The effects of sprint interval training on endothelial function in young men and women

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Graduate Program in Kinesiology
A thesis submitted in partial fulfillment of the requirements for the degree in Master of Science
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The effects of sprint interval training on endothelial function in young men and women

(Spine Title: SIT improves endothelial function in young women)

(Thesis format: Monograph)

By

Stephanie M. Reid

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science

The School of Graduate and Postdoctoral Studies
The University of Western Ontario
London, Ontario, Canada

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The thesis by

**Stephanie M. Reid**

titled:

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is accepted in partial fulfillment of the requirements for the degree of
Master of Science

____________________  ____________________________
Date  Chair of the Thesis Examination Board
ABSTRACT

The benefits of exercise training on endothelial function in the healthy young population are unclear. This study was designed to assess the effects of sprint interval training (SIT; 4-7 x 30-45 s all out exercise efforts separated by 4 min of recovery, repeated 3-4 times/wk over 6 wk) on endothelial function of the superficial femoral artery (SFA) in young recreationally active men and women. Twenty-one men and twenty women arrived at the laboratory after >3 h fast and were assigned randomly to SIT or Control (no training) groups. SFA diameter and blood velocity (BV) were assessed using Doppler ultrasound and imaging before and after 5 min of suprasystolic cuff inflation of the lower limb. Normalized flow mediated dilation (FMD) was analyzed as the maximal Δ in SFA diameter (\%) ÷ shear rate (mean BV x 8 ÷ SFA diameter: s⁻¹) measured every 30 s for 5 min following cuff release. Percent FMD (% FMD) was also calculated as the maximal Δ in SFA diameter (%). Measures were assessed before and following 6 weeks of SIT vs Control. After training, no changes in baseline SFA diameter (Women: P=0.61; Men: P=0.33), peak shear rate (30 sec average) after cuff release (Women: P=0.10; Men: P=0.22) or peak flow after cuff release (Women: P=0.15; Men: P=0.50) were observed. However, in the women SIT decreased time to peak dilation (Pre=163±68 vs Post=69±34 s; P=0.003), increased normalized FMD (SIT = 0.002±0.001 vs Control = 0.001±0.001 s⁻¹; P= 0.02), and increased % FMD (SIT = 6.5±3.2 vs Control = 2.5±1.3 %; P= 0.04). There were no changes observed in the men. These data suggest that SIT improved endothelial function in the women. The mechanisms responsible as well as the reasons for the observed gender differences require further study.

Key words: Flow mediated dilation, Doppler ultrasound, blood velocity
ACKNOWLEDGMENT

Dr. Peter Lemon, you have given me all the necessary tools to be a great scientist. I appreciate your love for scientific research and how you are able to provide feedback and insight to any scientific research project. Thank you for all your guidance and for being passionate about everything you do. I am grateful to have had the opportunity to work under your supervision.

To my colleagues in the Exercise Nutrition Research lab, thank you for support and help along the way. Particularly, I would like to thank Alan and Kristine. Alan, thank you for taking on this research project with me and for helping it become a success. Kristine, thank you for keeping me sane during thesis writing.

The Neurovascular Research lab (NVRL) was an integral part of my thesis research. Without the guidance and the kindness of many people in the NVRL my project would not have come into fruition. Particularly, I would like to thank Dr. Shoemaker and Dylan Olver. To Dr. Shoemaker, thank you for being a great teacher and for taking the time to oversee my research. To Dylan, thank you for helping me get this project started, for taking the time to provide guidance and be a mentor; you will undoubtedly be one of the future great scientific minds.

Lastly, and most importantly, I would like to thank my family. They have encouraged and supported all of my athletic and academic endeavors. They have equipped me with the confidence and ability to persevere through any situation. I am very thankful for my family and without them I could not be where I am today. So I dedicate this thesis to my mother Valrie, my father Lascelle, my sister’s Stacey and Sheryl and nephews, Andre, Nathaniel, Mekhi and Micah. I love them very much and without them this thesis would not be complete.
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<td>AUC</td>
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Chapter 1
# Introduction

The cardiovascular system is composed of three components: the heart, the blood vessels, and the blood and all three are important for cardiovascular health. In fact, blood vessel properties, specifically the function of the most inward layer - the endothelium, can be an early predictor of future cardiovascular health problems (Vita, 2005). Furthermore, research has shown that endothelial function is reduced in people with major risk factors for cardiovascular disease (CVD), such as high blood pressure, smoking, and diabetes (Celermajer et al., 1992; Lloyd-Jones et al., 2010). Also, it is known that the endothelial cell layer is vital to artery function, particularly the vasodilatory response and likely this explains the association with CVD.

The properties of arteries, such as their ability to vasodilate, involve several mechanisms. Shear stress (friction caused by blood flow against the vessel wall) or flow-mediated dilation is one of these. Shear stress stimulates receptors on the endothelial layer of arteries, which cause the release of a vasodilator substance, nitric oxide (NO). NO is a gas that affects several target cells including smooth muscle cells, endothelial cells, and inflammatory cells (Strijdom, Chamane, & Lochner, 2009) and it plays a major role in cardiovascular cell signaling. In the myocardium, it is involved with contractility and protects against ischemic injuries (Jones & Bolli, 2006). As such, NO is described as an ubiquitous vasodilator that offers cardioprotective properties (Cooke & Dzau, 1997).

The flow mediated dilation (FMD) technique has been established as a means of determining the endothelial function of arteries (nitric oxide mediated). Briefly, the FMD technique uses Doppler ultrasound to examine the reactive hyperemic response of an artery (via artery dilation) after artery blood flow has been occluded for a period of three to five minutes. This is a valuable technique because it provides a link between NO bioavailability and cardiovascular health (CH). Specifically, reduced bioavailability of NO is associated with reduced CH and an increased prevalence of developing CVD (Cooke & Dzau, 1997).

Currently, cardiovascular risk factors tend to occur at a much younger age than in previous years (Lloyd-Jones et al., 2010; Roger et al., 2012) and a new initiative of the American Heart Association (AHA) is focused on the prevention of various risk factors (diabetes, hypertension) in the pediatric population (Lloyd-Jones et al., 2010; Roger et
al., 2012). Some research even suggests that the offspring of parents with CVD are more likely to have endothelial dysfunction, despite showing no other signs of cardiovascular risk factors (Taddei et al., 1996). Thus, enhancing endothelial function in the younger healthy population may be cardioprotective.

There is a great deal of evidence suggesting that low to moderate intensity endurance exercise training can improve endothelium function (Green et al., 2004; Maiorana et al., 2003; Moyna & Thompson, 2004; Walsh et al., 2003) especially in cardiovascular at risk and/or diseased individuals. Further, regular exercise (light to moderate intensity endurance exercise) decreases cardiovascular risk by 30% in individuals with CVD (Thijssen et al., 2010). Conversely, it is less clear whether or not exercise enhances endothelial function in a young healthy population (Clarkson et al., 1999; Green et al., 2004; Maiorana et al., 2001). However, some suggest that matching experimental groups based on age, sex, and body composition may help decrease confounding variables (Thijssen et al., 2010). Also, there is a dearth of literature about exercise and endothelial function, particularly, in young healthy women. This may be in part due to the added variability that hormonal fluctuation of the menstrual cycle adds to endothelial function (Hayashi et al., 1995).

Interestingly, there is evidence in young healthy individuals to suggest that repeated high intensity exercise, such as sprint interval training [SIT – four to six, 30 s “all out” efforts separated by four min recovery between bouts, over several weeks produces similar metabolic adaptations as traditional endurance training (Burgomaster et al., 2007; Gibala et al., 2006). Moreover, a recent study found that SIT or endurance training cause similar adaptations in endothelial function of the popliteal artery in young healthy participants (Rakobowchuk et al., 2008). In addition, these investigators speculated that if the sprint bouts were of longer duration endothelial function might be enhanced further (Rakobowchuk et al., 2008). Certainly, the longer duration sprint bouts could increase the amount of shear stress stimulus and facilitate a greater enhancement of endothelial function but this proposed response has yet to be documented. Further, additional research is needed to assess the effects of SIT on the endothelial function of other conduit arteries (large blood vessels that provide a low resistant path for blood to flow to organs and/or limbs). Importantly, SIT is very time efficient and may enhance
endothelial function in young healthy individuals. If so, use of SIT could decrease the early onset of cardiovascular risk factors and improve cardiovascular health. This would be consistent with the AHA suggestion that improving cardiovascular health in the younger population is likely a better way to decrease the prevalence of CVD into the future, as opposed to focusing solely on managing and treating those who have already developed CVD (Roger et al., 2012).

The aim of this thesis was to further understand the effect of a longer duration SIT regimen (four – seven, 30 s to 45 s “all out” effort bouts; three times per week over six weeks) on superficial femoral artery endothelial function in young recreationally active healthy men and women. It was hypothesized that the longer exercise bout (up to 7 x 45 s) SIT protocol would improve endothelial function of the superficial femoral artery.
Chapter 2
2 Review of Literature

2.1 Vasculature

The cardiovascular system is composed of the heart, blood vessels, and the blood. Specifically, the blood vessels (the vasculature) are composed of arteries, veins, and capillaries. Arteries carry blood away from the heart, whereas veins carry blood towards the heart. With the exception of the pulmonary system, arteries transport oxygenated blood and veins transport deoxygenated blood (Nicpon-Marieb, 2004).

The structures of arteries and veins have some similarities. For example, the outermost layer of both is the tunica externa, the middle layer is the tunica media, and the inner layer is the tunica intima. The tunica intima is composed of the endothelium and a subendothelial layer. The endothelium layer makes contact with the blood. Arteries have a much thicker tunica media than veins and, in addition to the three layers, have two additional elastic layers called the internal and external lamina. The elastic components in arteries, particularly in the aorta, are necessary because of the pressure exerted on the walls when the blood is carried away from the heart (Nicpon-Marieb, 2004). As such, the aorta is sometimes referred to as an elastic conduit artery. The role of conduit arteries (superficial femoral artery, brachial artery, carotid artery, etc) is to provide a low resistance path for blood to flow to organs and/or limbs (Greenwald, 2007).

2.2 Regulation of Blood Circulation

There are several factors, such as blood flow and resistance, which play a role in the regulation of blood pressure and blood circulation. Blood flow is simply the volume of blood flowing through a vessel in a given timeframe (ml/min). As blood flows through the blood vessels, it exerts pressure on the vessel walls, expressed in millimeters of mercury (mmHg), typically. Systolic pressure represents the highest pressure exerted on the artery wall during the contraction phase of the cardiac cycle (systole) and diastolic blood pressure is the lowest pressure exerted on the artery wall during the relaxation phase (diastole). Resistance is another important component of blood circulation.

Resistance is the opposition to flow that is created as the blood travels through the blood vessel; this creates friction against the vessel wall. Interestingly, most of the resistance is found further away from the heart in the more peripheral arteries. Thus, resistance is
commonly referred to as peripheral resistance. In 1864, Jean Baptiste Poiseuille described the components that determine resistance (see equation below)

\[
\text{Resistance} = 8 \times \text{viscosity} \times \text{length} \times \text{blood flow} \\
= \pi \times \text{radius}^4
\]

where \(\pi = \text{mathematical pi (} \approx 3.14\)

Poiseuille’s Law (Pfitzner, 1976) states that resistance is affected by blood viscosity (thickness), vessel diameter, and vessel length. The longer the vessel and the greater the viscosity, the more resistance but both viscosity and vessel length remain rather constant so often these are unimportant factors. In contrast, blood vessel diameter is very dynamic; as the diameter decreases, friction or resistance increases. Further, resistance varies inversely with the fourth power of the vessel radius. Therefore, a doubling of vessel radius produces a large change (decrease) in resistance. Similarly, a halving the arterial radius results is a sixteen-fold increase in resistance. The largest changes in vessel diameter are observed in the smaller vessels, i.e., the arterioles. Consequently, it is the smaller downstream vasculature that determines the resistance to flow.

Hemodynamics, specifically describes the governing factors (blood pressure and resistance) that define blood flow. Furthermore, there are principles that describe the dynamics of blood flow. Ohm’s Law, first described by George Ohms in 1827, is a key principle that governs flow of all fluids (Schagrin, 1963). Ohm’s law, as applied to blood, states that blood flow is equal to the pressure differences between the arterial (high pressure) and the venous (low) pressure divided by blood flow resistance (R). Specifically, in arteries, the pressure gradient is the difference between any two points along the vessel.

\[
\text{Flow (ml \cdot min}^{-1}) = \frac{\text{change in pressure (}\Delta P\text{)}}{\text{Resistance (R)}}
\]
Lastly, arterial compliance and vascular conductance are elements that can change in response to fluctuations in blood flow. Vascular conductance (VC) is the capacity of blood to flow or the ability to transmit fluid. VC is equal to blood flow over change in pressure (Calbet et al., 2004). Furthermore, as VC is increased, resistance is decreased (see equation below).

\[
VC \text{ (ml } \cdot \text{ min}^{-1} \cdot \text{ mmHg}^{-1}) = \frac{\text{Flow (Q)}}{\text{change in pressure (\Delta P)}}
\]

Rearranged from Ohm’s Law

\[
R \text{ (mmHg } \cdot \text{ ml } \cdot \text{ min}^{-1} ) = \frac{\text{change in pressure (\Delta P)}}{\text{Flow (Q)}}
\]

Arterial compliance is a mechanical property of arteries. Basically, it is the ability of the arteries to stretch in response to changes in blood flow and/or pressure.

There are several important relationships that exist between blood flow, pressure, and resistance. A change in one component (resistance) initiates changes in another (pressure). Thus, it is clear that hemodynamics is an integrative process and that the ability of the vascular system to maintain a balance between blood flow, pressure and resistance is an important aspect of cardiovascular health.

