Blood Vessel Health: The Influence of Ageing and Aerobic Fitness

by

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Submitted in partial fulfilment of the requirements
for the degree of Master of Science

at

Dalhousie University
Halifax, Nova Scotia
June 2016

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Abstract

Optimal blood vessel function requires a balance between sympathetic neural vasoconstrictor activity (SNA) and endothelial-derived vasodilatory nitric oxide (NO) bioavailability. Ageing is associated with elevated resting SNA and reduced NO production. Aerobically-trained older adults (OT) have higher SNA compared with their untrained age-matched peers (OU). An explanation for higher SNA is unclear but may serve to 'buffer' a corresponding increase in NO production.

PURPOSE. To test the hypothesis that OT will: 1) have higher resting SNA than aerobic fitness-matched young adults (YA) and OU, 2) have similar endothelial-mediated NO production to YA, but greater than OU, and 3) observe a positive relationship between resting SNA and popliteal artery (PA) flow-mediated dilation (FMD).

METHODS. Resting common fibular nerve muscle SNA (MSNA, microneurography) and PA endothelial-dependent FMD were determined in 8 YA (23±2yrs, VO_{2peak} 49.2±9.4mLO_2/kg/min), 8 OU (63±6yrs, VO_{2peak} 28.1±5.2mLO_2/kg/min), and 8 OT (65±3years, VO_{2peak} 49.4±3.2mL O_2/kg/min).

RESULTS. MSNA was higher in OT than YA (71±20 vs. 29±19 bursts/100heartbeats; p=0.006) but not OU (43±32 bursts/100heartbeats; p=0.1). FMD was higher in both YA and OT (9.5±1.5% and 12.1±2.4%, respectively) than OU (6.7±1.1%, all, p<0.02). In OT, the correlation between MSNA and FMD was significant (r=0.89, p=0.003).

CONCLUSIONS. This study marks the first attempt to concurrently measure MSNA and FMD in older adults. Our data suggests that exaggerated resting MSNA levels in OT adults may be a compensatory mechanism to 'buffer' a corresponding increase in NO production to maintain vascular tone and arterial pressure.

WORD COUNT: 250

KEY WORDS: Endothelial function, muscle sympathetic nerve activity, ageing, aerobic fitness.

List of Abbreviations Used

ADMA- asymmetric dimethylarginine
ATP- adenosine triphosphate
BI- burst incidence
BF- burst frequency
BMI- body mass index
cGMP- cyclic guanosine monophosphate
CVD- cardiovascular disease
DBP- diastolic blood pressure
ECG- electrocardiography
EDD- endothelial-dependent dilation
EID- endothelial-independent dilation
eNOS- endothelial nitric oxide synthase
FMD- flow-mediated dilation
H^+- hydrogen ion
HR- heart rate
K^+- potassium ion
L-NMMA- *N*-monomethyl-L-arginine
LVC- leg vascular conductance
LVR- leg vascular resistance
MAP- mean arterial pressure
MLCK- myosin light chain kinase
MLCP- myosin light chain phosphatase
MSNA- muscle sympathetic nerve activity
NE- norepinephrine
NO- nitric oxide
NTG- nitroglycerin
NTS- nucleus of the solitary tract
OT- older trained
OU- older untrained
PAD- peripheral artery disease
Q- cardiac output
RER- respiratory exchange ratio
ROI-region of interest
SBP- systolic blood pressure
SNA- sympathetic nerve activity
SNS- sympathetic nervous system
SR_{AUC}- shear rate area under the curve
SV- stroke volume
TPR- total peripheral resistance
TVC- total vascular conductance
VCO_2- volume of carbon dioxide production.
VO_{2peak}- peak volume of oxygen consumption
VSM- vascular smooth muscle
YA- young adult

Acknowledgements

I would first like to thank my supervisor, Dr. Derek Kimmerly for his continuous support throughout my Masters work and research. His patience, support, and knowledge in the subject helped to steer me when I needed guidance, but he also allowed me to formulate this project into my own and was there when I needed anything at all. The learning that I have done in his lab throughout this process is invaluable and I leave this program a better student and researcher because of it.

Besides my supervisor, I would like to thank the rest of my thesis committee, Dr. Scott Grandy and Dr. Gail Dechman, for their insights and helping to guide and encourage me through this process.

Additionally, I would like to thank Dr. Saïd Mekary and all of the people at Acadia University who I had the pleasure to work with, for helping with my research and making the process so easy.

To all of the other individuals at Dalhousie who were there when I had questions or just needed to talk to someone: Dr. Lori Dithurbide, Dr. John Kozey, Dr. David Westwood, and Dr. Heather Neyedli- I cannot thank you enough.

All of the people who worked with me for countless hours in the lab and library, I can't name you all but especially thank you to Julia Kontak, Josh Goreham, Susan Robinson, and Julie Weir for keeping me sane these last years. Additionally the friends at home, Haydie Wood, Austin Zhang, Laura MacEachearn, Kira Swan, and Benia Nowak, words cannot describe how much your friendship and the countless laughs and beers we've shared have helped me, thank you!

Last, but definitely not least, thank you to my family. I really would not have been able to do any of this without your constant love and support, even from halfway across the world. Mom, Dad, David, and Elizabeth I love you so much and thank you for all you do putting up with me. Your continuous encouragement helped me every day. None of this would have been possible without any of you!

Thank you,

Diane Ramsay

Chapter 1: Introduction

1.1- Introduction

In developed, high-income countries, heart disease and stroke are the leading causes of death (132). In Canada alone, the cost of cardiovascular disease exceeds $23 billion per year (20) and there are currently 1.6 million Canadians living with the effects of heart disease or stroke (19). Changes to the cardiovascular system occur with ageing, however it has been well established that one cost-effective preventative measure to reduce the risk of cardiovascular disease and some of the detrimental effects that occur with ageing is to participate in regular physical activity (50, 161).

Healthy blood pressure regulation and blood flow distribution are achieved through an intricate balance between competing chemically- and neurally-mediated mechanisms. The release of nitric oxide (NO) from the endothelial cells that line blood vessels results in vascular smooth muscle (VSM) relaxation and subsequent vasodilation. Conversely, innervation from post-ganglionic sympathetic nerves to the VSM causes contraction (i.e., vasoconstriction). An imbalance of these mechanisms results in exaggerated vasoconstriction, and contributes to the progression of arterial hypertension and the development of cardiovascular disease (CVD) (70, 105, 200). Though this imbalance can happen during the ageing process, engaging in physical activity can mitigate these adverse effects.

Flow-mediated dilation (FMD) is one technique to measure endothelial-dependent NO bioavailability and endothelial-mediated vasodilatory function (67, 97, 186). FMD is a commonly used clinical test due to its non-invasive nature, and through the utilization of ultrasound imaging, FMD can be used to perform repeated, reliable measures with proper technician training (40). The FMD test is as predictive of future CVD events as traditional risk factors such as smoking, obesity, and diabetes (186, 216). FMD responses are typically higher in those who are active and younger, and decreases with age and sedentary lifestyle (11, 96, 114, 140).

Sympathetic nerve activity (SNA) is chronically heightened in humans with CVD such as hypertension (2) and congestive heart failure (52, 128) but also increases in humans as they age (120, 156). In humans, SNA directed towards skeletal muscle arterioles (i.e., muscle sympathetic nerve activity, MSNA) can be recorded with microelectrodes from superficial nerves using the microneurography technique (35, 175, 198). MSNA levels are typically lower in younger individuals, and rise with age (84, 156), potentially increasing the risk of hypertension and other CV issues (16, 90). By measuring the FMD response and the MSNA of an individual, two markers of their vascular function can be attained.

1.2- Nature of the Problem

Based on cross sectional data, sedentary ageing is associated with a lower FMD response (76), and higher resting levels of MSNA (122) compared to their younger peers. However, this pattern is altered in aerobically trained older adults. Specifically, older endurance trained athletes have greater FMD responses than their age-matched sedentary peers (37, 215) and similar to that of a younger healthy population (113, 140, 143). Counter-intuitively however, resting levels of MSNA of these older athletes is also higher than that of their sedentary counterparts (125). Possible reasoning for this is as a protective mechanism to ensure that these older, trained individuals do not develop hypotension, due to the associated increase in vasodilatory activity. However, the study which investigated the MSNA of the older trained individuals did not measure FMD, so the mechanism is not well understood (125). What is known is that individuals who exercise consistently throughout their lives have a lower risk of developing CVD (50, 161, 183) and so there is a complicated interaction between FMD and MSNA with regards to ageing and aerobic exercise.

There is only one known study which measured reactive hyperemia (with peripheral artery tonometry, a surrogate marker of endothelial function) and MSNA concurrently, while factoring in physical activity levels (180). This study found that in 10 young, healthy, normotensive participants, those with higher levels of MSNA had a significantly lower reactive hyperemia index ($r= -0.80$, $p=$

0.005) (180). Additionally, this study found a significant inverse relationship between MSNA and physical activity hours per week (r= -0.79, p=0.006) and a significant direct relationship between the reactive hyperemia index and physical activity hours per week (r= 0.83, p= 0.004) (180). This study did not directly measure endothelial function and was done with young healthy, participants, and so it is unknown if these results can be generalized to an aged population (180).

The aim of the current cross-sectional study was to quantify how aerobic fitness and healthy ageing influence the relationship between FMD and resting MSNA. This was the first known attempt at measuring these two variables concurrently in an aged population. This was accomplished by measuring aerobic fitness, FMD, and MSNA in three separate groups: 1) older untrained (OU), 2) older endurance-trained (OT), and 3) younger recreationally active (YA). The experimental design involved two study days. On Day 1 participants completed an incremental maximal aerobic exercise test on a cycle ergometer. On Day 2, resting MSNA recordings from the right common fibular nerve, as well as measurements of popliteal artery endothelial-dependent (i.e., FMD) and endothelial-independent dilation were completed. Endothelial-independent dilation was assessed following a sublingual spray of nitroglycerin. We hypothesized that the magnitude of the FMD response would be greatest in the YA group and lowest in the OU group. It was expected that resting MSNA would be lowest in the YA group and highest in the OT group. Finally, we hypothesized that there would be a direct and positive relationship between the magnitude of the FMD and resting MSNA levels in the OT group.

Chapter 2: Review of Literature

2.1- Overview of Vascular Structure and Function

Blood vessels vary in their anatomical characteristics, and all connect to carry blood throughout the body and through the vascular network. To bring oxygen to active skeletal muscles during exercise, blood leaves the heart through the largest vessels, the arteries, which flow into arterioles that branch into capillaries in the muscular bed where gas exchange occurs. Deoxygenated blood is then carried back to the heart through venules and then the larger veins (50). The most important blood vessels for the control of vascular tone and tissue perfusion are peripheral arteries and arterioles, due to their thick smooth muscle layer and their ability to change the diameter of their lumen (50).

Due to the high pressure the arteries and arterioles must withstand as they transport blood away from the heart, they are thick blood vessels and contain large amounts of collagen and elastin in order to allow the arteries to expand and recoil with each heart beat (166). The arteries have large amounts of collagen and elastin, meaning the passive elastic recoil of the arterioles is important as it helps to preserve energy, promotes laminar flow, and helps maintain a continuous flow of blood throughout the vascular tree (29). Arteries are composed of three layers, the tunica adventitia, tunica media, and tunica intima layers (Figure 1). The outer tunica adventitia (or tunica externa) layer is composed of collagen and contains the sympathetic nerve endings as well as the vascular blood supply (i.e., the vaso vasorum). The middle tunica media is composed mostly of vascular smooth muscle cells, embedded in a matrix of collagen, elastin and glycoproteins, which are responsible for the maintenance of arterial lumen diameter and vascular tone. The innermost layer (i.e., the tunica intima) consists of a single layer of endothelial cells (166).

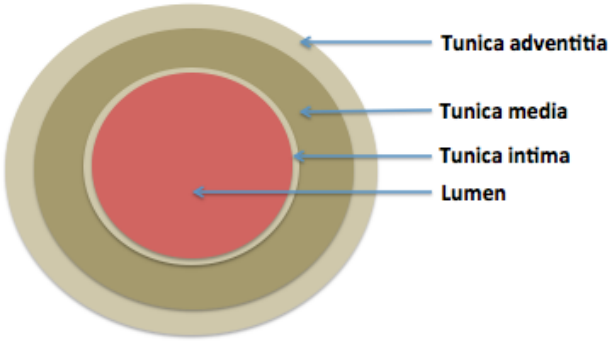

Figure 1. *Layers of an arterial blood vessel*

The vascular smooth muscle (VSM), which makes up the tunica media of blood vessels, is the contractile component of the arteries. The VSM is innervated by the sympathetic nervous system, which causes vasoconstriction via release of norepinephrine (NE). It is a sustained tonic contraction, which narrows the lumen of the blood vessel. The VSM cells are layered through the tunica media, with VSM filaments running diagonally alongside each other, without the visible striations that are present in skeletal muscle cells (see Figure 2). They have a single, central nucleus, and gap junctions connect adjacent cells for chemical and electrical communication (166). The relative thickness of VSM is greatest in small arteries and large arterioles, which is consequently where there is the highest density of sympathetic innervation exists (71).

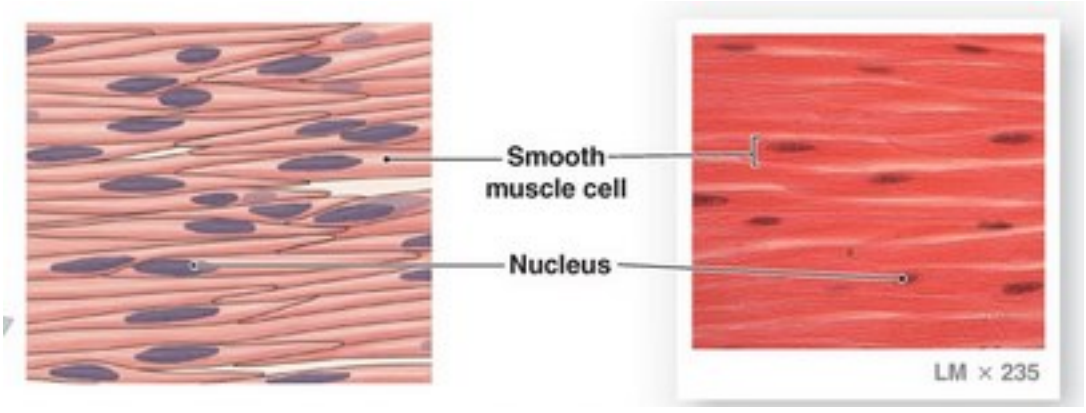

Figure 2. *Vascular smooth muscle cells* (162)

During the activation of the SNS, neuropeptide Y, ATP, epinephrine and NE are released from post-ganglionic sympathetic nerve endings into indentations in the VSM called calveoli, where they binds with their respective receptors on the VSM

(166) causing an increase in intracellular calcium concentrations. Calcium binds with calmodulin, a regulatory protein, and this calcium-calmodulin complex activates myosin light chain kinase (MLCK), an enzyme that phosphorylates the myosin head (50). This phosphorylation allows the myosin head to bind with actin and cause contraction in the VSM, leading to vasoconstriction (see Figure 3).

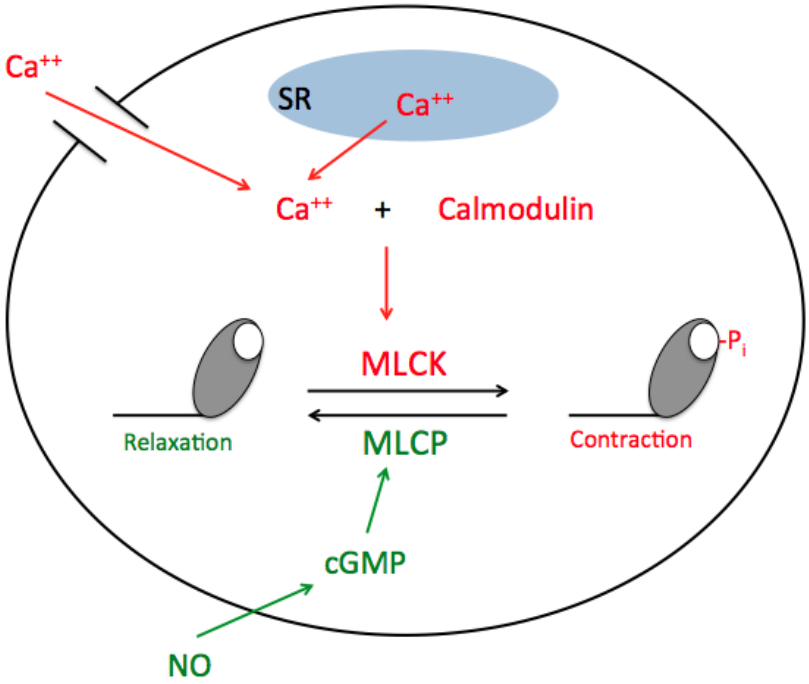

Figure 3. *Mechanism of vascular smooth muscle contraction and relaxation. SR- sarcoplasmic reticulum; Ca^{2+}- calcium; MLCK- myosin light chain kinase; MLCP- myosin light chain phosphatase; P_i- phosphate; NO- nitric oxide; cGMP- cyclic guanosine monophosphate.*

VSM relaxation occurs when the phosphate that was attached to the myosin light chain by MLCK is removed and the actin-myosin complex dissociates. Nitric oxide (NO) mediates this dilation through activation of cyclic guanosine monophosphate (cGMP). Increased cGMP concentrations in the VSM activates myosin light chain phosphatase (MLCP), the enzyme that removes the phosphate from the myosin light chain (131). Once this phosphate is removed, relaxation and vasodilation occur (see Figure 3). Additional mechanisms, which cause vasodilation through an NO-mediated pathway, also exist. For example, NO has the ability to blood extracellular calcium entry, therefore preventing contraction (101).

Additionally, NO may potentially directly open outward rectifying potassium channels, therefore causing hyperpolarization of the VSM cell and subsequent vasodilation (108).

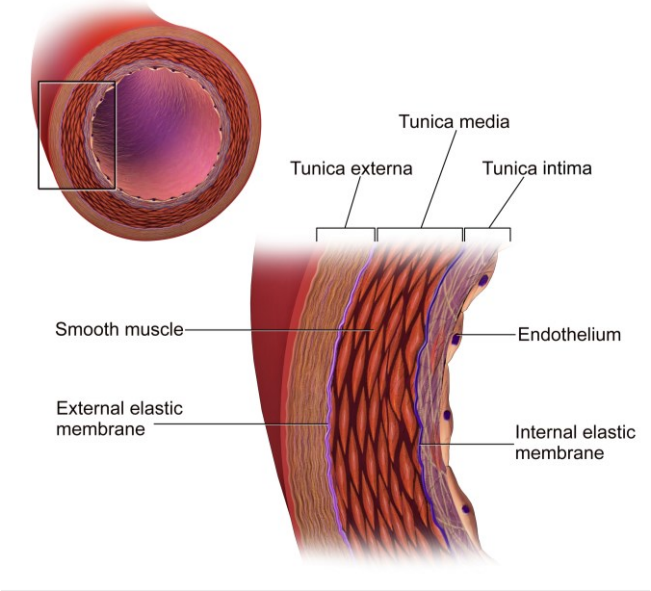

Figure 4. *The structure of an arterial blood vessel, showing the innermost endothelial layer* (10)

Moving inwards from the tunica media is the tunica intima, the innermost layer of the arteries and arterioles. Every blood vessel in the body, from the heart to the microscopic capillaries, is lined with vascular endothelial cells (see Figure 4). The vascular endothelium is a metabolically active organ system which secretes chemicals that regulate smooth muscle vascular tone such as the potent vasodilator nitric oxide (NO) (166, 174). The importance of an intact endothelium cannot be overstated. This is evident as acetylcholine, serotonin, NE, and histamine can all be NO agonists and cause VSM relaxation with an intact endothelial layer, but it has been found that when this layer is removed, these substances all cause VSM contraction (18). There are dilating factors other than NO such as prostaglandins, and endothelial-hyperpolarizing factors, which lead to the relaxation of VSM and dilation of arterial blood vessels (12, 71) however NO is one of the most potent (89). These regulatory dilatory factors are released in response to either chemical

stimuli (e.g. acetylcholine) (58) or physical stimuli (e.g. increase in shear stress on the blood vessel walls) (146). In addition to these dilatory chemicals, the endothelial cells release potent vasoconstrictors such as endothelin-1, thromboxane A_2, prostaglandin H_2, and angiotensin (39, 103). Endothelin-1 is the foremost contracting factor in the peripheral vasculature, and is released in response to thrombin, interleukin factor-1, epinephrine, angiotensin II, and vasopressin (103) in order to aid with blood clotting and to increase or maintain blood pressure.

The vascular endothelium plays other important roles within the cardiovascular system. Endothelial cells can help ensure that oxygenated blood is carried where it is required in the body. If local hypoxia is detected, cells increase their production of hypoxia-inducible factor-1, which in turn increases vascular endothelial growth factor, leading to proliferation of endothelial cells in a process known as angiogenesis (1). Endothelial cells are also pivotal to the immune and inflammatory response, controlling leukocyte movement from the blood stream into tissues and simultaneously dilating blood vessels at a site of injury or infection (174). Additionally, endothelial cells facilitate blood flow, which is done by providing a surface that inhibits platelet adhesion and clotting during normal, healthy function (28). This may change however if an injury occurs, transforming that area of the endothelium to a prothrombotic surface (28, 174).

