ACUTE RESPONSES AND CHRONIC ADAPTATIONS OF THE ARTERIAL SYSTEM TO SPRINT EXERCISE AND TRAINING

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By

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ABSTRACT

The present thesis examined the acute and chronic (training) hemodynamic responses to the unique exercise stimulus of high-intensity "sprint" interval exercise or training (SIT). Previous research has characterized the muscle metabolic and exercise performance adaptations to both short and medium term SIT, however the cardiovascular adjustments and adaptations have not been examined. As part of this thesis two studies were designed to permit evaluations of the chronic cardiovascular responses to a six-week SIT intervention protocol, while two separate studies examined the acute impact of a sprint exercise session on indices of vascular structure and function. Comparisons were made between the SIT and traditional endurance exercise training (ET) in the two exercise training studies, while comparisons were made between a single sprint and that of multiple sprints in the acute exercise studies. The subject population examined in this research was young healthy participants.

Our general hypothesis regarding the training adaptations was that similar changes of artery stiffness, vascular endothelial function, blood flow kinetics and oxygen uptake kinetics would occur following SIT compared to ET. Regarding the acute effects of a sprint exercise, we expected arterial stiffness to decrease in the exercising limbs and increase in the central arteries, similar to the responses observed previously immediately following endurance exercise, while we hypothesized that endothelial function would be decreased immediately following the exercise session because of the intense nature of the exercise. The overarching hypothesis guiding these specific hypothesis is that we believe that individual bouts of exercise impact on the arterial wall through the generation of a shear stimulus related to cyclic increases in blood flow and blood pressure. In the short-

term the acute response of the artery depends on the composition of the arterial wall and the local stimulus. Over time, functional and structural adjustments occur to normalize the impact of shear forces.

Training adaptations in vascular structure and function to SIT were similar to those observed with ET. Both exercise training methods stimulated improved peripheral artery stiffness and endothelial function. The rate of increase in oxygen uptake (kinetic response) was not improved with either training method. However, estimated myocardial demand was reduced with ET but not SIT, which indicates more favourable adaptation in central hemodynamics with ET.

Acute sprint exercise markedly reduced peripheral artery stiffness in the exercised limbs well into recovery (~45 minutes), which may benefit central hemodynamics after exercise completion. Sprint exercise also acutely decreased endothelial function, likely because of high oxidative stress generated during the exercise bout and may provide the ideal stimulus for endothelial adaptation.

In summary, this thesis highlights the chronic and acute effects of sprint interval exercise and training in young health individuals. The notion that sprint interval exercise provides equivalent benefits to the cardiovascular system as endurance exercise may be true in the peripheral circulation. However, further study focusing is required before the general acceptance of more favorable central hemodynamic effects from endurance exercise training.

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Format and Organization of Thesis

This thesis was prepared in the "sandwich format" as outlined in the School of Graduate Studies' Guide for the Preparation of Thesis. This thesis is comprised of four original research papers (Chapters 2-5), preceded by a general introduction and followed by a general discussion. Chapter 3 and 4 have been published or accepted in peer-reviewed journals. The remaining chapters have also been peer-reviewed and have been or will be submitted to peer reviewed journals with the candidate as first author.

Contribution to Papers with Multiple Authorship

Chapter 2

Publication

M. Rakobowchuk, S. Tanguay & M.J. MacDonald. Sprint interval and traditional endurance training fail to improve oxygen uptake, blood flow kinetic responses in young healthy participants.

Contribution

The experiments were coordinated and conducted by M. Rakobowchuk with assistance S. Tanguay. Training was coordinated by M. Rakobowchuk, K. R. Howarth and K.A. Burgomaster. The supervisor for this study was M.J. MacDonald. During the trials measures of oxygen uptake, arterial blood flow, pressure and resting cardiac parameters were either measured or monitored by M. Rakobowchuk. S. Tanguay acquired stroke velocity at the sternal notch using ultrasound. All data analyses were performed by M. Rakobowchuk except measures of aortic root diameter, which were analyzed by S. Tanguay under the supervision of M. Rakobowchuk. Statistical analyses and manuscript preparation were completed by M. Rakobowchuk.

Chapter 3

Publication

M. Rakobowchuk, S. Tanguay, K. A. Burgomaster, K. R. Howarth, M. J. Gibala & M. J. MacDonald (2008). Sprint interval and traditional endurance training induce similar

improvements in peripheral arterial stiffness and flow mediated dilation in healthy humans. *Am J Physiol Regulatory Integrative Comp. Physiol.* 295, R236-242.

Contribution

The experiments were coordinated and conducted by M. Rakobowchuk with assistance from the co-authors. The supervisor for this study was M.J. McDonald. S. Tanguay assisted with the collection of arterial tonometer measurements. The training program was conducted by M. Rakobowchuk, K.A. Burgomaster and K.R. Howarth. All data analyses were performed by M. Rakobowchuk including flow-mediated dilation and arterial distensibility. Statistical analyses and manuscript preparation were also completed by M. Rakobowchuk.

Chapter 4

Publication

M. Rakobowchuk, M. I. Stuckey, P.J. Millar, L. Gurr, & M.J. MacDonald. Effect of acute sprint interval exercise on central and peripheral artery distensibility in young healthy males (accepted). *European Journal of Applied Physiology.*

Contribution

M. Rakobowchuk coordinated and conducted the experiments with assistance from M.I. Stuckey and L. Gurr. The supervisor for this study was M.J. MacDonald. M.I. Stuckey acquired the femoral arterial tonometer signal, L. Gurr acquired the automated brachial artery blood pressures, and M. Rakobowchuk acquired all ultrasound images. All data analyses were completed by M. Rakobowchuk, including pulse wave velocity, femoral artery stiffness, and Wingate data. Statistical analyses and manuscript preparation were completed by M. Rakobowchuk.

Chapter 5

Publication

M. Rakobowchuk, L. Gurr, P.J. Millar & M.J. MacDonald. The effect of single and multiple sprint exercise on vascular reactivity and reactive hyperemia.

Contribution

The experiments were coordinated and conducted by M. Rakobowchuk with assistance from the co-authors. The supervisor for this study was M.J. MacDonald. During the trials all measures used in this study were take by M. Rakobowchuk. P.J. Millar took venous blood samples not used in the present study and L. Gurr helped with timing and image storage. All data analyses were performed by M. Rakobowchuk. Statistical analyses and manuscript preparation were also completed by M. Rakobowchuk.

Chapter 1

General Introduction

1.1 Introduction

In humans the cardiovascular interactions with, and responses to, both acute and chronic exercise provide a window into the regulatory control processes and the adaptive responses to stress. Work in this area began with simple measures of arterial blood pressure, heart rate, and estimates of oxygen consumption, both in cross-sectional comparisons of sedentary and active populations, as well as before and after exercise training (see review by Green, O'Driscoll, Joyner & Cable, 2008). Recent technological advances have facilitated more detailed, non-invasive determinations of many of these parameters at rest, during exercise and during the transition from both rest to exercise and low to high work rates. New methods of analysis have also enabled researchers to describe potential mechanisms for adjustments to the cardiovascular system with both acute exercise and chronic exercise training. These adjustments include heart rate and arterial pressure changes, and recent studies have expanded this list to include other independent predictors of vascular health, such as arterial stiffness, endothelial function, intima-media thickness, and left ventricular size and function.

The importance of evaluating exercise and the impact it has on the health and performance of the cardiovascular system relates to its' effectiveness, and to the multiple beneficial effects it confers. Exercise training not only improves outcomes in disease populations, but it may also be superior to routine interventional methods of

cardiovascular disease prevention and treatment (pharmacology and surgery) in certain populations at a much-reduced cost (Hambrecht *et al.*, 2004). From a health management perspective, exercise training provides a long-term strategy for the prevention and treatment of cardiovascular disease, however, time-commitment and psycho-sociological barriers continue to be limitations to widespread application in the "real-world" (Myers & Roth, 1997; Strutts, 2002). One potential strategy to overcome the obstacle of time-restriction is to increase the intensity of exercise in an interval pattern, while reducing the duration. Recent work suggests that this method of training, sprint interval training (SIT), provides similar muscle metabolic improvements compared to aerobic or endurance training (ET) (Burgomaster, Heigenhauser, & Gibala, 2006; Burgomaster *et al.*, 2008; Burgomaster, Hughes, Heigenhauser, Bradwell, & Gibala, 2005). Few studies have assessed the acute or chronic effect of SIT and sprint interval exercise on the cardiovascular system especially in comparison to endurance exercise training.

The current thesis addressed questions related to the impact of sprint exercise in the form of repeated, brief (30 second) "all-out" efforts (commonly referred to as a Wingate test) on arterial structure and function through a series of studies. Two of these studies examined the acute effects of sprint interval exercise while two examined chronic adaptations to SIT in comparison with endurance training (ET). Delineation should be made between this anaerobic method of exercising and commonly used aerobic interval exercise when placing the findings in context. This introductory chapter gives an overview of pertinent literature, including subtopics devoted to the impact of different types of acute and chronic exercise on the cardiovascular parameters: arterial

distensibility, vascular function, blood flow delivery, and oxygen uptake. Specific sections describe the regulatory processes responsible for alterations in these parameters with both acute and chronic exercise, such as nitric oxide mediated vasodilation, arterial collagen/elastin composition, bulk blood flow and regulation of aerobic metabolism. The format follows an integrative approach; however, subtopics are introduced to delineate the acute and chronic responses.

1.2 Structure and innervations of central and peripheral arteries

The arterial wall, both centrally and peripherally, is made up of three concentric regions or tunics: the tunica adventitia, tunica media, and tunica intima. Each of these regions is composed of varying amounts of elastin and collagen. The inner most layer, the intima is composed of a single layer of endothelial cells, which may be altered with age and disease, and is anchored to the internal elastic lamina with elastin and collagen (Nichols, McDonald, & O'Rourke, 2005). The media makes up the bulk of the vessel wall and is the portion of the wall that contributes most to the elastic properties of the artery. The media is composed of specific individual layers of elastin, collagen and smooth muscle that act like one homogeneous layer when experiencing tensile forces (Berne & Levy, 2001). The makeup of this layer ensures preferential loading of the elastin fibres at low pressure while collagen fibres become involved at higher pressures (O'Rourke, 2008). Lastly, the adventitia is a complex layer, which connects the vessel to surrounding tissues, nerves and its blood supply (Tortora & Derrickson, 2006).

The composition of the arterial wall varies along the length of the arterial tree. The structure of the wall varies from the aorta to the conduit vessels (muscular arteries)

to the arterioles. Collagen and elastin compose approximately 50% of the dry weight of all vessels; however, the relative contribution of elastin and collagen differs between central and peripheral arteries. Figure 1.1 outlines the differences in wall structure in different sections of the arterial tree (Harkness, Harkness, & McDonald, 1957):

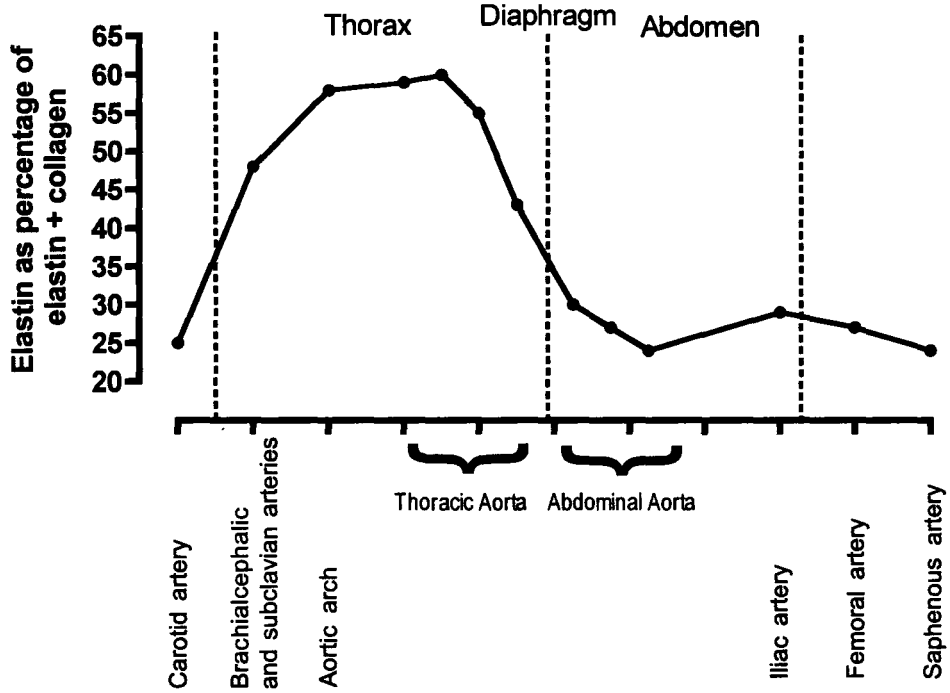

Figure 1.1 Composition of the arterial wall of a dog from the carotid artery to the saphenous artery. The elastin content is greatest in the thoracic portion with reductions noted both toward the head and in the abdomen. Once through the transition in the abdomen, there are no further reductions in elastin content. (Reproduced from Harkness et al. 1957).

Large elastic and medium-sized muscular arteries are two divisions used to describe arteries. The large elastic arteries cushion pulsatile blood ejection then act like a pressure reservoir during diastole. The high elastin content of these arteries enables this function (see Figure 1.1). The greater smooth muscle content of medium-sized muscular

arteries contributes to greater vasoconstriction and greater vasoreactivity compared with muscular arteries (Tortora & Derrickson, 2006). These muscular arteries are also referred to as distributing arteries since they branch widely to supply blood to various organ systems (Tortora & Derrickson, 2006).

1.3 Arterial distensibility and the acute effects of exercise

Arterial distensibility is the relative change in the cross-sectional area of a vessel for a given amount of pressure. Distensible arteries convert cardiac output, which is periodic and pulsatile, into laminar blood flow for muscle and end organs (Nichols et al., 2005). Without such dampening, nutrient, waste and blood gas exchange would be inefficient and potentially insufficient. Further, a distensible and compliant proximal aorta augments ventricular contraction efficiency. With aging and inactivity arterial distensibility decreases unless maintained by therapeutic methods, such as physical activity. Eventually, this decrease in arterial distensibility may lead to left ventricular hypertrophy and cardiac insufficiency (congestive heart failure) (Nichols et al., 2005). Arterial distensibility, like many physiological parameters, is regulated over both the long-term and the short-term by various feed-forward and feed-back mechanisms. However, controversy exists concerning the regional changes in arterial distensibility occurring during and after acute exercise. Several factors, including sympathetic nervous activity, circulating vasoactive substances, arterial blood pressure, and vessel dimensions acutely regulate arterial distensibility. Each of these factors has a differential effect dependent upon the vascular bed of interest.

Sympathetic nervous activity increases immediately before exercise in a feed-forward manner and remains elevated throughout exercise and into recovery. Distensibility may increase or decrease depending on the artery studied; however, sympathetic stimulation most often operates through adrenergic receptors located in the smooth muscle layer of the vessel causing vasoconstriction and thus decreased distensibility (Boutouyrie *et al.*, 1994; Failla *et al.*, 1999). In the descending aorta, sympathetic stimulation has little direct effect on artery distensibility but may lead to indirect decreases in distensibility associated with increased mean arterial pressure (MAP) (Sonesson, Vernersson, Hansen, & Lanne, 1997). Similarly, the proximal brachial (Bjarnegard, Ryden Ahlgren, Sonesson, & Lanne, 2004) and common carotid arteries (Pannier, Slama, London, Safar, & Cuche, 1995) show no change in distensibility with application of lower body negative pressure which induces sympathetic activation. Sympathetic stimulation has been shown to reduce arterial distensibility in distal arteries, which display muscular artery properties. Specifically, this effect may occur in the distal brachial artery (Bjarnegard et al., 2004), and was observed in the radial and femoral arteries (Failla et al., 1999).

Beyond sympathetic nervous activity, vasoactive substances contribute to the regulation of arterial distensibility during rest. Specifically, nitric oxide (NO), endothelin-1, prostaglandins, and oxidative stress all contribute to basal artery distensibility. NO is the most extensively studied of these vasoactive substances and NO synthase inhibition using NG-monomethyl-L-arginine (L-NMMA) either administered systemically or locally in various arteries is the method most often used. Numerous studies suggest a role

of NO in maintaining distensibility through smooth muscle relaxation (Fitch, Vergona, Sullivan, & Wang, 2001; Reid & Durham, 2002; Schmitt *et al.*, 2005; Wilkinson, MacCallum, Cockcroft, & Webb, 2002a; Wilkinson *et al.*). Specifically, NO mediates smooth muscle relaxation through a cAMP dependent mechanism, which stimulates endoplasmic reticulum sequestering of cytosolic Ca^{2+}(Reid & Durham, 2002). Studies in animal models showed central pulsewave velocity (PWV) increased upon systemic administration of N-nitro-L-arginine methyl ester (L-NAME), and that this was independent of changes in MAP (Fitch et al., 2001). Increased PWV occurs when arterial distensibility is decreased. After local infusion of L-NMMA into the artery they observed the same increase in central PWV (Wilkinson et al., 2002b). In human studies, systemic inhibition of NO synthesis reduced arterial distensibility, however, the increased MAP may have had influenced the results (Wilkinson et al., 2002a). Further studies involving localized inhibition of NO synthesis in the iliac artery showed that this segment relies on basal NO as a modulator of artery distensibility (Schmitt et al., 2005).

Oxidative stress, a result of an imbalance between free radical production and degradation (prooxidant), also regulates artery distensibility; however, there have been few human studies in this area. Wilkinson et al. (1999) showed improved augmentation index (AIx) following acute oral administration of ascorbic acid in healthy males, while Type 2 diabetic patients also saw similar benefits (Mullan, Young, Fee, & McCance, 2002). AIx is a calculated variable that represents the contribution of the peripheral pulse wave to the central blood pressure waveform and can be used to estimate changes in arterial distensibility. It is likely that acute reductions of oxidative stress induced by

ascorbic acid administration allow for greater NO bioavailability and subsequent smooth muscle relaxation, however, this awaits further study (Wilkinson *et al.*, 1999).

Acute exercise is a potent stimulus for the production of free radicals at several sites, and these free radicals transfer electrons to oxygen both in the cytosol and extracellularly (Jackson, Pye, & Palomero, 2007) thus contributing to oxidative stress and reduced NO bioavailability. Conversely, increases in blood flow and the resultant shear stress cause the release of various vasoactive substances such as NO, prostacyclin, and endothelial derived hyperpolarizing factor (EDHF). These vasoactive substances all have direct effects on vascular smooth muscle tone (Davies, 1995) and influence arterial stiffness.

Exercise augments arterial blood pressure, however, different types and intensities of exercise are associated with vastly different blood pressure responses. Specifically, during predominantly aerobic, large muscle mass exercise such as running or cycling, mean arterial pressure (MAP) increases in a somewhat linear fashion because of an increase in systolic blood pressure and a maintenance or a decrease in diastolic pressure (Nichols et al., 2005). The blood pressure responses are similar for high intensity exercise involving dynamic movements. However, exercise involving static or slow movements against high resistance result in large increases in both systolic and diastolic pressure, and therefore MAP (MacDougall *et al.*, 1992; MacDougall, Tuxen, Sale, Moroz, & Sutton, 1985).

With aerobic exercise the magnitude of the increase in blood pressure differs in central compared to peripheral arteries, resulting in increased pressure amplification

(ratio of peripheral pulse pressure:central pulse pressure, PPP:CPP) as the intensity of exercise increases (Sharman *et al.*, 2005). The effects of exercise type and peripheral blood pressure augmentation creates situations where artery distensibility responses can be quite different in different arterial segments. All factors which influence arterial distensibility are present to different degrees in different segments of the arterial tree (i.e. central vs. peripheral, active vs. nonactive tissue beds) and thereby produce acute alterations in artery distensibility which vary depending on the arterial segment and the activity of the surrounding tissue. Other factors that also influence arterial distensibility include the method and timing of measurement and the population studied.

To date, several studies have focused on the acute effects of cycling exercise on both central and peripheral arterial distensibility (Heffernan, Jae, Echols, Lepine, & Fernhall, 2007b; Kingwell, Berry, Cameron, Jennings, & Dart, 1997a; Naka *et al.*, 2003; Sugawara *et al.*, 2004; Sugawara *et al.*, 2003). The varying exercise protocols used in these studies include incremental exercise to exhaustion (Heffernan et al., 2007b; Kingwell et al., 1997a), moderate intensity continuous exercise (Naka et al., 2003), and brief (5 min) low intensity exercise (Sugawara et al., 2004; Sugawara et al., 2003). In each of these previous studies arterial distensibility following exercise was decreased and localized to the exercising limb (Sugawara et al., 2004; Sugawara et al., 2003), or described as whole-body (Kingwell et al., 1997a). The acute effects of high intensity exercise on arterial distensibility have not been examined.

The effects of acute exercise on vascular distensibility is equivocal. Exercise situations that stimulate high sympathetic nervous activity, high rates of cardiac output,

and high arterial blood flow are particularly complex. A better understanding of these factors during sprint exercise is warranted and may provide mechanistic insight in to chronic adaptations to sprint interval exercise training.

1.4 Chronic adaptations of artery distensibility with exercise training

Adaptations in arterial distensibility to chronic exercise training have appeared to be related to the mode of exercise performed during the training program and the type of artery examined. Endurance or predominantly aerobic exercise training often improves central artery distensibility (Cameron & Dart, 1994; Hayashi, Sugawara, Komine, Maeda, & Yokoi, 2005; Kakiyama *et al.*, 2005; Kawano, Tanaka, & Miyachi, 2006; Moreau, Donato, Seals, DeSouza, & Tanaka, 2003; Sugawara *et al.*, 2006; Tanaka *et al.*, 2000; Tordi *et al.*, 2006), while alterations in peripheral muscular arteries are rare (Thijssen, de Groot, Smits, & Hopman, 2007).

Studies in healthy middle-aged (53 ± 2 years) men have shown moderate exercise (~ 60-75% of max heart rate), of limited duration (30-45 minutes), at a relatively low frequency (4-6 days/week) increases arterial distensibility of central arteries after only twelve weeks. These increases were independent of changes in body mass, arterial blood pressure, heart rate or traditional blood markers of cardiovascular risk (Tanaka et al., 2000). A similar exercise training program induced increases in central artery distensibility in older women after 3 months (Moreau et al., 2003). The gains in arterial distensibility observed in the older women were beyond those attributable to hormone replacement therapy (Moreau et al., 2003). Furthermore, increases in central artery

distensibility have been noted after only 4 weeks of endurance exercise training in healthy young males (Cameron & Dart, 1994; Kakiyama et al., 2005), however, those with isolated systolic hypertension may be unresponsive (Ferrier *et al.*, 2001). Finally, most endurance exercise training studies that showed improvements in artery distensibility, have noted changes in the central arterial tree (aorta or carotid arteries) (Hayashi et al., 2005; Kawano et al., 2006; Sugawara et al., 2006; Tanaka et al., 2000; Tordi et al., 2006), while peripheral muscular arteries commonly showed no endurance exercise training induced improvements (Cook *et al.*, 2006; Hayashi et al., 2005; Petersen *et al.*, 2006; Tanaka, DeSouza, & Seals, 1998). The previous investigations of peripheral muscular artery distensibility were made in the relatively stiff common femoral artery (Cook et al., 2006; Hayashi et al., 2005; Petersen et al., 2006; Tanaka et al., 1998) and other less stiff peripheral muscular arteries may be more susceptible to change.

Numerous studies have evaluated the effects of resistance exercise training on arterial distensibility. The unique high arterial pressures (MacDougall et al., 1985) and low arterial flows that accompany resistance training create a situation where arterial modification may be different in comparison to endurance exercise training. Resistance training has been associated with both increased central artery stiffness (Bertovic *et al.*, 1999; Miyachi *et al.*, 2003; Miyachi *et al.*, 2004) or no change (Casey, Pierce, Howe, Mering, & Braith, 2007; Maeda *et al.*, 2006; Poelkens *et al.*, 2007; Rakobowchuk *et al.*, 2005a). The reasons for conflicting results regarding the effect of resistance training on central arterial stiffness are not clear, however, some suggest it is related to circulating vasoactive substances (Otsuki *et al.*, 2006; Otsuki *et al.*, 2007), the training volume

(Casey et al., 2007), or the resistance training method (Okamoto, Masuhara, & Ikuta, 2006). Specifically, increased levels of endothelin-1 have been noted with resistance training (Otsuki et al., 2006; Otsuki et al., 2007) while training that does not increase the training volume does not decrease distensibility (Casey et al., 2007). Finally, resistance training that incorporates mostly eccentric contractions did not alter stiffness (Okamoto et al., 2006). In summary, resistance exercise is a complex method of training that may alter central artery stiffness in certain situations.

From a cardiovascular perspective, there has been little research on the effects of high intensity sprint interval training (SIT). Alterations in artery stiffness with SIT have not been studied. The periodic high intensity and high rates of cardiac output, mean arterial pressure, and conduit artery blood flow experienced during SIT combine to create a unique training stimulus. Whether this situation creates an environment that induces increases or decreases in arterial stiffening requires further study.

1.5 Vascular reactivity and the acute effects of exercise

The primary target of injury from mechanical forces and processes related to cardiovascular risk, such as hypertension and aging, is the vascular endothelium (Moyna & Thompson, 2004). The health of the endothelium plays a pivotal role in the maintenance of vascular tone and reactivity (Moncada & Higgs, 1991). The distinction between artery stiffness and vascular reactivity is that vascular reactivity is the response to or change in vascular tone that occurs in reaction to a stimulus. Many if not all the factors described in section 1.3 as regulators of arterial distensibility also contribute to the

regulation of vascular reactivity. In experimental situations, the stimulus for assessment of vascular reactivity is most often an alteration in blood flow (reactive hyperemia) or an intra-arterial administration of acetylcholine; however, a stimulus that augments sympathetic nervous activity (i.e. mental stress or cold pressor test) can also be used to assess vascular reactivity. The larger the change in vascular tone observed in response to a stimulus, the more reactive the artery is considered to be and thus more responsive to physiological perturbations. Increased vascular responsiveness is most often considered to be a positive situation (Green, Maiorana, O'Driscoll, & Taylor, 2004) since many activities of daily living require changes in vascular tone. These changes assist in regulating the distribution of blood to different vascular beds in concert with the specific metabolic demands of the tissues receiving blood supply from different vessels.

In humans, researchers measure arterial reactivity using several techniques. The most commonly used technique is reactive hyperemia induced by cuff occlusion, which is termed flow-mediated dilation (FMD). Shear-stress caused by elevated blood flow after cuff release, induces activation of eNOS in endothelial cells. Specific guidelines have been established for the optimal assessment of FMD, however, these guidelines cover only the measurement of FMD in the brachial artery (Corretti *et al.*, 2002). The researchers who developed the technique chose this vessel because it is easily accessible and is similar in size to the coronary arteries (Corretti et al., 2002). However, more recently modifications involving measurement and correction for the shear stimulus have been proposed (Black, Cable, Thijssen, & Green, 2008; Pyke & Tschakovsky, 2007; Tschakovsky & Pyke, 2005). The current literature suggests that brachial FMD is an

effective bio-assay for NO-dependent vasodilation (Green, 2005); however, various studies show that other factors may alter the response (Betik, Luckham, & Hughson, 2004; Dyson, Shoemaker, & Hughson, 2006).

Other methods of measuring vascular reactivity include observing alterations of arterial diameter in response to mental or physical stress (Cortez-Cooper *et al.*, 2008; Kawano *et al.*, 2008), or measuring the alterations of pulsewave contour or pulsewave velocity following cuff occlusion and reperfusion (Lind, Pettersson, & Johansson, 2003; Naka, Tweddel, Doshi, Goodfellow, & Henderson, 2006). Each method has inherent strengths and weaknesses and these methods require further evaluation regarding their underlying mechanistic importance. Specifically, physical stress methods such as carotid artery reactivity (cold pressor test) lack a controlled stimulus and mechanistic understanding. The purported mechanism is flow induced vasodilation, however, no study has measured flow during the manoeuvre. Without this information, one cannot normalize the response to account for interindividual differences. The pulsewave contour method has also been questioned as it may not be completely NO dependent and skin blood flow influences this measure especially using a finger photoplethysmograph. The post occlusion change in PWV correlates with traditional FMD measures, it is more sensitive than FMD, is relatively reproducible and theoretically should represent NO mediated dilation of the extremity; however, this technique lacks specific validation.

Recently, a common practice has been to evaluate vascular reactivity in an acute exercise setting. A review by Padilla et al. (2007) suggests this may provide insight regarding various regulatory processes contributing to improved vascular reactivity with

exercise training. However, few studies have measured vascular reactivity in the acute exercise setting. Specifically, recent studies suggest both depressed (Rognmo et al. 2008; Harris, Padilla, Hanlon, Rink, & Wallace, 2008; McGowan *et al.*, 2006; Silvestro *et al.*, 2002) and augmented (Harris et al., 2008) brachial vascular reactivity following acute exercise. The mechanisms arise from the complex interaction of sympathetic nervous activity (Dyson et al., 2006), nitric oxide bioavailability (Silvestro et al., 2002), and oxidative stress responses (Silvestro et al., 2002) during exercise and their degradation/removal throughout the recovery from exercise. Like distensibility, vascular reactivity likely displays differential responses, which depend on the vascular bed studied and the timing of measurement following exercise. Animal studies suggest endothelial function may be reduced initially following exercise with an enhancement for up to 192 hours post exercise with training (Haram *et al.*, 2006). Whether this response is dependent on the type of exercise and the vascular bed examined and whether this occurs in humans requires further investigation.

1.6 Chronic adaptations of arterial vascular reactivity with exercise training

Endothelial dysfunction is the inability of an artery to dilate (less than 4%) in response to an increase in blood flow or stimulation of the endothelium via ACh. Those with coronary artery disease (Gokce *et al.*, 2002; Takase *et al.*, 1998), chronic heart failure (Gokce *et al.*, 2003; Hornig, Maier, & Drexler, 1996; Linke *et al.*, 2001) and apparently healthy elderly individuals with no overt signs of cardiovascular disease (Takase et al., 1998) often exhibit this endothelial dysfunction. In patients with coronary

artery disease brachial endothelial dysfunction correlates with disease progression of the coronary arteries (Gokce et al., 2003).