### 2.3 Cardiovascular Health

Over the last decade, cardiovascular disease (CVD) among adults in North America has been the leading cause of death and morbidity (Lloyd-Jones et al., 2010). As a result, a large emphasis has been placed both on understanding how to decrease CVD incidence as well as on ways to quantify the severity of CVD. A recent focus of the American Heart Association (AHA) is to improve the cardiovascular health (CH) of the entire population (Lloyd-Jones et al., 2010). A secondary focus of the AHA is to create initiatives to combat the major risk factors (increased obesity, prevalence of both hypertension and type 2 diabetes) in the pediatric population, because each is associated with the development of CVD (Roger et al., 2011). This new trend/focus suggests that improving CH in the younger population may help decrease the onset of cardiovascular risk factors that lead to CVD.
Alterations in vascular properties, such as decreased arterial compliance (the ability of the arteries to stretch in response to changes in blood flow and/or pressure) as well as the ability to vasodilate, can be useful in identifying early signs of CVD (O'Rourke & Hashimoto, 2008; Vita, 2005). Clearly, the endothelial layer in blood vessels plays a key role in the ability of arteries to dilate or constrict. Overall, the goal of improving CH is to reduce CVD via decreasing cardiovascular risk factors and to do so it is important to understand that many of these risks originate in the endothelial layer of the blood vessels.

2.4 The Endothelial Cell layer

The endothelium is the largest organ in the body and, as described above, is the layer in the blood vessel structure that makes contact with the blood. One role of the endothelium is to sense both mechanical and chemical stimuli associated with changes in blood flow and blood pressure. Further, it is clear that the endothelial layer is quite dynamic (Furchgott & Zawadzki, 1980). Seminal studies have described the role of the endothelial cell layer in the vasculature in relation to changes in blood flow (Berdeaux et al., 1994; Langille & O'Donnell, 1986; Pohl et al., 1986). These studies altered blood flow in the arteries of animals with intact and denuded endothelium. The increase in blood flow in the intact endothelium caused dilation, whereas in the denuded endothelium there was constriction. These data suggest that increases in blood flow cause an increase in diameter, which is both endothelial dependent and shear stress mediated (Berdeaux et al., 1994; Langille & O'Donnell, 1986). Basically, as blood flows, friction is created by the red blood cells making contact with the vessel wall; this friction is called shear stress. Further, studies have manipulated shear stress to validate that changes in arterial size were indeed shear stress mediated (Green et al., 2005; Pohl et al., 1986; Rubanyi, Romero, & Vanhoutte, 1986).

Shear stress causes the endothelium to release agents that trigger the inflammatory processes, regulate vasomotor function, and alter hemostasis (Endemann & Schiffrin, 2004). NO and prostacycline are vasodilatory substances produced by the endothelium. Specifically, NO is one of the most important endothelial derived relaxing factors (Palmer, Ferrige, & Moncada, 1987) and decreased amounts of NO are associated
with CVD (Creager et al., 1990). Clearly, the endothelial cell layer has a dynamic role in vascular function. Thus, it is understandable that dysfunction or damage to the endothelial cell layer has several implications for the onset of CVD.

Perhaps not surprisingly, hypertensive participants have a decreased ability for forearm vasodilation (Panza et al., 1990). Further, CVD and several cardiovascular risk factors are associated with a deficiency in vasodilation. For example, there are decreased vasodilation responses in type 1 (Beckman et al., 2003) and type 2 diabetics (Rizzoni et al., 2001), as well as in patients with coronary artery (Monnink et al., 2002) and/or peripheral vascular disease (Park, Charbonneau, & Schiffrin, 2001). Furthermore, in 1996 Taddei and colleagues, demonstrated that the normotensive children of hypertensive parents displayed endothelial dysfunction. Thus, not only is a decreased vasodilatory response seen in the vasculature of those with CVD or cardiovascular risk factors, it may precede the future development of CVD (Panza et al., 1990). Similarly, young adults and children with a high risk of developing atherosclerosis, but who demonstrated no clinical symptoms often have endothelial dysfunction (Celermajer et al., 1992). Other studies have even linked endothelial dysfunction to a sedentary lifestyle (Green et al., 2003), smoking (Oida et al., 2003), the metabolic syndrome, and dyslipidemia (Engler et al., 2003) even in the absence of obvious cardiovascular disease.

This dysfunction or damage to the endothelial cell layer causes the vasodilatory response to be hindered (particularly the response from rest to exercise). Clearly, the inability of the arteries to dilate or constrict in response to changes in blood flow, due to endothelial dysfunction, negatively affects vasomotor tone (the balance between vasodilation and vasoconstriction). Additionally, vasodilation of the conduit arteries is likely initiated at the microvasculature (via ascending vasodilation); so endothelial function in the conduit may also be indicative of the function of the downstream microvasculature (Ellis, Milkovich & Goldman, 2012; Moore, Rich, & Casellas, 1994;). Interestingly, the inability to dilate in response to an increase in blood flow is a predictor of the development of cardiovascular risk factors and/or disease and decreased vasodilation is apparent in several forms of CVD so it is important to understand the mechanisms associated with vasodilation.
2.5 Vasodilatory Mechanisms

Vasomotor tone describes the variety of stimuli that govern the vasoconstriction and vasodilation of blood vessels (Oluwole, McMillen, & Sumpio, 1995). Blood flow is determined by both vasomotor tone and changes in pressure gradient (Clifford, 2011). Furthermore, there is a direct relationship between skeletal muscle blood flow and metabolic demands (Clifford, 2011). Skeletal muscle contractions cause a rapid increase in blood flow to the active musculature in order to increase oxygen delivery and to remove the end products of accelerated metabolism. This is known as exercise hyperemia. Initiation and maintenance of vasodilation in the blood vessels of skeletal muscle associated with the exercise-induced hyperemia has five hypothesized mechanisms. These mechanisms include mechanical factors, biochemical (sometimes called conducted) hypothesis, red blood cell hypothesis, metabolic autoregulation, and flow mediated/endothelial responses. All these mechanisms are essential to vasomotor tone, which is integral in the control of local blood flow.

2.5.1 Mechanical Factors

The mechanical hypothesis of vasomotor control involves the muscle pump. The muscle pump refers to when skeletal muscle acts as a mechanical force that creates changes in the pressure gradient across the capillary bed. Specifically, contraction of skeletal muscle causes it to shorten, which changes the dimensions (bulge) of the muscle. This change in dimension squeezes the vasculature between/within the contracting muscles, which moves the blood in veins towards the heart and reduces arterial inflow changing the venous and arterial pressures (pressure gradient). Once the contraction is released, there is a moment where there is a decreased amount of blood in the veins. Thus, there is a rapid increase in blood flow from the arteries to the venous side upon release of the muscle contraction. Furthermore, the increase (widening) in the pressure gradient (arterial pressure to venous pressure) during muscle contraction and relaxation causes an increase in blood flow (Laughlin, 1987). The onset of the exercise-induced hyperemia response induced by the muscle pump occurs within one to two seconds of exercise (Clifford & Hellsten, 2004). The muscle pump mechanism is a significant
component associated with the rapid hyperemia that occurs from rest to exercise in the skeletal muscle.

Another theorized mechanical aspect of vasodilation is myogenic autoregulation; which is an active process of blood flow. It focuses on pressure differences inside and outside of the blood vessel that cause changes in vasomotor tone. Decreased intraluminal pressure stimulates mechanoreceptors that initiate a vasodilatory response (Clifford, 2011). Calcium ions (Ca$^{++}$) play an important role in myogenic tone in both the smooth muscle cells and the endothelial cells. Apparently, there is a competition for the control of intracellular Ca$^{++}$; in the endothelial cell increased Ca$^{++}$ activates endothelial relaxing factor NO (vasodilation), whereas in the smooth muscle cells it leads to vasoconstriction (Moore, Rich, & Casellas, 1994; Nordlander, 1989). The time course of this myogenic autoregulation vasodilatory response is approximately four to five seconds (Clifford, 2011). Studies have described the mechanical factors as the initial aspect of vasodilation. However, there are other chemical mechanisms involved (Laughlin, 1987; Sheriff, Rowell, & Scher, 1993).

### 2.5.2 Biochemical Hypothesis

The biochemical mechanisms of vasodilation are initiated by extracellular factors but are driven primarily by changes in intracellular ions (calcium and potassium). This response involves acetylcholine (Ach) which is a neurotransmitter found in both the peripheral and central nervous system (Nicpon-Marieb, 2004). In the peripheral nervous system, one of the functions of Ach is the activation of muscular contraction. When Ach is released from a nerve cell (neuron) at the neuromuscular junction, it initiates skeletal muscle contraction (Welsh & Segal, 1997). If Ach escapes (Ach spillover) from the neuromuscular junction (NMJ), it may diffuse into the blood vessels and cause vasodilation via both direct and indirect mechanisms (Welsh & Segal, 1997). Indirectly, the Ach inhibits the release of norepinephrine (NE) from surrounding adrenergic neurons and because NE is a vasoconstrictor the increase in Ach causes a decrease in vasoconstriction. Directly, Ach interacts with both the smooth muscle cell (in the tunica media) and endothelial cell layer of the blood vessels (Welsh & Segal, 1998). At the intracellular level, Ach binds to muscarinic receptors (M3) on the cell membrane of the
endothelial cell, which activates phospholipase C (PLC). PLC stimulates the conversion of membrane phospholipid inositol biphosphate (PiP2) to inositol triphosphate (IP3) and IP3 binds to the endoplasmic reticulum stimulating the release of endothelial Ca\(^{++}\) intracellularly (Clifford & Hellsten, 2004). This increase of calcium stimulates an increase in nitric oxide synthase (NOS) which catalyzes the production of NO (Palmer, Ferrige, & Moncada, 1987). NO then diffuses through the smooth muscle cell membrane layer, where it increases guanylate cyclase (GC) which converts guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP) (Strinden & Stellwagen, 1984). cGMP activates the calcium - ATPase pump (Ca\(^{++}\) ATPase) to release Ca\(^{++}\) and the release of Ca\(^{++}\) decreases Ca\(^{++}\) intracellularly (smooth muscle cell) causing relaxation (vasodilation). The production of cGMP also inhibits the activation of myosin light chain kinase (MLCK), a potent vasoconstrictor, via inhibition of PLC in the smooth muscle cell (Boron & Boulpaep, 2003).

A secondary method by which Ach induces vasodilation is via hyperpolarization of both smooth muscle cells and endothelial cells. Hyperpolarization is created when a cell becomes more negative on the inside (intracellular) when compared to the outside environment. The hyperpolarization creates an electrical gradient that can be passed along arterial walls to either endothelial cells or smooth muscle cells (Clifford, 2011). Hyperpolarization also initiates an onset of events that lead to calcium inhibition. Both the creation of an electrical gradient and calcium inhibition causes vasodilation of the smooth muscle cell (Welsh & Segal, 1998). The peak vasodilation response due to Ach is reached in (eight to ten seconds) after the onset of exercise-induced hyperemia (Clifford & Hellsten, 2004; Clifford, 2011).

2.5.3 Red Blood Cell Hypothesis

The third hypothesized vasodilatory mechanism involves the red blood cells (RBC). When oxygen is released from a RBC, it also releases both adenosine triphosphate (ATP) and NO (Bergfeld & Forrester, 1992; Stamler et al., 1997). When released ATP binds to the purinergic receptor (P2Y) on the membrane of the endothelial cell it activates PLC (Clifford, 2011). This causes the release of NO, which diffuses into smooth muscle cell and leads to the relaxation of SMC, as previously described.
2.5.4 Metabolic Autoregulation

The accumulation of metabolites (such as lactate, potassium ions, carbon dioxide, and adenosine) from the muscles in the interstitial space can also lead to hyperpolarization of smooth muscle cells (Clifford & Hellsten, 2004). Indirectly, metabolite accumulation increases vasodilation by inhibiting or decreasing smooth muscle cells sensitivity to norepinephrine (vasoconstrictor).

2.5.5 Flow Mediated/Endothelial Responses

The endothelial hypothesis involves shear stress and its effect on vasodilation. Shear stress occurs in blood vessels when the red blood cells rub or create friction along the endothelial layer. On the endothelial cell layer there are mechanoreceptors that sense changes in mechanical pressure or distortion. These receptors on the endothelial layer are called glycocalyx mechanoreceptors and are activated by blood flow induced (such as exercise – induced hyperemia) shear stress (Pohl et al., 1986; Rubanyi, Romero, & Vanhoutte, 1986). Apparently, there are two pathways that are initiated once the mechanoreceptors are activated. The first pathway begins with phospholipase A2 (PLA2) catalyzing the production of arachidonic acid (Boron & Boulpaep, 2003). Arachidonic acid is then catalyzed by cyclooxygenase into prostaglandin. One particular prostaglandin, protacyclin I2, interacts with smooth muscle cells via coupling with a G protein receptor on the smooth muscle cell membrane (Messina, Weiner, & Kaley, 1977). This coupling signals the activation of adenylate cyclase converting ATP to cyclic adenosine monophosphate (cAMP), reducing the influx of Ca++ via the calcium channel, and activating Ca++ ATPase (removes calcium) in the smooth muscle cell. The decrease in intracellular calcium leads to vasodilation.

The second pathway of the flow-mediated response begins with the G protein on the endothelial cell membrane activating PLC. The mechanisms by which PLC activated IP3 and increases Ca++ in the endothelial cell were described previously. However, the details of endothelial NO production remains unclear. Increased Ca++ in the endothelial cell increases NO synthase activity catalyzing the conversion of L-arginine and oxygen (O2) into NO and L-citrulline (Palmer, Ferrige, & Moncada, 1987). NO can diffuse into
the smooth muscle cell where it activates the conversion of GTP into cGMP (via guanylate cyclase) inducing relaxation of the smooth muscle cell (vasodilation). As such, the flow mediated vasodilatory response begins with a shear stress stimulus created by exercise hyperemia (see Figure 1). However, there are other ways to generate the flow mediate response in a resting state, particularly the NO flow-mediated response (Celermajer et al., 1992).

