ARTERIAL ADAPTATIONS TO SPRINT INTERVAL TRAINING

THE IMPACT OF SPRINT INTERVAL TRAINING ON ARTERIAL COMPLIANCE AND BRACHIAL ENDOTHELIAL FUNCTION IN YOUNG HEALTHY MALES

By

Jennifer L. Bartholomew, B.Sc. (Hons.)

A Thesis

Submitted to the School of Graduate Studies

In Partial Fulfillment of the Requirements

for the Degree

Master of Science

McMaster University

MASTER OF SCIENCE (2005) McMaster University

(Human Biodynamics) Hamilton, Ontario

TITLE: The Impact of Sprint Interval Training on Arterial Compliance and Brachial Endothelial Function in Young Healthy Males

AUTHOR: Jen Bartholomew, B.Sc. (University of Guelph)

SUPERVISOR: Maureen J. MacDonald, PhD.

NUMBER OF PAGES: xiii, 95

ABSTRACT

Increased arterial stiffness and vascular endothelial dysfunction have been identified as independent risk factors for the development and progression of cardiovascular disease. Traditional endurance training has been associated with elevated levels of central arterial compliance and an attenuation of cardiovascular events. As well, the positive benefits of aerobic-based training have been acknowledged as effective modulators of vascular endothelial function. To date, the impact of sprint interval training on cardiovascular health has not been evaluated. Furthermore, the mechanisms responsible for previously observed enhancements in endurance (750 kJ) performance following two weeks of sprint interval training remain unclear, but may be related to changes in vascular structure and function.

Nine young healthy males [age: 22 $\pm$ 0.5 (mean $\pm$ SEM)] participated in a two week sprint interval training program consisting of 4-6 30 second maximum effort exercise bouts performed every other day on a cycle ergometer. In addition, each participant was required to complete a 750 kJ time trial on a cycle ergometer as a measure of aerobic exercise performance before (PRE) and after (POST) training. Measurements of supine, resting carotid pulse pressure, carotid cross-sectional compliance, and brachial vascular endothelial function (using flow mediated dilation) were also acquired PRE and POST training.

Resting pulse pressure did not show any significant changes with exercise training (PRE = 48.6$\pm$1.6, POST = 52.4$\pm$2.5 mmHg, $p>0.05$). Mean brachial artery diameter was not changed with sprint interval training (PRE = 4.29$\pm$0.17, POST = 4.38$\pm$0.18 mm,

p>0.05); however, mean carotid artery diameter increased significantly PRE to POST (PRE = 6.40±0.15, POST = 6.49±0.14 mm, p=0.008). Carotid cross-sectional compliance did not change PRE to POST training (PRE = 0.164±0.010, POST = 0.162±0.007 mm^2/mmHg, p>0.05). Brachial vascular endothelial function measured using flow-mediated dilation did not show a significant change with sprint interval training, however a trend towards improvement was noted (PRE = 4.6±1.8, POST = 6.4±1.0 %, p=0.296). When normalized for shear rate (which was also unaltered with sprint interval training) there were no changes in endothelial function (PRE = 0.158±0.068, POST = 0.198 ± 0.034 %/S^{-1}, p>0.05). Average brachial post-occlusion blood flow was significantly enhanced following training possibly revealing enhanced resistance vessel function (PRE = 296.0±37.4, POST = 324.8±38.8 ml/min, p=0.04), despite no change in peak brachial blood flow (PRE = 332.0±42.3, POST = 362.6±45.7 ml/min, p>0.05). 750 kJ time trial performance was significantly enhanced with training (PRE = 62.8±4.9; POST = 55.84±3.55 min; p=0.006).

In conclusion, sprint interval training did not change resting carotid compliance or brachial endothelial function, despite significant improvements in aerobic performance (750 kJ). However, carotid resting diameters and brachial post occlusion blood flow were significantly increased PRE to POST and a trend towards improvement was seen for brachial flow mediated dilation. The exact mechanisms responsible for such changes remain unknown and require further investigation.

ACKNOWLEDGEMENTS

The past two years at McMaster have been filled with many excellent learning opportunities and countless good times. My achievements in graduate school would not have been possible without the support of several great individuals

First and foremost, I would like to thank my advisor Dr. Maureen MacDonald for opening her arms and adopting me as an EMRG member. Just when I thought it was time to hit the road, you picked me up and offered me a position on the blood flow team. Only someone as kind as you would be willing to take on a student you had never even met. You have given me the opportunity to experience independence, yet you always seem in reach when I need your guidance. You are a fantastic mentor and friend, and I only hope that one day I am able to embrace your attitude towards family and life.

M-dog, D-rock and Little Arbs……thanks for all great memories. Who thought grad school could actually be that much fun! I could always count on you guys to give me an escape (and many longs ones at that) from the books, or even just to do a little TV watchin'. Although the memories are too numerous to list, we must always remember: carpet cleaner, exam marking, and would you rather. My hopes for you all are that you may one day become official "gang" members.

Rako, where would I be without you?? Thank you so much for all of your help over the past year. You were always willing to lend a hand, ear, or even some brain power when I needed it…and let's be honest, that was pretty much every day! I really appreciate your honesty and even sometimes enjoy your sarcasm.

To the rest of the EMRG group, you guys are awesome! Let us never forget a few of our fondest moments: "snakin' it", "air guitaring", and betting on the ponies. Good luck to you all. I hope you make it out alive☺

I would also like to thank Stu and Marty for reminding me that there is more to exercise physiology than blood flow. Your enthusiasm for research and genuine interest in the well being of your students and colleagues is very apparent and much appreciated. I thank you for your leadership and motivation over the past 2 years.

Most importantly, I would like to thank my family. Mom, Dad, Mike, and Shawn, your support has been endless despite this seemingly long and very costly academic pathway I have chosen. You have always encouraged me to dream big and pursue my goals. Mom and Dad, I was hoping that the day I wrote this acknowledgements page I would have an answer to your favourite question "So what are you going to do when you're done??" I regret to say that I still have no idea☺

Later Skaters,
J-Bart

TABLE OF CONTENTS

Chapter 2: The Impact of Sprint Interval Training on Central Arterial Compliance and Brachial Endothelial Function in Young Healthy Males

LIST OF APPENDICES

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

SIT	Sprint interval training
SBP	Systolic blood pressure
DBP	Diastolic blood pressure
AC	Arterial Compliance
PP	Pulse Pressure
VSMC	Vascular smooth muscle cell
NO	Nitric Oxide
PWV	Pulse wave velocity
EF	Endothelial function
eNOS	Endothelial nitric oxide synthase
FBF	Forearm blood flow
FMD	Flow mediated dilation
ATP	Adenosine triphosphate
H+	Hydrogen ions
PCr	Phosphocreatine
PFK	Phosphofructokinase
PHOS	Phosphorylase
CAD	Coronary artery disease
MBV	Mean blood velocity
ROI	Region of interest
BV	Blood velocity

d	Diameter
r	Radius
v	Velocity
0	Angle of insonation
c	Speed of sound in tissue
f_d	Frequency difference
f_t	Transmitted frequency
f_r	Received frequency

CHAPTER 1

REVIEW OF LITERATURE

1.1 INTRODUCTION

In humans, cardiovascular function plays a central role in the maintenance of the internal environment. The circulatory system facilitates the exchange of molecules and ions between cells. This intercellular exchange supports cellular metabolism and permits the removal of waste products. Specifically, the arterial branches of the cardiovascular system work to move blood from the heart to various organs of the body. One particular function of arteries is to maintain a continuous supply of oxygen to skeletal muscles. Maintenance and optimization of this system is vital to human health and performance. Exercise is recognized as one of the most effective approaches in the prevention of cardiovascular dysfunction and disease. During exercise, the arterial system is subjected to various stimuli that are thought to evoke favourable structural and functional adaptations. Different exercise modalities produce different physical and chemical stresses on the arterial system. Sprint interval training (SIT) is an exercise modality characterized by short bouts of high intensity work that results in increased muscle oxidative potential (Burgomaster *et al.*, 2005); however, to date there is no literature examining the impact of SIT on measures of vascular structure and function.

Characteristics of the cardiovascular system are associated with the risk and occurrence of heart disease, and therefore, evaluation of cardiovascular structure and function are important (Arnett *et al.*, 1994; Moyna & Thompson, 2004; Tanaka *et al.*, 2000). Clinically, one of the most common measures of arterial health is resting blood

pressure. Due to the ease and accuracy of blood pressure measurements, physicians often rely on measures of systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial pressure (MAP) as the sole indicators of an individual's cardiovascular health. While elevated arterial pressure has been proven to be strongly correlated with the incidence of heart disease and other associated conditions (Kelly *et al.*, 1989), the assessment of additional structural and functional properties is valuable. Structural assessments of the arterial system may include measures of arterial compliance or stiffness, arterial diameter, arterial wall thickness, and arterial distensibility. Functional evaluations of the endothelial system may include measurements of dilation and constriction in arteries that are faced with various physical and chemical stimuli.

With aging there is an inherent reduction of both arterial structure and function that may lead to the progression of several disease processes including coronary artery disease, diabetes mellitus, and hypertension (Clarkson *et al.*, 1999; O'Rourke *et al.*, 2002). Past research has confirmed that measurements of arterial properties can identify individuals who are at risk of developing cardiovascular disease or who are already dealing with the problem (Adji & O'Rourke, 2004; Baguet *et al.*, 2003; Cameron *et al.*, 2002; Corretti *et al.*, 2002). As well, these values can be used as benchmarks to track the attenuation or even reversal of the negative consequences of these diseases. Once diagnosed with an ailment of this nature or identified as an individual who is predisposed, several therapeutic measures can be taken to help control the condition.

Exercise training has been acknowledged as an effective, non-pharmalogical therapy to treat some of the disease processes associated with the structure and function

of the arterial system. However, the most effective exercise modality to promote arterial health remains controversial. To date, the most popular form of exercise prescription has been aerobic or endurance-based training, with resistance training receiving more attention in the literature recently. The effects of SIT on the management of arterial health have yet to be investigated.

1.2 ARTERIAL COMPLIANCE

Arterial compliance (AC) is defined as the ability of an artery to accommodate an increase in volume (O'Rourke *et al.*, 2002). Also commonly referred to as arterial stiffness (the inverse of compliance), the term AC is used when describing changes at the arterial level in response to an intervention or therapy, however, both names are often used interchangeably (O'Rourke *et al.*, 2002). This vascular property is expressed as the change in diameter of an artery resulting from the difference between systolic and diastolic blood pressures during a heart cycle. The denominator of this equation is known as pulse pressure (PP) and can be used independently as a determinate of arterial stiffness (Dart & Kingwell, 2001). Elevated PP is increasingly being recognized as a risk factor for cardiovascular disease and therefore this measure is used regularly in clinical practice (Adji & O'Rourke, 2004; Dart & Kingwell, 2001; Kelly *et al.*, 1989). Although the determination of an individual's PP is a simple measurement that can yield valuable insight into arterial health, it does not directly measure the compliance of an artery (Bulpitt *et al.*, 1999). As a result, many scientists are relying on other non-invasive

techniques including pulse wave velocity (PWV) and ultrasound to acquire more comprehensive values of arterial structure (Woodman *et al.*, 2005).

Increased arterial stiffening is a hallmark of the aging process and indicative of many disease states such as diabetes, atherosclerosis, myocardial infarction, and heart failure (Seals, 2003). The mechanisms associated with the marked decline in the compliance of large elastic arteries involve both structural and functional alterations in properties of the arterial wall (Kingwell *et al.*, 2001).

1.2.1 Mechanisms of Arterial Stiffening

Arterial stiffening develops from a complex interaction between stable and dynamic changes involving structural and cellular elements of the vessel wall (Zieman *et al.*, 2005). Increased stiffness is not uniformly distributed, and does not affect all vessels in the same manner. It occurs most often in the central and conduit vessels, while sparing peripheral arteries (Benetos *et al.*, 1993). The central arterial system, consisting of the aorta and its major branches, is responsible for accommodating a relatively large volume of blood during systole and ensuring continuous blood flow throughout the remainder of the cardiac cycle (Dart & Kingwell, 2001), and therefore, is highly susceptible to decreases in function.

Structural changes are believed to play a major role in the age-associated declines in large artery compliance (Lakatta, 2002). The stability, resilience, and compliance of the vascular wall are dependent on the relative amounts of collagen and elastin present. These proteins provide structural integrity and elasticity within the extracellular matrix of the vessel wall. With aging, there are inherent inflammatory responses that lead to the

overproduction of abnormal collagen and diminished quantities of normal elastin (Zieman *et al.*, 2005).

Collagen molecules provide the tensile strength of the vessel wall, and are enzymatically cross-linked soon after their formation to render them insoluble to hydrolytic enzymes. Collagen has a slow hydrolytic turnover rate, and as a result, it is very susceptible to non-enzymatic gylcation cross-linking. This leads to increased collagen content (Zieman *et al.*, 2005). The increased concentration causes the arteries to become more rigid and non-compliant to the biological pulsation and flow of blood. Eventually this rigidity will lead to endothelial damage (Seals, 2003). Elastin molecules are stabilized by cross-linking, and disruption of these bonds contributes to the weakening of the elastic properties. Fragmentation of elastin causes a decrease in the density of these proteins and cues arterial remodeling (Lakatta, 2003). These molecular changes in elastin manifest as a thickening of the intima-media, as well as an increase in lumen diameter.

Arterial stiffness can also be caused by advanced glycation end products (AGE's), which result from non-enzymatic protein glycation to form irreversible cross links. Both collagen and elastin molecules are susceptible to AGE crosslinking, resulting in an accumulation of structurally inadequate molecules. AGE's may also affect endothelial cells by quenching nitric oxide (Zieman *et al.*, 2005).

In addition to structural changes, arterial stiffness is strongly affected by endothelial cell signaling and vascular smooth muscle cell (VSMC) tone. An increase in VSMC tone likely contributes to the decrease in compliance with age (Lakatta, 2002).

VSMC tone can be modified by paracrine mediators such as angiotensin II, endothelin-I, oxidative stress, and nitric oxide (NO) (DeSouza *et al.*, 2000). Decreased bio-availability of NO and an increase in local endothelin-I and angiotensin-II are proposed mechanisms (Seals, 2003).

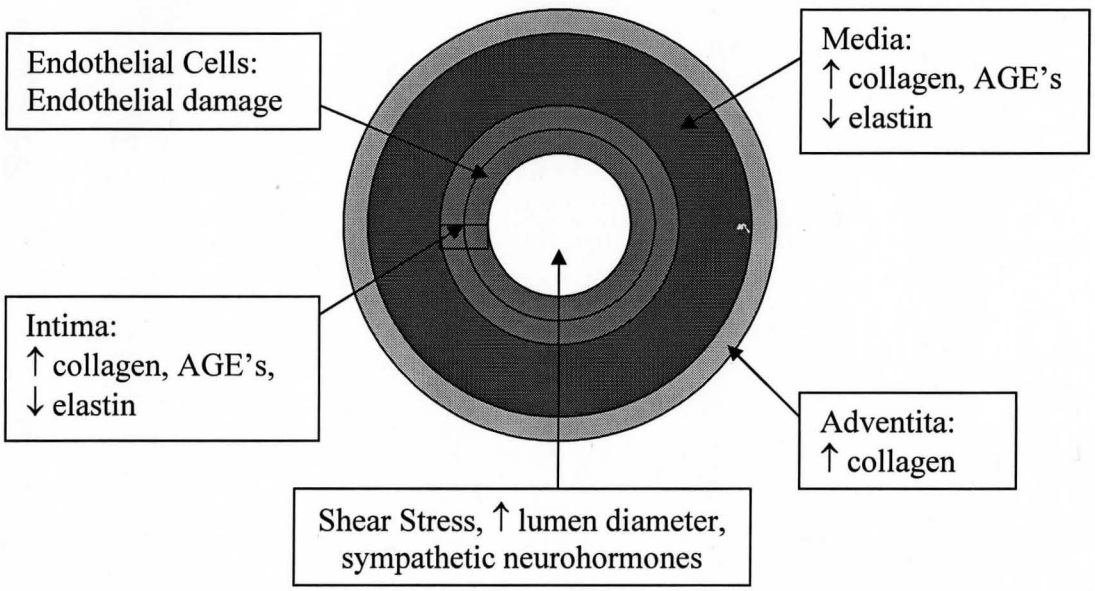

Figure 1.1: Summary of causes and locations of arterial stiffness (Adapted from (Zieman *et al.*, 2005)

1.2.2 Consequences of Decreased Arterial Compliance

The progressive reduction in the compliance of large elastic arteries is associated with several adverse adaptations in the vascular system that may ultimately lead to cardiovascular disease or mortality. Increases in SBP may be a direct effect of a less compliant circulatory system. Ejection of blood from the left ventricle to the aorta (stroke volume) may result in systolic hypertension if the aorta can not accommodate for the volume of blood. Also, the loss of elastic recoil ability reduces DBP, thus increasing

arterial PP. This type of chronic elevation in SBP may contribute to endothelial damage and accelerate the progression of cardiovascular disease (Seals, 2003).

Reductions in large artery stiffness can also lead to declines in cardiac structure and function. Specifically, with increases in large artery stiffness, the left ventricle faces many adverse effects such as hypertrophy, increased oxygen demand, and increased wall tension. Reduced left ventricular function and hypertrophy in an aging individual may place them at an increased risk of congestive heart failure (Seals, 2003).

The responsiveness of several key reflexes involved in the maintenance of circulatory homeostasis can be reduced with impaired AC. Decreased distensibility of the aorta and carotid sinuses in response to changes in intravascular pressure result in limited afferent signaling by baroreceptors to the central nervous system. The result is a smaller efferent response and decreases in cardiac vagal activity, consequently leading to common age-related disorders of the cardiovascular system (Monahan *et al.*, 2001).

The importance of compliant arterial circulation for minimizing cardiac work and providing adequate perfusion is large. Therefore, precise and reliable measurements are vital.

1.2.3 Measuring Arterial Compliance

Adequate measurement techniques are an essential prerequisite for establishing a definitive association between arterial function and cardiovascular health. Currently, no global method exists to assess arterial structure and function; however, there are a number of available approaches that are commonly used to reflect arterial status. The measurement of AC is one of the most popular choices to asses arterial health (Bulpitt *et*

al., 1999). The choice of evaluation must be considered carefully as different arterial segments exhibit differing characteristics and particular functional parameters may provide better association with specific pathophysiological processes (van der Heijden-Spek *et al.*, 2000). The competing presence of various techniques suggests that no one approach is universally accepted or applicable (Cameron *et al.*, 2002).

Measuring AC in humans is most often done using, non-invasive, in vivo measurements. Although once measured in vitro using excised arteries, it has more recently been determined that results derived from in vitro measures may not apply to undisturbed arteries (Arnett *et al.*, 1994). Two popular, robust methods that exist to assess AC in vivo are PWV and ultrasound imaging. While PWV estimates arterial stiffness indirectly through the assessment of the speed of a pulse wave propagating along a specific segment of a vessel, imaging techniques allow directly visualized arterial diameter changes. Since diameter changes are not able to provide adequate estimates of arterial stiffness (the degree of stretching depends on the pulse pressure within the artery), diameter change measurements are adjusted for the pressure within the artery.

Ease of applicability of pulse wave analysis has made this technique very popular, and described as the most common practice (Cameron *et al.*, 2002). PWV is measured as the difference between two recording sites in the line of pulse travel, and the delay between corresponding points on the wave. Blood pressure and flow are recorded concurrently at each site (O'Rourke *et al.*, 2002). For example, blood flow can be measured at the aortic root using Doppler ultrasound and simultaneously blood pressure can be obtained at a distal artery using arterial tonometry. The time difference between

the initial point of outflow and the blood pressure upstroke is compared to the distance the pulse wave has traveled (Vaitkevicius *et al.*, 1993).

PWV analysis does have certain drawbacks and limitations. Of particular concern is the indirect nature of this measurement. Pulse wave analysis can be affected by a vessel geometry, wall thickness, blood velocity, and distances to the principal reflection site (Cameron *et al.*, 2002). The specificity and accuracy of this technique limits its application. Because the length of the vessel of interest can influence this measure, there is room for error. The exact length of the conduit arteries varies from person to person due to anatomical differences. As well, PWV increases markedly with age in the large elastic arteries, however, it has not been shown to do the same in the upper limb muscular arteries (O'Rourke *et al.*, 2002). As well, the further the recording site is from the heart, the higher the PWV (Arnett *et al.*, 1994).

Ultrasound imaging combined with arterial tonometry is used to evaluate AC in a specific region or specific artery such as the carotid artery. The diameter of an artery during a heart cycle can be observed non-invasively using ultrasound. In vascular ultrasonogarphy, a continuous beam is transmitted from a probe containing two piezoelectric crystals. The transmitting crystals produce ultrasound at a fixed frequency and the receiving crystal responds to reflected waves and produces an output voltage. B-mode (brightness mode) ultrasound records the waves reflected from the tissue interfaces, to produce a two-dimensional picture (McGrath, 2002). From these images it is possible to track the change in vessel size over time or in response to a stimulus.

Blood pressures are concurrently measured when using ultrasound to assess AC. The development of high-fidelity, linear force transducers (tonometers) allow for non-invasive registration of continuous arterial blood pressure waveforms (Cameron *et al.*, 2002). An accurate blood pressure device has been created that combines an arterial tonometer at the radial artery with an oscillometric measurement of brachial artery blood pressure (Colin Medical). As well, Millar Instruments Inc. has developed a hand-held, pen like device that records pressure readings from various superficial arteries such as the carotid. This technique is comparable with intra-arterial measurements (Kelly *et al.*, 1989). By combining these three devices, (ultrasound imaging, Colin, and Millar) both central and peripheral AC can accurately be determined.

Ultrasound imaging is an advantageous technique due to its excellent technical precision and test reproducibility (Arnett *et al.*, 1994) however, like PWV, it also has its limitations. Ultrasound data acquisition may be restricted by participant effects such as respiratory movements, persistent swallowing and anatomical shape. In addition, there is great possibility of investigator error. Operator-dependent sources of missing data include incorrect identification of the vessel walls and errors in digitizing data for later analysis (Arnett *et al.*, 1994).

1.2.4 Arterial Compliance and Exercise Training

Regular exercise appears to modify favourably at least some of the negative consequences resulting from the age-associated reductions in arterial health (Seals, 2003). High levels of aerobic activity and training have been associated with elevated levels of central AC (Gates *et al.*, 2003; Kakiyama *et al.*, 1998; Vaitkevicius *et al.*, 1993).

Pioneering studies of this discipline that have investigated resting AC in healthy adults (age 21-96) suggest a strong correlation between high fitness levels ($\dot{V}O_{2max}$) and an attenuation of age-related decreases in AC (Vaitkevicius *et al.*, 1993). Subsequent to this investigation, Vaitkevicius and colleagues (1993) also determined that within this population, older, endurance trained males had lower PWV's and augmentation indices than their sedentary complements (Vaitkevicius *et al.*, 1993). In 1998, Kakiyama and colleagues found a similar positive relationship between regular exercise and central AC in healthy young men of varying fitness levels. These authors demonstrated that these relationships were dependent on the frequency, duration, and mode of their physical activity. This suggests that individuals with higher levels of habitual exercise will reap the greatest benefits (Kakiyama *et al.*, 1998).

Previously described cross-sectional evaluations have been backed by several longitudinal studies that support the association of increased AC and endurance training (Cameron & Dart, 1994; Ferrier *et al.*, 2001; Tanaka *et al.*, 2000). Studies focusing on healthy young males have shown that exercise of moderate intensity (60-75% of max heart rate), short duration (30-45 minutes/day) and moderate frequency (4-6 days/week) will increase central AC following twelve weeks of training (Tanaka et al., 2000). Cameron and colleagues also showed significant increases in AC in sedentary middle aged males following four weeks of moderate intensity cycle training three times per week (Cameron & Dart, 1994). This study was recognized for utilizing a protocol of limited duration yet demonstrating beneficial effects from the exercise.

Although the focus in this area of research has revolved around aerobic-type training, a few pieces of literature have examined the influence of resistance training on central AC (Rakobowchuk *et al.*, 2005a; Miyachi *et al.*, 2004; Miyachi *et al.*, 2003; Bertovic *et al.*, 1999). Resistance training is associated with abrupt and large pressor responses to the cardiovascular system. In marked contrast to the favourable effects noted from aerobic exercise, strength training sometimes shows adverse associations with improved arterial health.

In 1999 Bertovic and colleagues compared measures of arterial health (systemic AC, PWV, and aortic stiffness) in a cross-sectional design between strength-trained individuals and age matched sedentary controls. The primary finding was that systemic AC was significantly reduced, whereas PWV from the carotid to the femoral and the femoral to the dorsalis pedis was not different between groups. Bertovic suggests that this indicates stiffness was localized to the central elastic arteries (Bertovic *et al.*, 1999). In an attempt to reproduce these findings, Miyachi and colleagues (2003) conducted a cross-sectional analysis between sedentary and resistance trained individuals focusing on two different age groups. They hypothesized that because age exerts an independent effect in reducing AC in humans, an interaction between age and resistance training would exist. Two groups of men, young (20-39 yrs) and middle aged (40-60 yrs), were assigned to either the sedentary or resistance trained groups. Those participating in the strength training protocol were recruited from various fitness clubs and had been identified as persons who had been engaging in resistance exercise for more than two years. In both activity groups, central arterial (carotid) compliance was lower in the

middle-aged men compared to their young counterparts. Although a trend for reduced carotid compliance was apparent in young resistance-trained participants it did not reach significance (P=0.09). However, statistical significance was met in the middle aged resistance-trained men (Miyachi *et al.*, 2003). Recognizing that cross-sectional design has its limitations, Miyachi and colleagues (2004) determined that their previous worked should be confirmed prospectively with an intervention approach. Their original findings were replicated, and results support the notion that resistance training reduces central AC in healthy men (Miyachi *et al.*, 2004).

Most recently, Rakobowchuk and colleagues (2005) have demonstrated that 12 weeks of resistance training had no effect on central AC in young healthy men. This may suggest that the mechanisms responsible for reduced AC as previously reflected are not inherent to all resistance training programs or may require a prolonged stimulus (Rakobowchuk *et al.*, 2005a).

1.2.5 Mechanisms of Altered Arterial Compliance with Exercise Training

Any favourable influence of regular exercise should involve an attenuation or reversal of one or more of the mechanisms contributing to the age-associated reductions in arterial health. Mechanistic human studies related to compliance and exercise are lacking; however, several studies conducted in rats have given valuable insight. It is believed that both structural and functional adaptations contribute to the training induced enhancement of AC. With regards to structural adjustments, aerobic exercise training is associated with changes in the composition of the arterial wall such as increased levels of total elastin and reduced calcium content of elastin (Seals, 2003). In 1993 Matsuda and

colleagues measured the contents of rat aorta following endurance exercise training and found significantly increased levels of elastin content compared to sedentary controls. They speculate that the increase in elastin would positively contribute to total elasticity, thus increasing AC (Matsuda *et al.*, 1993).

The most likely mechanism for functional adaptations is related to alterations in vascular smooth muscle tone influenced by vascular-endothelium-dependent vasodilation (nitric oxide bioavailability) (Seals, 2003). Moderate-intensity aerobic exercise has been shown to restore the age-related loss of endothelium-dependent vasodilatory responsiveness in previously sedentary middle aged men (DeSouza *et al.*, 2000). The expanded nitric oxide (NO) availability developed with aerobic exercise training should tonically suppress VSMC tone in the arterial wall, thus increasing AC.

What physiological mechanisms might vindicate the reduced central AC in resistance trained men? A likely explanation may be the blood pressure responses during strength training which are known to reach levels as high as 320/250 mmHg (MacDougall *et al.*, 1992). This response may result in chronic increases in the smooth muscle content of the arterial wall and the load bearing properties of collagen and elastin (Miyachi *et al.*, 2003). Another possible mechanism is that the higher sympathetic nervous system activity in resistance trained individuals may act to reduce compliance by chronically restraining the arterial wall via increased vasoconstrictor tone (Pratley *et al.*, 1994).

1.3 VASCULAR ENDOTHELIAL FUNCTION

Endothelial function (EF) refers to the capacity of an artery to dilate in response to physical or chemical stimuli (Moyna & Thompson, 2004). The heart, and the vessels to which it supplies blood, are lined by a single layer of vascular endothelium which functions as an interface between circulating blood elements and the biological system. The endothelium is an essential contributor to the processes of homeostasis as it synthesizes and releases several active factors involved in the regulation of vascular tone (Vane *et al.*, 1990). Thus, the health of the endothelium plays a vital role in the maintenance of vascular tone and reactivity. Conduit artery endothelium is the primary site of injury resulting from the mechanical forces and processes related to aging and disease (Moyna & Thompson, 2004). Vascular injury provoked by pathophysiological stimuli reduces the bioavailability of endothelium-derived NO. As a result, the endothelium can become dysfunctional and may lead to impaired cardiovascular health. The ability to recognize these impairments and suggest putative therapies is clinically important.

In humans, advancing age is associated with a progressive impairment in endothelium dependent vasodilation (DeSouza *et al.*, 2000). Accumulating evidence suggests that endothelial dysfunction symbolizes one of the earliest events in the pathogenesis of cardiovascular disease (Fathi *et al.*, 2004). Specifically, endothelial dysfunction has been reported in individuals with coronary artery disease (Gokce *et al.*, 2002; Takase *et al.*, 1998), chronic heart failure (Gokce *et al.*, 2003), and even in persons

with no overt signs of cardiovascular disease (Takase *et al.*, 1998). Early detection of endothelial dysfunction is important in the battle against cardiovascular related events.

1.3.1 Pathophysiology of Endothelial Dysfunction

The hallmark of endothelial dysfunction is a reduction in the bioavailability of endothelium-derived NO at VSMC. There are three specific means by which NO availability can be altered. These include: (1) availability of NO precursor molecule L-arginine, (2) variations in the rate of NO synthesis which depends on endothelial nitric oxide synthase (eNOS) expression, and (3) differences in the rate of NO breakdown relative to available reactive oxygen species (Gielen & Hambrecht, 2001).

The availability of L-arginine to the active site of eNOS is dependent on several factors. First, there must be a positive supply and demand relationship of L-arginine. Endogenous synthesis of L-arginine occurs within the urea cycle, or sometimes via an independent intraendothelial pathway. Second, accumulation of L-arginine intracellularly controlled by cytokine-regulated transmembranous transport. Third, degradation of L-arginine by arginase or urea, and fourth, NOS from L-arginine may be blocked by its antagonist, asymmetric dimethyl arginine (ADMA) (Gielen & Hambrecht, 2001).

The activity of eNOS can be altered in response to an acute stimulus by structural alterations. Other long term influences have been shown to lower eNOS expression in endothelial cells such as hypoxia, low density lipoproteins, and tumor necrosis factors.

Endothelial dysfunction has also been associated with the occurrence of oxidative stress (Gielen & Hambrecht, 2001). Excessive production of reactive oxygen species that

cannot be combated by endogenous antioxidant defense mechanisms has been implicated in the pathogenesis of many cardiovascular diseases. This process is modulated by the bioavailability of NO, such that, when the bioavailability decreases, EF worsens. When an excess of free radicals such as superoxide anions are produced, they interact with NO molecules and cause them to become inactive vasodilators. This ultimately leads to a decreased bioavailability of NO and decreased EF (Cai & Harrison, 2000).

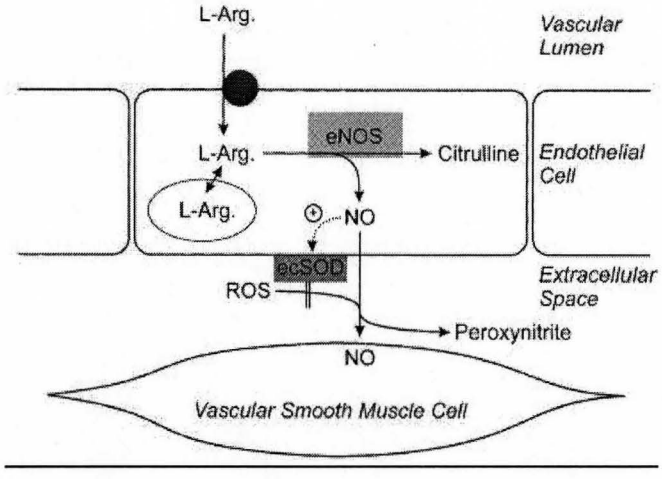

Figure 1.2: Pathways of L-arginine (L-Arg) transport, endothelial nitric oxide (NO) generation and extracellular NO breakdown (Taken from (Gielen & Hambrecht, 2001).

1.3.2 Measuring Endothelial Function and Reactivity

Cardiovascular disease is currently a leading cause of morbidity and mortality in the Western world (Smart & Marwick, 2004). This fact has provided a strong impetus for the development of methods that facilitate in vivo assessment of EF.

Endothelium-dependent vasodilation function, reflecting local bioavailability of NO, can be measured clinically in the peripheral and coronary circulation and refers to a

measure of endothelial cell response to stimulation (Verma *et al.*, 2003). Several invasive and non-invasive techniques have been developed to evaluate EF. Invasive techniques involve intracoronary or intrabrachial infusions of vasoacting agents and are still considered the gold standard for detection of endothelial dysfunction. Several non-invasive techniques have also been developed with comparable results and good reproducibility (Halcox & Deanfield, 2004).

In the coronary circulation, endothelial-dependent function can be assessed using quantitative coronary angiography which examines the changes in vascular diameter in response to an infusion of an endothelial-dependent vasodilator such as acetylcholine. Briefly, acetylcholine stimulates muscarinic receptors on the endothelium, which in turn activate eNOS resulting in the production of NO from its precursor L-arginine. Subsequently, L-arginine diffuses into adjacent muscle cells causing smooth muscle relaxation via cyclic guanosine monophosphates' stimulation of sarcoplasmic reticulum calcium uptake (Halcox & Deanfield, 2004). Coronary arteries with normal endothelium dilate in a dose-dependent manner, however, if the endothelial cells are dysfunctional, the infusion will cause a blunted NO-mediated vasodilatory response and sometimes even vasoconstriction (Verma *et al.*, 2003). EF can also be evaluated within the coronary circulation using intracoronary Doppler techniques to measure blood flow in response to a pharmalogical or physiological stimulus (Tousoulis *et al.*, 2005).

It has been shown that EF in the coronary arteries is closely related to EF in the peripheral arteries such as the brachial artery (Anderson *et al.*, 1995). Therefore, the technique of intracoronary infusions can also be applied in the brachial artery, which has

fewer potential dangers and complications. The methodology is very similar to that used in the coronary artery; however, in this case the dose-response relationship refers to changes in forearm blood flow (FBF). FBF is measured in the brachial artery using strain-gauge plethysmogrphy. During each FBF determination, the circulation of the hand is excluded for one minute, by inflation of a paediatric cuff around the wrist at suprasystolic blood pressure. After the determination of baseline FBF, acetylcholine is infused in the brachial artery with a gradually increasing infusion rate. FBF under acetylcholine infusion is measured, and the change in FBF from baseline represents an index of endothelium dependent dilation (Tousoulis *et al.*, 2005). Although this method is reliable, reproducible, and easily applied, it still remains an invasive method with potential side effects.

The ability to acquire measurements of EF in the peripheral arteries non-invasively is very appealing. Many recently developed non-invasive techniques are easily conducted on human participants and can be used as screening tests for identification of early onset cardiovascular disease (Verma *et al.*, 2003). These non-invasive techniques include venous occlusion plethysmography and Doppler ultrasound. Assessment of EF in conduit arteries such as the brachial artery is commonly completed using Doppler ultrasound. Peripheral arteries respond to various stimuli by adjusting vascular tone and regulating blood flow. Increased blood flow leads to an increased shear stress stimulus, causing augmented NO production and subsequently vasodilation. This vasodilatory response to shear stress is refered to as flow mediated dilation (FMD) and reflects the ability of the vascular endothelium to produce NO (Vane *et al.*, 1990).

Assessment of FMD involves the application of ischemia to an extremity for an extended period of time (4-8minutes), after which ultrasonography is used to image the artery of interest (Verma *et al.*, 2003). The brachial artery is imaged above the antecubital fossa in the longitudinal plane. The diameter of the artery is initially determined at rest, and blood flow is estimated by the pulsed Doppler velocity signal obtained from a sample volume of the brachial artery. Subsequently, ischemia is produced by inflating a cuff to a pressure of 200+ mmHg that is suspended around the distal forearm. After five minutes, the cuff is released causing a dramatic increase in FBF, leading to vasodilation of the brachial artery. The maximum blood flow velocity is acquired via mid-artery pulsed Doppler signal immediately, and after 15 seconds of release, while the maximum diameter of the brachial artery is determined 45-60 seconds following release. FMD is defined as the percentage change of the brachial artery from rest to 60 seconds post-ischemia (Tousoulis *et al.*, 2005). Peripheral EF measured with ultrasound has been well correlated with EF of the coronary circulation (Takase *et al.*, 1998).

1.3.3 Endothelial Function and Exercise Training

The available literature largely supports a reversal or attenuation of the age related reductions in EF with exercise therapy (Moyna & Thompson, 2004). Several studies have summarized the effects of exercise training of EF, and concluded that impaired EF is improved with aerobic exercise training. This has been proven in young healthy men via cross-sectional evaluation (DeSouza *et al.*, 2000; O'Sullivan, 2003), as well as through intervention studies (Clarkson *et al.*, 1999; Green *et al.*, 2002; Green *et al.*, 1994; Kingwell *et al.*, 1997; O'Sullivan, 2003). O'Sullivan and colleagues had healthy young

men perform five weeks of moderate aerobic exercise on a cycle ergometer consisting of three 30-minute sessions per week at 60% of $\dot{V}O_{2max}$. Significant improvements in endothelial-dependent responses of the brachial artery were observed after training indicating enhanced EF. These novel findings suggest that optimal benefits to the cardiovascular system may be obtained even after short-term (2-5 weeks) training protocols (O'Sullivan, 2003).

The benefits of aerobic-based exercise have also been recognized in the clinically impaired. Clinically impaired individuals have endothelial dysfunction as a consequence of aging or disease (Gokce *et al.*, 2003; Gokce *et al.*, 2002; Hambrecht *et al.*, 1998; Maiorana *et al.*, 2001a; Maiorana *et al.*, 2000; Walsh *et al.*, 2003). The diversity of these populations include patients with coronary artery disease (Gokce *et al.*, 2002; Walsh *et al.*, 2003), chronic heart failure (Maiorana *et al.*, 2000), and elderly athletes with (Taddei *et al.*, 2000). The use of exercise training as an intervention has been proven useful in each of these cohorts.

Although the support for beneficial effects of aerobic-type training is abundant in the literature, much less is understood about the effects of resistance training on EF. A series of investigations conducted by Maiorana and colleagues evaluated the combined effects of aerobic exercise and resistance training on EF using a circuit-type program which included both strength training and 45s cycling intervals. This influence was studied in patients with chronic heart failure (Maiorana *et al.*, 2000) as well as in persons suffering from Type 2 diabetes (Maiorana *et al.*, 2001a). Results indicated significant increases in brachial FMD following training in both cohorts. A limitation to this study

in regards to finding a definitive answer concerning resistance exercise was that the intensity (~60% of 1RM) and duration (1 set) of the training stimulus was limited. As well, it is certainly plausible that the aerobic component of the stimulus induced improvements in EF. A recent publication by Rakobowchuk and colleagues (Rakobowchuk *et al.*, 2005b) has utilized a prospective, longitudinal design to examine the impact of twelve weeks of whole body resistance training on EF in young, healthy, men. The participants trained five times per week, employing a repeating, split-body, three day cycle. Observations from this study included an increase in resting and peak FBF as well as greater mean brachial diameters following training. Despite these changes, no significant increases were noted in EF. This study was the first to show peripheral arterial remodeling does occur with resistance training in young healthy men; and the results suggest that arterial adaptations occurring from high-pressure loads may be quite different compared with those occurring from aerobic-based training (Rakobowchuk *et al.*, 2005a).

As mentioned previously, endothelial responses to FMD as a result of SIT have never been examined; however in 1999, Bergholm and colleagues studied the influence of intense aerobic-based exercise on EF. The participants completed a 60 minute ride on a cycle ergometer at 70% - 80% of their $\dot{V}O_2max$, 4 times per week. The elevated intensity and duration of the protocol made this stimulus unique from traditional aerobic training programs. After three months of training, they concluded that EF had decreased from baseline. The apparent reductions of endothelium-dependent dilation were related to reduced levels of circulating antioxidants likely due to a decrease in NO availability

(Bergholm *et al.*, 1999). A follow up study in 2003 by Goto et al. did not report impaired endothelial depended dilation with high intensity training, nor did they find any beneficial effects of the high intensity as compared with moderate intensity training (Goto *et al.*, 2003). We may propose from these findings that endothelial dependent dilation responses may be influenced by exercise stimulus in a parabolic fashion.

1.3.4 Mechanisms of Altered Endothelial Function with Exercise Training

Physical exercise augments blood flow to the exercised limbs and to the coronary circulation (Gielen & Hambrecht, 2001). In the absence of endothelial dysfunction these arteries dilate in response to the increased flow; however, if endothelial impairment is present, these vessels may constrict instead. Shear stress as a result of an acute bout of exercise is a potent physiologic stimulus for the release of NO, thus exercise training may chronically increase NO production mediated by an increase in the expression of eNOS (Clarkson *et al.*, 1999). This NO-induced vasodilation helps to move blood efficiently and restore EF. Alternatively, exercise-induced enhancements of EF may be attributed to an increased concentration of prostaglandins. Chronic increases in blood flow affect the release of prostaglandins, which play and important role in FMD (Hambrecht *et al.*, 1998).

Exercise training may also improve EF via an upregulation of antioxidant defense mechanisms. An acute bout of exercise causes an abundance of free radicals. In response, there is an adaptation of the cell to increase its inherent antioxidant defenses. In particular, there is an upregulation of the superoxide dismutase enzyme which combats the deleterious effects of the free radical production. This indirectly allows greater NO

bioavailability since interactions between NO and oxygen free radical species are reduced (Cai & Harrison, 2000).

1.4 SPRINT INTERVAL TRAINING

Sprint exercise, also referred to as anaerobic supra-maximal exercise, is characterized by a high-intensity effort that is sustained for a short period of time (Abernethy *et al.*, 1990). The peak power output generated during a single bout of sprint exercise can reach values three to four times higher than an individual's maximum aerobic power (Jacobs *et al.*, 1987). It is well established that SIT increases various performance measures (Burgomaster *et al.*, 2005; MacDougall *et al.*, 1998), as well as improves cardiovascular indices (Warburton *et al.*, 2004) however, the physiologic mechanisms for many of these improvements remain elusive.

1.4.1 Acute Physiological Responses to Sprint Exercise

The majority of research surrounding SIT has focused on the metabolic responses to high-intensity exercise, specifically, skeletal muscle energy metabolism. It is thought however, that metabolic and cardiovascular responses to SIT may occur in parallel, and that investigations into the time course and mechanisms are necessary for a complete understanding of the physiological effects of SIT.

A single bout of sprint exercise requires energy supply from all 3 available adenosine triphosphate (ATP) sources: phosphagen hydrolysis, glyco(geno)lysis, and oxidative phosphorylation. The estimated contribution from each of these energy sources are 25%, 55%, and 20% respectively during a 30-second maximum effort (Trump *et al.*,

1996). The majority of the total energy provision required for a bout of sprint exercise is needed during the first 5 seconds of exertion, during which time the non-oxidative contributors (phosphocreatine [PCr] hydrolysis and glycol[geno]lysis) provide nearly all the ATP required. However, over the subsequent 15 seconds of the sprint bout, PCr stores diminish and rate of gylco(geno)lysis decreases drastically due to negative modulators such as hydrogen ions (H+). This deficit is counteracted by an increase in ATP provision via oxidative metabolism (Parolin *et al.*, 1999).

When repeated bouts of sprint exercise are performed, as seen in a typical sprint training protocol, the contributions from the various energy sources are modified. Contrary to a single bout, PCr hydrolysis remains stable for the duration of a training session, whereas glyco(geno)lytic flux routinely attenuates after the first exercise bout (Trump *et al.*, 1996). In order to maintain power outputs representative of the initial bout despite a limited contribution of energy from glycolytic pathways, constant contributions from PCr hydrolysis and oxidative phosphorylation are required in subsequent sprint bouts. In 1994 it was estimated by Bogdanis and colleagues that energy contributions for aerobic pathways increased by 14% during successive exercise bouts (Bogdanis *et al.*, 1996).

Recovery intervals between sprint bouts are an important factor in skeletal muscle metabolism. Rest periods allow the muscle to resynthesize metabolites and replenish energy stores. It has been shown that PCr can be nearly resynthesized in as little as 2-minutes following intense, short-duration exercise (Bogdanis *et al.*, 1998). The recovery of PCr has been associated with peak power outputs during repeated sprint bouts

(Bogdanis *et al.*, 1998; Trump *et al.*, 1996). Despite the ability to replenish PCr stores, rest intervals do not appear capable of removing adequate concentrations of H^+ from the muscle, therefore causing decreased glyco(geno)lytic flux in later sprints due to inhibition by H^+. Based on this literature, it is recognized that varying training protocols used within this realm of research may lead to equivocal findings.

1.4.2 Physiological Adaptations to Sprint Interval Training

SIT typically results in an array of skeletal and cardiovascular adaptations. The adaptations of muscle to sprint training can be separated into metabolic and morphological changes. Enzyme variations represent major metabolic adaptations to sprint training, with the regulatory enzymes of all three energy systems showing signs of revision to training. These adaptations form the basis for many of the assumptions regarding metabolic mechanisms that underlie training induced enhancements in performance.

The topic of enzymatic adaptations to SIT is confounded by the idea that sprint exercise can induce increases in levels of oxidative enzymes, as well as the anticipated increases in gylcolytic enzyme levels such as phosphofructokinase (PFK) and glycogen phosphorylase (PHOS). The literature is consistent in reporting maximal activites of PFK activity in response to SIT (MacDougall *et al.*, 1998; Parra *et al.*, 2000); however, evidence supporting changes in other major glycolytic enzymes are inconsistent. Changes in the enzyme levels of both PHOS and lactate dehydrogenase are controversial. Research suggests that the rest intervals between training bouts may influence the

direction and magnitude of change in PHOS (MacDougall *et al.*, 1998; Parra *et al.*, 2000).

Despite popular belief, it was discovered that SIT could alter the enzymatic levels of oxidative regulators such as succinate dehydrogenase, citrate synthase, and malate dehydrogenase (Burgomaster *et al.*, 2005; MacDougall *et al.*, 1998). Originally a controversial topic, it was quickly determined that these oxidative enzyme changes were only consistent in training protocols that utilized sprint bouts lasting a minimum of 30-seconds. The correlation between duration and oxidative enzyme changes suggests that the gradual shift in reliance to oxidative energy provision during repeated bouts provides an adequate stimulus to increase the activity of the oxidative regulators.

Muscle morphological adaptations to SIT are controversial. Six weeks of SIT has been shown to increase the proportion of type IIA fibers by as much as 10% with parallel decreases in type I concentrations (Dawson *et al.*, 1998). Conversely, it has been noted that SIT results in elevated levels of type I fibres concomitant with a decrease in the percentage of type IIB fibers (Linossier *et al.*, 1993). These discrepancies within the literature are likely due to a lack of congruency in methodology.

The majority of studies that have investigated adaptations to SIT have focused on intramuscular adaptations, thus research focused on cardiovascular responses is limited. A comprehensive evaluation conducted by Bogdanis and colleagues in 1996 assessed the cardio-respiratory responses to a single 30-second exercise sprint cycling bout. They concluded that $\dot{V}O_2$ responses were parallel to energy provision theories, such that $\dot{V}O_2$ levels increased rapidly after the first 10 seconds of exercise (Bogdanis *et al.*, 1996). A

potential explanation for these elevated $\dot{V}O_2$ levels may be cardiovascular adaptations such as plasma volume expansion. It is believed that improved maximal aerobic performance is directly related to larger blood volumes (Warburton *et al.*, 2004). An increase in the amount of blood traveling to the vascular bed could theoretically augment oxygen delivery to the working muscles. It has been concluded that even a single session of high-intensity sprint exercise can increase plasma volume levels by 10% (Gillen *et al.*, 1991). Warburton and colleagues discovered that SIT-induced hypervolemia accounts for approximately 47% of the changes in $\dot{V}O_2max$ (Warburton *et al.*, 2004).

1.4.3 Effects of Sprint Interval Training on Performance

The literature is saturated with evidence suggesting that the implementation of SIT has the potential to significantly increase an array of both anaerobic and aerobic performance measures (Burgomaster *et al.*, 2005; MacDougall *et al.*, 1998). In regards to cardiovascular health, it is valuable to reflect on the changes in aerobic performance. It is already well known that by increasing aerobic capacity, one may acquire several cardiovascular health benefits. If an individual could improve their aerobic capacity via alternative exercise modalities such as SIT, it would be promising that the options for therapeutic remedies may expand.

In the past, the lone indicator of aerobic performance was a subject's peak oxygen uptake ($\dot{V}O_{2peak}$). It has been well documented that a strict anaerobic training protocol can results in significant increases to maximal oxygen consumption (Dawson *et al.*, 1998; MacDougall *et al.*, 1998). The disadvantage to the measurement of $\dot{V}O_{2peak}$ is that it does not conclusively tell us about performance at sub-maximum levels. Direct measures of

sub-maximal aerobic capacity include exercise tests to exhaustion and time trial performance tests. Burgomaster and colleagues concluded that 2 weeks of SIT on a cycle ergometer resulted in a 100% increase in exercise time to exhaustion (Burgomaster *et al.*, 2005). While this topic still lacks congruency, it appears that training at an intensity that exceeds $\dot{V}O_{2max}$ may be a more important component than the volume of training to stimulate increases in oxidative potential.

The paradoxical finding by Dawson and colleagues that aerobic capacity increased following SIT with negligible changes in oxidative enzyme levels raises the possibility that factors other than those within the muscle may underlie aerobic performance adaptations (Dawson *et al.*, 1998). Therefore, additional cardiovascular mechanisms require further investigation to gain a better understanding as to what is regulating these performance and health adaptations.

1.4.4 Sprint Interval Training and Cardiovascular Health

To date, there is a limited amount of research that has evaluated the influences of SIT on cardiovascular health. It seems that if an array of therapeutic exercise modalities were available to the clinical population more individuals may be able to find an approach that suits their lifestyle and health status. A recent investigation from the University of Alberta studied the effectiveness of high-intensity interval training for the rehabilitation of patients with coronary artery disease (CAD). Warburton and colleagues have recognized that although continuous aerobic exercise is a valuable, non-pharmalogical intervention to improve cardiovascular health, this form of training may be suboptimal for patients with CAD. Following sixteen weeks of high-intensity interval

training, Warburton et al. found greater improvements in aerobic exercise capacity, compared to traditional rehabilitation exercise programs, despite improvements in $\dot{V}O_{2max}$ with both programs. Also, it was determined that this type of training stimulus can be conducted with minimal risk and is tolerated well by functional CAD patients (Warburton *et al.*, 2005). This research has important implications for overall health status of individuals suffering from cardiovascular disease.

1.5 SUMMARY AND STATEMENT OF INTENT

It is well known that arterial health declines with age even in healthy individuals with no overt cardiovascular disease. Accurate and reliable measurements of vascular status are important in clinical practice, and the identification of effective interventions is vital. Conceivably, young adults who are at high risk could be identified before clinical cardiovascular complications develop. Ultimately, the range of options for appropriate primary intervention could be expanded. It has been shown that exercise training can help protect the arterial system against its age-associate declines, as well as halt disease progression. Throughout the literature aerobic exercise is commended for its positive effects on cardiovascular health, thus suggesting potential benefits for other exercise modalities. Research exists which examines the impact of resistance training, but to date, research investigating the effects of SIT on structural and functional attributes of the cardiovascular system is non-existent. Therefore, the purpose of the current study was to evaluate central AC, brachial EF, and performance after two weeks of SIT in a population of young healthy males.

CHAPTER 2

THE IMPACT OF SPRINT INTERVAL TRAINING ON CENTRAL ARTERIAL COMPLIANCE AND BRACHIAL ENDOTHELIAL FUNCTION IN HEALTHY YOUNG MALES

2.1 INTRODUCTION

The human arterial system is a complex network of vessels that works continuously to transport blood from the heart to the peripheries of the body. The health of the arterial system has been suggested to predict the likelihood of cardiovascular disease, and thus evaluation of its structure and function are of valuable clinical relevance. Specifically, the ability of the an artery to accommodate an increase in volume, known as arterial compliance (AC), has been implicated in the progression of coronary artery disease (Arnett, et al, 1994; Hodes et al., 1995; Rowe, 1987). Vascular endothelial dysfunction, which describes the inability of an artery to dilate in response to chemical or physical stimuli such as shear stress, has also been observed in patients with coronary artery disease (Gokce et al., 2002) and chronic heart failure (Gokce et al., 2003).

Strategies capable of reversing impairments in arterial structure and function have important clinical applications (Moyna & Thompson, 2004; Dart and Kingwell, 2001). Exercise training has been acknowledged as an effective, non-pharmalogical therapy that can attenuate, and even reverse, the deterioration of the arterial system; however, the most successful exercise modality remains controversial. To date, the focus of this

research has revolved around aerobic-based interventions such as endurance exercise as well as recent investigations of resistance exercise training. Until now, research has failed to examine the influence of a rather novel exercise stimulus, sprint interval training (SIT), on the structure and function of the arterial system.

Therefore, the purpose of the current investigation was to evaluate the impact of two weeks of high intensity, short duration exercise training on central AC and brachial endothelial function (EF) in a population of young healthy males. It was hypothesized that SIT wouldl result in training induced improvements in central AC and brachial EF. In addition, we postulate that this training stimulus will enhance aerobic cycling performance.

METHODS

2.2.1 Participants

Nine healthy young males with an average age of 21.9 $\pm$ 0.5 years (mean $\pm$ SEM) participated in this study. All participants were recreationally active, and at the time of the study were not involved in any other exercise training regimes. Exclusion criteria included any clinical signs of heart disease and prior participation in any sprint related studies in the past year. The experimental protocol was approved by the McMaster University Ethics Board and all participants provided informed consent prior to the initiation of the study.

Table 2.1 Participant Characteristics (Mean + SEM)

Age (years)	Weight (kg)	Height (m)	BMI (kg/m^2)
21.9 $\pm$ 0.5	77.1 $\pm$ 1.7	183 $\pm$ 2	23.1 $\pm$ 0.7

2.2.2 Training Protocol

The experimental training protocol required participants to undergo 2 weeks of SIT 3 days a week for a total of 6 sessions. Participants came to McMaster University to complete their training sessions in the Exercise Metabolism Research Laboratory. Sessions were limited to weekdays (Monday to Friday), and on weekends participants were instructed to refrain from any type of high intensity exercise. Each session consisted of a series of repeated 30-second Wingate tests each separated by 4 minutes of recovery. Participants completed a total of 6 training sessions, with the number of Wingate tests required in each session rising from 4 in sessions 1 and 2 up to 6 in sessions 5 and 6. The exercise sessions were structured as follows:

Table 2.2 Training Progression

Training Seesions 1 & 2	Training Sessions 3 & 4	Training Sessions 5 & 6
4 Wingates	5 Wingates	6 Wingates

Participants who partook in any other types of recreational activities were asked to maintain a standard level of participation for the duration of the study period. All participants completed an identical training program. Prior to commencement of the PRE-training testing, participants were involved in a series of familiarization sessions in which testing procedures and training protocols were outlined. As well, an introduction to the equipment and instrumentation that would be used for various aspects of the investigation was conducted.

2.2.2.1 Wingate Tests

Each training session consisted of repeated 30 s "all out" exercise efforts performed on an electronically braked cycle ergometer (Lode BV, Excalibur Sport V20, The Netherlands) against a resistance equivalent to 0.075 kg/kg body mass. This type of stimulus is often referred to as a Wingate test. Participants received instructions to begin pedaling as fast as possible against the ergometer's inertial resistance. Approximately 2 seconds later, the automated software program interfaced with the ergometer (Wingate Software Version 1.11, Lode BV) applied the appropriate load. Participants received verbal encouragement to continue pedaling as fast as possible for 30 s. During the 4 minutes of recovery, subjects were encouraged to remain seated on the bike and cycle at a very low cadence (<30 rmp) in order to reduce venous pooling in the lower extremities, and syncope.

2.2.3 Testing Procedures and Data Collection

Assessments of AC, brachial EF, and cycling performance over 30 km were conducted prior to (PRE) and following (POST) training. Measures of AC and brachial EF were taken twice at each time point to assess reproducibility. Each assessment was separated by at least 24 hours and was conducted a minimum of 12 hours prior to the commencement of the training program and at least 24 hours following their last training session. Subjects were asked to abstain from caffeine for 24 hours prior to testing and to fast for the final 4 hours before a testing session.

2.2.3.1 Arterial Compliance

Central AC was measured in the left carotid artery of all subjects. Synchronized measurements of arterial blood pressure and arterial diameter were used to assess compliance in the carotid artery. Blood pressure values were obtained beat by beat from the left common carotid artery, while ultrasound images were obtained from the right common carotid artery proximal to the bifurcation of the internal and external carotid arteries. Throughout testing, continuous measurements of radial artery blood pressure were obtained using applanation tonometry (CBM-7000, Colin Medical Instruments, San Antonio, TX, USA) while at the carotid artery, a continuous blood pressure waveform was collected via a pen like device containing a high-fidelity transducer (SPT-301, Millar Instruments Inc, Houston, TX, USA) that was held perpendicular to the vessel.

Images of the carotid artery were obtained using B-Mode ultrasound (System Five, GE Medical Systems, Horten, The Netherlands). A 10 MHz linear array probe was positioned longitudinally along the vessel of interest. The carotid artery was imaged approximately two cm proximal to the bifurcation that divides the vessel into the external and internal carotid arteries. Two video clips of ten complete heart cycles were obtained at each testing time point and stored digitally for later off-line analysis. Electrocardiography was used to acquire a continuous heart rate signal for each subject.

All measurements were input into a data acquisition board (ML795, ADInstruments, Colorado Springs, CO, USA) for analogue to digital conversion and stored on a desktop computer (IBM Netvista x86 compatible processor, White Plains, NY, USA) using Chart 5 software (ADInstruments, Colorado Springs, CO, USA). Analogue signals were

sampled at a frequency of 200 Hz. Alignment of the digital images with the continuous blood pressure measurements was confirmed by an external trigger which was initiated whenever a digital image was stored on the ultrasound machine and was depicted on the Chart files as a 1V pulse.

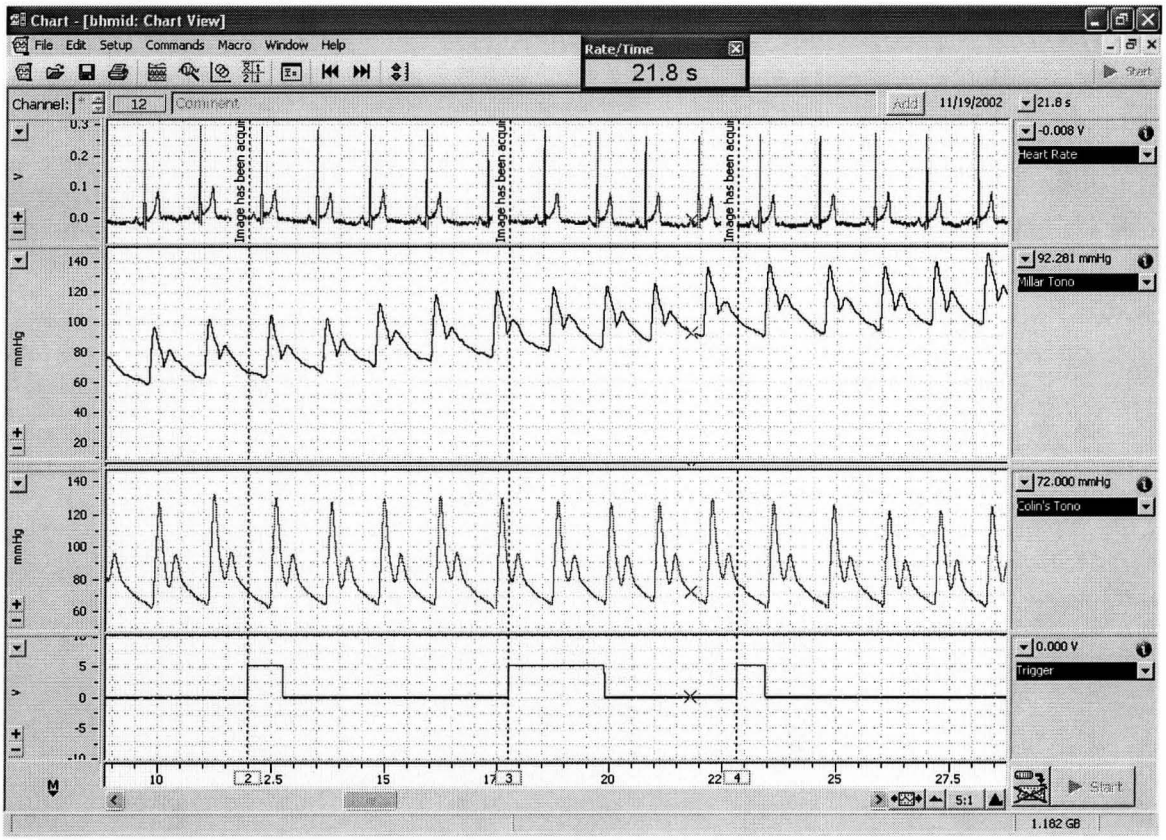

Figure 2.1 Screenshot of a typical Chart 5 data recording session. Arrows indicate the time points of ultrasound digital image storage recorded in channel 5. Channel 1 records continuous ECG tracing, channel 2 records continuous blood pressure at the carotid artery, and channel 3 records continuous blood pressure measured at the radial artery.

2.2.3.2 Brachial Endothelial Function

Brachial vascular EF was evaluated using the flow mediated dilation (FMD) technique. A cuff was applied to the forearm approximately 3 cm distal to the antecubital

fossa. The pneumatic cuff was inflated with a rapid inflation system (E20 and AG101, Hokanson, Bellevue, WA, USA) to a pressure of at least 200 mmHg to occlude blood supply to the brachial artery distal to the cuff. This occlusion was maintained for 5 minutes. A 10 MHz linear array ultrasound probe was positioned approximately 3-5 cm proximal to the antecubital fossa to obtain a longitudinal image of the brachial artery. Video recordings were obtained continuously from 15 seconds prior to release until 70 seconds post cuff deflation. As well, digital video clips were stored 15 seconds before release of the cuff and 70 seconds post release with B-mode ultrasound. In synchrony with the imaging of the brachial artery, mean blood velocity (MBV) was measured using the duplex function on the linear array probe.

Data was collected continuously from 15 seconds before the cuff was deflated until 15 seconds after release. All measurements of blood velocity were obtained at a pulse wave frequency of 4.0 MHz, using a velocity range gate of at least 500 cm/s, and a sample volume that enveloped the entire vessel beyond its walls. The insonation angle was recorded for subsequent analysis of the raw audio signal. The Doppler equation was used to determine red blood cell velocity:

Equation -1- $f_d = f_t - f_T = (2vf_t \cos 0)/c$

where:
f_d = frequency difference
f_t = transmitted frequency
f_T = received frequency
v = velocity
0 = angle of insonation
c = speed of sound in tissue

The raw blood velocity signal was output from the Doppler ultrasound into a transcranial Doppler system (Neurovision 500M TCD, Multigon Industries, Yonkers,

USA) which was used to process the raw signal. A fast Fourier transform was applied to the raw signal to determine the MBV. MBV values were corrected for the angle of insonation.

2.2.3.3 750 kJ Time Trial Performance Test

Each subject completed a 750 kJ (~30 km) time trial on an electronically braked cycle ergometer (LODE BV) prior to the training program and again upon conclusion. The resistance on the ergometer was dependent on the rider's cadence. A linearity factor of 0.6 was used. The ride was conducted in a private room which was free from outside distraction. Participants were equipped with a heart rate monitor (Polar A3, Lake Success, NY, USA). Subjects did not receive any visual or verbal feedback from which they could track their progress. The only acceptable form of entertainment allowed during the ride was a series of shuffled music to avoid subjects being able to identify their time course. As well as average heart rate obtained from the Polar system, the computer also gathered measures of mean running power and time to completion.

2.2.4 Data Analysis and Calculations

Digital images that were obtained via B-mode ultrasound were converted to DICOM (digital imaging and communication in medicine) compressed JPEG image files for further analysis of mean arterial diameters using automated software (AMS II, Chalmers University of Technology, Goteborg, Sweden). Data files containing blood pressure and MBV data were analyzed using Chart 5 software.

2.2.4.1 Arterial Diameters

Resting carotid and brachial arterial DICOM flies were loaded on a Pentium 4.6 personal computer and visualized using AMS II software. A specific reproducible portion of the image was identified for automated analysis by the investigator. The region of interest (ROI) for the carotid artery was identified ~ 2-3 cm proximal to the bifurcation of the carotid artery into internal and external branches, while the ROI for the brachial artery was identified by a visual landmark on the artery that could be tracked through the series of images in order to keep the ROI consistent between images. This technique ensured that measurements were taken at the same location on each artery of each individual subject. A rectangular frame was fit around the ROI to encompass both the near and far vessel wall. Once manually situated at the appropriate location, the automated software was able to track changes in the size of the artery as well as identify the mean diameter size from leading edge to leading edge throughout subsequent frames of the digital image. Figure 2.2 illustrates the diameter vessel wall segments used for measurement of mean arterial diameters:

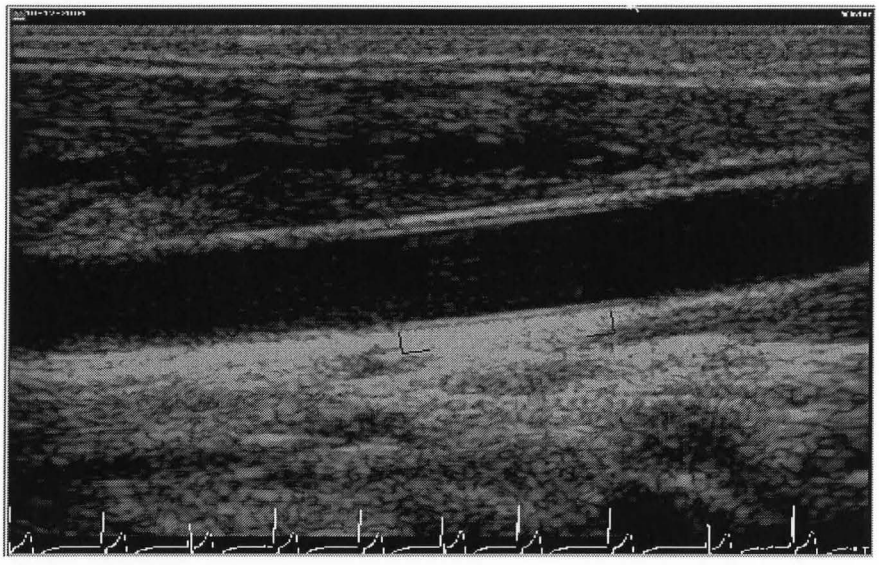

Figure 2.2 A diagram of the AMS II diameter analysis software. During automated detection, a rectangular box encompass the region of interest determined by the investigator and dotted lines are the automatically detected vessel walls. These markings are not visible in this depiction.

The automated software takes a minimum of one hundred measurements of the artery diameter in order to obtain a mean value for each frame of the digital clip. In order to obtain an overall measure of mean arterial diameter for one heart cycle, an average of the means for each frame, not including the first frame (because it is at the same time point as the last frame), is calculated. Furthermore, mean arterial diameters calculated for each heart cycle were then averaged to obtain mean arterial diameters for the carotid and brachial arteries at each time point of interest. In a few cases, the arterial images obtained were not sufficient to assess. However, because we had taken two images on two subsequent days, we had ample data to report both PRE and POST values for each participant.

Delta diameters, or the change in arterial diameter measurements, were acquired

from two separate heart cycle video clips. The automated edge-detection software program was able to identify the mean arterial diameter for each frame of each heart cycle as previously described. The maximal and minimal arterial diameters were used to determine changes in arterial diameter according to the following formula:

Equation -2- Δ Diameter = Maximal Diameter – Minimum Diameter

2.2.4.2 Arterial Tonometry Pulse Pressure

For the calculations of AC a measure of pulse pressure (PP) in the vessel of interest is required. These values were obtained using a pen like pressure assessment device (Millar) as previously described. Due to the sensitivity of this tool, the measurements required adjustments that were based on several assumptions. The first assumption is that both arterial diastolic blood pressure (DBP) and mean arterial pressure are the same or similar in all conduit arteries of the human body when in the supine position (Nicholas et al., 1998). However, systolic blood pressure (SBP) is not the same in all conduit arteries, and therefore, we must correct the signal obtained in the vessel of interest. The mean and minimum values obtained with the tonometer in the carotid artery were equated to the mean arterial and diastolic blood pressure values that were obtained via the radial tonometer (Colin). Subsequently, the maximum value obtained in the carotid artery waveform was used as an extrapolation point from which systolic blood pressure at the carotid artery could be obtained. Lastly, because DBP is assumed to be similar in all conduit arteries, this value was subtracted from the extrapolated SBP to obtain a measurement of PP in the carotid artery (Kelly et al., 1989).

2.2.4.3 Cross Sectional Compliance

Two measurements of carotid cross-sectional AC were calculated using an equation containing the previously obtained values of PP and delta diameters, and averaged to quantify cross-sectional compliance for each time point of interest. Cross-sectional compliance was calculated as follows:

Equation -3-

$$CSC = \frac{\Delta \text{ Area}}{PP} = \frac{\pi r^2_{max} - \pi r^2 min}{PP} = \frac{\pi(d_{max}/2)^2 - \pi(d_{min}/2)^2}{PP}$$

where,
CSC = cross-sectional compliance
PP = pulse pressure
r = radius of the artery
d = diameter of the artery
max = maximal value
min = minimum value

2.2.4.4 Brachial Flow Mediated Dilation

Maximal dilation of the brachial artery in the 53 to 70 s following deflation of the cuff was determined by analyzing digital images using the automated edge-detection software. Similar to the protocol previously described, a ROI was visually identified on each artery based on the resting images that had already been analyzed. Measurements of mean end-diastolic arterial diameter were taken for all beats the made up the ~16.7 s digital clip. The end-diastolic measurements were then averaged and used to calculate relative flow mediated dilation (FMD) of the brachial artery. This value was calculated as follows:

Equation -4- $$FMD = \frac{d_{\text{end diastolic FMD}} - d_{\text{end diastolic rest}}}{d_{\text{end diastolic rest}}} \times 100\%$$

where, d = diameter
FMD = flow mediated dilation

2.2.4.5 Post Occlusion Blood Flow and Shear Rate

Two measures of blood flow and shear rate were determined for each subject at each time point. A 10-second average as well as peak blood flow and wall shear rate was calculated using the blood velocity values that were recorded following brachial occlusion. Beat by beat average blood velocity was determined as the area under the curve from R to R points on the simultaneously recorded ECG channel. A 10-second average post occlusion blood velocity was defined as the average blood velocity for ten seconds following cuff deflation excluding the first beat. Peak MBV was defined as the highest single beat average blood velocity after exclusion of the first beat following cuff deflation. Blood flow was calculated as a product of blood velocity and vessel area as described below.

Equation -5- $$FBF_{10\text{-s avg}} = \pi(d/2)^2 \times BV_{10\text{-s avg}} \times 60\ s$$

where, FBF = forearm blood flow
d = resting brachial diameter
BV = blood velocity

$$FBF_{peak} = \pi(d/2)^2 \times BV_{peak} \times 60\ s$$

where, FBF = forearm blood flow
d = resting brachial diameter
BV = blood velocity

Wall shear rates were calculated again as both a 10-second average as well as peak based on the blood velocity measurements described previously. The following equations were used:

Equation -6- $$Shear_{peak} = \frac{4 \times BV_{peak}}{d_{mean}}$$

where, BV = blood velocity
d_{mean} = average heart cycle diameter

$$Shear_{10\text{-}s} = \frac{4 \times BV_{10\text{-}s}}{d_{mean}}$$

where, BV = blood velocity
d_{mean} = average heart cycle diameter

2.2.4.6 Normalized Endothelial Dependent FMD

Endothelial dependent FMD previously calculated (eq.5), was normalized for the average shear rate experienced during the post occlusion reactive hyperaemic response. The following equation was used to calculate a normalized FMD of the brachial artery:

Equation -7- $$\text{Normalized FMD} = \frac{FMD}{Shear_{10\text{-}s}}$$

where, FMD = flow mediated dilation

2.2.5 Statistics

All variables were evaluated using a repeated measures analysis of variance (ANOVA) with two time points of interest (PRE and POST). Significance for all analysis was set at $P \leq 0.05$. All values are presented as mean $\pm$ standard error of the mean (SEM).

2.3 RESULTS

2.3.1 Resting Arterial Diameter

Resting mean brachial artery diameters did not change (P>0.05) following two weeks of SIT (Figure 2.3b. Table 2.3); however, the resting mean carotid artery diameter increased significantly (P< 0.01) after the training protocol (Figure 2.3a, Table 2.3).

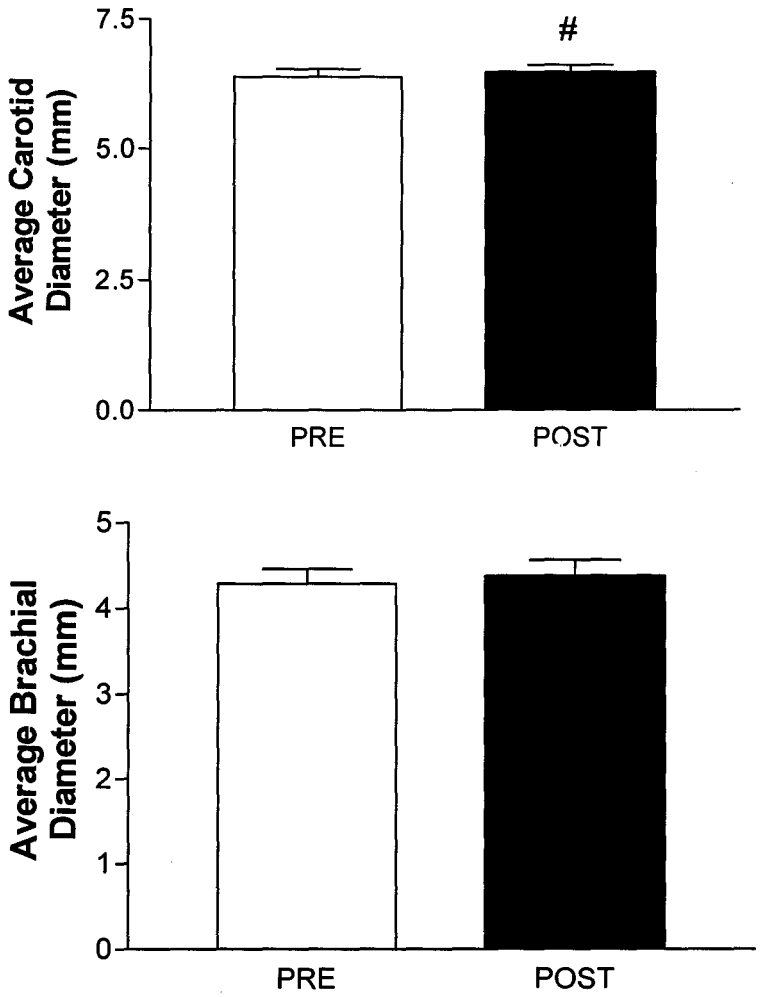

Figure 2.3 Mean arterial diameters at PRE and POST training time points. (a) illustrates mean carotid diameter while (b) illustrates mean brachial diameter. # denotes a significant difference from PRE values (P<0.01).

Table 2.3 Resting Mean Arterial Diameters PRE and POST

	PRE	POST
Carotid	6.4 ± 0.2	6.5 ± 0.1 #
Brachial	4.3 ± 0.2	4.4 ± 0.2

All values are mean ± SEM in mm, # denotes significant difference from Pre values ($P<0.05$)

2.3.2 Resting Pulse Pressure

PP measured at the carotid artery did not show a significant change ($P>0.05$) following training (Figure 2.4, Table 2.4).

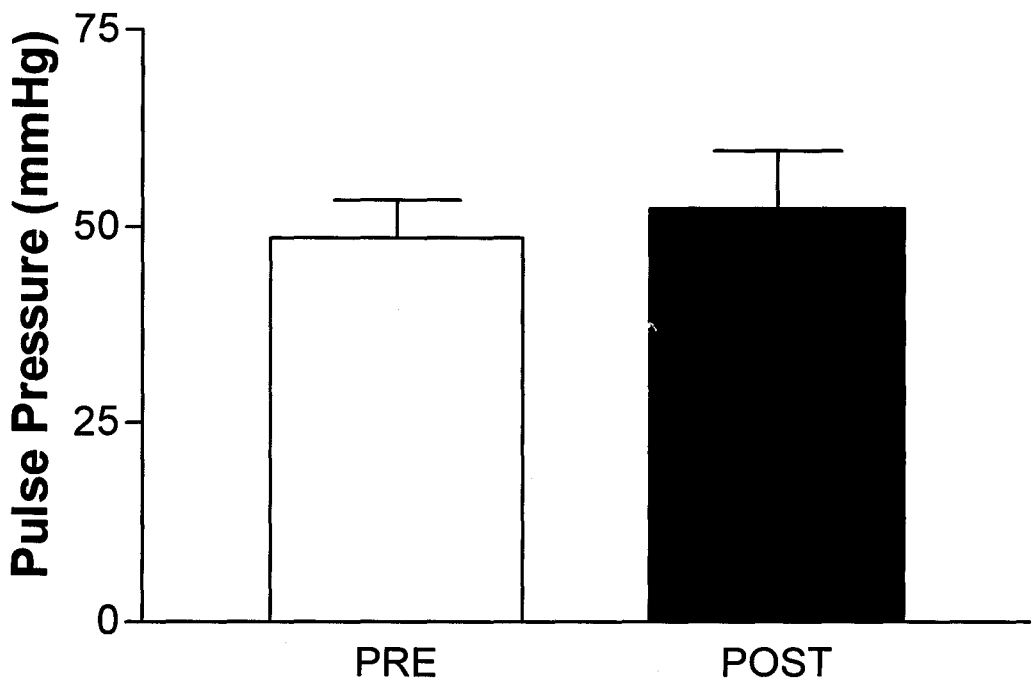

Figure 2.4 Resting PP measurements at the carotid artery at PRE and POST training time points. No significant difference was noted following training.

Table 2.4 Resting Pulse Pressure PRE and POST

	PRE	POST
Carotid	48.6 ± 4.9	52.4 ± 7.4

All values are mean ± SEM in mmHg

2.3.3 Cross-Sectional Arterial Compliance

Cross-sectional compliance measured at the carotid artery did not change significantly ($P>0.05$) in response to the training protocol (Figure 2.5, Table 2.5).

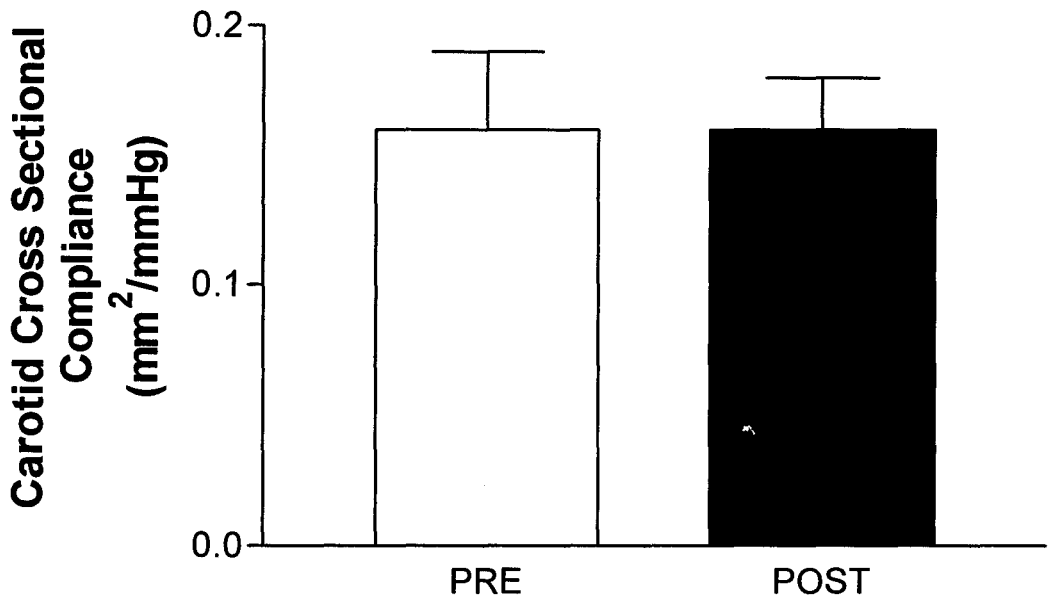

Figure 2.5 Cross-sectional compliance PRE and POST training time points. No significant differences were found following training.

Table 2.5 Resting Cross-Sectional Compliance PRE and POST

	PRE	POST
CSC (mm2/Hg)	0.16 ± 0.03	0.16 ± 0.02

All values are mean ± SEM in mm2/mmHg

2.3.4 Brachial FMD Responses

Peak (Figure 2.6a, Table 2.6) brachial artery blood flow following 4.5 minutes of occlusion did not change ($P>0.05$) as a result of two weeks of SIT; however, average brachial blood following cuff release were significantly ($P\leq0.05$) elevated POST training (Figure 2.6b, Table 2.6).

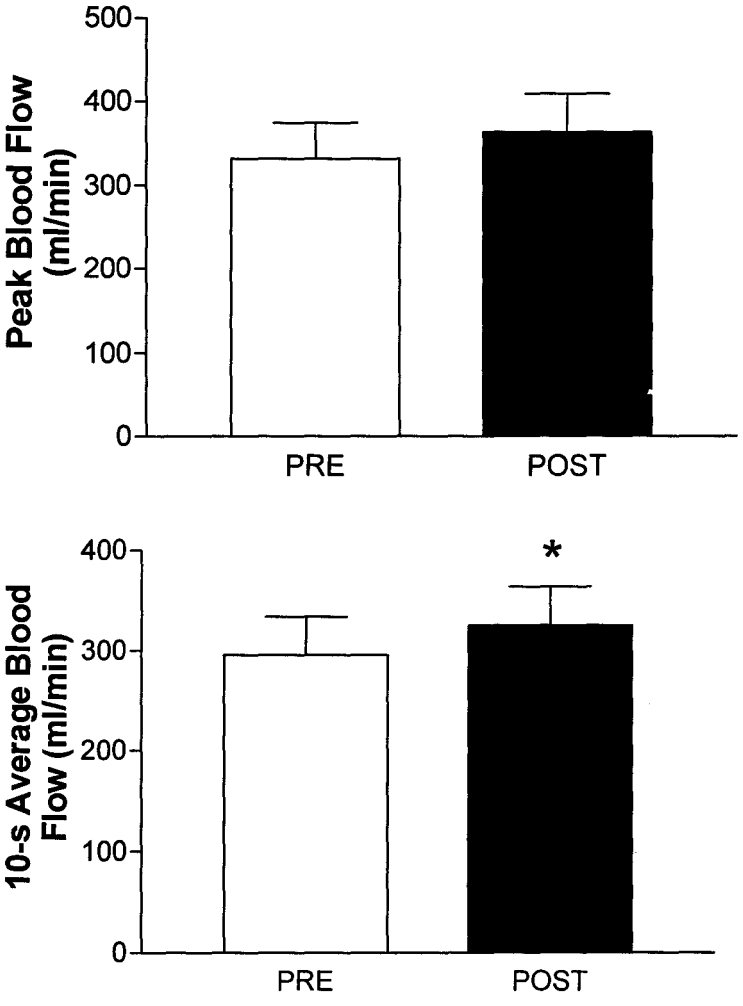

Figure 2.6 Post occlusion blood flow responses in the brachial artery PRE and POST training time points. (a) illustrates peak blood flow changes following training while (b) illustrates 10-s mean blood flow changes following training. # denotes a significant difference from PRE values ($P<0.05$).

Post occlusion 10-average (Figure 2.7a, Table 2.6) and peak (Figure 2.7b, Table 2.7) arterial wall shear rates in response to 4.5 minutes of forearm occlusion were not different (P>0.05) PRE versus POST. Relative FMD (Figure 2.8a, Table 2.6) of the brachial artery was not altered (P>0.05) by training. In addition, when the relative FMD was normalized for mean wall shear rate (Figure 2.8b, Table 2.6), no significant differences (P>0.05) were noted.

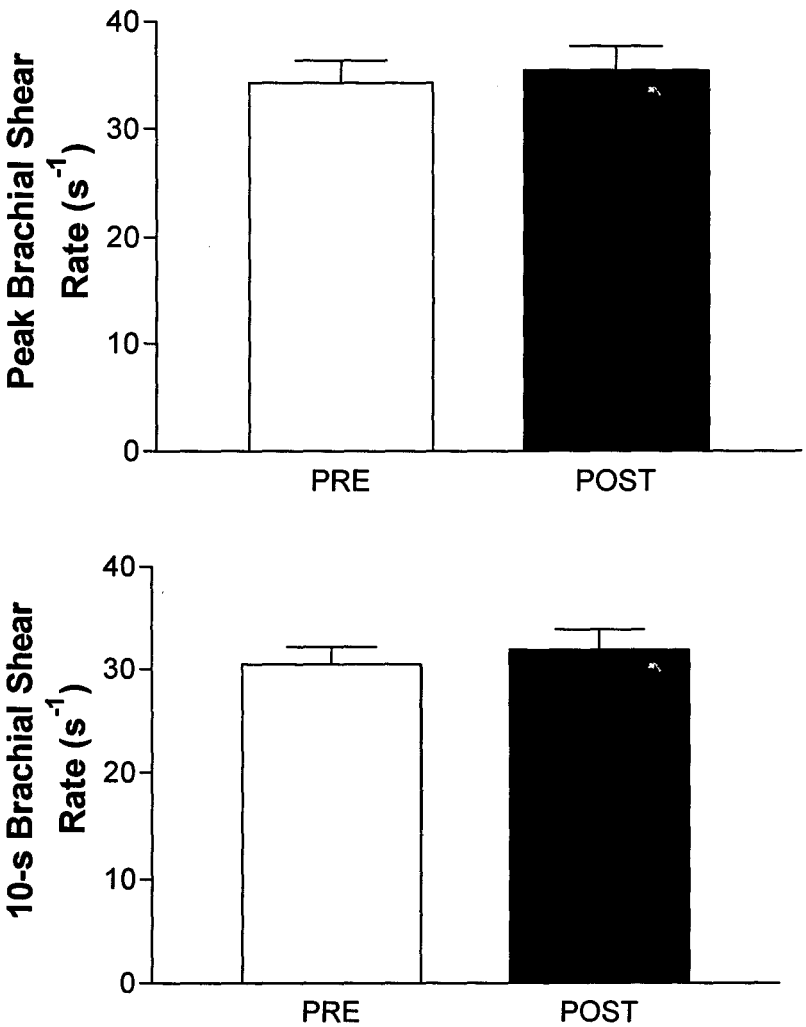

Figure 2.7 Brachial blood flow responses following 4.5 minutes of forearm occlusion PRE and POST training time points. (a) illustrates 10-s average blood flow while (b) illustrates peak one beat blood flow. No significant changes were found.

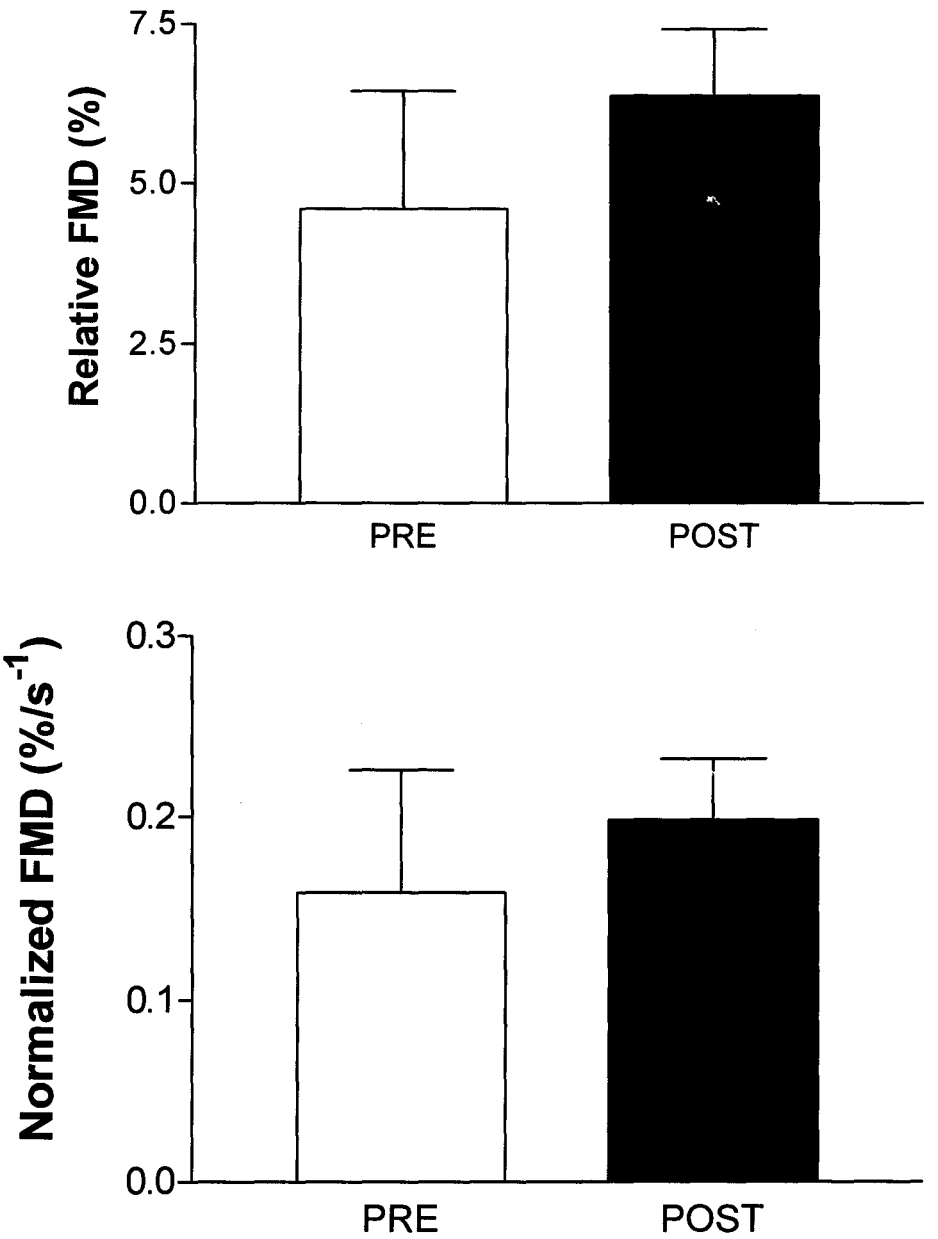

Figure 2.8 Relative and normalized brachial FMD at PRE and POST training time points. (a) illustrates the relative change in brachial artery diameter while (b) illustrates the relative change in brachial artery diameter when normalized for 10-s mean wall shear rates. No significant differences were noted following training.

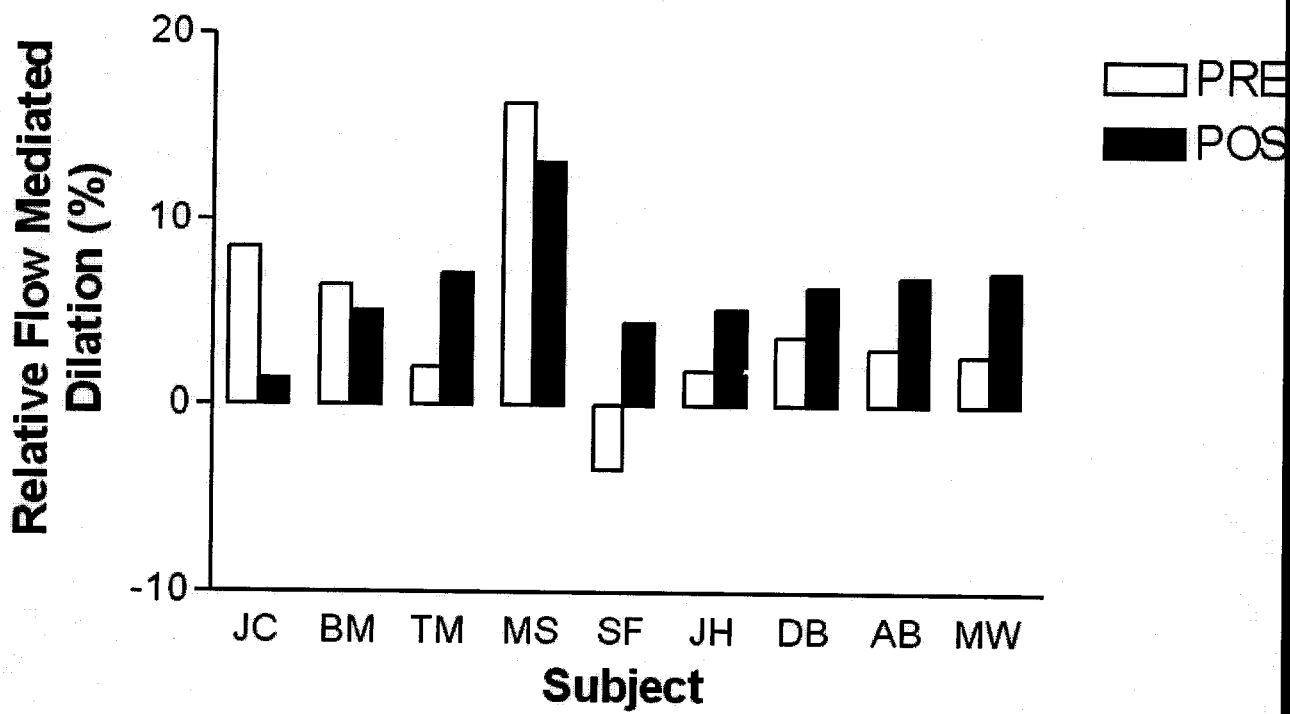

Figure 2.9: Relative flow mediated dilation changes at the brachial artery PRE and POST for each individual participant.

Table 2.6 Brachial Flow Mediated Dilation Responses PRE and POST

	PRE	POST
Resting Brachial Blood Flow (ml/min)	38.7 ± 6.5	39.2 ± 4.5
Peak Brachial Blood Flow (ml/min)	332.0 ± 42.3	363.6 ± 45.8
10-s Average Blood Flow (ml/min)	295.9± 37.4	324.8 ± 38.8 #
Peak Shear Rate (s-1)	34.3 ± 2.1	35.5 ± 2.3
10-s Average Shear Rate (s-1)	30.5 ± 1.7	31.9 ± 2.0
Relative Flow Mediated Dilation (%)	4.6 ± 1.8	6.4 ± 1.05
Normailized Flow Mediated Dilation (%/s-1)	0.16 ± 0.07	0.20 ± 0.03

All values are mean ± SEM, # denotes significant difference from Pre values ($P<0.05$)

2.3.5 Time Trial Performance

The time required to complete a 750 kJ time trial on a cycle ergometer decreased significantly ($P \leq 0.01$) following the training stimulus (Figure 2.10, Table 2.7).

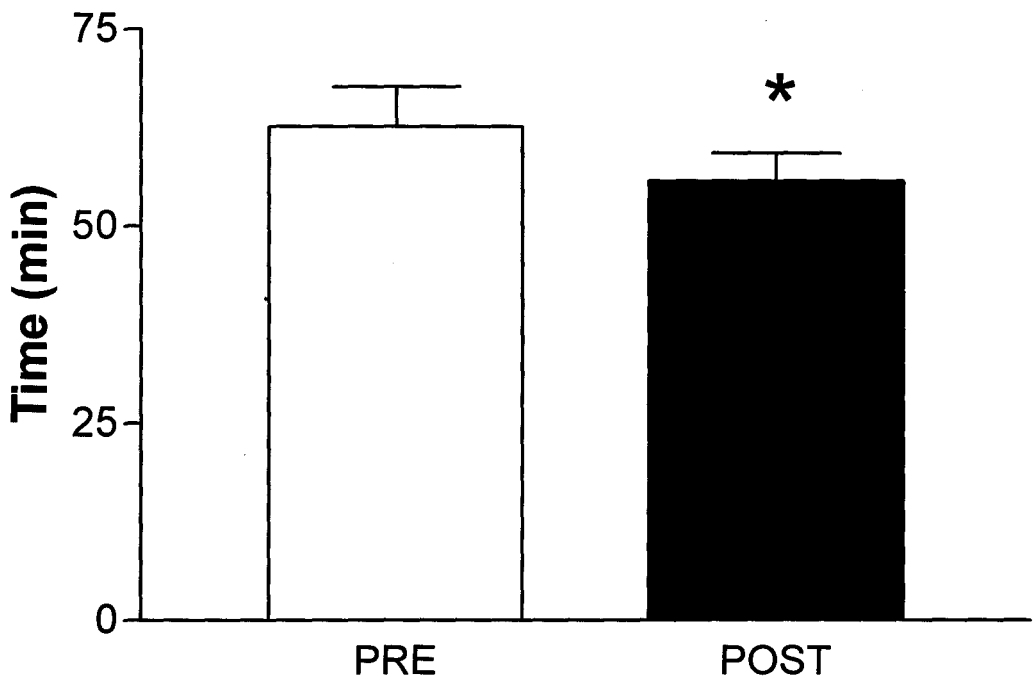

Figure 2.10 Performance on a 750 kJ time trial at PRE and POST training time points. Performance is depicted as time to completion in minutes. # a significant difference from PRE values ($P<0.01$).

Table 2.7 Peformance Time for 750 kJ Time Trial PRE and POST

	PRE	POST
Time to Completion	62.8 ± 4.9	55.9 ± 3.5 #

All values are mean ± SEM in minutes, # denotes significant difference from Pre values ($P<0.05$)

2.4 DISCUSSION

The purpose of this investigation was to evaluate the effects of two weeks of SIT on central AC, brachial EF, and cycling performance over 750 kJ in young healthy men. The most relevant findings were that AC and EF did not change in response to the training stimulus despite a significant improvement in endurance performance. However, significant increases in resting carotid diameter and post hyperemic brachial blood flow were noted POST training. As well, a strong trend towards improvements in brachial FMD was observed. This study was the first to examine the impact of SIT on arterial structure and function and thus comparative data does not exist.

2.4.1 Sprint Interval Training and Resting Arterial Diameter

These results show that short term (2 wk) SIT in healthy young men can induce an increase in resting carotid diameter. These unique alterations may have been mediated through persistent chemical stimuli that affect arterial tone and/or structural changes to the vessel itself.

Structural remodeling of a vessel is influenced by a number of mechanisms. Although our data does not permit insight into definitive answers, it is likely that structural changes are regulated by the transient shear stresses during exercise. With an acute bout of sprint-exercise, blood flow is increased to maintain adequate supply to the working organs. This augmented blood flow response causes increased levels of shear stress on the vascular endothelium which results in the release of NO and subsequent diffusion to underlying tissues. NO can also result in arterial smooth muscle relaxation, and ultimately remodeling (Tronc *et al.*, 1996). Lloyd and colleagues have determined

the importance of NO in this process by inhibiting eNOS via L-NAME and failing to observe changes in arterial remodeling in response to high rates of induced blood flow (Lloyd *et al.*, 2001).

Increased arterial diameters may also be the product of a chronic reduction in sympathetic activation and/or changes in alternative regulators of vasoconstriction or dilation. We have reason to believe this mechanism is less likely as we did not note any significant changes in pulse pressure, suggesting that baroreceptors did not alter their firing patterns.

Evidence to support our finding of increased resting carotid artery diameters with exercise training does not exist, thus future studies of greater duration and equally intense protocols are needed to support these findings.

2.4.2 Sprint Interval Training and Central Arterial Compliance

We have demonstrated that two weeks of SIT does not affect central AC in young healthy men. Despite a significant increase in carotid artery diameter and improvements in endurance performance, SIT failed to improve resting large-artery mechanical properties or blood pressure.

Because classical endurance exercise training programs have been shown to improve both blood pressure and arterial stiffness in healthy young subjects, it seems likely that modifications in arterial stiffness are dependent on the intensity and duration of the exercise (Tanaka *et al.*, 2000). Previous literature examining the impact of resistance training also found a lack of improvement in vascular compliance (Bertovic *et al.*, 1999; Rakobowchuk *et al.*, 2005b). It is important to note, however, that unlike

observations made by Miyachi and colleagues (Miyachi *et al.*, 2004; Miyachi *et al.*, 2003) with regards to resistance exercise training, SIT does not appear to be associated with any negative influences on the vascular system. There are numerous factors which likely contribute to our null findings including the duration of the exercise training stimulus, the artery of choice for obtaining these measures, and the time course of experimental procedures.

AC is mainly determined by the intrinsic elastic properties of an artery (Tanaka *et al.*, 2000). The elements of the arterial wall that determine its compliance are the composition of collagen and elastin (structural) and vasoconstrictor tone induced by smooth muscle cells (functional). Because changes in the structural properties of the arterial wall are believed to occur over many years, it is likely that just two weeks of exercise training is not long enough to incur structural adaptations. Animal studies have shown that structural alterations account for elevated compliance when the training programs are extended over several months (Koutsis *et al.*, 1995; Matsuda *et al.*, 1989).

The observation of increased resting carotid arterial diameter seems to be in contrast to the lack of change in carotid arterial compliance. If smooth muscle relaxation plays a role in the baseline diameter you may also expect it to influence the elasticity of the artery. The smooth muscle layer of the carotid artery, is however, relatively thin and any impact of changes in its tone may be apparent at rest, yet absent when the distending pressure is taken into account, as is done when calculating AC.

As mentioned previously, this is the first investigation to examine the effects of SIT on AC and EF. Prior to this study, an investigation by Laughlin and colleagues

(Laughlin *et al.*, 2004) reported adaptations within rat vasculature following SIT that were time-dependent. Previous animal work demonstrates changes in the vasodilatory capacity of the arteries and arterioles feeding the gastrocnemius muscle in response to SIT; however, these alterations were noted in only in select regions and at differing times (Laughlin *et al.*, 2004). From this, the authors suggest that the time course of vascular adaptation may vary between different arterioles. Therefore, perhaps if we had taken measures of AC at alternative sites, we may have observed changes that went unnoticed via measurements at the carotid artery.

Measurements of arterial diameters and compliance were taken in the carotid artery for three specific reasons. First, we were evaluating the training effects at the central level and therefore the carotid artery was appropriate. Second, due to its elastic nature, the carotid artery is thought to be more likely to adapt to an exercise training stimulus and show signs of improvement in structure and function than alternative conduit arteries. Third, imaging of the carotid artery can be done with ease and is generally less variable than at other arteries (Cameron *et al.*, 2002). However, it could be postulated that if we had obtained measurements in the artery of the exercising limb (femoral artery) we may have seen improvements due to the high blood flow responses to the working muscles.

The time course of experimental procedures may have also influenced the AC outcome values. The constituents that derive AC, particularly blood pressure, are highly impacted by any type of moderate to high intensity exercise. Unfortunately, during this investigation we had a few (n=3) individuals who completed their last SIT session within

24 hours of their first POST training testing session. This occurred because of conflicting testing schedules with other researchers. It could be speculated that the high intensity sprint exercise piggy backed with the POST testing measurement sessions influenced the blood pressure response, and ultimately the AC of select participants.

2.4.3 Sprint Interval Training and Endothelial Function

Changes in brachial FMD did not occur with SIT; however, post-occlusion average blood flow responses were significantly increased as a result of the training program. Shear rates were unchanged with SIT when represented as either a peak or 10-s mean. Taken together, these findings indicate a lack of changes in FMD with SIT of two weeks in duration. Therefore, brachial artery EF was not significantly altered by SIT; however, an apparent trend towards improvement in FMD was noted.

Although EF did not change within the brachial (conduit) artery, endothelial-dependent function may have been improved at the level of the resistance vessels, reflected as an increase in post-occlusion blood flow with training. The augmented hyperemic blood flow response to 4.5 minutes of forearm occlusion may also be related to improved resistance vessel function, or resistance vessel proliferation and muscle hypertrophy. The most likely explanation relates to enhanced resistance vessel function; however, because measurements were obtained from a conduit feed artery, one cannot infer what is happening at the destination vessels downstream.

Increased resistance vessel function has been noted previously in studies evaluating the combined effects of aerobic and resistance training (Maiorana *et al.*, 2001a; Maiorana *et al.*, 2000) While these results are not conclusive, clinically they are

intriguing as blood flow is controlled at the level of the resistance vessels. Several disease states including peripheral artery disease (Bauer *et al.*, 2004) and congestive heart failure (Belardinelli *et al.*, 1997) are characterized by limited blood flow in response to physiological stimuli; thus, SIT may be a valuable therapeutic tool to enhance resistance vessel function. It should also be noted that changes in resistance vessel function may not be apparent in the conduit arteries supplying the vascular beds displaying these enhancements. Therefore, it is likely that underlying improvements are occurring, just not large enough to be detected in the brachial artery. In addition, it is likely that the coronary arteries may have improved their dilation capacity due to the chronic shear stress experienced during SIT, as compared to the brachial artery which likely did not experience an equivalent chronic stimulus. Anderson and colleagues suggest that while the relationship between the brachial and coronary vasodilatory response is significant, the degree of correlation is not strong enough to predict the magnitude of response in one artery from the magnitude of response in the other ($r = 0.36$) (Anderson *et al.*, 1995). This implies that in fact there may have been improvements within the coronary circulation that were not evident in measurements obtained from the brachial artery.

An alternative explanation regarding the lack of improvement in brachial artery vascular EF may be that the population studied exhibited healthy, intact vascular endothelium prior to the commencement of training. It is difficult to stimulate enhancements in a system that is fully functional; and therefore, improvements may not have been possible. Other exercise training studies have noted this possibility (Maiorana *et al.*, 2001b).

Although it was not noted at the site of interest, we could postulate that the total downstream cross-sectional area increased through greater resistance vessel dilation in response to blood flow occlusion. Alternatively, capillary/arteriolar proliferation may have lead to increased post occlusion blood flow. Although it has not been investigated in regards to SIT, increased capillary density has been observed following resistance training (Green *et al.*, 1999). Capillary density may also coincide with muscle hypertrophy in the forearm. An increase in skeletal muscle mass distal to the site of occlusion would generate a greater metabolic stimulus for amplified blood flow upon release of the cuff.

It remains to be determined whether further improvements in FMD could be achieved with a longer SIT protocol, or in a population of older subjects. As well, further research is required to determine if SIT can improve vascular function in association with other cardiovascular risk factors.

2.4.4 Sprint Interval Training and Performance

One of the most relevant findings to be extracted from this research is that two weeks of SIT can significantly improve endurance exercise capacity. These results are consistent with previous research that has also demonstrated increased time to exhaustion in intense aerobic exercise following 6 sessions of sprint training (Burgomaster *et al.*, 2005). While our goal was to identify cardiovascular adaptations to SIT, we appreciate that many other factors influence changes in performance following training. Due to our null hypothesis, we may conclude that aerobic performance enhancements may not be the result of improvements at the level of arterial structure or function. Alternative

possibilities, though not measured in our investigation, include improved oxidative metabolism, increased plasma and stroke volume, and an increased concentration of type II muscle fibres.

Increases in oxidative enzymes such as citrate synthase contribute to improved oxidative potential of the muscle. Increases in citrate synthase levels upwards of 30% have been recorded following two weeks of SIT (Burgomaster *et al.*, 2005), and are consistent with other studies implementing similar sprint exercise protocols (MacDougall *et al.*, 1998; Parra *et al.*, 2000). Although the exact mechanism by which these enzymatic increases occur is not precisely defined, it is likely in response to the increased demand for ATP provision. Oxidative pathways become an important source of fuel (ATP) during repeated sprint efforts (Bogdanis *et al.*, 1996; Trump *et al.*, 1996). As well, the short recovery time (4 minutes) between exercise bouts may not be long enough to allow complete recovery of anaerobic energy stores, thus reliance on oxidative pathways becomes greater.

While it has been speculated that changes in arterial structure and function are not regulators of aerobic performance, other cardio-respiratory factors may be influential. Increases in plasma volume as well as stroke volume have been recognized following interval training programs (Gillen *et al.*, 1991; Warburton *ct al.*, 2004). Gillen et al. reported a 10% increase in plasma volume expansion in a single session of sprint training (Gillen *et al.*, 1991). Recently, Warburton and colleagues supported these results by identifying significant increases in plasma volume and stroke volume following twelve weeks of interval training (Warburton *et al.*, 2004). Although these mechanisms seem

acceptable, no empirical evidence has been produced to suggest these factors directly result in improved aerobic performance.

It is also conceivable that two weeks of high intensity cycle training will lead to increases in leg strength that carries over to improve time trial performance. Researchers believe that as the result of SIT, individuals have a greater percentage of type IIA muscle fibres and type II fibre area (Dawson *et al.*, 1998). Type IIA fibres are associated with high levels of both glycolytic and oxidative enzyme activity, and therefore, this adaptation is parallel with an increase in citrate synthase activity.

2.4.5 Limitations and Future Direction

The current investigation addresses many questions regarding the effects of SIT and vascular characteristics; however, limitations within this study do exist. These shortcomings include measurement techniques and technical limitations, the study population of choice, and the time course of experimental procedures.

Measurement techniques used within this examination can be susceptible to variability and investigator error. While care was taken to obtain the most accurate and reliable measures possible, there is always the chance of technical error. The use of ultrasound to acquire visual images of arteries has been shown to be very reliable and reproducible, however, there is the possibility for investigator error. It is essential that a steady image of the artery is maintained throughout the study, and that the relative positions and anatomic landmarks are noted for every measurement.

The FMD technique to assess EF is also vulnerable to criticisms. Ultrasonographic assessment of the brachial artery is uniquely challenging and must be performed by a well trained individual. Several technical aspects affect the precision of this technique such as comfort of the examiner, transducer movement, and anatomical landmarking. Another drawback is the lack of control that exists over shear stress that is placed on the endothelium on a day-to-day basis. Recently it has been suggested that development of a technique that allows a constant shear stimulus should be implemented (Pyke *et al.*, 2004). As well, measurements of forearm post-ischemia blood flow should be represented relative to tissue volume. This may give a better indication of the extent of muscle hypertrophy as a result of exercise training, or whether the increases in blood flow response following ischemia are actually the result of improved resistance vessel dilation.

The sample cohort used may limit our investigation. Because this was novel research in the area of SIT, it was necessary to recruit healthy, able bodied participants. The risks involved in using older or clinically unwell subjects have not been determined. Unfortunately, inherent with this population, are strong, intact arterial systems. Common sense reminds us that it is difficult to fix something that is not broken, or improve a system that is fully functional. As a result, even an intense training protocol may not lend to enhancements of these individual's cardiovascular systems. In addition, extrapolation of the results to other cohorts such as the elderly, females, or patients with cardiovascular disease is limited at best. Based on our findings that SIT does not appear to have any adverse effects on vascular structure and function, it seems appropriate to expand the

participant pool and consider investigations of SIT in middle aged and older individuals, as well as those at high risk for cardiovascular events.

The time course of experimental procedures was variable between subjects within this investigation. Due to organizational disruptions, as well as participant's lack of availability, four of the nine participants were required to perform their POST 750 kJ time trial prior to undergoing their POST assessments of resting AC and EF, while the rest of the group completed the performance test following the completion of all other testing procedures. As well, two participants underwent their POST testing within 24 hours of their last training session. Unfortunately, the lack of research on SIT limits our knowledge regarding the acute responses of the cardiovascular system to sprint exercise. If enhancements in AC and/or EF had been noted, we may have speculated that we were actually seeing the lingering effects of a single bout of sprint exercise, or of a 750 kJ performance test, rather than a true training effect. Follow up studies should ensure a consistent and time-sensitive schedule incorporating any known facts about the time course of cardiovascular responses to exercise training. As well, a short and simple pilot study that should be conducted in the near future is to examine the vascular response to a single bout of SIT and determine how long the effects, if any, remain.

From this preliminary research we can only speculate what may be limiting vascular enhancements, and generate future research ideas to address the unanswered questions. The novelty of SIT research provides an abundance of intriguing future research investigations. Simple manipulations of the training protocol, study population,

and time course of events could prove to be very valuable in demonstrating that SIT can be beneficial to the human cardiovascular system.

2.5 CONCLUSIONS

In conclusion, these findings reveal that two weeks of SIT does not affect central AC or brachial EF in young healthy men, despite improvements in aerobic exercise capacity. Also observed were increases in carotid artery diameter and post occlusion blood flow. While not conclusive, we may speculate that SIT does elicit a positive effect to the arterial system due to significant improvements noted within this system. It is important to emphasize that SIT was not interpreted as a negative mediator, and that there is no evidence to suggest that SIT should not be used as part of a regular exercise program. Future studies are needed to gain additional insight into the time course of cardiovascular responses to SIT, as well as the impact of SIT on a wide range of populations.

Reference List

Abergel, E., Linhart, A., Chatellier, G., Gariepy, J., Ducardonnet, A., Diebold, B., & Menard, J. (1998). Vascular and cardiac remodeling in world class professional cyclists. *Am.Heart J.* 136, 818-823.

Abernethy, P. J., Thayer, R., & Taylor, A. W. (1990). Acute and chronic responses of skeletal muscle to endurance and sprint exercise. A review. *Sports Med.* 10, 365-389.

Adji, A. & O'Rourke, M. F. (2004). Determination of central aortic systolic and pulse pressure from the radial artery pressure waveform. *Blood Press Monit.* 9, 115-121.

Anderson, T. J., Uehata, A., Gerhard, M. D., Meredith, I. T., Knab, S., Delagrange, D., Lieberman, E. H., Ganz, P., Creager, M. A., Yeung, A. C., & . (1995). Close relation of endothelial function in the human coronary and peripheral circulations. *J.Am.Coll.Cardiol.* 26, 1235-1241.

Arnett, D. K., Evans, G. W., & Riley, W. A. (1994). Arterial stiffness: a new cardiovascular risk factor? *Am.J.Epidemiol.* 140, 669-682.

Baguet, J. P., Kingwell, B. A., Dart, A. L., Shaw, J., Ferrier, K. E., & Jennings, G. L. (2003). Analysis of the regional pulse wave velocity by Doppler: methodology and reproducibility. *J.Hum.Hypertens.* 17, 407-412.

Bauer, T. A., Brass, E. P., & Hiatt, W. R. (2004). Impaired muscle oxygen use at onset of exercise in peripheral arterial disease. *J.Vasc.Surg.* 40, 488-493.

Belardinelli, R., Barstow, T. J., Nguyen, P., & Wasserman, K. (1997). Skeletal muscle oxygenation and oxygen uptake kinetics following constant work rate exercise in chronic congestive heart failure. *Am.J.Cardiol.* 80, 1319-1324.

Benetos, A., Laurent, S., Hoeks, A. P., Boutouyrie, P. H., & Safar, M. E. (1993). Arterial alterations with aging and high blood pressure. A noninvasive study of carotid and femoral arteries. *Arterioscler.Thromb.* 13, 90-97.

Bergholm, R., Makimattila, S., Valkonen, M., Liu, M. L., Lahdenpera, S., Taskinen, M. R., Sovijarvi, A., Malmberg, P., & Yki-Jarvinen, H. (1999). Intense physical training decreases circulating antioxidants and endothelium-dependent vasodilatation in vivo. *Atherosclerosis* 145, 341-349.

Bertovic, D. A., Waddell, T. K., Gatzka, C. D., Cameron, J. D., Dart, A. M., & Kingwell, B. A. (1999). Muscular strength training is associated with low arterial compliance and high pulse pressure. *Hypertension* 33, 1385-1391.

Bogdanis, G. C., Nevill, M. E., Boobis, L. H., & Lakomy, H. K. (1996). Contribution of phosphocreatine and aerobic metabolism to energy supply during repeated sprint exercise. *J.Appl.Physiol* 80, 876-884.

Bogdanis, G. C., Nevill, M. E., Lakomy, H. K., & Boobis, L. H. (1998). Power output and muscle metabolism during and following recovery from 10 and 20 s of maximal sprint exercise in humans. *Acta Physiol Scand.* 163, 261-272.

Bulpitt, C. J., Cameron, J. D., Rajkumar, C., Armstrong, S., Connor, M., Joshi, J., Lyons, D., Moioli, O., & Nihoyannopoulos, P. (1999). The effect of age on vascular compliance in man: which are the appropriate measures? *J.Hum.Hypertens.* 13, 753-758.

Burgomaster, K. A., Hughes, S. C., Heigenhauser, G. J., Bradwell, S. N., & Gibala, M. J. (2005). Six Sessions of Sprint Interval Training Increases Muscle Oxidative Potential and Cycle Endurance Capacity in Humans. *J.Appl.Physiol.*

Cai, H. & Harrison, D. G. (2000). Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. *Circ.Res.* 87, 840-844.

Cameron, J. D. & Dart, A. M. (1994). Exercise training increases total systemic arterial compliance in humans. *Am.J.Physiol* 266, H693-H701.

Cameron, J. D., Gatzka, C. D., & Kingwell, B. A. (2002). Assessment of large artery function. *Coron.Artery Dis.* 13, 405-413.

Clarkson, P., Montgomery, H. E., Mullen, M. J., Donald, A. E., Powe, A. J., Bull, T., Jubb, M., World, M., & Deanfield, J. E. (1999). Exercise training enhances endothelial function in young men. *J.Am.Coll.Cardiol.* 33, 1379-1385.

Corretti, M. C., Anderson, T. J., Benjamin, E. J., Celermajer, D., Charbonneau, F., Creager, M. A., Deanfield, J., Drexler, H., Gerhard-Herman, M., Herrington, D., Vallance, P., Vita, J., & Vogel, R. (2002). Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J.Am.Coll.Cardiol.* 39, 257-265.

Dart, A. M. & Kingwell, B. A. (2001). Pulse pressure--a review of mechanisms and clinical relevance. *J.Am.Coll.Cardiol.* 37, 975-984.

Dawson, B., Fitzsimons, M., Green, S., Goodman, C., Carey, M., & Cole, K. (1998). Changes in performance, muscle metabolites, enzymes and fibre types after short sprint training. *Eur.J.Appl.Physiol Occup.Physiol* 78, 163-169.

DeSouza, C. A., Shapiro, L. F., Clevenger, C. M., Dinenno, F. A., Monahan, K. D., Tanaka, H., & Seals, D. R. (2000). Regular aerobic exercise prevents and restores age-related declines in endothelium-dependent vasodilation in healthy men. *Circulation* 102, 1351-1357.

Fathi, R., Haluska, B., Isbel, N., Short, L., & Marwick, T. H. (2004). The relative importance of vascular structure and function in predicting cardiovascular events. *J.Am.Coll.Cardiol.* 43, 616-623.

Ferrier, K. E., Waddell, T. K., Gatzka, C. D., Cameron, J. D., Dart, A. M., & Kingwell, B. A. (2001). Aerobic exercise training does not modify large-artery compliance in isolated systolic hypertension. *Hypertension* 38, 222-226.

Gates, P. E., Tanaka, H., Graves, J., & Seals, D. R. (2003). Left ventricular structure and diastolic function with human ageing. Relation to habitual exercise and arterial stiffness. *Eur.Heart J.* 24, 2213-2220.

Gielen, S. & Hambrecht, R. (2001). Effects of exercise training on vascular function and myocardial perfusion. *Cardiol.Clin.* 19, 357-368.

Gillen, C. M., Lee, R., Mack, G. W., Tomaselli, C. M., Nishiyasu, T., & Nadel, E. R. (1991). Plasma volume expansion in humans after a single intense exercise protocol. *J.Appl.Physiol* 71, 1914-1920.

Gokce, N., Keaney, J. F., Jr., Hunter, L. M., Watkins, M. T., Nedeljkovic, Z. S., Menzoian, J. O., & Vita, J. A. (2003). Predictive value of noninvasively determined endothelial dysfunction for long-term cardiovascular events in patients with peripheral vascular disease. *J.Am.Coll.Cardiol.* 41, 1769-1775.

Gokce, N., Vita, J. A., Bader, D. S., Sherman, D. L., Hunter, L. M., Holbrook, M., O'Malley, C., Keaney, J. F., Jr., & Balady, G. J. (2002). Effect of exercise on upper and lower extremity endothelial function in patients with coronary artery disease. *Am.J.Cardiol.* 90, 124-127.

Goto, C., Higashi, Y., Kimura, M., Noma, K., Hara, K., Nakagawa, K., Kawamura, M., Chayama, K., Yoshizumi, M., & Nara, I. (2003). Effect of different intensities of exercise on endothelium-dependent vasodilation in humans: role of endothelium-dependent nitric oxide and oxidative stress. *Circulation* 108, 530-535.

Green, D., Cheetham, C., Mavaddat, L., Watts, K., Best, M., Taylor, R., & O'Driscoll, G. (2002). Effect of lower limb exercise on forearm vascular function: contribution of nitric oxide. *Am.J.Physiol Heart Circ.Physiol* 283, H899-H907.

Green, D. J., Cable, N. T., Fox, C., Rankin, J. M., & Taylor, R. R. (1994). Modification of forearm resistance vessels by exercise training in young men. *J.Appl.Physiol* 77, 1829-1833.

Green, H., Goreham, C., Ouyang, J., Ball-Burnett, M., & Ranney, D. (1999). Regulation of fiber size, oxidative potential, and capillarization in human muscle by resistance exercise. *Am.J.Physiol* 276, R591-R596.

Halcox, J. P. & Deanfield, J. E. (2004). Endothelial cell function testing: how does the method help us in evaluating vascular status? *Acta Paediatr.Suppl* 93, 48-54.

Hambrecht, R., Fiehn, E., Weigl, C., Gielen, S., Hamann, C., Kaiser, R., Yu, J., Adams, V., Niebauer, J., & Schuler, G. (1998). Regular physical exercise corrects endothelial dysfunction and improves exercise capacity in patients with chronic heart failure. *Circulation* 98, 2709-2715.

Jacobs, I., Esbjornsson, M., Sylven, C., Holm, I., & Jansson, E. (1987). Sprint training effects on muscle myoglobin, enzymes, fiber types, and blood lactate. *Med.Sci.Sports Exerc.* 19, 368-374.

Kakiyama, T., Matsuda, M., & Koseki, S. (1998). Effect of physical activity on the distensibility of the aortic wall in healthy males. *Angiology* 49, 749-757.

Kelly, R., Hayward, C., Avolio, A., & O'Rourke, M. (1989). Noninvasive determination of age-related changes in the human arterial pulse. *Circulation* 80, 1652-1659.

Kingwell, B. A., Medley, T. L., Waddell, T. K., Cole, T. J., Dart, A. M., & Jennings, G. L. (2001). Large artery stiffness: structural and genetic aspects. *Clin.Exp.Pharmacol.Physiol* 28, 1040-1043.

Kingwell, B. A., Sherrard, B., Jennings, G. L., & Dart, A. M. (1997). Four weeks of cycle training increases basal production of nitric oxide from the forearm. *Am.J.Physiol* 272, H1070-H1077.

Koutsis, G., Kadi, F., Vandewalle, H., Lechat, P., Hadjiisky, P., & Monod, H. (1995). Effects of an endurance training programme on the passive and noradrenaline-activated compliances of rat aorta. *Eur.J.Appl.Physiol Occup.Physiol* 71, 173-179.

Lakatta, E. G. (2002). Age-associated cardiovascular changes in health: impact on cardiovascular disease in older persons. *Heart Fail.Rev.* 7, 29-49.

Lakatta, E. G. (2003). Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: Part III: cellular and molecular clues to heart and arterial aging. *Circulation* 107, 490-497.

Laughlin, M. H., Woodman, C. R., Schrage, W. G., Gute, D., & Price, E. M. (2004). Interval sprint training enhances endothelial function and eNOS content in some arteries that perfuse white gastrocnemius muscle. *J.Appl.Physiol* 96, 233-244.

Linossier, M. T., Denis, C., Dormois, D., Geyssant, A., & Lacour, J. R. (1993). Ergometric and metabolic adaptation to a 5-s sprint training programme. *Eur.J.Appl.Physiol Occup.Physiol* 67, 408-414.

Lloyd, P. G., Yang, H. T., & Terjung, R. L. (2001). Arteriogenesis and angiogenesis in rat ischemic hindlimb: role of nitric oxide. *Am.J.Physiol Heart Circ.Physiol* 281, H2528-H2538.

MacDougall, J. D., Hicks, A. L., MacDonald, J. R., McKelvie, R. S., Green, H. J., & Smith, K. M. (1998). Muscle performance and enzymatic adaptations to sprint interval training. *J.Appl.Physiol* 84, 2138-2142.

MacDougall, J. D., McKelvie, R. S., Moroz, D. E., Sale, D. G., McCartney, N., & Buick, F. (1992). Factors affecting blood pressure during heavy weight lifting and static contractions. *J.Appl.Physiol* 73, 1590-1597.

Maiorana, A., O'Driscoll, G., Cheetham, C., Dembo, L., Stanton, K., Goodman, C., Taylor, R., & Green, D. (2001a). The effect of combined aerobic and resistance exercise training on vascular function in type 2 diabetes. *J.Am.Coll.Cardiol.* 38, 860-866.

Maiorana, A., O'Driscoll, G., Dembo, L., Cheetham, C., Goodman, C., Taylor, R., & Green, D. (2000). Effect of aerobic and resistance exercise training on vascular function in heart failure. *Am.J.Physiol Heart Circ.Physiol* 279, H1999-H2005.

Maiorana, A., O'Driscoll, G., Dembo, L., Goodman, C., Taylor, R., & Green, D. (2001b). Exercise training, vascular function, and functional capacity in middle-aged subjects. *Med.Sci.Sports Exerc.* 33, 2022-2028.

Matsuda, M., Nosaka, T., Sato, M., Iijima, J., Ohshima, N., & Fukushima, H. (1989). Effects of exercise training on biochemical and biomechanical properties of rat aorta. *Angiology* 40, 51-58.

Matsuda, M., Nosaka, T., Sato, M., & Ohshima, N. (1993). Effects of physical exercise on the elasticity and elastic components of the rat aorta. *Eur.J.Appl.Physiol Occup.Physiol* 66, 122-126.

McGrath, B. P. (2002). Imaging of arteries. *Coron.Artery Dis.* 13, 399-403.

Miyachi, M., Donato, A. J., Yamamoto, K., Takahashi, K., Gates, P. E., Moreau, K. L., & Tanaka, H. (2003). Greater age-related reductions in central arterial compliance in resistance-trained men. *Hypertension* 41, 130-135.

Miyachi, M., Kawano, H., Sugawara, J., Takahashi, K., Hayashi, K., Yamazaki, K., Tabata, I., & Tanaka, H. (2004). Unfavorable effects of resistance training on central arterial compliance: a randomized intervention study. *Circulation* 110, 2858-2863.

Monahan, K. D., Tanaka, H., Dinenno, F. A., & Seals, D. R. (2001). Central arterial compliance is associated with age- and habitual exercise-related differences in cardiovagal baroreflex sensitivity. *Circulation* 104, 1627-1632.

Moyna, N. M. & Thompson, P. D. (2004). The effect of physical activity on endothelial function in man. *Acta Physiol Scand.* 180, 113-123.

O'Rourke, M. F., Staessen, J. A., Vlachopoulos, C., Duprez, D., & Plante, G. E. (2002). Clinical applications of arterial stiffness; definitions and reference values. *Am.J.Hypertens.* 15, 426-444.

O'Sullivan, S. E. (2003). The effects of exercise training on markers of endothelial function in young healthy men. *Int.J.Sports Med.* 24, 404-409.

Parolin, M. L., Chesley, A., Matsos, M. P., Spriet, L. L., Jones, N. L., & Heigenhauser, G. J. (1999). Regulation of skeletal muscle glycogen phosphorylase and PDH during maximal intermittent exercise. *Am.J.Physiol* 277, E890-E900.

Parra, J., Cadefau, J. A., Rodas, G., Amigo, N., & Cusso, R. (2000). The distribution of rest periods affects performance and adaptations of energy metabolism induced by high-intensity training in human muscle. *Acta Physiol Scand.* 169, 157-165.

Pratley, R., Nicklas, B., Rubin, M., Miller, J., Smith, A., Smith, M., Hurley, B., & Goldberg, A. (1994). Strength training increases resting metabolic rate and norepinephrine levels in healthy 50- to 65-yr-old men. *J.Appl.Physiol* 76, 133-137.

Pyke, K. E., Dwyer, E. M., & Tschakovsky, M. E. (2004). Impact of controlling shear rate on flow-mediated dilation responses in the brachial artery of humans. *J.Appl.Physiol* 97, 499-508.

Rakobowchuk, M., McGowan, C. L., de Groot, P. C., Bruinsma, D., Hartman, J. W., Phillips, S. M., & Macdonald, M. J. (2005a). Effect of whole body resistance training on arterial compliance in young healthy males. *Exp.Physiol.*

Rakobowchuk, M., McGowan, C. L., de Groot, P. C., Hartman, J. W., Phillips, S. M., & Macdonald, M. J. (2005b). Endothelial function of young healthy males following whole body resistance training. *J.Appl.Physiol* 98, 2185-2190.

Seals, D. R. (2003). Habitual exercise and the age-associated decline in large artery compliance. *Exerc.Sport Sci.Rev.* 31, 68-72.

Smart, N. & Marwick, T. H. (2004). Exercise training for patients with heart failure: a systematic review of factors that improve mortality and morbidity. *Am.J.Med.* 116, 693-706.

Taddei, S., Galetta, F., Virdis, A., Ghiadoni, L., Salvetti, G., Franzoni, F., Giusti, C., & Salvetti, A. (2000). Physical activity prevents age-related impairment in nitric oxide availability in elderly athletes. *Circulation* 101, 2896-2901.

Takase, B., Uehata, A., Akima, T., Nagai, T., Nishioka, T., Hamabe, A., Satomura, K., Ohsuzu, F., & Kurita, A. (1998). Endothelium-dependent flow-mediated vasodilation in coronary and brachial arteries in suspected coronary artery disease. *Am.J.Cardiol.* 82, 1535-1538.

Tanaka, H., Dinenno, F. A., Monahan, K. D., Clevenger, C. M., DeSouza, C. A., & Seals, D. R. (2000). Aging, habitual exercise, and dynamic arterial compliance. *Circulation* 102, 1270-1275.

Tousoulis, D., Antoniades, C., & Stefanadis, C. (2005). Evaluating endothelial function in humans: a guide to invasive and non-invasive techniques. *Heart* 91, 553-558.

Tronc, F., Wassef, M., Esposito, B., Henrion, D., Glagov, S., & Tedgui, A. (1996). Role of NO in flow-induced remodeling of the rabbit common carotid artery. *Arterioscler.Thromb.Vasc.Biol.* 16, 1256-1262.

Trump, M. E., Heigenhauser, G. J., Putman, C. T., & Spriet, L. L. (1996). Importance of muscle phosphocreatine during intermittent maximal cycling. *J.Appl.Physiol* 80, 1574-1580.

Vaitkevicius, P. V., Fleg, J. L., Engel, J. H., O'Connor, F. C., Wright, J. G., Lakatta, L. E., Yin, F. C., & Lakatta, E. G. (1993). Effects of age and aerobic capacity on arterial stiffness in healthy adults. *Circulation* 88, 1456-1462.

van der Heijden-Spek JJ, Staessen, J. A., Fagard, R. H., Hoeks, A. P., Boudier, H. A., & Van Bortel, L. M. (2000). Effect of age on brachial artery wall properties differs from the aorta and is gender dependent: a population study. *Hypertension* 35, 637-642.

Vane, J. R., Anggard, E. E., & Botting, R. M. (1990). Regulatory functions of the vascular endothelium. *N.Engl.J.Med.* 323, 27-36.

Verma, S., Buchanan, M. R., & Anderson, T. J. (2003). Endothelial function testing as a biomarker of vascular disease. *Circulation* 108, 2054-2059.

Walsh, J. H., Bilsborough, W., Maiorana, A., Best, M., O'Driscoll, G. J., Taylor, R. R., & Green, D. J. (2003). Exercise training improves conduit vessel function in patients with coronary artery disease. *J.Appl.Physiol* 95, 20-25.

Warburton, D. E., Haykowsky, M. J., Quinney, H. A., Blackmore, D., Teo, K. K., Taylor, D. A., McGavock, J., & Humen, D. P. (2004). Blood volume expansion and cardiorespiratory function: effects of training modality. *Med.Sci.Sports Exerc.* 36, 991-1000.

Warburton, D. E., McKenzie, D. C., Haykowsky, M. J., Taylor, A., Shoemaker, P., Ignaszewski, A. P., & Chan, S. Y. (2005). Effectiveness of High-Intensity Interval Training for the Rehabilitation of Patients With Coronary Artery Disease. *Am.J.Cardiol.* 95, 1080-1084.

Woodman, R. J., Kingwell, B. A., Beilin, L. J., Hamilton, S. E., Dart, A. M., & Watts, G. F. (2005). Assessment of central and peripheral arterial stiffness: studies indicating the need to use a combination of techniques. *Am.J.Hypertens.* 18, 249-260.

Zieman, S. J., Melenovsky, V., & Kass, D. A. (2005). Mechanisms, pathophysiology, and therapy of arterial stiffness. *Arterioscler.Thromb.Vasc.Biol.* 25, 932-943.

Appendix A
Subject Information and Consent Form

EXERCISE METABOLISM RESEARCH GROUP
DEPARTMENT OF KINESIOLOGY – MCMASTER UNIVERSITY

INFORMATION & CONSENT TO PARTICIPATE IN RESEARCH

THE IMPACT OF SPRINT INTERVAL TRAINING ON ARTERIAL STRUCTURE AND FUNCTION

You are being asked to participate in a research study being conducted by the investigators listed below. Prior to participation, you are asked to read this consent form which outlines the purpose and testing procedures used in this study. Unless otherwise stated, all testing and experimental procedures will be conducted in the Exercise Metabolism Research Laboratory, RM A103, Ivor Wynn Centre.

INVESTIGATOR	DEPARTMENT	CONTACT
Dr. Maureen MacDonald	Kinesiology, AB115	x.2358o
Jen Bartholomew	Kinesiology, A103	x.27037
Heath D'Sa	Kinesiology, A103	x.27037

PURPOSE

Exercise is known to increase blood flow to exercising muscle and decrease blood flow to other areas of the body. The impact of high intensity, short duration exercise training has not been examined with regards to arterial structure and function. To gain a better understanding of how sprint interval training affects the mechanisms that control blood flow, we will examine endothelial function at the brachial artery and artery compliance and vessel geometry of the carotid artery during rest.

DESCRIPTION OF TESTING PROCEDURES

Subjects will be required to visit the lab on several different occasions and perform the following tests:

(A) 30km Time Trial on a Cycle Ergometer
(B) Resting Measures of Arterial Compliance and Endothelial Function
(C) Wingate Training Tests on a Cycle Ergometer

30 Km Time Trial

Subjects will be required to visit the lab on two occasions to complete a performance ride on a cycle ergometer. This is a timed test to examine how subjects perform PRE and POST training.

Arterial Compliance

Arterial compliance at the carotid artery will be assessed by using a combination of Doppler ultrasound and blood pressure measurements. Doppler ultrasound will acquire visual images of the carotid artery at rest. This technique is totally non-invasive and will not cause any discomfort. Blood pressure will be recorded using applanation tonometry. This process involves a cuff being placed around the arm. It is inflated by an automated system and record pressures continuously. As well, a small pen like device will be applied on the skins surface directly above the carotid artery. Force transducers generate a pressure waveform. There is very little risk involved with these procedures unless the cuff was left inflated in excess of 45 minutes.

Endothelial Function
Endothelial function at the brachial artery is assessed using a technique known as flow mediated dilation. In this process, a cuff will be placed around the forearm and inflated to a pressure of 200 mmHg for approximately 5 minutes. Before inflation, blood velocity will be measured using pulsed Doppler ultrasound. Just before deflation and for 1 minute following, blood velocity will be measured again with the same technique. There is very little risk involved in this procedure unless the cuff is left inflated in excess of 45 minutes.

Wingate Tests
Subjects will be required to visit the lab every other day for 2 weeks to complete their training sessions. Each training session will consist of a series of wingate bike tests which increase in number with each training sessions starting at 4 wingates up to a maximum of 6. Each trial is separated by 4 minutes of recovery. Each wingate trial involves 30 seconds of maximal cycling where the subjects will pedal as fast as they can for the entire 30 seconds.

POTENTIAL RISKS AND DISCOMFORTS
All of the procedures will be completely non-invasive. There is no danger associated with wingate training, although some fatigue may be experienced.

BENEFITS & REMUNERATION
In participating in this project you realize that there are no direct benefits to you. You will receive an honorarium appropriate fro the amount of time that you put into this project. $200 will be received upon completion of the study to compensate you for your time commitment (~ 10 hours).

CONFIDENTIALITY
All data collected during this study will remain confidential and stored in secure offices and on authorized computers. You should be aware that the results of this study will be made available to the scientific community through publication in a scientific journal. Your name or any reference to you as an individual will not be used in compiling or publishing these results. Additionally, you will have access to your own data as well as the group data when it becomes available if you are interested.

PARTICIPATION & WITHDRAWL

You can choose whether to participate in this study or not. You should be aware that your participation in this study would in no way affect your academic performance in any course offered within the Department of Kinesiology. You may exercise the option of removing your data from the study if you wish. You may also refuse to answer any questions posed to you during the study and still remain as a subject in the study. The investigators reserve the right to withdraw you from the study if they believe that circumstances have arisen which warrant doing so.

RIGHTS OF RESEARCH PARTICIPANTS

You will receive a signed copy of this ethics form. You may withdraw your consent to participate in this study at any time, and you may also discontinue participation at any time without penalty. In signing this consent form or in participating in this study you are not waiving any legal claims. This study has been reviewed and has received clearance from the McMaster University Research Ethic Board (MREB). If you have any further questions regarding your rights as a research participant contact Dr. Maureen MacDonald.

I HAVE READ AND UNDERSTAND THE ABOVE EXPLANATION OF THE PURPOSE AND PROCEDURES OF THE PROJECT. I HAVE ALSO RECEIVED A SIGNED COPY OF THE INFORMATION AND CONSENT FORM. MY QUESTIONS HAVE BEEN ANSWERED TO MY SATISFACTION AND I AGREE TO PARTICIPATE IN THIS STUDY.

SIGNATURE: _____ DATE: _____

PRINTED NAME: _____

WITNESS: _____ DATE: _____

PRINTED NAME: _____

INVESTIGATOR

In my judgment, the participant voluntarily and knowingly gave informed consent and possesses the legal capacity to give informed consent and participate in this research study.

SIGNATURE: _____ DATE: _____

Appendix B
ANOVA Summary Tables

Mean Carotid Arterial Diameter - All Subjects						
Effect	df Effect	MS Effect	df Error	MS Error	F-ratio	p-level
TIME	1	0.0401	8	0.0032	12.38	0.007862

Mean Brachial Arterial Diameter - All Subjects						
Effect	df Effect	MS Effect	df Error	MS Error	F-ratio	p-level
TIME	1	0.038	8	0.04	0.936	0.362

Pulse Pressure - All Subjects						
Effect	df Effect	MS Effect	df Error	MS Error	F-ratio	p-level
TIME	1	66.88	8	29.52	2.266	0.17068

Carotid Cross-Sectional Compliance - All Subjects						
Effect	df Effect	MS Effect	df Error	MS Error	F-ratio	p-level
TIME	1	0.000007	8	0.000396	0.017	0.89

Resting Brachial Blood Flow - All Subjects						
Effect	df Effect	MS Effect	df Error	MS Error	F-ratio	p-level
TIME	1	1.16	8	129.65	0.009	0.927

10s Average Brachail FMD Blood Flow - All Subjects						
Effect	df Effect	MS Effect	df Error	MS Error	F-ratio	p-level
TIME	1	3752	8	654	5.734	0.044

Peak Beat Brachial FMD Blood Flow - All Subjects						
Effect	df Effect	MS Effect	df Error	MS Error	F-ratio	p-level
TIME	1	4500	8	1021	4.408	0.069

10s Average Brachial FMD Shear Rate - All Subjects						
Effect	df Effect	MS Effect	df Error	MS Error	F-ratio	p-level
TIME	1	8.9	8	23.37	0.381	0.554

Peak Beat Brachial FMD Shear Rate - All Subjects						
Effect	df Effect	MS Effect	df Error	MS Error	F-ratio	p-level
TIME	1	6.24	8	33.19	0.1879	0.676

Relative Flow Mediated Dilation - All Subjects						
Effect	df Effect	MS Effect	df Error	MS Error	F-ratio	p-level
TIME	1	14.086	8	11.27	1.25	0.296

Normalized Flow Mediated Dilation - All Subjects						
Effect	df Effect	MS Effect	df Error	MS Error	F-ratio	p-level
TIME	1	0.007	8	0.014	0.508	0.496

Time Trial Performance - All Subjects						
Effect	df Effect	MS Effect	df Error	MS Error	F-ratio	p-level
TIME	1	217.74	8	16.13	13.496	0.006274

Appendix C
Raw Data

RAW DATA: RESTING PULSE PRESSURE

CAROTID ARTERY (mm)

SUBJECT	PRE	POST
JC	51.2	65.5
BM	42.4	48.5
TM	50.4	50.4
MS	47.5	55.6
SF	53.3	52.2
JH	42.3	43.3
DB	43.1	54.2
AB	54.1	42.6
MW	53.0	59.5
MEAN	**48.6**	**52.4**
SD	**4.9**	**7.4**
SEM	**1.6**	**2.5**

RAW DATA: MEAN ARTERIAL DIAMETERS

CAROTID ARTERY (mm)

SUBJECT	PRE	POST
JC	6.68	6.80
BM	5.52	5.68
TM	6.73	6.83
MS	6.95	6.95
SF	6.59	6.52
JH	5.97	6.13
DB	6.58	6.68
AB	6.09	6.25
MW	6.47	6.60
MEAN	**6.40**	**6.49**
SD	**0.45**	**0.41**
SEM	**0.15**	**0.14**

BRACHIAL ARTERY (mm)

SUBJECT	PRE	POST
JC	4.32	4.73
BM	4.21	4.20
TM	5.25	5.27
MS	3.30	3.40
SF	4.65	4.73
JH	4.15	3.94
DB	4.34	4.27
AB	4.35	4.19
MW	4.03	4.71
MEAN	**4.29**	**4.38**
SD	**0.52**	**0.55**
SEM	**0.17**	**0.18**

RAW DATA: CROSS-SECTIONAL COMPLIANCE

CAROTID ARTERY ($mm^2/mmHg$)

SUBJECT	PRE	POST
JC	0.193	0.176
BM	0.173	0.157
TM	0.150	0.174
MS	0.214	0.160
SF	0.163	0.180
JH	0.172	0.148
DB	0.163	0.190
AB	0.120	0.135
MW	0.129	0.137
MEAN	**0.164**	**0.162**
SD	**0.029**	**0.020**
SEM	**0.010**	**0.007**

RAW DATA: RESTING BLOOD FLOW

RESTING BRACHIAL BLOOD FLOW (ml/min)

SUBJECT	PRE	POST
JC	72.5	49.5
BM	51.2	46.7
TM	62.7	48.9
MS	16.4	17.7
SF	30.8	47.3
JH	19.4	26.0
DB	39.3	29.8
AB	30.0	30.5
MW	26.4	56.8
MEAN	**38.7**	**39.2**
SD	**19.5**	**13.4**
SEM	**6.5**	**4.5**

RAW DATA: POST OCCLUSION BLOOD FLOW

10-SECOND AVERAGE BRACHIAL BLOOD FLOW (ml/min)

SUBJECT	PRE	POST
JC	369.9	340.4
BM	207.7	234.7
TM	492.0	507.5
MS	112.9	149.3
SF	359.8	457.5
JH	208.8	201.0
DB	344.5	374.1
AB	307.3	342.8
MW	260.9	316.0
MEAN	**296.0**	**324.8**
SD	**112.2**	**116.4**
SEM	**37.4**	**38.8**

PEAK BEAT BRACHIAL BLOOD FLOW (ml/min)

SUBJECT	PRE	POST
JC	430.1	365.2
BM	226.9	264.1
TM	549.7	599.6
MS	135.5	161.5
SF	409.2	490.7
JH	225.6	213.5
DB	389.9	435.5
AB	329.1	377.4
MW	291.9	356.1
MEAN	**332.0**	**362.6**
SD	**127.0**	**137.2**
SEM	**42.3**	**45.7**

RAW DATA: POST OCCLUSION SHEAR RATES

10-SECOND AVERAGE BRACHIAL SHEAR RATE (S^{-1})

SUBJECT	PRE	POST
JC	39.0	27.3
BM	23.6	26.9
TM	28.8	29.4
MS	26.6	32.3
SF	30.4	36.7
JH	24.7	27.8
DB	35.7	40.8
AB	31.7	40.0
MW	33.7	25.7
MEAN	**30.5**	**31.9**
SD	**5.1**	**5.9**
SEM	**1.7**	**2.0**

PEAK BEAT BRACHIAL SHEAR RATE (S^{-1})

SUBJECT	PRE	POST
JC	45.4	29.3
BM	25.8	30.3
TM	32.2	34.8
MS	32.1	34.9
SF	34.5	39.4
JH	26.7	29.6
DB	40.5	47.5
AB	33.9	44.1
MW	37.7	29.7
MEAN	**34.3**	**35.5**
SD	**6.2**	**6.8**
SEM	**2.1**	**2.3**

RAW DATA: FLOW MEDIATED DILATION RESPONSES

RELATIVE FLOW MEDIATED DILATION (%)		
SUBJECT	**PRE**	**POST**
JC	8.5	1.4
BM	6.5	5.1
TM	2.0	7.2
MS	16.4	13.1
SF	-3.4	4.5
JH	1.8	5.2
DB	3.7	6.4
AB	3.1	7.0
MW	2.7	7.3
MEAN	**4.6**	**6.4**
SD	**5.5**	**3.1**
SEM	**1.8**	**1.0**

NORMALIZED FLOW MEDIATED DILATION (%/$^{S-1}$)		
SUBJECT	**PRE**	**POST**
JC	0.218	0.052
BM	0.277	0.148
TM	0.070	0.244
MS	0.616	0.407
SF	-0.113	0.123
JH	0.074	0.188
DB	0.103	0.157
AB	0.098	0.179
MW	0.081	0.284
MEAN	**0.158**	**0.198**
SD	**0.203**	**0.103**
SEM	**0.068**	**0.034**

RAW DATA: PERFORMANCE TIME ON 750 KJ TIME TRIAL

PERFORMANCE TIME (min)		
SUBJECT	**PRE**	**POST**
JC	60.42	53.78
BM	76.28	69.1
TM	42.63	40.9
MS	44.77	41.6
SF	63.15	59.03
JH	60.15	49.63
DB	89.57	69.3
AB	70.42	64.16
MW	57.83	55.1
MEAN	**62.80185**	**55.8463**
SD	**14.68697**	**10.6408**
SEM	**4.895657**	**3.54693**