The endothelial cells are vital for controlling vasodilation, triggered locally by an increase in anterograde shear stress. Anterograde shear stress is induced by an increase in forward blood flow through arteries and arterioles, with an associated increase in the frictional forces that red blood cells induce upon the endothelial cells lining the lumen. As blood flow increases or flows faster through the lumen, the force caused by the red blood cells rubbing against the endothelial cells is detected by cilia-like mechanoreceptors (33). This shear stress is by far the most important stimulus for NO release and subsequent vasodilation (21). As illustrated in Figure 5, this shear stress opens calcium channels on the endothelial cells, and the increased intracellular calcium activates the enzyme endothelial nitric oxide synthase (eNOS), which separates NO from the amino acid L-arginine (33, 71). This

NO then rapidly diffuses into the VSM, where it stimulates soluble guanylate cyclase, leading to an increase in cGMP concentration, which induces VSM relaxation, as described in detail above (see Figure 3).

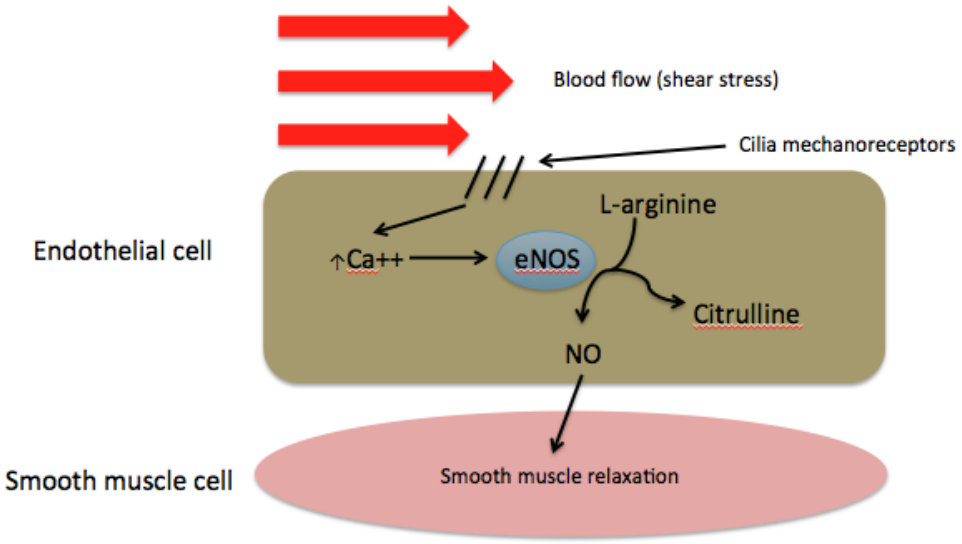

Figure 5. *Nitric oxide production cascade stimulated by shear stress on arterial endothelial cells. Ca++, calcium; eNOS, endothelial nitric oxide synthase; NO, nitric oxide.*

Nitric oxide (NO) also has a myriad of other roles in the body. It has roles in neuronal activity, metabolism, and immune responses (59) and its actions can differ based on the local environment and concentration of NO (34). Within the cardiovascular system, in addition to regulating vascular tone, NO regulates local cell growth, protects vessels from potential injury from circulating substances in the blood, and regulates inflammatory and reparative responses to local injury (21). Decreased NO bioactivity in the endothelium has been linked with increased oxidation of lipoproteins and the formation of foam cells, the precursor to atherosclerotic plaque (21).

2.1.1- Measurement of Endothelial-Dependent Dilation, Endothelial-Independent Dilation, and Muscle Sympathetic Nerve Activity

Endothelial-Dependent Dilation: The FMD technique can be utilized to quantify the degree of endothelium-dependent dilation (EDD) induced by the actions of NO

(166). FMD is most commonly performed on the brachial, radial, or femoral arteries due to their superficial anatomy (118). An ultrasound machine is used to collect transverse images of the artery and a pressure cuff is placed distal to where these arterial lumen diameter measurements are being recorded (136, 145). Images of the artery are measured before, during, and after a period of blood flow occlusion with the pressure cuff inflated to a level above systolic pressure. This period of occlusion leads to an increase in blood flow after the cuff is released, in a process known as reactive hyperemia, which causes an increase in shear stress on the endothelium. As outlined previously, shear stress is the stress placed by the frictional forces of the blood flowing through the vessels on the vessel walls and it is calculated with the use of the Hagen-Poiseuille equation (137). The equation is:

Resistance to blood flow= $(\eta * L)/r^4$, where η is the blood viscosity, L is the length of the vessel, and r is the radius of the vessel (109).

As the blood viscosity and length of the vessel of interest do not significantly change during an FMD session, changes in resistance to blood flow are driven by changes in the radius of the vessel. The increase in shear stress induces an increase in NO release and causes VSM relaxation and arterial dilation, increasing the arterial lumen diameter and decreasing resistance to blood flow (134).

The FMD response is quantified as a change in the arterial lumen diameter from rest, and it is expressed as both the absolute change (mm) and relative change (percentage, %) (186). A greater amount of arterial dilation indicates a healthier endothelium, whereas a diminished FMD response is indicative of endothelial dysfunction (136). Normal values vary dependent upon the artery in which FMD is performed, however brachial values, the most commonly studied artery, generally range from about 6-10% FMD in healthy individuals, while arteries with a smaller baseline arterial lumen diameter have a larger percentage change (186, 187). In a large study (*n*= 435) by Shechter et al. (2009), brachial FMD responses below the group median of 10.7% were significantly linked with negative CV events, such as all-cause mortality, non-fatal myocardial infarctions, heart failure, stroke, and

coronary artery bypass grafting (p= 0.007) in middle-aged (54 ± 12 years) adults (159). Additionally, Perrone-Filardi et al. (2009) found that a diminished brachial artery FMD (<6.0 ± 2.3%) was a significant predictor of other markers of coronary artery disease (139). Baseline artery diameters have also been shown to differ with age and sex. Sandgren et al. (1998) found larger baseline popliteal artery diameters as individuals age and also found that males have a larger average baseline lumen diameter than women (153).

Endothelial-Independent Dilation: The importance of the endothelial cells cannot be understated, but the reactivity of the VSM is also extremely important. As outlined in Figure 3 and Figure 5, the diffusion of NO into the VSM causes the activation of cGMP and subsequent relaxation of the VSM and dilation of the arteries. The administration of nitroglycerin (NTG) can be used to assess the reactivity of the VSM, independent of the reactivity of the endothelial cells. Endothelial-independent dilation (EID) can be measured when NTG is administered, as NTG is an NO donor, which activates guanalyl cyclase and dilates the blood vessels (82).

Ultrasound images of the arteries at baseline and after administration of NTG are used to assess the change in lumen diameter, and larger amounts of dilation are linked with higher EID and VSM reactivity (140, 219). A typical response to NTG administration in the brachial artery is ~18-28% dilation in healthy individuals (76, 140) and in the brachial artery the peak response is typically found 3-4 minutes after administration (30, 43). The pattern observed with FMD induced EDD and its relationship to baseline lumen diameter is also present with NTG induced EID, wherein arteries with a smaller baseline lumen diameter exhibit a larger percentage change in lumen diameter (187).

Muscle Sympathetic Nerve Activity: The NO-mediated dilation that occurs in the arteries is counteracted by sympathetic nerve activity that induce vasoconstriction (166). Resting levels of sympathetic activity to the arterioles located within skeletal muscle beds (muscle sympathetic nerve activity, MSNA) can be measured using a technique called microneurography. Microneurography directly measures efferent postganglionic nerve activity in human peripheral nerves by measuring the

frequency of neural impulses (198). This technique involves inserting a microelectrode directly into a fascicle containing post-ganglionic sympathetic nerves, while inserting a second reference electrode subcutaneously a few centimeters away. The reference electrode has no insulation, while the active needle has insulation everywhere except for on its tip, to detect the sympathetic neural action potentials. Nerve discharges are identified as the difference in voltage between these two electrodes. These nerve impulses are then amplified (~75 000 times), bandpass filtered (500-2000 Hz), full wave rectified, and integrated (0.1 second time constant) to produce a mean neurogram (see Figure 11 and more details in Section 2.3) and it has been shown that these measures correlate with overall sympathetic nerve activity (106, 180, 198).

An intricate balance between resting levels of MSNA and the vasodilatory action of the endothelium helps to maintain resting vascular tone in the arteries. The relationship between these competing factors changes during healthy ageing to favor higher levels of sympathetic activity (75, 84, 189). With aerobic exercise training during ageing, this is balanced by enhanced endothelial function (55, 158, 202). The following sections will highlight these mechanisms, the literature exploring the measurement of these factors, and current knowledge on the influence of healthy ageing and aerobic exercise on NO-mediated vasodilation and SNS vasoconstriction.

2.2- Vasodilation, Nitric Oxide, and Flow-Mediated Dilation

Celermajer, et al. in 1992, first introduced flow-mediated dilation as a method to measure endothelial function in conduit arteries (24). This method involves the use of a pressure cuff placed distal to the location where arterial dilation will be measured and inducing a period of ischemia (186). This was developed after it had been established by Furchgott that the endothelium produces vasodilators and that there was a strong link between an intact endothelial lining, shear stress, and the amount of dilation (142, 165). The aim of the FMD procedure is to temporarily cut off blood flow with a pressure cuff, and when this cuff is released, there is a subsequent large increase in blood flow and shear stress, will cause endothelial-

dependent vasodilation (24, 30, 186). This is caused by the hypoxia and metabolite build up (e.g., K^+, CO_2, H^+) in the tissue being occluded, leading to dilation of downstream arterioles and a decrease in vascular resistance (42, 186). The diameter of the lumen of the artery of interest is measured before, during, and after the cuff inflation, and the baseline arterial lumen diameter is compared to the largest dilation measured after the cuff deflation. This change is expressed as either an absolute or percentage change from baseline, and compared to norms for the individual's age, sex, and the artery being measured (186).

The results of an FMD assessment can be an important indicator of overall vascular health (91). FMD measures have been shown to be related to CVD independent of numerous other cardiovascular risk factors (such as cigarette smoking, increased age, male gender, and larger vessel size) in asymptomatic patients (23). Using a subset from the Framingham Heart Study, it was determined that relative FMD was inversely related to age, systolic blood pressure, body mass index (BMI), lipid-lowering medication, and smoking, and it was positively related to female gender, prior exercise, and heart rate (9). FMD also strongly predicts cardiovascular events in patients with cardiovascular disease (CVD), and is at least as predictive as traditional risk factors in this population (83, 186). It is also an independent predictor of cardiovascular events in patients with peripheral artery disease (PAD) (15). Based on this published literature, it is evident that FMD is an important measure of endothelial function and overall cardiovascular health.

Flow-mediated dilation uses high-resolution 2D-mode or B-mode ultrasound as the preferred instrument for measuring arterial lumen diameter as this uses a linear array of transducers to simultaneously scan a plane in the body that can then be viewed as a two-dimensional image (220). B-mode ultrasound is cost-effective, noninvasive, and reproducible with proper technician training (40, 186, 212). When the artery is viewed in the longitudinal plane, the double lines of Pignoli are visible (141) leading to precise measurements of arterial lumen diameter (Figure 6). Measures of arterial lumen diameter are made more precise and reliable through the use of automated edge-detection software, which has been shown to increase reliability and decrease inter-technician variability (31, 40, 186, 212).

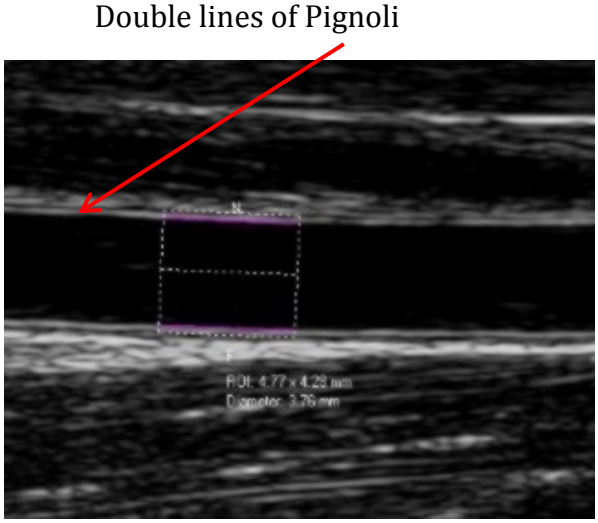

Figure 6. *Longitudinal view of the artery with B-mode ultrasound, with automated edge-detection software being used* (111).

Additionally, because of the importance of shear rate to the FMD response (134, 145, 146), duplex ultrasound is recommended in order to obtain simultaneous measures of pulsed-wave Doppler velocity signals as well as B-mode images. Due to the simultaneous recording of both signals and the differing requirements for each (B-mode optimized at 90 degrees while, pulse-waved Doppler optimized at 0 degrees) (98, 220) a compromise must be made to achieve both valid images and valid Doppler velocity signals. Since unreliable calculations of pulsed-wave Doppler flow velocity occur when insonation angle is >60 degrees (see Doppler equation in Figure 7A below), an insonation angle of ≤60 degrees is recommended (98, 186, 220). An example of this insonation angle and the ultrasound screen during recording are depicted in Figures 7A and 7B, respectively.

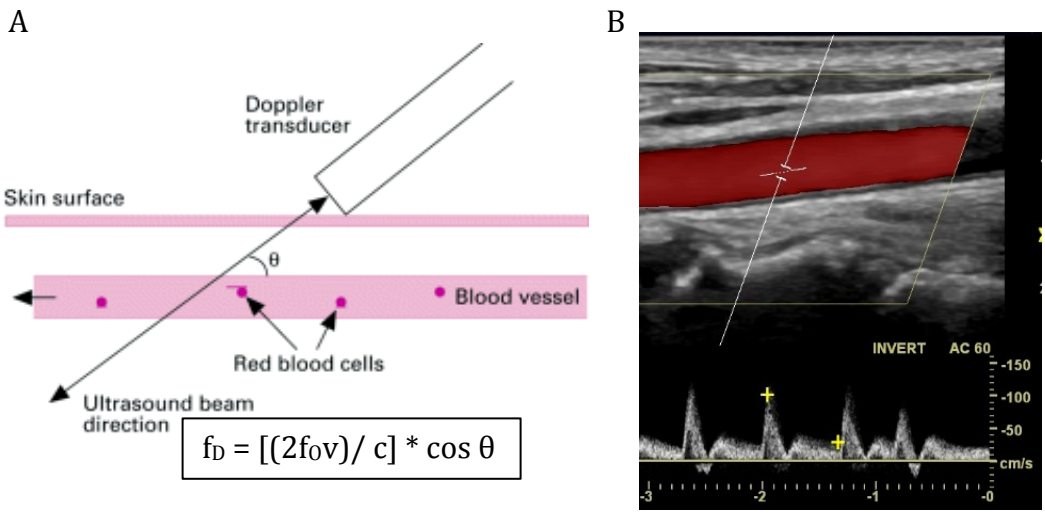

Figure 7. *The duplex B-mode ultrasound set up used for flow-mediated dilation; A. Insonation angle of ≤60 degrees is recommended (θ) in order to calculate the correct blood velocity using the Doppler shift equation, where f_D is the Doppler frequency, f_0 is the transmitting frequency, v is the flow velocity, and c is the speed of sound* (85)*; B. View on the duplex ultrasound screen depicting both the cross section of the artery and the blood flow velocity* (210)*.*

During recording of blood flow velocity, it is important to note the size of the ultrasound sample from which the pulsed-wave Doppler is being calculated. Interchanging a small sample size from the middle of the arterial lumen, which contains the highest velocity of red blood cells due to the laminar flow pattern, and a larger sample encompassing more of the lower velocity red blood cells which flow closer to the vessel walls will change the results and is not possible (47) (see Figure 8A-B). Therefore, the published guidelines recommend choosing one method and using this consistently within a study to improve validity of results (186). For the present study, a large ultrasound sample size encompassing the entire blood vessel lumen was used (as shown in Figure 8B).

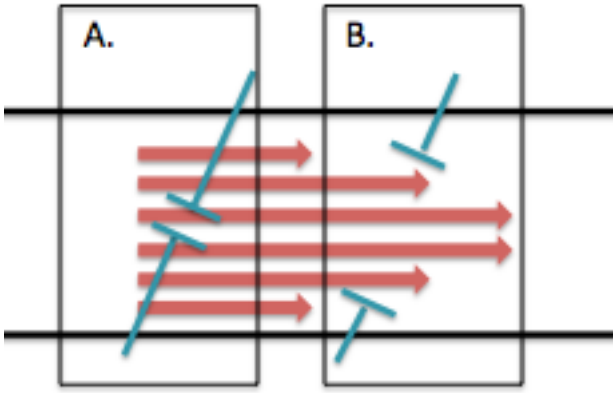

Figure 8. *Different sample sizes affect measurements of blood velocity in the arteries.* ***A.*** *Smaller sample size encompassing flow in the center of the vessel, where flow is moving faster due to the laminar flow profile of blood vessels.* ***B.*** *Larger sample size encompassing flow from the entire vessel, including both the faster flow in the center of the vessel and slower flow close to the arterial walls.*

Flow-mediated dilation has been used in numerous studies and manipulated in various ways to elicit different responses in the artery of interest. In order to measure endothelium-dependent NO bioavailability, many considerations must be accounted for. Changes in the length of the arterial occlusion, position of the occlusion cuff, direction (anterograde versus retrograde) and magnitude of the shear stress all elicit changes in FMD responses, indicating there may be numerous mechanisms acting concurrently during arterial dilation (30, 146, 170, 186). It is therefore important to use a technique to measure FMD that is solely NO-mediated, in order to determine the bioavailability of this potent vasodilator and provide an accurate index of vascular endothelial health.

2.2.1- Length of Occlusion, Position of Cuff, and Nitric Oxide-Mediated Dilation

Although there are other chemical signals that can cause arterial dilation, it has been confirmed that NO is the predominant mediator of dilation during FMD when there is strict adherence to published guidelines (170, 186). While not all studies have shown that nitric oxide is necessary to elicit an FMD response (144) the majority of studies have (42, 51, 53, 88, 97, 186). For example, Joannides et al. (1995) found that radial artery dilation was converted to constriction after 3 minutes of distal occlusion when looking at the FMD response before (3.6 ± 0.8%)

and after (-2.8 ± 0.4%) the administration of *N*-monomethyl-L-arginine (L-NMMA), an eNOS blocker (88). Additionally, Kooijman et al. (2008) found similar results in the femoral artery following administration of L-NMMA. Specifically, the FMD response after 5 minutes of distal occlusion changed from 4.2 ± 0.3% to 1.0 ± 0.2% from pre- to post- L-NMMA administration (97). Though the reduction or eradication of dilation with the infusion of L-NMMA shows that FMD is NO-mediated, there are many variables that must be controlled in order to illicit a fully NO-mediated, endothelium-dependent response.

The length of time that blood flow is occluded can drastically change the measured FMD response. Mullen et al. (2001) found that after 15 minutes of cuff inflation, the FMD response of the radial artery was significantly greater than the response after 5 minutes (118). Although there was no difference in blood flow velocity after the longer occlusion, the duration of the reactive hyperemia was significantly larger, leading to a greater total blood flow volume and therefore a greater total shear stress, the stimulus for NO production (118). Additionally, studies have found that infusion of L-NMMA abolished the FMD response in arteries in response to a short occlusion (5 minutes) but does not affect the response after a longer occlusion (15 minutes) (10, 118). This evidence indicates that after longer periods of occlusion, there may be different mechanisms working either alongside or instead of the initial NO-mediated dilation. Other vasodilators, such as prostaglandin, which activates the adenylyl cyclase pathway and increases VSM dilation factors such as cyclic adenosine monophosphate (21, 103) may be activated. Endothelial cells, in response to shear stress, also release prostaglandin, but in contrast to NO, it is additionally released during hypoxia, explaining why a longer period of occlusion would not be affected by L-NMMA infusion (103).

In addition to the length of occlusion being important, it has also been discovered that the position of the cuff during occlusion results in differing FMD responses. Both Betik et al. (2004) and Doshi et al. (2001) found an increased FMD response in the brachial artery with a proximal cuff position compared to a distal cuff position, despite similar peak flow stimuli (10, 42). This increased response is likely due to the increased tissue volume being occluded and the action of local

metabolites. It was further confirmed by Doshi et al. (2001) that this larger proximal occlusion was not solely NO-mediated. Specifically, L-NMMA infusion abolished the FMD response elicited by distal occlusion but did not affect the FMD response from proximal occlusion (42). In a meta-analysis by Green et al. (2011), they concluded that distal cuff placement during FMD was 100% dependent upon NO, while proximal cuff placement was only 40% dependent upon NO, using changes observed with either a saline control or L-NMMA infusion (67). Vasodilators such as prostaglandins, adenosine, and eicosatrienoic acid may be activated locally during proximal occlusion to maintain a basal level of vasodilation in the arteries (21, 42, 103).