![Flow-Mediated Hypothesis Diagram](image)

**Figure 1:** Flow mediated response mechanisms. AC = Adenylate Cyclase; ATP = Adenosine Triphosphate; Ca = calcium; cAMP = cyclic adenosine monophosphate; cGMP = cyclic guanosine monophosphate; ED = endothelial cell layer; eNOS = nitric oxide synthase; ER = endoplasmic reticulum; GCM = glycopcalyx mechanoreceptors; Gq, Gs = guanine nucleotide binding protein, q and s polypeptide; GTP = guanosine triphosphate; IP3 = inositol triphosphate; IP = prostacyclin receptor; O2 = oxygen; P2Y = purinergic receptor; PGI2 = prostaglandin; PI2P = phospholipid inositol biphosphate; PLA2 = phospholipase A2

(Adapted from Clifford, 2011)
2.6 Methods to Assess Endothelial Function

2.6.1 Flow Mediation Dilation

As previously mentioned, there are several proposed mechanisms for blood vessel dilation and there are techniques available to quantify these vasodilatory mechanisms. One such technique is called flow mediated dilation (FMD). This technique was first developed in 1992 by Celermajer and colleagues and is used typically to assess endothelial cell layer function in blood vessels. It uses occlusion-induced hyperemia to generate shear stress and noninvasive ultrasound to assess the FMD shear stress response of the endothelium. The FMD technique has been correlated with artery (brachial, carotid, femoral) endothelial function and several guidelines have been established to standardize the technique (Harris et al., 2010; Thijssen et al., 2011). During the FMD test, a suprasystolic cuff occlusion of the artery (brachial, carotid, femoral) causes a circulatory arrest. After a standard amount of time (usually three to five minutes), which is based on the artery size, the cuff is released and blood flow is measured. A five-minute cuff occlusion of the superficial femoral artery has been shown to produce vasodilation that is largely NO-dependent (Kooijman et al., 2008). The occlusion induced hyperemia (or reactive hyperemia) increases shear stress along the vessel wall, which activates the glycocalyx mechanoreceptors on the endothelial cell layer. This initiates the NO flow mediated vasodilator process (previously described). After some experimental treatment, this occlusion-induced vasodilation of the conduit artery can be compared to baseline diameter of the artery and expressed as % FMD.

Of particular importance is the relationship between FMD and the endothelial derived bioavailability of NO; the greater the % FMD (increase in diameter), as well as the rate at which the vessel is able to increase its diameter in response to the reactive hyperemia suggests increased bioavailability of NO (Doshi et al., 2001; Kooijman et al., 2008; Thijssen et al., 2010a). Likewise, studies (Green, 2005) have found that FMD reflects NO mediated endothelial function and that reduced bioavailability of endothelial derived NO is associated with endothelial dysfunction, which, as mentioned, is an early indication of atherosclerosis (Green et al., 2003). Thus, the measurement of FMD is
important because it allows for the detection of the early onset of CV risk factors. Finally, to maintain the reliability and reproducibility of FMD there are several precautions that must be taken before testing.

2.7 Testing Procedures for FMD

There are several subject specific factors that are important to consider for the accuracy of FMD testing including: medications (such as both tobacco and caffeine), menstrual cycle, food intake, prior exercise, room temperature, and even time of day.

2.7.1 Medication

Medications can have both indirect and direct effects on blood vessels. So, it is important that subjects refrain from taking any medication for greater than four half lives of the drug (Corretti et al., 2002). Nonsteroidal anti-inflammatory drugs (NSAIDS) should be avoided one to three days before, as well.

2.7.2 Tobacco Use

Tobacco use has many documented negative effects on cardiovascular health. Of particular interest with FMD testing, is that smoking can reduce endothelial function (Shimokawa et al., 1996). As such, cessation of smoking or exposure to second hand smoking for greater than 12 hours prior FMD testing is recommended.

2.7.3 Caffeine

Caffeine decreases smooth muscle relaxation because of its ability to inhibit soluble guanylate cyclase (Strinden & Stellwagen, 1984). Guanylate cyclase, as previously described, is an enzyme involved in the cascade of events that lead to vasodilation. Consequently, caffeine should be avoided for at least 12 hours before FMD testing.

2.7.4 Menstrual Cycle

Elevated endogenous estrogen and progesterone concentration have been associated with increased endothelial NO synthase activity (Hayashi et al., 1995). When testing premenopausal women, testing should be done at the same time of the menstrual
cycle for all participants. Furthermore, when testing for sex differences, all premenopausal women participants should be tested during the early follicular phase of the menstrual (day 1 to day 7 of the menstrual cycle), due to the low circulating estrogen and progesterone concentrations at this time (Hashimoto et al., 1995).

2.7.5 Food Intake

Studies have shown that consuming a high fat or high carbohydrate meal prior to testing can attenuate the FMD response in participants (Padilla et al., 2006). Further, it has been suggested that post meal hypertriglyceridemia leads to acute endothelial dysfunction (Bae et al., 2001). Consequently, the most common procedure for FMD measurement is to test under fasting conditions of > three hours.

2.7.6 Familiarization

For accurate FMD testing, participants should be relaxed and familiar to the testing protocol. All participants should be introduced to the testing procedure on a separate day, prior to the FMD testing. On the testing day each participant should be given >20 minutes of quiet resting time in the testing position (supine, prone) in a temperature controlled room (22 to 24 degrees Celsius). These precautions are necessary to decrease the possibility of sympathetic or orthostatic responses, which could affect the baseline diameter measurements of the artery (Thijssen et al., 2011).

2.7.7 Time of Day and Exercise

For valid and repeatable FMD testing, when assessing endothelial function changes in a research study design, it is also important to perform FMD testing for each participant at the same time of day due to diurnal effects on FMD measurements (Jarvisalo et al., 2006). Lastly, as previously mentioned, acute exercise induced hyperemia causes vasodilation and has been shown to improve FMD measures in participants (Clarkson et al., 1999). As such, there should be at least 12 hours of abstinence from exercise prior to FMD testing.

When all these considerations are considered and, assuming accurate measures of artery diameter and blood velocity, FMD can be an accurate and reproducible test
Typically, artery diameter and blood velocity are determined using Doppler ultrasound.

2.8 Ultrasound

Doppler ultrasound and Brightness mode (B-mode) ultrasound imaging can be used to assess changes in blood flow, blood velocity, blood vessel images, and shear stress. Briefly, an ultrasound transducer contains piezoelectric crystals that when connected to electrical sources transmit high frequency sound waves (Ultrasound: Frequency greater than 20,000 Hertz) (Fronek, 1989). The sound waves reflect off materials in proportion to the materials density. Ultrasound is based on the strength of the signal that is returned to the transducer. When ultrasound is reflected off moving red blood cells, the difference between the emitted frequency and the frequency that is reflected back to the transducer is called the Doppler shift (Kremkau, 1990). The Doppler shift allows an image or blood velocity waveform to be constructed and then displayed onto a screen. Both the artery image and blood velocity waveform are crucial aspects of FMD.

During FMD testing arterial diameter is assessed with B-mode ultrasound imaging. Doppler ultrasound is used to assess the blood velocity waveform of the artery. To achieve the most accurate representation of the Doppler shift, the angle of insonation should be 60 degrees for Doppler ultrasound and 90 degrees for B mode image ultrasound (Fronek, 1989). Further, when measuring the artery diameter change from the cuff induced reactive hyperemia, the placement of the suprasystolic cuff should be distal to the transducer (Doshi et al., 2001) because studies have shown that the placement of the cuff distal to the transducer is likely to produce a FMD response that is NO dependent (Doshi et al., 2001). Finally, when doing a repeated measures study, physical anatomical landmarks such as the anterior iliac crest, should be used to keep the location of the artery measures constant for both the pre- and post measures (Thijssen et al., 2011). This is critical because as you travel along an artery, the blood vessel diameter can change. So, if artery diameter measures of the superficial femoral artery are taken 10 centimeters below the iliac crest for the FMD pre-measures and then for the FMD post measures, the superficial femoral artery diameter is taken say 20 centimeters below the iliac crest, any
changes in the SFA diameter may be caused by the location rather than the study intervention.

2.9 Exercise as an Intervention

With recent standardization and precision of the FMD methodology (Harris et al., 2010; Pyke & Tschakovsky, 2005; Thijssen et al., 2011) FMD has been used widely in studies to examine the various interventions that can alter vascular function and cardiovascular risk factors (Hopkins et al., 2009; Kawano et al., 1997; Kawano et al., 2009; Liuni et al., 2010). One intervention of particular interest is exercise. Recently, the American College of Sport Medicine (ASCM) has released a new initiative; “Exercise is Medicine” (Garber et al., 2011) in part because exercise is associated with a 30% reduction in cardiovascular risk (Thijssen et al., 2010) and is integral in the maintenance of good CH (Haskell et al., 2007).

Landmark studies (Langille & O'Donnell, 1986; Pohl et al., 1986) have established that increases in blood flow cause an increase in arterial diameter, which is endothelial dependent and shear stress mediated. Hambrecht and colleagues (2003) provided further evidence that increases in blood flow via endurance exercise training (four weeks) increased shear stress, which increased artery diameter (via %FMD) in the exercise group when compared to the non-exercise group. Furthermore, they also extracted a portion of the artery and were able to measure an increased NO synthase protein expression and phosphorylation of endothelial NOS (eNOS) in the exercise trained group vs the non-exercise group. This, as well as several other studies have established that endurance exercise training improves endothelial function in vivo by increasing NO synthase protein expression and the phosphorylation of its enzyme in cardiovascular disease and/or at risk individuals (Berdeaux et al., 1994; Hambrecht et al., 2003; Langille & O'Donnell, 1986; Laughlin & Roseguini, 2008; Thijssen et al., 2008). These effects are mediated by increased shear stress (Tinken et al., 2010) and ultimately; result in enhanced NO bioavailability in the endothelial cell layer with training. However, the findings for endothelial adaptations with endurance exercise training in healthy asymptomatic individuals are not as clear.
Maiorana and colleagues conducted two identical training studies investigating the effects of endurance exercise training on endothelial function in healthy (study 1) and heart failure (study 2) participants and found contradictory results (Maiorana, O’Driscoll, Cheetham et al., 2001; Maiorana, O’Driscoll, Dembo et al., 2001). The heart failure participants showed an increase in endothelial function while there was no change in endothelial function in the healthy participants. Similarly, other studies have reported no change in endothelial function with endurance exercise training (Green et al., 2004; Maiorana et al., 2003). Particularly, interesting is one study by Bergholm and colleagues (1999) that revealed that 12 weeks of higher intensity endurance training (70 to 80% of VO$_{2\text{max}}$) increased oxidative stress, which could affect endothelial function adversely. However, Goto and colleagues (2003) reported that despite an increase in oxidative stress, 12 weeks of higher intensity endurance training (75% of VO$_{2\text{max}}$) did not decrease endothelial function. To the contrary, some studies have shown endurance and resistance training exercise-induced improvements in vascular function in healthy participants (Clarkson et al., 1999; Rakobowchuk et al., 2005). Thus, there continues to be disparity in the literature about the benefits of exercise training on endothelial vascular function in healthy subjects.

Thijssen and colleagues (2001) suggest that some of the inconsistencies that exist with exercise training and vascular function in healthy subjects may be due to training modalities, exercise intensity, and baseline vascular function. Most studies have used aerobic (endurance) types of exercise at low to moderate intensities as the training stimulus for vascular function adaptations. However, current guidelines state that both the general health and aerobic fitness benefits of exercise are likely exercise intensity driven. Shorter, but more intense exercise is better at eliciting the health benefits associated with regular exercise (Tremblay et al., 2011). Similarly, there is evidence to suggest that repeated high intensity exercise, such as sprint interval training (Gibala et al., 2006) produces similar metabolic adaptations (Burgomaster et al., 2007), exercise performance and body fat losses (MacPherson et al, 2011), as those seen with traditional endurance training. In contrast, with the exception of one study, little is known about the effects of sprint interval exercise training on endothelial function (Rakobowchuk et al., 2008).
Typically, SIT involves ~four-eight repeated “all out” effort sprints (cycling, running) separated by four min recovery periods of rest or low intensity exercise. The “all out” effort sprints can vary in duration, but the most common duration is 30 s (Burgomaster et al., 2005; Hazell et al., 2010; MacPherson et al., 2011). Clearly, SIT is a time efficient mode of exercise. Importantly, despite, the time efficiency and beneficial metabolic/performance/body fat loss adaptations of SIT when compared to endurance training, there are few studies that have focused on the vascular adaptations of SIT, particularly endothelial function.

Recently, endothelial function of the popliteal artery, a common site of atherosclerosis, (Debasso et al., 2004) was evaluated following both endurance training and SIT (cycling) (Rakobowchuk et al., 2008; Rakobowchuk et al., 2009). A significant increase in endothelial function in both the endurance training and SIT group was observed and it was suggested that performing the same training study on participants with decreased vascular function might elicit an even greater adaptation. Rakobowchuk and colleagues also evaluated carotid artery distensibility (a measure of cardiac performance/central adaptation) in both the SIT and endurance training groups and found no improvements in central distensibility. They suggested that a longer bout duration training stimuli may be required to elicit central adaptations. Additionally, MacPherson and colleagues, compared six weeks of SIT (running) to endurance training in young men and found that both groups improved their VO\textsubscript{2max} after training. However, the improvements in VO\textsubscript{2max} in the SIT group were due primarily to an increase in arterial-mixed venous O\textsubscript{2} (a - vO\textsubscript{2}) difference (training adaptation at the muscle/ peripheral adaptation). Whereas, the improvements in VO\textsubscript{2max} in the endurance training group were due primarily to an increase in maximal cardiac output (increase in heart stroke volume/central adaptation). Thus, both Rakobowchuk and colleagues and MacPherson and colleagues’ studies revealed that four to six, 30 s sprints (three times per week) over six weeks are not sufficient to elicit central adaptations. As such, increasing the duration of each sprint bout as well as the number of sprint bouts may lead to a central adaptation that would increase shear stress and mediate a greater improvement in endothelial function.
Interestingly, like the popliteal, recent studies suggest that the superficial femoral artery may also be a site for the early onset of atherosclerosis (McDermott et al., 2011). Further, unlike the popliteal artery, the superficial femoral artery is a larger artery that supplies blood to both the thigh and lower leg. This difference in arterial size may produce different inward vascular structure remodeling with interventions (Thijssen et al., 2008). Furthermore, unlike the popliteal artery, the superficial femoral artery has been researched and established as a conduit artery that is NO dependent in nature and appropriate to evaluated endothelial function via the FMD technique (Kooijman et al., 2008; Thijssen et al., 2011). Consequently, to better understand the endothelial function adaptations of the lower limb conduit arteries to SIT, examinations of various lower limb arteries are necessary.

