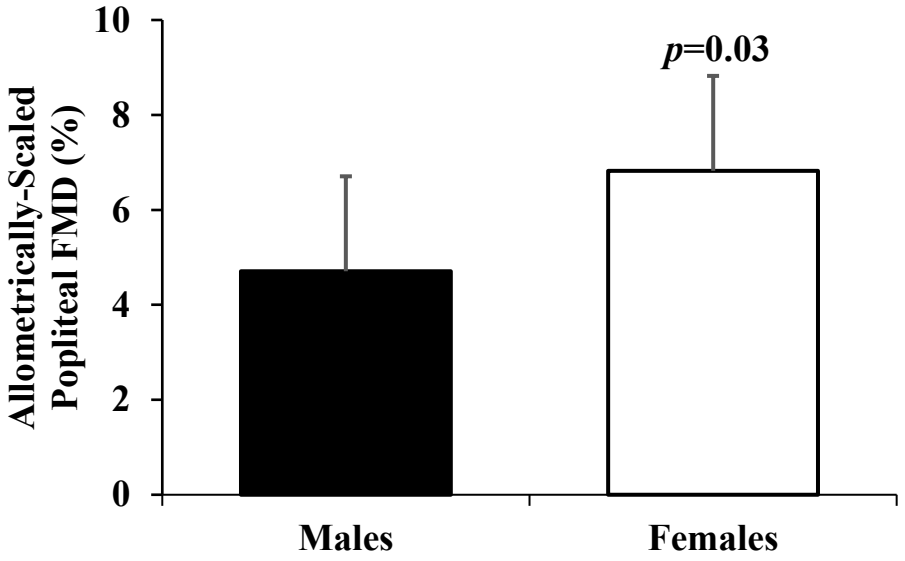

Figure 4-4: *Females exhibited greater popliteal artery flow-mediated dilation following allometric scaling for baseline diameter. Comparisons were made by least significant differences post-hoc testing from an analysis of covariance. Data are presented as estimated means ± standard deviation, therefore individual data points are not available. FMD, flow-mediated dilation.*

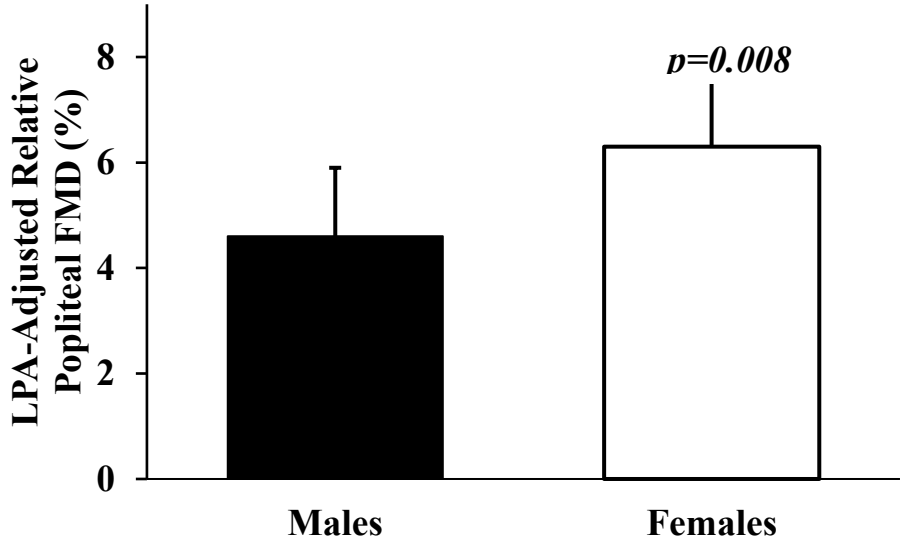

Figure 4-5: *Females exhibited greater popliteal artery flow-mediated dilation) following adjusting for weekly levels of light physical activity (LPA) using an analysis of covariance. Data are presented as estimated means ± standard deviation, therefore individual data points are not available. LPA, light-intensity physical activity; FMD, flow-mediated dilation.*

4.2.4 Popliteal Artery NMD Test

Compared to females, males had larger baseline (0.63 ± 0.08 cm vs. 0.51 ± 0.06 cm, p=0.001) and peak nitroglycerin-mediated diameters (0.69 ± 0.09 cm vs. 0.58 ± 0.06 cm, p=0.002). Males and females exhibited similar absolute POP-NMD (0.064 ± 0.02 cm vs. 0.069 ± 0.02 cm, p=0.61), relative POP-NMD responses (11.9 ± 3.9% vs. 9.4 ± 2.5%, p=0.07; Figure 4-6), and following allometric scaling to baseline diameter (11.9 ± 3.7% vs. 12.0 ± 3.3 %, p=0.95; Figure 4-7).

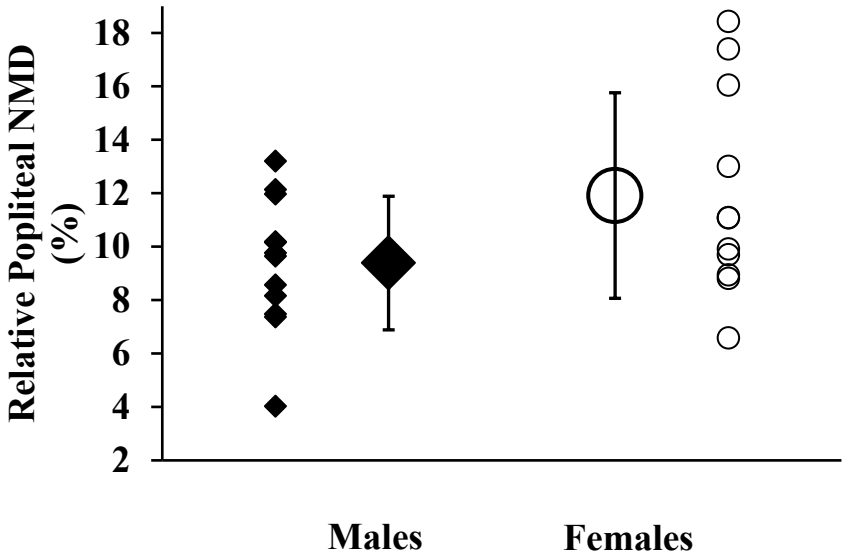

Figure 4-6: *Similar popliteal artery nitroglycerin-mediated dilation responses between males and females, as assessed by an independent t-test (p=0.07). Group means ± standard deviations are displayed as the larger symbols and individual data points are depicted as diamonds and circles for males and females, respectively. NMD, nitroglycerin-mediated dilation*

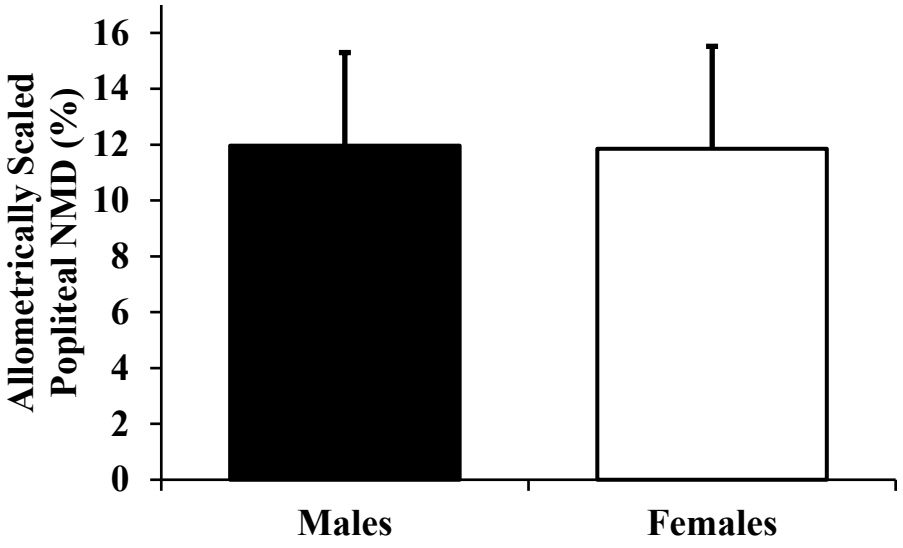

Figure 4-7: *Males and females exhibited similar popliteal artery nitroglycerin-mediated dilation following allometric scaling for baseline diameter. Comparisons were made by least significant differences post-hoc testing from an analysis of covariance (p=0.95). Data are presented as estimated means ± standard deviations, therefore individual data points are not available. NMD, nitroglycerin-mediated dilation.*

5 Discussion

The primary purpose of this study was to investigate sex differences in upper- and lower-limb arterial endothelial-dependent vasodilatory function in healthy young individuals with objectively measured aerobic fitness, habitual physical activity and sedentary behavior time. Males exhibited greater relative VO_2peak and females spent more time engaged in LPA, while MVPA and sedentary time were similar between males and females. It was hypothesized that males would exhibit greater brachial and popliteal FMD responses compared to females after allometrically scaling for baseline diameter. In contrast to our hypothesis, males and females exhibited similar allometrically-scaled BA-FMD responses. Furthermore, females had superior allometrically-scaled POP-FMD despite eliciting a similar shear rate stimulus (i.e., SR_{AUC}). A secondary aim was to uncover relationships between arterial health and measures of habitual behaviours and aerobic fitness in the pooled sample. In our physically active cohort (pooled sample average, 467 ± 160 min•week^{-1} of MVPA), there was only a positive relationship between weekly LPA and relative POP-FMD. Altogether, our findings suggest that potential sex differences in FMD may differ between the upper versus lower-limb arterial beds, and that in the pooled sample, habitual LPA may be more important for lower-limb endothelial function than other objective measures such as habitual MVPA, sedentary time and aerobic fitness.

5.1 Brachial Artery Flow-Mediated Dilation Responses

Investigation into sex differences in BA-FMD began when Celermajer et al., (1994) observed life course discrepancies in the timeline of BA-FMD reductions between males and females. Specifically, males experienced attenuated BA-FMD responses earlier in life (40's) compared to females (50's), which coincided with the start of menopause and reduced estrogen production (Celermajer et al. 1994). Since then, numerous studies have reported conflicting

results regarding sex difference when expressing relative FMD (% increase from baseline). Some studies have reported augmented BA-FMD in pre-menopausal females (Celermajer et al. 1994; Juonala et al. 2008; Nishiyama et al. 2008; Yao et al. 2014; Holder et al. 2019), whereas others have demonstrated similar BA-FMD responses between young males and females (Hashimoto et al. 1995; Harris et al. 2012; Shenouda et al. 2018). The present study documented smaller resting arterial diameters in females (Table 4-2), which aligns with previous reports (Nishiyama et al. 2008; Harris et al. 2012; Shenouda et al. 2018; Holder et al. 2019). It is well established that an inverse relationship exists between resting diameter and relative FMD responses (Thijssen et al. 2008b, 2008a). However, only two studies have identified and controlled for the smaller baseline diameters in females (Juonala et al. 2008; Shenouda et al. 2018). Juonala et al. (2008) reported greater relative BA-FMD in females, which was reversed after accounting for the sex difference in baseline diameter (Juonala et al. 2008). This was recently corroborated by Shenouda et al. (2018) who also demonstrated greater BA-FMD in males after correcting for their larger baseline diameter (Shenouda et al. 2018). In contrast, there were no differences in relative BA-FMD between the sexes in the present study, which remained similar after allometric scaling (Figure 4-2). Potential confounding differences between the current study and those by Juonala et al. (2008) and Shenouda et al. (2018) include the inclusion of objectively measured aerobic fitness, habitual physical activity and sedentary behaviours, which were not considered in these previous reports. Specifically, females from the present study spent more time engaged in weekly LPA, which may partly explain these discrepant findings. Though others have demonstrated that BA-FMD remains unchanged following either a one week reduction in daily step count (i.e., 10 000 – 5 000 steps•day^{-1}) (Boyle et al. 2013) or during an acute bout of prolonged sitting (Thosar et al. 2014), these studies were only investigated in males. As such, sex differences in the BA-FMD

response to acute and chronic changes in physical activity and sedentary behaviours are warranted. Considering that lower limb arteries are more directly influenced by the altered blood flow and shear stress responses associated with sedentary behaviours and traditional modes of physical activity (e.g. walking, cycling), perhaps it is more relevant to identify how these factors influence sex differences in popliteal artery endothelial function.

5.2 Popliteal Artery Flow-Mediated Dilation Responses

Historically, FMD has been performed predominantly in the BA due to the predictive information pertaining to future cardiovascular event risk (Inaba et al. 2010; Green et al. 2011; Matsuzawa et al. 2015) and strong correlation to coronary artery endothelial health (Broxterman et al. 2019). However, the POP is a clinically relevant conduit artery due to its increased susceptibility for atherosclerosis (Aboyans et al. 2011). As highlighted above, lower limb arteries experience large fluctuations in blood flow and shear stress during periods of (in)activity (Boyle et al. 2013; Padilla and Fadel 2017; Teixeira et al. 2017), highlighting the importance of capturing habitual physical activity intensity and sedentary time when measuring POP-FMD. To date, only one study has assessed sex differences in both the BA and POP (Nishiyama et al. 2008). They observed similar absolute FMD responses in both arteries, which aligns well with the present findings (Table 4-3). However, they observed greater BA-FMD in females (8.7 ± 1.1 vs. 6.7 ± 0.6%, $p<0.05$) but no difference in POP-FMD (5.4 ± 0.8 vs. 4.4 ± 0.6%, no p-value reported) when expressed as a relative increase from baseline (Nishiyama et al. 2008). This is in contrast to our findings as we observed no sex difference in relative BA-FMD (Figure 4-1) and greater POP-FMD in females compared to males (Figure 4-3). This observed difference between males and females persisted following allometric scaling for baseline diameter (Figure 4-4). Nishiyama et al. (2008) also observed sex differences in baseline diameter in both arteries, but

they only normalized the FMD responses to the shear rate stimulus (SR_{AUC}) without performing allometric scaling. Two other recent investigations have also reported no sex differences in relative POP-FMD responses (Vranish et al. 2017; O'Brien et al. 2019), which contrasts with this study. Importantly, Vranish et al. (2017) failed to account for potential differences in time spent engaged in LPA, MVPA and/or sedentary time between the sexes. Furthermore, while the study by O'Brien et al. (2019) did include objectively measured habitual MVPA and sedentary time, there were no differences between males and females. Although the two groups in the present study had similar MVPA and sedentary time, the females engaged in greater amounts of LPA (Table 4-1). This was accompanied by a positive relationship between LPA and relative POP-FMD in the pooled sample. As such, an ANCOVA was performed with LPA as the covariate, but POP-FMD was still greater in the females compared to males (p=0.008). This suggests that a true sex difference does in fact exist in this arterial bed, which was not mediated by weekly volume of LPA. There were no significant relationships between MPA, VPA, MVPA, or sedentary time and relative POP-FMD (Table 4-2). This is somewhat surprising as national physical activity guidelines recommend that adults achieve ≥150 minutes of MVPA per week and do not provide recommendations surrounding LPA (Warburton et al. 2010; 2018 Physical Activity Guidelines Advisory Committee 2018). On average, this group of individuals achieved more than double the national average according to Statistics Canada. Specifically, males achieved nearly double, whereas females tripled, the national average in time per week spent engaged in MVPA (259 and 168 min•week^{-1}, respectively) (Statistics Canada, 2019). However, because this group is already accumulating approximately three times the recommended amount of weekly MVPA, it is reasonable to suggest that any additional time spent at light intensities

may provide additional benefits from local changes in shear stress exposure that accompanies locomotion.

5.3 Popliteal Artery Nitroglycerin-Mediated Dilation Responses

To our knowledge, no study has compared POP-NMD between young males and females. Our results indicate that sex differences in vascular smooth muscle sensitivity do not exist in this group of healthy adults. This is in agreement with previous studies performing BA-NMD in males and females (Hashimoto et al. 1995; Black et al. 2009; Shenouda et al. 2018). Interestingly, Shenouda et al. (2018) reported that naturally menstruating females exhibited reduced BA-NMD compared to males and females using oral contraceptives. Although our sample size is smaller than Shenouda et al. (2018), who studied 15 females who were naturally menstruating and 18 females using combination oral contraceptives, we compared relative POP-NMD between females naturally menstruating (n=5) against those using combination oral contraceptives (n=5). We observed that these groups of females were similar (p=0.94), and these data did not meet the criteria for allometric scaling.

5.4 Sex Hormones

Prior studies investigating sex differences in arterial health have proposed that the cyclical fluctuations of E2 throughout the menstrual cycle favourably influences the endothelium (Hashimoto et al. 1995; Yao et al. 2014; Vranish et al. 2017). Mechanistic work has indicated that E2 acts in both genomic and non-genomic pathways, facilitating an anti-atherogenic environment via enhanced antioxidative capabilities, reduced ET-1 concentrations and heightened NO-bioavailability via upregulation of eNOS (Mendelsohn 2002; White 2002; Chakrabarti et al. 2014). There is also evidence from rodents suggesting that E2 (via the alpha E2 receptor) plays a crucial role in blood flow-induced remodelling of resistance arteries.

Specifically, ovariectomized rats who were given E2 supplementation experienced hyperemia-induced increases in arterial diameter versus ovariectomized rats without E2 supplementation (Tarhouni et al. 2013). Although females were studied in the early-follicular phase (or placebo phase for oral contraceptive users) to minimize the influence of E2 levels, we cannot rule out the possibility that females still had higher E2 levels then males that may have influenced their enhanced POP-FMD responses. Furthermore, while concentrations of synthetic E2 are reduced during the placebo phase in oral contraceptive users, endogenous levels of E2 increase to concentrations higher than what they would receive synthetically, but typically not until the 6th and 7th day following menstruation/into the placebo phase (Willis et al. 2006). As such, it is unlikely that this would have affected our results as female participants using oral contraceptives were tested in days 1-5 of their placebo phase.

5.5 Aerobic Fitness and Arterial Endothelial Health

The lower POP-FMD responses observed in males occurred despite them exhibiting a greater level of aerobic fitness than females (Table 4-1). However, when comparing the relative VO_2peak values with the sex- and age-based normative aerobic fitness categories reported by the Fitness Registry and the Importance of Exercise National Database (FRIEND), both groups fell between the 70th and 80th percentiles (Kaminsky et al. 2017). The lack of relationship observed between relative BA-FMD and relative VO_2peak (Table 4-2) is consistent with a recent study in young males (Bell et al. 2017). The null relationship observed between relative POP-FMD and relative VO_2peak (Table 4-2) was contrary to the secondary hypothesis that more aerobically fit individuals would have had a greater exposure time to increased shear stress levels during traditional lower-limb modes of exercise. However, relative VO_2peak was not correlated to weekly levels of LPA (r=-0.12, p=0.56) or MVPA (r=-0.03, p=0.89), highlighting common

misconceptions that measures of habitual MVPA and aerobic fitness are equivocal. This is supported by existing evidence that indicates that aerobic fitness involves a genetic component (Bouchard et al. 1999) so it may not be reflective of their habitual activity levels. Furthermore, while we monitored habitual activity that was reflective of a 'normal' week, this does not capture the long-term conditioning versus de-conditioning that occurs with regular exercise and inactivity, respectively (Coyle et al. 1984).

5.6 Limitations and Strengths

There are some limitations to the present study that should be noted. Firstly, the population we studied were highly active young adults so these findings cannot be generalized to the average young Canadian, and other less active populations. Future studies should investigate whether the findings reported in the present study extend to less active populations such as those with chronic diseases and older adults. Moreover, the possibility that these individuals changed their behaviours in response to wearing activity monitors (i.e., Hawthorne effect) (Wickstrom and Bendix 2000) may also be a limitation. However, the participants were instructed not to change their habitual activities (i.e., start a new exercise training regime) during the study.

Additionally, the group of pre-menopausal females was composed of those who were naturally menstruating (n=5), using an intrauterine device (n=2**)** and taking vaginal (n=1) or oral contraceptives (n=5). However, it was recently highlighted by Shenouda and colleagues (2018) that endothelial function was similar between young females who were naturally menstruating and those using oral contraceptives (Shenouda et al. 2018). Additionally, 2 females were using copper intrauterine devices, which prevent pregnancy via non-hormonal mechanisms (Stanford and Mikolajczyk 2002). It has been previously demonstrated that BA-FMD and BA-NMD are unchanged after 12 months of copper IUD use compared to baseline (Selim and Hussein 2013).

One other female was using a vaginal contraceptive ring, which releases similar hormonal dosages (vaginally) as oral contraceptives and has been shown to increase BA-FMD in the active phase compared to the inactive phase (Torgrimson et al. 2009). However, alike those taking contraceptives orally, we tested females when exogenous estrogen concentrations were at a nadir. Therefore, we combined copper IUD users with those who naturally menstruate to compare relative FMD between females who use hormonal (oral/vaginal) contraceptives (n=6) versus those who do not (n=7). Though our sample size was low, there were no differences in the BA- (p=0.68) or POP-FMD (p=0.13) and POP-NMD (p=0.92) when comparing the groups. However, we recommend that future studies investigating sex differences consider including groups of females using various methods of contraceptives similar to Shenouda et al. (2018) (i.e., intrauterine devices, oral and non-oral combination contraceptives).

Lastly, there were a number of measures including relative BA-FMD (p=0.09) and POP-NMD (p=0.07) that approached statistical significance. With a greater sample size (~20 per group) we may have seen sex differences in these measures. However, these measures may be considered less important compared to BA-FMD and POP-NMD following allometric scaling for differences in baseline diameter, where it was clear that sex differences did not exist (p=0.75, and p=0.95, respectively).

Despite our limitations, this study also had several strengths. First and foremost, this represents the only study to assess vascular function in both the upper- and lower-limb while objectively measuring habitual PA, sitting time and aerobic fitness. Future studies should incorporate objective measurements of these cardiovascular disease risk factors when making between-group comparisons. Additionally, only one study has previously investigated sex differences in the BA and POP simultaneously (Nishiyama et al. 2008). Because both the BA

and POP have clinical utility, it may beneficial to evaluate both arteries to allow for a more comprehensive assessment of overall vascular health.

5.7 Conclusion

This study adds to the growing evidence regarding sex differences in peripheral artery vasodilator function. These findings suggest that sex differences exist in POP-, but not BA-FMD, in highly active young adults that achieve nearly double (males) to triple (females) the national average for weekly MVPA. Interestingly, the observed sex difference existed in an atherosclerosis-susceptible artery. This information may lend further support as to why pre-menopausal females generally experience reduced incidence for cardiovascular disease compared to age-matched males. Greater POP-FMD in females persisted after considering between-group differences in weekly LPA. This was accompanied by a positive relationship between LPA and POP-FMD, suggesting that weekly engagement in LPA may be important for POP-FMD. Future studies should determine if the findings in this study extend to males and females at risk for cardiovascular disease, and in individuals who are less physically active and more representative of the general population.

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Appendix A: Ethics Approval

DALHOUSIE UNIVERSITY
Research Services

Health Sciences Research Ethics Board
Letter of Approval

July 31, 2018

Jarrett Johns
Health\School of Health and Human Performance

Dear Jarrett,

REB #: 2018-4518
Project Title: The Relationship Between Physical Activity Level and Blood Vessel Health in Young Adults: Impact of Sex and the Menstrual Phase

Effective Date: July 31, 2018
Expiry Date: July 31, 2019

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

Sincerely,

Dr. Tannis Jurgens, Chair

Appendix B: Health History Questionnaire

Health History Questionnaire

AGE:

PARTICIPANT I.D. (Code assigned by research team)

Instructions to calculate your body mass index (BMI). If your BMI is greater than 30 kg/m^2 you will not be eligible to participate in the study.

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

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

3. Please calculate your approximate BMI:

BMI (kg/m^2) = $\frac{\text{kg}}{(\text{m})*(\text{m})}$ = _____ = _____

These questions are designed to determine your eligibility for the study. If you answer 'Yes" to any question you will not be able to participate in the study.

1. Have you smoked or consumed any nicotine containing product daily within the past 6 months? Yes No

2. Have you been prescribed blood pressure medication? Yes No

3. Do you have a history of cardiovascular, respiratory or metabolic diseases? Yes No

4. Are you <u>not</u> between the ages of 18 and 30? Yes No

For females only:

5. Is there a possibility that you may be pregnant and/or are you breastfeeding? Yes No

6. Do you have an irregular menstrual cycle? (i.e., not menstruating consistently within the first 7 days of a 28-day cycle). Yes No

Appendix C: Physical Activity Readiness Questionnaire Plus (PAR-Q+)

2017 PAR-Q+

The Physical Activity Readiness Questionnaire for Everyone

The health benefits of regular physical activity are clear; more people should engage in physical activity every day of the week. Participating in physical activity is very safe for MOST people. This questionnaire will tell you whether it is necessary for you to seek further advice from your doctor OR a qualified exercise professional before becoming more physically active.

GENERAL HEALTH QUESTIONS

Please read the 7 questions below carefully and answer each one honestly: check YES or NO.	YES	NO
1) Has your doctor ever said that you have a heart condition ☐ OR high blood pressure ☐?	☐	☐
2) Do you feel pain in your chest at rest, during your daily activities of living, OR when you do physical activity?	☐	☐
3) Do you lose balance because of dizziness OR have you lost consciousness in the last 12 months? Please answer NO if your dizziness was associated with over-breathing (including during vigorous exercise).	☐	☐
4) Have you ever been diagnosed with another chronic medical condition (other than heart disease or high blood pressure)? PLEASE LIST CONDITION(S) HERE: _____	☐	☐
5) Are you currently taking prescribed medications for a chronic medical condition? PLEASE LIST CONDITION(S) AND MEDICATIONS HERE: _____	☐	☐
6) Do you currently have (or have had within the past 12 months) a bone, joint, or soft tissue (muscle, ligament, or tendon) problem that could be made worse by becoming more physically active? Please answer NO if you had a problem in the past, but it does not limit your current ability to be physically active. PLEASE LIST CONDITION(S) HERE: _____	☐	☐
7) Has your doctor ever said that you should only do medically supervised physical activity?	☐	☐

If you answered NO to all of the questions above, you are cleared for physical activity.
Go to Page 4 to sign the PARTICIPANT DECLARATION. You do not need to complete Pages 2 and 3.

- Start becoming much more physically active – start slowly and build up gradually.
- Follow International Physical Activity Guidelines for your age (www.who.int/dietphysicalactivity/en/).
- You may take part in a health and fitness appraisal.
- If you are over the age of 45 yr and NOT accustomed to regular vigorous to maximal effort exercise, consult a qualified exercise professional before engaging in this intensity of exercise.
- If you have any further questions, contact a qualified exercise professional.

If you answered YES to one or more of the questions above, COMPLETE PAGES 2 AND 3.

Delay becoming more active if:

- You have a temporary illness such as a cold or fever; it is best to wait until you feel better.
- You are pregnant - talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the ePARmed-X+ at www.eparmedx.com before becoming more physically active.
- Your health changes - answer the questions on Pages 2 and 3 of this document and/or talk to your doctor or a qualified exercise professional before continuing with any physical activity program.

01-01-2017

2017 PAR-Q+

FOLLOW-UP QUESTIONS ABOUT YOUR MEDICAL CONDITION(S)

1. Do you have Arthritis, Osteoporosis, or Back Problems?

If the above condition(s) is/are present, answer questions 1a-1c — If **NO** ☐ go to question 2

1a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
1b.	Do you have joint problems causing pain, a recent fracture or fracture caused by osteoporosis or cancer, displaced vertebra (e.g., spondylolisthesis), and/or spondylolysis/pars defect (a crack in the bony ring on the back of the spinal column)?	YES ☐ NO ☐
1c.	Have you had steroid injections or taken steroid tablets regularly for more than 3 months?	YES ☐ NO ☐

2. Do you currently have Cancer of any kind?

If the above condition(s) is/are present, answer questions 2a-2b — If **NO** ☐ go to question 3

2a.	Does your cancer diagnosis include any of the following types: lung/bronchogenic, multiple myeloma (cancer of plasma cells), head, and/or neck?	YES ☐ NO ☐
2b.	Are you currently receiving cancer therapy (such as chemotheraphy or radiotherapy)?	YES ☐ NO ☐

3. Do you have a Heart or Cardiovascular Condition? *This includes Coronary Artery Disease, Heart Failure, Diagnosed Abnormality of Heart Rhythm*

If the above condition(s) is/are present, answer questions 3a-3d — If **NO** ☐ go to question 4

3a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
3b.	Do you have an irregular heart beat that requires medical management? (e.g., atrial fibrillation, premature ventricular contraction)	YES ☐ NO ☐
3c.	Do you have chronic heart failure?	YES ☐ NO ☐
3d.	Do you have diagnosed coronary artery (cardiovascular) disease and have not participated in regular physical activity in the last 2 months?	YES ☐ NO ☐

4. Do you have High Blood Pressure?

If the above condition(s) is/are present, answer questions 4a-4b — If **NO** ☐ go to question 5

4a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
4b.	Do you have a resting blood pressure equal to or greater than 160/90 mmHg with or without medication? (Answer **YES** if you do not know your resting blood pressure)	YES ☐ NO ☐

5. Do you have any Metabolic Conditions? *This includes Type 1 Diabetes, Type 2 Diabetes, Pre-Diabetes*

If the above condition(s) is/are present, answer questions 5a-5e — If **NO** ☐ go to question 6

5a.	Do you often have difficulty controlling your blood sugar levels with foods, medications, or other physician-prescribed therapies?	YES ☐ NO ☐
5b.	Do you often suffer from signs and symptoms of low blood sugar (hypoglycemia) following exercise and/or during activities of daily living? Signs of hypoglycemia may include shakiness, nervousness, unusual irritability, abnormal sweating, dizziness or light-headedness, mental confusion, difficulty speaking, weakness, or sleepiness.	YES ☐ NO ☐
5c.	Do you have any signs or symptoms of diabetes complications such as heart or vascular disease and/or complications affecting your eyes, kidneys, **OR** the sensation in your toes and feet?	YES ☐ NO ☐
5d.	Do you have other metabolic conditions (such as current pregnancy-related diabetes, chronic kidney disease, or liver problems)?	YES ☐ NO ☐
5e.	Are you planning to engage in what for you is unusually high (or vigorous) intensity exercise in the near future?	YES ☐ NO ☐

2017 PAR-Q+

6. **Do you have any Mental Health Problems or Learning Difficulties?** *This includes Alzheimer's, Dementia, Depression, Anxiety Disorder, Eating Disorder, Psychotic Disorder, Intellectual Disability, Down Syndrome*

If the above condition(s) is/are present, answer questions 6a-6b — If **NO** ☐ go to question 7

6a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
6b.	Do you have Down Syndrome **AND** back problems affecting nerves or muscles?	YES ☐ NO ☐

7. **Do you have a Respiratory Disease?** *This includes Chronic Obstructive Pulmonary Disease, Asthma, Pulmonary High Blood Pressure*

If the above condition(s) is/are present, answer questions 7a-7d — If **NO** ☐ go to question 8

7a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
7b.	Has your doctor ever said your blood oxygen level is low at rest or during exercise and/or that you require supplemental oxygen therapy?	YES ☐ NO ☐
7c.	If asthmatic, do you currently have symptoms of chest tightness, wheezing, laboured breathing, consistent cough (more than 2 days/week), or have you used your rescue medication more than twice in the last week?	YES ☐ NO ☐
7d.	Has your doctor ever said you have high blood pressure in the blood vessels of your lungs?	YES ☐ NO ☐

8. **Do you have a Spinal Cord Injury?** *This includes Tetraplegia and Paraplegia*

If the above condition(s) is/are present, answer questions 8a-8c — If **NO** ☐ go to question 9

8a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
8b.	Do you commonly exhibit low resting blood pressure significant enough to cause dizziness, light-headedness, and/or fainting?	YES ☐ NO ☐
8c.	Has your physician indicated that you exhibit sudden bouts of high blood pressure (known as Autonomic Dysreflexia)?	YES ☐ NO ☐

9. **Have you had a Stroke?** *This includes Transient Ischemic Attack (TIA) or Cerebrovascular Event*

If the above condition(s) is/are present, answer questions 9a-9c — If **NO** ☐ go to question 10

9a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
9b.	Do you have any impairment in walking or mobility?	YES ☐ NO ☐
9c.	Have you experienced a stroke or impairment in nerves or muscles in the past 6 months?	YES ☐ NO ☐

10. **Do you have any other medical condition not listed above or do you have two or more medical conditions?**

If you have other medical conditions, answer questions 10a-10c — If **NO** ☐ read the Page 4 recommendations

10a.	Have you experienced a blackout, fainted, or lost consciousness as a result of a head injury within the last 12 months **OR** have you had a diagnosed concussion within the last 12 months?	YES ☐ NO ☐
10b.	Do you have a medical condition that is not listed (such as epilepsy, neurological conditions, kidney problems)?	YES ☐ NO ☐
10c.	Do you currently live with two or more medical conditions?	YES ☐ NO ☐

PLEASE LIST YOUR MEDICAL CONDITION(S) AND ANY RELATED MEDICATIONS HERE: _____

_____

GO to Page 4 for recommendations about your current medical condition(s) and sign the PARTICIPANT DECLARATION.

2017 PAR-Q+

If you answered NO to all of the follow-up questions about your medical condition, you are ready to become more physically active - sign the PARTICIPANT DECLARATION below:

- It is advised that you consult a qualified exercise professional to help you develop a safe and effective physical activity plan to meet your health needs.
- You are encouraged to start slowly and build up gradually - 20 to 60 minutes of low to moderate intensity exercise, 3-5 days per week including aerobic and muscle strengthening exercises.
- As you progress, you should aim to accumulate 150 minutes or more of moderate intensity physical activity per week.
- If you are over the age of 45 yr and **NOT** accustomed to regular vigorous to maximal effort exercise, consult a qualified exercise professional before engaging in this intensity of exercise.

If you answered YES to one or more of the follow-up questions about your medical condition:

You should seek further information before becoming more physically active or engaging in a fitness appraisal. You should complete the specially designed online screening and exercise recommendations program - the **ePARmed-X+** at **www.eparmedx.com** and/or visit a qualified exercise professional to work through the ePARmed-X+ and for further information.

Delay becoming more active if:

- You have a temporary illness such as a cold or fever; it is best to wait until you feel better.
- You are pregnant - talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the **ePARmed-X+** at **www.eparmedx.com** before becoming more physically active.
- Your health changes - talk to your doctor or qualified exercise professional before continuing with any physical activity program.

- You are encouraged to photocopy the PAR-Q+. You must use the entire questionnaire and NO changes are permitted.
- The authors, the PAR-Q+ Collaboration, partner organizations, and their agents assume no liability for persons who undertake physical activity and/or make use of the PAR-Q+ or ePARmed-X+. If in doubt after completing the questionnaire, consult your doctor prior to physical activity.

PARTICIPANT DECLARATION

- All persons who have completed the PAR-Q+ please read and sign the declaration below.
- If you are less than the legal age required for consent or require the assent of a care provider, your parent, guardian or care provider must also sign this form.

I, the undersigned, have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that a Trustee (such as my employer, community/fitness centre, health care provider, or other designate) may retain a copy of this form for their records. In these instances, the Trustee will be required to adhere to local, national, and international guidelines regarding the storage of personal health information ensuring that the Trustee maintains the privacy of the information and does not misuse or wrongfully disclose such information.

NAME _____ DATE _____

SIGNATURE _____ WITNESS _____

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER _____

For more information, please contact:

www.eparmedx.com
Email: eparmedx@gmail.com

Citation for this PAR-Q+
Warburton DER, Jamnik VK, Bredin SSD, and Gledhill N on behalf of the PAR-Q+ Collaboration.
The Physical Activity Readiness Questionnaire for Everyone (PAR-Q+) and Electronic Physical Activity Readiness Medical Examination (ePARmed-X+). Health & Fitness Journal of Canada 4(2):3-23, 2011.

The PAR-Q+ was created using the evidence-based AGREE process (1) by the PAR-Q+ Collaboration chaired by Dr. Darren E. R. Warburton with Dr. Norman Gledhill, Dr. Veronica Jamnik, and Dr. Donald C. McKenzie (2). Production of this document has been made possible through financial contributions from the Public Health Agency of Canada and the BC Ministry of Health Services. The views expressed herein do not necessarily represent the views of the Public Health Agency of Canada or the BC Ministry of Health Services.

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01-01-2017

Appendix D: Consent Form

INFORMATION LETTER

Project Title: The Relationship Between Physical Activity Level and Blood Vessel Health in Young, Healthy Adults

You are invited to take part in a research study being conducted by Jarrett Johns, a MSc Kinesiology candidate, Myles O'Brien, a MSc Kinesiology candidate and Amanda Bungay, a BSc Kinesiology honours candidate at Dalhousie University, School of Health and Human Performance, Division of Kinesiology. Your participation in this study is voluntary and you may withdraw from the study at any time. If you are a student, there will be no impact on your studies or academic evaluation if you decide not to participate in the research. The information below tells you about what you will be asked to do and about any benefit, risk, or discomfort that you might experience.

We will contact you again after you receive this information to review the eligibility requirements with you and set up an appointment for your testing session if you are deemed eligible and still wish to participate.

Who will be conducting the research?

The co-investigators will be Jarrett Johns, MSc Kinesiology candidate, Myles O'Brien, MSC Kinesiology candidate and Amanda Bungay, BSc Kinesiology honours candidate. from the School of Health and Human Performance, Division of Kinesiology at Dalhousie University. The project will be under the supervision of Dr. Derek Kimmerly who can be contacted via email (dskimmerly@dal.ca) or telephone at (902) 494-1164. Jarrett Johns can be contacted via email (j.johns@dal.ca), Myles O'Brien can be contacted via email (myles.obrien@dal.ca) and Amanda Bungay can be contacted via email (a.bungay@dal.ca) should you have any questions or concerns about the study.

Purpose and Outline of the Research Study

Regular physical activity is beneficial to blood vessel health in older males and females, independent of their aerobic fitness level and how much time they spend sitting. Regular physical activity is also beneficial for younger adults but the relationship between physical activity level, aerobic fitness level and sitting time has not been investigated. We also do not

know if there are differences in the relationship between physical activity levels, sitting time and blood vessel health between young males and females. As such, the purpose of this study is to determine if there are differences in blood vessel health based on weekly physical activity levels and time spent sitting in young adults, and if there are differences between younger males and females.

Who Can Participate in the Research Study?

You may participate in this study if you are between 18-30 years old and do not have a chronic condition or disease that affects the heart or blood vessels.

You will be included in the study if you:

- Are currently a healthy adult between the ages of 18-30 years old
- If you are a woman, you have a regular menstrual (i.e. menstruate between days 1 to 7 of a 28-day cycle).

You will be excluded from the study if you:

- Are pregnant or breastfeeding (females only).
    - Hormones associated with pregnancy and breastfeeding are known to alter blood vessel function and could affect the results of some tests done in this study.
- Have regularly (i.e., daily) smoked cigarettes or consumed any nicotine containing product within the past 6 months.
    - Smoking is known to affect cardiovascular health and could affect the results of some tests done in this study.
- Have a resting blood pressure >140/90 mmHg or have ever been prescribed blood pressure mediation.
    - Someone with high blood pressure is more likely to have impaired blood vessel function and could affect some test results done in this study. We can measure your blood pressure for you in the lab.
- Have a history of cardiovascular, respiratory or metabolic diseases.
    - These conditions may classify you has unhealthy–the results of unhealthy participants are not needed for this study.
- Have a body mass index > 30 kg/m^2.
    - Someone with a BMI over 30 kg/m^2 is more likely to have blood vessel impairments, which could affect some test results in this study. Please see the 'Health History Questionnaire' to determine how to estimate your body mass index.
- Are currently taking blood pressure medication.
    - Blood pressure medication can alter blood pressure and blood flow, which would affect the results of some of the tests in our study.

What You Will Be Asked to Do?

Participants will visit the lab twice taking approximately 3.5 hours of your time. In the first session we will ask you to complete some questionnaires about your current health, physical and sedentary activity patterns. We will measure your height, weight, and blood pressure (upper left arm) and explain all the equipment and procedures used for the study. We will then equip you with two different types of physical activity monitors (four monitors total). Three of the monitors will be placed on the front of your shin, the front of your right thigh, and on the right side of your lower ribs using a clear medical film. You should not worry about these monitors and maintain your daily routine as the monitor can be worn during showers, swimming, and exercise. The other monitor will be equipped at your waist by clipping onto your belt or pant. We ask that you remove this during sleep time, and during bathing/swimming. You will wear the monitors for 7 days after your first visit. We will also ask you to estimate the time you went to sleep, time you woke up, and hours you wore the device on your waist each day, in a sheet provided to you.

In the second session, you will come into the lab and we will retrieve your physical activity monitors. If you are unable to return the monitors after this initial visit we will make arrangements to pick them up from you. We will determine your eligibility based on your physical activity levels, using information from the monitors you were wearing. Once eligible, you will be given a quick tour of the laboratory and review the tests again, we will also answer any questions you may have. After the tour, we will direct you to a private change room so you may change into comfortable and loose clothing (so we can use the ultrasound to measure the arteries at the back of your knee and upper arm).

We will then set you up for measurements of heart rate, blood pressure and lower leg and upper arm blood flow. We will then give you the option to attach two sticky pads to yourself or have us do it for you (one will go on your shoulder, and the other on your ribs) to record heart rate (electrocardiogram). We will measure blood pressure by a blood pressure cuff on your upper left arm and a finger blood pressure cuff on either your index or middle finger. To measure blood vessel health, we will then use an ultrasound machine to record artery blood flow behind your knee. We will then place a blood pressure cuff around your lower leg and inflate it for 5 minutes. You may feel some discomfort, but it will subside immediately after the cuff is released, there are no long-term side effects of this test. We will record your artery blood flow for 5 minutes after the cuff is deflated. We will then repeat this procedure on the upper arm by inflating a cuff on your forearm for 5 minutes and use the ultrasound to measure artery blood flow for 5 minutes on your upper arm.

Once your blood flow has returned to resting levels (5-10 minutes), we will then examine how your artery relaxes after a single spray of nitroglycerin spray under your tongue. We will then use the ultrasound to measure your lower leg, without the cuff.

After another short period of rest, we will ask you to perform a maximal cycling test to assess your fitness level. We will equip you with a chest heart rate monitor and a breathing mask attached to a tube so we can measure heart rate, and gas exchange, respectively. Once you are comfortable on the bicycle, you will warm up for 5 minutes on the bicycle. We will then gradually increase the difficulty (power output) every minute (20 watts) until you reach exhaustion. The test is over when you can no longer continue cycling at a cadence of 50 pedal revolutions per minute. You can decide to stop the test at any point without giving a reason.

Possible Benefits, Risks and Discomforts

Information regarding your general cardiovascular health including resting blood pressure, heart rate, and lower leg blood flow will be provided to you upon request.

Finger Blood Pressure Measurements: You may experience slight discomfort as a result of these measurements, including sensations of “pins and needles” in your finger. When in use, the cuffs will inflate with air and you should feel it gently squeeze your finger. Your finger may turn slightly blue and feel numb or tingly when this cuff is inflated. The symptoms go away once the cuff pressure is reduced. Some people may feel slight pain in this procedure. Pain is not expected to be any worse than what you would feel when a physician takes your blood pressure. If you have felt pain due to blood pressure cuffs in the past, you may not want to take part in this study.

Automated Upper Arm Blood Pressure Measurements: Slight discomfort may be experienced by the participant as the blood pressure cuff is inflated, which may or may not include feelings of "pins and needles" in the participant's hand. This sensation should be relieved as soon as the cuff has been deflated.

Tests of Blood Vessel Function: How your popliteal (located behind your knee) and brachial arteries (located in your upper arm) responds to a stimulus will be measured by taking pictures of it with an ultrasound. The ultrasound is non-invasive with minimal risks. In rare cases, you may experience a slight rash from the ultrasound gel. There is no known medical risk associated with a 5-minute period of blood flow stoppage to one’s leg/arm, and the technique has been used on thousands of people worldwide. Nevertheless, during the 5-minute no-flow period you may feel numbness, a cold sensation, tightness and there may be a slight bluing in foot/hand or lower leg/forearm colour. All of these symptoms will go away once we have released the cuff after 5 minutes.

Nitroglycerin Testing: The reported possible side effects of nitroglycerin administration include: dizziness, light-headedness, or fainting when sitting up or standing; headache; rash; nausea or vomiting; fast heartbeat; or flushing of the face and neck. The most likely side-effect will be dizziness or light-headedness. These side effects occur in less than 5% of people. However, the administration of nitroglycerin while you are lying down should minimize this risk. If, however,

dizziness or light-headedness does occur, it should quickly subside. We will measure your blood pressure after nitroglycerin administration and ask you to remain lying down until your blood pressure is at the same level as it was before the nitroglycerin spray was provided.

Cycle exercise testing: You may experience some discomfort such as shortness of breath, light-headedness, dizziness, or leg fatigue. Some people report feeling restricted or claustrophobic from the face mask used in this test, but we can assure you will be getting sufficient oxygen. You will engage in a 5-minute warmup before the test and minimum 5-minute cooldown after the test to reduce discomfort and side effects. We will be beside you to watch for signs of distress or poor perfusion as well, and we will stop the test immediately if any signs show. You can decide to stop the test at any time without giving a reason.

Pictured above are the two breathing apparatus options to use during the maximal cycle exercise test: The face mask (left) or the mouth piece and nose clamp (right). We will show you both during visit 1.

Physical and Sedentary Activity Monitors: The device measuring your activity will be placed via a thin, clear medical film called Tegederm™ dressing on the front of your thigh for 7 days (males) or 14 days (females). This may become itchy, or have some associated redness surrounding the area. Mild discomfort may be experienced by the skin and/or body hair surrounding the area. The other device is worn around your waist and measures you steps, similar to a pedometer.

Steps have been taken to ensure that all procedures will be performed with minimal risks of any adverse health effects. Throughout the study we will be recording numerous measures of your health. If for any reason we find information that may show a possible health risk (e.g. high resting blood pressure), we will explain the issue to you and strongly recommend that you visit

your family doctor or a qualified physician. If this occurs, you may no longer be eligible to participate in the study.

Privacy and Confidentiality

Information that you provide to us will be kept private. Only the research team at Dalhousie University will have access to this information. We will describe and share our findings in a class presentation and thesis while also vying for publishing in an academic journal. We will be very careful to only talk about group results so that no one will be identified. This means that ***you will not be identified in any way in our reports***. The people who work with your information have special training and have an obligation to keep all research information private. Also, we will use a participant number (not your name) in our written and computerized records. Any documents containing identifying information will be locked in a secure filing cabinet within a locked room. All electronic records will be kept secure on a lab dedicated, password-protected computer that will be kept in a locked room (or on a Dalhousie University secure server) and all written documents will be stored in a locked cabinet where only the supervising professor will have access.

If You Decide to Stop Participating

You are free to leave the study at any time. If you decide to stop participating at any point in the study, you can also decide whether or not you want any of the information that you have contributed up to that point to be removed. You can also decide to have your information removed up to 2 weeks after the final testing.

How to Obtain Results?

You can obtain either group results or your individual results by including your contact information at the end of the signature page and we will send them to you via your preferred method.

Questions

We are happy to talk with you about any questions or concerns you may have about your participation in this research study. Please contact Jarrett Johns at j.johns@dal.ca, Myles O'Brien at myles.obrien@dal.ca, Amanda Bungay at a.bungay@dal.ca, or Dr. Derek Kimmerly at dskimmerly@dal.ca or (902) 494-2570, at any time. We will also tell you if any new information comes up that could affect your decision to participate.

If you have any ethical concerns about your participation in this research, you may also contact Catherine Connors, Director, Research Ethics, Dalhousie University at (902) 494-1462, or email: ethics@dal.ca

CONSENT FOR STUDY PARTICIPATION

Project Title: The Relationship Between Physical Activity Level and Blood Vessel Health in Young, Healthy Adults

I, _____ have read the explanation about this study. I have been given the opportunity to discuss it and my questions have been answered to my satisfaction. I agree to take part in this study. However, I realize that my participation is voluntary and that I am free to withdraw from the study while it is ongoing, and up to 2 weeks following testing.

_____ _____

Participant's Signature *DATE*

_____ _____

Print Name of Participant *DATE*

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

If you would like to be contacted with results from this study, please give us your contact information below and indicate what results you would like to receive.

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

_____

_____

_____

Please send me (please circle one):

GROUP RESULT **INDIVIDUAL RESULTS** **BOTH**