2.2.2- Shear Stress and Blood Flow Direction and Flow-Mediated Dilation

As shear stress is the main stimulus for NO release in the arteries, any changes in the direction (i.e., retrograde versus anterograde) or magnitude of blood flow affects the FMD response. Retrograde (reverse) flow can have a detrimental effect on endothelial health, and it has been shown that in young healthy human subjects, an increase in retrograde shear rate decreases FMD in a dose-dependent manner in the brachial artery (155, 188, 189) and in the superficial femoral artery (155). The retrograde flow may increase the expression of the endothelium-derived vasoconstrictor endothelin-1 and decrease the expression of eNOS (218), therefore contributing to endothelial dysfunction in the long term. While acute bouts of retrograde shear decreases FMD in young healthy individuals, it has been found that these same stimuli do not impair endothelial function in older individuals (155) suggesting that subjects with already diminished EDD, as seen in older individuals, may be less responsive to the same stimuli.

Along with the direction of the shear stress stimulus, the magnitude also affects the FMD response. The total shear stimulus area under the curve (SR_{AUC}) has been shown to be a more important determinant of FMD than the peak amplitude of the shear stimulus, with the SR_{AUC} being measured from the point of cuff deflation until the point of maximal dilation in the artery (147). Additionally, there are individual factors such as different baseline diameters or different responses to the same stimulus (Figure 9), which can be controlled with normalization of the FMD response (outlined in Section 3.5). All of the above evidence displays the large effect of any small changes in either the administration of the FMD or the analysis of the results, and therefore the importance of following published guidelines when measuring NO-induced FMD (170, 186).

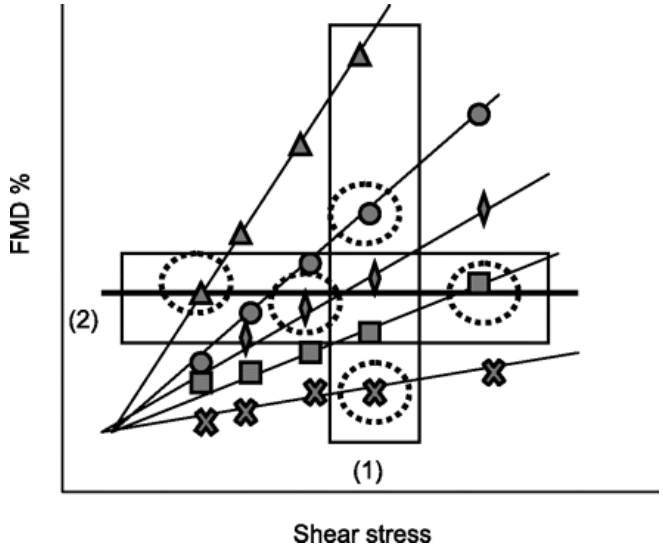

Figure 9. *A graphical example depicting the effects of individual differences on shear rate on percentage flow-mediated dilation. The vertical box (1) depicts individuals with different FMD% response to the same shear stress, while the horizontal box (2) depicts individuals with the same FMD% response to different shear stress stimuli* (186)*. FMD%, percentage change in arterial lumen diameter from baseline after a flow-mediated dilation test.*

2.2.3- Between Limb and Between Artery Flow-Mediated Dilation Heterogeneity

While the majority of studies utilize upper limb arteries to measure FMD, lower limb FMD is also of importance. There is a limb difference in FMD responses, with

the femoral artery showing a decreased FMD response to the same shear rate as compared to the brachial artery in healthy subjects (214). This difference in limb responses has been attributed to the effects of constantly higher blood pressures in the leg due to the hydrostatic effect of gravity (122). Additionally, differences in muscle mass and sustained increases in blood flow to the lower limbs due to locomotion may lead the lower limb arteries to be less responsive to the same magnitude of stimuli as the upper limb (215). Subjects with early stages of peripheral artery disease (PAD) have also been shown to have a reduced FMD response in the leg compared to the arm, showing impairment of leg circulation may occur before impairment of arm circulation (138, 179). The possibility of using the non-invasive FMD method for early detection of PAD is clinically important in addition to its usefulness as a predictor of future cardiovascular events (186).

In addition to limb differences, absolute FMD differences between arteries of the same limb have been found. Thijssen et al. (2008) measured FMD in the brachial, radial, common femoral, superficial femoral, and popliteal arteries, and found that baseline (i.e. resting) artery lumen diameter was inversely related to the peak FMD response (187). The exact mechanism for this difference is unclear, but it was suggested that the blunted FMD responses in larger arteries might be related to differences in the magnitude of the shear rate between arteries or the differing wall-to-lumen ratio that exists in arteries of different sizes (187, 192).

It has been hypothesized that this difference in shear rates between arteries and between individuals can be accounted for by normalizing the relative FMD change by dividing it by the shear rate. Pyke et al. (2004) and Padilla et al. (2008) have both found that controlling for shear rate decreases both the between subject variability and abolishes within artery FMD responses due to differing baseline diameters (134, 145). This normalization however, does not eliminate all differences in FMD between different arteries, as FMD differences were still visible in different arteries with only glyceryl trinitrate administration (a vasodilator) and no associated differences in shear rate (187). Furthermore, evidence has been presented questioning the mathematical appropriateness of this normalization

method, due to the relationship between baseline lumen diameter and differences in arterial lumen diameter, the two variables used to calculate FMD (6–8, 187, 199).

Atkinson (2014) has suggested the use of allometric scaling to isolate the FMD response and remove any confounding factors before analysis is performed, therefore removing the need for normalization (8). Briefly, allometric scaling is a process by which both the baseline and peak arterial diameters are logarithmically transformed, and then the slope of the regression between these two variables is determined. The slope of this line is determined to be the allometric scaling exponent, and an ANCOVA is performed to determine the new changes in diameters, and the scaled FMD change can be calculated. This method has been used for FMD values many times with the methods described in depth numerous times (5–8). Additionally, more details are available in Section 3.5- Data Analysis. As the debate surrounding the normalization vs. allometric scaling of the FMD response is still on going, both methods will be reported in the present study.

2.2.4- Ageing, Sex Differences, and Flow-Mediated Dilation

Sex hormones have a dramatic effect on endothelial function (85) with female circulating sex hormones, specifically estradiol, hypothesized to have vasoprotective properties (76). It has also been shown that FMD measures can vary during different phases of the menstrual cycle, as different sex hormones rise and fall, with the highest FMD measures being recorded during the phases where estradiol levels were highest (76). The lower incidences of CVD and improved endothelial function that are present in premenopausal women, compared to men and post-menopausal women, may be due in part to the vasoprotective properties of female sex hormones (66, 133).

The mechanism for the endothelial dysfunction that develops with ageing is not fully known but there is evidence for both structural changes to the blood vessels and also chemical changes. With increasing age, FMD decreases independent of other factors that affect vascular health (69, 157, 183, 213). With age, arterial remodeling has been shown to occur, with increases in the arterial wall thickness and increases in the wall-to-lumen ratio (69, 119). As Green et al. (2010) found,

these changes affect limbs differently, with the popliteal artery having larger increases in wall thickness and baseline lumen diameter than the brachial artery (69) and the popliteal artery baseline lumen diameter increasing steadily with increasing age (153). This is important, as it has been shown that increased arterial wall thickness leads to a lower FMD response (119, 192) and a larger baseline lumen diameter is also related to a lower FMD response (123, 187, 213). Additionally, arterial elasticity decreases with age, due to changes in the elastin and collagen which make up the arterial walls (154). This makes it harder for the arteries to dilate in response to stimuli, potentially further impairing FMD responses in older individual (158, 196). These structural changes that occur may contribute to the declines in FMD responses observed with age.

In addition to structural changes, there are chemical changes that occur with increased age. The mechanisms are not completely clear, but there is evidence to indicate strong links to NO-mediated functions (59, 71, 179). The following changes occur with age and may contribute to these chemical changes and associated decline in endothelial function:

- An increase in asymmetric dimethylarginine (ADMA) concentrations, an inhibitor of endothelial nitric oxide synthase (eNOS), along with a concurrent decrease in platelet NO responsiveness (179),
- An increase in the oxidation of NO by free radicals, especially by nicotinamide adenine dinucleotide phosphate (NADPH), a major source of the reactive oxygen species during oxidative stress or cardiovascular disease states (21, 53),
- Oxidative stress increases with age (3) and increases in oxidative stress are linked with CVD and vascular dysfunction (38), potentially by decreasing the bioavailability of NO (57, 157),
- A decrease in soluble guanylyl cyclase, which produces cGMP during VSM relaxation, is also associated with increasing age (27, 94) which may lead to decreased VSM sensitivity to NO.

This evidence shows that the impaired NO synthesis, increased NO oxidation, and decreased NO sensitivity associated with ageing may contribute to the large incidence of CVD and endothelial dysfunction observed in older populations. It is evident that functional NO-mediated pathways and endothelial health are vital during the ageing process in order to maintain good cardiovascular health.

In summary, during the ageing process, there is an increased risk of CVD, due to many mechanisms but the two most important seem to be decreased elasticity of the arteries and increased incidence of endothelial dysfunction (55, 161). Brachial artery FMD progressively declines with age after approximately 40 years in men and 50 years in women regardless of disease status (23). Similar declines are also observed in the lower limbs with age, but studies have found blunted responses in femoral artery FMD compared to brachial artery FMD in older men (215) but not in older, post-menopausal women (138), indicating an intricate interaction is occurring between sex hormones and age with regards to endothelial function (66).

2.2.5- Exercise and Flow-Mediated Dilation

Exercise is commonly prescribed for either the prevention or treatment of cardiovascular disease, and it is known that regular aerobic exercise is associated with less stiffening of the arteries with age (116). Exercise can not only prevent this natural stiffening, but can restore compliance in previously sedentary men (140, 183) along with in individuals with type 2 diabetes (104), coronary artery disease (206), and hypercholesterolemia (207). Additionally, exercise can decrease the incidence of endothelial dysfunction, and there is evidence that regular aerobic exercise can prevent age-related decreases in EDD in healthy men (37, 46, 140). Some studies have found no improvements with exercise training, but this may be due to small sample size or insufficient length of training program (190). Additionally, it has been shown that even acute bouts of inactivity can be detrimental to EDD. One prolonged period of sitting, e.g. 3 hours, can significantly decrease FMD in the superficial femoral artery, however this decrease can be avoided by taking breaks during sitting time (194). Furthermore, Boyle et al. (2013) found a significant decrease in popliteal but not brachial artery FMD after 5

days of reduced activity (≤5000 steps/day), indicating a greater reduction may be due to decreases in the amount of shear stress and blood flow the popliteal artery experienced throughout the duration of the study (14). Boyle et al. (2013) also found an increase in endothelial microparticles, which are linked with endothelial apoptosis, during the 5 days of inactivity (14). In longer-term studies, the type of aerobic exercise does not seem to matter; both sprint interval and endurance training induce similar changes in arterial stiffness and FMD in healthy young participants (150). The volume of aerobic training required to see improvements is unknown, however studies have observed a strong direct relationship between the number of physical activity hours per week and the reactive hyperemia index, or the ability of the artery to dilate in response to an increase in blood flow, in healthy participants (180). This phenomenon of improving FMD in healthy participants has also been studied in rats, and it has been shown that exercise training improves the inhibition of sympathetic vasoconstriction via a nitric oxide-mediated pathway, known as functional sympatholysis (86, 112).

Age-related declines in physiological function seem inevitable, but most negative effects associated with increased age can be prevented or delayed with regular exercise (143, 161). In a recent meta-analysis, Montero et al. (2014) showed that FMD responses in master athletes were significantly higher than their age-matched sedentary controls (113). Pierce et al. (2011) also found that the FMD of middle-aged/older men who had been involved in vigorous aerobic activity at least 5 times/week for ≥45min/session for at least 5 years had ~50% higher brachial artery FMD than their sedentary peers (140). Additionally, it has been found that older athletes have similar plasma nitrate concentrations to that of younger athletes (56). There is also evidence that exercise may produce limb specific local responses to long-term training, potentially due to differences in shear rate (62, 65). Rowley et al. (2011) found improvements in the brachial artery of elite racquet sport athletes in their dominant arm, but not their non-dominant arm, and no difference in the lower-limb arteries as compared to healthy controls (152).

The mechanism for this perseverance of NO and EDD in older athletes is poorly understood. It has been shown that higher fitness levels in postmenopausal women are associated with lower ADMA levels (182) and older athletes have higher antioxidant capacities and higher FMD responses (57), two of the ageing mechanisms that have been linked with endothelial dysfunction. Improvements in FMD can also be observed in older adults who had previously been sedentary. Following a 6-week exercise program, previously sedentary older men saw a significant improvement in brachial FMD (215) and 8 weeks of brisk walking can improve the FMD response in older men (140), indicating vascular plasticity and the ability to improve endothelial function still exists even in older populations. Using FMD to assess EDD in athletes of different ages and comparing this to other markers of vascular health can help to explain this protective mechanism, which has important implications in the prevention or treatment of cardiovascular disease.

2.2.6- Endothelial-Independent Dilation, Sex, Ageing, and Exercise

Blood vessel health is not only dependent upon the function of the endothelial cells that line the vessels, but also on the function of vascular smooth muscle (VSM) that makes up the middle media layer of the arterial wall. The administration of nitroglycerin (NTG) can be used to assess the health of the VSM. NTG is a donor of NO, which will activate cGMP, leading to relaxation and dilation of the arteries and veins (43, 61, 167). This dilation can be measured with an ultrasound machine, taking images both before and after administration of NTG and calculating the difference (30).

The EID has been shown to be similar between men and women of the same age during days 1-7 of the female's menstrual cycle (76), as shown in Figure 10A. Normal values for EID in the brachial artery in healthy participants range from 18-28% dilation (76, 140), much higher than the values obtained from brachial artery FMD (a measure of EDD).

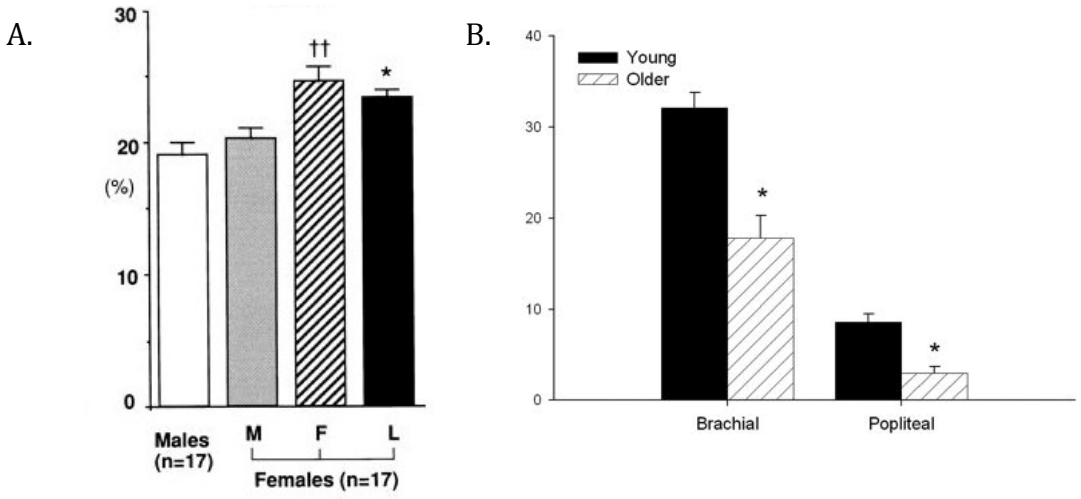

Figure 10. *Effects of sex and age on nitroglycerin (NTG) induced endothelial-independent dilation (EID).* ***A.*** *The effects of sex and menstrual cycle on EID of the brachial artery after NTG administration; M= menstrual phase, F= follicular phase, L= luteal phase, *p<0.05 vs M, ††p<0.01 vs males and M* (76)*;* ***B.*** *The effects of age on EID of the brachial and popliteal arteries after NTG administration; *p<0.05 vs young* (138)*.*

VSM reactivity and therefore EID declines with age in all vessels (11, 114, 138), as seen in Figure 10B. A recent meta-analysis by Montero et al. (2015) has further solidified that VSM function in peripheral resistance vessels declines with age, a contributor to the lower EID observed (114, 217). VSM reactivity and EID are even lower in individuals with diseases states such as peripheral artery disease (96), angina (26), and hypercalcemia (121). Exercise does not affect EID as it does EDD, with no improvements seen in the values of EID with either exercise training interventions (11, 140) or in trained athletes compared to sedentary controls (68, 140). This may be due to irreversible changes to the VSM or the sensitivity of VSM to NO diffusion that occur with ageing, though the exact mechanisms are unknown (114).

2.3- Vasoconstriction, the Sympathetic Nervous System, and Muscle Sympathetic Nerve Activity

Mean arterial pressure (MAP) is a tightly regulated variable and important for ensuring adequate blood flow of vital organs (161, 208). MAP is the product of

cardiac output (Q) and total peripheral resistance (TPR). Both of these variables are under control of the arterial baroreflex and autonomic nervous system. Arterial baroreceptors are located in the aortic arch and the carotid sinus. Since cardiac output is the product of both heart rate (HR) and stroke volume (SV), changes to either parameter can also influence MAP. Heart rate is primarily controlled by the parasympathetic or vagal nervous system while the sympathetic nervous system is largely responsible for changes in vascular resistance. At rest, the arterial baroreflex helps to maintain a high level of vagal innervation to the heart and relatively low levels of sympathetic neural activity to the heart and blood vessels.

The arterial baroreflex has two separate efferent arms, the cardiac (or cardiac-vagal) baroreflex and the sympathetic baroreflex (209). The cardiac baroreflex can be determined by assessing the relationship between HR (or RR interval) to a given change in MAP. The sympathetic baroreflex is determined by assessing the relationship between diastolic blood pressure and vasoconstrictor sympathetic nerve activity (209).

Baroreceptors detect elevations in MAP via a corresponding increase in arterial stretch. This increases afferent neural information to the medullary cardiovascular center where the action potentials synapse on the nucleus of the solitary tract (NTS) (209). The cardiac-vagal arm of the arterial baroreflex is then processed through the nucleus ambiguus and dorsal motor nucleus, which act upon the sinoatrial node of the heart, to reduce HR and decrease MAP (209). If the frequency of baroreceptor afferent firing decreases due to a drop in MAP, the NTS will increase sympathetic nerve activity to the heart and blood vessels from the rostral ventrolateral medulla by removing inhibitory input from the caudal ventrolateral medulla producing tachycardia, and peripheral arterial vasoconstriction (209). An opposite set of responses occurs during transient decreases in MAP (209).

Dutoit et al. (2010) examined the relationship between the two efferent arms of the arterial baroreflex and found there was no relationship between them (44). The study investigated the responses of 53 normotensive, healthy adults (24 ± 1 year)

to rapid changes in MAP stimulated by sequential administration of nitroprusside and phenylephrine while monitoring heart rate, arterial blood pressure, and efferent sympathetic signals to the blood vessels (MSNA) (44). Cardiac-vagal and sympathetic baroreflexes were examined, and no correlation was found between MSNA and the RR interval (r= -0.13) nor MSNA and HR (r= 0.21) (44). These results indicate that each arm of the arterial baroreflex is under separate control and are both important in regulating MAP (44, 209).

Muscle sympathetic nerve activity (MSNA), the efferent sympathetic signals to the blood vessels, can be measured through a process known as microneurography (203, 209). Microneurography was first reported as a method to measure peripheral nerve traffic by Vallbo and Hagbarth in 1966 and since then, the instruments used have dramatically improved (198). In humans, microneurography recordings of superficial nerves are the only way to directly measure vasoconstrictor sympathetic nerve activity signals to arteries located within skin and skeletal muscle beds (124) and is a common procedure used to examine sympathetic nerve activity in superficial nerves (35, 36, 198).

Direct recordings of MSNA are made using sterilized unipolar tungsten microelectrodes. An insulated recording electrode is inserted percutaneously into a superficial muscle nerve fascicle (active microelectrode) and an uninsulated electrode is also subcutaneously inserted 2-3cm from the active recording site (reference microelectrode). The active microelectrode records activity in un-myelinated postganglionic fibres and nerve discharges are calculated by the difference in voltage between the recording electrode and the reference electrode (84), with a differential pre-amplifier secured near the recording site. The active electrode measures the raw signal, which is amplified and band-pass filtered (500-2000Hz). The signal is then full wave rectified and integrated (0.1 second time constant) to produce a mean voltage neurogram of the recorded MSNA (Figure 11). From this mean voltage neurogram, “bursts” of multi-unit sympathetic activity can be determined and MSNA quantified as burst frequency (bursts/minute), burst incidence (bursts/100 heart beats), and burst amplitude.

As microneurography is a measurement of the neural signal and activity in the superficial muscle sympathetic nerves, the actual level of vasoconstrictor activity in an individual blood vessel cannot be directly measured. There are other factors that interact with the vessels to control the arterial tone. The concentration of the neurotransmitters NE, adenosine triphosphate (ATP), or neuropeptide Y, and the sensitivity of their receptors (α_1-adrenergic receptors, P2x or P2y receptors, and Y1 receptors, respectively) can influence the amount of vasoconstriction (71, 198, 201). Additionally, local factors such as carbon dioxide, hydrogen ions, potassium ions, and other local metabolites, as well as circulating factors such as epinephrine and angiotensin II, may influence the levels of vasoconstriction (50, 110).