2.10 Summary, Purpose and Hypothesis

The function of the blood vessels, in particular the endothelial cell layer, is an important component of cardiovascular health. The FMD technique is a reproducible means to quantify NO mediated endothelial function (Ghiadoni et al., 2012; Thijssen et al., 2011). Various interventions, including exercise training (primarily endurance) have been used to improve endothelial function in different populations. However, with the exception of a few recent studies on high intensity exercise, the most common form of exercise used to increase endothelial function have been low to moderate intensity (endurance type) exercise.

The aim of this study was to investigate how a longer duration run SIT program (four–seven, 30 s to 45 s “all out” effort bouts; three times per week over six weeks) affects both vascular function (endothelial function) and hemodynamics of the superficial femoral artery in a young healthy population. It was hypothesized that the longer exercise bout (up to 7 x 45 s) SIT protocol would improve endothelial function of the superficial femoral artery, quantified via an increased response to reactive hyperaemia (Flow mediated dilation).
Chapter 3
3 Methods

3.1 Participants

Participants (n = 41) volunteered for the study. Each was given a survey to assess his or her Cardiovascular Health (CH; see Appendix A) and those with cardiovascular risk factors or diabetes were excluded from the study. All were recreationally active (not engaged in an organized exercise program more than two sessions per week) and were deemed able to participate in physical activity via the Physical Activity Readiness Questionnaire (PAR-Q) (Thomas et al., 1992). The project was approved by the Health Science Research Ethics Board at the University of Western Ontario and both the experimental procedures and any risks were explained fully prior to the any data collection. All participants provided written, informed consent.

3.1.1 Familiarization

All participants completed a familiarization session that included all experimental procedures prior to the baseline testing to minimize any learning effect on the measures.

3.2 Baseline Test

The baseline testing took place during a one-hour visit to the Neurovascular Research Lab (NVRL). Most participants were pre- and post tested at the same time of day (within 3 hours). Sixteen participants were measured during the morning and 25 during the afternoon. Changes in schedules created inconsistencies in the pre- and post testing for three participants. They were tested within 4, 5 and 5 hours, respectively.

All measures were taken following a ≥3 h fast, ≥12 h abstinence from caffeine, alcohol, nicotine or vigorous physical activity in a temperature-controlled (23-24 °C) room. Female participants were measured during the early follicular phase of the menstrual cycle. Unfortunately, this necessitated some variability in time following the last training session for the post testing measures. For example, some female participants were tested the day after their last sprint interval exercise bout (Early: 1-4 days after last exercise bout) and others were tested up to two weeks after the last sprint interval exercise bout (Late: 5-14 days after last exercise bout). Lastly, due to injury or irregular menstrual cycle duration, two participants’ post testing measures were done before (one
week prior) the end of the six weeks of sprint interval training. The aforementioned methods for baseline testing were based on previous research (Thijssen et al., 2011).

3.2.1 Cardiac Cycle and Resting Blood Pressure

During 20 min of supine rest each participant’s cardiac cycle was monitored with a standard two–lead (three electrodes) electrocardiogram (ECG) sampled at 1,000 Hz via a computer-based data acquisition and analysis system (PowerLab; ADInstruments). Blood pressure was assessed continuously from the middle finger of the right hand using the volume clamp technique (Finometer; Finapres Medical Systems BV). The Finometer blood pressure values were validated via three manual sphygmomanometer measures with a single investigator. The finger arterial pressure values collected from the Finometer were integrated into the non-linear three-element model or inverse anti-resonance model, and corrected for differences in waveforms between finger-to-brachial pressure to estimate mean arterial pressure (MAP) (Zamir et al., 2007). Both cardiac cycle and blood pressure were measured continuously throughout the entire hour testing session. MAP and cardiac cycle data was collected via a computer-based data acquisition and analysis system (PowerLab; ADInstruments).

3.2.2 Flow Mediated Dilation (FMD) and Arterial Characteristics

The assessment of vascular function and the hemodynamics of the superficial femoral artery (SFA) were achieved with the flow-mediated dilation (FMD) technique (see figure 2). SFA images and blood velocity (BV) were assessed with ultrasound imaging (4 MHz Doppler ultrasound, 10 MHz Doppler ultrasound imaging, GE Vivid 7) before and after 5 min of suprasystolic cuff inflation of the lower leg (Thijssen et al., 2011). Specifically, the cuff was positioned over the tibial tuberosity of the right leg and inflated to ≥200 mmHg to occlude the SFA (Doshi et al., 2001). The entire FMD procedure included 12 minutes of SFA BV measurements (2 min of baseline, 5 min of cuff inflation and 5 min after cuff release). The angle of insonation was held at ≤60° for all participants. SFA images were taken at baseline (before cuff inflation), as well as every 30 s (up to 5 min) following cuff release. All SFA images were taken with the 10
MHz Doppler imaging probe positioned 30-35 cm from the iliac crest. Again the Powerlab system was used for acquisition and analysis of the SFA blood velocity.

Figure 2: Flow Mediated Dilation (FMD): Protocol
3.2.3  Sprint Interval Exercise Training (SIT)

The six weeks of SIT consisted of three sessions per week. Sessions were separated by at least one day. Participants exercised in a temperature controlled (21 °C) room. All sprint bouts were completed on a manually driven treadmill (Desmo Pro, Woodway®, Wisconsin, USA), so that the participant was the power source for the running belt. The training protocol was as follows:

<table>
<thead>
<tr>
<th>Day</th>
<th>*Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
<th>Week 5</th>
<th>Week 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4 reps x 30 s</td>
<td>4 reps x 35 s</td>
<td>4 reps x 45 s</td>
<td>5 reps x 45 s</td>
<td>6 reps x 45 s</td>
<td>7 reps x 45 s</td>
</tr>
<tr>
<td>2</td>
<td>4 reps x 30 s</td>
<td>4 reps x 40 s</td>
<td>4 reps x 45 s</td>
<td>5 reps x 45 s</td>
<td>6 reps x 45 s</td>
<td>7 reps x 45 s</td>
</tr>
<tr>
<td>3</td>
<td>4 reps x 35 s</td>
<td>4 reps x 40 s</td>
<td>4 reps x 45 s</td>
<td>5 reps x 45 s</td>
<td>6 reps x 45 s</td>
<td>7 reps x 45 s</td>
</tr>
</tbody>
</table>

* All sprint bouts were separated by four minutes of rest
* Training was adapted from previous research (Macpherson et al., 2011)

3.2.4  Body Composition

Body composition was assessed in the Exercise Nutrition Research Lab (ENRL) via whole body densitometry using air displacement plethysmography (Bod Pod®, Life Measurements, Concord, CA). All testing procedures were in agreement with the manufacturer’s instructions as detailed in the manual and thoracic gas volume was estimated for all participants using the predictive equation of the Bod Pod® software. The system software was used to complete all calculations.

3.3  SFA Diameter and Blood Flow Data Analysis

The terms blood flow, blood velocity, shear stress and shear rate are commonly used to describe blood vessel waveforms and the reactive hyperemia response of FMD and so, it is important that the definition of these terms are clear. As previously described, blood flow (ml•min⁻¹) is the volume of blood that is circulating per unit of
time. Whereas, blood velocity (cm\(\cdot\)s\(^{-1}\)) is the distance that blood is travelling with respect to time. However, flow can be related to velocity by the following equation:

Flow = blood velocity \(\times\) vessel cross-sectional area;

where, cross-sectional area of the vessel = \(\pi \times \text{radius}^2\)

Shear stress is the friction that is created as the red blood cells rub or move along the endothelial cell layer of blood vessels. Shear stress takes into account the changes in blood viscosity, whereas, shear rate assumes that blood viscosity does not significantly change from person to person. Shear rate and shear stress differ in how they are calculated:

Shear rate = \(8 \times \frac{\text{blood velocity}}{\text{diameter of blood vessel}}\)

Shear stress = \(8 \times \frac{\text{viscosity} \times \text{blood velocity}}{\text{diameter of blood vessel}}\)

Recent studies have shown that the addition of blood viscosity measures in the shear stress equation, does not significantly impact on the FMD outcome measures (Padilla et al., 2008) so for the current study blood viscosity was not measured.

Digital calipers were used to obtain the luminal diameters of the SFA. SFA diameters were assessed as the average of three digital caliper measurements. The same investigator measured all SFA diameters. All SFA diameters were determined at end diastole of the cardiac cycle. Diastolic arterial diameters are a better representation of the actual size of the artery because at rest, diastole is considered to be 2/3 of the cardiac cycle vs systole which is 1/3 (Radegran, 1997). Peak SFA diameter and time to peak (TTP) SFA diameter were also recorded. TTP diameter was calculated as the time in seconds (with a 30 s resolution), from cuff release to when peak SFA diameter occurred (Thijssen et al., 2011). The peak SFA diameter after cuff release when compared to the baseline SFA diameter was used to express relative change or percent (%) FMD (Celermajer et al., 1992). The equation used for % FMD is as follows:

\%
\text{FMD} = \left(\frac{\text{peak SFA diameter} - \text{baseline SFA diameter}}{\text{baseline SFA diameter}}\right) \times 100
To further quantify the reactive hyperemia associated with FMD, SFA blood flow (BF) and shear rate (SR) were calculated. In order to avoid underestimating the SR stimulus, SR was averaged over 3 (30 s resolution of SFA diameters were used) and 30 s and calculated as follows (Harris et al., 2010; Pyke & Tschakovsky, 2007):

\[
BF (\text{ml}\cdot\text{min}^{-1}) = \pi \times r^2 \times \text{SFA BV} \times 60;
\]

where, \( r \) = radius

Shear rate : \( SR (s^{-1}) = 8 \times \text{SFA BV}/\text{SFA diameter} \)

It has been suggested that a specific time frame interval be used for SR area under the curve (AUC). The time interval from time of cuff release to peak artery dilation is likely the best time interval to use to accurately quantify the SR stimulus because the SR created by the five minute cuff inflation is the stimulus that drives the dilation of the artery (Pyke & Tschakovsky, 2005). So to quantify the FMD stimulus, SR data were plotted vs time and using the area under the curve (AUC; trapezoid method) of 1) the SR from 15 to 45 s after cuff release and 2) the SR from cuff release to peak SFA diameter (Thijssen et al., 2011).

\[
\text{SR area under the curve =}
\]

\[(0.5) \times [(t1 + t5) / 2] + t2 + t3 + t4 \quad (\text{Pyke & Tschakovsky, 2007})\]

Where \( t \) = the shear rate at 30 s time intervals

Further, the amount of SR stimulus that is induced by the five minute suprasystolic cuff inflation with FMD can vary between subjects (Pyke & Tschakovsky, 2005; Pyke & Tschakovsky, 2007). So, in order to standardize the FMD for the differing amounts of SR stimulus for each subject, FMD measurements were normalized as follows:

Normalized FMD:

\[
\text{Peak} = \% \text{FMD/ SR}
\]

\[
\text{AUC 1} = \% \text{FMD/ SR AUC (15 - 45 s)}
\]
AUC 2= % FMD/ SR AUC (cuff release - SFA peak diameter)

3.4 Statistical Analyses

Statistical analyses were performed using Sigma Stat for Windows (version 3.5). Statistics were calculated separately for men and women. A two way ANOVA was used to test significance between the groups and time points, with a post-hoc Tukey test, where necessary. The level of significance was set at p < 0.05. Data are presented as means ± standard deviation. Due to limitations with the VO_{2peak} testing equipment (used to determine if SIT was an effective training regimen) and adherence to the training (see results and discussion), a one-way ANOVA was done on the VO_{2peak} results of the treatment group to determine if a treatment effect was evident.
Chapter 4
4 Results

4.1 Participant Characteristics

4.1.1 Women

Twenty women participated in the study. Fourteen women were randomly assigned to the SIT group and six to the control group. All anthropometric measures were similar between groups at baseline (Table 1).

Table 1 Anthropometric measures SIT vs control.

<table>
<thead>
<tr>
<th></th>
<th>Women (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SIT (n = 14)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>24 ± 4</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>165 ± 5</td>
</tr>
<tr>
<td>Mass (kg)</td>
<td>65.3 ± 7.2</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>47.0 ± 3.4</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>18.3 ± 6.2</td>
</tr>
<tr>
<td>% Fat (%)</td>
<td>27.5 ± 6.6</td>
</tr>
</tbody>
</table>

Values are means ± SD

4.2 Training

4.2.1 VO\textsubscript{2peak} of Female Participants

Two women in the SIT group and two women in the control group were excluded from the VO\textsubscript{2peak} measures results due to nonadherence to training, injury, and malfunction of testing equipment, respectively.