Almost without exception, endurance exercise training improves impaired or reduced endothelial function and this has been demonstrated in both cross-sectional studies of young healthy men (O'Sullivan, 2003), and following intervention studies (Clarkson *et al.*, 1999; D. Green *et al.*, 2002; D. J. Green, Cable, Fox, Rankin, & Taylor, 1994; Kingwell, Sherrard, Jennings, & Dart, 1997b; O'Sullivan, 2003). Furthermore, exercise limited to the lower limbs also improves endothelial function systemically (D. Green et al., 2002). One exception to these observations was an intervention study performed in middle-aged participants, in which no endothelial function improvements were observed after 12 weeks of training (Maiorana *et al.*, 2001b). This equivocal finding was attributed by the authors to the low intensity exercise stimulus and to the possibility that the participants had fully functional endothelia before exercise training (Maiorana et al., 2001b). The exercise training stimulus was identical to previous studies in type 2 diabetes patients, which involved 15 exercises, 7 of which were resistance exercises interspersed with 8 stations of aerobic exercise. Participants completed 45s of exercise at each station and all aerobic exercises were performed between 70-85% of heart rate max (HRmax). The authors concluded that for training to have a beneficial effect on vascular reactivity, it must be of an adequate intensity (Maiorana et al., 2001b).

Endurance training improves vascular reactivity in populations exhibiting decreased or dysfunctional baseline endothelial function (Gokce et al., 2003; Gokce et al., 2002; Hambrecht *et al.*, 1998; Hornig et al., 1996; Linke et al., 2001; Maiorana *et al.*,

2001a; Maiorana *et al.*, 2000; Walsh *et al.*, 2003) and in cross-sectional studies in men of different ages (DeSouza *et al.*, 2000; Taddei *et al.*, 2000). The diversity of these populations includes patients with coronary artery disease (Gokce et al., 2002; Walsh et al., 2003), chronic heart failure (Hornig et al., 1996; Linke et al., 2001; Maiorana et al., 2000), and the elderly with endothelial dysfunction (Taddei et al., 2000).

Interestingly, intense aerobic exercise training may impair endothelial function (Bergholm *et al.*, 1999; Goto *et al.*, 2003). Bergholm and colleagues (1999) noted reduced endothelial-dependent dilation following three months of running. The intensity and duration of the training programs were higher than other studies in the area with a frequency of four times per week at 70-80% VO_{2max} for a period of 60 minutes. This endothelium-dependent dilation may relate to reduced antioxidant levels in the circulation and potentially reduced NO bioavailability (Bergholm et al., 1999). Although a more recent study did not observe impaired endothelial-dependent dilation with high-intensity training (5-7 days per week, 30 minutes at 75% VO_{2max}), it did not find a beneficial effect compared to exercise training of a moderate intensity (Goto et al., 2003). These findings suggest a complex relationship between the exercise training stimulus (intensity and frequency) and vascular reactivity.

Three studies have evaluated the effect of exclusive resistance exercise training on conduit artery vascular reactivity, and they invariably show no changes with training (Cortez-Cooper et al., 2008; Kawano et al., 2008; Rakobowchuk *et al.*, 2005b). These evaluations were conducted in the brachial artery using FMD (Rakobowchuk et al., 2005b) and in the carotid artery using cold pressor stimulation to stimulate reactivity

changes (Cortez-Cooper et al., 2008; Kawano et al., 2008). Increased reactive hyperemia (Rakobowchuk et al., 2005b) and increased basal blood flow (Miyachi, Tanaka, Kawano, Okajima, & Tabata, 2005) following resistance exercise training suggested, however, some increases in vascular function at the level of the resistance vessels.

Although the effects of high intensity endurance training on vascular reactivity have been studied, SIT has not been evaluated for its effects on vascular reactivity. The extreme oxidative stress (Cuevas *et al.*, 2005) and high rates of blood flow observed during and following sprint exercise (Hussain, Smith, Medbak, Wood, & Whipp, 1996) provide unique stimuli that may instigate positive adaptations.

1.7 Kinetic responses of the cardiovascular system with exercise training

Numerous organ systems respond in an exponential fashion to an alteration in demand for aerobic metabolism (Hughson, Tschakovsky, & Houston, 2001). Systems that adapt quickly and reach a new steady state with limited reliance on inefficient processes are most effective in maintaining homeostasis. Therefore, characterizing the response to a step increase in metabolic demand is useful in studies of the integrative nature of the cardiovascular system (Fukuba, Hayashi, Koga, & Yoshida, 2002; Fukuba *et al.*, 2004; Gerbino, Ward, & Whipp, 1996; Hughson, Cochrane, & Butler, 1993; Hughson & Kowalchuk, 1991; Hughson et al., 2001)). The acute changes in the dynamic response of oxygen uptake or what are referred to as the kinetics of oxygen uptake (VO_2 kinetics) have been studied following priming exercise (prior high intensity exercise) (Burnley, Jones, Carter, & Doust, 2000; Jones *et al.*, 2008; M. MacDonald, Pedersen, & Hughson,

1997; Tordi, Perrey, Harvey, & Hughson, 2003; Wilkerson, Koppo, Barstow, & Jones, 2004), during perfusion pressure differences (Hughson et al., 1993; MacDonald, Shoemaker, Tschakovsky, & Hughson, 1998), and hyperoxia, or hypoxia (MacDonald, Tarnopolsky, & Hughson, 2000). Several studies have evaluated the effects of disease and sedentary lifestyles on VO_2 kinetics (Brandenburg *et al.*, 1999; Casaburi *et al.*, 1997; Otsuka, Kurihara, Fujii, Fujimoto, & Yoshikawa, 1997), while a limited number of studies have evaluated the effects of training (Cerretelli, Pendergast, Paganelli, & Rennie, 1979; Hagberg, Hickson, Ehsani, & Holloszy, 1980; Hickson, Bomze, & Hollozy, 1978, Phillips et al. 1995; Krustrup et al. 2004, Shoemaker et al. 1996). The VO_2 kinetic response has been divided into several phases dependent upon the intensity of the transition either from rest to exercise or from exercise to exercise. Specifically, researchers believe the phase II component of the response is the most important parameter since it most closely matches the rate of muscle oxygen uptake (Jones and Poole, 2005).

To obtain a more comprehensive understanding of the regulation of VO_2 kinetics, numerous studies have investigated the kinetic responses of other variables such as cardiac output, and exercising limb blood flow. The following sections briefly outline the various training induced adaptations of these parameters.

1.8 Oxygen uptake kinetics (VO_2) with exercise training

Initial studies showed that VO_2 kinetics improved with aerobic based interventions (Cerretelli, Pendergast, Paganelli, & Rennie, 1979; Hagberg, Hickson, Ehsani, & Holloszy, 1980; Hickson, Bomze, & Hollozy, 1978), however, these

investigators limited their analysis to describing the general response characteristics. At the time, researchers did not know that the response to a step increase in work-rate had several independent components, also these components were not discernable from the measurement techniques available. More recent work in sedentary (Phillips, Green, MacDonald, & Hughson, 1995) and diseased populations (Brandenburg *et al.*, 1999; Casaburi *et al.*, 1997; Otsuka, Kurihara, Fujii, Fujimoto, & Yoshikawa, 1997) showed that improved VO_2 kinetics primarily resulted from accelerated phase II (primary component) kinetics. Phase II kinetics have been related most often with muscle VO_2 kinetics (Grassi *et al.*, 1996) and may be altered as a result of either improved blood flow delivery (Krustrup, Hellsten, & Bangsbo, 2004; Shoemaker, Phillips, Green, & Hughson, 1996) or reduced metabolic inertia (Grassi, 2001). Endurance training alone or endurance training supplemented with interval training has been shown to effectively reduce the phase II rate constant (τ_2), which infers greater aerobic metabolism throughout a transition between low and high metabolic demand.

Phillips et al. (1995) conducted the most comprehensive aerobic training study that tracked VO_2 kinetics. They noted improvements as early as 4 days after the commencement of training and this observation was independent of changes in the maximal activity of citrate synthase, a marker of aerobic potential. Further, studies expanded on these findings showing that improved performance correlated with phase II improvements (Demarle *et al.*, 2001) and that even during high intensity constant work-rate tests, a faster phase II was apparent (Billat, Mille-Hamard, Demarle, & Koralsztein, 2002). The only study to evaluate VO_2 kinetics following exclusive high intensity

interval training involved unilateral knee-extensor training (Krustrup et al., 2004). In this study, seven weeks of unilateral knee-extensor training improved muscle VO_2 kinetic responses both during low to moderate work-rate transitions and during low to high work-rate transitions (Krustrup et al., 2004).

Blood flow kinetic responses have received limited attention in the context of exercise training. Improved blood flow kinetics may be one possible mechanism that would explain improved VO_2 kinetics with exercise training (Krustrup et al., 2004; Shoemaker et al., 1996). Shoemaker et al. (1996) were the first to measure blood flow kinetics before and after aerobic exercise training involving 8-10 two-hour training sessions for 2 weeks at 65 % of VO_{2peak} and found a reduced time to 63% of the response. This improvement seemed to be linked to improved cardiac output kinetics; however, there were no significant alterations in the speed of the response although the amplitude at t =20s and the total amplitude were greater after endurance training (Shoemaker et al., 1996). With high intensity interval training, Krustrup et al. (2004) showed that training involving 1 minute intervals at an estimated work-rate of 150% of VO_{2peak} improved blood flow delivery (faster kinetic responses and higher amplitudes) during transitions from low to high and low to moderate intensities.

In summary, blood flow kinetics are linked to cardiac output kinetics and may potentially explain improvements in VO_2 kinetics noted with training. The effects of SIT on these parameters have not been evaluated and would provide insight into observed improvements in exercise performance with SIT (Burgomaster et al., 2006; Burgomaster et al., 2005) and VO_{2peak} (Burgomaster et al., 2006; Burgomaster et al., 2008;

Burgomaster et al., 2005).

1.9 Ventricular vascular coupling and exercise

The characteristics of the vasculature and particularly the arterial vasculature ultimately translate proximally toward the heart. Specifically the arterial vasculature determines the net arterial load through a combination of peripheral vascular resistance, total arterial compliance, characteristic impedance and the duration of systole and diastole (Chantler, Lakatta and Najjar, 2008). The initial work of Sunagawa and colleagues effectively created a measure of this net arterial load currently referred to as arterial elastance (E_A). The elastance of the left ventricle (E_{LV}) is also measurable using various techniques and when combined with the E_A this ratio provides an estimate of the interaction of these two parts of the cardiovascular system (Sunagawa, Maughan, Burkhoff, & Sagawa, 1983).

The focus of this thesis is the arterial vascular adaptations and acute responses to sprint exercise and training, however we acknowledge the interactive role of arterial function and stiffness on the left ventricle. Exercise acutely alters E_A and E_{LV} (Little and Chen, 1993) and these parameters may be altered with training. The impact of these alterations is beyond the scope of this thesis but warrants further attention. Previous research describes rapid transitions from rest to strenuous exercise reduce left ventricular performance even in healthy young individuals (Foster, Anholm, Hellman, Carpenter, Pollock, & Schmidt, 1981) and may be a result of an inappropriate E_A/E_{LV} relationship that could be improved with a proper warm-up (Foster, Dymond, Carpenter, & Schmidt, 1982).

1.10 Literature review general summary

The current thesis aims to describe the both the chronic cardiovascular adaptations to SIT in comparison to more widely studied ET methods and the acute effects of SIT exercise on vascular function during the post-exercise recovery period. In our investigations of both the chronic and acute responses, we placed particular emphasis on arterial stiffness and vascular endothelial function. As described, these attributes are important since decreases in both of these parameters are precursors to vascular disease progression and represent some of the first discernable dysfunctions of the cardiovascular system in progressive cardiovascular disease. Unchecked, endothelial dysfunction and arterial stiffness can progress toward vascular disease and increase the risk of cardiovascular morbidity.

This literature review emphasized the chronic effects of endurance exercise training and resistance exercise training on vascular endothelial function and arterial stiffness. As well, the effects of SIT on cardiovascular fitness, exercise performance, and muscle metabolic adaptations were described. However, the chronic effects of SIT training on the arterial vasculature remain an area of interest and potential research.

The literature review also contains detailed descriptions of the divergent impact of endurance exercise and resistance exercise on acute adjustments of arterial endothelial function and stiffness. The acute adjustments to vascular characteristics affect cardiac work, arterial blood pressure regulation, and orthostatic intolerance during the recovery period. Investigations which challenge the cardiovascular system also provide insight into mechanisms contributing to long-term cardiovascular adaptations. Studies aimed at

understanding and describing these acute and chronic responses have not been conducted to date, and the background for the current state of knowledge in the area is outlined in the literature review.

1.11 General hypotheses and aims of the thesis

In planning the current thesi, the overarching theme was that sprint interval exercise generates specific cardiovascular challenges which result in both acute adjustments and chronic adaptations to vascular structure and function. The cardiovascular impact of sprint interval exercise has not been studied beyond measures of cardiorespiratory fitness or tests of exercise performance. The general hypothesis of the current thesis was that similar to ET, SIT would markedly increase cardiorespiratory and vascular endothelial function, decrease arterial stiffness both in peripheral and central arteries, and speed the delivery and use of oxygen during step changes in work intensity. Acutely following sprint interval exercise, we expected similar alterations in both central and peripheral arterial stiffness and vascular function as have been observed with endurance exercise. In general, we expected very similar adaptations and acute adjustments of the vascular system following SIT and acute sprint exercise to that of endurance type exercise and training.

The main research questions of this thesis were addressed using two studies involving exercise training interventions while the acute responses were addressed using separate acute exercise intervention studies. All studies were completed in young healthy populations to minimize confounding factors. The subsequent four chapters of the thesis begin with the chronic studies followed by the acute studies since they were completed in

this order and the rationale for completing acute studies developed from a desire to determine regulatory mechanisms associated with the observed changes with training. The thesis also contains an accompanying appendix describing methodologies and techniques used in the thesis.

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Chapter 2

Sprint interval and traditional endurance training fail to improve oxygen uptake and blood flow kinetic responses in young healthy participants

2.1 INTRODUCTION

Transitions from rest or low intensity activities to higher intensities occur frequently during activities of daily living. Metabolically efficient work-rate transitions are characterized by rapid adjustments to a new steady state with minimal perturbation to the system. Oxidative metabolism is the principle means through which skeletal muscle cells generate the ATP required to maintain normal function. However, during transitions non-oxidative metabolism supplements this process to ensure maintenance of ATP supply and "an oxygen deficit" (i.e. the difference between ATP demand and oxidative ATP production) is incurred, the magnitude of which depends on whether aerobic metabolism can adequately generate ATP. Determining interventions that may attenuate the oxygen deficit during transitions in energy demand are warranted. One effective strategy to reduce oxygen deficit is endurance exercise training (Brandenburg *et al.*, 1999; Casaburi *et al.*, 1997; Otsuka, Kurihara, Fujii, Fujimoto, & Yoshikawa, 1997; Phillips, Green, MacDonald, & Hughson, 1995).

Oxygen uptake kinetics (VO_2 kinetics) are accelerated after aerobic exercise training (Cerretelli, Pendergast, Paganelli, & Rennie, 1979; Hagberg, Hickson, Ehsani, & Holloszy, 1980; Hickson, Bomze, & Holloszy, 1978). Phillips et al. (1995) showed that VO_2 kinetics were accelerated as early as 4 days after the initiation of an endurance

training protocol and that further increases were noted at 8 and 30 days of training. Studies in previously trained individuals also demonstrated that increasing the intensity of training can reduce the phase II time constant (τ_2) (Demarle *et al.*, 2001; Norris & Petersen, 1998). It should be noted that the increased intensity was a result of incorporating interval-training sessions into the endurance training routines of these subjects.

The effectiveness of training may be related to the method (interval vs. continuous) used and one might hypothesize that using an interval based program would be the ideal method to improve oxygen uptake kinetics since participants would experience multiple work-rate transitions during each exercise session. Exclusive interval training has been used in recent studies both to improve fitness in both previously sedentary individuals (Burgomaster, Heigenhauser, & Gibala, 2006; Burgomaster *et al.*, 2008; Burgomaster, Hughes, Heigenhauser, Bradwell, & Gibala, 2005) and those with significant disease (Warburton *et al.*, 2005; Wisloff *et al.*, 2007). However, few studies have evaluated the impact of interval training on cardiovascular kinetic responses (Billat, Mille-Hamard, Demarle, & Koralsztein, 2002; Krustrup, Hellsten, & Bangsbo, 2004). A study by Billat et al. (2002) demonstrated that as few as 2 interval sessions per week for four weeks were required to accelerate the τ_2 during transitions from moderate to heavy exercise. While recent work by Krustrup et al. (2004), showed that muscle oxygen uptake and blood flow kinetics were accelerated during unilateral kicking transitions from light to moderate and light to heavy kicking intensities when training involved unilateral leg interval training 3-5 times per week for seven weeks. However, the small muscle mass

involved in the training may yield unique results not easily extrapolated to whole-body exercise training methods.

Previous studies examining the mechanisms that may account for accelerated VO_2 kinetics upon transition from rest-to-work or work-to-work, have shown enhanced blood flow and thus O_2 delivery following interval and endurance training (Krustrup et al., 2004; Shoemaker, Phillips, Green, & Hughson, 1996). In any case, these studies suggest that both continuous and aerobic interval training can enhance blood flow kinetics and potentially speed VO_2 kinetics. Furthermore, capillarization enhancements through proliferation or function may be involved, at least, with interval training (Krustrup et al., 2004).

Recently, efforts to characterize the physiological responses to whole body sprint interval training (SIT) have shown enhanced muscle oxidative potential (Burgomaster et al., 2008; Burgomaster et al., 2005), aerobic fitness (Burgomaster et al., 2008), and peripheral artery function and structure (Rakobowchuk *et al.*, 2008). As well, this method of exercise has been promoted as a time-efficient strategy to promote improved muscle oxidative capacity. However to date, no study has evaluated whether this method of training improves VO_2 or blood flow kinetics.

Therefore, the purpose of the current study was to evaluate whether whole body SIT accelerates VO_2 and blood flow kinetics at the onset of transitions from light to heavy kicking exercise to a similar degree as traditional endurance training (ET). The heavy exercise domain was selected since previous studies have shown that both traditional ET and aerobic interval training have the potential to accelerate the kinetic

responses of VO_2 during these transitions (Krustrup et al., 2004; Shoemaker et al., 1996). We hypothesized that SIT would be as effective as ET in accelerating the primary or phase II component time constant and that blood flow and cardiac output kinetics would be accelerated in parallel. We also hypothesized that heart rate would demonstrate classic training adaptations in that it would be reduced throughout the transition from low to heavy intensity kicking exercise while stroke volume would be augmented.

2.2 METHODS

2.2.1 Participants

Twenty young healthy men and women (n = 5 men and 5 women per group) volunteered for the study (Table 2.1). A preliminary screening process was employed to establish that subjects: (a) were free of risk factors associated with cardiovascular, pulmonary or metabolic disease; (b) were deemed safe to begin a physical activity program; and (c) other than activities of daily living, were not engaged in a regular training program (i.e. 2 sessions per week, 30 min per session, for at least 1 year prior to the study including recreational activity such as sport or leisure activities). Other exclusion criteria included cardiovascular disease, diabetes, obesity, hypertension (resting blood pressure > 140/90 mmHg), medication use, and smoking as assessed through pre-testing screening. The experimental procedures and potential risks were fully explained to the subjects prior to the study, and all subjects provided written, informed consent. Hamilton Health Sciences Research/McMaster University Faculty of Health Sciences Ethics Board approved the experimental protocol.

2.2.2 Pre-experimental procedures

Subjects initially performed a progressive exercise test (increasing 1 W every 2 s) on an electronically braked cycle ergometer (Lode BV, Excalibur Sport V2.0, the Netherlands) in order to determine their peak oxygen uptake using an on-line gas collection system (Moxus Modular VO2 System, AEI Technologies Inc., Pittsburgh, PA, USA). The value used for VO_{2peak} corresponded to the highest value achieved over a 30 s collection period. All subjects also performed a 30 s test of all out effort (Wingate Test) on the same cycle ergometer against a resistance equivalent to 0.075 kg • (kg body mass)$^{-1}$. After the familiarization procedures, subjects were assigned to either a SIT group or an ET group in a matched fashion based on sex and VO_{2peak}.

On a separate day, subjects performed a progressive exercise test to exhaustion (initial workload of 15W increasing 10W every minute) on a custom designed electronically braked kicking ergometer in order to determine their peak oxygen uptake, using an on-line gas collection system (Moxus Modular VO2 System, AEI Technologies Inc., Pittsburgh, PA, USA). The value used for $VO_{2peakkicking}$ corresponded to the highest value achieved over a 30 s collection period.

Table 2.1 Subject resting characteristics over the course of 6 weeks of either sprint interval or endurance training.

	Sprint (n=10)		Endurance (n=10)	
	PRE	POST	PRE	POST
Age (years)	23.6 ± 3.2	___	23.0 ± 2.4	___
Height (cm)	171.2 ± 7.3	___	175.2 ± 12.1	___
Weight (kg)	69.1 ± 9.4	68.3 ± 8.9	75.4 ± 13.3	74.9 ± 12.7
BMI ($kg \cdot m^{-2}$)	23.6 ± 3.0	23.3 ± 3.0	24.3 ± 2.1	24.2 ± 2.0
Heart Rate (bpm)	57 ± 8	56 ± 5	65 ± 10.0	62 ± 8.0
Brachial DBP (mmHg)	63 ± 5	63 ± 6	66 ± 5	65 ± 5
Brachial SBP (mmHg)	112 ± 9	114 ± 10	124 ± 14	121 ± 13
Brachial MAP (mmHg)	80 ± 6	80 ± 7	85 ± 7	83 ± 7

Data are mean ± SD. Where, BMI is body mass index, BP is blood pressure, D is diastolic, S is systolic and MAP is mean arterial pressure

2.2.3 Training protocol

ET consisted of continuous cycling on an ergometer, 5 days per week (Monday–Friday) for 6 weeks, at a power output corresponding to 65% VO_{2peak}. Subjects performed 40 min of exercise per training session for the first 2 weeks. Exercise time was increased to 50 min per session during weeks 3 and 4, and subjects performed 60 min of exercise per session during the final 2 weeks. VO_{2peak} tests were re-administered after 3 weeks of training and training loads were adjusted in order to maintain a training intensity equivalent to 65% VO_{2peak}. SIT consisted of repeated Wingate tests on an ergometer 3 days per week (Monday, Wednesday and Friday) for 6 weeks. The number of Wingate

tests performed during each training session increased from four during week 1 and 2, to five during week 3 and 4, and finally to six during week 5 and 6. For all training sessions, the recovery interval between Wingate tests was fixed at 4.5 min, during which time subjects cycled at a low cadence (< 50 r.p.m.) against a light resistance (30 W) to reduce venous pooling in the lower extremities and minimize feelings of light-headedness or nausea. The ET program was based on general guidelines recommended by a leading public health agency (ACSM, 2001) whereas the SIT program was modeled on recent studies conducted in our laboratory that have examined metabolic and performance adaptations to low-volume, high-intensity interval training (Burgomaster *et al.*, 2007; Burgomaster et al., 2006; Burgomaster et al., 2008; Burgomaster et al., 2005; Gibala *et al.*, 2006). By design, the protocols differed substantially in terms of total training volume and time commitment in order to evaluate vascular adaptations to two diverse training programs.

2.2.4 Experimental sessions

All participants arrived at the laboratory at the same time of the day for all testing sessions. Time of testing was specific to each subject with some participants arriving in the morning and others in the afternoon. Female participants were tested in the same phase of their individual menstrual cycle to control for this potential confounding factor. Due to the pragmatic constraints of scheduling and the need to perform metabolic measurements (Burgomaster et al., 2008) within a reasonable time, 2 participants were tested during the luteal phase while the remaining 8 were tested during the follicular phase. Prior to arriving in the lab participants were instructed to abstain from caffeine and

no participant was taking medication or using nicotine products for at least 12 hours. Testing sessions were performed 4 hours postprandial following the consumption of a commercially available standardized meal replacement drink (237ml BOOST®, Mead Johnson Nutritionals, Ottawa, ON, Canada). The kicking ergometer apparatus was located in a temperature controlled (22-24°C) room and subjects completed all trials in the upright position and water was available *ad libitum*. Kicking trials were conducted twice prior to training and at 48 and 72 hours following their final exercise session.

2.2.5 Kicking ergometer sessions

Participants completed 3 kicking transition trials per exercise session each separated by 10 minutes of recovery. Each transition trial consisted of 2 minutes of rest followed by an initial transition to a low work-rate of 15 W, which was maintained for 2 minutes. After 2 minutes of kicking another square-wave transition to the heavy kicking intensity exercise occurred and was maintained for 5 minutes. The heavy kicking exercise was subject specific at 80% of $VO_{2peak\ kicking}$, which corresponded to ~ 50% of the difference between gas exchange threshold and $VO_{2peak\ kicking}$. All subjects completed the kicking exercise tests at the same absolute intensity prior to and after the completion of training.

Throughout the transitions, electrocardiography was used to record ventricular depolarization via a one lead set-up (V_5 configuration) (Powerlab model ML2340, ADInstruments, Colorado Springs, USA), while simultaneous measurements of continuous mean arterial blood pressure were acquired by photoplethsmography (Finapres Ohmeda 2300, Amsterdam, The Netherlands) with the finger placed at the level

of the common femoral artery. Both signals were acquired and recorded using commercially available hardware (Powerlab model ML795, ADInstruments, Colorado Springs, USA) and software (Chart 5, ADInstruments, Colorado Springs, CO, USA). Breath-by-breath ventilation (V_E) and pulmonary gas exchange were continuously measured using an on-line gas collection system (Moxus Modular VO2 System, AEI Technologies Inc., Pittsburgh, PA, USA) to determine minute ventilation (V_E), VO_2, carbon dioxide output (VCO_2) and the respiratory exchange ratio (RER). Before each test, the gas analyzers were calibrated to precision-analyzed gases of known concentrations and a 3L syringe was used to calibrate the flow turbine.

2.2.6 Femoral blood flow acquisition

Blood flow was calculated in a subset of eleven subjects (6-ET and 5-SIT) in whom, it was possible to image the common femoral artery for continuous measurement of blood flow via pulsed Doppler ultrasound. Throughout the kicking sessions, the left common femoral artery was insonated using duplex mode imaging and Doppler ultrasound (System FiVe, GE Medical Systems, Horten, Norway) to acquire beat by beat measurements of blood flow. Longitudinal images and Doppler signals were acquired using a 10 MHz duplex linear (PW= 4 MHz) array probe positioned distal to the inguinal ligament and ~2cm proximal to the bifurcation of the common femoral artery into its' profundus and superficial branches. The raw audio signal corresponding to blood velocity was output from the Doppler ultrasound system to an external spectral analysis system (model Neurovision 500M TCD, Multigon Industries, Yonkers, NY) which applies a fast Fourier transform to the raw audio signal to determine MBV continuously. Blood

velocity was corrected for insonation angle during post acquisition analysis. Mean blood velocity (MBV), like all other physiological signals, was acquired and recorded using the previously described Powerlab system.

As mentioned, B-mode images were acquired simultaneously to Doppler signals and collected continuously via sVHS videotape and analyzed for artery diameter with ultrasonic calipers. Maximal and minimal vessel diameters of 2 sequential heart cycles throughout the transition from light to heavy kicking exercise were determined. Specifically, two heart cycles immediately before the transition and every 10s during the first minute of exercise, and then at 1-minute intervals to the end of exercise were used. These diameter data were fit with an exponential regression to obtain an average response and to reduce random error. Following this, the regressions were used to determine the diameter at all time-points throughout the transition from light to heavy kicking exercise in accordance with MBV data for the calculation of mean blood flow (MBF = femoral artery cross-sectional area x MBV).

2.2.7 Cardiac output acquisition

Cardiac output was acquired using a combination of B-mode imaging of the aortic root measured at rest and continuous wave Doppler ultrasound during the kicking transitions. A total of nine full heart cycle high-resolution (60 Hz) video clips were obtained in the parasternal long axis orientation while subjects were positioned in the left lateral decubitus position using a 2.5 MHz phased array probe (System FiVe, GE Medical Systems, Horten, The Netherlands). Two additional M-mode images were taken according to the American Society of Echocardiography guidelines when the orientation

of the heart was appropriate. Digital video clips and appropriate M-mode images were subsequently transferred for offline analysis using commercially available software (Echopac 6.4.2, GE Medical Systems, Horten, The Netherlands). Ultrasonic calipers were used to measure aortic root diameter in triplicate at both end-diastole and end systole. A weighted mean (1/3 systolic + 2/3 diastolic) was used to calculate mean aortic diameter.

Continuous-wave Doppler was used to acquire aortic root blood velocity throughout the transitions from light to heavy kicking exercise. A 2.0 MHz continuous-wave probe (GE Vingmed CFM-800, Horten, Norway) was positioned at the suprasternal notch with an orientation that produced the greatest positive blood velocity signal. A fast Fourier transform was applied to the signal internal to the ultrasound machine. Plug flow was assumed at the aortic root and therefore, the positive blood velocity envelope was output from the Doppler to the data acquisition system (Powerlab model ML795, ADInstruments, Colorado Springs, USA) simultaneous to all other cardiovascular parameters.

2.2.8 Data analysis

For the purposes of the current study, the transition from light to heavy kicking exercise was used since previous investigations found that transitions below the gas exchange threshold showed no training related adaptations in this population during kicking exercise (Krustrup et al., 2004). Breath-by-breath VO_2 data were interpolated and averaged every second to allow multiple trials to be time aligned to the point of transition between light and heavy kicking exercise using commercially available software (Matlab

R2007a, The Mathworks Inc., Natick, MA, USA). The transitions (4-6 PRE and 6 POST) were then averaged to increase the signal to noise ratio and the underlying physiological responses. In most participants PRE training, 6 transitions were used; however, two of the transitions from two participants were lost due to data storage issues.

Blood velocity data were visually inspected for signal integrity and portions of poor signal quality were excluded from the raw data. Blood velocity data was subsequently filtered to remove non-physiological variation due to movement using the method described by Ferreira et al. (2006). Briefly, previous work using spectral analysis of the blood velocity signal determined that frequencies above 0.2 Hz corresponded to movement and heart rate artifact and that applying a band-pass filter to these data effectively removed this inherent noise (Ferreira, Harper, & Barstow, 2006). This observation was confirmed in the present data set using spectral analysis of the blood velocity signal (Rakobowchuk unpublished observations). As such, the data were filtered using a 0.2 Hz low-pass filter. The filtered data were then interpolated and averaged every second to allow multiple trials to be time aligned to the point of transition between light and heavy kicking exercise using commercially available software (Matlab R2007a, The Mathworks Inc., Natick, MA, USA).

Cardiac output (Q) data were analyzed similar to femoral artery blood velocity data in that, the signal was visually inspected for aberrant beats. Beat-by-beat time-velocity integrals (TVI) were determined from the raw data and subsequently interpolated, averaged every second and time aligned to the start of the transition. ECG was used to determine heart rate continuously. Similar to TVI, heart rate was determined

beat by beat, interpolated, averaged every second and time aligned to the start of the transition using commercially available software (Matlab R2007a, The Mathworks Inc., Natick, MA, USA). Calculation of Q was determined using the following equations:

$$SV = TVI \times (\text{Aortic mean diameter}/2)^2 \times \pi$$

$$Q = HR \times SV$$

Where SV is stroke volume, TVI is the time-velocity integral and HR is heart rate.

Finally, estimates of myocardial demand were obtained by calculating the tension time index (TTI), which is the integral of the systolic portion of the blood pressure curve, while rate pressure product (RPP = TTI x HR) was also determined throughout each of the transitions. Similar to VO_2, MBF, and Q, the RPP and TTI were determined beat by beat, interpolated, averaged every second and time aligned to the start of the transition using commercially available software (Matlab R2007a, The Mathworks Inc., Natick, MA, USA).

2.2.9 Modeling and averaging of cardiovascular parameters

Graphical overlay of the average pre and post training VO_2, Q, HR, SV, TTI, RPP and MAP profiles of each individual subject, revealed no obvious kinetic differences. Therefore, rather than determine the kinetic responses, a discrete time-point analysis was conducted. A local mean of 5s surrounding the times of -30, -15, 15, 30, 60, 120, 180, and 240s relative to the initiation of the transition were determined for each subject.

Modeling of blood flow, which was calculated as previously described, was performed using nonlinear regression techniques of eleven participants with high-quality data. A two-component exponential pattern was observed in all but 2 of the subjects who

displayed a late rise of blood flow and as such, were fit with a three-component exponential both before and after training. Both participants who displayed 3 component kinetic responses were in the ET group.

The three-component model equation incorporates the one and two-component models and is as follows:

$$Y(t) = G_{bl} + G_1 \bullet (1-e^{-(t-TD_1)/\tau_1}) + G_2 \bullet (1-e^{-(t-TD_2)/\tau_2}) + G_3 \bullet (1-e^{-(t-TD_3)/\tau_3})$$

Where G_{bl} is the baseline light intensity exercise value, TD_1, TD_2 and are the time delays from the onset of the transition to the first, second and third components; G_1 is the change in the amplitude above G_{bl} within the first component, G_2 is the change in amplitude above G_1 within the second component and G_3 represents the change in amplitude above G_2 within the third component at end exercise; τ_1, τ_2, and τ_3 are the time constants for each component. As previously described by Hughson et al. (1988), in the models TD_1 was constrained to be equal to, or greater than zero. Time to 63% of the total response was calculated using the following equation:

$$T_{63\%} = (G_1/(G_1+G_2+G_3) \times (TD_1 + \tau_1) + (G_2/(G_1+G_2+G_3) \times (TD_2 + \tau_2) (G_3/(G_1+G_2+G_3) \times (TD_3 + \tau_3)$$

Where G, TD and τ are the gains, time delays, and time constants as described above. The $T_{63\%}$ is equivalent to the mean response time (MRT). The following figure (Figure 2.1) illustrates the parameters and a simple response to an abrupt change in work-rate.

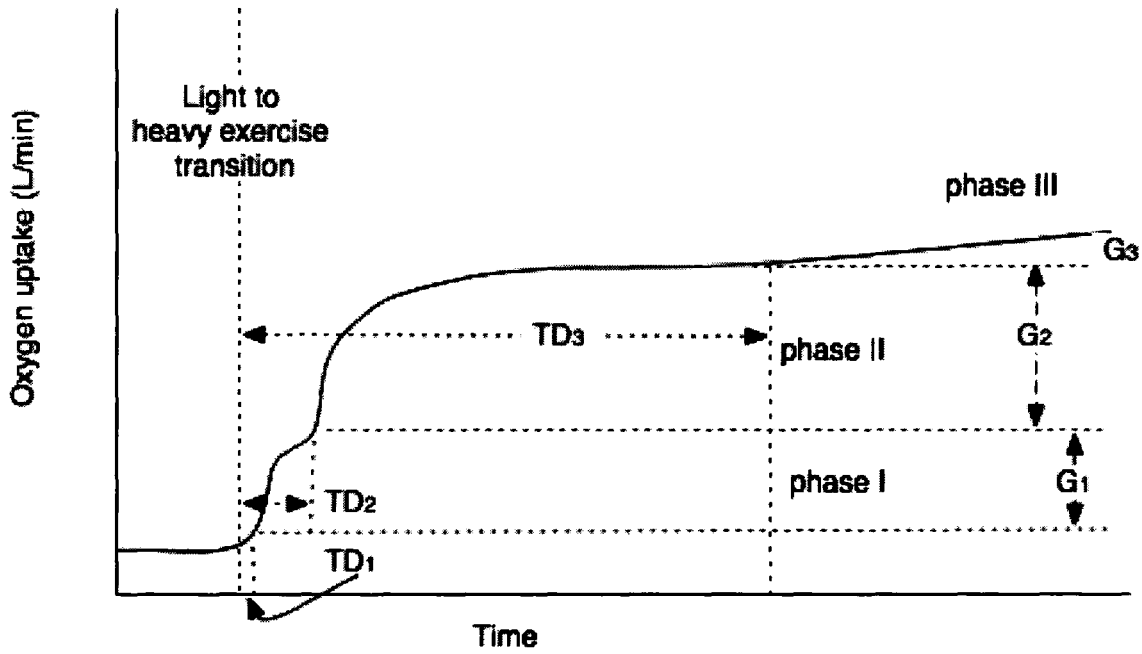

Figure 2.1 A representation of an abrupt change in work-rate from light to heavy exercise and the various parameters used to model the response. TD corresponds to the various time delays, G corresponds to the various gains of each phase. Not illustrated are the time constants that describe the rate of change of each phase and are denoted typically as τ.

2.2.10 Statistical analysis

Discrete measurements of VO_2, Q, SV, TTI, RPP and MAP were analyzed using a three-way mixed analysis of variance, with the repeated factors "Time" (-15, -30, 15, 30, 60, 120, 180, and 240s) and "training" (PRE vs. POST), and the between factor "Group" (SIT vs. ET) using commercially available software (SPSS 11.0 for Mac OS X, SPSS Inc. Chicago, IL). HR was analyzed using a 2-way repeated measures analysis of variance with the repeated measures of "time" (-15, -30, 15,30,60,120,180 and 240s) and "training" (PRE vs. POST) individually on each group. The change score between PRE and POST was calculated and used to compare the groups using independent t-tests at each time point. Parameters derived from the fitting of the femoral blood flow data were

compared using a 2-way repeated measures analysis of variance, with the repeated factor "training" (PRE vs. POST) and the between factor "Group" (SIT vs. ET). When a significant main effect was noted, Bonferroni corrected pair-wise comparisons were made to determine differences. Significance for all analysis was set at $P \leq 0.05$. All values are presented as mean ± standard error of the mean (SEM). Blood flow parameters were only compared over the first 2 phases and MRT was only calculated over the first 2 phases since only 2 data sets required the 3 component model.

2.3 RESULTS

2.3.1 Evidence of a training effect and average weekly work

Training increased VO_{2peak}, with no difference between groups (SIT: PRE 41± 2, POST 44 ± 2, ET: PRE 41 ± 2, POST 45 ± 2 $ml \cdot kg^{-1} \cdot min^{-1}$) (Burgomaster et al., 2008). As previously described, training reduced exercising HR and improved Wingate peak power with no differences between groups (Burgomaster et al., 2008). The SIT group performed on average 225 kJ of work per week while the ET group performed on average 2250 kJ of work per week. The average workload for the sprint intervals was ~500W while the ET workload was ~150 W.

2.3.2 Heart rate and arterial blood pressure responses during light to heavy kicking exercise transitions

Heart rate reductions with training were analyzed separately and revealed some difference between the SIT and ET. Specifically, both groups showed reduced HR during heavy kicking exercise; however, before the transition (light exercise) only the ET group

displayed reduced HR (Figure 2.2). When the change of heart rate with training was calculated at each time point and compared between the SIT and ET there were main effects for time indicating a greater difference at the end exercise time-point (240s) compared to one of the low intensity measures prior to the transition (-15s) (Post hoc for Time $p=0.049$). All other time-points were not different. There was also a group effect indicating a greater overall HR reduction in the endurance group (Figure 2.2c, $p=0.048$).

Mean arterial pressure responses were not altered with training and showed similar increases throughout the transition from light to heavy kicking exercise (main effect for Time, $p<0.001$, Figure 2.3).

2.3.3 VO_2 during light to heavy kicking exercise transitions

Oxygen uptake increased throughout the transition from stable levels observed during light exercise (-30 and -15s) (main effect for Time, $p<0.001$, Figure 2.4). There was a significant Time x Group interaction, in which the ET group had higher oxygen uptake 120 and 180s of exercise compared to the SIT group. However, this difference was observed both before and after training (no Time x Training x Group interaction $p>0.05$)

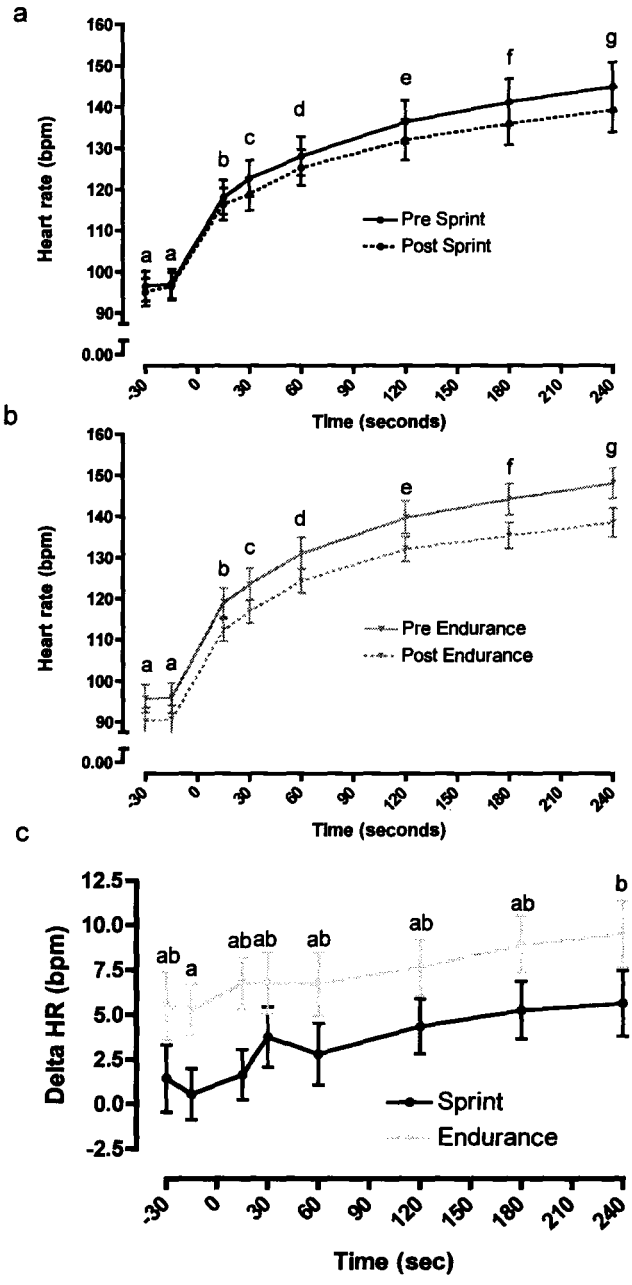

Figure 2.2 Average heart rate response from light (-30s to 0s) to heavy (0s to 240s) kicking exercise before and after training. Training resulted in a significant reduction in both the ET and SIT groups. Means with different letters are significantly different from each other. * Indicates a significant difference with training.

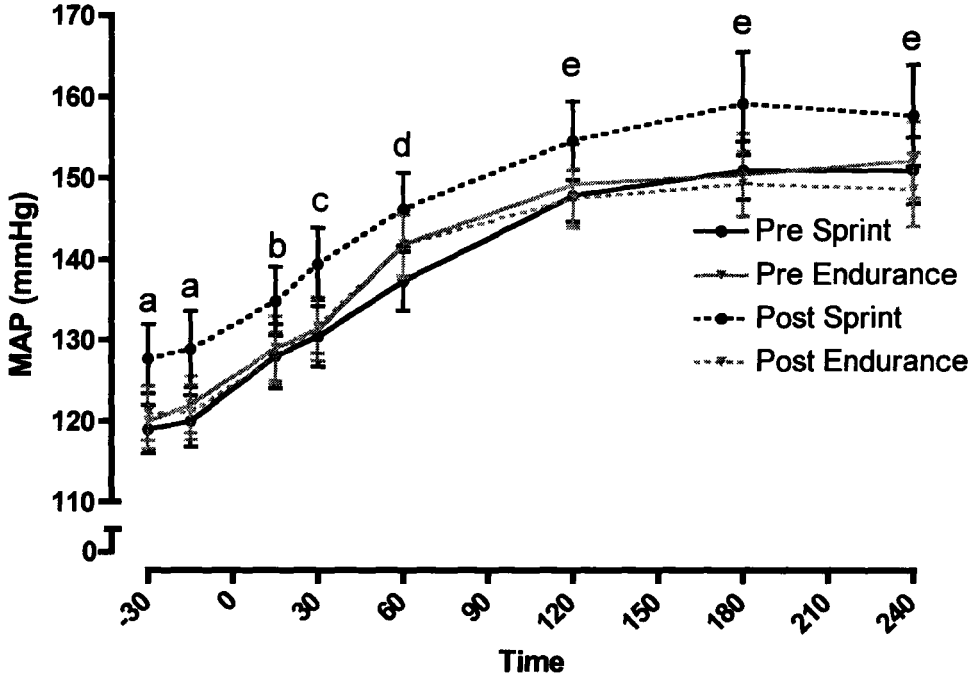

Figure 2.3 Average mean arterial pressure response from light (-30s to 0s) to heavy (0s to 240s) kicking exercise before and after training. Means with different letters are significantly different from each other. No significant differences were noted with training.

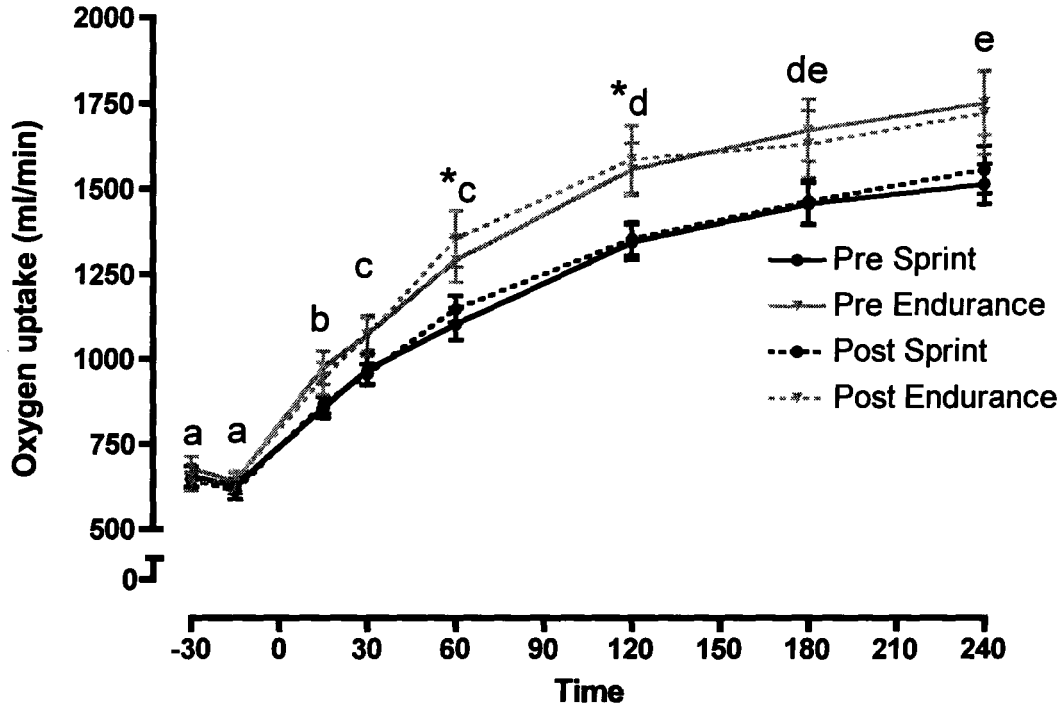

Figure 2.4 Average VO_2 response from light (-30s to 0s) to heavy (0s to 240s) kicking exercise before and after training. Means with different letters are significantly different from each other. * Indicates significant difference between the SIT and ET at specific time points. No significant differences were noted with training in either group.

2.3.4 Femoral artery blood flow kinetic responses during light to heavy kicking exercise transitions

Femoral artery blood flow kinetic response parameters are summarized in Table 2.2. MRT ($p=0.28$), G_1 ($p=0.59$), G_2 ($p=0.28$) were not altered with either sprint or endurance training. Baseline blood flow during the last 60 seconds of light exercise just prior to the transition (G_{bl}), showed a Training x Group interaction with the ET group having lower femoral blood flow at baseline post training, while the SIT group had similar levels of flow pre and post training. TD_1 also exhibited a Training x Group interaction with the sprint group having an increased TD_1 post training. Finally, G_2 exhibited a Training x Group interaction with the endurance group having an increased G_2 post training which was also greater than that of the sprint group at the post training time-point. No differences were noted in any the other parameters. It should be noted that G_3, TD_3 and τ_3 were not compared since only 2 participants displayed this type of response. The summary of the blood flow response is illustrated in Figure 2.5.

2.3.5 Cardiac output, stroke volume and estimated heart work during light to heavy kicking exercise transitions

Cardiac output increased from baseline (-30 and -15s) to reach a stable plateau at 180s following the transition (main effect for Time, $p<0.001$, Figure 2.6). Stoke volume was elevated at all time points following training (main effect for Training, $p=0.038$, Figure 2.7).

TTI increased from stable baseline to reach steady-state levels at 180 and 240 seconds in both SIT and ET groups. There were no differences with training ($p= 0.58$, Figure 2.8). RPP also increased throughout the transition above baseline levels. There was a significant Time x Training interaction indicating that after training there was a plateau between 120 and 240 seconds which was not observed before training ($p<0.01$, Figure 2.9a). There was also a significant Training x Group interaction with the ET exhibiting reduced RPP post training compared to pre training ($p=0.012$, Figure 2.9b), whereas the SIT group showed no changes with training ($p=0.70$, Figure 2.9b)

Table 2.2 Blood flow kinetics parameters during transitions from light to heavy kicking exercise

	Sprint		Endurance	
	PRE	POST	PRE	POST
MRT (sec)	183.5 ± 47.5	245.1 ± 55.1	163.7 ± 86.9	158.8 ± 63.4
G_{bl}, L/min	1.31 ± 0.05	1.27 ± 0.13	1.47 ± 0.11	0.98 ± 0.04*
G_t, ml/min	1.30 ± 0.18	1.18 ± 0.03	1.55 ± 0.26	2.16 ± 0.0.27*
G_1, L/min	0.58 ± 0.16	0.46 ± 0.09	0.50 ± 0.11	0.76 ± 0.06
TD_1, s	3.7 ± 0.7	6.8 ± 2.0*	3.9 ± 0.6	2.25 ± 0.5
τ_1, s	7.5 ± 2.4	7.8 ± 4.0	5.5 ± 1.6	7.8 ± 1.8
G_2, L/min	0.71 ± 0.10	0.72 ± 0.12	0.72 ± 0.17	0.99 ± 0.23
TD_2, s	28.9 ± 8.6	34.9 ± 12.0	20.9 ± 4.5	24.2 ± 3.8
τ_2	55.5 ± 17.8	53.9 ± 10.6	42.4 ± 18.7	52.9 ± 17.7
G_3, L/min			0.98 ± 0.02	1.21 ± 0.32
TD_3, s			107.8 ± 22.4	171.1 ± 22.7
τ_3, s			233.3 ± 63.3	169.45 ± 0.69.2

Data are means ± SEM. G_{bl} is light intensity exercise blood flow; G_t is the total gain; G_1, G_2 and $G_3$are the phase I, II and III gains; TD_1, TD_2 and TD_3 are the phase I, II and III time delays; τ_1, τ_2, and τ_3 are the phase I, II and III time constants. * Indicates a significant effect for training $p<0.05$. Eleven subjects were included with 5 in the SIT group and 6 in the ET group.

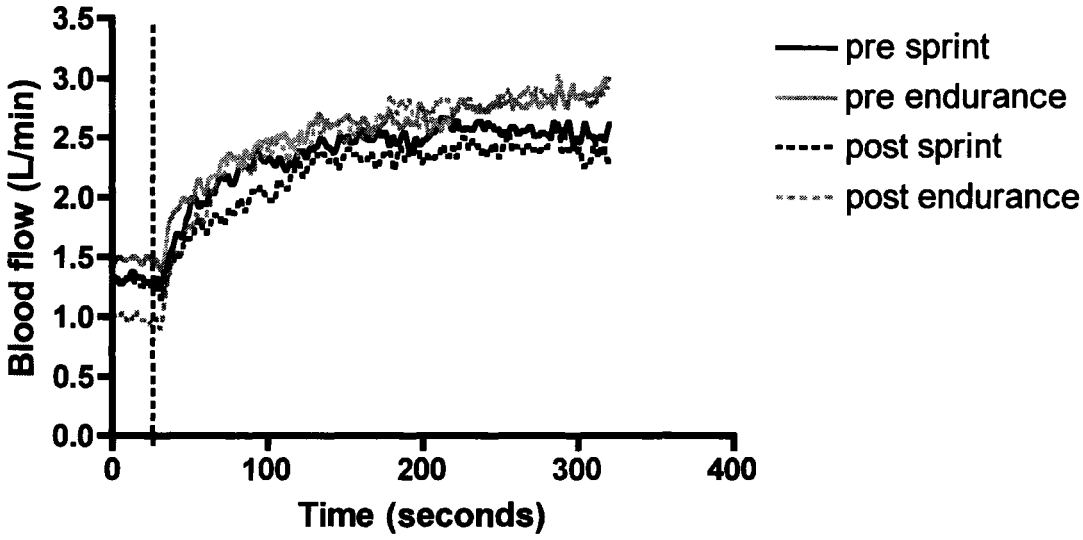

Figure 2.5 Blood flow kinetics before and after sprint interval and endurance training.

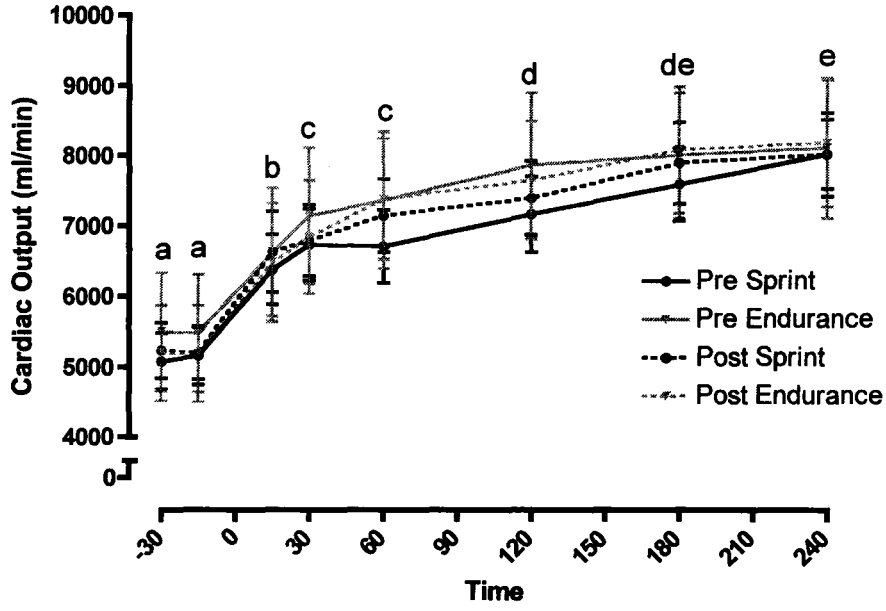

Figure 2.6 Average cardiac output response from light (-30s to 0s) to heavy (0s to 240s) kicking exercise before and after training. Means with different letters are significantly different from each other. No significant differences were noted with training in either group.

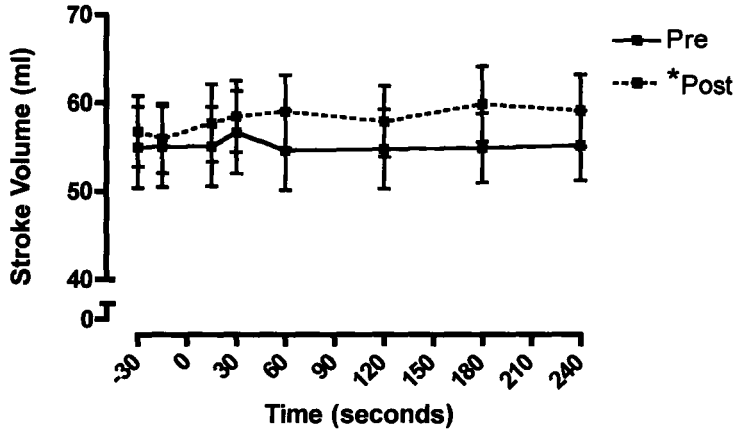

Figure 2.7 Average stroke volume response from light (-30s to 0s) to heavy (0s to 240s) kicking exercise before and after training. Main effect for training indicating a greater stroke volume following training, with no difference noted between groups.

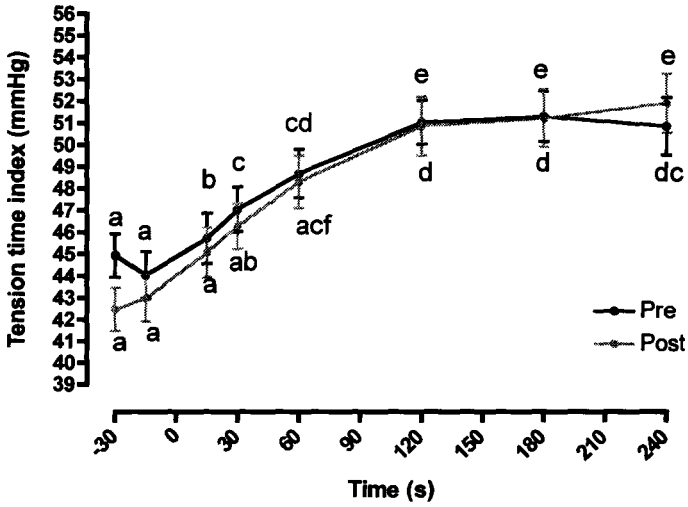

Figure 2.8 Average tension time index response from light (-30s to 0s) to heavy (0s to 240s) kicking exercise before and after training. Means with different letters are significantly different from one another with the letters above the means corresponding to Pre training and letters below corresponding to Post training.

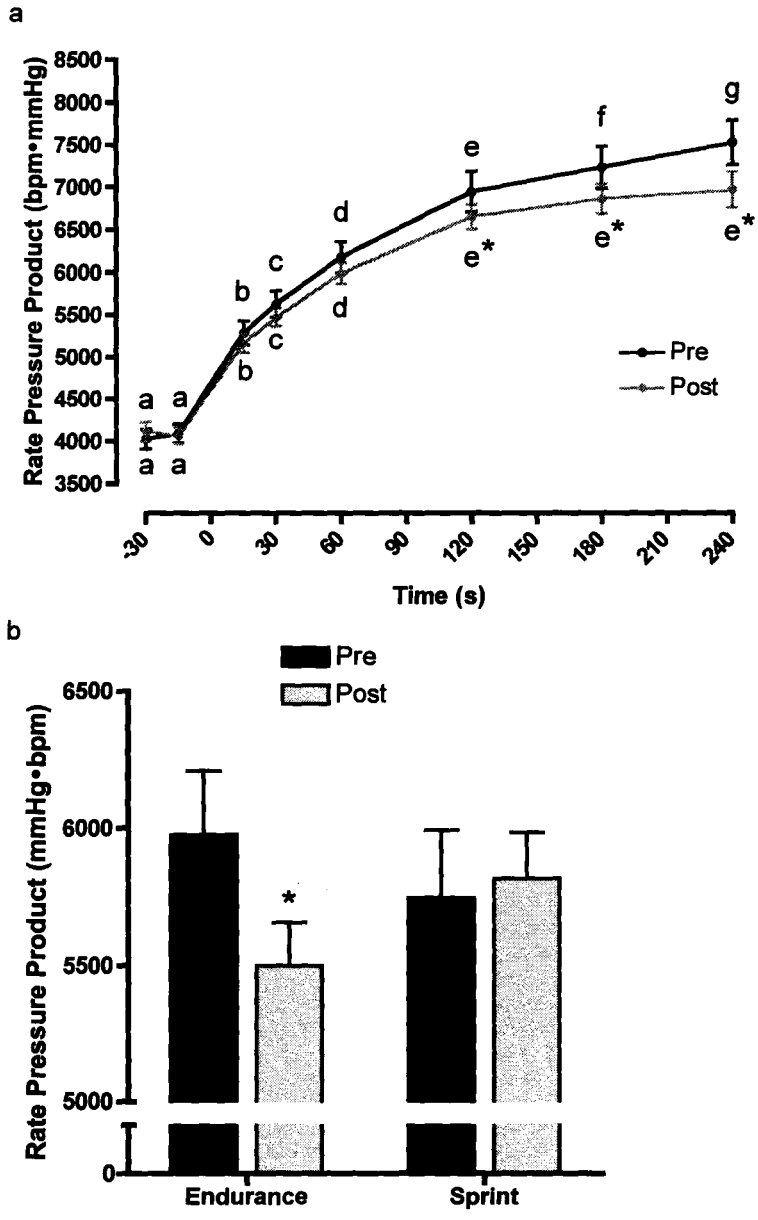

Figure 2.9 Average rate pressure product a) response from light (-30s to 0s) to heavy (0s to 240s) kicking exercise before and after training. Means with different letters are significantly different from each other, while b) describes the training x group interaction with * indicating a significant reduction of RPP with training in the ET group.

2.4 DISCUSSION

The current study is the first to compare the training effects of SIT and ET on cardiovascular parameters during submaximal exercise transitions. Major interesting observations relate to central aspects of the cardiovascular system. First, there was a reduction of resting, submaximal (light) and high intensity submaximal (heavy) heart rates with concomitant increases in stroke volume. Although both groups displayed some cardiac efficiency improvements, only the ET group showed decreased RPP with training. Other noteworthy findings include an increased VO_{2max}, however this was not accompanied by improved phase II VO_2, or blood flow kinetics, which was contrary to our hypothesis.

2.4.1 Oxygen delivery with training

We hypothesized in the current study that we would see an improvement or speeding of the blood flow and VO_2 uptake response to a step transition from a light to heavy kicking exercise level. However, this was not apparent. In the ET group, baseline blood flow was lower following training however, the responses of the various components were not greatly altered and the main variable of interest, the phase II flow response was unaltered in either group. These findings do not support those of Shoemaker et al. (1996) or Krustrup et al. (2004) who showed speeding of the blood flow response with exercise training. It is possible that the current study may be limited by the rest intervals between transitions. If the first transition from light to heavy kicking exercise was great enough to induce a persistent metabolic acidosis similar to previous studies examining the effects of prior exercise (MacDonald, Pedersen, & Hughson, 1997;

Paterson, Kowalchuk, & Paterson, 2005; Rossiter *et al.*, 2001), then averaging of all three kicking transitions on each day may have obscured training induced changes. This is supported by the fact that magnitude of the training induced speeding of both blood flow and VO_2 kinetics is not much greater than the effects of prior heavy exercise (MacDonald et al., 1997; Phillips et al., 1995). Therefore, it is possible that larger rest periods between kicking trials were needed in order to observe training induced changes.

2.4.2 Oxygen consumption with ET and SIT

We hypothesized that VO_2 kinetics would be accelerated following training in both the ET and SIT groups. This was not observed in the current study unlike that of Krustrup et al. (2004) who noted enhanced oxygen consumption at the muscle level with unilateral high intensity aerobic interval training during high intensity transitions. Methodological differences between the two studies may account for the contrasting results. First, the study by Krustrup et al. (2004) used a training model specific to the muscle being tested (i.e. knee extensor training and transitions). Therefore, a training specificity effect may be one difference. Second, we used a measure of whole-body VO_2 rather than at the level of the muscle. Although the phase II VO_2 response measured at the mouth is believed to be very similar to the exercising muscle VO_2 (Jones & Poole, 2005), it is possible that small changes in muscle level kinetics could not be resolved using whole body measures. This may be especially true at high intensities when the VO_2 response is complex. Further breakdown of subjects into groups who responded to training and those who did not may reveal differences, however the lack of statistical power as a result of further breakdown of the groups likely precludes this possibility.

In the current study, the ET group also did not show an accelerated VO_2 response with training. This is contrary to the work of Phillips et al. (1995), who demonstrated faster kinetics as early as 4 days after the initiation of training. Once again, this difference may be related to training specificity in that the participants in the current study were trained using a cycle ergometer and tested using a kicking ergometer. As well, the VO_2 requirements of cycling at the same relative intensity compared to kicking are markedly greater. Potentially, if the VO_2 kinetics trials in the current investigation were performed on a cycle ergometer, a greater signal to noise ratio might be evident due to the higher VO_2 amplitude achieved during each transition. This greater signal to noise ratio would allow smaller differences in VO_2 kinetic responses to be resolved.

From a metabolic control perspective, the findings of the current study do raise an important point. Previous studies show that this type of training increases the active fraction of the rate determining enzyme for carboxyl group entry into the TCA cycle (PDHa), reduces muscle glycogen depletion and reduces lactate accumulation during constant load exercise (Burgomaster et al., 2006). It had also been proposed that PDHa might have a regulatory role in the time course of oxidative metabolism during transitions of work rate (Howlett, Heigenhauser, Hultman, Hollidge-Horvat, & Spriet, 1999). The results of the current study provides evidence that PDHa may not be the rate determining process of oxidative metabolism at the start of exercise since VO_2 kinetics were not altered; even though the training stimuli likely enhanced PDH activation (Burgomaster et al., 2006).

2.4.3 Central cardiovascular responses to training evident during exercise

As outlined, both training methods effectively reduced steady-state heart rate during heavy kicking exercise. However, ET also reduced light exercise HR, which was not the case with SIT. This is the first time that SIT has been shown to reduce exercising heart rate and importantly, there were no overall differences in the noted reductions between SIT and ET groups (no Group x Training interaction). Previous work has shown no changes in exercising HR with short-term SIT (Burgomaster et al., 2006), however a study involving lower intensity intervals showed similar heart rate reduction during constant load exercise (Fox *et al.*, 1973). Beyond heart rate, the current study was able to establish that stroke volume is increased with both training methods. This has been proposed previously with high intensity interval training (Fox et al., 1973) and observed with ET (Levy, Cerqueira, Abrass, Schwartz, & Stratton, 1993; Matsuda *et al.*, 1983).

One unique difference between the training responses of the two groups studied was the reduction of the exercising RPP in the endurance-trained group while this estimate of myocardial demand was not altered with SIT. This adaptation may be related to the magnitude of the volume load experienced by each group. Since the ET group was training for longer periods, and more frequently, it is likely that the heart experienced a much greater volume load during ET in comparison with SIT. Since both groups reduced their submaximal exercising heart rates with training, the RPP differences between the groups must be related to a differential effect of training on the integrated systolic portion of the pressure curve in that ET either maintained or reduced this area while SIT increased this area. No significant Group x Training interaction was noted with the TTI

(the integral of the systolic portion of the pressure curve) however there was a trend ($p=0.13$) with the SIT group showing a general increase with training. Mechanistically, changes of the TTI are related to either a longer period of systole and/or a greater systolic peak. This may be related to increased arterial stiffness of the central vessels, or an early return of the reflected wave from the lower body (Nichols, McDonald, & O'Rourke, 2005). Further study that evaluates arterial compliance during exercise before and after SIT in large central arteries is required to confirm this hypothetical idea.

2.4.4 Limitations and context

There are several limitations to the data presented in this study. First, the calculation of the tension time index and the rate pressure product is derived from finger plethysmography. This method does not provide values that are true estimates of myocardial demand and can only be used to estimate the relative change in myocardial demand noted with training. As well, it may be argued that the placement of the finger at the level of the leg would contribute to inaccuracies regarding the absolute value of the TTI. We acknowledge that this is likely, however; as outlined this method can only provide a relative measure with training due to pulse pressure amplification throughout the arterial tree (Nichols et al., 2005), thus any additional discrepancy between measures at the heart and those calculated in the present study would be present all time points regardless of finger placement. It may be argued that a transfer function would be a more appropriate systolic integral to use however; the validity of the transferred blood pressure waveform is questionable during exercise.

2.4.5 Summary

In conclusion, the present study demonstrates that heart rate reductions are evident both during repeated submaximal exercise following a time-efficient sprint interval training method and that these alterations were similar to those of endurance training. However, myocardial demand during exercise transitions was attenuated with ET while no changes were noted with SIT. As well, it should be noted that as previously shown, maximal oxygen uptake was improved; however, submaximally, no adaptation of the oxidative system in response to a step increase in work rate was evident. From a clinical and cardiac rehabilitation perspective, SIT training is effective in attenuating exercise heart rate responses; however, some of the benefits of ET in reducing cardiac work seem superior to those of SIT with relatively short-term training.

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Chapter 3

Sprint interval and traditional endurance training induce similar improvements in peripheral arterial stiffness and flow mediated dilation in healthy humans

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3.1 INTRODUCTION

The distensibility of the arterial tree has an important regulatory impact on cardiac performance, perfusion and homeostasis (Kingwell, 2002). A stiff arterial tree is also associated with adverse cardiovascular events (Boutouyrie *et al.*, 2002). As well, decreased peripheral artery distensibility impacts coronary circulation through quicker pulsewave reflection, which augments systolic pressure while concomitantly lowering diastolic pressure and thus coronary perfusion pressure (Safar & Lacolley, 2007).

Traditional moderate-intensity exercise training improves central artery distensibility in populations with impaired vasculature (Hayashi, Sugawara, Komine, Maeda, & Yokoi, 2005; Sugawara *et al.*, 2006; Tanaka *et al.*, 2000) and most training studies showing improvements of artery distensibility have noted changes in the central arterial tree (aorta or carotid arteries) (Hayashi et al., 2005; Kawano, Tanaka, & Miyachi, 2006; Sugawara et al., 2006; Tanaka et al., 2000; Tordi *et al.*, 2006) while peripheral muscular arteries commonly show no exercise training induced improvements (Cook *et al.*, 2006; Hayashi et al., 2005; Petersen *et al.*, 2006; Tanaka, DeSouza, & Seals, 1998). However, these investigations of peripheral muscular artery distensibility were made in the relatively stiff common femoral artery (Cook et al., 2006; Hayashi et al., 2005; Petersen et al., 2006; Tanaka et al., 1998).

Brachial endothelial function is a surrogate indicator of coronary endothelial function and an independent measure of atherosclerotic disease risk (Schachinger, Britten, & Zeiher, 2000; Suwaidi *et al.*, 2000; Vita, 2005). As well, coronary artery disease patients exhibit reduced popliteal artery flow-mediated dilation (Angerer, Negut, Stork, & von Schacky, 2001). Similar to artery distensibility, exercise training is a potent stimulus that improves brachial flow-mediated dilation and endothelial function in young healthy (Clarkson *et al.*, 1999; DeSouza *et al.*, 2000) and diseased populations (Maiorana *et al.*, 2000; Maiorana *et al.*, 2001). The popliteal artery, unlike the brachial artery, is a common site of peripheral vascular disease and displays unique elastic-like properties (Debasso *et al.*, 2004). To date, it has not been established whether exercise training can improve the structural and functional properties of this disease prone artery. In models of integrated vascular physiology, structure and function are tightly linked with decreased peripheral artery distensibility and endothelial function often occurring in concert, thereby creating an environment where disease progression accelerates. A recent review highlights the importance of exercise training in modifying traditional cardiovascular risk factors such as hypercholesterolemia and hypertension (Green, O'Driscoll, Joyner, & Cable, 2008). However, 40% of the reduction of cardiovascular disease risk attributed to exercise cannot be explained by modifications of the mentioned risk factors (Green et al., 2008). Other vascular indices such as artery endothelial function and distensibility may provide useful information about the link between exercise stimuli and cardiovascular risk reduction (Green et al., 2008).

Recently, there has been renewed interest in interval training models, particularly

sprint interval (above 100% peak aerobic power) training because of evidence that the ensuing metabolic adaptations mirror those observed after traditional endurance training (Burgomaster *et al.*, 2007; Burgomaster, Heigenhauser, & Gibala, 2006; Burgomaster, Hughes, Heigenhauser, Bradwell, & Gibala, 2005; MacDougall *et al.*, 1998). High-intensity interval training has been shown to accelerate the kinetic responses of leg blood flow and oxygen uptake at the onset of high intensity single leg kicking exercise, indicating a more efficient cardiovascular response system (Krustrup, Hellsten, & Bangsbo, 2004). As well, studies in rodents reveal many alterations in vascular structure and function and eNOS protein expression with this type of training (Laughlin, Woodman, Schrage, Gute, & Price, 2004). However, the mechanisms responsible for these adaptations have not been fully examined. We suspect that changes in vascular structure and function may impact the kinetic responses of skeletal muscle blood flow at the onset of exercise.

Therefore, the purpose of the current study was to evaluate whether 6 weeks of high intensity, low volume, sprint interval training (SIT) improves central (carotid) artery distensibility and, peripheral (popliteal) artery distensibility and endothelial function in the trained legs to the same extent as high-volume, moderate intensity endurance training (ET). We hypothesized that central artery distensibility would increase to a similar degree with both training methods (ET and SIT). Further we hypothesized that popliteal artery distensibility would improve in concert with enhanced endothelial function indicating improved peripheral vascular structure and function. Note that metabolic and performance adaptations to the training protocol have been previously described in a

separate publication (Burgomaster *et al.*, 2008).

3.2 METHODS

3.2.1 Subjects

Twenty young healthy men and women (n = 5 men and 5 women per group) volunteered for the study (Table 3.1). A preliminary screening process was employed to establish that subjects: (a) were free of risk factors associated with cardiovascular, pulmonary or metabolic disease; (b) were deemed safe to begin a physical activity program; and (c) other than activities of daily living, were not engaged in a regular training program (i.e. 2 sessions per week and 30 min per session, for at least 1 year prior to the study including recreational activity such as sport or leisure activities). Other exclusion criteria included cardiovascular disease, diabetes, obesity, hypertension (resting blood pressure > 140/90 mmHg), medication use, and smoking as assessed through pre-testing screening. The experimental procedures and potential risks were fully explained to the subjects prior to the study, and all subjects provided written, informed consent. Hamilton Health Sciences Research Ethics Board approved the experimental protocol.

Table 3.1 Subject characteristics over the course of 6 weeks of either sprint interval or endurance training.

	Sprint (n=10)		Endurance (n=10)	
	PRE	POST	PRE	POST
Age (years)	23.6 ± 3.2	___	23.0 ± 2.4	___
Height (cm)	171.2 ± 7.3	___	175.2 ± 12.1	___
Weight (kg)	69.1 ± 9.4	68.3 ± 8.9	75.4 ± 13.3	74.9 ± 12.7
BMI ($kg \cdot m^{-2}$)	23.6 ± 3.0	23.3 ± 3.0	24.3 ± 2.1	24.2 ± 2.0
Heart Rate (bpm)	57 ± 8	56 ± 5	65 ± 10.0	62 ± 8.0
Brachial DBP (mmHg)	63 ± 5	63 ± 6	66 ± 5	65 ± 5
Brachial SBP (mmHg)	112 ± 9	114 ± 10	124 ± 14	121 ± 13
Brachial MAP (mmHg)	80 ± 6	80 ± 7	85 ± 7	83 ± 7

Data are mean ± SD. Where, BMI is body mass index, BP is blood pressure, D is diastolic, S is systolic and MAP is mean arterial pressure

3.2.2 Pre-experimental procedures

Subjects initially performed a progressive exercise test (increasing 1 W every 2 s) on an electronically braked cycle ergometer (Lode BV, Excalibur Sport V2.0, the Netherlands) in order to determine their peak oxygen uptake using an on-line gas collection system (Moxus Modular VO2 System, AEI Technologies Inc., Pittsburgh, PA, USA). The value used for VO_{2peak} corresponded to the highest value achieved over a 30 s collection period. All subjects also performed a 30 s test of all out effort (Wingate Test) on the same cycle ergometer against a resistance equivalent to 0.075 kg • (kg body mass)$^{-1}$. After the familiarization procedures, subjects were randomly assigned to either a SIT) group or an ET group in a matched fashion based on sex and VO_{2peak}.

3.2.3 Training protocol

ET consisted of continuous cycling on an ergometer, 5 days per week (Monday–Friday) for 6 weeks, at a power output corresponding to 65% VO_{2peak}. Subjects performed 40 min of exercise per training session for the first 2 weeks. Exercise time was increased to 50 min per session during weeks 3 and 4, and subjects performed 60 min of exercise per session during the final 2 weeks. VO_{2peak} tests were re-administered after 3 weeks of training and training loads were adjusted in order to maintain a training intensity equivalent to 65% VO_{2peak}. SIT consisted of repeated Wingate Tests on an ergometer 3 days per week (Monday, Wednesday and Friday) for 6 weeks. The number of Wingate Tests performed during each training session increased from four during week 1 and 2, to five during week 3 and 4, and finally to six during week 5 and 6. For all training sessions,

the recovery interval between Wingate Tests was fixed at 4.5 min, during which time subjects cycled at a low cadence (< 50 r.p.m.) against a light resistance (30 W) to reduce venous pooling in the lower extremities and minimize feelings of light-headedness or nausea. The ET program was based on general guidelines recommended by a leading public health agency (ACSM, 2001) whereas the SIT program was modeled on recent studies conducted in our laboratory that have examined metabolic and performance adaptations to low-volume, high-intensity interval training (Burgomaster et al., 2007; Burgomaster et al., 2006; Burgomaster et al., 2008; Burgomaster et al., 2005; Gibala *et al.*, 2006). By design, the protocols differed substantially in terms of total training volume and time commitment in order to evaluate vascular adaptations to two diverse training programs.

3.2.4 Vascular assessment sessions

All participants arrived at the laboratory at the same time of the day (at all testing sessions) for all vascular assessments. Time of testing was specific to each subject with some participants arriving in the morning and others in the afternoon. Female participants were tested in the same phase of their individual menstrual cycle to control for this potential confounding factor. Due to the pragmatic constraints of scheduling and the need to perform metabolic measurements (Burgomaster et al., 2008) within a reasonable time, 2 participants were tested during the luteal phase while the remaining 8 were tested during the follicular phase. Further, the 2 participants tested during the luteal phase were subsequently removed from the endothelial function data set due to poor image quality. Therefore, all female participants included in the endothelial function portion of the

experiment were in the follicular phase at both pre and post testing time points. Prior to arriving in the lab participants were instructed to abstain from caffeine and no participant was taking medication or using nicotine products for at least 12 hours. Testing sessions were performed 4 hours postprandial following the consumption of a commercially available standardized meal replacement drink (237ml BOOST®, Mead Johnson Nutritionals, Ottawa, ON, Canada) to control for the acute effects of diet. Measurements were taken while subjects were in the supine position in a temperature controlled (22-24°C) room. Vascular measurements were conducted twice prior to the initiation of training and at 48 and 72 hours following their final exercise session. All measurements were taken following a 20-min supine rest period. Because no differences were noted between the 2 PRE and 2 POST testing sessions, the average of these tests was used for subsequent analysis.

Resting heart rate, central and peripheral arterial blood pressure

Electrocardiography was used to record ventricular depolarization via a one lead set-up (V_5 configuration), while simultaneous measurements of continuous brachial blood pressure were acquired by automated applanation tonometry (model CBM-7000, Colin Medical Instruments, San Antonio, USA). Both signals were acquired and recorded using commercially available hardware (Powerlab model ML795, ADInstruments, Colorado Springs, USA) and software (Chart 5, ADInstruments, Colorado Springs, CO, USA).

3.2.5 Direct arterial distensibility

Measurements of vascular structure and artery distensibility were determined using two methods by the same investigator (MR) who has 6 years of experience imaging

the peripheral vasculature in similar research applications. Arterial distensibility in the vessel of interest (carotid or popliteal artery) was assessed directly using a combination of Ultrasound imaging (System FiVe, GE Medical Systems, Horten, The Netherlands) for the measurement of lumen diameter and vessel specific blood pressure via applanation tonometry (model SPT-301, Millar Instruments Inc., Texas, USA) or automated oscillatory cuff. These methods have been previously described (Rakobowchuk *et al.*, 2005), but have been modified slightly. Briefly, the same investigator throughout the protocol imaged the carotid artery and used baseline images as visual feedback to ensure similar ultrasound probe placement and imaging of the common carotid artery 2-3cm proximal to the bifurcation. At the popliteal artery, the designated peripheral artery, measurements were made either proximal or distal to the branching of the middle genicular artery, yet consistent within each subject as verified by visualization of landmarks. This variation between subjects was needed to ensure the highest possible image quality.

Two ultrasound video clips of ten heart cycles each were acquired at a frame rate of 15 frames per second, simultaneous to measurements of carotid or ankle pulse pressure. Simultaneous to imaging at the carotid artery, a hand-held pressure transducer (model SPT-301, Millar Instruments Inc., Texas, USA) sensitive to hold-down pressure, was held against the carotid artery to acquire arterial blood pressure waveforms while simultaneous measurements of continuous absolute brachial blood pressure was obtained for the purpose of calibrating the carotid waveforms to diastolic and mean pressures (model CBM-7000, Colin Medical Instruments, San Antonio, USA). The Colin model

CBM-7000 device combines blood pressure from a brachial cuff and a wrist sensor to determine beat-to-beat brachial blood pressure. The device calibrates the radial blood pressure waveform to the brachial cuff derived blood pressure thus giving the equivalent of beat-to-beat brachial blood pressure. This modification of previous methods (Kelly, Hayward, Avolio, & O'Rourke, 1989) simply provides brachial blood pressure for each beat so that carotid blood pressure calibration is beat specific. Briefly, it was assumed both DBP and MAP are similar in all conduit arteries when an individual is in the supine position while SBP is amplified through the arterial tree (Nichols, Hartley, McDonald, & O'Rourke, 1998). The mean and minimum BP values obtained from the carotid waveform were equated to the MAP and DBP of the radial artery. The maximum BP waveform value recorded in the carotid artery was then used as an extrapolation point from the calibrated MAP and DBP. For popliteal measurements, ankle pulse pressure from 2 ankle cuff (model CBM-7000, Colin Medical Instruments, San Antonio, USA) derived measurements was used because brachial cuff values do not correlate with posterior tibial pressures due to pulsewave amplification.

All video clips used to determine artery distensibility were analyzed by the same investigator using a semi-automated edge detection software program (AMS II, Chalmers University of Technology, Göteborg, Sweden).

The twenty measurements of diameter change were subsequently used to calculate distensibility. The following equation was used to calculate distensibility (O'Rourke, Staessen, Vlachopoulos, Duprez, & Plante, 2002):

$$\text{Dist} = \frac{\pi(d_{max}/2)^2 - \pi(d_{min}/2)^2}{\pi(d_{min}/2)^2 \bullet PP}$$

Where, Dist is distensibility, d_{max} is maximum diameter, d_{min} is minimum diameter and PP is pulse pressure.

3.2.6 Vascular structure measurements

Arterial diameters acquired for arterial distensibility measurements were also used to determine resting vascular structure. Measurements of minimum, maximum and mean arterial diameter were determined from carotid and popliteal arteries as described above. Mean arterial diameter was determined using a weighted average calculation (1/3 x systolic diameter + 2/3 x diastolic diameter). Intima-media thickness (IMT) was also determined from carotid images. The average of twenty frames was used to determine IMT from images taken at end-diastole and each frame consisted of between 150-200 measures of IMT within a designated region of interest (AMS II, Chalmers University of Technology, Göteborg, Sweden).

3.2.7 Vascular function of the popliteal artery

Flow mediated dilation (FMD) was used to assess vascular function in the legs at the popliteal artery using a combination of Doppler Ultrasound and B-mode imaging. Participants were positioned prone throughout the FMD protocol. Briefly, a pneumatic cuff connected to a rapid inflation system (model E20 and AG101, Hokanson, Bellevue, WA) was placed around the leg 2-3 cm distal to the popliteal fossa. The cuff was inflated to a pressure of at least 250 mmHg to ensure complete occlusion of the popliteal artery. Occlusion was maintained for a period of 5-min. Longitudinal popliteal artery images and

blood velocity measurements were made using a 10 MHz (18 participants) or 5MHz (2 participants) linear array pulse Doppler ultrasound probe (System FiVe, GE Medical Systems, Horten, Norway) which was positioned ~3-5 cm proximal to the popliteal fossa either 2 cm proximal or distal to the branching of the middle genicular artery. This was consistent between testing days within each subject to ensure maximal image quality. All pre images were available to the ultrasonographer and displayed on an additional monitor throughout subsequent testing to ensure identical probe placement. Continuous video recording of the image of the popliteal artery was obtained from15s prior to cuff deflation until 4 min following cuff deflation. In addition, a single heart cycle digital video clip was stored at 15s intervals from 30s to 4 min following cuff deflation. This digital video clip contained images acquired at a rate of 15Hz. Simultaneous to the imaging of the popliteal artery, mean blood velocity (MBV) was obtained using the duplex function of the previously described linear array probe 15s prior to cuff deflation until 25s after cuff release to determine peak and mean post-occlusion blood flow and shear rates. The raw audio signal corresponding to blood velocity was output from the Doppler ultrasound system to an external spectral analysis system (model Neurovision 500M TCD, Multigon Industries, Yonkers, NY) which applies a fast Fourier transform (FFT) to the raw audio signal to determine MBV continuously. Blood velocity was corrected for insonation angle during post acquisition analysis. MBV, like all other physiological signals, was acquired and recorded using the previously described Powerlab system.

3.2.8 Image analysis of relative flow mediated dilation

Using semi-automated analysis software (AMS II, Chalmers University of

Technology, Göteborg, Sweden) diameters at end-diastole were acquired from leading edge to leading edge from all images acquired for 4 min post occlusion. The maximal post-occlusion end-diastolic value was compared to resting end-diastolic diameters and expressed as a relative change. The following equation was used to calculate relative FMD (Corretti *et al.*, 2002):

$$FMD = \frac{FMD_{\text{peak end diastolic diameter}} - Resting_{\text{end diastolic diameter}} \times 100}{Resting_{\text{end diastolic diameter}}}$$

3.2.9 Post-occlusion reactive hyperemia

As previously described, blood velocity measurements were acquired 15s prior to until 25s following cuff release. Mean blood flow was calculated (vessel cross-sectional area x MBV) and used to quantify the hyperaemic response. Also, mean wall shear rate (MWSR) was determined as:

$$MWSR = \frac{4 \times MBV}{\text{mean diameter.}}$$

Where MBV is mean blood velocity.

3.2.9 Normalized flow mediated diameter

Resultant measurements of flow-mediated dilation were normalized to the average MWSR during the first 25 s after cuff release since the amount of dilation is dependent on the resultant hyperaemic flow stimulus as represented by mean wall shear rate (Pyke & Tschakovsky, 2007). The following equation was used:

$$\text{normalized FMD} = \frac{\text{relative FMD}}{MWSR_{25s}}$$

3.2.10 Reproducibility of measurements

The reproducibility of the measurements in our laboratory was determined in the 20 participants of this study through evaluation of all measures at 2 time-points prior to training separated by 5-7 days. All participants underwent identical procedures to those outlined above on both of these testing days. Carotid diameter, pulse pressure, and distensibility showed very good reproducibility with coefficients of variation of 2%, 8% and 8% respectively. Popliteal diameter, pulse pressure and distensibility also showed good reproducibility with coefficients of variation of 2%, 8%, and 18%, respectively. Measurements of IMT also showed very good reproducibility with a CV of 5%. Relative popliteal flow mediated dilatation also showed reproducibility with a CV of 28% in 16 participants.

3.2.11 Data Analysis and statistics

Data are expressed as mean ± SD. Measures acquired twice prior to and twice following training were averaged. All variables were analyzed using a two-way mixed analysis of variance, with the repeated factor "Time" (PRE vs POST) and the between factor "Group" (SIT vs ET) using commercially available software (SPSS 11.0 for Mac OS X, SPSS Inc. Chicago, IL). Significance for all analysis was set at $P \leq 0.05$. Analyses of popliteal parameters were performed on 18 rather than 20 participants due to image quality issues with one participant from each group, which were removed from the dataset. Analysis of FMD was performed on 16 participants rather than 20 because of image quality issues with two participants from each of the groups, which were removed from the dataset.

3.3 RESULTS

3.3.1 Evidence of a training effect and average weekly work

Training increased VO_{2peak}, with no difference between groups (SIT: PRE 41± 2, POST 44 ± 2, ET: PRE 41 ± 2, POST 45 ± 2) (Burgomaster et al., 2008). As previously described, training reduced steady-state exercising HR and improved Wingate peak power with no differences between groups (Burgomaster et al., 2008). The SIT group performed on average 225 kJ of work per week while the ET group performed on average 2250 kJ of work per week. The average workload for the sprint intervals was ~500W while the ET workload was ~150 W.

3.3.2 Heart rate and resting arterial blood pressure

Resting heart rate ($p=0.16$) and brachial blood pressure were not significantly altered (SBP $p=0.69$, DBP $p=0.38$) with training in either group (Table 1).

3.3.3 Arterial distensibility and structure

Popliteal artery distensibility was increased after training in both groups ($p<0.01$, main effect for time) while differences of carotid artery distensibility were not statistically significant (Figure 3.1, $p=0.29$). Ankle pulse pressure was not statistically altered with training in either group (Table 3.2, $p=0.41$). However, the change in popliteal cross-sectional area within each heart cycle (delta CSA) increased after training in both groups (Table 3.2, $p<0.01$). Resting arterial structure, as estimated by mean diameter, was not statistically different with training in either the carotid ($p=0.10$) or popliteal arteries ($p=0.10$) (Table 3.2). IMT was not statistically different after training (Table 3.2,

p=0.69)).

3.3.4 Vascular function assessed by flow mediated dilation

Absolute popliteal artery flow mediated dilation was improved with training (PRE: 0.28 ± 0.03 mm vs. 0.36 ± 0.03mm); however, it did not reach statistically significant levels (p=0.06). When normalized to resting end diastolic diameter popliteal relative flow mediated dilation increased after training in both groups (p=0.05) (Figure 3.2a). The enhanced endothelial-dependent dilation was also apparent in both groups after normalization to post-occlusion MWSR (Figure 3.2b, p=0.047).

Resting blood flow in the popliteal artery was not statistically altered with training (Table 3.3). Post occlusion blood flow and MWSR following cuff release evaluated during the flow mediated dilation test were also not statistically different (Table 3.3, p=0.23).

Table 3.2 Vascular structure changes with sprint interval or endurance training.

	Sprint (n=10)		Endurance (n=10)	
	PRE	POST	PRE	POST
Carotid IMT (mm)	0.43 ± 0.04	0.42 ± 0.04	0.46 ± 0.06	0.46 ± 0.06
Carotid Mean Dia (mm)	6.3 ± 0.3	6.3 ± 0.3	6.2 ± 0.5	6.1 ± 0.5
Carotid Delta CSA within the heart cycle (mm^2)	7.0 ± 0.4	6.6 ± 0.4	6.5 ± 0.4	6.4 ± 0.4
Carotid PP (mmHg)	39 ± 8	40 ± 9	42 ± 8	43 ± 7
Popliteal Mean Dia (mm)	5.5 ± 0.5	5.3 ± 0.6	5.6 ± 0.8	5.4 ± 0.8
Popliteal Delta CSA within the heart cycle (mm^2)	2.0 ± 0.5	3.0 ± 1.2*	2.4 ± 1.1	3.4 ± 1.4*
Ankle PP (mmHg)	60 ± 8	61 ± 6	64 ± 5	66 ± 6

Data are mean ± SD, * $p \leq 0.05$ versus PRE, main effect for time. Where, Dia is diameter, CSA is cross-sectional area, and PP is pulse pressure

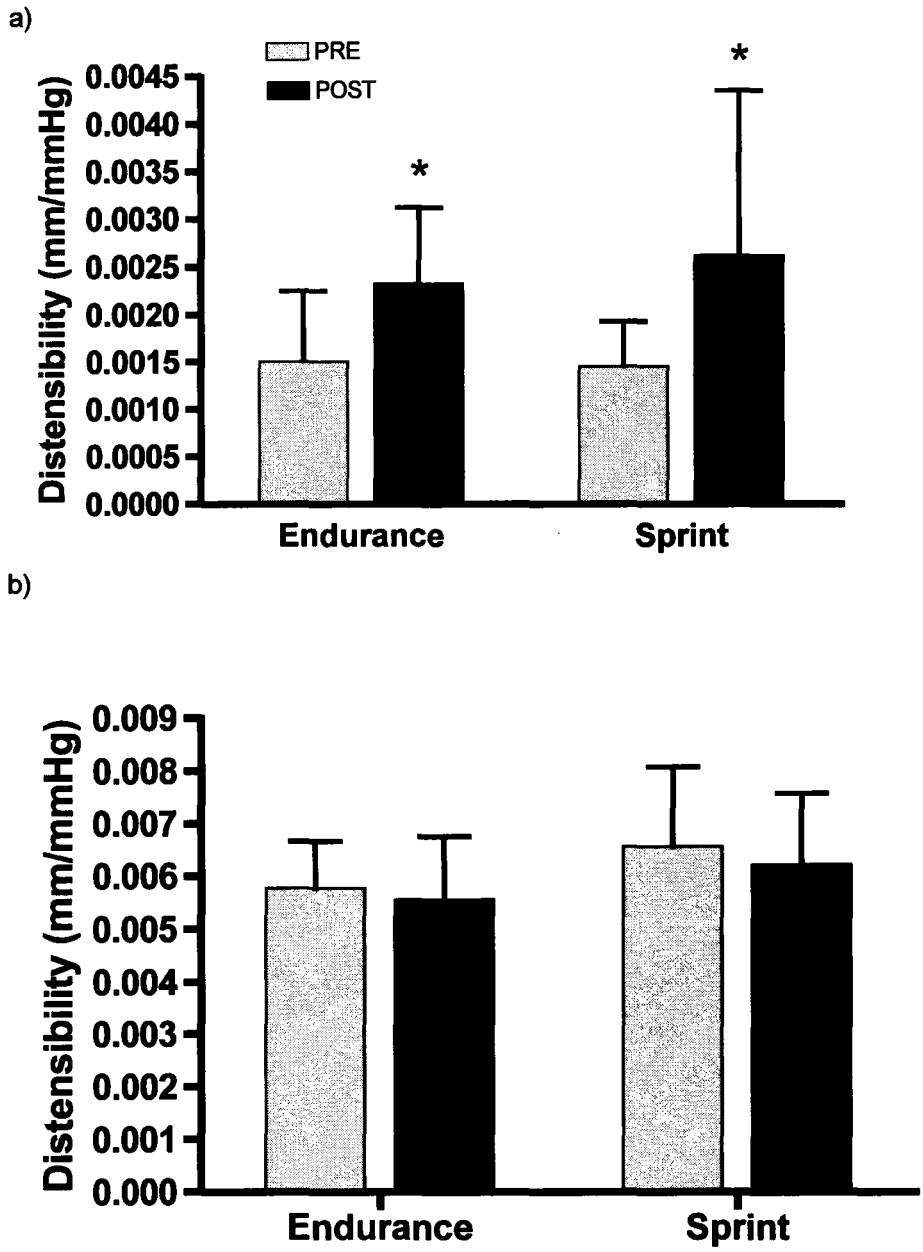

Figure 3.1 Arterial distensibility of the a) popliteal and b) carotid arteries before and after 6 weeks of either sprint interval (SIT) or endurance training (ET). Popliteal artery distensibility was higher after training in both groups, whereas carotid artery distensibility was unchanged. Values are mean ± SD, n = 10 per group. * $P<0.05$ versus PRE, main effect for TIME.

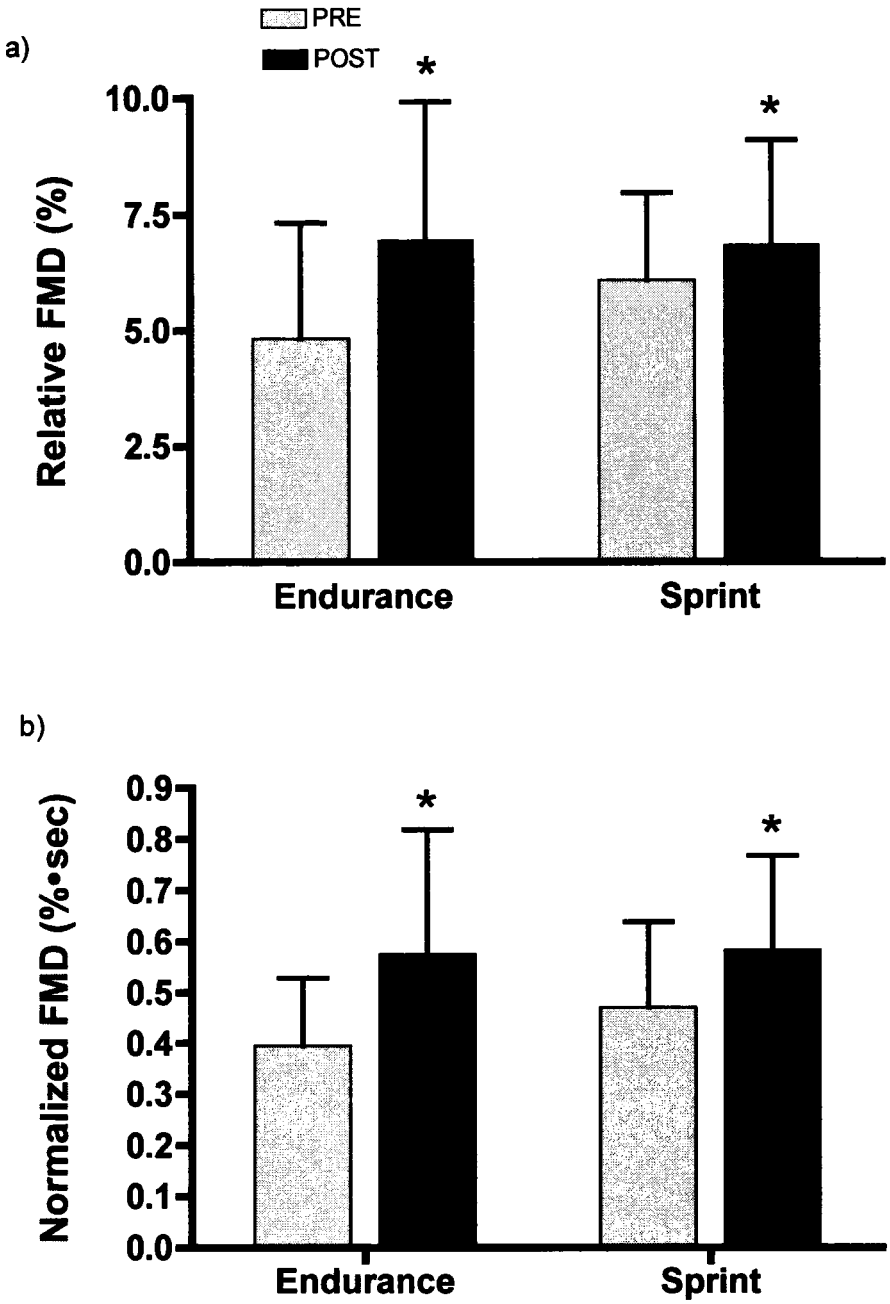

Figure 3.2 Relative (a) and normalized (b) flow mediated dilation of the popliteal artery before and after 6 weeks of either sprint interval (SIT) or endurance training (ET). Relative and normalized popliteal FMD was higher after training in both groups. Values are mean ± SD, n=8 per group. * $P<0.05$ versus PRE, main effect for TIME.

Table 3.3 Flow and shear rate at rest and following cuff release (FMD protocol) before and after sprint interval or endurance training

	Sprint (n=10)		Endurance (n=10)	
	PRE	POST	PRE	POST
REST				
Popliteal MBV ($cm \cdot sec^{-1}$)	1.2 ± 0.4	1.4 ± 0.4	1.6 ± 0.8	1.6 ± 1.1
Popliteal MBF ($ml \cdot min^{-1}$)	17 ± 6	18 ± 7	25 ± 16	24 ± 24
Popliteal MWSR (sec^{-1})	0.9 ± 0.3	1.0 ± 0.4	1.2 ± 0.7	1.1 ± 0.6
REACTIVE HYPERAEMIA				
Popliteal MBV_{25s} ($cm \cdot sec^{-1}$)	17.9 ± 1.8	16.7 ± 1.5	18.4 ± 1.7	16.5 ± 1.4
Popliteal MBF_{25s} ($ml \cdot min^{-1}$)	244 ± 48	226± 76	250 ± 100	216 ± 52
Popliteal $MWSR_{25s}$ (sec^{-1})	13.5 ± 3.9	12.5 ± 2.7	13.9 ± 4.8	12.8 ± 4.2

Data are mean ± SD, Where, MBV is mean blood velocity, MBF is mean blood flow, MWSR is mean wall shear rate. Reactive hyperaemic variables are the 25s average.

3.4 DISCUSSION

To our knowledge, this is the first study to show that both low-volume SIT and traditional high volume ET improve popliteal artery distensibility and endothelial function to the same extent in young healthy men and women. As previously reported, the time commitment and total training volumes were much lower with SIT (Burgomaster et al., 2008) compared to ET. As well, contrary to recent studies of high-intensity training (Bergholm *et al.*, 1999; Goto *et al.*, 2003), endothelial function improved with SIT. Finally, the increases in VO_{2peak} of ~10% show the effectiveness of both ET and SIT training programs.

3.4.1 Alterations of peripheral arterial distensibility

The current observation of improved popliteal artery distensibility is unique because peripheral distensibility is not often measured and in cases when it has been examined there is usually no indication of change with exercise training (Cook et al., 2006; Hayashi et al., 2005; Petersen et al., 2006). Only one study that evaluated stiffness in the superficial femoral artery showed improvements with training in older participants (Thijssen, de Groot, Smits, & Hopman, 2007). Possible reasons for the contrasting findings may include differences in exercise modes (rowers) (Cook et al., 2006; Petersen et al., 2006), and populations (elderly) (Hayashi et al., 2005). Recent evidence suggests the popliteal artery has some structural and functional characteristics of an elastic artery rather than a muscular artery since it exhibits age-related stiffening (Debasso et al., 2004). The popliteal artery may therefore be a site of significant vascular disease and

may be a prime target for exercise intervention.

The mechanisms responsible for training induced improvements in popliteal artery distensibility may be local alterations in vessel wall structural and/or alterations in vascular tone. Substances such as endothelin-1 (ET-1), nitric oxide (NO), prostaglandins, or reactive oxygen species (ROS) regulate resting vascular tone along side sympathetic output and are influenced by training (Goto et al., 2003; Maeda *et al.*, 2001; Tordi et al., 2006). Specifically, ET has been shown to decrease ET-1 (Maeda et al., 2001) increase basal NO levels (Tordi et al., 2006) and reduce basal ROS levels (Goto et al., 2003), which combine to improve vascular tone and potentially artery stiffness. Structural alterations such as collagen/elastin ratios, reductions of uncoiled collagen fibres, and fewer frayed elastic fibres may contribute to an altered extracellular matrix and a less stiff artery (Zieman, Melenovsky, & Kass, 2005). Finally, basal sympathetic tone, which has been shown to acutely regulate distensibility (Sonesson, Vernersson, Hansen, & Lanne, 1997), may be altered. Resting diameters of both the popliteal and carotid artery and resting brachial blood pressure were not statistically different with either exercise training program, limiting the likelihood that basal sympathetic nervous tone accounts for the observed improvements in popliteal distensibility.

3.4.2 Unaltered central artery distensibility

In central arteries like the carotid artery, age-associated stiffening is often reversed or attenuated with ET (Hayashi et al., 2005; Sugawara et al., 2006; Tanaka et al., 2000; Tordi et al., 2006) and with combined endurance-strength training (Cook et al., 2006). However, similar to the current study, young sedentary participants do not exhibit

differences compared to endurance-trained athletes (Tanaka et al., 2000). Our participants had relatively high levels of baseline carotid distensibility compared to older cohorts leaving little room for improvement with training (Tanaka et al., 2000). This is evident from our carotid distensibility measures, which were quite high and similar to those of healthy young subjects previously reported by Miyachi et al. (Miyachi *et al.*, 2004) (~0.007 mm/mmHg).

3.4.3 Structural adaptations- IMT, artery diameter size

Contrary to our hypothesis, resting central and peripheral artery structure estimated using end-diastolic diameters and carotid IMT were not statistically altered with either training program in the current study. Similar to previous research, IMT in our group was low at baseline, which likely explains why there was no reduction (Tanaka *et al.*, 2002). ET lasting 6 weeks has been shown previously to result in increased cross-sectional area of the common femoral artery (Miyachi, Iemitsu, Okutsu, & Onodera, 1998). Contrary to these results, our study did not show an increased popliteal artery diameter which may be related to our observation of improved distensibility or a different time-course of adaptation specific to this artery (Jasperse & Laughlin, 2006).

3.4.4 Endothelial function in the exercised limb

Popliteal artery endothelial function, another measure associated with cardiovascular disease, was improved equally with both training methods. Relative and normalized FMD increased while the mean hyperaemic blood flow response to occlusion was not statistically different with training. Enhanced FMD accompanied by no change in post-occlusion blood flow points specifically to a training induced improvement. Had

there been an increase of the shear stimulus following training, the stimulus may have been the cause of increased FMD rather than exercise training. Mechanistically, improved endothelial function likely relates to reduced oxidative stress (Goto et al., 2003), an improved antioxidant defense system (Rush, Laughlin, Woodman, & Price, 2000) (circulating and reactive oxygen species scavenging enzyme capacity) or an upregulation of endothelial nitric oxide synthase (eNOS) gene expression (Hambrecht *et al.*, 2003). All of these factors would improve NO bioavailability upon shear-induced endothelial stimulation and have been noted previously with training.

The current observation of improved popliteal FMD contrasts with the results of two previous studies in healthy young populations (Bergholm et al., 1999; Goto et al., 2003) that have shown no improvement (Goto et al., 2003) or reduced endothelial function (Bergholm et al., 1999). In the current study, vascular assessment sessions were conducted at 48 and 72 hours following the last training session. This delay before measurement was designed to limit the effects of oxidative stress induced by the final training session on FMD, which has been noted by other researchers following ischemia inducing exercise (McGowan *et al.*, 2006; Silvestro *et al.*, 2002). The stimulus used to evaluate endothelial function was different. Previous studies used invasive measures (intra-arterial Ach infusion) specific to endothelial derived NO release (Bergholm et al., 1999; Goto et al., 2003), while the current study used flow mediated shear, which may cause the release of other vasoactive substances in the popliteal artery other than NO. Finally, we evaluated endothelial function in the vasculature of the trained limb rather than a non-trained limb and specifically a conduit artery rather than the resistance vessels,

as was the case with both previous studies (Bergholm et al., 1999; Goto et al., 2003). It is likely that SIT training in the current study resulted in eNOS protein upregulation specific to this vessel that also contributed to greater local NO bioavailability beyond improved or maintained antioxidant defenses.

3.4.5 Mechanistic insight into improved aerobic performance

As described previously VO_{2peak} was improved in the current study to the same degree with SIT and ET (Burgomaster et al., 2008). Structural and functional vascular improvements may contribute to better performance noted previously with high intensity training (Burgomaster et al., 2005; Krustrup et al., 2004). Greater peripheral artery distensibility and enhanced endothelial-dependent vasodilation at the onset of exercise may facilitate greater oxygen availability through more efficient blood delivery; however, further study is needed to determine cause and effect.

3.4.6 Study limitations and interpretations

Although the magnitudes of the adaptations in vascular structure and function were similar between SIT and ET, the required dose of ET needed to improve popliteal artery endothelial function and distensibility is unknown and requires specific dose-response research. We also acknowledge that SIT is not practical for some populations as it requires high levels of motivation and possibly supervised training facilities. Therefore, further studies that determine ideal interval exercise intensities specific for each disease condition are warranted. In fact, initial studies in patients with coronary artery disease and chronic heart failure show that the effectiveness of a high-intensity training regime may be greater compared to traditional ET methods (Warburton *et al.*, 2005; Wisloff *et*

al., 2007) and high intensity aerobic interval training is now being recommended for several disease populations.

As well, we acknowledge that normalization of FMD to the area under the flow curve until the time of maximal dilation is the optimal method (Pyke & Tschakovsky, 2007). We were unable to capture the full post occlusion blood flow response due to technical limitations; however, given the lack of difference between our pre and post hyperaemic responses ($MWSR_{25s}$), we believe it is reasonable to assume we have accounted for most of the stimulus through normalization to the shear stimulus for the first 25 s after cuff release.

3.4.7 Perspectives and Significance

Both training protocols improved VO_{2peak} yet the actual total work performed over the period of 6 weeks was much different highlighting the time-efficiency of SIT. From a vascular health perspective this study shows that the beneficial effects of exercise on prognostic indicators of cardiovascular disease, such as peripheral artery distensibility and endothelial function, are modified effectively with either ET or SIT. Extension of interval training has already begun in populations with compromised health such as those with coronary artery disease (Warburton et al., 2005), chronic obstructive pulmonary disease (Coppoolse *et al.*, 1999), and chronic heart failure (Wisloff et al., 2007). Whether the vascular benefits outlined in the present study are apparent in these populations awaits further attention.

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Chapter 4

Effect of acute sprint interval exercise on central and peripheral artery distensibility in young healthy males

4.1 INTRODUCTION

Long-term, moderate intensity exercise training increased central artery distensibility in populations with reduced baseline central elasticity (Cameron & Dart, 1994; Hayashi, Sugawara, Komine, Maeda, & Yokoi, 2005; Sugawara *et al.*, 2006; Tanaka *et al.*, 2000). Contrary to these observations, younger populations tended to have higher baseline central artery distensibility and showed less propensity for training related increases (Rakobowchuk *et al.*, 2008; Tanaka et al., 2000). As well, previous research demonstrated that a sprint interval training program based on repeated Wingate exercise bouts improved popliteal artery function and structure (Rakobowchuk et al., 2008), skeletal muscle metabolic efficiency (Burgomaster *et al.*, 2007; Burgomaster *et al.*, 2008), and aerobic fitness and performance (Burgomaster et al., 2007; Burgomaster, Heigenhauser, & Gibala, 2006; Burgomaster et al., 2008; Burgomaster, Hughes, Heigenhauser, Bradwell, & Gibala, 2005). In young healthy individuals, while popliteal artery distensibility increased, carotid artery distensibility did not change following 6 weeks of either sprint interval or endurance exercise training (Rakobowchuk et al., 2008).

Although researchers often think of central stiffness as the prime target for intervention, recent evidence suggests that in aged and hypertensive populations irreversible structural alterations may limit the beneficial effects of exercise training in

central arteries (O'Rourke, 2008). However, exercise may acutely reduce peripheral PWV, which would briefly reduce central artery augmentation and potentially cardiac work through delayed and diminished pulsewave reflection (O'Rourke, 2008). If an exercise stimulus were applied on a daily basis, the chronic effect would be similar to the positive effects of daily pharmacological administration on resistance vessel vasodilation (O'Rourke, 2008).

To date, several studies examined the acute effects of cycling exercise on both central and peripheral arterial distensibility (Heffernan, Jae, Echols, Lepine, & Fernhall, 2007b; Kingwell, Berry, Cameron, Jennings, & Dart, 1997; Naka *et al.*, 2003; Sugawara *et al.*, 2004; Sugawara *et al.*, 2003). The varying exercise protocols used in these studies included incremental exercise to exhaustion (Heffernan et al., 2007b; Naka et al., 2003), moderate intensity continuous exercise (Kingwell et al., 1997), and brief (5 min) low intensity exercise (Sugawara et al., 2004; Sugawara et al., 2003), however none of these or other studies examined high intensity exercise. In each of these previous studies, acute increases in arterial distensibility were observed following exercise that either was localized to the exercising limb (Sugawara et al., 2004; Sugawara et al., 2003), or was described as whole-body (Kingwell et al., 1997).

As mentioned, acute increases in artery distensibility may be mechanistically linked to the increased arterial distensibility observed with chronic exercise training (Naka et al., 2003). To date no study has determined whether there is an intensity related dose-response relationship that may impact peripheral and central artery distensibility after acute sprint exercise. Furthermore, it is possible that the elevated blood flow and

blood pressure observed following high intensity exercise (Hussain, Smith, Medbak, Wood, & Whipp, 1996) may not cause beneficial changes in arterial distensibility similar to those observed with endurance exercise (Heffernan et al., 2007b; Kingwell et al., 1997; Naka et al., 2003; Sugawara et al., 2004; Sugawara et al., 2003).

Examining the acute vascular responses to high intensity exercise may provide information on the mechanisms responsible for improvements in vascular health and function associated with chronic exercise training (Kingwell et al., 1997) and will determine if acute sprint exercise causes similar increases in peripheral distensibility to that noted with acute aerobic exercise.

The aim of the present study was to examine the effects of acute sprint interval exercise on central and peripheral artery distensibility. We hypothesized that both a single sprint interval and multiple sprint intervals would acutely increase whole-leg peripheral artery distensibility. Furthermore, we hypothesized that following exercise central distensibility would decrease but would rapidly return to baseline values. We examined whether multiple sprint session would lead to greater increases in peripheral arterial distensibility in the exercised limb and greater decreases in central arterial distensibility compared to a single sprint session.

4.2 METHODS

4.2.1 Participants

Nine young healthy males (height: 1.81 ± 0.07 m, weight: 77.5 ± 6.8 kg, age: 20.1 ± 1.2 y, VO_{2peak} : 46.3 ± 5.8 $ml \cdot kg^{-1} \cdot min^{-1}$ (mean ± SD)) volunteered for the study. All participants were recreationally active (no more than 2 days of exercise per week),

normotensive (<140/90 mm Hg), non-smokers, and non-obese. No participant was on medication or taking vitamin, mineral, or herbal supplements. The experimental procedures and potential risks were fully explained to the participants prior to the study, and all participants provided written, informed consent to the protocol as approved by Hamilton Health Sciences Research Ethics Board.

4.2.2 Study design

Participants attended three sessions: (1) a familiarization and VO_{2peak} assessment followed by either (2) a single sprint interval (1 Wingate) or (3) a multiple sprint interval (4 Wingates) exercise session with the order of the last 2 sessions randomized. Participants also attended 2 additional sessions in the laboratory setting for additional experiments not associated with this investigation.

4.2.3 Familiarization session

The familiarization session, performed at least 4 days before the first sprint interval exercise session, consisted of a progressive exercise test on an electronically braked ergometer (Lode BV, Excalibur Sport V2.0, the Netherlands) to obtain an estimate of VO_{2peak}. The test consisted of three initial two-minute stages, during which the resistance was increased by 50W at the onset of each stage. Thereafter, the resistance was increased by 25W each minute until volitional fatigue, determined by failure to maintain a cadence of 40 revolutions per minute (RPM). The highest oxygen uptake achieved over a 30-s collection period using an online gas collection system (Moxus Modular VO_2 System, AEI Technologies Inc., Pittsburgh, PA, USA) was considered the

VO_{2peak}. Following a 20-minute rest period, the same cycle ergometer was used to familiarize participants to the Wingate protocol. Using the body mass of each participant, a 30-s "all-out" cycling effort was performed against a resistance that was equal to 0.075 kg • (kg body mass)$^{-1}$.

4.2.4 Experimental exercise sessions

Participants arrived at the lab at the same time of day either beginning at 8:00 AM or 1:00 PM for both sprint interval exercise sessions. The only difference between the two exercise sessions was the number of Wingate tests (1 vs. 4) performed. Sessions were performed 4 hours postprandial following the consumption of a commercially available standardized meal replacement drink (237ml BOOST®, Mead Johnson Nutritionals, Ottawa, ON, Canada). Measurements were taken while participants were in the supine position in a temperature controlled (22-24°C) room. Participants were asked to abstain from exercise for at least 24 hours, as well as alcohol and caffeine for at least 12 hours prior to each experimental session.

4.2.5 Experimental Design

Direct measurements of superficial femoral artery distensibility and measurements of central and lower limb PWV were determined at three serial time points each separated by 15 minutes to establish baseline central and peripheral artery distensibility. Following the completion of each exercise session, measurements were made for a period of 60 minutes. Discrete measurements of superficial femoral artery distensibility (ultrasound method) and brachial blood pressure were taken at the three time points outlined above as well as 2, 15, 30, 45 and 60 minutes following exercise.

The duration of the entire protocol was 2 hours for the single sprint interval session and 2.5 hours for the multiple sprint interval session.

4.2.6 Single or multiple sprint interval exercise

The single sprint interval session was preceded by a warm-up of 2 minutes of cycling at 40W. A standard Wingate protocol was completed as described above, with vocal encouragement throughout. The multiple sprint interval session involved the same 2 minute warm-up followed by four standard Wingate protocols interspersed with recovery intervals of 4.5 minutes of low cadence (<50 r.p.m.) cycling against a resistance of 40W. After completion of the last high intensity period of exercise, participants were immediately (within 30s) assisted to the supine position for the remainder of the experimental protocol.

4.2.7 Heart rate and blood pressure

ECG was acquired using a one lead system (V_5 configuration) and used to calculate heart rate beat-to-beat. Heart rate was then averaged to give one value for rest and 1-minute values for the 60 minutes post-exercise. Brachial blood pressure (Dinamap Pro 100, Critikon LLC, Tampa, Florida, USA) was also obtained at discrete times points outlined above at the same time as ultrasound measurements of superficial femoral artery distensibility. The average of 2 measurements was taken and reported at all time points.

4.2.8 Measurement of artery distensibility using ultrasound

Measurement of superficial femoral artery distensibility was acquired using a combination of ultrasound imaging at the superficial femoral artery and oscillometric measurements of brachial arterial blood pressure (Dinamap Pro 100, Critikon LLC,

Tampa, Florida, USA). Acquisition of direct femoral artery pressure via applanation tonometry was not possible/reliable, while ethical limitations precluded using indwelling catheters to obtain artery specific blood pressures; therefore, brachial blood pressure was used as a surrogate.

B-mode ultrasound images of the superficial femoral artery were taken ~2cm distal to the bifurcation of the common femoral artery (System FiVe, GE Medical Systems, Horten, The Netherlands). All imaging was performed by the same operator (MR), who had access to all previous images throughout each testing day to ensure identical probe placement.

At the three discrete time-points before exercise (R15, R30 and R45 minutes) and at the 6 discrete time-points following exercise (2, 15, 30, 45, 60 minutes) digital images from 5 consecutive complete heart cycles were acquired in DICOM format (15Hz) at the superficial femoral artery. These images were acquired at the same time as measures of brachial blood pressure.

Since arterial diameter may have been altered with the strenuous nature of the exercise, distensibility was calculated according to the following equation:

-1- $$Dist = \frac{[\pi(d_{max}/2)^2 - \pi(d_{min}/2)^2]}{PP \cdot \pi(d_{min}/2)^2}$$

Where, Dist is distensibility, PP is pulse pressure, d_{max} is the maximum diameter, and d_{min} is the minimum diameter.

To account for the effect of pre-to-post exercise changes in blood pressure on distensibility, B-stiffness index was calculated according to the following equation:

-2- $SI = \ln(SBP/DBP) \times (\Delta CSA/CSA_{min})$

Where, SBP is systolic blood pressure, DBP is diastolic blood pressure, and ΔCSA is the change in cross-sectional area, and CSA_{min} is the minimum cross-sectional area.

4.2.9 Measurement of PWV

To get a detailed timeline of the central and peripheral changes in arterial distensibility, measurements of PWV were obtained at several sites. During the resting period before exercise, PWV was measured for 5 minutes continuously at the 3 time-points outlined above (15-20, 30-35, 45-50 minutes before exercise). Following the completion of the exercise session, PWV was determined continuously from 2-60 minutes by detecting simultaneously the ECG, pulse waves by applanation tonometry (model SPT-301, Millar Instruments Inc., Texas, USA) at the common femoral artery and by infrared (IR) blood volume detection device at the dorsalis pedis artery (Model No. MLT1020PPG, ADInstruments, Colorado Springs, USA). All signals were acquired simultaneously at 2000Hz using a commercially available data acquisition system (Powerlab model ML795, ADInstruments, Colorado Springs, USA) and software (Chart v5.5.3, ADInstruments, Colorado Springs, USA).

4.2.10 PWV signal analysis

Tonometer and IR signals were band-pass filtered (1-10 Hz) to remove non-physiological variations (Nichols, McDonald, & O'Rourke, 2005). Once filtered, the foot of each wave was determined during each heart cycle by the identification of the inflection point. The inflection point was determined from the first derivative. Specifically, the time when the derivative changed from negative to positive was

determined from both signals. Central pulse transit time was determined as the time delay between the R wave peak and the foot of the femoral artery blood pressure wave. Peripheral pulse transit time was determined as the time delay between the foot of the femoral artery pulse wave and the foot of the dorsalis pedis artery volume change. Central and peripheral pulsewave velocities were then calculated using the distance between measurement sites estimated from standard equations (Munakata, Ito, Nunokawa, & Yoshinaga, 2003).

As described earlier, 5 minutes of continuous data were obtained at each pre exercise time point. Once all waveforms were acquired, outliers related to poor signal acquisition or ectopic beats were removed from the dataset. From this data an average was calculated. Since there were no significant differences between the three separate 5-minute rest period averages, they were pooled and a single value was used to represent the resting time point. Measurements taken after exercise were also visually inspected for ectopic beats and signal quality and aberrant beats were removed. The remaining suitable beats (>90% of the total beats collected) were one minute averaged to provide a single value at each minute for comparison (2-60 minutes).

4.2.11 Reproducibility of the measurements

Since all participants attended the lab on two separate occasions separated by at least 7 days, we were able to complete between day comparisons of the resting measurements as determined by the coefficient of variation which was calculated using the following equation:

$$CV_{subject} = \frac{\text{stdev of measures}}{\text{mean of the 2 days}} \times 100$$

$$CV_{measure} = \frac{\text{sum of all subjects' CV}}{\text{total number of subjects}}$$

Where $CV_{subject}$ is the coefficient of variation of the individual subject, stdev is the standard deviation.

Coefficient of variation for resting central PWV and peripheral PWV were 2% and 10%, respectively. Coefficient of variation for resting superficial femoral artery distensibility and stiffness index were 15%, and 13%, respectively. These values are higher than those of previously reported carotid artery CV of 8% in our laboratory (Rakobowchuk et al., 2008), likely due to the variability of ultrasound measurements of diameter in the femoral artery compared to carotid artery.

4.2.12 Statistical analysis

Data are expressed as mean ± SEM. All variables except total work, mean and peak power were analyzed using a two-way within repeated measures analysis of variance, with the factors "time" (rest, 2, 15, 30, 45, 60 minutes) and "trial" (single vs. multiple sprint interval sessions) using commercially available software (Sigma Stat 3.1, Systat, San Jose, CA, USA). When a significant effect was noted, a Holm-Sidak post hoc test was used for subsequent analysis. Significance for all analyses was set at $P \leq 0.05$. Total work, peak and mean power performed during each exercise session were compared using a dependent t-test.

4.3 RESULTS

4.3.1 Wingate performance

Mean power during the single sprint interval session was 653 ± 22 W, while mean power over the multiple sprint intervals were 670 ± 20, 570 ± 29, 504 ± 30, and 482 ± 29

W, respectively. Peak power during the single sprint interval was 1058 ± 49 W, while peak power over the multiple sprint intervals were 1090 ± 63, 1015 ± 66, 888 ± 70 and 841, ± 63 W, respectively. There were no significant differences between the mean ($p=0.16$) and peak powers ($p=0.22$) attained during the single sprint interval and the first interval of the multiple sprint interval session. There was a significant difference between the total work performed during each exercise session (single sprint interval: 19.8 ± 0.6 kJ, multiple sprint intervals: 102.4 ± 2.7 kJ, $p<0.001$).

4.3.2 Heart rate and blood pressure

Heart rate was elevated throughout the recovery period following both exercise sessions ($P<0.05$, main effect for Time) (Figure 4.1). Brachial artery blood pressure values are shown in Figure 4.2. Systolic blood pressure was elevated immediately post exercise ($p<0.01$) with no differences noted between trials (main effect for Time). Diastolic blood pressure followed the same pattern with an elevation at the immediately post exercise time-point that returned to baseline by 15 minutes of recovery (main effect for Time, $p<0.01$).

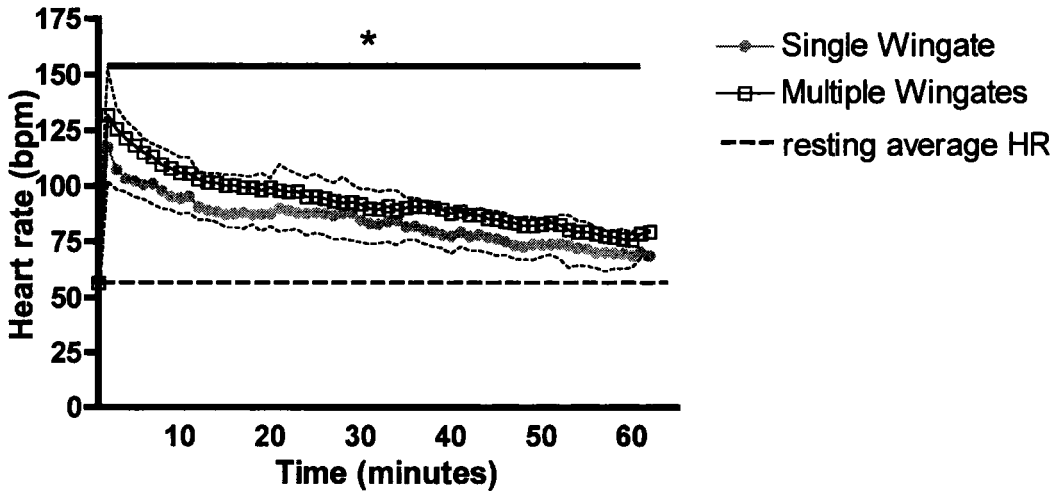

Figure 4.1 Measurements of heart rate throughout recovery from single or multiple sprint interval tests. Measures are mean ± upper and lower limit of SEM (broken lines). * indicates significant difference from PRE at $p<0.05$.

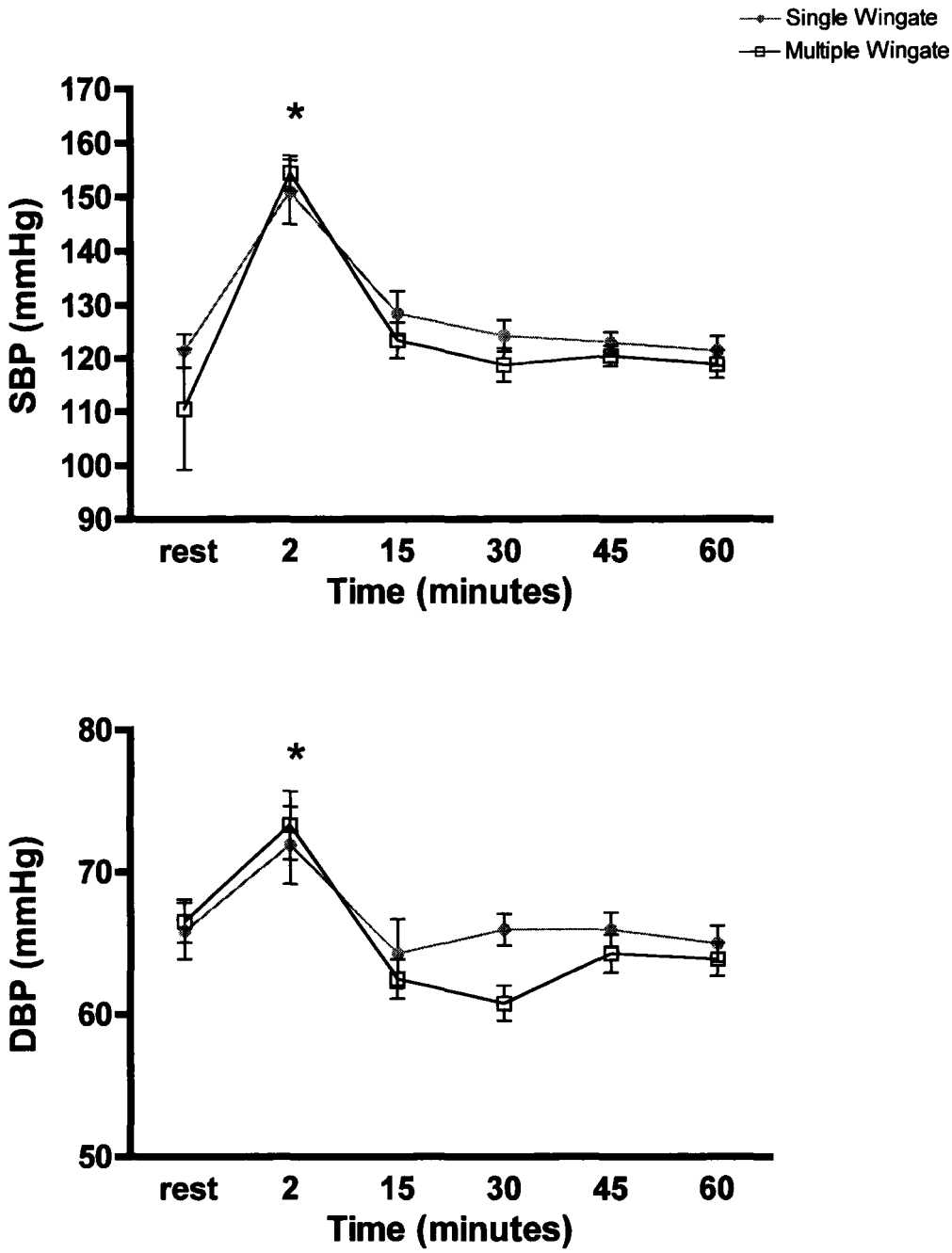

Figure 4.2 Measurements of a) systolic and b) diastolic brachial arterial blood pressure before and at discrete time points following single or multiple sprint interval sessions. Measures are mean ± SEM. * indicates significant difference from PRE at $p<0.05$.

4.3.3 Distensibility and stiffness index of the superficial femoral artery

At the superficial femoral artery, distensibility was not different from rest at any time point in recovery (Figure 4.3). When differences in arterial pressure were taken into account using the β-stiffness index there was a trend for increased stiffness immediately following exercise; however, this did not reach statistical significance ($p=0.06$) (Figure 4.3).

4.3.4 Central and lower limb PWV

Central PWV was increased, indicating decreased distensibility, immediately following both the single and multiple sprint interval sessions and returned to baseline resting levels by 20 minutes of recovery (main effect for Time, $p<0.001$). In contrast, lower limb PWV was reduced immediately, indicating increased distensibility, following both exercise sessions and returned to resting levels by 44 minutes of recovery (main effect for Time, $p<0.001$) (Figure 4.4).

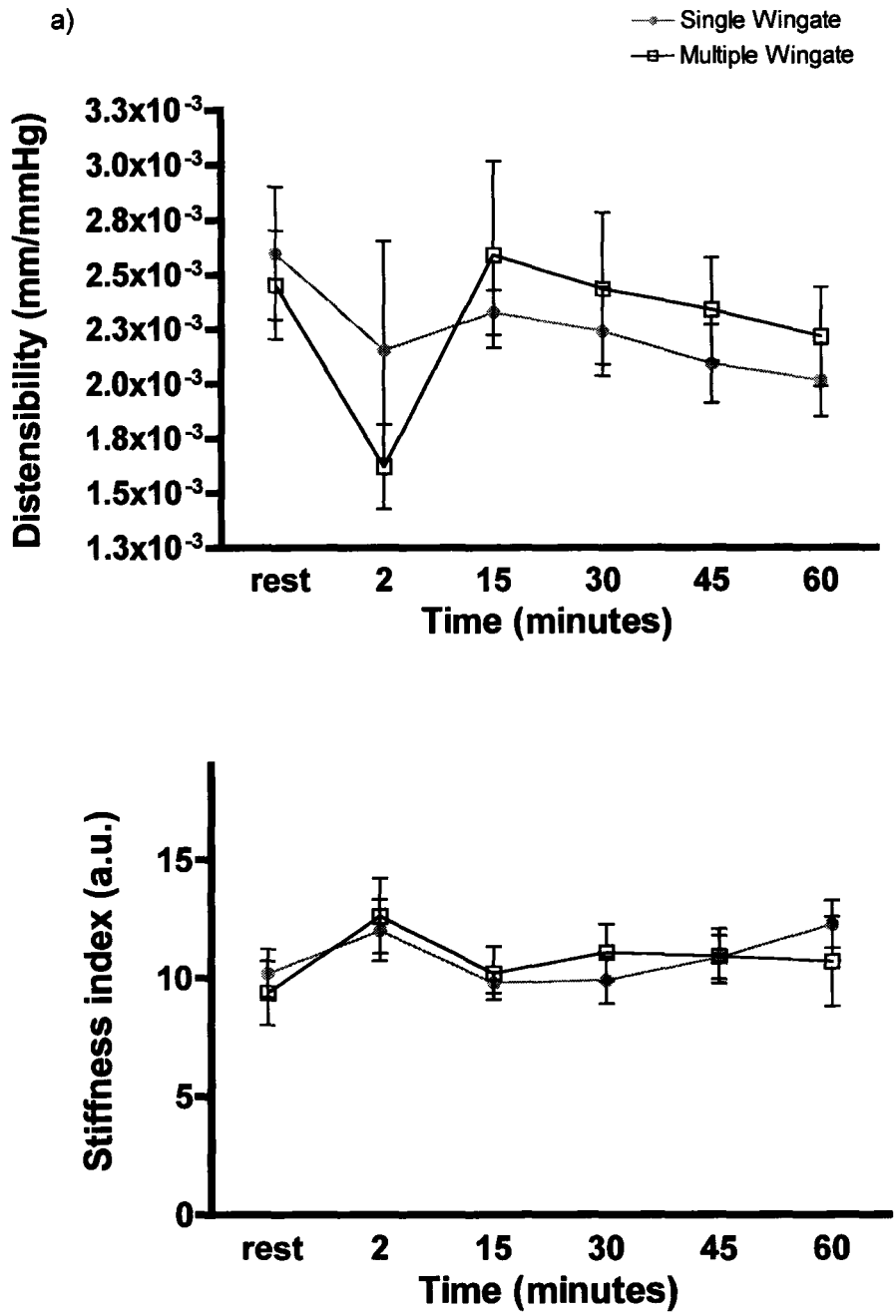

Figure 4.3 Measurements of superficial femoral a) distensibility and b) stiffness index before and at discrete time points following single or multiple sprint interval sessions. Measures are mean ± SEM. Note: immediately post shows a trend (p=0.06) for increased stiffness.

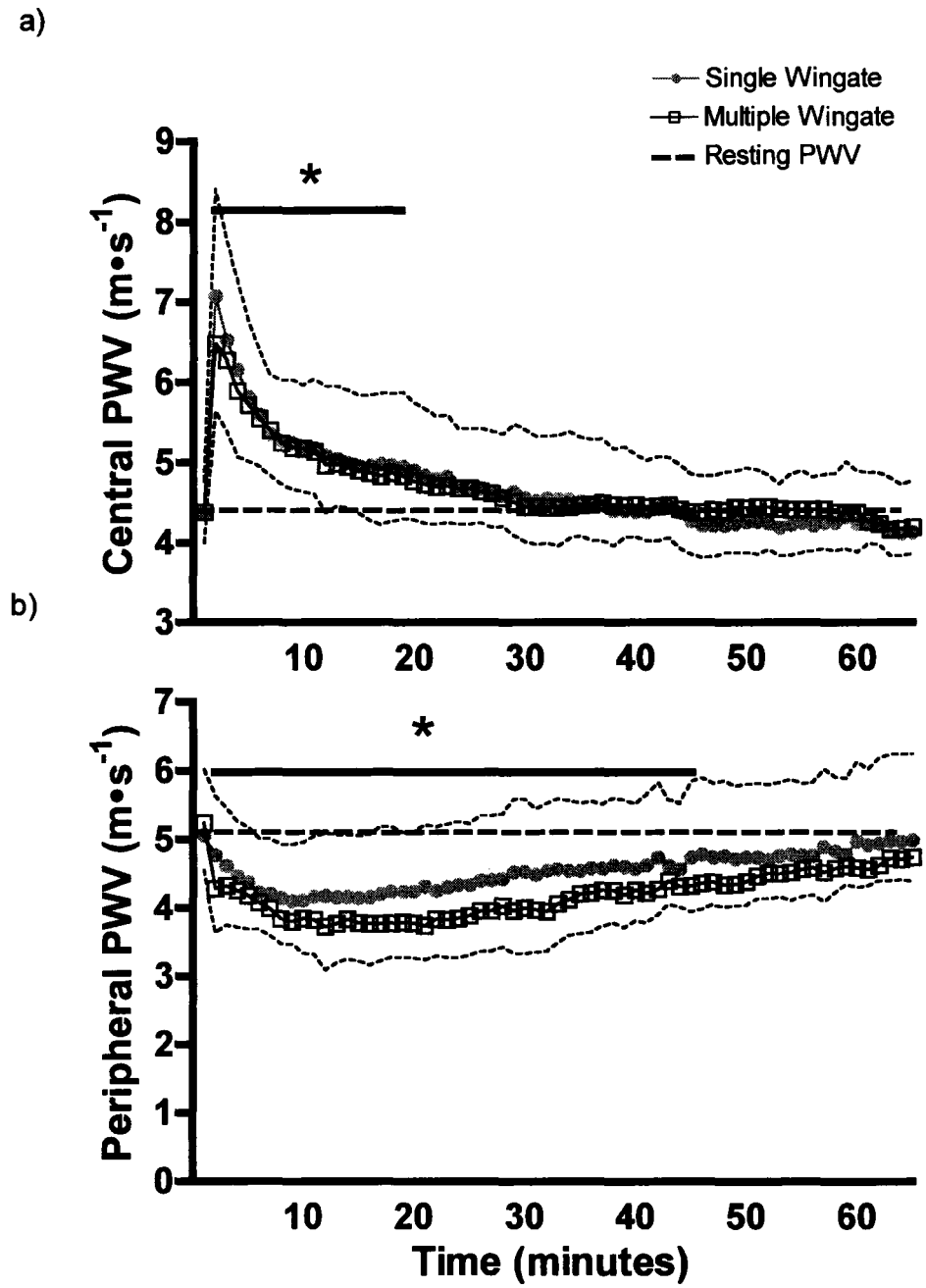

Figure 4.4 Measurements of a) central and b) peripheral pulsewave velocity (PWV) before and continuously following single or multiple sprint interval sessions. Measures are mean ± upper and lower limit of SEM (broken lines). * indicates significant difference from PRE at $p<0.05$.

4.4 DISCUSSION

To our knowledge, this is the first study that has examined the acute effects of both a single bout and repeated bouts of high intensity interval exercise on post exercise arterial distensibility. This area of research is interesting and important because people are using similar types of exercise sessions for athletic training and in the rehabilitation setting (Coppoolse *et al.*, 1999; Warburton *et al.*, 2005; Wisloff *et al.*, 2007). Unique results of the current study include the finding of transiently reduced central distensibility and a trend for reduced superficial femoral artery stiffness during early recovery. Our observation of increased peripheral artery distensibility compliments previous research that showed increased arterial distensibility in the exercised limb following aerobic cycling exercise (Heffernan et al., 2007b; Kingwell et al., 1997; Naka et al., 2003; Sugawara et al., 2003). We saw no dose-response differences in any artery stiffness variables between the single and multiple sprint interval sessions suggesting that a single "all-out" exercise task may evoke maximal changes in artery distensibility during recovery. Since we observed no dose-response effects, subsequent sections will not discuss outcomes from this perspective.

4.4.1 Central artery distensibility

Few studies have examined the distensibility of central arteries following cycling exercise (Kingwell et al., 1997). Our results suggest that sprint exercise may be more similar to resistance exercise regarding central PWV since we observed a reduction, indicating decreased central aortic distensibility, during recovery. Specifically, previous studies involving brief and prolonged low or moderate intensity cycling show unchanged

(Heffernan et al., 2007b; Munir *et al.*, 2008; Sugawara et al., 2003) or increased central artery distensibility during recovery (Kingwell et al., 1997) while central distensibility has been shown to decrease following resistance exercise (Devan *et al.*, 2005; Heffernan, Collier, Kelly, Jae, & Fernhall, 2007a; Heffernan, Jae, Edwards, Kelly, & Fernhall, 2007c). The differences between previous results and those in the present study may relate to the intensity of exercise. However, they do not likely relate to the duration of exercise since we observed decreased central distensibility of an analogous magnitude after both the single and multiple sprint interval exercise sessions. Alternatively, these contrasting results may relate to the timing of central measurements of stiffness following endurance exercise, which might have been too late into recovery in previous studies (Kingwell et al., 1997). A recent study further supported this fact since central stiffness increased during aerobic exercise (Lydakis *et al.*, 2008).

Mechanisms that may account for increased central stiffness during and after exercise include: lingering sympathetic adrenergic vasoconstrictor tone because of elevated circulating catecholamine levels (Greer, McLean, & Graham, 1998), acute impairment of endothelial function (Sugawara *et al.*, 2007), vasoconstriction via endothelin-1 release (Wray *et al.*, 2007), and a more distended artery resulting from increased mean arterial pressure (Nichols et al., 2005). This area of research requires further studies that follow the time-course of sympathetic tone or central and peripheral endothelial function throughout recovery to fully delineate potential mechanisms.

A recent study suggested that increased exercise induced vasodilation reduces central pressure augmentation, as determined from peripheral waveform analysis, which

may ensure better ventricular-vascular coupling during exercise (Munir et al., 2008). Further, Munir et al. (2008) suggested that central artery distensibility had little influence on aortic input impedance since central PWV was not different during recovery, despite reduced central pressure augmentation. In contrast, the current study suggests that during the first ~20 minutes following very high intensity exercise increased central PWV may return the reflected pulse wave to the proximal aorta earlier during systole compared with resting conditions. The relative contribution of the early increased central PWV compared with a dampened reflected pulse wave requires further study immediately following high intensity exercise, to determine which mechanism is dominant.

Several previous investigations have used the traditional carotid-femoral PWV as an index of central (aortic) PWV (Heffernan et al., 2007b; Munir et al., 2008) following brief or maximal aerobic cycling exercise and noted equivocal results. The differences between these studies and the current findings may relate to this method of determining central PWV, which we believe may not be entirely suitable when used in an acute exercise setting.

The carotid to femoral artery PWV technique requires the two arterial paths (left ventricle to carotid and left ventricle to femoral) be in the same hemodynamic state (Nichols et al., 2005). However, during exercise, blood flow through the carotid artery does not increase substantially (~25% increase at maximal exercise) (Secher, Seifert, & Van Lieshout, 2008) and the artery may vasoconstrict (Giller, Giller, Cooper, & Hatab, 2000). Vasoconstriction would increase carotid artery stiffness and concomitantly increase PWV in this segment, while the high flows in the aorta would decrease PWV in

that segment. The local flow differences could lead to errors in the estimation of true aortic PWV from the carotid to femoral PWV segment (see Figure 4.5). To avoid this potential error, we performed a direct evaluation of the entire aorta to femoral segment by determining the time delay between the R wave of the ECG trace and the foot of the subsequent pressure wave at the femoral artery. This ensures we are measuring PWV over a segment of the arterial tree that experiences similar flow changes following exercise.

Several recent studies have used a generalized transfer function to estimate central aortic pressure waveforms, and variables related to central artery stiffness during (Lydakis et al., 2008) and following exercise (Sharman *et al.*, 2006). During aerobic exercise the central arterial tree stiffens, and this may relate to increased MAP (Lydakis et al., 2008; Sharman et al., 2006). Though we did not measure central arterial stiffness during exercise, our post exercise observations likely involve central artery stiffening that has continued into recovery. These results suggest that early central artery stiffening is a transient phenomenon, and the effect this has on cardiac work requires further study.

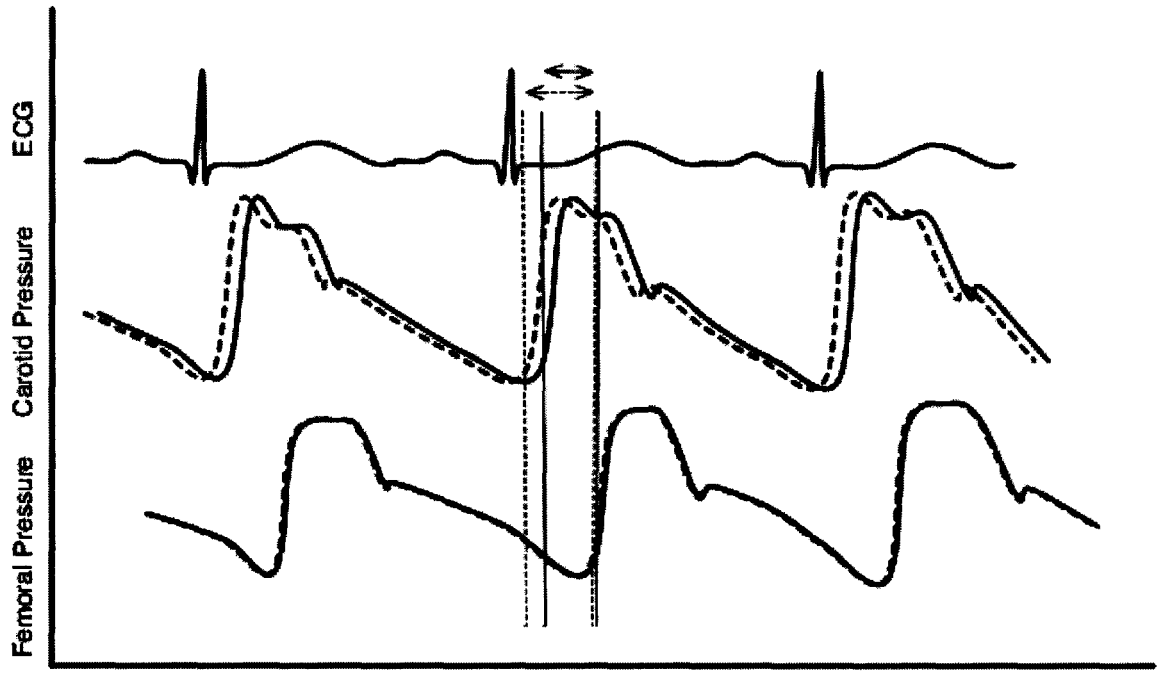

Figure 4.5 Illustration of the effect of local vasoconstriction at the carotid artery on measurements of carotid-femoral pulse wave velocity. Arrows indicate relative pulse transit time (PTT) under conditions of rest (solid arrow) and following local vasoconstriction as experienced with exercise (dashed arrow). Note the lengthening of PTT (dashed arrow) with local vasoconstriction. This interpretation would falsely show an increased central distensibility through an artificial decrease in PWV. The alternative of measuring central PWV from the ECG avoids this potential issue.

4.4.2 Exercised limb artery distensibility

The present study involved both direct ultrasound (superficial femoral artery) and indirect (PWV) measurements of artery distensibility in the exercised limb. This is the only study to our knowledge, combining direct artery specific and indirect whole limb measurements of distensibility in the same study protocol. Similar to previous studies (Kingwell et al., 1997; Naka et al., 2003; Sugawara et al., 2003), we observed increased distensibility in the exercised limb determined from PWV, likely mediated by changes in circulating vasoactive substances like nitric oxide and prostaglandins, altered blood pH, and metabolite accumulation (Kingwell et al., 1997; Naka et al., 2003; Sugawara et al., 2003). Since levels of vasoconstrictor tone are high in this vasculature at rest (Nichols et al., 2005), contributing to relatively fast PWV, we expected a large and prolonged reduction during flow induced vasorelaxation and metabolite induced vasodilation.

When we measured the superficial femoral artery stiffness index, we observed a trend ($p=0.06$) toward increased stiffness immediately following exercise. This observation may seem to contradict our leg PWV results, however, it is possible local metabolic factors play only a minor role early during recovery at this site while persistently elevated levels of MAP likely dominate. It is also possible that lingering high concentrations of norepinephrine and oxidative stress impede NO bioavailability in this arterial segment leading to transiently reduced distensibility that dissipates once norepinephrine and oxidative stress return to baseline levels. Evidence for high levels of circulating catecholamines following high intensity sprint interval exercise has been previously described (Greer et al., 1998). Maybe if we had made direct measures of

stiffness distal to the working muscle mass, such as the popliteal artery, we would have observed increased distensibility to coincide with the leg PWV findings.

4.4.3 Limitations

We acknowledge the pulse pressures used to determine femoral artery distensibility were taken at a site different from our images and that this does not conform to recommended guidelines (i.e. in the same vessel). Since participants were their own controls (repeated measures design) and positioned supine, it is reasonable to assume that these factors did not affect the trends but may influence the absolute values. We do acknowledge that the absolute values of superficial distensibility may not be accurate, however, relative changes in artery distensibility would be discernable from our results. We also acknowledge that a "sham" or time control could potentially strengthen our research design; however, we believe the magnitude and similarity of the effects to previous literature (Naka et al., 2003) precludes this requirement.

4.4.4 Conclusions

In summary, both a single sprint and a multiple sprint session acutely decreased central artery distensibility and increased peripheral artery distensibility during recovery. These results indicate that brief (30s), extremely high intensity exercise transiently increases central artery stiffness, either through persistent increases of MAP or circulating vasoactive substances. Whereas metabolite induced vasodilation likely reduces peripheral stiffness well into recovery reducing central blood pressure augmentation. This persistent reduction of peripheral PWV may be a non-pharmacological method to reduce central blood pressure and cardiac work.

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Chapter 5

The effect of single and multiple sprint exercise on vascular reactivity and reactive hyperemia

5.1 INTRODUCTION

Exercise training studies have established that long-term, moderate intensity, exercise training improves endothelial dependent vascular function in those with vasculature dysfunction (Hayashi, Sugawara, Komine, Maeda, & Yokoi, 2005; Sugawara *et al.*, 2006; Tanaka *et al.*, 2000) while high intensity exercise training improves vascular function in conduit arteries of vascular beds involved in the training (Rakobowchuk *et al.*, 2008). Previous studies showing no improvements in vascular function with high intensity exercise training (Goto *et al.*, 2003), suggest that persistently elevated oxidative stress levels may lead to reduced vascular function with high intensity training. It has also been suggested that examining the acute vascular responses to exercise may identify mechanisms responsible for observed improvements in vascular health and function associated with chronic exercise training (Kingwell, Berry, Cameron, Jennings, & Dart, 1997).

Few studies have evaluated the acute effects of exercise on vascular function. The first studies to employ a repeated evaluation of flow mediated dilation (FMD) before and after acute bouts of exercise showed that FMD was reduced following ischemic handgrip exercise (McGowan et al., 2006) or aerobic exercise (Silvestro et al., 2002). However, with training (McGowan et al., 2006) and with the administration of vitamin C (Silvestro

et al., 2002) this reduction was attenuated. Recently, Harris et al (2008) suggested that, in certain populations, chronic moderate intensity exercise training may improve vascular function immediately after exercise. However, this observation was limited to obese participants who were engaged in regular physical activity (Harris, Padilla, Hanlon, Rink, & Wallace, 2008). Other studies examining the acute changes in vascular function with exercise employed the exercise bout as a countermeasure for hyperlipidemically induced decreases in vascular function. In these studies, moderate exercise attenuated the reduction of vascular function observed following the consumption of a high fat meal (Padilla, Harris, & Wallace, 2007). One cross-sectional study examined the effects of high intensity acute exercise (5 intervals of 5 minutes at 90% of VO_{2peak}) on endothelial function in a group of endurance trained athletes and compared them to sedentary matched controls (Rognmo et al., 2008). At one hour following exercise, both groups exhibited a transient reduction of FMD that returned to baseline by 24 hours (Rognmo et al., 2008).

To date no study has determined whether high intensity exercise acutely alters vascular function in healthy young adults and whether acute responses with exercise are linked to adaptations that occur with chronic exercise training (Rakobowchuk et al., 2008). Therefore, the purpose of the current study was to monitor vascular function before and after two different bouts of acute sprint exercise. We hypothesized that, similar to previous work involving ischemic exercise (McGowan et al., 2006; Rognmo et al., 2008), sprint exercise would acutely reduce vascular reactivity during recovery and that this reduction would be greater with increased exercise volume (i.e. 4 Wingates vs. 1

Wingate).

5.2 METHODS

5.2.1 Participants

Nine young healthy males (height; 1.81 ± 0.06 m, weight: 77.6 ± 6.4 kg, 20.1 ± 1.2 y (mean ± SD)), volunteered for the study. A preliminary screening process was employed to establish that subjects: (a) were free of risk factors associated with cardiovascular, pulmonary or metabolic disease; (b) were deemed safe to engage in physical activity; and (c) other than activities of daily living, were not engaged in a regular training program (i.e. 2 sessions per week and 30 min per session, for at least 1 year prior to the study including recreational activity such as sport or leisure activities). Other exclusion criteria included cardiovascular disease, diabetes, obesity, hypertension (resting blood pressure > 140/90 mmHg), medication use, and smoking as assessed through pre-testing screening. The experimental procedures and potential risks were fully explained to the subjects prior to the study, and all subjects provided written, informed consent. Hamilton Health Sciences Research Ethics Board approved the experimental protocol.

5.2.2 Study design

Participants attended three sessions: (1) a familiarization and VO_{2peak} assessment followed by either (2) a single sprint interval (1 Wingate) or (3) a multiple sprint interval (4 Wingates) exercise session with the order of the last 2 sessions randomized.

5.2.3 Familiarization session

The familiarization session was performed at least 4 days before the first sprint

interval exercise session and consisted of a progressive exercise test on an electronically braked ergometer (Lode BV, Excalibur Sport V2.0, the Netherlands) to obtain a measure of VO_{2peak}. The test consisted of three initial 2-minute stages, where the resistance increased by 50W at each successive stage then by 25W at each subsequent stage until volitional fatigue was reached. As previously described, the highest oxygen uptake achieved over a 30-s collection period using an online gas collection system (Moxus Modular VO_2 System, AEI Technologies Inc., Pittsburgh, PA, USA) was determined and deemed the VO_{2peak}. Following a 20-minute rest period the same cycle ergometer was used to familiarize participants to the Wingate protocol. Using the body mass of each participant, a 30-s "all-out" cycling effort was performed against a resistance that was equal to 0.075 kg • (kg body mass)$^{-1}$.

5.2.4 Experimental exercise sessions

Participants arrived at the lab at the same time of day for both sprint interval exercise sessions. The only difference between the two exercise sessions was the number of Wingate tests (1 vs. 4) performed. Sessions were performed 4 h postprandial following the consumption of a commercially available standardized meal replacement drink (237ml BOOST®, Mead Johnson Nutritionals, Ottawa, ON, Canada) to control the acute effects of diet. Measurements were taken while subjects were supine in a temperature controlled (22-24˚C) room. Also, participants were asked to abstain from exercise for at least 24 hours and alcohol and caffeine for at least 12 hours prior to each experimental session and no subject was taking nicotine products.

5.2.5 Experimental procedures

Pulse wave velocity was evaluated at rest and following lower limb reactive hyperaemia before each sprint exercise session and at 30, 60 and 120 minutes during recovery. Details of the reactive hyperaemia test are as follows:

Participants were instrumented with one lead ECG (V_5 configuration), an infrared blood volume detection device (Model No. MLT1020PPG, ADinstruments, Colorado Springs, USA), and a hand-held applanation tonometry probe (model SPT-301, Millar Instruments Inc., Texas, USA). The IR device was positioned over the dosalis pedis artery, while the tonometer was positioned over the common femoral artery. Positioning of the tonometer probe was confirmed through simultaneous ultrasound imaging of the common femoral artery bifurcation (System FiVe, GE Medical Instruments, Gronigen, The Netherlands). All signals were acquired simultaneously at 2000Hz using a commercially available data acquisition system (Powerlab model ML795, ADInstruments, Colorado Springs, USA) and software (Chart v5.5.3, ADInstruments, Colorado Springs, USA).

5.2.6 Reactive hyperaemia testing procedures

Vascular reactivity was measured using methods outlined by Naka et al. (2006), modified to assess reactivity of the lower extremities. The concept is similar to that of flow mediated dilation techniques using ultrasound, however rather than monitoring arterial diameter size, changes in pulse wave velocity over the length of the extremity are monitored throughout the post occlusion period. This method has been shown to correspond well with simultaneously acquired measurements of flow mediated dilation in the brachial artery during rest situations (Naka, Tweddel, Doshi, Goodfellow, &

Henderson, 2006). The benefits of this method are that it is more sensitive than ultrasound acquired FMD and provides a measure of vascular reactivity across a large segment of the vasculature compared to traditional flow mediated dilation which is limited to a small section of the brachial, popliteal or superficial femoral artery.

Peripheral pulsewave velocity was determined prior to limb occlusion (pre-occlusion) and compared to post occlusion measures. Pre-occlusion measures were the average of 30s of continuously acquired values taken in the minute before cuff occlusion. Pulse waves and blood volume waveforms were recorded both at the femoral artery and at the dorsalis pedis artery, respectively. A pneumatic cuff was applied around the thigh ~5 cm proximal to the patella and inflated 50 mmHg above systolic blood pressure for a period of 10 minutes. Upon release of the cuff, pulse waves and blood volume waveforms were acquired and recorded continuously for 10 minutes post occlusion.

From the acquired pulse and blood volume waveforms, the foot of the wave was determined by phase velocity theory. It has been established that high-frequency components of the blood pressure or blood volume change waveform are derived mainly from the sharp systolic upstroke and are ~30Hz. Therefore a band-pass filter (5-30Hz) was applied to extract the data corresponding to the systolic upstroke. This method is similar to that of commercially available systems although the process is not automated (Sugawara *et al.*, 2004). In addition, the ECG was used as a reference to ensure proper identification of the foot of the wave based on expected transit times (ie. 200ms<PTT>500ms).

Measurements were also visually inspected for ectopic beats and signal quality and

aberrant beats were removed and subsequently fit to a fine Lowess curve (GraphPad Prism version 4.00 for Windows, GraphPad Software, San Diego California USA). From these curves, the maximum pulse transit time (lowest PWV) was determined and used to quantify the vascular response in comparison to resting measures using the following equation.

$$\text{Relative FM-PWV (\%)} = \frac{PWV_{post\text{-}oclussion} - PWV_{pre\text{-}occlusion}}{PWV_{pre\text{-}occlusion}} \times 100$$

where FM-PWV is flow mediated pulsewave velocity.

PWVs were calculated using the distance between measurement sites estimated from standard equations (Munakata, Ito, Nunokawa, & Yoshinaga, 2003).

5.2.7 Post occlusion blood flow

Post occlusion blood flow was acquired simultaneous to the PWV measurement. Longitudinal superficial femoral artery images and blood velocity measurements were made using a 10 MHz linear array pulse Doppler ultrasound probe (System FiVe, GE Medical Systems, Horten, Norway) which was positioned ~2-3 cm distal to the bifurcation of the common femoral artery into its' branches. Continuous video recording of the image of the superficial femoral artery was obtained from 15s prior to cuff deflation until 4 min following cuff deflation. Simultaneous to the imaging of the superficial femoral artery, mean blood velocity (MBV) was obtained using the duplex function of the previously described linear array probe 15s prior to cuff deflation until 10 minutes after cuff release of which 200 seconds were used to determine mean post-occlusion blood flow, mean shear rate and blood flow area under the curve (AUC). The raw audio signal corresponding to blood velocity was output from the Doppler ultrasound

system to an external spectral analysis system (model Neurovision 500M TCD, Multigon Industries, Yonkers, NY), which applies a fast Fourier transform (FFT) to the raw audio signal to determine MBV continuously. Blood velocity was corrected for insonation angle during post acquisition analysis. MBV, like all other physiological signals, was acquired and recorded using the previously described Powerlab system.

5.2.8 Post-occlusion reactive hyperemia

Mean blood flow was calculated (vessel cross-sectional area x MBV) and used to quantify the hyperaemic response. Also, mean wall shear rate (MWSR) was determined as:

$$\text{MWSR} = \frac{4 \times \text{MBV}}{\text{mean diameter.}}$$

where MBV is mean blood velocity.

Mean vessel diameter was determined from 3 separate heart cycles acquired at 5 second intervals immediately following deflation of the occlusion cuff (5,10 and 15s post occlusion). Diameter was not assessed for the duration of the post-occlusion period since the magnitude of the increase in diameter was marginal following the first 15 s and any changes would not create a large discrepancy in flow calculation since most of the change in blood flow is the result of changes in blood velocity and not diameter. Three measures of end systolic and end diastolic lumen diameter were determined using electronic callipers. Mean arterial diameter was calculated using a weighted average equation (1/3 x systolic diameter + 2/3 x diastolic diameter). The error in the reproducibility of this method in our laboratory is <3% in the superficial femoral artery.

Blood flow responses were averaged every 3 seconds to reduce beat to beat

variability and the area under the hyperaemic curve from 0 to 200 seconds was calculated. Prior to deciding on this specific duration, each participant's hyperaemic curve was plotted and visually inspected to determine what was the longest duration before blood flow returned to baseline values. It was determined that in every subject at all time-points, the hyperaemic response was finished by ~180-190s. Therefore, 200s was chosen as the end point for blood flow area under the curve determination to ensure the full hyperaemic response was used.

5.2.9 Single or multiple Wingate exercise

The single sprint interval session was preceded by a warm-up of 2 minutes of cycling at 40W. A standard Wingate protocol was completed as described above, with vocal encouragement throughout. Multiple sprint interval sessions involved the same 2 minute warm-up which was followed by four standard Wingate protocols interspersed with recovery intervals of 4.5 minutes of low cadence (<50 r.p.m.) cycling at a work-rate of 40W. After completion of the exercise session, participants were immediately (within 30s) assisted to the supine position for the remainder of the experimental protocol.

5.2.10 Statistical analysis

Data are expressed as mean ± SEM. All variables except total work, mean and peak power were analyzed using a two-way within repeated measures analysis of variance, with the factors "time" (rest, recovery 30, 60 and 120 minutes) and "trial" (single vs. multiple Wingate) using commercially available software (SPSS 11.0 for Mac OS X, SPSS Inc. Chicago, IL). When a significant effect was noted, a Bonferroni post hoc test was used for subsequent analysis. Significance for all analyses was set at $P \leq 0.05$.

Total work, peak and mean power performed during each exercise session were compared using a dependent t-test.

5.3 RESULTS

5.3.1 Wingate performance

Mean power during the 1 Wingate session was 675 ± 18 W, while mean power over the 4 Wingates were 659 ± 23, 596 ± 24, 517 ± 26, and 493 ± 24 W, respectively. Peak power during the 1 Wingate was 1112 ± 54 W, while peak power over the 4 Wingates were 1111 ± 42, 1044 ± 60, 921 ± 56 and 851 ± 60 W, respectively. There were no significant differences between the mean ($p=0.27$) and peak powers ($p=0.94$) attained during the 1 Wingate and the first Wingate test of the 4 Wingate session. There was a significant difference between the total work performed during each exercise session (single: 20.3 ± 0.6 kJ, multiple: 103.9 ± 2.5 kJ, $p<0.001$).

5.3.2 Heart rate

Resting heart rate prior to each occlusion protocol showed a time x trial interaction ($p=0.04$, Figure 5.1). Heart rate was significantly elevated above resting values at both 30 minutes Post and 60 minutes Post and returned to levels not significantly different from rest by 120 minutes of recovery during both trials. As well, the HR at 30 minutes Post was significantly higher following the 4 Wingate trial compared to the 1 Wingate trial ($p=0.05$, Figure 5.1).

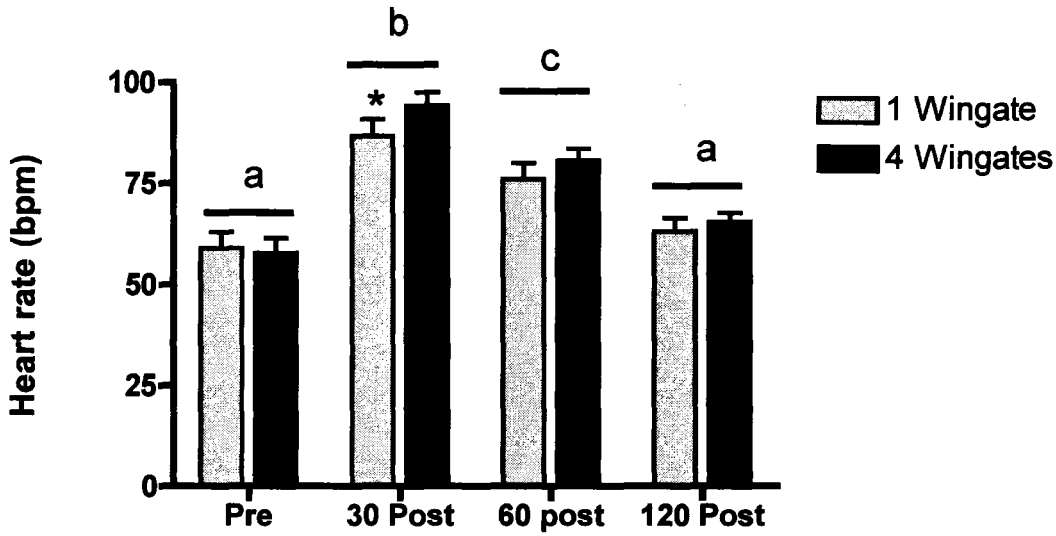

Figure 5.1 Heart rate at rest and following different sprint exercise sessions. Means with different letters are significantly different from each other ($p<0.05$). * Indicates significantly different means at the same time point.

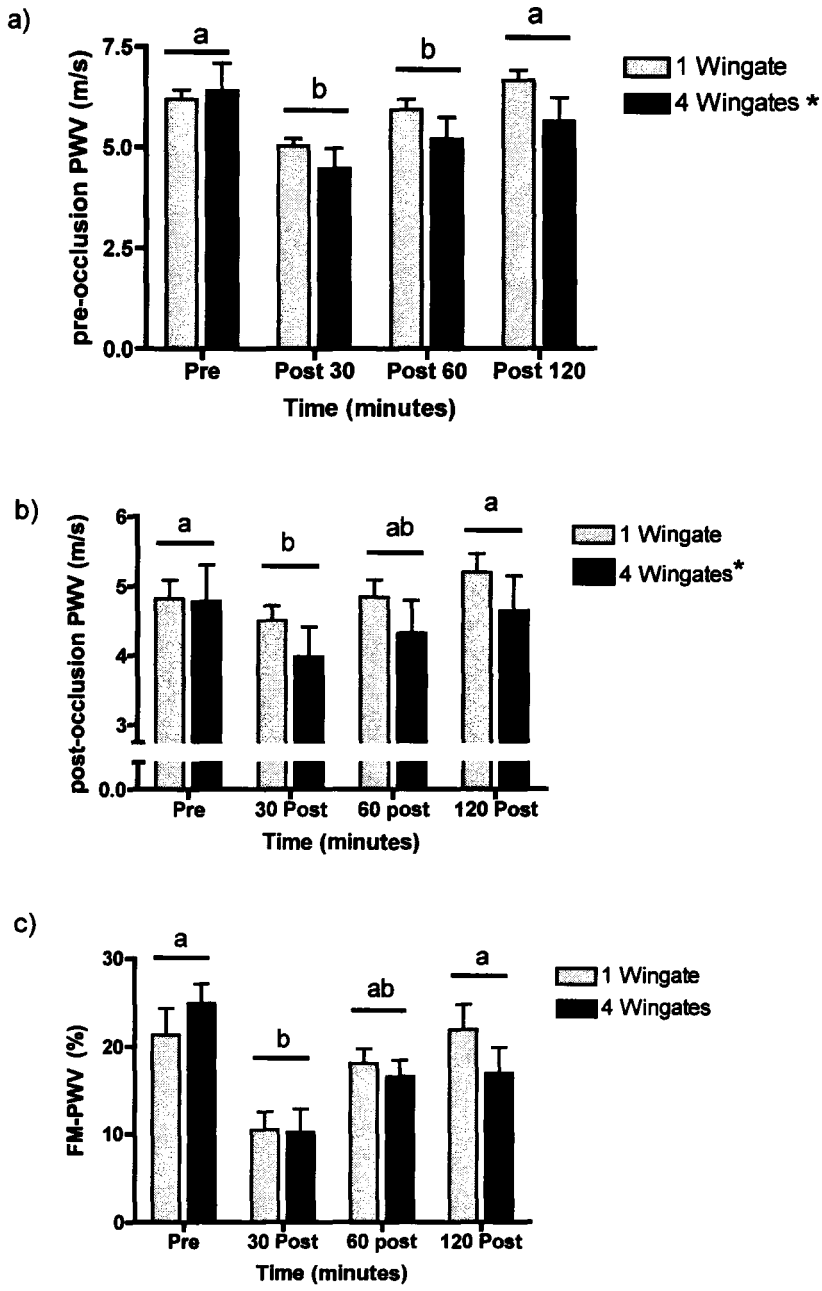

Figure 5.2 Pulsewave velocity a) pre-occlusion in the lower limb (peripheral) following either 1 Wingate or 4 Wingates and b) post-occlusion c) as a relative change from pre to post occlusion. Means with different letters are significantly different from each other ($p<0.05$). * Indicates significantly different main effect for trial.

5.3.3 PWV

Peripheral pre-occlusion PWV showed a significant main effect of time ($p=0.001$) and trial ($p<0.001$); however, there was no interaction ($p=0.30$). The 4 Wingate trial resulted in larger reductions in peripheral pre-occlusion PWV compared to the 1 Wingate trial for all time points. At 30 ($p<0.01$) and 60 minutes Post ($p<0.04$) sprint exercise, PWV was significantly reduced compared to Pre and 120 minutes Post (Figure 5.2a) for both the 1 and 4 Wingate trials.

5.3.4 Flow mediated changes in lower limb PWV

There was a main effect for time in flow mediated changes in lower limb PWV during both the 1 and 4 Wingate trials in which the 30 minute Post value was significantly decreased indicating less acceleration of the pulse wave in response to occlusion and therefore less dilation compared to the PRE and 60 minute Post time points ($p<0.01$, Figure 5.2c).

5.3.5 Mean blood flow, mean shear rate and area under the curve (AUC) hyperaemic blood curve

Mean blood flow and AUC following occlusion showed significant main effects for both time ($p=0.008$) and trial ($p=0.05$); however, the interaction did not reach statistical significance ($p=0.06$). Mean blood flow was greater following occlusion during the 4 Wingate trial (main effect for trial), while Post 30 and 60 were significantly greater than all other time points for both the 1 and 4 Wingate trials (main effect of time) (Figure 5.3a). Similar patterns were observed with average shear rate, which showed significant

main effects for both time ($p=0.014$) and trial ($p=<0.001$, Figure 5.3b); however, the interaction did not reach statistical significance ($p=0.12$). Average shear rate was greater during the 4 Wingate trial (main effect for trial), while Post 30 was significantly greater than all other tests, and Post 60 was significantly greater than Post 120.

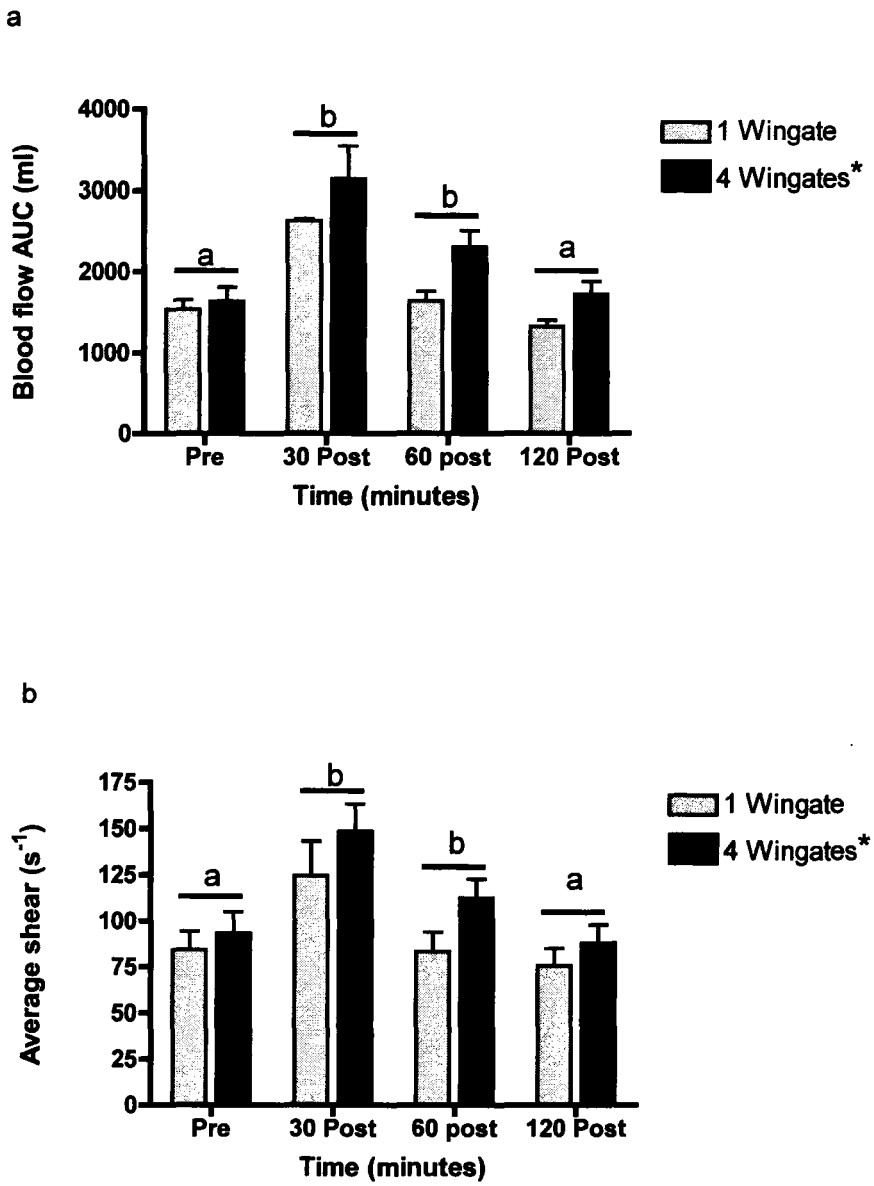

Figure 5.3 Hyperaemic responses after cuff deflation (0 to 200s) before (Pre) and after (30, 60 and 120 Post) an exercise session of 1 or 4 Wingates. The hyperaemic response as measured by a) the area under the curve (AUC) of the hyperaemic blood flow response and b) the average shear rate throughout the 200 seconds following cuff deflation are displayed. Means with different letters are significantly different from each other ($p<0.05$). * Indicates significantly different main effect for trial with the 4 Wingate trial having greater blood flow and shear rates.

5.4 DISCUSSION

To our knowledge, this is the first study to show that as little as 30 seconds of extremely high-intensity exercise can acutely decrease vascular reactivity. Furthermore, this form of exercise causes reductions that are apparent for 30 minutes into recovery. Secondary to this reduction of vascular function, blood flow over a 200 second period following cuff release is substantially greater following exercise compared to before exercise.

Of particular interest is the observation of acutely reduced vascular function, which supports our hypothesis. These findings also support those of McGowan and colleagues (2006) who observed acutely reduced brachial flow mediated dilation following isometric handgrip exercise both before and after training. It also confirms reduced FMD in endurance-trained athletes following acute one hour after exercise (Rognmo et al. 2008). However, our results contrast studies in which brachial flow mediated dilation was augmented following exercise in active obese participants (Harris et al., 2008). Several differences between the present study and that of Harris and colleagues (2008) may explain these divergent findings and they relate to their results. First, following both high and low intensity exercise, the active obese subjects displayed reduced baseline arterial diameters in comparison to pre exercise diameters, which were likely the result of vasoconstriction. This reduction in baseline diameter indicates that the brachial artery was not in the same vascular state before and after exercise, prior to the commencement of occlusion. The authors interpreted an increased FMD response following exercise as enhanced endothelial function, however, we believe that this

interpretation is incorrect since the absolute diameter achieved after cuff release was not likely different compared to before exercise. Greater upper body vasoconstriction occurred with exercise and was abolished with reactive hyperemia and therefore gave the appearance of enhanced endothelial function.

Another potential confounding factor in the Harris et al. (2008) study is that no attempt was made to normalize their FMD values to the shear stimulus, which has been shown to specifically modify the magnitude of the FMD response. Several studies suggest normalization of the FMD response to the AUC of the shear stimulus (Black, Cable, Thijssen, & Green, 2008; Pyke & Tschakovsky, 2007). Recent work by Black et al. (2008) specifically demonstrates that without normalization to the area under the hyperaemic shear curve comparisons between populations (old, young, trained and untrained) yield inappropriate results for comparison (Black et al., 2008).

Similar to previous studies where baseline diameters had returned to normal (McGowan et al., 2006; Silvestro *et al.*, 2006; Silvestro et al., 2002; Rognmo et al. 2008), we observed reduced vascular reactivity post exercise. There was no significant difference in the vascular reactivity response between trials (1 Wingate vs. 4 Wingates) indicating that 1 Wingate is sufficient to stimulate a maximal acute reduction in vascular reactivity. Mechanistically, the acute reduction in vascular reactivity is likely due to the extreme oxidative stress induced by the exercise session (Rognmo et al. 2008; Silvestro et al., 2006; Silvestro et al., 2002). Previously, when antioxidant therapy was administered before exercise, reductions of vascular function were attenuated (Silvestro et al., 2002). Whether chronic antioxidant administration with high intensity training provides a

beneficial effect requires further study.

Peripheral PWV was reduced, indicating increased distensibility, at both of the time points at which reduced vascular reactivity was observed. We do not discount the possibility that the vascular reactivity were influenced by the enhanced distensibility at 30 and 60 minutes post exercise, which is similar to the previous study in this thesis.

5.4.1 Post occlusion reactive hyperemia

The premise of a reactive hyperemia test is to ensure that maximal blood flows are induced, thus previous studies of brachial reactive hyperemia have concluded that 5 minutes of occlusion is sufficient in maximizing this response (Corretti *et al.*, 2002). Evaluation of reactive hyperemia in the lower limb has received less attention and there are currently no specific guidelines recommending specific occlusion durations to ensure maximal hyperaemic flow. As well, it has been suggested that the peak hyperaemic response can be used as a surrogate measure of arteriolar structure (Green *et al.*, 2004). However, this depends on the effectiveness of the protocol at dilating all downstream arterioles since they provide the majority of the resistance to flow. Our results show that currently used methods (Bleeker *et al.*, 2005; de Groot, Crozier, Rakobowchuk, Hopman, & MacDonald, 2005) involving occlusion durations of between 10 and 12 minutes are not sufficient and that further metabolic stress through prior exercise alters the magnitude and duration of the shear stimulus depending on how long after exercise one evaluates vascular function. This variability in shear produced for a 10 minute occlusion period may indicate that maximal dilation was not achieved with the occlusion protocol employed. These findings of increased shear at 30 and 60 minutes post exercise may also

be interpreted as enhanced resistance vessel function following intense exercise. This may be true; however, this acute alteration had returned to normal by 120 minutes post exercise. It is likely that repeated hyperaemia induced by exercise training leads to chronic changes in resistance vessel function (Higashi et al., 1999).

Regarding the relationship between the vascular reactivity measures and the flow stimulus, we emphasize that the direction of change in these variables was opposite throughout the post exercise period. At time-points when vascular reactivity decreased, there was concomitant increase in the average shear. Although our vascular reactivity values were not normalized to our shear stimulus due to physiological dissimilarities between the measurement of the stimulus and the response, this general comparison indicates that the reduction of vascular reactivity was not the result of a reduced stimulus. Rather this reduced vascular reactivity was observed despite an augmented shear stimulus.

5.4.2 Conclusion

The results of the present study provide evidence that high intensity exercise acutely reduces vascular reactivity in the exercising muscle bed. These reductions in reactivity occurred despite augmented shear rates at the same time points. From a clinical perspective, the results emphasize the effects of acute exercise, and oxidative stress induced by exercise, on measures of vascular reactivity. Over periods of training, these small reductions likely stimulate adaptive responses, which result in long-term beneficial adaptations similar to those previously shown by Rakobowchuk et al. (2008) with both endurance and sprint interval training.

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Chapter 6

General Conclusions

6.1 Introduction

The following chapter describes the overall key findings outlined in the previous four chapters. As well, recommendations for future research are outlined specifically focusing on further studies on the impact of high intensity exercise on the cardiovascular system in different populations.

6.2 Impact of sprint interval training on oxygen uptake, blood flow and cardiac output kinetics in comparison to traditional endurance training

Recently, studies used high intensity interval training both as a method to improve fitness in both young healthy individuals (Burgomaster, Heigenhauser, & Gibala, 2006; Burgomaster *et al.*, 2008; Burgomaster, Hughes, Heigenhauser, Bradwell, & Gibala, 2005), and those with significant disease (Warburton *et al.*, 2005; Wisloff *et al.*, 2007). As well, recent short-term training studies involving SIT reported enhanced muscle oxidative potential (Burgomaster et al., 2008; Burgomaster et al., 2005), and greater skeletal muscle monocarboxylate transporter content (both MCT4 and MCT1) (Burgomaster *et al.*, 2007) following training. These muscle and whole body adaptations and improved performance suggest enhanced cardiovascular performance, such as the delivery, and distribution of blood flow, with this method of training. Previous training studies demonstrated rapid improvements of VO_2 kinetics in sedentary participants after

as little as 4 days of aerobic training (Phillips, Green, MacDonald, & Hughson, 1995) and that studies involving as few as 2 sessions of aerobic interval training per week (intervals performed below maximal aerobic power) also accelerated phase II VO_2 kinetics (Billat, Mille-Hamard, Demarle, & Koralsztein, 2002).

The study outlined in Chapter 2 showed improvements of VO_{2peak} were not associated with accelerated VO_2 kinetics, at least in young healthy participants. Since VO_2 kinetic responses were not altered, it is not surprising that there was no alteration of putative regulators of VO_2 kinetics such as cardiac output kinetics and blood flow kinetics. This differs from previous studies, which showed that blood flow kinetics were faster following either endurance training (Shoemaker, Phillips, Green, & Hughson, 1996) or unilateral interval high intensity interval training (Krustrup, Hellsten, & Bangsbo, 2004).

Endurance exercise training reduced both exercising heart rate and cardiac work during high intensity exercise. This reduced cardiac work (rate pressure product) suggests reduced cardiac oxygen uptake. In the sprint interval trained participants, cardiac work was unchanged despite reductions of exercising heart rate, which suggests greater average systolic pressure. These results provide evidence that both methods are beneficial, and ideally one should combine them to gain the time-efficiency of SIT and the cardiac adaptations of ET.

6.2.1 Future Directions

Numerous future studies are warranted especially regarding central cardiovascular

and cardiac adaptations to sprint interval training in comparison to moderate intensity prolonged training. Mechanisms underlying the reduced heart rates noted with both training methods need to be determined since they may be structural or neurological and may relate specifically to the method of exercise training. To evaluate this possibility, echocardiography, or an equivalent imaging technique would be required at rest and throughout exercise. The mechanisms responsible for the discrepancies in estimated cardiac work following training also need to be addressed. Specifically, during exercise we need to determine whether average systolic pressure is changed and what mechanisms contribute to any alterations, such as arterial distensibility. It is likely that invasive arterial pressure measurements combined with beat-by-beat stroke velocity measurements would provide an ideal investigation method.

Long-term training also requires attention, since sprint interval training of 6 weeks may be too short to cause the same volume loads associated with endurance training noted in the current study. As well, this area needs further research that determines whether completely sedentary participants or people in diseased populations, who are starting at low baseline levels of vascular function and cardiorespiratory fitness, benefit from the time-efficient effects of sprint interval training.

6.3 Chronic adaptations of artery stiffness and peripheral vascular endothelial function with sprint and endurance training

Chapter 3 describes the exercise training induced adaptations of the arterial vasculature in response to both sprint and endurance exercise training. Previous research

into the vascular effects of endurance exercise training has established that central arteries, such as the carotid artery, become more distensible with endurance exercise training, particularly in participants who have stiff arteries before the intervention (Cameron & Dart, 1994; Kakiyama, Matsuda, & Koseki, 1998; Kakiyama *et al.*, 2005; Moreau, Gavin, Plum, & Seals, 2006; Tanaka *et al.*, 2000) or following retraining in athletes (Tordi *et al.*, 2006). While training studies in both healthy (Clarkson *et al.*, 1999; O'Sullivan, 2003), and diseased populations (Gokce *et al.*, 2003; Gokce *et al.*, 2002; Hambrecht *et al.*, 1998; Hornig, Maier, & Drexler, 1996; Linke *et al.*, 2001; Maiorana *et al.*, 2001; Maiorana *et al.*, 2000; Walsh *et al.*, 2003) show enhanced endothelial function.

When focusing on the intensity of the training protocols, studies have found that high intensity aerobic training had no effect (Goto *et al.*, 2003) or even reduced acetylcholine mediated vascular reactivity in the brachila artery (Bergholm *et al.*, 1999). Contrary to these previous findings with high intensity training, our results indicated that sprint interval training and endurance training both improved flow mediated dilation in the exercise trained limbs to the same degree. As well, both endurance and sprint interval training improved artery distensibility in the popliteal artery. As previously described, the structural degradation of the elastic arterial system with aging and disease may be irreversible; however, the effect of acute exercise on the pulsewave propagation and reflection may be an ideal intervention strategy. Delaying and diminishing the magnitude of the reflected pulsewave could reduce central pressure augmentation and provide short-term benefits similar to pharmacological treatment (O'Rourke, 2008).

Several markers of vascular health were unchanged by either endurance or sprint

training, such as popliteal artery size, carotid artery intima-media thickness (IMT) and carotid artery stiffness. There was a strong indication that these parameters were unresponsive to training because of a "ceiling" or "floor" effect as our participants were not completely sedentary at the start of the study. Our participants did not display elevated baseline IMT values (Tanaka *et al.*, 2002) and carotid artery distensibility was already relatively high prior to training (Miyachi *et al.*, 2004). Contrary to previous studies (Miyachi, Iemitsu, Okutsu, & Onodera, 1998) our study did not show an increase in popliteal artery diameter with exercise training, which could relate to our observation of improved distensibility or a different time-course of adaptation specific to this artery (Jasperse & Laughlin, 2006)

6.3.1 Future Directions

Future studies should focus on both cellular and molecular mechanisms involved in these vascular adaptations, the long-term training responses, and the acute effects of sprint exercise before and after training. The vascular biopsy technique could provide a window into exercise training adaptations and would be an ideal tool. This technique could provide detailed information about both the long-term stable training adaptations to the nitric oxide system, superoxide dismutase system, and many other putative regulators of vascular health and inflammation (Colombo *et al.*, 2002; Onat *et al.*, 2007).

The goal of SIT is to provide a time-efficient strategy that provides similar health benefits as traditional moderate intensity endurance training. Study populations with vascular disease should be a focus of future research, especially in reference to health

benefits. Future studies in diseased and aged populations should include longer training durations, and measures of traditional as well as more recently determined independent risk factors for vascular disease (endothelial function, artery stiffness).

6.3.2 The integration of adaptations and their influence on integrative cardiovascular responses to exercise

Chapters 2 and 3 may be compared regarding the interaction of vascular adaptations and their impact on overall cardiovascular responses to exercise. Since both studies involved the same participants, some general conclusions may be drawn regarding the effects of endothelial function and arterial stiffness. Chapter 3 described a number of adaptations observed at rest in both the SIT and ET groups. Specifically, peripheral artery stiffness and endothelial function, as well as aerobic fitness were improved. From these observations one would expect an improvement of the various kinetic parameters determined in Chapter 2 if these vascular parameters.

In general, alterations of these parameters did not affect the kinetic responses in either blood flow or oxygen uptake. This suggests alternative adaptations such and increased arterial diameter and/or altered central vascular stiffness may be required in this population to alter flow and oxygen kinetics and ultimately aerobic metabolism. Future research should aim to determine whether alterations of artery size and central stiffness impact blood flow and oxygen uptake kinetics through cross-sectional and acute intervention studies.

6.4 Acute alterations of artery stiffness following sprint exercise

Recent research has focused on acute changes in arterial distensibility following both aerobic and resistance exercise with cycling exercise being the most common exercise method evaluated (Heffernan, Jae, Echols, Lepine, & Fernhall, 2007b; Kingwell, Berry, Cameron, Jennings, & Dart, 1997; Naka *et al.*, 2003; Sugawara *et al.*, 2004; Sugawara *et al.*, 2003). Exercise protocols used previously have included incremental exercise to exhaustion (Heffernan et al., 2007b; Kingwell et al., 1997), moderate intensity continuous exercise (Naka et al., 2003), and brief (5 min) low intensity exercise (Sugawara et al., 2004; Sugawara et al., 2003). In each of these previous studies arterial distensibility acutely increased in the exercised limb (Sugawara et al., 2004; Sugawara et al., 2003), throughout the body (Kingwell et al., 1997) or in non-exercised vascular beds after a delay during recovery (Naka et al., 2003).

DeVan et al. (2005) evaluated the acute effects of resistance exercise on arterial distensibility in the carotid artery and observed a significant reduction up to 30 minutes into recovery. In a series of studies, Heffernan and colleagues studied the acute effects of resistance exercise on both central and peripheral arteries and showed analogous results (Heffernan, Collier, Kelly, Jae, & Fernhall, 2007a; Heffernan, Jae, Edwards, Kelly, & Fernhall, 2007c; Heffernan *et al.*, 2006). Peripherally, local metabolic vasodilation following resistance exercise may have augmented artery distensibility (Heffernan et al., 2006). While central artery stiffness has been attributed to the Valsalva manoeuvre when performing resistance exercise movements (Heffernan et al., 2007a; Heffernan et al.,

2007c).

The results of the study presented in Chapter 4 represent the first data that follows the acute effects of a single sprint as well as that of a multiple sprint exercise session on arterial distensibility. This study showed that acute sprint exercise presents a unique vascular stress that acutely stiffens central arteries while increasing peripheral artery distensibility. The acute stiffening of central arteries mirrors that noted with acute resistance exercise (Devan *et al.*, 2005; Heffernan et al., 2007a) and is relatively short lived (ending at 20 minutes). Recently, studies indicate increased central artery stiffness during incremental aerobic exercise (Lydakis *et al.*, 2008a) and static exercise (Lydakis *et al.*, 2008b); however, by 10 minutes of recovery this effect dissipates (Munir *et al.*, 2008). Elevated arterial blood pressure, sustained latent smooth muscle contraction resulting from elevated circulating catecholamines (Greer, McLean, & Graham, 1998) or other circulating vasoconstrictors such as endothelin-1 (Wray *et al.*, 2007) may cause this brief stiffening of the central vasculature.

In the periphery, the marked and prolonged (up to 44 minutes) reduction of PWV (indicating increased distensibility) likely results from smooth muscle relaxation mediated by circulating vasoactive substances. Previous studies demonstrate increased superficial femoral leg blood flow up to 120 minutes into recovery (Hussain, Smith, Medbak, Wood, & Whipp, 1996). Elevated blood flow during recovery likely ensures an elevated shear stimulus of the endothelium lining leg blood vessels and thereby continued eNOS activity and NO production, as well as the release other vasoactive substance from the endothelium.

Clinically, reduced artery stiffness and enhanced endothelial function in this distal vascular bed may reduce or delay the reflected pulsewave, which could subsequently reduce cardiac impedance and cardiac work (O'Rourke, 2008). Recent literature suggests that in aged and diseased populations targeted modification of peripheral arteries may be ideal since central arteries, may be unresponsive to exercise training or pharmacological interventions because of their structural composition (O'Rourke, 2008). Whether this acute effect can be sustained with chronic exercise training remains an important question.

6.4.1 Future Directions

The results of Chapter 4 provided an overview of the acute effects of sprint exercise on artery distensibility both centrally and in the peripheral exercised limb. Although this study evaluated several mechanisms, others require further study to determine their contribution to the noted effects. Several suggested mechanisms for the changes with acute exercise included sympathetic nervous activity and circulating vasoactive substances. Pre and Post exercise evaluations of muscle sympathetic nervous activity and measurements of circulating levels of oxidative stress, nitric oxide products (NOx), endothelin-1, and antioxidant capacity would provide further insight. As well, we require studies, which describe the acute response of arterial distensibility to SIT or modified SIT in other populations, before adoption of this method of training especially in diseased or aged populations.

Recent studies have attempted to gain insight into the effect of acute exercise on central parameters of blood pressure and augmentation using non-invasive techniques

(Lydakis et al., 2008a; Lydakis et al., 2008b; Munir et al., 2008), however, these studies used indirect methods. We suggest using imaging techniques so that researchers can evaluate many central cardiovascular effects, such as cardiac performance, immediately post-exercise, and into recovery. Future studies evaluating vascular-ventricular coupling and efficiency with exercise combining these indirect (Lydakis et al., 2008a; Lydakis et al., 2008b; Munir et al., 2008) and direct methods could provide a better understanding of the interaction of arterial vascular stiffness with cardiac work during recovery from acute exercise.

6.5 Acute alterations of vascular reactivity following single or multiple sprint exercise

The study in Chapter 5 evaluated the acute alterations of vascular reactivity and reactive hyperaemic blood flow following both a single and a multiple sprint exercise session. Vascular reactivity provides a surrogate measure of nitric oxide mediated dilatory capacity and provides insight about vascular health (Corretti *et al.*, 2002). Previous research observed acutely impaired vascular endothelial function following ischemia inducing isometric handgrip contractions (McGowan *et al.*, 2006). Moderate intensity prolonged cycling also induced an acute impairment of vascular reactivity that was eliminated with antioxidant therapy (Silvestro *et al.*, 2006; Silvestro *et al.*, 2002). Studies in murine models indicate that exercise acutely and rapidly increases eNOS transcription and enhances endothelial function compared with pre-exercise for up to 48 hours after an initial reduction (up to 6h post exercise) before training. After training 6 weeks of training, up to 192h endothelial functionis enhanced and no acute reduction was

observed (Haram *et al.*, 2006). Haram et al. (2006) suggested that the acute increases in oxidative stress both quench NO, and the by-product peroxinitrile inhibits eNOS activity (Haram et al., 2006). By preincubation of excised vessels with superoxide dismutase they effectively eliminated the post-exercise oxidative stress and alleviated any transient endothelial dysfunction (Haram et al., 2006). Overall, this suggested that the vascular system must first experience acute endothelial dysfunction to adapt with training.

The study presented in Chapter 5 demonstrated acute reductions in vascular reactivity following both a single sprint exercise session, and a multiple sprint interval exercise session. This is the first data in humans, to our knowledge, involving sprint exercise and the vascular effects in the immediate post exercise period up to 2 hours. Similar to work with animal models (Haram et al., 2006) and other human studies involving acute exercise (McGowan et al., 2006; Silvestro et al., 2006; Silvestro et al., 2002; Rognmo et al. 2008), we observed reduced vascular reactivity in the immediate post exercise period. There were no differences between the two exercise protocols suggesting that 30s of "all-out" exercise provides a substantial oxidative stress that impairs vascular reactivity. These data also provide evidence that sprint exercise has the potential to induce vascular adaptations if used in a progressive training regime, since similar adaptations are noted in murine studies (Haram et al., 2006).

6.5.1 Future Directions

The study described in Chapter 5 highlights the potential effects of acute exercise on vascular reactivity in the immediate post exercise period. This research area requires additional studies using invasive methods conducted over a more prolonged period of up

to 2 days to elucidate mechanisms and to determine whether those in healthy human populations mirror animal model investigations. Since one study suggested enhanced endothelial function following exercise in active obese individuals (Harris, Padilla, Hanlon, Rink, & Wallace, 2008), further study to determine the timeline of these adaptations are needed. Particularly, the study by Harris et al. (2008) suggested enhanced endothelial function as early as 1-hour post exercise. This immediate response may relate to the phosphorylation (Hambrecht *et al.*, 2003) of the eNOS complex and thus enhanced activation following exercise since eNOS protein expression changes are unlikely at such an early time point post exercise.

This research area also requires studies that examine the timeline and characterise the dose response characteristics of stimuli that would alter the eNOS system with exercise, and newly developed techniques may provide the necessary tools. One technique in particular is the vascular endothelial biopsy technique (Colombo et al., 2002). Using this technique, researchers could measure the protein expression of various enzymes involved in vascular endothelial function and could determine the aforementioned time-course and stimuli required for endothelial adaptations with exercise.

6.5.2 Interaction of acute alterations of artery stiffness on arterial function

Chapters 4 and 5 involved the same participants completing all 4 testing days with an emphasis on separate vascular properties altered acutely following sprint exercise. The techniques used throughout these experiments were similar and allow for general comparisons. The stiffness and function of arteries interact in situations of altered

vascular tone. Exercise acutely alters vascular tone and this is a regional effect as evidenced by the results of Chapter 4, which showed increased stiffness in the central vasculature and decreased stiffness of the vasculature of the exercised limb. This reduction of peripheral vascular stiffness, which is a consequence of reduced vascular tone, likely limits further reductions of vascular tone and stiffness when stimulated to relax by flow mediated vascular shear stress. Therefore, a loss of vascular reactivity at the start of recovery may be a simple "ceiling effect" and a direct result of exercise induced low vascular tone.

Future studies need to specifically take into account the possibility that measures of vascular reactivity are being influenced by altered vascular tone. Further, many current methods, including FM-PWV and FMD, only provide a simple evaluation of vascular reactivity without proper

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APPENDIX A

Techniques and Methods

Appendix A

7.1 Non-invasive vascular measurement techniques in the human cardiovascular system

Throughout the different parts of this thesis document, a variety of non-invasive techniques are used to characterize structural and functional aspects of the cardiovascular system both at rest and during transitions between various intensities of exercise. These techniques involve measurements based on imaging and Doppler Ultrasound, arterial applanation tonometry, oscillatory automated cuff blood pressure, and photoplethysmography. Each of these techniques has inherent limitations that need to be discussed.

7.1.1 Imaging and Doppler ultrasound at rest and during exercise

Imaging ultrasound has been widely used in research and clinical settings to quantify structural components of various tissues in the human body. The density of the tissue surrounding blood vessels and heart chambers is quite different from blood allowing precise measurements throughout the cardiac cycle in both conduit arteries and cardiac tissue. The basic principle of imaging or 'B-mode' ultrasound is that high frequency sound waves are transmitted intermittently and received (pulsed) using a single transducer. If the transducer is paired with a position sensor, a two-dimensional image of a vessel can be created in 'real-time' as portions of the image with reduced sound reflection appear dark (blood) or bright (vessel walls).

The resolution of these images depends on both the ultrasound frequency and the speed of the sound waves through tissue (~1500 m/s) (Hedrick, Hykes, & Starchman, 2005). Thus, the resolution of typical ultrasound images obtained in the carotid artery is

in the range of 0.3-0.15mm (wavelength (λ) = speed of sound in tissue/ frequency) (Hedrick et al., 2005). However, semi-automated edge detection software improves the precision of this measurement considerably.

If an object is moving, Doppler velocimetry can be used to determine its' speed relative to the transducer. This is especially useful when measuring blood velocity. The following equation provides the basis of blood velocity measurement *in vivo* using Doppler ultrasound (Kremkau, 2006):

$$f_d = f_t - f_r = (2vf_t \cos 0)/c$$

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

When combined, 'B-mode' and Doppler ultrasound enables the estimation and monitoring of volume flow continuously. However, determining mean blood velocity measurements require processing large quantities of blood velocity data since each red blood cell (RBC) either deflects or reflects the Doppler signal providing a large number of Doppler shift frequencies and a highly variable signal with multiple frequency characteristics. It is common practise to determine the mean of Doppler velocimetry using frequency deconstruction (Nichols, McDonald, & O'Rourke, 2005). Most often, a fast Fourier transform (FFT) is used to determine the mean velocity at a high temporal resolution (as small as 5.0 ms). In our hands, a dedicated personal computer with specific software (Neurovision 500M TCD, Multigon Industries, Yonkers, NY) determines mean

blood velocity in 'real-time' providing blood velocity throughout the heart cycle.

Ultrasound imaging and Doppler are commonly used to measure artery distensibility (common carotid, superficial femoral and popliteal arteries), rest and exercising blood velocities, shear rates and blood flows. Exercising blood flow measurement was first described by Walloe and Wesche (1988) and is widely used throughout the field of exercise physiology (MacDonald, Shoemaker, Tschakovsky, & Hughson, 1998; MacDonald, Tarnopolsky, & Hughson, 2000; Pyke & Tschakovsky, 2007; Radegran, 1997, 1999; Rakobowchuk *et al.*, 2005a; Rakobowchuk *et al.*, 2005b; Shoemaker, Phillips, Green, & Hughson, 1996; Shoemaker, Pozeg, & Hughson, 1996; Sugawara *et al.*, 2004; Walloe & Wesche, 1988). It has been validated in comparison to invasively measured methods and has the advantage of providing high temporal resolution (Radegran, 1997, 1999).

Reviews highlight the limitations of Doppler ultrasound and some of these limitations will be outlined. First, insonated vessels must be relatively linear as tortuosity violates the stipulation of using Doppler ultrasound and measurements should be made at a distance from bifurcations (Nichols et al., 2005). These requirements relate to the accuracy of determining the insonation angle. At bifurcations and along tortuous sections of a vessel, turbulent blood flow alters the actual direction of RBCs within the region of interest making the measured isonation angle relative to the vector of the RBCs unknown. Second, insonation of the vessel is subject to inherent overestimation since the whole cross-section of the vessel is rarely insonated using current techniques in large conduit arteries (Hedrick et al., 2005). Third, the accuracy of mean blood velocity (MBV)

determined by FFT is hindered by a violation of steady-state required when performing an FFT (Nichols et al., 2005). Since blood velocity is rarely in a steady state, this assumption is violated when quantifying blood velocity signals. Each of these limitations is acknowledged and each may impact the absolute measures of blood velocity and subsequent estimations of blood flow and shear rates; however, the experiment designs used throughout the thesis are repeated measures. As such, measurement error is likely equal across all conditions and time.

7.1.2 Arterial applanation tonometry to determine arterial pressure wave contours

Recently non-invasive methods to determine arterial pressure have been developed based on applanation tonometry. This technique involves flattening (applanating) a superficial artery against an underlying solid (bone or cartilage) so that the tangential pressures internal to the artery are transmitted to an external sensor and recorded (Kelly, Hayward, Avolio, & O'Rourke, 1989). This technique has been validated against intra-arterial pressure throughout the frequency range of the physiological arterial pressure signal (Kelly et al., 1989) and produces a pulse contour that is virtually identical to that recorded intra-arterially.

The current thesis often uses two different applanation devices; one identical to that of Kelly et al. (1989) used at the carotid artery to determine pulse pressure (model SPT-301, Millar Instruments Inc., Texas, USA) and another radial tonometer which is automated and calibrated to cuff measured brachial artery blood pressure (model CBM-7000, Colin Medical Instruments, San Antonio, USA). Combined with measures of arterial diameter, artery distensibility measurements that conform to established

guidelines (O'Rourke, Staessen, Vlachopoulos, Duprez, & Plante, 2002) were acquired and analyzed.

Limitations of this technique involve the calibration of the carotid pulse pressure and proper applanation of the carotid artery (O'Rourke, 2008). Appropriate calibration involves invasive blood pressure calibration since one of the major sources of error with this method is calibration of the signal to non-invasive blood pressure cuffs (Sharman *et al.*, 2006). However, this is rarely appropriate and simply using an invasive method to measure blood pressure would preclude a need for applanation tonometry. Therefore, though non-invasive calibration is not ideal, it is more appropriate than no calibration. Nichols et al. (2005) outlined a group of criteria for acceptance of a tonometer signal and they are outlined as follows:

1. A sharp upstroke of the pulse from its diastolic nadir
2. A steep, uninterrupted rise of pressure to a peak some 100ms after the foot of the wave
3. A second, late systolic shoulder (or rarely a second peak) between the first peak and incisura.
4. A sharp inflection to identify the incisura, which identifies aortic valve closure.
5. A second pressure rise in early diastole (only when the second systolic shoulder is close to the incisura).
6. A near exponential decline in pressure during the latter part of diastole before onset of the next pulse.

These criteria are not often a problem in young healthy participants such as those examined in the current thesis (Nichols et al., 2005).

7.1.3 Arterial blood pressure measured with photoplethysmography

Commonly known as the commercial product Finapres (Finapres 2300, Ohmeda, Englewood, Colorado, USA) or Finometer (Finometer, Finapres Medical Systems BV,

Amsterdam, The Netherlands), this non-invasive method for acquiring blood pressure is relatively easy to use and provides reproducible measurement of arterial pressure within the standards outlined by the U.S. Federal Dood and Drug Administration. This device uses the volume clamp method described by Pénaz (van Egmond, Hasenbos, & Crul, 1985). A small finger cuff is attached to an appropriately selected finger after the finger pressure compared to brachial blood pressure measurement and deemed similar. An infrared transmitter and receiver present on the interior of the cuff and an automated servo-adjusted pneumatic cuff monitors the infrared signal continuously and the cuff pressure is adjusted quickly to maintain a constant infrared signal (Imholz *et al.*, 1988). The device continuously monitors the cuff pressure needed to maintain the constant infrared signal and relays this information to the user since this pressure theoretically should be very close to that of absolute blood pressure. Inaccuracies arise when the cuff is placed on an inappropriate finger (too large or too small) the hand is not placed at the level of the heart, or the patient is experiencing vasoconstriction (Imholz, Wieling, van Montfrans, & Wesseling, 1998).

In summary this device provides a good measure of pressure when appropriately applied and used in combination with other methods of blood pressure assessment.

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APPENDIX B
Power Calculations

Appendix B

8.1 Power calculations for specific variables in studies 1 to 4

Statistical power calculations were made for primary outcome variables in each study based *post hoc* although *a priori* power analysis was conducted prior to each study. In the case of *a priori* power analysis, the chosen variables used to determine statistical power were generally those with the greatest variability in our laboratory when previous data was available and within the field when these data were not available. The following sections give an overview of the *post hoc* power analysis of each study.

8.1.1 Power calculations for the integrative cardiovascular response study (Chapter 2)

All power analyses were performed with an alpha level of 0.05. The variables of interest in the integrative cardiovascular response study include: blood flow kinetic phase II time constant, oxygen uptake discrete measures, cardiac output discrete measures and heart rate average response. Further the measure of rate pressure product was also calculated with regards to the interaction of training and group. The following table displays the power values for each variable.

Table 8.1 Variables of interest and the observed power calculated from the results of Chapter 2

Variable of interest	Observed statistical power
Phase II blood flow kinetics training main effect	0.061
Oxygen uptake training effect	0.051
Cardiac output training main effect and training by time interaction	0.050 and 0.404
Rate pressure product interaction of training and group	0.555
Stroke volume main effect for training	0.564
Heart rate training main effect for SIT and ET	1.000 (SIT) and 1.000 (ET)

8.1.2 Power calculations for the resting vascular parameters (Chapter 3)

All power analyses were performed with an alpha level of 0.05. The variables of interest in the resting vascular parameters study following SIT or ET include: carotid artery distensibility, popliteal artery distensibility and FMD, carotid IMT, and carotid PP. The following table displays the power values for each variable.

Table 8.2 Variables of interest and the observed power calculated from the results of Chapter 3

Variable of interest	Observed statistical power
Carotid artery distensibility	0.18
Popliteal artery distensibility	0.871
Carotid artery intima-media thickness	0.068
Popliteal flow-mediated dilation	0.500
Carotid artery pulse pressure	0.142

8.1.3 Power calculations for Chapter 4 on acute arterial stiffness following sprint exercise

All power analyses were performed with an alpha level of 0.05. The variables of interest in this chapter include: central and peripheral PWV, heart rate, MAP, and superficial femoral artery stiffness. The following table displays the power values for each variable.

Table 8.3 Variables of interest and the observed power calculated from the results of Chapter 4

Variable of interest	Observed statistical power
Central pulsewave velocity	1.000
Peripheral pulsewave velocity	1.000
Heart rate	1.000
Mean arterial pressure	1.000
Superficial femoral artery stiffness index	0.682

8.1.4 Power calculations for Chapter 4 on acute vascular reactivity following sprint exercise

All power analyses were performed with an alpha level of 0.05. The variables of interest in this chapter include: baseline peripheral PWV, post occlusion PWV, FM-PWV, post occlusion average shear rate, and the area under the blood flow curve. The following table displays the power values for each variable.

Table 8.4 Variables of interest and the observed power calculated from the results of Chapter 5

Variable of interest	Observed statistical power
Baseline pulsewave velocity time and trial main effects	1.000 and 0.985
Post occlusion pulsewave velocity time and trial main effects	0.999 and 0.540
Flow-mediated pulsewave velocity time and trial main effects	0.996 and 0.060
Post occlusion average shear rate time and trial main effects	1.000 and 0.527
Hyperaemic blood flow area under the curve	1.000 and 0.786.