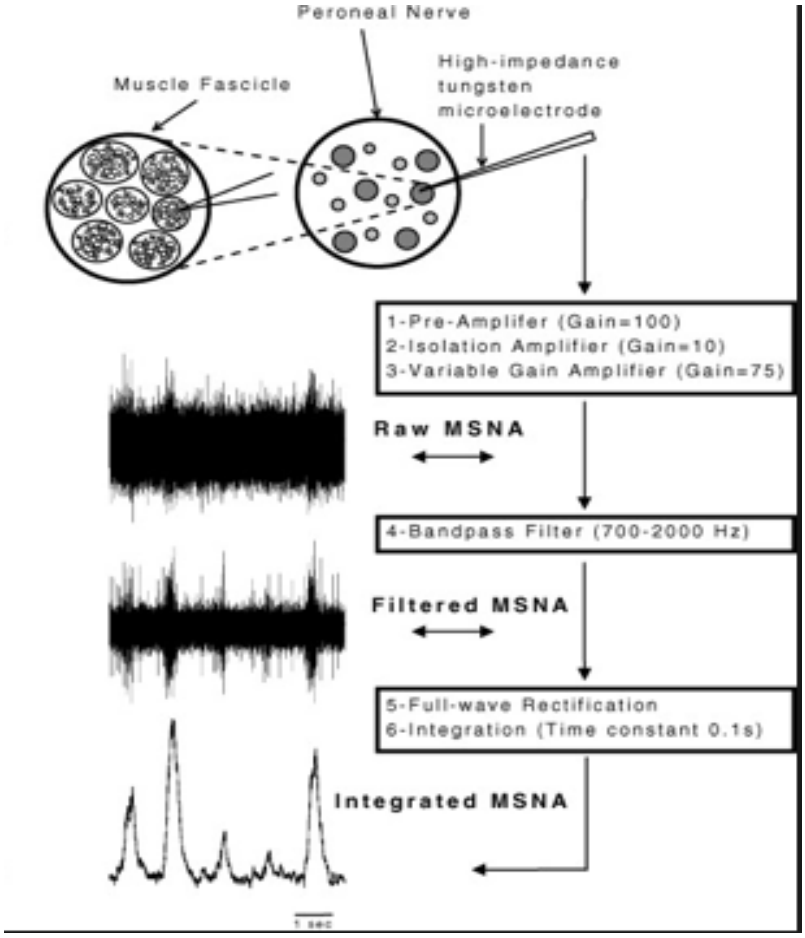

Figure 11. *The process of obtaining a muscle sympathetic nerve activity recording and producing the final mean voltage neurogram* (198).

There are other potential difficulties, for example the risk of collecting data from mixed muscle and skin sympathetic sites or movement of the electrode's tip during data collection (203). There are maneuvers that induce sympathetic activity, which may be used to verify that data are being collected from a muscle sympathetic nerve site, for example observing the sympathetic nervous system response to an expiratory apnea (Figure 12) or a Valsalva maneuver. Additionally, MSNA measures exhibit cardiac synchrony, so every MSNA burst will be associated with a preceding R-wave from an electrocardiography (ECG) (Figure 12).

Microneurography is a reliable tool to measure sympathetic neural signals (106, 198) and its measures are usually closely linked to levels of norepinephrine elsewhere in the body, another measure of sympathetic nervous system activity (22).

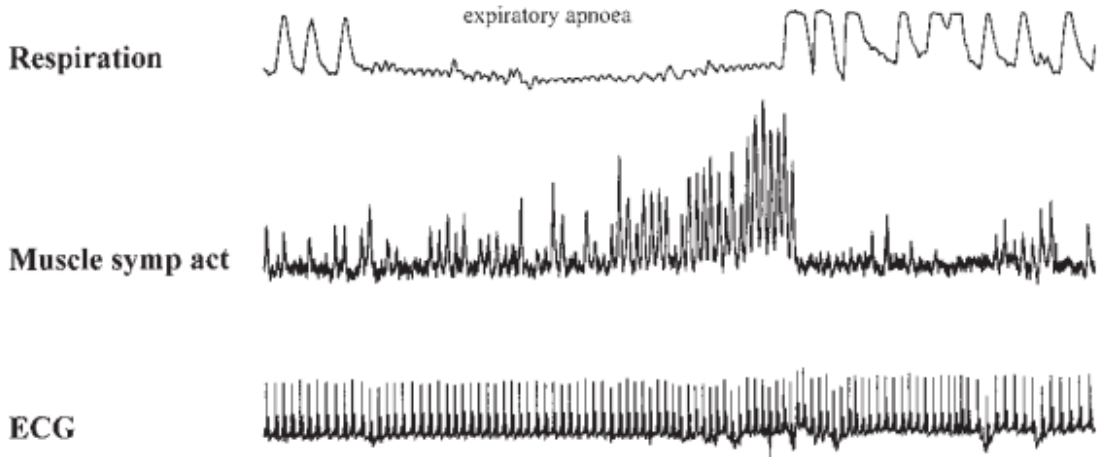

Figure 12. *Example of the respiration movements, integrated muscle sympathetic nerve activity, and electrocardiography measures during an expiratory apnea. The MSNA activity increases towards the end of the expiratory apnea, as sympathetic activity increases* (204).

2.3.1- Age, Sex, Exercise, and Muscle Sympathetic Nerve Activity

The amount of sympathetic nerve activity and the recorded MSNA signals can vary greatly depending on age and sex, with an intricate interaction between the two. Resting sympathetic activity has been shown to be higher in younger (i.e., < 50 years) men than women of the same age (124). After the age of ~50-59, when

comparing men and post-menopausal women, the difference is abolished, with no significant difference observed between sexes (107). Increased sympathetic outflow has been linked to cardiovascular issues such as chronic heart failure and high blood pressure in older individuals (2, 52, 105) and sympathetic outflow and rates of CVD are lower in pre-menopausal women (133), which supports the hypothesis of a cardio-protective mechanism of female sex hormones. With ageing, MSNA recordings of sympathetic neural output increase (84, 158) and so the levels of resting sympathetic activity progressively increase with age and sex as follows: young women < young men < older women < older men (124, 172) with levels of MSNA becoming non-significant between older women and older men around ages ~50-59 (107).

Young, healthy individuals who participate in aerobic exercise training have lower (87, 151) or similar (22, 127, 128, 178) MSNA compared to their sedentary peers. This is closely linked to the changes that occur to the NO-mediated pathway, as found in studies using both humans and rats (51, 86, 112). Mizuno et al. (2014) measured SNA and vascular conductance before and after an exercise training intervention, and observed that along with decreases in SNA, the improvements seen with conductance after the training program were attenuated when the rats were administered an eNOS-blockade (112), showing the links between the SNS and NO-mediated vasodilatory pathway. Individuals who participate in long-term aerobic exercise while they age have been shown to have increased levels of MSNA compared to age matched sedentary adults (125) but the literature about this and the associated mechanisms is lacking. This phenomenon is important to understand vascular health and how it changes with ageing and exercise.

2.4- Flow-Mediated Dilation and Muscle Sympathetic Nerve Activity Interactions

Along with working alongside the endothelium to maintain vascular tone, the sympathetic nervous system interacts with the vascular smooth muscle to maintain a fine balance between vasodilation and vasoconstriction. It has been shown that changes in sympathetic activity lead to corresponding changes in endothelial function. A study by Hijmering et al. (2002) induced sympathetic stimulation in

healthy participants with baroreceptor unloading via lower body negative pressure (80). The participants lay supine within an airtight box from their iliac crest down, after which suction was applied until their heart rate had increased by 15% or the pressure difference was -20 mmHg, inducing sympathetic activation. It was found that the brachial FMD response was significantly higher before (8.3 ± 3.4%) than after the sympathetic stimulation (3.6 ± 3.5%) (80). The link between increased SNA and decreased FMD response has been further solidified by many additional studies with similar results (45, 102, 168, 189). Additionally, Padilla et al. (2010) has shown that acute increases in MSNA lead to increased retrograde blood flow (135) and retrograde flow has also been linked with diminished FMD responses (155, 188). It has also been shown that the decreased FMD in the superficial femoral artery in older individuals is at least partially attributed to increased SNA (189) as the differences in FMD responses of older and younger men were decreased with reductions in sympathetic activity in the older men.

A study by Sverrisdottir et al. (2010) utilized concurrent measurements of MSNA and finger arterial pulse wave amplitude and reactive hyperemia index (a surrogate marker of endothelial function) (180). The results showed a strong indirect relationship between sympathetic nerve activity in the fibular nerve and upper arm arterial tone in a young, healthy population ($r= -0.8$, $p= 0.005$), confirming the close linkage between these two competing signals to endothelial cells and vascular smooth muscle (180). Furthermore, increased sympathetic tone, along with diminished endothelial function, has been observed in patients with chronic conditions such as hypertension (136) and congestive heart failure (54, 92, 99), supporting the intricate and important relationship between these two measures of blood vessel resistance. However the exact mechanism of this relationship, especially how it relates to exercise and ageing, remains unknown.

The study conducted by Sverrisdottir et al. (2010) was the first and only known published literature measuring both MSNA and arterial tone concurrently, yet this study utilized the fibular nerve for MSNA measures and the upper limb for arterial tone measures (180). Since these two variables were measured in two different limbs this may decrease the consistency of the results, as these limbs undergo

different stressors on a daily basis (the lower limbs experience increased hydrostatic pressure from gravitational forces and increased blood flow from locomotion) (123). This leads to differing FMD responses (123, 214) and it is unknown if measures taken from these two different limbs are comparable. MSNA responses are found to be relatively similar among different limbs (175), however, the current study measured both MSNA and FMD from the same limb, therefore eliminating the variability associated with FMD from different limbs (187, 191). MSNA was measured from the common fibular nerve while FMD was collected from the popliteal artery. This is especially important, as it will shed light on any potential site-specific exercise adaptations. For example most people engage in lower limb driven aerobic exercises, such as running and cycling, and so it is logical to study the relationship in these lower limbs where the greatest adaptations are expected to occur.

It has been well documented that both FMD and MSNA change with ageing and also are affected by habitual aerobic exercise (23, 37, 84, 87, 125, 143, 158, 180, 181, 183). As detailed in these studies and previously, sedentary ageing is associated with an increase in MSNA and a decrease in FMD. This is not the relationship observed with people who habitually exercise as they age, where greater FMD is evident along with greater MSNA when compared with their sedentary peers (56, 84, 113, 125), which may be a protective strategy against hypotension. The mechanism for this is unknown, but there is a lower incidence of CVD in aged individuals who habitually participate in aerobic exercise (158, 161, 183) therefore it may not be detrimental to vascular health. Sverrisdottir et al. (2010) published the first study with concurrent arterial tone and MSNA measures on young, healthy, normotensive adults (180) but it is not known how applicable these findings are to an aged population. Additionally, the effect of aerobic fitness on the relationship between MSNA and endothelial function in older populations is not known.

The present study marked the first known attempt at collecting simultaneous FMD and MSNA measures in an older population in order to quantify the interaction between resting levels of MSNA and FMD in the lower limb, and how

this interaction changes as a function of healthy ageing and aerobic fitness. This information will help elucidate the mechanisms associated with vascular health throughout the course of life and the influence that regular aerobic exercise has on this relationship. The interaction between resting levels of sympathetic vasoconstrictor outflow and EDD was investigated by studying three groups of participants. There were two groups of older adults (55-80 years), one untrained (OU) and one endurance-trained (OT). Endurance-trained individuals had a VO_{2peak} score that places them in the "Excellent" or "Superior" category for their age and sex based on published norms by Heyward (2010) (79). The last group were a group of younger (18-40 years) adults (YA) who were healthy and recreationally active as well as matched for aerobic fitness with the OT group. Aerobic fitness level, FMD from the popliteal artery, endothelial independent dilation via nitroglycerin spray, and resting MSNA from the common fibular nerve were measured in each group. With this experimental design, the effects of age (YA vs. OT) and aerobic fitness (OU vs. OT) on the relationship between FMD and resting MSNA were determined.

We hypothesized the following relationships based on the previously summarized research:

- FMD: OU < OT < YA
- NTG: OT = OU < YA
- MSNA: YA < OU < OT
- FMD and MSNA: YA- negative relationship; OT and OU- positive relationship
- VO_{2peak} and FMD: YA, OT, and OU- positive relationship
- VO_{2peak} and MSNA: YA- no relationship; OT and OU- positive relationship

Chapter 3: Methods

3.1- Participants

All experimental procedures and protocols were approved by both the Dalhousie University Health Sciences (Appendix A) and Acadia University (Appendix B) Research Ethics Boards. All subjects provided verbal and written informed consent before participation (Appendix C). Recruitment was conducted via poster advertisements (Appendix D) and word of mouth. Eight young (23 ± 2 years), 8 older untrained (63 ± 6 years), and 8 older aerobically-trained (65 ± 3 years) adults participated in the study, and all demographic data are presented in Table 1. All subjects were screened for neurological, cardiovascular, and metabolic diseases using a health history questionnaire (Appendix E) and deemed capable of safely performing maximal aerobic exercise using the Physical Activity Readiness Questionnaire for Everyone (PAR-Q+, Appendix F). All participants were non-hypertensive (resting systolic blood pressure <140 mmHg and diastolic blood pressure <90 mmHg), which was confirmed via seated brachial artery measurements using an automated patient monitor (Carescape™ v100, General Electric Healthcare). Subjects were non-diabetic, not taking over-the-counter or prescription drugs with primary or secondary cardiovascular effects (e.g., anti-hypertensives, anti-coagulants, statins, anti-depressants, etc.), and did not use tobacco-containing products. Subjects were non-obese (body mass index < $30 kg/m^2$) as calculated from measurements of height (to nearest 0.1 cm) and body mass (to nearest 0.1 kg) using a stadiometer and physicians scale, respectively. Young women were tested during the menstrual phase (days 1-7) of their cycle or during the placebo phase if using oral contraceptives. Women taking hormone replacement therapy or who had recently taken hormone replacement therapy were excluded. All participants were familiarized with the equipment, experimental protocol, and testing procedures before the testing visit.

3.2- Experimental Design

Participants reported to the laboratory for two days of testing. Day 1 consisted of the informed consent process and a graded maximal exercise test on a cycle ergometer to determine peak oxygen consumption (VO_{2peak}). Day 2 was the main testing day and involved recordings of MSNA, the FMD and NTG tests of vascular function with participants positioned in the supine (MSNA recordings), prone or lateral decubitus (FMD and NTG tests) position. The order of these tests were standardized such that MSNA recordings were first and the NTG test was last.

There was a minimum of 48 hours separating Days 1 and 2. On both days of testing, participants were asked to arrive having avoided consumption of alcohol and caffeine products for 24 hours, strenuous physical exertion for 24 hours, and refrained from food or drink for 3 hours, and consumed ~500 mL of water ~3-4 hours prior to testing. Prior to Day 2 participants abstained from eating for 6-hours, as well as, consuming caffeine, nicotine, chocolate, kiwi, saturated fats, folic acid supplements, antioxidant and multivitamin supplements, and strenuous physical activity for 24 hours, in order to prevent any external influences on either endothelial or sympathetic nervous system function, as outlined previously (186, 203). All testing was performed in a thermoneutral laboratory (21°C).

3.3- Experimental Protocols and Measurements

Graded Exercise Test. Participants completed an incremental cycle exercise test (Lode Excalibur Sport, Groningen, The Netherlands) to determine VO_{2peak}. Specifically, following a 5 minute warm-up period of easy cycling (20-50W), workload increased by 15-20 W per minute. Upon completion of the test, the workload was immediately reduced to the warm-up level for a 5 minute cool-down period. The initial warm-up workload and increment range were dependent on the subjective aerobic fitness level of each participant with the goal of producing exhaustion within 8-12 minutes. Whole-body O_2 uptake and expired CO_2 were measured via a mixing chamber connected in series to a commercial metabolic system (TrueOne® 2400, Parvomedics Inc., Sandy, UT). Respiratory exchange ratio (RER) was calculated as the volume of CO_2 (VCO_2) divided by VO_2. Participants

were equipped with a chest strap heart rate monitor (T34 Heart Rate Transmission, Polar, Lachine, Quebec), and indicated their Rating of Perceived Exertion (RPE) on a scale of 6-20 (Appendix F) every 2 minutes (13). VO_2 and VCO_2 data were averaged every 15 seconds. To be considered a 'true' maximal exercise test, 2 of the following 4 criteria must be have been observed (110): 1) an increase in $VO_2 \leq 150$ml/min despite a change in workload, 2) a final RPE score ≥17, 3) a maximal HR ≥95% of their age-predicted maximum heart rate [207 – (0.67×age)] (184), and 4) an RER ≥1.10.

Incremental cycling protocols have been shown to be valid measures of VO_{2peak} in healthy adults (49, 171) as well as in healthy older adults (32). Aerobic fitness levels (i.e., Poor–Superior) were classified based on published age- and sex-based normative VO_{2peak} values (79). To be considered aerobically-trained, older adults had to have a classification of "Excellent" or "Superior".

Cardiovascular measures. Continuous resting HR was determined throughout the MSNA, FMD, and NTG tests from lead II of a standard bipolar electrocardiogram. Beat-to-beat arterial blood pressure was obtained using photoplethysmography (Portapres®, Finapres Medical Systems B.V, Amsterdam, The Netherlands) from the middle or index finger of the right hand held at heart level. Automated brachial artery blood pressures (Carescape™ v100, General Electric Healthcare) were also measured periodically and used to verify absolute finger blood pressure measurements. If discrepancies between the brachial and finger arterial pressure were found, the finger arterial pressure was calibrated to the brachial pressure.

MSNA Recordings. Multi-unit post-ganglionic MSNA was recorded using standard microneurographic techniques (197, 198, 203). Briefly, the right common fibular nerve was mapped using percutaneous electrical stimulation posterior to the fibular head (203). A 2MΩ unipolar tungsten microelectrode (FHC; Bowdoin, MA), connected to an isolated pre-amplifier, was inserted percutaneously into a muscle fascicle. A non-insulated reference microelectrode was positioned 2-3cm subcutaneously from the active recording site. Neural signals were amplified (~75,000 ×), bandpass filtered (500-2000 Hz), full-wave rectified, and integrated

(0.1 second time constant) to obtain a mean voltage neurogram (662C-4 Nerve Traffic Analysis System, University of Iowa Bioengineering, Iowa City, IA). MSNA recordings were identified by the presence of cardiac synchronicity, responsiveness to an end-expiratory apnea and/or Valsalva's maneuver, and lack of response to arousal or skin stimulation. Once a stable MSNA signal was obtained, participants rested quietly in a darkened room for a minimum of 10 minutes.

All cardiovascular and MSNA data were recorded on a dedicated data acquisition system (PowerLab, ADInstruments) using compatible software (LabChart, ADInstruments). The electrocardiogram was sampled at 1000 Hz, and the Portapres® waveform and MSNA mean voltage neurogram at 400 Hz.

Popliteal artery FMD. The right lower leg was imaged while participants were in either the prone or lateral decubitus position, depending on which position was most comfortable. Heart rate and finger blood pressure were recorded throughout the test. A pressure cuff attached to a rapid cuff inflation system (E20 and AG101, Hokanson®, Bellevue, WA) was positioned at the mid-calf level (~10cm distal to the popliteal fossa). The artery was imaged proximal to the bifurcation (i.e., at or slightly above the popliteal fossa) using a 12 MHz multi-frequency linear array probe attached to a high-resolution ultrasound machine (Vivid i, General Electric Healthcare). Doppler blood flow velocity was measured using an insonation angle <60° that was held constant throughout the study. The sample volume was adjusted for each participant such that the anterior to the posterior intima were included, as recommended in published guidelines (186).

After the subject had rested for a minimum of 10 minutes, resting popliteal artery lumen diameter and velocity were measured for a minimum of two minutes before inflation of the pneumatic cuff. The pressure cuff was rapidly inflated to 250 mmHg for 5 minutes. Continuous arterial lumen diameter and blood flow velocity recordings were collected throughout the cuff inflation period. Upon release of cuff pressure, popliteal arterial lumen diameter and velocity recordings continued for an additional 5 minutes.

Popliteal artery endothelial-independent vasodilation. A minimum of 10 minutes following the FMD assessment, once it was confirmed that blood flow velocities had

returned to resting levels, participants underwent assessment of endothelial-independent dilation via administration of 0.4mg of sublingual nitroglycerin (NTG), a NO donor eliciting maximal dilation representative of vascular smooth muscle function. This rest period allowed popliteal blood flow and arterial lumen diameter to return to resting values. Following a one minute baseline recording period, popliteal lumen diameter and blood flow velocity were continuously measured for 10 minutes after NTG administration.

3.4- Data Analyses

Cardiovascular data. The Portapres® system uses the Modelflow® method in the Beatscope® program to calculate beat-to-beat SV, Q and TPR based on corrected changes in flow and impedance measured non-invasively from the finger through the use of an infrared photo-plethysmography sensor (195, 211). The age, sex, weight, and height of the participants were inputted into the model to calculate these variables. MAP was calculated from the raw Portapres® waveform as ⅓ systolic + ⅔ diastolic pressure. The blood flow velocity was calculated from the Doppler audio information from the GE Vivid i ultrasound, which was processed into a blood velocity signal by a custom interface unit via Fourier transform with a calibrated scale (77) and sampled in real time at 400 Hz (PowerLab, ADInstruments). Leg blood flow was then calculated, first converting the blood flow velocity from cm/sec to L/min ($6x10^4$ conversion factor), and then multiplied by the circular area (i.e., πr^2). Leg vascular resistance (LVR) was calculated by dividing MAP by leg blood flow. Total vascular conductance (TVC) and leg vascular conductance (LVC) were calculated as the inverse of TPR and LVR, respectively.

The resting cardiovascular variables were calculated during the supine rest period, and mean values over the final 5 minutes of supine rest were used to represent the resting variables.

Sympathetic data. MSNA was quantified as burst frequency (BF, bursts per minute) and burst incidence (BI, bursts per 100 heart beats). The MSNA data were analyzed within LabChart (ADInstruments). Impulses were identified and marked when they were pulse-synchronous, and at least 2-3 times larger than any other

surrounding background noise. The bursts were then counted and quantified based on either bursts/minute (burst frequency, BF) or bursts/100 heartbeats (burst incidence, BI). The units in which MSNA are expressed can become important when making comparisons between groups. In order to help compare MSNA between different groups who may have different resting heart rates MSNA BI was used for all between group comparisons. However, MSNA BF was used for all correlations due to the importance of the frequency of impulses on biologic variables (such as FMD or VO_{2peak}) regardless of the resting heart rate.

Popliteal FMD data. The video signal from the Vivid i was exported directly onto a MacIntosh laptop via a video graphics array converter (Epiphan Systems Inc., VGA 2 USB, Ottawa) for offline analysis. Post-test analyses of popliteal lumen diameter were performed using automated edge-detection software (FMD Studio, Cardiovascular Suite, Institute of Clinical Physiology, Pisa, Italy). Briefly, a region of interest (ROI) was selected along the artery wall, with the edge of the wall automatically determined as the point where the pixel density changed the most rapidly. Each test was reviewed and edited when needed to ensure that arterial lumen diameter measurements were always calculated from the intima-lumen interface at the anterior and posterior vessel walls (Figure 13). Arterial lumen diameter was measured continuously during the baseline, occlusion, and post-occlusion phases. FMD was calculated as the percentage change in lumen diameter from baseline to the peak lumen diameter obtained during the 5 minute period following pneumatic cuff occlusion.

For the calculation of popliteal blood flow velocity, a ROI was selected around the Doppler waveform. The automated program detected the peak of the waveform and the mean velocity across the cardiac cycle (Figure 13, Box 4). The system was calibrated prior to any testing. The velocity was calibrated by the principal investigator before testing began, with a ROI selected around the area on the screen where the Doppler wave form would be present and calibration of the x-axis (time) and y-axis (flow velocity) was completed with the ultrasound machine. The lumen diameter was calibrated by the principal investigator once a clear image of the artery was achieved, with the scale of the ultrasound screen being selected so

that CV Suite could calculate arterial lumen diameter. The lumen diameter and blood velocity data were time-aligned. Popliteal blod flow and shear shear were calculated using the following formulea:

Blood flow (mL/minute) = $Vm * \pi * (d^2/4)$; where Vm is mean blood velocity (cm/sec) and d is mean arterial lumen diameter (cm)

Shear rate (s^{-1}) = $(4 * Vm) / d$; where Vm is mean blood velocity (cm/sec) and d is mean arterial lumen diameter (cm)

The FMD data were normalized to the shear rate stimulus responsible for the arterial dilation. The area under the shear rate curve (SR_{AUC}) was calculated between the time of cuff deflation to the point of maximal dilation. This normalization process minimizes the influence of individual variability in blood flow (and hence shear stress) on NO production and the resulting maximal vasodilatory response, as recommended by recent findings (134, 147).

Additionally, due to the known correlation between baseline lumen diameter and the FMD response (187), Atkinson and colleagues (2013a, 2013b, 2014a, 2014b, 2015) have suggested the use of allometric scaling to remove any confounding factors before analysis is performed (4–8). This accounts for any changes in FMD that may be related to baseline lumen diameter and not endothelial function (187, 199). First, both the baseline and peak arterial diameters were logarithmically transformed, and the slope of the regression between these two variables determined. The slope of this line was used as the allometric scaling exponent, and an ANCOVA was performed with the group as the fixed factor, and the logarithmically transformed baseline lumen diameter as the covariate. After this, the data were transformed back into a percentage change. This was done to remove any variation in the FMD responses solely due to differences in baseline lumen diameter.

Popliteal artery endothelial-independent vasodilation data: Post-test analysis of changes in popliteal artery lumen diameter following NTG administration was also

performed using FMD Studio. A ROI was selected along the artery wall and each test was reviewed and edited as required (Figure 14). Endothelial-independent dilation was calculated as the percentage change in lumen diameter from baseline to the peak lumen diameter obtained during the 10 minute period following NTG administration.

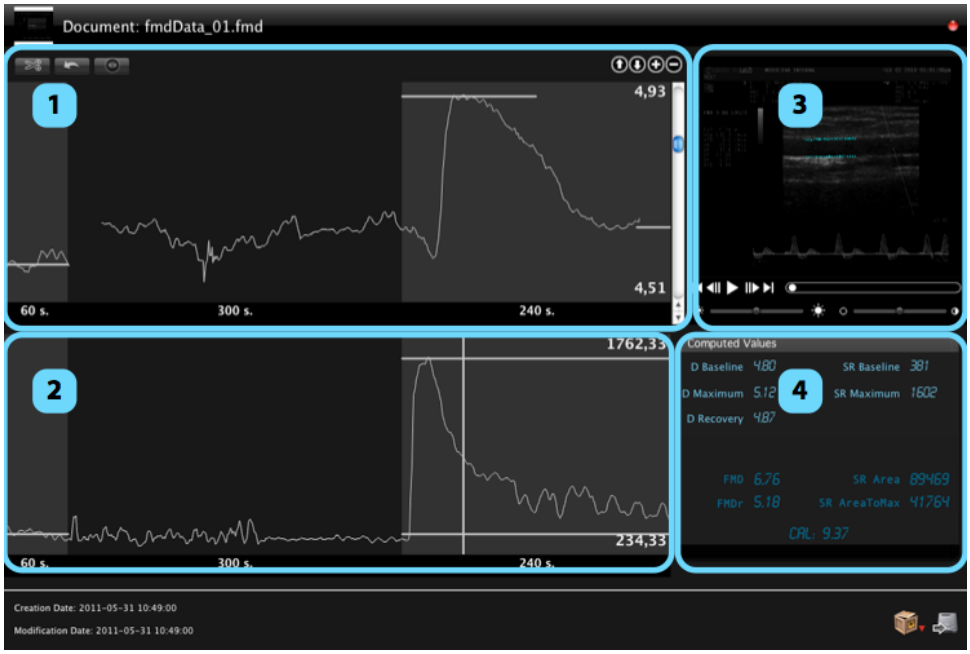

Figure 13. *Example of the FMD Studio review screen with: 1) Mean diameter chart configured for the current study with a 120 second baseline, 300 second occlusion, and 300 second post-inclusion data collection period; 2) Time averaged positive shear rate chart; 3) Video window- shows real time video recorded during the FMD study; 4) Results display- summary of baseline, maximum, and recovery diameters, baseline and maximum shear rate, FMD% and shear rate AUC. FMD- flow-mediated dilation; AUC- area under the curve* (148)

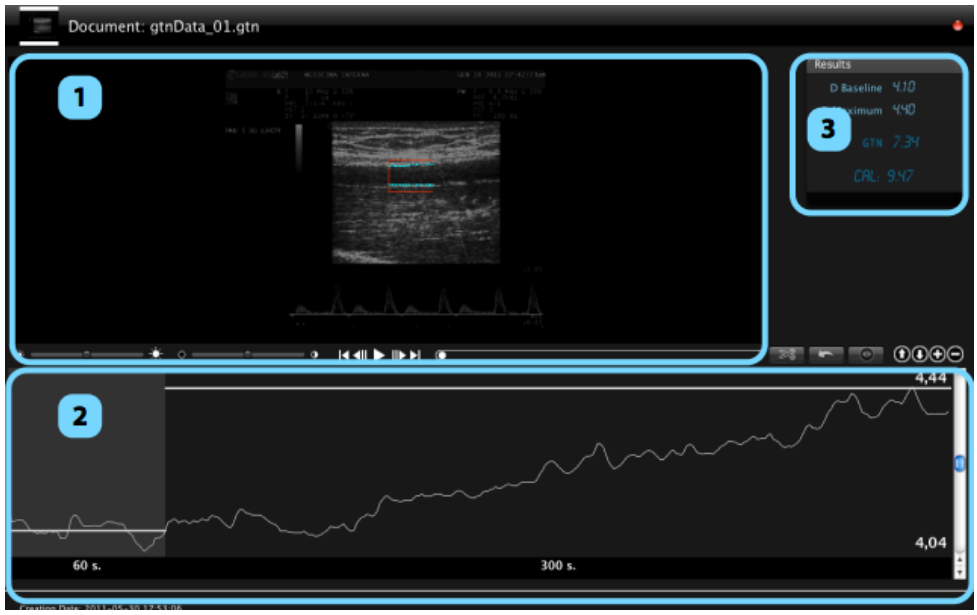

Figure 14. *The set up of the review screen of the NTG analysis with: 1) Video window- shows real time video recorded during the NTG study; 2) Mean diameter chart which was configured for the current study with a 60 second baseline and a 300 second post-spray data collection period; 3) Results display- summary of baseline and maximum diameters, with the calculated percentage increase in lumen diameter. NTG, nitroglycerin* (148)

3.5- Statistical Analysis

Statistical Package for the Social Sciences 22 (SPSS 22, Chicago, IL) was used for all statistical analyses. Statistical significance was set at $p < 0.05$ and all data were expressed as means ± standard deviations. Descriptive statistics were used to summarize the participant demographics. Normality was tested for using the Shapiro-Wilke test and if normality was violated, an independent samples Kruskal-Wallace non-parametric analysis of variance (ANOVA) was used. If normality was not violated, a one-way ANOVA was used to determine differences in resting cardiovascular measures between the three groups. Post-hoc analyses were performed using the Bonferroni correction. The assumption of sphericity was assessed using Mauchly's Test. If this assumption was violated, the Greenhouse-Geisser correction to the degrees of freedom was applied.

Additionally, linear regression analyses were used to assess the relationships between VO_{2peak} and MSNA BF, VO_{2peak} and FMD, and MSNA and FMD were investigated. All analyses were conducted within each group and for all participants as a whole.

Chapter 4: Results

4.1- Participant Physical Characteristics

All participant physical characteristics are outlined in Table 1. Final analysis included n= 8 (7 male, 1 female) for the YA group, n= 8 (3 male, 5 female) for the OU group, and n= 8 (7 male, 1 female) for the OT group.

The OU group had a significantly lower VO_{2peak} than the YA and OT groups (both, $p< 0.001$) as shown in Figure 15A. There was no significant difference in the VO_{2peak} between the YA and OT groups ($p= 1.000$) (Fig. 15A).

Table 1. *Participant physical characteristics*

	Young Adults (*n=8*)	Older Untrained (*n=8*)	Older Trained (*n=8*)
Age, years	23 ± 2	63 ± 6*	65 ± 3*
Height, m	1.75 ± 0.04	1.69 ± 0.10	1.69 ± 0.06
Weight, kg	74.5 ± 10.1	75.3 ± 8.7	72.6 ± 12.1
BMI, kg/m^2	24.3 ± 3.1	26.4 ± 3.1	25.2 ± 3.1
Absolute VO_{2peak}, L/min	3.71 ± 0.91	2.12 ± 0.69*†	3.59 ± 0.69
Percent Predicted VO_{2peak}, %	115 ± 20	130 ± 36†	177 ± 23*

BMI, body mass index; VO_{2peak}, peak volume of oxygen consumption. *, $p< 0.05$ vs. Young Adults; †, $p< 0.05$ vs. Older Trained

Resting cardiovascular variables are presented in Table 2. Portapres® data could not be recorded from one participant (male) in the YA group. Therefore, YA group SV, Q, TVR, and TVC data were determined from 7 participants. There were no significant between-group differences (all, $p > 0.15$) in HR (Fig. 15B), SBP, Q, TVR, and TVC (Table 2). The OT group had significantly higher (all, $p < 0.02$) DBP and MAP than the YA group and had significantly higher SV (Fig. 15C) than both the YA and OU groups (both, $p < 0.03$).

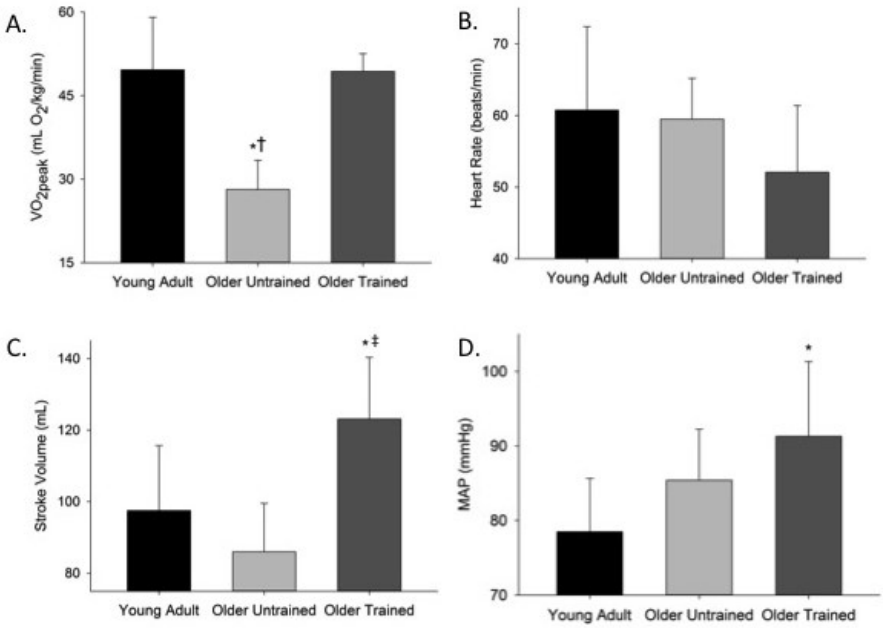

Figure 15. ***Relative VO_{2peak} (A), resting heart rate (B), stroke volume (C), and mean arterial pressure (D) for all groups.*** Data are presented as means ± standard deviations. MAP, mean arterial pressure. *, p< 0.05 vs. Young Adult; †, p< 0.05 vs. Older Trained; ‡, p <0.05 vs. Older Untrained.

Table 2. *Participant resting cardiovascular characteristics*

	Young Adults	Older Untrained	Older Trained
Systolic blood pressure, mmHg	112 ± 5	118 ± 12	120 ± 11
Diastolic blood pressure, mmHg	63 ± 9	70 ± 5	78 ± 11*
Cardiac Output, L/min	6.0 ± 1.6	5.1 ± 1.0	6.4 ± 1.4
Total Peripheral Resistance, mmHg/L/min	12.7 ± 4.5	16.3 ± 4.2	14.9 ± 3.7
Total Vascular Conductance, mL/min/mmHg	7.7 ± 1.8	6.1 ± 1.2	7.1 ± 1.6
Resting Leg Blood Flow, mL/min	55.0 ± 21.2	55.0 ± 15.7	53.8 ± 10.1
Resting Blood Flow Velocity, cm/s	3.3 ± 1.6	3.3 ± 1.3	2.7 ± 0.4

Data are means ± standard deviations. Young adult, n=7 for stroke volume, cardiac output, total peripheral resistance, and total vascular conductance. *, p< 0.05 vs. Young Adult.

4.2- Resting Muscle Sympathetic Nerve Activity

We were unsuccessful finding a stable MSNA signal in one participant (female) from the OU group. As such, OU group MSNA data are from 7 participants only. The OT group had a significantly higher ($p = 0.006$) MSNA BI than the YA group (71 ± 20 vs. 29 ± 19 bursts/100 heart beats), as shown in Figure 16A. There were no significant differences in MSNA BI between the YA and OU ($p= 0.80$) nor the OU and OT groups ($p= 0.10$) (Fig. 16A). Similarly, the OT group had a significantly higher ($p = 0.02$) MSNA BF than the YA group (37 ± 12 vs. 17 ± 12 burst/min, Fig. 16B). There were no significant differences (all, $p > 0.24$) in MSNA BF between the YA and OU, nor the OU and OT groups (Fig. 16B).

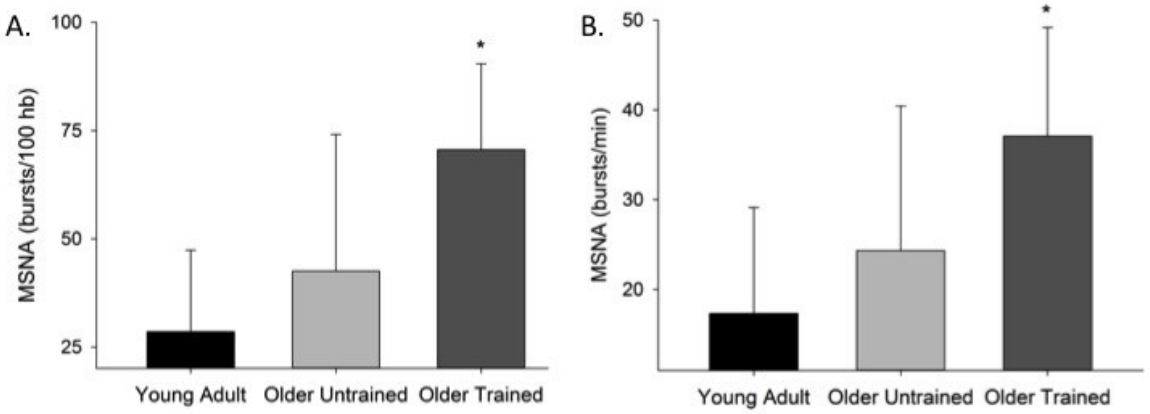

Figure 16. ***Resting muscle sympathetic nerve activity burst incidence (A) and burst frequency (B) for all groups.*** Data are presented as means ± standard deviations. Older Untrained, n=7. MSNA, muscle sympathetic nerve activity; hb, heart beats. *, $p< 0.05$ vs. Young Adult

4.3- Popliteal Artery Endothelial-Dependent Dilation

The OU group had a significantly lower (Both $p < 0.03$) relative FMD response (6.7 ± 1.1%) than YA (9.4 ± 1.5%) and OT (11.5 ± 2.5%) groups (Fig. 17A). There was a trend towards significance in the relative FMD response between the YA and OT groups ($p= 0.09$, Fig. 17A). The absolute FMD-mediated lumen diameter increase was significantly greater (all, $p < 0.04$) in the OT group (0.78 ± 0.17 mm) than both the YA (0.58 ± 0.16 mm) and the OU (0.41 ± 0.07 mm) groups (Fig. 17B). There was a trend ($p= 0.07$) between YA and OU groups (Fig. 17B). When

normalized to the shear rate until peak lumen diameter (area under the curve, SR_{AUC}), the OU group had a significantly lower (all, $p < 0.03$) FMD response (7.5 ± 1.4%) than both the YA group (10.6 ± 2.0%) and the OT group (13.2 ± 2.8%) (Fig. 17C). Furthermore, there was a near-significant difference ($p = 0.07$) between the YA and OT groups (Fig. 17C). When allometrically scaled according to published guidelines (5–8, 54), the OT group FMD response (11.8 ± 2.3%) was significantly higher (all, $p < 0.04$) than the YA (9.4 ± 1.5%) and OU groups (6.7 ± 1.1%) (Fig. 17D). The allometrically scaled FMD response for the YA group was also significantly higher ($p = 0.02$) than the OU group (Fig. 17D).

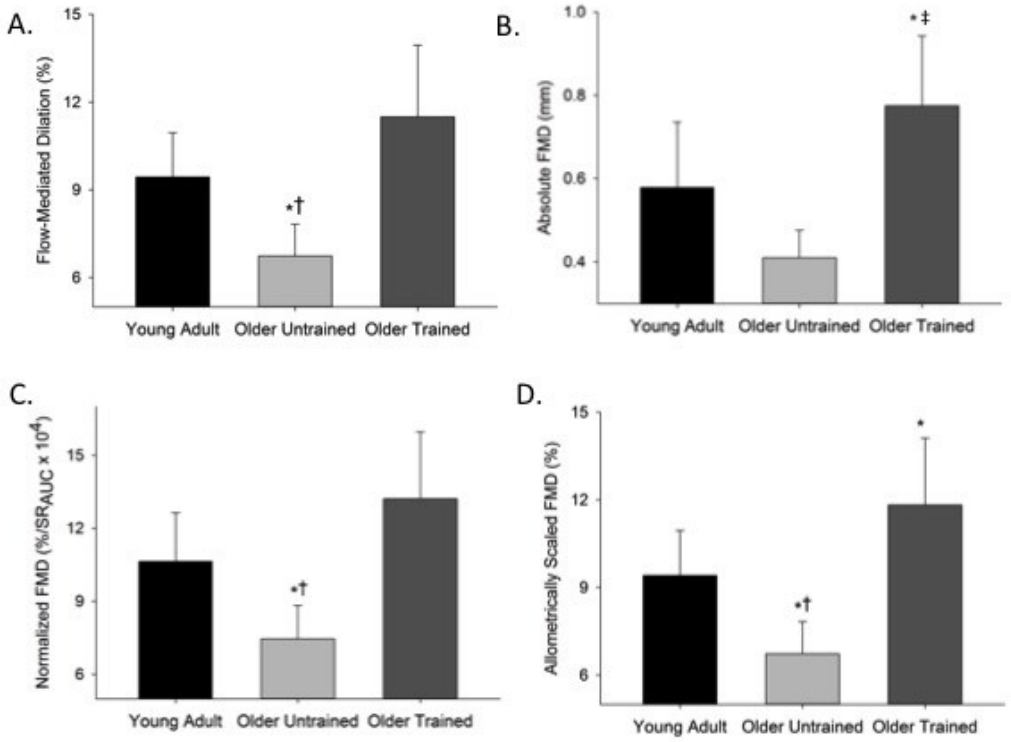

Figure 17. ***Relative (A), Absolute (B), Normalized (C), and Allometrically scaled (D) flow-mediated dilation responses for all groups.*** Data are presented as means ± standard deviations. FMD, flow-mediated dilation; SR_{AUC}, shear rate area under the curve. *, $p < 0.05$ vs. Young Adult; †, $p < 0.05$ vs. Older Trained; ‡, $p < 0.05$ vs. Older Untrained.

There were no significant differences (all, $p > 0.69$) between groups for baseline and maximum popliteal artery lumen diameter, shear rate until peak lumen diameter (SR_{AUC}), nor time to peak dilation, as shown in Table 3.

Table 3. *Subject popliteal artery flow-mediated dilation characteristics*

	Young Adults	Older Untrained	Older Trained
Baseline Diameter, mm	6.2± 1.5	6.6 ± 1.6	6.5 ± 0.7
Maximum Diameter, mm	6.7 ± 1.6	6.7 ± 1.7	7.3 ± 0.8
SR_{AUC}	8935 ± 945	9156 ± 1335	9183 ± 925
FMD Time to Peak, s	109 ± 15	119 ± 19	115 ± 16

FMD, flow-mediated dilation; SR_{AUC}, shear rate area under the curve.

4.4- Popliteal Artery Endothelial-Independent Dilation

The current analysis includes data from two separate studies conducted at Dalhousie University and Acadia University. Data from eight participants were used from an initial study at Acadia, and a further eight participants were recruited through Acadia and testing was completed there for the current project. The initial study however did not have ethical approval for the use of NTG for assessment of endothelial-independent dilation and so this was only collected on seven YA, eight OU individuals, and one OT individual.

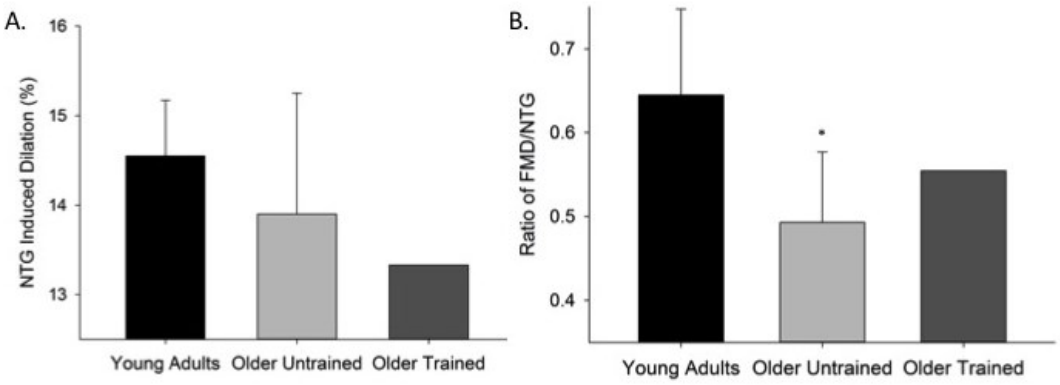

Figure 18. ***Resting endothelial-independent dilation (A) and ratio of flow-mediated dilation to endothelial-independent dilation (B) for all groups.*** Data are presented as means ± standard deviations. Young Adults, n=7, Older Untrained, n=8, Older Trained, n=1. NTG, nitroglycerin; FMD, flow-mediated dilation; *, $p < 0.05$ vs. Young Adult

There was no significant difference in the NTG percentage response between the YA and OU groups (p= 0.81, Fig. 18A).

The ratio of FMD dilation to NTG induced dilation was significantly higher in the YA group than the OU group (p= 0.03, Fig. 18B).

4.5- Resting Muscle Sympathetic Nerve Activity and VO_{2peak} Correlation

There was a no significant relationship between MSNA BF and VO_{2peak} in the YA group (p= 0.13, r= -0.58, Fig. 19A), though there was a negative trend. There was a positive trend but no significant relationship between MSNA BF and VO_{2peak} in the OU (p= 0.18, r= 0.57, Fig. 19B) and OT groups (p= 0.34, r=0.39, Fig. 19C). When combining all groups together, there was no relationship between MSNA BF and VO_{2peak} (p= 0.79, r= 0.06, Fig. 19D).

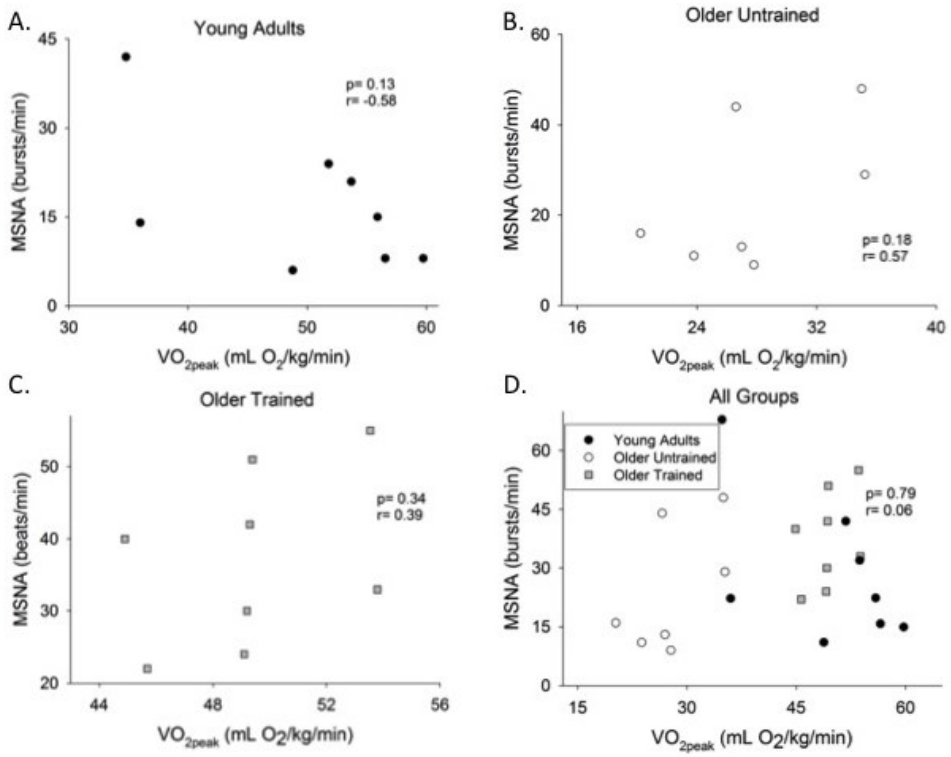

Figure 19. ***Relationship between muscle sympathetic nerve activity burst incidence and VO_{2peak} in young adults (A), older untrained (B), older trained (C), and all groups (D).*** Older untrained, n=7. MSNA, muscle sympathetic nerve activity; min, minutes.

4.6- Resting Popliteal Artery Flow-Mediated Dilation and VO_{2peak} Correlation

There was a significant inverse relationship between relative FMD and VO_{2peak} in the YA group (p= 0.01, r=-0.62, Fig. 20A) while there was no relationship between relative FMD and VO_{2peak} in the OU group (p= 0.58, r= 0.25, Fig. 20B). There was a significant positive relationship between relative FMD and VO_{2peak} in the OT group (p= 0.03, r=0.76, Fig. 20C). Overall, there was a significant positive relationship between relative FMD and VO_{2peak} (p= 0.004, r=0.57, Fig. 20D).

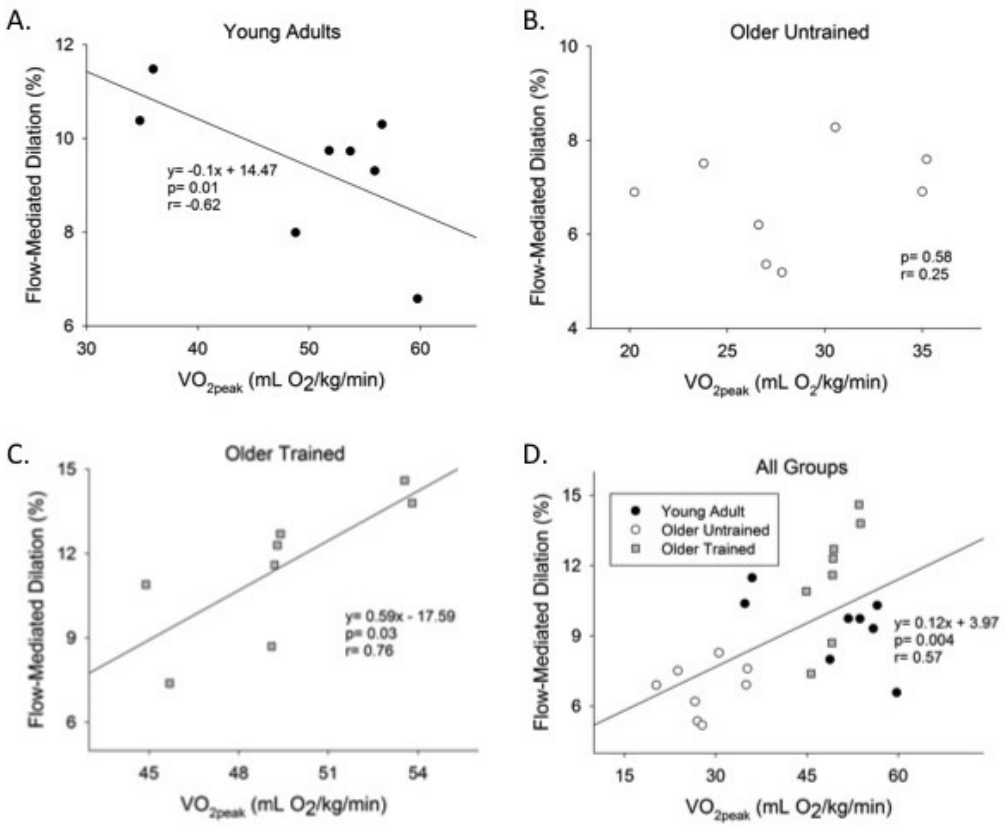

Figure 20. Relative f*low-mediated dilation versus VO_{2peak} in young adults (A), older untrained (B), older trained (C), and all groups (D).* VO_{2peak}, peak volume of oxygen consumption.

4.7- Muscle Sympathetic Nerve Activity and Relative FMD Interactions

There was no relationship between MSNA BF and relative FMD in the YA group (p=0.43, r=0.43, Fig. 21A) nor between MSNA BF and relative FMD in the OU group (p= 0.56, r=0.27, Fig. 21B). There was a significant positive relationship between MSNA BF and relative FMD in the OT group (p= 0.02, r=0.79, Fig. 21C). Overall,

there was a significant positive relationship between MSNA BF and relative FMD (p= 0.01, r=0.51, Fig. 21D)

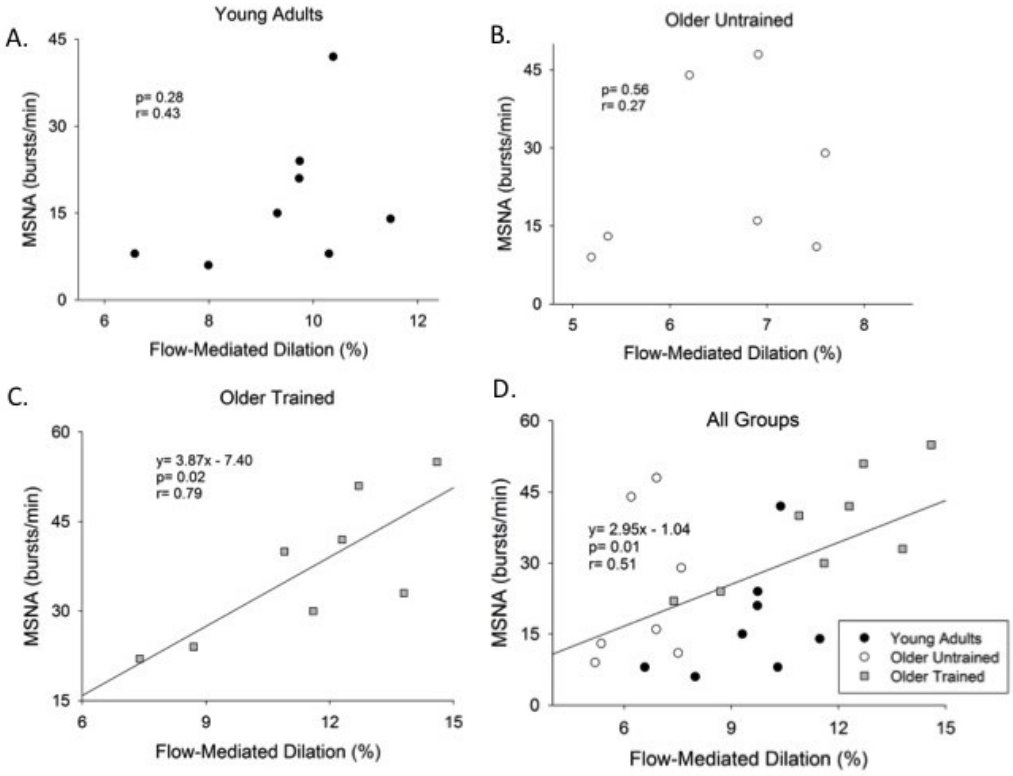

Figure 21. ***Muscle sympathetic nerve activity vs. flow-mediated dilation in young adults (A), older untrained (B), older trained (C), and all groups (D).*** Older untrained, n=7. MSNA, muscle sympathetic nerve activity; min, minutes.

Chapter 5: Discussion

The main objective of this study was to investigate the relationship between aerobic fitness, ageing, MSNA, and FMD. The main hypotheses were:

1) FMD: YA < OT < OU

2) MSNA: YA < OU < OT

3) FMD and MSNA: YA- negative relationship; OT and OU- positive relationship

4) VO_{2peak} and FMD: YA, OT, and OU- positive relationship

5) VO_{2peak} and MSNA: YA- no relationship; OT and OU- positive relationship.

The main results of the study were as follows:

1) The relative flow-mediated dilation response was significantly lower in OU than both YA and OT;

2) MSNA BI and burst BF were significantly higher in OT individuals than the YA fitness-matched individuals;

3) There was a significant relationship between MSNA BF and relative FMD in the OT group and in the overall relationship with all groups;

4) There was a significant correlation between relative FMD and VO_{2peak} in the OT group and when looking at all groups combined, there was a significant relationship between VO_{2peak} and relative FMD;

5) There was a negative trend between VO_{2peak} and MSNA BF in young adults, while there was a positive trend between VO_{2peak} and MSNA BF in both the OU and OT groups.

5.2- The Influence of Aerobic Fitness on MSNA

Muscle sympathetic nerve activity (MSNA) is a direct measure of SNA and has been shown to possess high intra-individual reproducibility (25, 203, 205). Compared to other measures of SNA such as heart rate variability or plasma levels of norepinephrine, MSNA is sensitive to changes in beat-to-beat arterial pressure and is the most reliable direct way to assess SNA (48, 128). Additionally, MSNA has been shown to have greater short and long term reproducibility than direct measurements of plasma noradrenaline (63).

Within the YA group, there was no relationship between VO_{2peak} and MSNA BF (Fig.17A). The literature regarding this relationship between VO_{2peak} and MSNA has shown either lower (64, 151) or similar (22, 178) SNA in more aerobically fit individuals after a training program was implemented. Additionally, Carter and Ray (2015) completed a meta-analysis of current literature and found that the majority of studies they examined (8 out of 9) measured no change in MSNA before and after aerobic training in healthy adults (22). In cross-sectional studies, no differences in resting MSNA were found when comparing trained and untrained young adults (178). Svedenhag et al. (1984) found that in participants with a mean age of 22 years, despite differences in training status, the untrained group had an MSNA BI of 34 ± 7 and an MSNA BF of 22 ± 4, whereas the trained group had an MSNA BI of 35 ± 7 and an MSNA BF of 20 ± 4 (both, $p > 0.05$) (178). Though the present study was cross-sectional in design, the lack of relationship observed in the present study between MSNA BF and VO_{2peak} in young adults, supports the data that differences in aerobic fitness in young adults is not correlated with differences in MSNA.

The relationship between VO_{2peak} and MSNA in older, untrained individuals was investigated by Notarius et al. (1999) in a group of 17 healthy individuals aged 44 ± 4 years (127). They found the relationship between the MSNA BF and VO_{2peak} was insignificant in this population (127), which corresponds with the results in the present study (Fig. 19B-C). The effect of training on the relationship between aerobic fitness and MSNA was investigated by Sheldahl et al. (1994), and this showed that with a short-term (12 week) aerobic training intervention in older individuals (54 ± 8 years), there was a significant increase in VO_{2peak} ($p<0.05$) but no change in MSNA BF (23.1 ± 2.3 to 23.6 ± 1.9 bursts/min) (160).

In a study by Studinger et al. (2009), young, healthy (24±3 years, VO_{2peak} 52.1±6.6mL O_2/kg/min) men were compared to older male masters athlete (60 ± 4 years, VO_{2peak} 49.2 ± 7.6mL O_2/kg/min) of similar fitness levels (172). These groups were of similar age and fitness level to those in the current study. The older masters athletes had significantly higher MSNA BI than the younger men (34±13 bursts/100heartbeats vs. 19±8 bursts/100heartbeats; $p<0.05$) (172). The relationships from the present study are in accordance with these previous

findings, however the present results are higher for each group. With the present results, there was a significant increase from YA to OT in both MSNA BI (29 ± 19 vs. 71 ± 20 bursts/100heartbeats) and BF (17 ± 12 vs. 37 ± 12 bursts/minute) (Fig. 16A-B).

In the present study, there was not a significant increase in MSNA BI and BF with age, potentially due to the small sample size or large variability, as it has previously been measured that MSNA increases with age, regardless of disease state (74, 84, 124). For example, Hart et al. (2009) found lower MSNA BI in young (26±1 year) versus older (60±2 years) men (41±3 vs. 64±4 bursts/100heartbeats; $p<0.05$), however this cross-sectional study did not control for fitness level (74). It was hypothesized that MSNA would increase with age in part due to decreased baroreflex sensitivity (72, 100, 173, 209), and a potential factor could be that the barosensory vessels (e.g. aortic arch and carotid arteries) become more stiff with age, due to structural changes in the blood vessel (172). Arterial baroreceptors are mechanical sensors in the aortic and carotid arteries which detect beat-to-beat changes in blood pressure, via detecting arterial stretch, and making adjustments based on a central set-point if the pressure is too high or too low (50, 110). Typically, higher pressures and greater stretch detected by the baroreceptors lead to higher afferent firing rates, which inhibits MSNA in order to lower arterial pressure (209). If the baroreceptors are less sensitive, as occurs with ageing (129), this would lead to less inhibition of MSNA. As such, older adults may have greater MSNA at the same mean arterial pressure as younger adults.

Additionally, due to increased arterial stiffness, which occurs with age due to increases in collagen and decreases in elastin in the arteries (158, 196), the changes in beat-to-beat pressure are not transduced as efficiently, leading to decreased baroreceptor control of blood pressure and less inhibition of MSNA (73, 129, 172). Furthermore, decreased adrenergic receptor sensitivity on the VSM, associated with increasing age (114), would mean that higher levels of MSNA would be needed to maintain the same levels of vascular tone in this older population. In a cross sectional study, Hogikyan & Supiano (1994) found greater plasma NE levels and decreased adrenergic responsiveness in older adults (65 ± 1 year) versus younger

adults (23 ± 1 year) (81). These changes may lead to an increase in MSNA in older individuals.

Neither MSNA BI nor MSNA BF were significantly higher in the OT than the OU group and the OU group was not significantly higher than the YA group, in contradiction with our hypothesis (Fig. 16A-B). There are a few variables that may contribute to this. Firstly, the OU group may not have been a truly 'untrained' group of individuals. When comparing this group to the YA group, the range of VO_{2peak} values in the OU group was from Poor-Good, while in the YA group this range was from Poor-Superior (79). Additionally, as there was a wide range of fitness levels from Poor-Good (79) within the OU group, some in the 'Good' category should not be considered untrained and therefore the difference between the OU and OT groups may not have been enough to elicit a significant difference. In older untrained and trained adults, Ng et al. (1994) found MSNA BI values of 52 ± 5 bursts/100heartbeats in the untrained group, and 75 ± 4 bursts/100heartbeats in the aerobically trained group (125). It is evident that though the means are similar, the large standard deviation values in present study (YA: 29 ± 19 bursts/100heartbeats, OU: 43 ± 32 bursts/100heartbeats, OT: 71 ± 20 bursts/100heartbeats) may be contribute to a lack of significance between groups.

5.3 The Influence of Aerobic Fitness on FMD

Flow-mediated dilation is an accurate measure of EDD and endothelial function (149, 170, 186). Lower levels of FMD are associated with hypertension (136), heart failure (92), and as predictive of future cardiovascular events as traditional risk factors (9, 216). Due to its non-invasive nature using an ultrasound machine and automated software, FMD is easily performed with proper technician training and has high inter-technician reliability (31, 40, 186). The relative FMD values of the YA group were consistent with popliteal FMD values obtained from young, healthy individuals in other studies, ranging from ~7-11% dilation in the (14, 187, 191).