4.2.2 VO\textsubscript{2peak}

There was no significant group by time interaction in VO\textsubscript{2peak} (p = 0.28), (SIT: pre-, 45.3 ± 4.4: post 48.1 ± 3.8; Control: pre-, 51.5 ± 4.1: post, 51.6 ± 3.1 ml•kg\textsuperscript{-1}•min\textsuperscript{-1}; mean ± SD). There was a main effect for group (p = 0.03) but no main effect of time (p = 0.25) perhaps indicating that the SIT group’s VO\textsubscript{2peak} (46.7 ± 4.2 ml•kg\textsuperscript{-1}•min\textsuperscript{-1}) was significantly lower than the control group (51.6 ± 3.4 ml•kg\textsuperscript{-1}•min\textsuperscript{-1}) or that there was an increase with SIT. An independent analysis of groups (one way ANOVA) revealed a significant increase in VO\textsubscript{2peak} (p = 0.05) within the SIT group (pre- to post).
4.3 Baseline Mean Arterial Pressure (MAP)

There was no significant group by time interaction (p = 0.96) in baseline resting (supine position) MAP (SIT: pre-, 85.0 ± 6.4: post, 85 ± 6.1; Control: pre-, 83.0 ± 6.3: post, 84.0 ± 8.4 mmHg). Further, there was no main effect for group (p = 0.48) or time (p = 0.80).

4.4 Baseline Blood Flow Measures

There was no significant group by time interaction in baseline blood flow (p = 0.86) or baseline shear rate (p = 0.73), (blood flow SIT: pre-, 60.0 ± 27.0: post 53.0 ± 17.5; Control: pre-, 48.0 ± 15.2: post, 55.9 ± 29.0 ml•min\(^{-1}\)), (Shear rate SIT: pre-, 50.0 ± 23.3: post, 60.3 ± 27.3: Control: pre-, 41.0 ± 17.3: post, 47.9 ± 32.2 s\(^{-1}\)). There was no main effect for group (blood flow: p = 0.27), (shear rate: p = 0.32) or time (blood flow: p = 0.29), (shear rate: p = 0.18).

4.5 Baseline Diameter

Likewise, there was no significant group by time interaction in baseline SFA diameter (p = 0.21) between groups (SIT: pre-, 5.97 ± 0.50: post, 5.82 ± 0.56; Control: pre-, 5.96 ± 0.51: post, 6.03 ± 0.63 mm). There was no main effect for group (p = 0.92) or time (p = 0.61).

4.6 Peak Blood Flow Measures (three second average)

There was no significant group by time interaction in the three second average peak blood flow (p = 0.44) or the three second average peak shear rate (p = 0.11) (Blood flow SIT: pre-, 647 ± 200: post 849 ± 211; Control: pre-, 713 ± 250: post, 801 ± 313 ml•min\(^{-1}\)), (Shear rate SIT: pre-, 534 ± 125: post, 749 ± 219; Control: pre-, 575 ± 207: post, 600± 85 s\(^{-1}\)). There was no main effect for group (blood flow: p = 0.97), (shear rate: p = 0.40). However, there was a main effect of time (blood flow: p = 0.03), (shear rate: p = 0.05). Likely, the increase in peak shear rate and peak blood flow over time was driven by the SIT group as peak shear rate increased by 215 s\(^{-1}\) in SIT group vs 25 s\(^{-1}\) in the control group. Further, the peak blood flow in the SIT group increased by 202 ml•min\(^{-1}\) vs 88 ml•min\(^{-1}\) in the control group.
4.7 Peak blood flow measures (30 second average)

There was no significant group by time interaction in the 30 second average peak blood flow \((p = 0.16)\) or the 30 second average shear rate \((p = 0.36)\), (Blood flow SIT: pre-, 477 ± 181: post 643 ± 176; Control: pre-, 541 ± 217: post, 543 ± 190 ml*min\(^{-1}\)), (Shear rate SIT: pre-, 370 ± 111: post, 499 ± 159: Control: pre-, 407 ± 146: post, 445 ± 131 s\(^{-1}\)). There was no main effect for group (blood flow: \(p = 0.80\)), (shear rate: \(p = 0.86\)) or time (blood flow: \(p = 0.15\)), (shear rate: \(p = 0.11\)).

4.8 Peak Diameter

Likewise, there was no significant group by time interaction \((p = 0.58)\) in peak SFA diameter (SIT: pre-, 6.22 ± 0.50: post, 6.20 ± 0.54; Control: pre-, 6.30 ± 0.60: post, 6.18 ± 0.66 mm). There was no main effect for group \((p = 0.92)\) or time \((p = 0.47)\).

4.9 Flow Mediated Dilation

4.9.1 FMD

There was a significant group by time interaction in \%FMD \((p =0.03)\) between groups. A Tukey’s HSD poc hoc test revealed a significantly greater \((p = 0.04)\) \%FMD in the SIT group (post, 6.5 ± 3.2 \%) vs control (2.5 ± 1.3\%) (Fig 3). There was no main effect for training status \((p = 0.22)\) or time \((p = 0.56)\).

![Flow mediated dilation (FMD)](image)

**Figure 3.** FMD before and after 6 weeks of SIT vs control. Values are means±SD \((n = 20)\). * Significantly different post training values between groups \((p<0.05)\)
4.9.2 FMD: Peak (normalized)

There was no significant group by time interaction (p = 0.10) in %FMD normalized for peak shear rate (SIT: pre-, 0.011± 0.008: post, 0.014 ± 0.005; Control: pre-, 0.011 ± 0.010: post, 0.006 ± 0.003 a u). There was no main effect for training status (p = 0.19) or time (p = 0.56).

4.9.3 FMD: AUC (normalized)

There was a significant group by time interaction (p = 0.03) in % FMD normalized for the shear rate area under the curve (AUC) from cuff release to peak dilation between groups. A Tukey’s HSD poc hoc test revealed a significantly greater (p = 0.02) %FMD:AUC for the SIT group (0.002 ± 0.001 a u) vs the control group (0.001 ± 0.001 a u) (Fig 4). There was also a significant (p = 0.01) increase in %FMD:AUC, post training (pre-, 0.001 ± 0.001: post, 0.002 ± 0.001) in the SIT group. There was no main effect for training status (p = 0.22) or time (p = 0.56).

Figure 4. FMD:AUC before and after 6 weeks of SIT vs control. Values are means±SD (n = 20). * Significantly different (p<0.05).
4.9.4 FMD: AUC15-45 s (Normalized)

There was no significant group by time interaction (p = 0.34) in %FMD normalized for the shear rate area under the curve (AUC) from 15 second after cuff release to 45 seconds (SIT: pre-, 0.003 ± 0.003; post, 0.004 ± 0.002; Control: pre-, 0.003 ± 0.003; post, 0.002 ± 0.001 a u ). There was no main effect for training status (p = 0.28) or time (p = 0.50).

4.9.5 Time to Peak Dilation

There was a significant group by time interaction (p = 0.02) in time to peak dilation (TTPD) after cuff release. A Tukey’s HSD poc hoc test revealed a significant decrease (p = 0.03) in TTPD in the SIT group (post, 69 ± 34 s) when compared to pre- (pre-, 163 ± 68 s) training (Fig 5). There was no main effect for training status (p = 0.80) but there was a main effect of time (p = 0.003).

![Time to peak dilation (TTPD)](image)

**Figure 5.** TTPD before and after 6 weeks of SIT vs control. Values are means±SD (n = 20). * Significantly different (p<0.05).
4.9.6 Area under the Shear Rate Curve (AUC\textsubscript{SR})

There was no significant group by time interaction in area under the shear rate curve (AUC\textsubscript{SR}) (p = 0.84) for AUC\textsubscript{SR} (SIT: pre-, 4021.5 ± 1382.0: post, 3663.7 ± 1548.4; Control: pre-, 3471.6 ± 1622.5: post, 3279.5 ± 1303.6 s\textsuperscript{-1}). There was no main effect for training status (p = 0.45) or time (p = 0.49).

4.10 Participant Characteristics

4.10.1 Men

Twenty-one men participated in the study. Fifteen men were randomly assigned to the SIT group and six were to the control group. All anthropometric values were similar at baseline (Table 2).

Table 2: Anthropometric measures of SIT vs control. Values are means±SD.

<table>
<thead>
<tr>
<th></th>
<th>Men (n = 21)</th>
<th>SIT (n = 15)</th>
<th>Control (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>22 ± 5.0</td>
<td>24 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>178 ± 8.0</td>
<td>175 ± 6.0</td>
<td></td>
</tr>
<tr>
<td>Mass (kg)</td>
<td>78.8 ± 10.7</td>
<td>77.5 ± 7.7</td>
<td></td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>67.0 ± 7.4</td>
<td>65.8 ± 8.8</td>
<td></td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>11.7 ± 6.4</td>
<td>11.7 ± 3.3</td>
<td></td>
</tr>
<tr>
<td>% Fat (%)</td>
<td>14.4 ± 6.6</td>
<td>15.3 ± 5.1</td>
<td></td>
</tr>
</tbody>
</table>

4.11 Training

4.11.1 VO\textsubscript{2peak}

There was a significant group by time interaction (p = 0.02) in VO\textsubscript{2peak}. A Tukey’s HSD poc hoc test revealed a significantly greater (p = 0.039) VO\textsubscript{2peak} with SIT (pre-, 54.4 ± 6.8 vs post, 56.8 ± 6.7 ml•kg\textsuperscript{-1}•min\textsuperscript{-1}) (Fig 6). There was no main effect for group (p = 0.83) or time (p = 0.80).
Figure 6. $\text{VO}_2\text{peak}$ before and after 6 weeks of SIT vs no training in men. Values are means±SD (n =20). *Significantly different (p < 0.05)

4.12 Baseline Mean Arterial Pressure (MAP)

There was no significant group by time interaction (p = 0.23) in resting (supine position) baseline MAP (SIT: pre-, 81 ± 10; post, 83 ± 8; Control: pre-, 86 ± 9; post, 84 ± 12 mmHg). There was no main effect for group (p = 0.48) or time (p = 0.80).

4.13 Baseline Blood Flow Measures

There was no significant group by time interaction in baseline blood flow (p = 0.19) or baseline shear rate (p = 0.15) (Blood flow SIT: pre-, 77.8 ± 30; post 103 ± 41; Control: pre-, 84 ± 35.; post, 88 ± 30 ml•min$^{-1}$), (Shear rate SIT: pre-, 41 ± 18; post, 58 ± 26; Control: pre-, 47 ± 22; post, 49 ± 18 s$^{-1}$; mean ± SD). There was no main effect for group (blood flow: p = 0.74), (shear rate: p = 0.88) or time (blood flow: p = 0.08), (shear rate: p = 0.09).
4.14 Baseline Diameter

Likewise, there was no significant group by time interaction (p = 0.33) in baseline SFA diameter (SIT: pre-, 6.90 ± 0.57: post, 6.80 ± 0.45; Control: pre-, 6.74 ± 0.33: post, 6.79 ± 0.44 mm). There was no main effect for group (p = 0.69) or time (p = 0.71).

4.15 Peak Blood Flow Measures (three second average)

There was no significant group by time interaction in the three second average peak blood flow (p = 0.30) or the three second average peak shear rate (p = 0.30) (Blood flow SIT: pre-, 787 ± 300: post 1008 ± 398; Control: pre-, 824 ± 258: post, 837 ± 101 ml•min⁻¹), (Shear rate SIT: pre-, 406 ± 150: post, 535 ± 198; Control: pre-, 575 ± 207: post, 600± 85.0 s⁻¹). There was no main effect for group (blood flow: p = 0.59), (shear rate: p = 0.59) or time (blood flow: p = 0.24), (shear rate: p = 0.24).

4.16 Peak Blood Flow Measures (30 second average)

There was no significant group by time interaction in the 30 second average peak blood flow (p = 0.16) or the 30 second average shear rate (p = 0.36) (Blood flow SIT: pre-, 614 ± 259: post 764 ± 299; Control: pre-, 576 ± 158: post, 624 ± 77.0 ml•min⁻¹), (Shear rate SIT: pre-, 300 ± 123: post, 390 ± 140: Control: pre-, 318 ± 113: post, 316 ± 71.0 s⁻¹). There was no main effect for group (blood flow: p = 0.37), (shear rate: p = 0.55) or time (blood flow: p = 0.19), (shear rate: p = 0.24).

4.17 Peak Diameter

Likewise, there was no significant group by time interaction (p = 0.21) in peak SFA diameter (SIT: pre-, 7.24 ± 0.68: post, 7.13 ± 0.47; Control: pre-, 6.99 ± 0.30: post, 7.17 ± 0.52 mm). There was no main effect for group (p = 0.67) or time (p = 0.78).

4.18 Flow Mediated Dilation

4.18.1 FMD

There was no significant group by time interaction (p =0.47) in %FMD (SIT: pre-, 4.9 ± 3.7: post, 4.9 ± 3.0; Control: pre-, 3.7 ± 1.7: post, 5.5 ± 2.7 %). There was no main effect for group (p = 0.92) or time (p = 0.33).
4.18.2  FMD: Peak (normalized)

There was no significant group by time interaction (p = 0.38) in %FMD normalized for peak shear rate (SIT: pre-, 0.018 ± 0.017134; post, 0.014 ± 0.011; Control: pre-, 0.014 ± 0.012; post, 0.019 ± 0.012 a.u.). There was no main effect for group (p = 0.93) or time (p = 0.99).

4.18.3  FMD: AUC (normalized)

There was no significant group by time interaction (p = 0.93) in %FMD:AUC (SIT: pre-, 0.002 ± 0.002; post, 0.002 ± 0.001; Control: pre-, 0.002 ± 0.003; post, 0.002 ± 0.001 a.u.). There was no main effect for group (p = 0.83) or time (p = 0.80).

4.18.4  FMD: AUC15-45 s (normalized)

There was no significant group by time interaction (p = 0.47) in %FMD:AUC 15-45 (SIT: pre-, 0.005 ± 0.009; post, 0.004 ± 0.003; Control: pre-, 0.004 ± 0.003; post, 0.005 ± 0.003 a.u.). There was no main effect for group (p = 0.90) or time (p = 0.96).

4.18.5  Time to Peak Dilation

There was no significant group by time interaction (p = 0.09) in TTPD (SIT: pre-, 146 ± 78; post, 104 ± 73; Control: pre-, 110 ± 78; post, 165 ± 89 s). There was no main effect for group (p = 0.52) or time (p = 0.97).

4.18.6  Area under the Shear Rate Curve (AUC_{SR})

There was no significant group by time interaction (p = 0.93) in area under the shear rate curve (AUC_{SR}) (SIT: pre-, 3684.7 ± 2630.9; post, 3813.8 ± 2021.2; Control: pre-, 3477.4 ± 2270.6; post, 3486.8 ± 1089.5 s⁻¹). There was no main effect for group (p = 0.76) or time (p = 0.92).

4.19  Challenges with Pre- and Post Testing Time

Men: AM vs PM (Diurnal effects)

There was no diurnal effects between groups (AM vs PM) in any hemodynamic or endothelial function measure (See Table 3). There was no main effect for group or time (See Table 3).
Table 3. Systematical analysis of the possible diurnal effects on testing measures (Male participants only). Values are means±SD

<table>
<thead>
<tr>
<th></th>
<th>Testing time of Day (Group) vs. Time (pre-/post)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AM: Morning (n=11)</td>
</tr>
<tr>
<td><strong>MAP (mmHg)</strong></td>
<td></td>
</tr>
<tr>
<td>Pre-</td>
<td>82.2 ± 10.9</td>
</tr>
<tr>
<td>Post</td>
<td>78.3 ± 4.3</td>
</tr>
<tr>
<td>Interaction</td>
<td>P = 0.82</td>
</tr>
<tr>
<td>Group</td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td></td>
</tr>
<tr>
<td><strong>Baseline Blood flow (ml/min)</strong></td>
<td></td>
</tr>
<tr>
<td>Pre-</td>
<td>64 ± 19.7</td>
</tr>
<tr>
<td>Post</td>
<td>114.6 ± 28.9</td>
</tr>
<tr>
<td>Interaction</td>
<td>P = 0.14</td>
</tr>
<tr>
<td>Group</td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td></td>
</tr>
<tr>
<td><strong>Baseline Shear rate (s⁻¹)</strong></td>
<td></td>
</tr>
<tr>
<td>Pre-</td>
<td>33.1 ± 11.3</td>
</tr>
<tr>
<td>Post</td>
<td>69.4 ± 10.2</td>
</tr>
<tr>
<td>Interaction</td>
<td>P = 0.52</td>
</tr>
<tr>
<td>Group</td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td></td>
</tr>
<tr>
<td><strong>Baseline diameter (mm)</strong></td>
<td></td>
</tr>
<tr>
<td>Pre-</td>
<td>6.9 ± 0.5</td>
</tr>
<tr>
<td>Post</td>
<td>6.8 ± 0.4</td>
</tr>
<tr>
<td>Interaction</td>
<td>P = 0.52</td>
</tr>
<tr>
<td>Group</td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td></td>
</tr>
<tr>
<td><strong>Peak diameter (mm)</strong></td>
<td></td>
</tr>
<tr>
<td>Pre-</td>
<td>7.3 ± 0.7</td>
</tr>
<tr>
<td>Post</td>
<td>7.2 ± 0.5</td>
</tr>
<tr>
<td>Interaction</td>
<td>P = 0.72</td>
</tr>
<tr>
<td>Group</td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td></td>
</tr>
<tr>
<td><strong>Peak flow: 30 s average (s⁻¹)</strong></td>
<td></td>
</tr>
<tr>
<td>Pre-</td>
<td>525.2 ± 177.3</td>
</tr>
<tr>
<td>Post</td>
<td>793.7 ± 358</td>
</tr>
<tr>
<td>Interaction</td>
<td>P = 0.50</td>
</tr>
<tr>
<td>Group</td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td></td>
</tr>
<tr>
<td><strong>Peak flow 3 s average ()</strong></td>
<td></td>
</tr>
<tr>
<td>Pre-</td>
<td>698.4 ± 226.1</td>
</tr>
<tr>
<td>Post</td>
<td>984.1 ± 448</td>
</tr>
<tr>
<td>Interaction</td>
<td>P = 0.45</td>
</tr>
<tr>
<td>Group</td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td></td>
</tr>
<tr>
<td><strong>FMD (%)</strong></td>
<td>4.4 ± 4.1</td>
</tr>
<tr>
<td><strong>Peak Shear: 30 s average (s⁻¹)</strong></td>
<td></td>
</tr>
<tr>
<td>Pre-</td>
<td>259.1 ± 100.8</td>
</tr>
<tr>
<td>Post</td>
<td>440 ± 126.7</td>
</tr>
<tr>
<td>Interaction</td>
<td>P = 0.35</td>
</tr>
<tr>
<td>Group</td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td></td>
</tr>
<tr>
<td><strong>Peak Shear: 3 s average (s⁻¹)</strong></td>
<td></td>
</tr>
<tr>
<td>Pre-</td>
<td>359.5 ± 128.5</td>
</tr>
<tr>
<td>Post</td>
<td>567.6 ± 156.2</td>
</tr>
<tr>
<td>Interaction</td>
<td>P = 0.35</td>
</tr>
<tr>
<td>Group</td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td></td>
</tr>
<tr>
<td><strong>FMD: Peak (s⁻¹)</strong></td>
<td>0.02 ± 0.02</td>
</tr>
<tr>
<td><strong>FMD: AUC (s⁻¹)</strong></td>
<td>0.001 ± 0.002</td>
</tr>
<tr>
<td><strong>FMD: AUC 15-45 (s⁻¹)</strong></td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td><strong>Time to Peak Dilation (TTPD)</strong></td>
<td>163.6 ± 72.7</td>
</tr>
<tr>
<td><strong>Baseline diameter (mm)</strong></td>
<td>6.9 ± 0.5</td>
</tr>
<tr>
<td><strong>Peak diameter (mm)</strong></td>
<td>7.3 ± 0.7</td>
</tr>
<tr>
<td><strong>Peak flow: 30 s average (s⁻¹)</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Peak flow 3 s average ()</strong></td>
<td>698.4 ± 226.1</td>
</tr>
<tr>
<td><strong>FMD (%)</strong></td>
<td>4.4 ± 4.1</td>
</tr>
<tr>
<td><strong>Peak Shear: 30 s average (s⁻¹)</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Peak Shear: 3 s average (s⁻¹)</strong></td>
<td></td>
</tr>
<tr>
<td><strong>FMD: Peak (s⁻¹)</strong></td>
<td>0.02 ± 0.02</td>
</tr>
<tr>
<td><strong>FMD: AUC (s⁻¹)</strong></td>
<td>0.001 ± 0.002</td>
</tr>
<tr>
<td><strong>FMD: AUC 15-45 (s⁻¹)</strong></td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td><strong>Time to Peak Dilation (TTPD)</strong></td>
<td>163.6 ± 72.7</td>
</tr>
</tbody>
</table>
Women: early vs. late effects.

Baseline Blood Flow measures

There was a significant group by time interaction (p = 0.05) in baseline blood flow (Early: pre-, 47.3 ± 19.1: post, 67.2 ± 5.86; Late: pre-, 66.6 ± 28.5: post, 64.3 ± 21.8 ml•min⁻¹) but no significant interaction (p = 0.08) in baseline shear rate (Early: pre-, 38 ± 19: post, 64 ± 28.8; Late: pre-, 56.2 ± 23.8: post, 58.3 ± 28 s⁻¹). There was no main effect for group (blood flow p = 0.76) (shear rate p = 0.40) but there was a main effect of time (blood flow p = 0.01) (shear rate p = 0.01).

There was no significant group by time interaction (early vs. late) in any other hemodynamic or endothelial function measures (See Table 4). There was no main effect for group or time (See Table 4).

Table 4. Systematical analysis of the amount of days after the last SIT bout and post testing measures due to menstrual cycle (female participants only). Values are means±SD

<table>
<thead>
<tr>
<th>Testing day after SIT (Group) vs. Time (pre-/post)</th>
<th>Early (1-4 days post SIT) (n= 5)</th>
<th>Late (5-14 days post SIT) (n= 9)</th>
<th>Main effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-</td>
<td>Post</td>
<td>Pre-</td>
<td>Post</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>87.6 ± 9.4</td>
<td>88.2 ± 6.4</td>
<td>83.9 ± 4.0</td>
</tr>
<tr>
<td>Baseline Blood flow (ml•min⁻¹)</td>
<td>47.3 ± 19*</td>
<td>67.2 ± 5.9</td>
<td>66 ± 28.5</td>
</tr>
<tr>
<td>Baseline Shear rate (s⁻¹)</td>
<td>38 ± 19.0</td>
<td>64 ± 28.8</td>
<td>56.2 ± 23.8</td>
</tr>
<tr>
<td>Baseline diameter (mm)</td>
<td>6.1 ± 0.5</td>
<td>5.8 ± 0.7</td>
<td>5.9 ± 0.5</td>
</tr>
<tr>
<td>Peak diameter (mm)</td>
<td>6.4 ± 0.7</td>
<td>6.3 ± 0.6</td>
<td>6.1 ± 0.5</td>
</tr>
<tr>
<td>Peak flow: 30 s average (s⁻¹)</td>
<td>512.6 ± 209</td>
<td>764.3 ± 206.7</td>
<td>457.1 ± 174</td>
</tr>
<tr>
<td>Peak flow</td>
<td>734.5 ± 942 ± 245</td>
<td>640.8 ± 796.8 ±</td>
<td>P = 0.40</td>
</tr>
<tr>
<td></td>
<td>3 s average ()</td>
<td>448</td>
<td>195.2</td>
</tr>
<tr>
<td>----------------------</td>
<td>----------------</td>
<td>-----</td>
<td>-------</td>
</tr>
<tr>
<td>FMD (%)</td>
<td>5.6 ± 3.6</td>
<td>8.7 ± 3.4</td>
<td>3.4 ± 2.6</td>
</tr>
<tr>
<td>Peak Shear: 30 s average (s(^{-1}))</td>
<td>363.2 ± 149.4</td>
<td>560.2 ± 191.8</td>
<td>374.3 ± 94.7</td>
</tr>
<tr>
<td>Peak Shear: 3 s average (s(^{-1}))</td>
<td>555.1 ± 169</td>
<td>843.5 ± 250.1</td>
<td>522.2 ± 102.5</td>
</tr>
<tr>
<td>FMD: Peak (s(^{-1}))</td>
<td>0.02 ± 0.01</td>
<td>0.02 ± 0.01</td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td>FMD: AUC (s(^{-1}))</td>
<td>0.001 ± 0.001</td>
<td>0.002 ± 0.001</td>
<td>0.001 ± 0.001</td>
</tr>
<tr>
<td>FMD: AUC 15-45 (s(^{-1}))</td>
<td>0.01 ± 0.003</td>
<td>0.004 ± 0.002</td>
<td>0.002 ± 0.002</td>
</tr>
<tr>
<td>Time to Peak Dilation (TTPD)</td>
<td>180 ± 70.4</td>
<td>60 ± 52</td>
<td>153 ± 69.5</td>
</tr>
</tbody>
</table>
Chapter 5
5 Discussion

5.1 Overview and Major Findings

The purpose of this study was to determine the effects of longer exercise bout (up to 7 x 45 s) SIT protocol on superficial femoral artery (SFA) endothelial function and hemodynamics in young healthy recreationally active men and women. To do so, flow mediated dilation (FMD), blood flow, shear rate, vessel diameter, and time to peak dilation after cuff release (TTPD) of the SFA was assessed in control and SIT groups (separated by gender). A Finometer was used to collect mean arterial pressure (MAP). It was hypothesized that the longer exercise bout (up to 7 x 45 s) SIT protocol would improve endothelial function and hemodynamic properties of the SFA in the SIT group in both men and women compared to the control group. The key findings of this research study were that, despite observing no statistically significant changes in baseline shear, baseline flow, baseline SFA diameter, MAP, peak SFA diameter, peak shear rate, shear rate AUC, %FMD:AUC15-45, or peak blood flow, %flow mediated dilation (%FMD), flow mediated dilation normalized by shear rate area under the curve (FMD:AUC), and time to peak dilation all improved in the female SIT group compared to the control female group. This suggests that endothelial function improved despite no significant changes in baseline hemodynamics. In contrast, these findings were not observed in the men; there were no statistically significant differences in the endothelial function and hemodynamic measures between the male SIT group compared to the male control group.

Specifically, the current study revealed that SIT increased %FMD in the women. Similar results were also found in %FMD:AUC. This is important because %FMD:AUC takes into account the differences in shear stimulus between each person and normalizes the shear stimulus across the group. Likely, %FMD normalized for the shear stimulus is a more definitive measure of endothelial function (Pyke & Tschakovsky, 2005). These findings are very interesting because there was no statistically significant increase in peak, area under the curve (AUC), or baseline shear rate between the SIT group and the control group. However, time to peak dilation after cuff release decreased significantly in the SIT group but not in the control group. Thus, SIT improved endothelial function (via %FMD, FMD:AUC) and improved the SFA vasodilatory response to the shear rate.
stimulus (via decrease TTPD). In addition, because the NO is the endothelial derived releasing factor that often mediates the vasodilatory response of arteries, these data suggest that SIT might produce an increased NO bioavailability and/or an increased NO sensitivity in the SFA endothelium in the women studied. Interestingly, the same response was not apparent in the male participants.