The current study found a significant negative relationship between FMD and VO_{2peak} (Fig 20A). This is in contrast to a systematic review by Montero (2015) of

the relationship between VO_{2peak} and relative FMD in young healthy individuals. This review showed a moderate positive relationship between these two variables, but it is important to note that the majority of studies in this review (90%) were conducted using upper limb measures (115). It is known that there are differences in relative FMD results between limbs, with the lower limbs exhibiting blunted responses due to day to day differences in hydrostatic pressure (123, 191) and so the results of the present study, which used the popliteal artery, may not be applicable to these data. Additionally, Wang et al. (2014) found no link between young male endurance capacity, VO_{2max}, and endothelial function (208).

Increases in aerobic fitness can improve FMD in populations with previously diminished endothelial function, as observed in a longitudinal studies in older healthy and clinical populations (62, 140, 215). Endothelial dysfunction is typically associated with increasing age (56, 123, 157). This is due to increases in baseline arterial lumen diameter that occurs with age, driven by increases in intima-media thickness (153), as well as increases in arterial stiffness due to diminished collagen and elastin function (60). In addition, decreases in NO bioavailability (154) and VSM responsiveness (114) have been linked with lower EDD. FMD responses in older, sedentary adults are consistently significantly lower than younger adults (157, 158, 217), which is consistent with the findings from the present study. The FMD% of the OU group was significantly lower than both the YA group and the OT group.

Aerobic fitness has been shown to be extremely beneficial for endothelial function, as long-term aerobically trained older individuals exhibit similar endothelial function to young adults (37, 56, 57, 140). Our results are consistent with this previous research (113, 140), as the relative FMD responses in the OT group were significantly higher than the OU group (Fig. 21B-C). For example, Pierce et al. (2011) found that middle-aged and older adults who were aerobically trained had about 50% higher brachial artery FMD% than their age-matched sedentary controls (6.4 ± 0.4% vs. 4.3 ± 0.3%, $p < 0.001$) (140). There are both chemically-mediated and structurally-mediated changes which occur which may contribute to

the improved FMD% in older aerobically trained individuals, as compared to their sedentary counterparts.

Typically with ageing, increased oxidative stress and inflammation can lead to decreased NO bioavailability and a reduction in NO signal transduction (55, 154). However, in exercise trained individuals, these impairments are attenuated (57, 158). Eskurza et al. (2004) found that acute vitamin C infusion improved the brachial FMD response in older sedentary men (4.6 ± 0.2% to 6.7 ± 0.3%, $p < 0.005$), but not in young men or aerobically trained older men (46). This indicates that the exercise-mediated improvements in antioxidant capacity play a role in the protection of vascular function with ageing.

Additionally, the ageing associated increases in inflammation are decreased with aerobic exercise training (154, 157). The proinflammatory transcription factor NF-κB increases with sedentary ageing (41), stimulating the production of superoxides, which reduce the bioavailability of NO (154, 202). Walker at al. (2014) found the NF-κB inhibitor salsalate and found that it improved brachial FMD in older sedentary adults (4.0 ± 0.7% to 6.8 ± 0.7±, $p < 0.001$), but not in young adults or aerobically trained older adults (202). This supports the hypothesis that aerobic fitness can helps to preserve endothelial function by preventing the inflammation typically associated with ageing.

In addition to these chemically-mediated changes in vascular function, there are structural changes with exercise training in older adults which help to maintain FMD% at the levels of young adults. With ageing, baseline arterial lumen diameter increases (11, 69). Though increased baseline lumen diameter is associated typically with decreased FMD responses (11, 187), Montero et al. (2014) investigated the baseline diameters of both younger and older sedentary and trained individuals (113). This review found older aerobically trained athletes had similar increases in baseline lumen diameter to their sedentary peers, however they had significantly higher FMD% responses (113). The reason for this discrepancy between groups was thought to be due to other changes within the arteries, such as with inflammation and oxidative stress, or with the structural collagen and elastin (55, 154, 158, 202). Baseline arterial lumen diameter was

measured in the present study and was found not to be different between groups (Table 3). This lack of significant differences could be due to large inter-individual variability as well as limb differences in vascular remodeling (69). The review from Montero et al. (2014) utilized only studies which investigated brachial artery FMD (113). As it is known that even in sedentary controls, the popliteal artery, undergoes more extensive remodeling (126), this helps to explain the lack of significant differences in baseline lumen diameter between the YA and both the OU and OT groups in the present study.

In addition to changes in the lumen diameter of the artery, changes with age and exercise in the stiffness of the artery help to improve FMD responses in older trained individuals. Age-associated increases in stiffness are due to a combined effect of increased VSM tone and changes to the make-up of the artery (154, 196). There is an increase in collagen and a decrease in elastin which typically occurs with age (60). However, older aerobically trained individuals have less stiff arteries than their sedentary peers (158) due to a combination of increased elastin, decreases in collagen, and decreases in oxidative stress which damage these fibres (154, 158). Though arterial stiffness was not measured in the current study, decreases in stiffness are linked to increases in FMD responses in older individuals (95, 158), may have contributed to the significantly higher FMD% responses measured in the OT group than the OU group (Fig. 17A).

There has been much debate surrounding the appropriate method for reporting FMD results. For this reason, relative FMD, absolute FMD, FMD normalized to SR_{AUC}, and allometrically scaled FMD% were all reported in the present study, as outlined in published guidelines (7, 8, 146, 147, 185, 186). Similar results were found when the FMD was reported as both relative FMD (Fig. 17A) and normalized FMD (Fig. 17C) with the OU group having significantly lower values than both the YA and OT groups and no significant difference found between the YA and OT groups. When expressed as absolute FMD (Fig. 17B), the OT group had a significantly higher response than both the YA and OU groups, while there was no significant difference between the YA and OU groups. Lastly, when allometrically scaled (Fig. 17D), the OT group had a significantly higher response than both the YA and OU groups and

the YA group also had a significantly higher response than the OU group. This may be attributed to the fact that though the YA and OT groups were fitness matched, the OT participants may have been participating in more regular physical activity, while the YA group, though healthy, may only have been recreationally active. This is hypothesized due to the wide range of VO_{2peak} values observed. One of the main factors responsible for inducing the enhanced relative FMD after aerobic exercise training is the increased shear stress on the endothelial cells (55, 57, 68, 140, 218). The endothelium of the OT group would have been exposed to this stressor more frequently if they were more highly trained, inducing adaptations, which are conducive to increased FMD response.

These FMD results were all similar, with the OU group FMD significantly lower than their OT peers regardless how the results are expressed (Fig. 17A-D). The YA relative FMD response was significantly higher than the OU response, with three of the four methods (relative FMD, normalized FMD, and allometrically scaled FMD; Fig. 17 A, C, D, respectively) and significantly lower than the OT response with two of the four methods (absolute FMD and allometrically scaled FMD; Fig. 17 B, D, respectively). This may be due to differences in aerobic fitness, as the OT group had all VO_{2peak} values within the 'Superior' category for their age and sex, whereas the YA group had VO_{2peak} values ranging from Poor-Superior (79). Additionally, the volume of training and training status of these groups are unknown. Investigating individuals with similar training statuses may help address this issue.

5.4- Relationship Between MSNA and FMD

The present study found no significant relationship between FMD and MSNA BF in both the YA and OU groups (Fig. 21A and B, respectively). However there was a significant positive relationship between these two variables in the OT group (Fig. 21C) and also between these variables in all three groups (Fig. 21D).

As detailed above, β-adrenergic receptor sensitivity and baroreflex sensitivity decrease with age (72, 173, 209). However, these two mechanisms, which help to control blood pressure, are still well maintained in the YA group. For this reason, any changes in blood pressure that occur in young adults, as measured via arterial

stretch by arterial baroreceptors, can be easily adjusted. In older individuals, due to decreases in these two control mechanisms, the β-adrenergic vasodilator receptor sensitivity (73) and arterial baroreflex sensitivity to changes in blood pressure (209), maintenance of arterial tone through other means becomes more important. Therefore, this may help to explain why there is an increase in MSNA associated with higher fitness levels in older individuals, as a mechanism to increase constriction and maintain arterial tone.

Older individuals who are aerobically trained have increased endothelial function, and demonstrate increased MSNA compared to their sedentary peers (51, 125, 177). It was hypothesized that the increase in MSNA in this population was a mechanism to help protect from hypotension and to help maintain arterial tone. Additionally, it has been hypothesized that higher MSNA helps to maintain appropriate blood pressure and cerebral perfusion in older adults (129). As the long-term effects of exercise on the endothelium occur, such as increased NO bioavailability and vascular remodeling, the increased MSNA acts to prevent the blood vessels from excessive vasodilation, helping to maintain basal limb blood flow both at rest and during exercise (173). This hypothesis is supported by the present research, as there was no significant difference in leg blood flow between any of the groups (Table 2) despite the increase in EDD in the YA and OT groups compared to the OU group.

Higher levels of nitrates have been associated with higher levels of MSNA in young, healthy participants (163). The additive effect of increased age and increased nitric oxide bioavailability due to a higher aerobic fitness level may help to explain why there is an associated increase in MSNA as FMD increases in the OT group. NO can be a direct mediator of enhanced sympatholysis, and physical activity through ageing can prevent age-related declines in functional sympatholysis (117, 130). In addition, the increased MAP observed in the OT group in the current study further supports this hypothesis (Fig. 15D). As increased MSNA is a systemic effect, this will induce constriction though the entire vasculature. Endothelial adaptations may only occur in the active limbs (126, 152, 191), as this is where the stimulus for change, the increased shear stress (33), is taking place. As

such, in order to maintain limb blood flow to these limbs where adaptations have occurred, the subsequent increase in MSNA may constrict vessels that may not be at risk from this excessive vasodilation. MSNA is not correlated with MAP in young, healthy participants (25, 176) however these variables become closely linked in older individuals (16, 73) and this may be a byproduct of this relationship.

The changes in the relationship between MSNA and blood pressure may be due to decreased β-adrenergic vasodilator receptor sensitivity and baroreflex sensitivity, which decrease with age (72, 173, 209). β-adrenergic receptors are located on the vascular smooth muscle, and detect chemical signals, such as from acetylcholine, which induce relaxation and subsequent vasodilation (217). The arterial baroreflex helps to maintain arterial tone as it detects beat-to-beat arterial stretch, and subsequently increases or inhibits MSNA based on the pressure (166, 172). Hart et al. (2011) used a β-blocker to observe the effects on the relationship between MSNA and MAP as well as MSNA and TPR in young and post-menopausal women (72). It was found that in young women, after administration of the β-blocker, the relationships between MSNA and MAP as well as MSNA and TPR became significant ($r=0.58$, $p<0.05$ and $r=0.59$, $p<0.05$), whereas there was no change in the relationship for older women (72). This supports the hypothesis that the adrenergic receptors are important in helping to maintain normal blood pressure measures in young adults, but other mechanisms must be utilized in older adults (16, 72, 90), such as MSNA. Additionally, Sugawara et al. (2007) found that a 3-month exercise intervention significantly improved adrenergic receptor responsiveness to phentolamine, an α-adrenergic blocker ($p <0.05$) in older adults (60 ± 3 years) (173). This increased adrenergic receptor responsiveness with aerobic fitness in older adults, accompanied by a decrease in the ability to control blood pressure with the arterial baroreflex, helps to explain why MSNA increases with increasing FMD in older aerobically trained adults. It may possibly be used as a 'buffer' for the increased endothelial function associated with aerobic exercise.

The potent vasoconstrictor endothelin-1 increases with age, which may cause additional increases in vasoconstriction (103, 157). With exercise training however, Thijssen et al. (2007) has shown an associated increase in leg blood flow

in older men (aged 67-76 years), which is at least partially due to decreased endothelin-1 levels (193). As such, with increased aerobic fitness, and the associated increase in endothelial function and EDD, a decrease in endothelin-1 would allow for potentially more vasodilation. This may be beneficial during exercise, as increased leg blood flow and oxygen-delivery may increase performance, however this would also increase the risk of the older trained individuals developing hypotension. As such, MSNA may also increase in the OT group due to decreases in endothelin-1 vasoconstriction. Endothelin-1 levels were not however measured in the present study.

In healthy, young adults, increased SNA is linked with lower endothelial function both in studies which used transient increases in SNA (4, 80, 135), as well as in the only study to date which has concurrently measured MSNA and the reactive hyperemia index in young healthy participants (180). Increases in MSNA lead to increased vasoconstriction, which then is exhibited in a lower endothelial-dependent dilation, as the endothelial cells would have to overcome higher VSM constriction. Additionally, it has been shown that higher levels of MSNA are associated with higher levels of NO release in young, healthy individuals (163), in order to help counteract the increased MSNA.

When investigating how these variables change with exercise, Sverrisdottir et al. (2010) found that increased hours of physical activity per week in young adults was linked to lower MSNA and increased reactive hyperemia index (180). These results are not fully supported by the present findings. While there was no relationship between MSNA BF and VO_{2peak} in the YA group (Fig. 19A), there was also a significant decrease in relative FMD as VO_{2peak} increased (Fig. 20A). These findings may be linked to previously mentioned potential differences in the level of aerobic fitness within the YA group and how this may affect relative FMD as well as studies which have found no link between aerobic capacity and endothelial function (208).

The current research supports previous work done with MSNA, ageing, and aerobic fitness which have found increased MSNA to be associated with higher aerobic fitness levels in highly trained older athletes (125). This is in contrast to

older individuals who are sedentary, as they have been found to have decreased endothelial function (140, 157, 217) and lower MSNA (124, 125) than their aerobically trained counterparts. This may be counterintuitive, as increased MSNA is closely linked with cardiovascular diseases and aerobic exercise helps to prevent cardiovascular disease, however it is hypothesized, and supported by the current study, that this increased MSNA may be a byproduct of an increase in EDD with improved aerobic fitness in order to maintain arterial tone.

5.5- Limitations and Future Directions

A limitation of the present study was though YA and OT groups were VO_{2peak}-matched, the range of aerobic fitness levels in the YA group may have influenced some variables. As increased shear stress from increased blood flow during exercise is the main driver of endothelial function adaptations, if the YA participants were participating in less physical activity their overall magnitude of shear stress would be less. This may help explain the negative correlation observed in this study between VO_{2peak} and relative FMD, whereas others have found a positive correlation (56, 180) or no correlation (208). Other factors can influence endothelial function, such as body composition and diet (17, 93, 164). Obesity has been linked to lower exercise-induced FMD in young males as compared to their lean colleagues (164). Additionally, FMD has been shown to be impaired after a diet high in saturated fats (93), whereas diets high in antioxidants and folic acid have been shown to have improved endothelial function (17). Therefore, future studies should match groups for the volume of aerobic exercise, diet, and body composition, to observe how this would influence the results.

The current study also did not measure any other factors that may affect vascular tone and NO bioavailability, such as levels of endothelin-1, reactive oxygen species, and asymmetric dimethylarginine (38, 103, 179, 182). Therefore, differences in vasodilatory function may be influenced by more than just changes in NO in the present study. Additionally, MSNA is only a measure of neural signal and cannot tell us about the amount or type of neurotransmitter released nor the sensitivity of the receptors for those transmitters. Future studies should measure

levels of all of these substances that affect arterial tone or use chemical agents, such as norepinephrine (α-receptor agonist) or phentolamine (α-receptor blocker), as used by La Favor et al. (2014) to test NO-mediated dilation or α-adrenergic vasoconstrictor receptor sensitivity (51), in order to compare these same groups.

There are well studied differences in EDD between sexes, with female circulating sex hormones, specifically estradiol, hypothesized to have vascular protective properties (11, 51, 76, 140, 169). This is supported by lower incidences of CVD and improved endothelial function in premenopausal women, as well as in women taking hormone replacement therapy (149), compared to men and post-menopausal women (66, 133). The YA and OT groups were not only fitness matched but also sex matched (7 males, 1 female per group), however the OU group was not sex matched (3 males, 5 females). Adherence to published guidelines during the present study was done to minimize any potential sex-differences. For example, young females were tested on days 1-7 of their menstrual cycle, when circulating sex hormones are the lowest as previously recommended (133, 186). Additionally, all females in the OU group were post-menopausal, which theoretically should abolish any sex-related differences in EDD (66). Despite this, there is the potential for sex-related differences in endothelial function to exist between the groups.

Though all of the women were post-menopausal, changes in vascular health occur at different rates in men and women. Men begin to see increases in blood pressure earlier, but post-menopausal women's blood pressure increases more rapidly (16, 73, 120). Future studies should investigate further the sex-specific affects of aerobic fitness on MSNA and endothelial-dependent dilation.

The present study could be strengthened through recruitment of larger sample sizes, which would help increase the statistical power. Additionally, due to the pooling of two different data sets for the present analysis, endothelial-independent dilation was not assessed in enough participants to draw conclusions. The endothelial-independent (i.e., NTG test) data are available in Appendix H, but future studies should implement this protocol in order to assess vascular smooth muscle reactivity, which will help make conclusions about blood vessel health (43, 91).

Lastly, the present study only investigated aerobic fitness in healthy individuals and so more work needs to be done to determine the mode and volume of training to see results and how this work translates to clinical populations, as these are the individuals who will see the most benefits from exercise interventions and prescription.

Furthermore, the current study was cross-sectional in nature and did not control for diet, body composition, and volume of physical activity, all of which can have effects on either FMD or MSNA or both (17, 57, 93, 128, 156). This means that many confounding factors may exist within our study population. A better study design would be a longitudinal design looking at the pre- and post-aerobic training differences in MSNA, FMD, and how this relationship changes in older individuals. Additionally, studies should be conducted in clinical populations who may benefit the most from these interventions. These populations have heightened MSNA and reduced FMD, therefore studies should be conducted to investigate the effects of aerobic training on MSNA and FMD in these groups.

5.6- Conclusion

The present study demonstrated the first known concurrent collection of direct measures of sympathetic nerve activity and endothelial dependent dilation in older trained and untrained individual. The main result of the study was the strong positive correlation between MSNA BF and relative FMD in the OT group.

The intricate linkage between FMD and MSNA in respect to ageing and aerobic fitness level has been highlighted through the results of this study. The relationship between VO_{2peak} and MSNA BF was insignificant in all groups, however the relationship between FMD% and MSNA BF was significant in the OT group. As aerobic fitness levels increase, the subsequent increase in NO and reductions in inflammation and increases in antioxidant capacity of the arteries leads to improved EDD (55, 57). In older aerobically individuals, who have decreased adrenergic responsiveness (72) and decreased arterial baroreflex sensitivity (129, 209), in order to maintain blood flow and MAP, this is balanced by an increase in MSNA. Increases in MSNA are not associated with increased FMD% in YA as this

group maintains a higher adrenergic responsiveness and improved arterial baroreflex sensitivity than older individuals (81, 209), and so blood flow and MAP are maintained through these mechanisms. This balance is not seen in the OU group, as their EDD is not enhanced and therefore additionally increases in MSNA, above that typically observed with ageing (75, 124), are not needed.

As EDD increases in older individuals as a benefit of aerobic exercise, the subsequent increase in MSNA is a potential mechanism to help this group buffer these endothelial increases in vasodilatory capacity and maintain arterial tone. The understanding of this relationship and its influence on blood vessel health and blood pressure has implications for the understanding of cardiovascular disease and developing targeted treatments for hypertension. More studies are needed to determine the volume of exercise training necessary to elicit changes, longitudinal studies investigating the influence of other variables such as diet and body composition, and if these same patterns of increased FMD and MSNA with improved aerobic fitness are true in clinical populations who may benefit the most from interventions, such as those suffering from heart failure.

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Appendix A- Dalhousie University Research Ethics Board Approval Letter

Health Sciences Research Ethics Board
Letter of Approval

June 24, 2014

Ms Diane Ramsay
Health Professions\Health & Human Performance

Dear Diane,

REB #: 2014-3254
Project Title: Blood Vessel Health: The Influence of Ageing and Aerobic Fitness

Effective Date: June 24, 2014
Expiry Date: June 24, 2015

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. Brenda Beagan, Chair

Appendix B- Acadia University Research Ethics Board Approval Letter

Research Ethics Board

Acadia University Box 181
Wolfville, Nova Scotia
Canada B4P 2R6
Email: smaitzen@acadiau.ca
Telephone: (902) 585-1407
http://reb.acadiau.ca

12 July 2015

Professor Said Mekary
School of Kinesiology
Acadia University

Re: "Blood Vessel Health: Influence of Ageing and Aerobic Fitness" (REB 15-33)

Dear Professor Mekary,

At its meeting of 9 July 2015, the Acadia University Research Ethics Board granted ethics approval to your above-referenced research proposal. In the judgment of Dr. Conor Vibert, a Representative of Faculty on the Board, the proposed research poses no more than minimal risk of harm to research subjects. Accordingly, your application received delegated ethics review and approval by Dr. Vibert and subsequent ratification by the entire Board, as provided for in Articles 2.9 and 6.12 of the Tri-Council Policy Statement (TCPS2) governing research on human subjects.

This approval is for a term of one year. If your project will not conclude before 12 July 2016, please contact me at that time for an extension of this term of approval. Please inform me of any significant changes to the research before they are implemented. Please also note this additional requirement: In accordance with Article 6.14 of TCPS2, the Board must be promptly notified when each project concludes; an email notification sent to me will suffice.

The Board extends its best wishes for a successful project.

Sincerely,

[redacted]

Stephen Maitzen, Ph.D.
Chair, Research Ethics Board

Appendix C- Informed Consent Form

Project Title: Blood Vessel Health: The Influence of Ageing and Aerobic Fitness

We invite you to take part in a research study being conducted by Diane Ramsay, a student at Dalhousie University, as part of her MSc Kinesiology degree. The supervisor for this study is Dr. Derek Kimmerly, who has been conducting human exercise physiology research for over 14 years. Participation in this research is up to you and you can leave the study at any time. There will be no negative impact on your studies or your access to health care 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 benefits, risks, or discomforts that you might experience. You should discuss any questions you have about this study with Diane Ramsay.

Who Is Conducting the Research Study?

INVESTIGATOR:	DEPARTMENT/DIVISION:	CONTACT:
Diane Ramsay	School of Health and Human Performance/Kinesiology	diane.ramsay@dal.ca
Derek S. Kimmerly, PhD	School of Health and Human Performance/Kinesiology	(902) 494-2570 dskimmerly@dal.ca

Purpose and Outline of the Research Study

The goal of this research is to find out more about the relationship between age, fitness level, and the health and function of a person's blood vessels and nervous system. This will be done by measuring your aerobic fitness level, resting nervous system activity, and blood vessel response to a change in blood flow. This will help us better understand the interactions between these functions and how aerobic fitness changes the normal process that occurs with ageing.

Who Can Participate in the Research Study?

You will not be eligible for this study if you:

- Are **not** between the ages of 18 and 40 or 55 and 80
- Have a resting blood pressure >140/90 mmHg
- Are a current or recent (within the past 6 months) smoker
- Have been diagnosed with a medical condition that affects the nervous or cardiorespiratory systems or a condition which affects your metabolism (i.e. diabetes)
- Have a body mass index of >30kg/m^2

- Have been prescribed high blood pressure or any other cardiovascular medications
- Have a fear of needles
- Are pregnant or breastfeeding

What You Will Be Asked to Do?

To help us understand how differences in aerobic fitness levels change the blood vessels and nervous system, we will ask you to come in for 2 sessions with a total time commitment of about 4 hours. The sessions must be separated by at least 48 hours. During the first session, you will first complete and sign this informed consent form. Following this your height and weight will be collected as well as your resting blood pressure to ensure you are eligible to participate. Following this we will get you to complete a Physical Activity Readiness Questionnaire and a Health History Questionnaire. You will be shown the equipment we will use in the study and you will have a chance to ask any questions. After this we will direct you to a private change room if you need to change into comfortable clothing for the test. We will then fit you with a heart rate monitor and a mask that goes over your mouth and nose that will measure the levels of oxygen and carbon dioxide you breathe out. If you are uncomfortable wearing a mask that covers both your nose and mouth, you have the option to wear an individual mouthpiece with a nose clip. We will then ask you to warm up and then perform a maximal exercise test on a stationary bike. This will include a 5 minute warm-up, and then we will increase the resistance every minute until you feel you have reached your maximum effort. After this test is done and you have completed a cool down, you will be free to go. This session will take approximately 1 hour.

During the second session, we will first direct you to a private change room if you need to change into comfortable clothing for the duration of the test. The first part of this session will include placing three small electrodes on your upper body to monitor your heart rate, followed by fitting you with a belt around your chest that will measure your breathing frequency. To monitor your blood pressure, we will be fitting you with a small cuff around your index or middle finger as well as a cuff around your upper left arm. After this set-up, we will be taking direct measurements of your nerve activity of your lower leg. A very small, sterile microelectrode (about the width of a hair) will be inserted through your skin and positioned just under the skin about 2-3cm from the nerve site. This will be followed by the placement of a second tiny electrode, through your skin, into the nerve. This second electrode may be moved around slightly, until an appropriate recording site is found. Once an appropriate site is found, the microelectrode will remain in your skin for the whole experiment. In most people, a good site can be found in 20-30 minutes, but in some people either a good site cannot be found or their nerve activity it so low that a good site cannot be determined. If a good site cannot be located within an hour, the procedure will stop.

After we have collected this data, we will then look at the ability of an artery in your lower leg to relax by taking pictures of it with an ultrasound machine after two separate tests. First, we will take images of your artery at rest, with you lying on your stomach while we spread a water-based gel over the space behind your leg and collect resting measurements with the ultrasound probe for ~5 minutes. After, we will place a pressure cuff around your leg, which we will inflate to a high level in order to temporarily stop the

blood flow to your foot and lower leg, while taking pictures with the ultrasound. After 5 minutes, we will deflate the cuff and blood flow will return to normal after a few minutes, while still taking pictures of the artery for 5 minutes after the cuff is released in order to see how much bigger it gets. After this is done, you will rest for a minimum of 10 minutes while we wait for the artery diameter and blood flow to return to resting levels. We will then administer a single spray (0.4 mg) of nitroglycerin under your tongue and continue to measure your artery size and blood flow for an additional 10 minutes. This session will take approximately 3 hours.

Possible Benefits, Risks, and Discomforts

Presented here are the potential risks and discomforts that may arise throughout the study:

a) Maximal Exercise Testing: The mask worn while testing will allow you to breath in room air while exhaling air into our analyzers. The mask will stay on for the duration of the test and may cause some discomfort, especially at higher intensities of exercise. If you are uncomfortable wearing a mask that covers both your nose and mouth, you make wear an individual mouth piece with a nose clip. An aerobic exercise test is safe and all people who are helping to run the study are trained in first aid and have access to a defibrillator as well as a phone in the small chance that it is needed. You may experience some moderate pain and discomfort, along with shortness of breath and tiredness when completing the test, as with any type of maximal exercise. This will go away once the exercise stops.

b) Heart Rate Measurements (with electrodes): Small sticky patches will be placed on your chest, shoulders, and ribcage. The patches will record the electrical activity of your heart. A small rash may develop on the skin after you remove the patches. This is due to the adhesive but should disappear within 48 hours.

c) Finger Blood Pressure Measurements: You may experience slight discomfort as a result of these measurements, including sensations of "pins and needles" in your fingers. When in use, the cuffs will inflate with air and you should feel it gently squeeze your fingers. Your fingers 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.

d) Upper Arm Blood Pressure Measurements: Blood pressure will be measured from the brachial artery by fitting a pressure cuff around your upper arm. The cuff will be inflated to a pressure automatically by a machine and then released slowly to determine your blood pressure. There is some physical discomfort associated with the pressure that this cuff will exert on your arm. You may experience a tingling sensation in your left hand and the skin may turn slightly bluish in colour. These symptoms should subside shortly after the cuff is deflated. 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.

e) Nerve Activity in the Lower Leg: A technique called microneurography will be used to measure the nerve activity in your fibular nerve, just below your knee. First, the position of your nerve will be mapped out using a small pen-shaped instrument that emits a small electrical pulse. This will cause the muscles in your lower leg to twitch or tingle, which may be a strange sensation but will not be painful and the sensations will disappear when the stimulation stops. After this, two small microelectrodes will be placed just under your skin close to your knee. Microneurography has been used in over 2000 experiments worldwide with only 3 known complications in the last 40 years, the worst of which was a slight weakness in the leg muscles lasting 2 to 6 months. Dr. Derek Kimmerly will be performing all microneurography procedures, and he has performed over 400 microneurographic procedures since 2001. You may feel a small pinch when the electrode is being inserted, but once it is inserted, there should be no discomfort. You may notice a mild ache in the muscles of your right leg for a few days after the procedure. ***We recommend that you restrain from heavy physical activity with the right***

leg for 24 hours after the testing. Also, we recommend that no pressure be placed over the site for the 24 hours following the experiment.

f) Tests of Blood Vessel Health: How your popliteal artery (located behind your knee) 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. When the blood flow is cut off to the lower leg for 5 minutes, you may feel some tingling, numbness, a cold sensation, or have a slight bluing of your foot or lower leg. All of these symptoms will go away once we have released the cuff. There is no known medical risk with cutting of blood flow for 5 minutes and this technique has been used on thousands of people.

The reported possible side effects of nitroglycerin administration include: dizziness, lightheadedness, 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 lightheadedness. However the administration of nitroglycerin while you are lying down should minimize this risk. If, however, dizziness or lightheadedness 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.

Throughout the testing we will be taking numerous measures of your cardiovascular health. If for any reason we find information that may show a possible health risk, we will explain the issue to you and strongly recommend that you visit your family doctor. You will no longer be eligible to participate in the study.

Compensation/Reimbursement

There is no compensation for being part of this research study. However, information regarding your general cardiovascular health including resting blood pressure and heart rate will be provided to you upon request.

If you are bringing your own vehicle to the study, the cost of parking will be covered. We will meet you at the entrance of the building and pay for your parking meter. Additionally, we will make sure that the meter is paid for the whole time you are in the lab participating in the study.

If you answer "yes" to one of the questions on the Physical Activity Readiness Questionnaire, we may give you a form to be filled out by your doctor before you start the study. This form is used to get your doctors approval before you participate. If there is a fee associated with your doctor completing this form that is not covered by either the province's or your own health care plan, we regrettably cannot reimburse you for this.

Privacy and Confidentiality

All information collected during this study will remain confidential. It will be stored in locked offices and on secured computers. Only the principal investigator and her supervisor will have access. You should know that the results of this study will be made available to the scientific community. This will happen through publication in a scientific journal. Neither your name nor any reference to you will be used in creating or publishing these results. 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, you may have access to your own data, as well as the group data when it becomes available and if you are interested. To ensure confidentiality of participant information each participant will be assigned an identification code. Each code and its files will be labeled and stored in a secured file folder.

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 you want any of the information that you have contributed up to that point to be removed or if you will allow us to use that information. You can also decide to have your information removed up until the time of study publication and/or presentation. After that time, it will become impossible for us to remove it because it will already be analyzed.

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 Diane Ramsay at diane.ramsay@dal.ca or Dr. Derek Kimmerly at dskimmerly@dal.ca or 902-494-2570 at any time with questions, comments, or concerns about the research study. 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

Signature Page

Project Title: Blood Vessel Health: The Influence of Ageing and Aerobic Fitness

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 at any time.

_____ _____

Participant's Signature DATE

_____

Print Name of Participant

_____ _____

Signature of Witness DATE

I would like to receive a copy of group results or my individual results (please indicate):

_____ _____

Participant's Signature DATE

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

_____

Please send me (please circle):

GROUP RESULTS INDIVIDUALS RESULTS BOTH

Appendix D- Information Poster

WANT TO LEARN MORE ABOUT YOUR AEROBIC FITNESS AND BLOOD VESSEL HEALTH?

Are you healthy and between the ages of 18-40 or 55-80?

You could participate in research looking at how blood vessel health changes with ageing and aerobic fitness

You will not be eligible if you:

- ✓ *are pregnant or breast feeding*
- ✓ *are a smoker or have quit smoking within the past six months*
- ✓ *have disorders that affect the heart, blood vessels or lungs*
- ✓ *have been told by a doctor to not engage in moderate-vigorous exercise*

A Master's of Kinesiology candidate enrolled in the School of Health and Human Performance at Dalhousie University is currently recruiting participants for this study. You would be required to attend two study sessions at Dalhousie University. The first session would take approximately 1.5 hours while the second would take approximately 3.5 hours. If you are interested, please contact us at your earliest convenience:

NAME: Diane Ramsay

E-MAIL: diane.ramsay@dal.ca

Blood Vessel Health Study diane.ramsay@dal.ca	Blood Vessel Health Study diane.ramsay@dal.ca	Blood Vessel Health Study diane.ramsay@dal.ca	Blood Vessel Health Study diane.ramsay@dal.ca	Blood Vessel Health Study diane.ramsay@dal.ca	Blood Vessel Health Study diane.ramsay@dal.ca	Blood Vessel Health Study diane.ramsay@dal.ca

Appendix E- Health History Questionnaire

1. *Age:* _____
2. *Sex*
   a) *Male*
   b) *Female*
3. *What is your approximate weight (kilograms)?* _____
   To convert from pounds to kilograms, multiply by 0.454

4. *What is your approximate height (meters)?* _____
   To convert from inches to meters, multiple by 0.0254

5. *Please calculate your approximate BMI:*

   BMI= kg/(meters)^2 = _____

6. *Do you currently have any physician diagnosed conditions or diseases?*
   a) *Yes*
   b) *No*

If yes please list the conditions here:

_____
_____

7. *Are you currently taking any prescription or over the counter medications?*
   a) *Yes*
   b) *No*

If yes please list the medications here:

_____
_____

8. *If you are female, are you currently pregnant?*

a) *Yes*

b) *No*

9. *If applicable, when was the date of your last period?*

   _____

10. *At any point in the past six months, were you a regular smoker?*

    a) *Yes*

    b) *No*

11. *How many days per week do you perform aerobic exercise at a moderate intensity or higher i.e. such that you have a rapid heart rate and increase in breathing? For example- brisk walking or cycling*

    _____

12. *How long do these sessions of aerobic exercise typically last?*

    _____

13. *Do you participate in any endurance based sports (eg. Soccer, running, swimming)?*

    _____

14. *Would you say you are active more, less, or the same amount now than five years ago?*

    _____

Appendix F- Physical Activity Readiness Questionnaire for Everyone (PAR-Q+, from the Canadian Society of Exercise Physiologists)

CSEP approved Sept 12 2011 version

PAR-Q+

The Physical Activity Readiness Questionnaire for Everyone

Regular physical activity is fun and healthy, and more people should become more physically active every day of the week. Being more physically active 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.

SECTION 1 - GENERAL HEALTH

	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)?	☐	☐
5.	Are you currently taking prescribed medications for a chronic medical condition?	☐	☐
6.	Do you have a bone or joint problem that could be made worse by becoming more physically active? Please answer NO if you had a joint problem in the past, but it does not limit your current ability to be physically active. For example, knee, ankle, shoulder or other.	☐	☐
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 Section 3 to sign the form. You do not need to complete Section 2.

- Start becoming much more physically active – start slowly and build up gradually.
- Follow the Canadian Physical Activity Guidelines for your age (www.csep.ca/guidelines).
- You may take part in a health and fitness appraisal.
- If you have any further questions, contact a qualified exercise professional such as a CSEP Certified Exercise Physiologist® (CSEP-CEP) or CSEP Certified Personal Trainer® (CSEP-CPT).
- If you are over the age of 45 yrs. and NOT accustomed to regular vigorous physical activity, please consult a qualified exercise professional (CSEP-CEP) before engaging in maximal effort exercise.

If you answered YES to one or more of the questions above, please GO TO SECTION 2.

Delay becoming more active if:

- You are not feeling well because of a temporary illness such as a cold or fever – wait until you feel better
- You are pregnant – talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the PARmed-X for Pregnancy before becoming more physically active OR
- Your health changes – please answer the questions on Section 2 of this document and/or talk to your doctor or qualified exercise professional (CSEP-CEP or CSEP-CPT) before continuing with any physical activity programme.

CSEP | SCPE

SECTION 2 - CHRONIC MEDICAL CONDITIONS

Please read the questions below carefully and answer each one honestly: check YES or NO.			YES	NO
1.	Do you have Arthritis, Osteoporosis, or Back Problems?		☐ If yes, 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)	☐	☐
	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)?	☐	☐
	1c.	Have you had steroid injections or taken steroid tablets regularly for more than 3 months?	☐	☐
2.	Do you have Cancer of any kind?		☐ If yes, 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 neck?	☐	☐
	2b.	Are you currently receiving cancer therapy (such as chemotherapy or radiotherapy)?	☐	☐
3.	Do you have Heart Disease or Cardiovascular Disease? This includes Coronary Artery Disease, High Blood Pressure, Heart Failure, Diagnosed Abnormality of Heart Rhythm		☐ If yes, answer questions 3a-3e	☐ 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)	☐	☐
	3b.	Do you have an irregular heart beat that requires medical management? (e.g. atrial brillation, premature ventricular contraction)	☐	☐
	3c.	Do you have chronic heart failure?	☐	☐
	3d.	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)	☐	☐
	3e.	Do you have diagnosed coronary artery (cardiovascular) disease and have not participated in regular physical activity in the last 2 months?	☐	☐
4.	Do you have any Metabolic Conditions? This includes Type 1 Diabetes, Type 2 Diabetes, Pre-Diabetes		☐ If yes, answer questions 4a-4c	☐ If no, go to question 5
	4a.	Is your blood sugar often above 13.0 mmol/L? (Answer YES if you are not sure)	☐	☐
	4b.	Do you have any signs or symptoms of diabetes complications such as heart or vascular disease and/or complications affecting your eyes, kidneys, and the sensation in your toes and feet?	☐	☐
	4c.	Do you have other metabolic conditions (such as thyroid disorders, pregnancy-related diabetes, chronic kidney disease, liver problems)?	☐	☐
5.	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 yes, answer questions 5a-5b	☐ If no, go to question 6
	5a.	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)	☐	☐
	5b.	Do you also have back problems affecting nerves or muscles?	☐	☐

Please read the questions below carefully and answer each one honestly: check YES or NO.			YES	NO
6.	Do you have a Respiratory Disease? This includes Chronic Obstructive Pulmonary Disease, Asthma, Pulmonary High Blood Pressure		☐ If yes, answer questions 6a-6d	☐ 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)	☐	☐
	6b.	Has your doctor ever said your blood oxygen level is low at rest or during exercise and/or that you require supplemental oxygen therapy?	☐	☐
	6c.	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?	☐	☐
	6d.	Has your doctor ever said you have high blood pressure in the blood vessels of your lungs?	☐	☐
7.	Do you have a Spinal Cord Injury? This includes Tetraplegia and Paraplegia		☐ If yes, answer questions 7a-7c	☐ 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)	☐	☐
	7b.	Do you commonly exhibit low resting blood pressure significant enough to cause dizziness, light-headedness, and/or fainting?	☐	☐
	7c.	Has your physician indicated that you exhibit sudden bouts of high blood pressure (known as Autonomic Dysreflexia)?	☐	☐
8.	Have you had a Stroke? This includes Transient Ischemic Attack (TIA) or Cerebrovascular Event		☐ If yes, answer questions 8a-c	☐ 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)	☐	☐
	8b.	Do you have any impairment in walking or mobility?	☐	☐
	8c.	Have you experienced a stroke or impairment in nerves or muscles in the past 6 months?	☐	☐
9.	Do you have any other medical condition not listed above or do you live with two chronic conditions?		☐ If yes, answer questions 9a-c	☐ If no, read the advice on page 4
	9a.	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?	☐	☐
	9b.	Do you have a medical condition that is not listed (such as epilepsy, neurological conditions, kidney problems)?	☐	☐
	9c.	Do you currently live with two chronic conditions?	☐	☐

Please proceed to Page 4 for recommendations for your current medical condition and sign this document.

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:

- It is advised that you consult a qualified exercise professional (e.g., a CSEP-CEP or CSEP-CPT) 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 – 20-60 min. 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 yrs. and NOT accustomed to regular vigorous physical activity, please consult a qualified exercise professional (CSEP-CEP) before engaging in maximal effort exercise.

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

- You should seek further information from a licensed health care professional before becoming more physically active or engaging in a fitness appraisal and/or visit a or qualified exercise professional (CSEP-CEP) for further information.

Delay becoming more active if:

- You are not feeling well because of a temporary illness such as a cold or fever – wait until you feel better
- You are pregnant - talk to your health care practitioner, your physician, a qualified exercise profesional, and/or complete the PARmed-X for Pregnancy before becoming more physically active OR
- Your health changes - please talk to your doctor or qualified exercise professional (CSEP-CEP) before continuing with any physical activity programme.

SECTION 3 - DECLARATION

- You are encouraged to photocopy the PAR-Q+. You must use the entire questionnaire and NO changes are permitted.
- The Canadian Society for Exercise Physiology, the PAR-Q+ Collaboration, and their agents assume no liability for persons who undertake physical activity. If in doubt after completing the questionnaire, consult your doctor prior to physical activity.
- 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.
- Please read and sign the declaration below:

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 they maintain the privacy of the information and do not misuse or wrongfully disclose such information.

NAME _____ DATE _____

SIGNATURE _____ WITNESS _____

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER _____

For more information, please contact:
Canadian Society for Exercise Physiology
www.csep.ca

KEY REFERENCES
1. Jamnik VJ, Warburton DER, Makarski J, McKenzie DC, Shephard RJ, Stone J, and Gledhill N. Enhancing the cost-effectiveness of clearance for physical activity participation: background and overall process. APNM 36(S1):S3-S13, 2011.
2. Warburton DER, Gledhill N, Jamnik VK, Bredin SSD, McKenzie DC, Stone J, Charlesworth S, and Shephard RJ. Evidence-based risk assessment and recommendations for physical activity clearance; Consensus Document. APNM 36(S1):S266-s298, 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 BC Ministry of Health Services.

CSEP approved Sept 12 2011 version

Appendix G- Borg Rating of Perceived Exertion Scale

Rating	Perceived Exertion
6	No exertion
7	Extremely light
8	
9	Very light
10	
11	Light
12	
13	Somewhat hard
14	
15	Hard
16	
17	Very hard
18	
19	Extremely hard
20	Maximal exertion