A possible explanation for inconsistencies in endothelial dependent improvements in the women compared to men in the current study may be related to differences in oxidative stress. Specifically, Laughlin and Roseguini (2008) suggest that the elevated oxidative stress and inflammatory response after high intensity exercise training may blunt the shear stress and hemodynamic mediated beneficial adaptations of exercise. This observation is important because even though, SIT is a self-limiting “all out” effort; the men’s absolute speeds were on average 4 km faster than the women. Consequently, the men may have reached a higher threshold of oxidative stress and thus, had a greater inflammatory response after SIT. If so, this could blunt any enhancement in endothelial function (Bergholm et al., 1999; Laughlin & Roseguini, 2008). However, this is not likely as relative exercise intensity determines oxidative stress. Previous studies have characterized the intensity of SIT at upwards of 250 percent of VO$_{2\text{max}}$ (Burgomaster, Heigenhauser, & Gibala, 2006) so it is reasonable to suspect that SIT would be associated with significant oxidative stress (Bergholm et al., 1999; Goto et al., 2003). However, Goto and colleagues (2003) measured oxidative stress in men after 12 weeks of endurance training (70-80% of VO$_{2\text{max}}$) and reported an increase in oxidative stress without endothelial dysfunction, although at much lower exercise intensity than the current study. Regardless, the idea that increased oxidative stress can elicit endothelial dysfunction needs future study especially because there is also evidence that high intensity exercise improves antioxidant defenses against oxidative stress (Atalay et al., 1996; Bloomer, 2007; Ellosua et al., 2003; Gomez-Cabrera, Vina, & Ji, 2009; Sen, Atalay, & Hanninen, 1994). Other possible explanations for the observed gender inconsistencies include structural (changes in resting artery diameter) adaptations in the artery, differences in the time course of both structural and functional changes in the artery, and/or perhaps even differences in pre-study training status.
Classically, structural arterial remodeling with exercise is characterized by increased resting artery diameter (Langille & O'Donnell, 1986; Rodbard, 1975) but is likely assessed more accurately via ischemic handgrip exercise or nitroglycerin vasodilation methodology (Naylor et al., 2005). In 2008, Tinken and colleagues used both, the maximal artery diameter vasodilation technique (Naylor et al., 2005) and the FMD technique to assess structural and functional arterial changes in the brachial and popliteal artery every two weeks over eight weeks of moderate exercise training (cycling and running for 30 minutes at 80% of heart rate reserve) in healthy men, respectively (Tinken et al., 2008). Tinken and colleagues reported that arterial structure (remodeling) and arterial function (endothelial function) were significantly improved in both the popliteal and brachial artery after two and four weeks of exercise training when compared to baseline. These increases in arterial function persisted all the way out to week six in the popliteal artery. However, at week eight arterial function in both arteries returned to baseline levels. Interestingly, maximal arterial diameter (structural remodeling) was statistically greater compared to baseline after two weeks and persisted all the way out to week eight of exercise training. These time course data validate the hypothesis proposed earlier by Laughlin and colleagues, that functional vascular adaptations precede structural remodeling (Laughlin et al., 1996) and are of relevance here because structural remodeling (that normalizes shear stress) likely would abolish any improvement in vascular function measured with FMD. As mentioned, SFA functional changes were not observed in the men in our study. However, of course, the SIT studied was much more intense than the exercise training used in the Tinken et al. (2008) study. Perhaps the intensity of the SIT drove rapid (occurring at week two and four) endothelial function improvements in the SFA of men in our SIT group that then returned to baseline by week six. If this was the case, the post measure assessment of endothelial function (via FMD) after six weeks of SIT would yield no changes in endothelial function, despite endothelial function improvements at week two and four. Unfortunately, Tinken and colleagues did not include women participants in their study so no gender comparison is possible (Tinken et al., 2008); however, increased circulating estrogen concentrations have been associated with an increased NO synthase (Hayashi et al., 1995). Consequently, fluctuating estrogen concentrations across the menstrual cycle may affect
the time course of adaptation to SIT exercise-induced structural and functional arterial changes in young women when compared to young men. Future studies are needed to confirm these observations.

Interestingly, structural and functional adaptations are not limited to the arteries of the dominant exercising muscle (Clarkson et al., 1999; Green et al., 2004). These studies have demonstrated that brachial endothelial function can be improved following lower body exercise. Further, others observed a significant increase in peak forearm blood flow during bicycle training, which included very minimal upper limb movement (Silber, McLaughlin, & Sinoway, 1991). They concluded that the vascular adaptations to training are not specific to the limb involved in the exercise and that training adaptations may be dependent upon the amount of mass muscle involved (Silber, McLaughlin, & Sinoway, 1991). Of course, our study involved a very large muscle mass but we did not assess brachial artery endothelial function. Despite, the lower body dominant exercise of SIT, assessment of the brachial artery might have been informative. However, a recent retrospective analysis of the relationship between vasodilatory function in upper and lower limbs revealed that upper limb vasodilator function in healthy humans may not be predictive of that of the lower limbs (Thijssen, Rowley et al., 2011). Importantly, this study assessed baseline resting vascular function (via FMD) in young untrained men (Thijssen, Rowley et al., 2011) which, as mentioned, may not be an accurate representation of the endothelial adaptations that occur throughout the entire vasculature of healthy men with training. Clearly much more work is necessary to sort this all out. Regardless, functional adaptations might occur in conduit arteries of muscle not predominantly involved in the exercise training.

As previously mentioned, exercise training leads to an increased bioavailability of NO (Laughlin & Roseguini, 2008) and NO bioavailability is an important marker of endothelial dependent function. Interestingly, NO bioavailability may be vessel dependent (Green et al., 2004; Green et al., 2011; Thijssen et al., 2010). Larger conduit vessels have a greater capacity for NO production (Laughlin et al., 2003a; Laughlin et al., 2003b). Furthermore, if NO bioavailability is vessel dependent, it may also be variable from person to person. Green and colleagues suggest that differences in baseline vascular function may provide insight into the inconsistencies in outcomes of endothelial function
in healthy populations (Green et al., 2011). The current study did not assess participants based on baseline vascular function. Rather, participants were grouped based on sex and assigned randomly to either the SIT group or the control group. Grouping the participants based on sex and baseline vascular function is likely an approach that would add clarity to the current findings.

Interestingly, training status as well as sport specificity may also alter NO bioavailability. Studies performed on the arteries of athletes and physically fit individuals suggest that exercise training induces larger arterial diameters than their age matched controls (Green et al., 2012). Furthermore, limb dominant sport athletes such as tennis and squash players, have larger arterial diameters in their dominant arm (Green et al., 1996; Naylor et al., 2006; Sinoway et al., 1986; Zeppilli et al., 1995). However, enhanced arterial function (via FMD) was not significantly greater in the arteries of athletes compared to controls. Green and colleagues suggest that functional changes in the arteries of well-trained individuals may have already been determined due to previous arterial remodeling (Green et al., 2011). In the current study young recreationally active men and women were recruited. Their level of physical activity was assessed using a participant survey (Appendix A). Of the 21 female participants only one was a varsity level athlete. Conversely, eight of the 15 male SIT group participants and three of the six male control group participants were involved in highly competitive rugby, soccer, mixed martial arts, weightlifting, and/or rowing prior to our SIT study. Despite, the emphasis placed on all participants eliminating all other sources of training besides the SIT for the six weeks of the experiment; it is possible that the men’s previous training status caused chronic arterial remodeling (larger SFA diameter). If so, the arterial remodeling would likely normalize the SIT induced shear stress, and could explain the observed no change in endothelial function (via FMD) in the male participants. This is possible because popliteal artery endothelium function improved in young inactive men and women (not engaged in regular exercise more than 2 times X 30min per week for at least 1 year prior to the study) with SIT (Rakobowchuk et al., 2008).
5.2 Future Direction

To further document the endothelial functional adaptations of SIT in the SFA of young recreationally healthy men and women research needs to focus on several areas. First, both the structural and functional vascular adaptations of the conduit arteries via SIT need to be better understood. The use of maximal artery diameter measures to assess vascular structural changes should help determine baseline vascular function in healthy individuals. Likewise, a better grouping and assessment of prior training history of participants would likely decrease confounding variability of training status on both arterial vascular function and structural remodeling. Second, recent studies have focused on the time course of vascular and structural adaptation of exercise training in young healthy men (Thijssen et al., 2010; Tinken et al., 2008). However, to our knowledge, there are no research studies on the time course of functional and structural arterial adaptations of exercise training in healthy young women so more study of women is needed.

Third, to assess the more mechanistic side of SIT in vascular health, the use of both in vivo and in vitro studies such as the studies by Hambrecht et al. (2003) and Laughlin et al. (1996) to determine the NO synthase protein expression, eNOS phosphorylation, oxidative stress and antioxidants associated with SIT would likely help decrease the discrepancies with endothelial function improvement and exercise in healthy populations. This would establish if SIT induces an upregulation of NO synthase protein by increasing phosphorylation of eNOS, as previously described by Hambrecht and colleagues (2003) and/or if there are oxidative and antioxidant species that play a role in vascular adaptations at the cellular level. The ability to establish arterial NO bioavailability prior to exercise training would be another interesting approach to establishing baseline vascular function.

Fourth, increases in blood flow patterns induce shear stress. Blood flow patterns consist of both antegrade (forward) and retrograde (backward) flow and both are variable. Recently, several studies (Green et al., 2005; Thijssen et al., 2009; Tinken et al., 2009) investigated whether lower body exercise induced changes in blood flow patterns (amount of retrograde and antegrade blood flow) affect the upper limb endothelial function and reported an increase in NO release. Others suggest that higher intensity
exercise produces increased blood flow and shear rate that may result in different endothelial adaptations compared to less intense exercise (Thijssen et al., 2010). Moreover, endothelial cell phenotype (a cell’s ability to integrate and transduce stimuli from its surrounding environment) is sensitive to flow and shear stress patterns; so it is likely that different exercise intensity stimuli may result in different endothelial adaptations (Laughlin & Roseguini, 2008; Laughlin, Newcomer, & Bender, 2008). Future SIT investigations should assess both upper and lower limb vascular function, as well as retrograde and antegrade blood flow patterns in order to provide a better understanding of any systemic vascular or endothelial (phenotype) adaptations.

5.3 Limitations

One investigator manually took all the artery diameter measurements. Three diameter measures were taken and then averaged to represent the artery diameter during the diastolic portion of the cardiac cycle. Several studies suggest that manual analysis is subject to substantial observer error (Black, Cable, Thijssen, & Green, 2008). The gold standard for diameter measurement is the use of computerized Edge detection software (Black, Cable, Thijssen, & Green, 2008; Thijssen., 2011) but this software was not available for this study. However, despite this limitation statistically significant changes were found in %FMD and FMD:AUC suggesting that the manual diameter measurements were sensitive enough to detect change.

Peak shear rate (averaged over three seconds) increased over time from pre- to post measures for all female participants. This suggests that there was no difference in peak shear rate after cuff release between the SIT group and the control group. However, this significant increase in peak shear rate may be due to measurement error as follows. During the FMD technique, a cuff is placed around the lower leg to occlude the SFA artery. Upon release of the cuff following five minutes of occlusion, the ultrasound technician must be able to quickly adjust the probe to the new position of the artery. The ability to quickly adjust the probe to the new artery position without losing the detection of blood flow is a skill that improves with repetition. So it is possible that the increase in peak shear rate (averaged over three seconds) was due to improvement in probe placement technique during the post training measurements.
5.3.1 Challenges with the VO\textsubscript{2peak} Measures

As previously mentioned SIT is a time efficient mode of training that has produced many similar adaptations when compared to more traditional endurance training. Previous studies with SIT have shown increases in VO\textsubscript{2PEAK/MAX} after four and six weeks (Burgomaster et al., 2005; Burgomaster et al., 2007; Gibala et al., 2006; Hazell et al., 2010; Macpherson, Hazell et al., 2011; Rakobowchuk et al., 2008). Furthermore, most studies with SIT and high intensity exercise training only used four to six sets of 30 s exercise bouts and still saw a significant increase in VO\textsubscript{2PEAK/MAX} (Burgomaster et al., 2005; Burgomaster et al., 2007; Gibala et al., 2006; Hazell et al., 2010; Macpherson et al., 2011; Rakobowchuk et al., 2008). The current study used a longer duration SIT protocol, with sprint bouts increasing from 30 to 45 s and sets of sprints increasing from six to seven repetitions of sprints. The male SIT group increased their VO\textsubscript{2PEAK} significantly after six weeks of SIT when compared to the control group but the women did not. The female participants results were likely affected by the smaller sample size as four female (two from the SIT group and two from the control) were removed due to injury, lack of adherence to the SIT protocol, and data that was lost due to technical error (corrupted files). Furthermore, of the four females in the control group, three participants improved their VO\textsubscript{2peak}, which is not representative of a typical control group. Likely, the aforementioned limitations (smaller sample size and control group improvements) contributed to a type II statistical error. As a result, a one way ANOVA was performed on the SIT group for the VO\textsubscript{2peak} data and revealed a significant training effect.

Regardless, a recent exercise training study by Tinken et al. (2008) saw enhanced endothelial function with only a modest change in VO\textsubscript{2max}.

5.3.2 Challenges with Pre- and Post Testing Time

Due to changes in schedules, there were inconsistencies in the pre- and post testing of 3 male participants. This is important, as diurnal effects have been observed on FMD measurements (Harris et al., 2010). However, statistical analysis of the FMD measures between those tested in the morning and those tested in the afternoon in our study revealed no significant differences. This suggests that the variability of the testing time of the 3 male participants did not confound the FMD measures.
Lastly, to be able to control for the effects of estrogen on FMD, the female participants were pre- and post tested during the early follicular phase of the menstrual cycle. Controlling for the effects of estrogen on FMD, created variability in the number of days between the last sprint interval exercise bout and the post testing measures. For example, five females were post tested early (1 to 4 days) after their last sprint interval exercise bout and nine females were tested late (5 to 14 days) after their last sprint interval exercise bout. Statistical analyses revealed a decrease in baseline blood flow (post SIT) in the females tested late (5-14) that was statistically significant. Interestingly, none of these results (late testing vs. early testing) were in conflict with the previously described female results (SIT vs. control: increased FMD, FMD:AUC and decreased TTP dilation). Suggesting that the variability with the testing time after SIT (due to the menstrual cycle) did not add confounding variability to measures of endothelial function.

### 5.4 Summary and Conclusion

Currently, western society has reached a level of physical inactivity that far outweighs prior generations (Booth, Chakravarthy, & Spangenburg, 2002). Further, it is clear that physical inactivity is a risk factor for various cardiovascular diseases such as atherosclerosis. Likely, disease of conduit arteries as assessed via endothelial dysfunction is an early manifestation of atherosclerosis (Vita, 2005). Several studies have revealed that endurance exercise training is an intervention that can improve endothelial vascular function in a diseased or at risk population (Green et al., 2004; Maiorana et al., 2003; Moyna & Thompson, 2004; Walsh et al., 2003). However, studies on exercise training and endothelial vascular function in healthy populations are far less common and the results are inconsistent (Green et al., 2011). Further, there is a large void of research on the effects of exercise training on the endothelial vascular function of young healthy women. Interestingly, moderate intensity endurance exercise is used commonly as the training stimulus to improve endothelial function in both healthy and diseased populations, despite research that denotes many similar and/or greater training adaptations with SIT (Burgomaster, Heigenhauser, & Gibala, 2006; Green et al., 2011; Macpherson et al., 2011; Maiorana et al., 2001; Thijssen et al., 2011; Tinken et al., 2010). However, high intensity exercise training induces greater oxidative stress, which could be
positive (via its increased antioxidant defense) or negative (via blunted endothelial function) (Atalay et al., 1996; Bloomer, 2007; Elosua et al., 2003; Gomez-Cabrera, Vina, & Ji, 2009; Laughlin & Roseguini, 2008; Sen, Atalay, & Hanninen, 1994).

The current study examined the effects of SIT on endothelial vascular function (via FMD) in 41 young recreationally active men and women. It was hypothesized that the longer exercise bout (up to 7 x 45 s) SIT protocol would improve SFA endothelial function and hemodynamic properties. In the women, SIT decreased time to peak dilation and increased flow mediated dilation. Surprisingly, SIT had no effect on any endothelial function or hemodynamic measures in the men.

These results suggest that SIT improved endothelial vascular function in young recreationally active women. This improvement could be due to changes in the NO response. The observed gender difference may be due to differences in the men’s baseline vascular function attributed to exercise training status, greater oxidative stress, and/or vascular structural remodeling that prevented endothelial vascular functional improvements in the male participants. Future studies on the effects of SIT on endothelial vascular function in young healthy individuals are needed to establish the blood flow pattern (retrograde and antegrade) associated with SIT, in order to quantify any associated shear stress stimulus as well as to document whether increases in endothelial phenotype sensitivity contribute to different vascular adaptations. Longitudinal training studies that include measures of vascular structural remodeling would be welcomed to assess possible gender differences in structural and vascular adaptations with time.

In conclusion, six weeks of SIT improved endothelial function (perhaps mediated by increased NO response) in young women. However, six weeks of SIT did not improve endothelial function in young men.
References


Shimokawa, H., Yasutake, H., Fujii, K., Owada, M. K., Nakaie, R., Fukumoto, Y., et al. (1996). The importance of the hyperpolarizing mechanism increases as the vessel size...


Appendix A

Participant Information

Subject I.D.: ____________ Date: ________________
Age: _______________ Height: ___________ Weight: _______________
Smoker: Yes / No Ethnic Background: ____________________________

Medical History (please check any and all that apply)

- Family history of heart disease
- Heart murmur
- Phlebitis
- Other heart disorder (please specify)

- Family history of stroke
- Migraines
- Sinus problems
- Hypertension
- Diabetes
- Raynaud’s syndrome
- Polycystic ovary syndrome
- Seizures
- Digestive problems
- Asthma
- Bronchitis
- Other respiratory disorder (please specify)

Have you ever fainted? Yes / No
If yes, under what circumstances:

________________________________________________________________________

Are you taking any medications? Yes / No
If yes, please specify:

________________________________________________________________________

Have you consumed alcohol or any caffeinated beverages in the last 12 hours? Yes / No
If yes, please specify the quantity:

________________________________________________________________________

Have you had any major surgeries, illnesses or injuries? Yes / No
If yes, please specify (include dates): ____________________________________________

Are you physically active?  Yes / No

If yes, please specify the type, frequency, and typical duration of exercise:

______________________________________________________________________________

How long have you been physically active?

______________________________________________________________________________

Age of menarche:  ____________________
Appendix B
Certificate of Approval of Human Ethics

Use of Human Participants - Ethics Approval Notice

Principal Investigator: Dr. Peter Leman
Review Number: 17006E
Review Level: Delegated
Approved Local Adult Participants: 30
Approved Local Minor Participants: 0
Protocol Title: The effect of sprint interval training (SIT) on selected cardiovascular measures and exercise performance in men and women.
Department & Institution: Kinetics, University of Western Ontario
Sponsor:
Ethics Approval Date: June 06, 2011
Expires Date: July 31, 2012
Documents Reviewed & Approved: 1
Documents Received for Information:

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This is to notify you that the University of Western Ontario Research Ethics Board for Health Sciences Research involving Human Subjects (HSREB) which is organized and operates according to the Tri-Council Policy Statement: Ethical Conduct of Research Involving Humans and the Health Canada/CIHI Good Clinical Practice Practice: Consolidated Guidelines; and the applicable laws and regulations of Ontario has reviewed and granted approval to the above referenced revision(s) or amendment(s) on the approval date noted above. The membership of this REB also complies with the membership requirements for REB's as defined in Division 5 of the Food and Drug Regulations.

The ethics approval for this study shall remain valid until the expiry date noted above unless timely and acceptable responses to the HSREB's periodic requests for surveillance and monitoring information. If you require an updated approval notice prior to that time you must request it using the UWO Updated Approval Request Form.

Members of the HSREB who are named as investigators in research studies, or declare a conflict of interest, do not participate in discussion related to, nor vote on, such studies when they are presented to the HSREB.

The Chair of the HSREB is Dr. Joseph Gilbert. The UWO HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

This is an official document. Please retain the original in your files.

The University of Western Ontario
Office of Research Ethics
Support Services Building Room 5156 • London, Ontario • CANADA • N6A 3K7
PH: 519-661-3036 • F: 519-850-2466 • ethics@uwo.ca • www.uwo.ca/research/ethics
Appendix C

Letter of Information
LETTER OF INFORMATION REGARDING RESEARCH

Title of Study: The effect of sprint interval training on selected cardiovascular measures and exercise performance in men and women

You are being invited to participate in a research study conducted by P.W.R. Lemon (PhD), Stephanie Reid (BSc), Alan Smith (BSc), Terry Olver (MSc), Alex Stevens (BHK), and Craig Hamilton (BSc), from the Exercise Nutrition Research Laboratory in the School of Kinesiology at the University of Western Ontario.

If you have any questions or concerns about the research, please feel free to contact Stephanie, Alan, Terry, Alex or Craig at XXXXXXX or Dr. Lemon XXXXXXX

PURPOSE OF THE STUDY

The purpose of this research experiment is to determine if six weeks of sprint interval training (SIT) will improve selected cardiovascular measures and exercise performance in men and women.

INCLUSION/EXCLUSION CRITERIA

In order to be eligible to participate in this study you must be a healthy, 18 to 35 year old male or female.

You will be excluded from this study if you are: injured, diabetic, currently undergoing a similar training program or have any contraindications to this type of exercise.

PROCEDURES

If you volunteer to participate in this study, we will ask you to do the following things:

1. Complete a health survey (PAR-Q) to assess your current physical capability.
2. Record a 3-day dietary record for the weeks that pre- and post-training testing are completed.
3. Undergo an assessment of various cardiovascular and exercise measures, before and after training, that will include: determination of body composition (BodPod® - involves sitting comfortably in a chamber for about 5 minutes while the space your body takes up is measured), a multi-stage exercise test on a treadmill (maximal oxygen uptake test), blood pressure, femoral blood flow, cardiac output, pulse wave velocity, blood velocity, arterial compliance, femoral artery diameter, heart rate, and a 5 minute run on a manually driven treadmill.

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4. Complete 6 weeks of sprint interval training (3 days/week). Each session will consist of 4 (wk 1 and 2), 5 (wk 3 and 4), or 6 (wk 5 and 6) 1 minute “all out” running efforts on a manually driven treadmill, with each effort separated by 4 minutes of no exercise.

Testing will be conducted in the Exercise Nutrition Research Laboratory, the Neurovascular Research Laboratory, and the Canadian Centre for Activity and Aging. Females will be tested during the beginning of the menstrual cycle to ensure hormone levels are consistent during testing. Women will be asked to keep track of their menstrual cycle so that testing will occur during the first seven days of the menstrual cycle. Whereby, the first day of menstruation marks day one of the menstrual cycle.

TIME INVOLVED

The study will take place over eight weeks. There will be six weeks of training and 2 weeks of testing (pre- and post-training).

Pre- and post-training testing will take place during the first and eighth week of the study and will take place over 4 days. Day one will involve being familiarized with the procedures that will be done and completion of a dietary record. On day two, body composition and maximal oxygen uptake tests will be completed. On day three, resting cardiovascular measures will be taken (blood pressure, femoral blood flow, cardiac output, pulse wave velocity, blood velocity, arterial compliance, and femoral artery diameter). On day four, a 5 minute run on a manually driven treadmill will be completed.

Training will take place from week 2 to week 7. It will occur 3 days per week. The number of intervals per session will increase over the course of the training program. Subjects will complete 4 intervals per session during weeks 2 and 3, 5 intervals during weeks 4 and 5, and 6 intervals during weeks 6 and 7. At the end of weeks 3 and 5 subjects will be reassessed for changes in resting cardiovascular measures (blood pressure, femoral blood flow, and cardiac output). A recovery drink will be given after each training session.

POTENTIAL RISK AND DISCOMFORTS

All exercise involves some health risk (primarily cardiovascular or hydration-related) but lack of activity has been shown to be more hazardous to one’s health. Further, these concerns are much reduced in young, healthy individuals. This type of exercise is completed by many Western students in kinesiology classes, in intramural sports, and by Mustang athletes. Participants will be encouraged to adequately hydrate. There are no risks associated with the finger cuff method for measuring blood pressure. When the cuff is inflated subjects may experience numbness but this is reversed immediately upon deflation of the cuff. Adhesive electrodes will be applied to the skin for ECG. Some individuals may experience a slight skin reaction in response to the adhesive. This typically clears up in a few days. There are no known risks associated with ultrasound.
This typically clears up in a few days. There are no known risks associated with ultrasound.

POTENTIAL BENEFITS

Your aerobic fitness will likely improve using this training protocol. Society may benefit from this study. It is unknown how SIT affects cardiovascular health measures. If we find SIT to be beneficial to cardiovascular health this would provide people with an alternate, effective mode of training.

COMPENSATION

You will not be compensated for your participation in this study.

CONFIDENTIALITY

Any information that is obtained in connection with this study that can identify you will remain confidential and will be disclosed only with your permission. This information will be collected on a master list that will be kept in a password protected file with access to only the investigators in this study. All data will be collapsed before results are printed (only group averages and variability). All participants will be assigned an arbitrary number to ensure anonymity. Mean data will be stored in a password protected file for comparison with future studies. Raw data will not be released to any other parties.

PARTICIPATION AND WITHDRAWAL

You can choose whether to be in this study or not. If you are a student and you volunteer, you may withdraw at any time without any effect on your status at UWO. If you are not a student at UWO, you may withdraw from the study at any time. You may also refuse to answer any questions you feel are inappropriate and still remain in the study. The investigator may withdraw you from this research if circumstances arise which warrant doing so. Participation in this study is voluntary.

FEEDBACK OF THE RESULTS OF THIS STUDY TO THE SUBJECTS

We plan to publish this study in a reputable academic journal upon the completion of the research. The information published in a journal or subsequent studies will not identify you in any way. Copies of such articles will be available upon request.

SUBSEQUENT USE OF DATA
You will receive a copy of the consent form after it has been signed. You do not waive any legal rights by signing the consent form.

This letter is for you to keep. If you have any questions about this research project, feel free to call us (Dr. Peter Lemon / Stephanie Reid, Alan Smith, Terry Olver, Alex Stevens or Craig Hamilton –XXXXXXX for clarification. Further, if you have any questions about the conduct of this study or your rights as a research subject you may contact the Office of Research Ethics at The University of Western Ontario at XXXXXXX or at XXXXXX@uwo.ca.

Sincerely,

Dr. Peter Lemon / Stephanie Reid / Alan Smith / Terry Olver / Alex Stevens / Craig Hamilton
Principal Investigators
RM 2235 3M Centre UWO, Exercise Nutrition Research Laboratory

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Appendix D

Consent to Participate in Research

INFORMED CONSENT STATEMENT
The effect of sprint interval training on selected cardiovascular measures and exercise performance in men and women

Investigator: P.W.R. Lemon (Stephanie Reid and Alan Smith)

I have read the accompanying "Letter of Information", have had the nature of the study explained to me and I agree to participate. All questions have been answered to my satisfaction.

By signing below, I agree to participate in this study.

Name of Participant (please print):

_______________________________________

Signature of Participant:

________________________________________ Date: ________________________________

Name of Person Obtaining Informed Consent:

_______________________________________

Signature of Person Obtaining Informed Consent:

________________________________________ Date: ________________________________

RE-RECRUITMENT IN FUTURE STUDIES
If you wish to participate in future studies in the Exercise Nutrition Research Lab, please include your current contact information below.

I wish to be contacted for future studies in the Exercise Nutrition Research Laboratory.

Yes_____ (check mark), No _____ (check mark)

Date:________________________

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Initials
Curriculum Vitae

Stephanie M Reid

Post-secondary Education: Winthrop University
Rock Hill, SC USA
BSc Major: Biology Minor: Chemistry
2004-2008

Western University
London, Ontario, Canada
MSc Kinesiology: Integrative Physiology
2010-2012

Honours: Western University
Western Graduate Research Scholarship
School of Kinesiology

Related Work Experience: Graduate Teaching Assistant
The University of Western Ontario
2010-2012

Published Abstracts:

