University of Alberta

The effect of aerobic fitness on the cardiovascular and sympathetic nervous system response to physiological stress at rest and during dynamic exercise

by

Duncan Andrew Raymond

A thesis submitted to the Faculty of Graduate Studies and Research
in partial fulfillment of the requirements for the degree of

Master of Science

Faculty of Physical Education and Recreation

Fall 2012
Edmonton, Alberta

Abstract

A cardio-protective adaptation associated with aerobic fitness may be an attenuated sympathetic nervous system (SNS) and cardiovascular response to stress. The hypothesis that the cardiovascular and SNS responses to physiological stress at rest and during exercise would be a function of maximal aerobic capacity (VO_2max) was investigated. Young males performed ramp cycling exercise to determine VO_2max and were then assigned to low (n=8), mid (n=8) and high (n=7) aerobic fitness groups. The physiological responses to a cold-pressor test and isometric handgrip exercise were measured at rest and during moderate- and heavy-intensity knee-extension (KE) exercise. Highly fit subjects had lower resting muscle sympathetic nerve activity (MSNA), but a larger MSNA response to physiological stress at rest. The cardiovascular response to stress at rest or during KE exercise was not altered by aerobic fitness. Heavy-intensity KE exercise attenuated leg vasoconstriction in response to physiological stress by a similar magnitude in all groups.

Acknowledgement

I'd first like to thank my supervisor Dr. Darren DeLorey. His guidance was instrumental throughout the entire process of my master's degree. Without the knowledge gained, training in our laboratory and overall vision for the study, I would have had a much lesser graduate student experience.

I would also like to thank both Dr. Kelvin Jones from the University of Alberta and Dr. Craig Steinback from the University of Calgary. Their technical expertise was paramount in this project, not to mention the numerous hours spent explaining and showing me how to analyze these data.

I would be remiss if I did not give an enormous extension of gratitude to Tim Just, Natalie Chua, Ryan Roszko, Nick Jendzjowsky, Scott MacLeod and Emily Ainsley; all of whom put in extensive hours in the laboratory, helping make this project possible. Without their help and dedication, this study would not have been possible.

I'd like to thank my committee members - Dr. Michael Kennedy and Dr. Linda McCargar. I sincerely appreciate the time and feedback they've provided me while outlining my study and in pursuit of my degree.

Finally, I would like to acknowledge and thank all the participants who graciously volunteered their time. These studies could not happen if it was not for them.

Table of Contents

List of Tables

List of Figures

Figure 2-11. Individual representative tracing of the cardiovascular (heart rate, HR; mean arterial pressure, MAP; limb blood flow, LBF; femoral vascular conductance, FVC) response to the cold-pressor test (CPT) during knee-extension exercise. The CPT was initiated at 480 s, was 180 s in duration and was followed by 60 s of recovery. Each data point represents a 5 second average.

Figure 2-12. Percent change of heart rate (HR) in response to the cold-pressor test (CPT) at rest, and during moderate- and heavy-intensity knee-extension exercise in low (n=7), mid (n=8) and high (n=7) aerobic fitness groups. ψ Indicates a significant difference between rest and exercise. Values are mean ± SD.

Figure 2-13. Percent change of mean arterial pressure (MAP) in response to the cold-pressor test (CPT) at rest, and during moderate- and heavy-intensity knee-extension exercise in low (n=7), mid (n=8) and high (n=7) aerobic fitness groups. ψ Indicates a significant difference between rest and exercise. ϕ Indicates a significant difference between all exercise intensities. Values are mean ± SD.

Figure 2-14. Percent change of femoral vascular conductance (FVC) in response to the cold-pressor test (CPT) at rest, and during moderate- and heavy-intensity knee-extension exercise in low (n=7), mid (n=8) and high (n=7) aerobic fitness groups. γ Indicates a significant difference between heavy-intensity exercise, and rest and moderate-intensity exercise. Values are mean ± SD.

Figure 2-15. Magnitude of sympatholysis (expressed as the difference in %ΔFVC in response to CPT during exercise, from the %ΔFVC during CPT at rest) in response to the cold-pressor test (CPT) during moderate- and heavy-intensity knee-extension (KE) exercise in low (n=7), mid (n=8) and high (n=7) aerobic fitness groups. χ Indicates a significant difference between moderate- and heavy-intensity KE exercise. Values are mean ± SD.

Figure 2-16. Individual representative tracing of the cardiovascular (heart rate, HR; mean arterial pressure, MAP; limb blood flow, LBF; femoral vascular conductance, FVC) response to the IHG at rest. The IHG was initiated at 300 s, was 180 s in duration and was followed by 60 s of recovery. Each data point represents a 5 second average.

Figure 2-17. Percent change of muscle sympathetic nerve activity (MSNA) burst frequency in response to isometric handgrip (IHG) at rest in low (n=5), mid (n=6) and high (n=6) aerobic fitness groups. $^{+}$ Significant difference from both low and mid fitness groups ($p< 0.05$). Values are mean ± SD.

Figure 2-18. Percent change of muscle sympathetic nerve activity (MSNA) burst incidence in response to isometric handgrip (IHG) at rest in low (n=5), mid (n=6) and high (n=6) aerobic fitness groups. $^{++}$ Significant difference between high aerobic fitness and mid aerobic fitness group ($p< 0.05$). Values are mean ± SD.

Figure 2-19. Sympathetic vascular transduction (SVT) during isometric handgrip (IHG) at rest in low (n=5), mid (n=6) and high (n=6) aerobic fitness groups. ## Significant difference from high and low aerobic fitness groups ($p < 0.05$). Values are mean ± SD.

Figure 2-20. Individual representative tracing of the cardiovascular (heart rate, HR; mean arterial pressure, MAP; limb blood flow, LBF; femoral vascular conductance, FVC) response to isometric handgrip (IHG) during knee-extension exercise. The IHG was initiated at 480 s, was 180 s in duration and was followed by 60 s of recovery. Each data point represents a 5 second average.

Figure 2-21. Percent change of heart rate (HR) in response to isometric handgrip (IHG) at rest, and during moderate- and heavy-intensity knee-extension exercise in low (n=8), mid (n=7) and high (n=7) aerobic fitness groups. ψ Indicates a significant difference between rest and exercise. Values are mean ± SD.

Figure 2-22. Percent change of mean arterial pressure (MAP) in response to isometric handgrip (IHG) at rest, and during moderate- and heavy-intensity knee-extension exercise in low (n=8), mid (n=7) and high (n=7) aerobic fitness groups. ϕ Indicates a significant difference between all exercise intensities. Values are mean ± SD.

Figure 2-23. Percent change of femoral vascular conductance (FVC) in response to isometric handgrip (IHG) at rest, and during moderate- and heavy-intensity knee-extension exercise in low (n=8), mid (n=7) and high (n=7) aerobic fitness groups. γ Indicates a significant difference between heavy-intensity exercise, and rest and moderate-intensity exercise. Values are mean ± SD.

Figure 2-24. Magnitude of sympatholysis (expressed as the difference in %ΔFVC in response to IHG during exercise, from the %ΔFVC during IHG at rest) in response to isometric handgrip (IHG) during moderate- and heavy-intensity knee-extension (KE) exercise in low (n=8), mid (n=7) and high (n=7) aerobic fitness groups. χ Indicates a significant difference between moderate- and heavy-intensity KE exercise. Values are mean ± SD.

List of Abbreviations

ABP - arterial blood pressure (mmHg)

ATP - adenosine triphosphate

BP - blood pressure (mmHg)

CPT - cold-pressor test

ECG - electrocardiogram

FBV - femoral artery blood velocity

FVC - femoral vascular conductance ($L \cdot min^{-1} \cdot mmHg^{-1}$)

FVR - femoral vascular resistance ($mmHg \cdot L^{-1} \cdot min^{-1}$)

HR - heart rate ($beats \cdot min^{-1}$)

IHG - isometric handgrip

KE - knee extension

KEmax - knee-extensor max

LBF - limb blood flow ($L \cdot min^{-1}$)

MAP - mean arterial pressure (mmHg)

MBV - mean blood velocity ($cm \cdot s^{-1}$)

MSNA - muscle sympathetic nerve activity

MVC - maximum voluntary contraction (N)

NE - norepinephrine

NO - nitric oxide

PE - phenylephrine

Q - cardiac output ($L \cdot min^{-1}$)

RER - respiratory exchange ratio

SV - stroke volume ($mL \cdot min^{-1}$)

SVT - sympathetic vascular transduction (Δ FVC (%)$\cdot\Delta$ Burst Frequency (%)$^{-1}$)

TPR - total peripheral resistance ($mmHg \cdot L^{-1} \cdot min^{-1}$)

VCO_2 - volume of carbon dioxide ($L \cdot min^{-1}$)

VE – minute ventilation ($L \cdot min^{-1}$)

VO_2 - volume of oxygen ($L \cdot min^{-1}$) or ($mL \cdot kg^{-1} \cdot min^{-1}$)

VO_2max - maximal oxygen consumption ($L \cdot min^{-1}$) or ($mL \cdot kg^{-1} \cdot min^{-1}$)

VT - ventilatory threshold

W - watts

80% VT - 80% of ventilatory threshold, moderate knee-extension intensity

Δ50% - midway (50%) between VT and KEmax, high knee-extension intensity

CHAPTER 1: Introduction

1.1 Introduction

1.1.1 Overview

Cardiovascular disease is the leading cause of premature mortality, not only in Canada, where it claims the lives of ~ 72,000 Canadians annually, but also throughout the Western world (Pub Health Agency, 2004). In addition to risk factors such as smoking and high blood cholesterol, a sedentary (physically inactive) lifestyle is a major modifiable risk factor for cardiovascular disease (Lakatta & Levy, 2003). Indeed, the risk of being sedentary may exceed the relative risk attributed to more traditional risk factors such as smoking, obesity and hypertension (Green et al., 2008; Mora et al., 2007).

A sedentary lifestyle can lead to various physiological adaptations that may compromise an individual's functional independence and health. Vascular dysfunction, elevated blood pressure, and insulin resistance are just some of the negative health effects associated with a sedentary lifestyle that may be associated with the development of chronic disease (Forcier et al., 2006).

In contrast, regular physical activity and aerobic fitness appear to have favorable effects on cardiovascular function, and have been linked to a reduced risk of cardiovascular disease (Blair et al., 1989). Surprisingly, ~ 60% of the reduction in cardiovascular disease risk associated with exercise-training cannot be attributed to a reduction of traditional, modifiable cardiovascular disease risk-factors, such as weight loss and a reduction in blood pressure and blood lipids (Green et al., 2008; Mora et al., 2007).

One potential mechanism that may be associated with the cardio-protective effects of regular exercise and aerobic fitness is an attenuated response to physiological and psychological stress. The physiological response to exercise is similar to that of stress and it has been argued that repeated exposure to the stress of exercise may lead to an attenuated physiological response to other stressors. Sothmann and colleagues (1996) have described this as the "cross-stressor adaptation". Specifically, it has been argued that people who regularly engage in physical activity would have a reduced physiological reactivity to stress relative to a sedentary person (Sothmann et al., 1996). A blunted physiological reactivity to stress may be cardio-protective, as associations between the physiological response to stress, repeated and or chronic exposure to stress and cardiovascular disease have been documented (Krantz & Manuck, 1984; Manuck, 1994; McEwen, 1998).

The physiological response to stress is predominately mediated by the sympathetic, cardiovascular and hormonal systems. In response to stress, sympathetic nervous system activity increases and elicits numerous physiological responses, including an increase in heart rate (HR) and blood pressure, dilation of respiratory bronchioles and inhibition of non-vital bodily functions such as peristalsis (Brodal, 2003). The functional consequence of an increase in sympathetic nervous system outflow to the peripheral vasculature is vasoconstriction, elevated vascular resistance and blood pressure and a reduction of tissue blood flow (Rowell, 1993). An increase in peripheral vasoconstriction may also influence the dynamic regulation of skeletal muscle blood flow at rest and during exercise as the balance between sympathetic nervous system mediated vasoconstriction and local vasodilation may be altered (Rowell, 1993).

During exercise there is substantial vasodilation in skeletal muscle to accommodate the requisite increase in oxygen delivery needed to meet the elevated O_2 demand (Delp, 1999). Concomitantly, sympathetic nerve activity increases to exercising and non-active tissues in a manner dependent upon the intensity of exercise (DiCarlo et al., 1996; Hajduczok et al., 1991; Hill et al., 1996; O'Hagan et al., 1993; Savard et al., 1987; Savard et al., 1989).

The presence of tonic sympathetic nervous system mediated vasoconstriction in the skeletal muscle vascular bed is well established, and vasoconstriction in exercising skeletal muscle must balance the robust local vasodilation in order to maintain arterial blood pressure (Buckwalter et al., 1997; Rowell, 1993). However, the vascular response to muscle sympathetic nerve activity (MSNA) appears to be blunted in exercising skeletal muscle, a phenomenon known as functional sympatholysis (Remensnyder et al., 1962). Indeed, several investigations have reported that the vascular response to sympathetic stimulation is attenuated from rest to exercise and the magnitude of attenuation appears to increase as the intensity of exercise increases (Buckwalter & Clifford, 1999; Buckwalter et al., 2001; Joyner et al., 1992; Rosenmeier et al., 2003; Ruble et al., 2002). While the mechanism responsible for the diminished vascular response to sympathetic nerve activity has not been determined, an attenuation of post-synaptic adrenergic and non-adrenergic receptor responsiveness has been implicated (Anderson & Faber, 1991; Buckwalter et al., 2001; Dinenno & Joyner, 2003; Rosenmeier et al., 2003; Thomas et al., 1994; Thomas et al., 1998). A reduction of post-synaptic receptor responsiveness may be caused by acidosis, local hypoxia, skeletal muscle metabolite production and local temperature, all of which may change in response to exercise (Dinenno, 2003; Hansen et

al., 2000; Saltin et al., 1998; Thomas & Segal, 2004). However, to date, none of the above factors have been shown to mediate sympatholysis. Activation of adenosine triphosphate (ATP)-sensitive K^+ channels has been shown to attenuate sympathetic vasoconstriction, suggesting that sympatholysis may be influenced by cellular energy levels (Tateishi & Faber, 1995; Thomas et al., 1997). Evidence is also accumulating that nitric oxide (NO) may be involved in the blunting of sympathetic vasoconstriction, because removal of the endothelium or NO-synthase blockade enhances the vascular response to sympathetic stimulation (Chavoshan et al., 2002; Dinenno & Joyner, 2004; Nase & Boegehold, 1996; Nase & Boegehold, 1997; Sander et al., 2000; Tesfamariam & Cohen, 1988; Tesfamariam et al., 1987; Thomas et al., 1998; Thomas & Victor, 1998; Thomas et al., 2001). Aerobic fitness and/or regular physical activity is known to improve the vasodilator capacity of the skeletal muscle vasculature and improve the matching of local O_2 delivery to O_2 demand (Lash & Bohlen, 1992; Martin et al., 1991). The improved vasodilation appears to be associated with increased NO bioavailability (Sun et al., 1994; Sun et al., 2002) and/or NO-related signaling mechanisms (Koller et al., 1995).

Whether aerobic fitness influences the magnitude of functional sympatholysis has not been elucidated. An augmented ability to blunt sympathetic vasoconstriction in active skeletal muscle may facilitate the matching of O_2 delivery to O_2 demand and augment exercise capacity. Numerous groups have investigated vasoconstriction and sympatholysis in exercising skeletal muscle (Dinenno & Joyner, 2003; Hansen et al., 1996; Joyner et al., 1992; Keller et al., 2003; O'Leary et al., 1991; Richardson et al., 1995; Rosenmeier et al., 2003; Ruble et al., 2000; Ruble et al., 2002; Shoemaker et al.,

1997; Thomas et al., 1994; Thomas et al., 1997; Thomas et al., 1998; Tschakovsky et al., 2002), however the effect of aerobic fitness on the regulation of sympathetic vasoconstriction during exercise has received limited attention.

A potential weakness of previous investigations in this area is that we are not aware of any study that has investigated both the sympathetic and vascular responses to physiological stress simultaneously in the same subjects. Moreover, previous studies have investigated the effects of aerobic fitness across a limited range of aerobic fitness levels.

1.1.2 Sympathetic nervous system

The sympathetic nervous system, a branch of the autonomic nervous system, regulates numerous physiological functions throughout the body. These include blood pressure, tissue blood flow, temperature regulation, water balance, peristalsis and heart rate (Brodal, 2003). The sympathetic nervous system aids in maintaining homeostasis during stress and can up- or down-regulate homeostatic mechanisms when faced with a stressor (Brodal, 2003). Sympathetic nerve pathways consist of two neurons arranged in series that innervate tissues throughout the body. Pre-ganglionic neurons originate in the central nervous system and project from the thoracolumbar area of the vertebral column (T1-L2), to sympathetic ganglia that lie outside the central nervous system in two chains that run along the sides of the vertebral column (Brodal, 2003). At the ganglion, the pre-ganglionic neuron synapses with post-ganglionic fibers. Most sympathetic pathways have short pre-ganglionic nerves and long post-ganglionic nerves. All pre-ganglionic fibers are cholinergic, and thus use acetylcholine as their neurotransmitter, which binds to

nicotinic cholinergic receptors on the post-ganglionic fibers (Brodal, 2003). Subsequently, post-ganglionic fibers release neurotransmitters, which bind to post-synaptic receptors on peripheral tissues to affect target tissues (Brodal, 2003).

The sympathetic nervous system innervates the heart and vascular tree (i.e. arteries, arterioles, veins) and is important to the regulation of cardiac function, tissue blood flow and the maintenance of systemic blood pressure (Buckwalter et al., 2001). The sympathetic nervous system innervates the heart through β_1- and β_2-adrenergic receptors, which increase HR and myocardial contractility, resulting in an increased cardiac output (Thomas, 2011). The sympathetic nerves exert their influence on peripheral blood vessels through neurotransmitters binding to post-synaptic receptors on vascular smooth muscle. It is well established that efferent MSNA produces tonic vasoconstriction in the resting skeletal muscle vascular bed (Rowell, 1993). The magnitude of sympathetic nervous system vasoconstriction varies as a function of sympathetic nerve activity; with increased activity producing increased vascular resistance, increased blood pressure and reduced tissue blood flow, whereas less sympathetic activity causes vascular tone to decline, decreased vascular resistance and systemic arterial blood pressure and increased tissue blood flow. Sympathetic vasoconstriction is opposed by local vasodilation, and vascular tone and tissue perfusion are therefore regulated by a dynamic balance between local vasodilation and sympathetic vasoconstriction (Rowell, 1993).

Exposure to stress results in increased efferent sympathetic nerve activity. An increased frequency of firing and increased amplitude of individual bursts of nerve activity results in the release of larger quantities of neurotransmitters. Within the

cardiovascular system, this increased nerve activity increases arterial blood pressure, local vascular resistance and HR (Rowell, 1993).

1.1.3 Sympathetic activity and vasoconstriction at rest

The maintenance of arterial vascular resistance is of paramount importance to the distribution of cardiac output, the regulation of tissue perfusion and maintenance of arterial pressure. MSNA occurs in bursts of nerve impulses (Hagbarth & Vallbo, 1968; Johnson & Gilbey, 1996; Sundlof & Wallin, 1977) that typically occur during diastole. This nerve activity causes the release of neurotransmitter from the nerve terminal, which binds to post-synaptic receptors on vascular smooth muscle causing vasoconstriction. Evidence has shown that different frequencies of neural activity determine the type and quantity of neurotransmitter released (Bradley et al., 2003; Elam et al., 2003; Tanaka et al., 2003). Specifically, low frequency bursting patterns tend to release ATP followed by norepinephrine (NE) release, mid-frequency bursting patterns favour NE release while high-frequency bursting favours neuropeptide Y (NPY) release (Evans & Cunnane, 1992; Haniuda et al., 1997; Pernow et al., 1989; Ren & Burnstock, 1997). It had previously been thought that only post-synaptic α_1-adrenergic receptors would bind with NE to produce sympathetic vasoconstriction; however, it is now established that NE also binds to α_2-adrenergic receptors, and that the neurotransmitters ATP and NPY bind to purinergic (P2X) and NPY receptors, respectively and produce vasoconstriction.

Effect of aerobic fitness and exercise training on sympathetic vasoconstriction at rest

It is well established that efferent MSNA produces tonic vasoconstriction in the resting skeletal muscle vascular bed (Rowell, 1993). However, the effect of aerobic fitness on efferent MSNA has yet to be resolved, as the available scientific evidence is limited and contradictory. The effect of aerobic fitness training has been shown to increase, decrease and have no effect on MSNA at rest (Grassi et al., 1994; Ray, 1999; Ray & Carter, 2010; Sinoway et al., 1996; Seals, 1991; Svedenhag et al., 1984). In a healthy older adult population, Ng et al. (1994) found that aerobically trained individuals, compared to age-matched sedentary adults, had elevated levels of resting MSNA.

The functional consequence of MSNA in the vasculature is vasoconstriction. The magnitude of the constrictor response for a given level of sympathetic outflow can be influenced by the responsiveness of post-synaptic vascular receptors. It has been suggested that repeated exposure to elevated levels of sympathetic outflow during exercise may down-regulate post-synaptic receptor responsiveness following exercise training (Smith et al., 2007). Indeed, Donato et al. (2007) reported that α-adrenergic receptor responsiveness was attenuated after exercise training in isolated rat skeletal muscle arterioles. In contrast, Lash (1998) reported that in treadmill trained rats (9-10 weeks, 30 $m \cdot min^{-1}$, 1.5° incline, 90 $min \cdot day^{-1}$) adrenergic receptor-mediated vasoconstriction was enhanced in the spinotrapezius muscle. At high doses of NE and epinephrine, Lash (1998) noted more constriction in trained rats as compared to sedentary rats in terminal-feed arteries and first-order arterioles, suggesting that adrenergic receptors responsiveness may increase following exercise-training. Consistent with this notion, Jendzjowsky and DeLorey (2012) recently reported that 4 weeks of exercise

training increased vasoconstriction in response to sympathetic nerve stimulation in rats. The magnitude of vasoconstriction in response to sympathetic stimulation (lumbar sympathetic chain stimulation) increased in a training-intensity dependent manner, indicating that the intensity of training may influence the effects of exercise training on vascular responsiveness. Spier et al. (1999) reported that a single exercise bout did not attenuate the responsiveness abdominal aorta rings to vasoconstrictor agonists, but saw a decreased responsiveness to NE after 10 weeks of training, suggesting that the duration of training may also influence the effects of exercise training on vascular responsiveness. These studies demonstrate that exercise training can affect vascular responsiveness, however the available evidence is limited and contradictory and our understanding of the dose- response relationship between exercise training and vascular adaptations is very limited. Therefore, at this time, it is not possible to arrive at a consensus on the effects of exercise training on the regulation of MSNA and sympathetic vascular responsiveness and further investigation in this area is warranted.

1.1.4 Sympathetic activity and vasoconstriction during exercise

During exercise the vascular bed of active skeletal muscles dilates to facilitate an increase in blood flow and oxygen delivery (Delp, 1999). Concomitantly, sympathetic nerve activity increases to exercising and non-active tissues as a function of the exercise intensity (DiCarlo et al., 1996; Hill et al., 1996; O'Hagan et al., 1993; Savard et al., 1987; Savard et al., 1989). Buckwalter and colleagues (1997) suggested that the increased MSNA might cause neurally-mediated vasodilation. To test this hypothesis, Buckwalter et al. (1997) infused beta-adrenergic and muscarinic receptor antagonists into the skeletal

muscle vascular bed of chronically instrumented canines. The blood flow response to exercise was similar prior to and following receptor antagonism, demonstrating that sympathetic outflow was not producing local vasodilation. Subsequent investigations have demonstrated the presence of tonic vasoconstriction in the vasculature of exercising muscle even during intense exercise (Buckwalter & Clifford, 1999; O'Leary et al., 1997). The presence of tonic vasoconstriction in exercising skeletal muscle appears counter-intuitive, however it is currently believed that tonic vasoconstriction in exercising skeletal muscle must balance the robust local vasodilation that occurs with exercise in order to maintain systemic arterial blood pressure.

Although sympathetic restraint of active skeletal muscle blood flow has been documented, it has also been demonstrated that the effectiveness of sympathetic outflow is attenuated in active compared to resting skeletal muscle. Remensnyder et al. (1962) first termed this attenuated vascular response to augmented sympathetic outflow during exercise "functional sympatholysis". The mechanism(s) responsible for functional sympatholysis have not been identified. However, considerable evidence suggests that the responsiveness of post-synaptic adrenergic receptors may decline during exercise (Anderson & Faber, 1991; Buckwalter et al., 2001; Remensnyder et al., 1962; Rosenmeier et al., 2003; Ruble et al., 2002; Thomas et al., 1997).

Effect of aerobic fitness and exercise-training on sympathetic vasoconstriction during exercise

Due to technical limitations associated with the microneurography technique, no direct measurements of MSNA to active muscle have been made during dynamic

exercise. However, experiments that have measured MSNA in an inactive muscle during exercise have provided some insight on the effects of exercise training on MSNA during exercise. Sinoway et al. (1996) have shown that 4 weeks of unilateral forearm exercise training attenuated the increase in MSNA and NE spillover in response to non-fatiguing (25% MVC) rhythmic forearm exercise. Somers et al. (1992) also reported a significant attenuation of the MSNA response to isometric handgrip exercise following 6 weeks of handgrip exercise training. Consistent with these findings, Ray (1999) reported that the increase in MSNA during single-leg knee extension exercise was attenuated following 6 weeks of one-legged cycle exercise training. In contrast, Ng et al. (1994) and Seals (1991) both reported no difference in MSNA in response to isometric handgrip exercise between physically trained and untrained subjects.

The effect of exercise training on vascular responsiveness in contracting muscle has received relatively little attention in the scientific literature. Recently, O'Sullivan and Bell (2001) found that the heart rate and blood pressure responses to sustained isometric handgrip exercise were attenuated in aerobically trained ($VO_2max = 68 \pm 3$ $mL \cdot kg^{-1} \cdot min^{-1}$) compared to untrained subjects ($VO_2max = 54 \pm 2$ $mL \cdot kg^{-1} \cdot min^{-1}$). Subsequently, the "untrained" subjects completed five weeks of moderate-intensity aerobic exercise training, which significantly increased maximal aerobic capacity and caused a blunting of their HR and blood pressure in response to isometric handgrip exercise when compared to their pre-training response. These findings suggest that aerobic exercise training may attenuate the hemodynamic response to isometric exercise. Wray and colleagues (2007) also compared the central hemodynamic and the leg and arm vascular response to acute physiological stress, a cold-pressor test (CPT), during both

dynamic knee-extension and handgrip exercise in aerobically fit cyclists (VO_2max = 64 ± 2 mL·kg^{-1}·min^{-1}) and sedentary (VO_2max = 40 ± 2 mL·kg^{-1}·min^{-1}) subjects. During knee extension exercise, the CPT caused similar increases in mean arterial pressure and HR, and decreases in leg vascular conductance in the sedentary and trained cyclists, whereas during handgrip exercise the CPT produced a smaller decrease in arm vascular conductance in sedentary compared to trained cyclists. These findings suggest that exercise training may produce limb-specific changes in vascular responsiveness.

Overall, the effect of aerobic fitness on the cardiovascular and sympathetic nervous system responses to stress is inconclusive. As such, the effect of aerobic fitness on the cardiovascular and sympathetic response to stress remains to be fully characterized.

1.2 Purpose

The purpose of this thesis was to investigate the effect of aerobic fitness [defined by the subjects maximal oxygen consumption (VO_2max)] on the sympathetic nervous system and cardiovascular response to acute physiological stress at rest and during dynamic exercise in humans.

This thesis investigated whether the aerobic fitness of an individual: 1) influenced the magnitude of the sympathetic nervous system response to acute physiological stress under resting conditions, and 2) influenced the cardiovascular response (including HR, blood pressure and leg vascular) to acute physiological stress at rest and during moderate- and heavy-intensity exercise.

1.3 Hypothesis

It was hypothesized that the magnitude of the sympathetic and cardiovascular responses to acute physiological stress would decline as a function of aerobic fitness at rest and during exercise.

1.4 Significance

Regular physical activity is commonly prescribed by doctors and recommended by national public health agencies as a treatment and preventative measure for cardiovascular disease. However, our understanding of the mechanisms responsible for the cardio-protective effects of physical activity and aerobic fitness is limited. Paterson et al. (2007) have argued that simply being "physically active" may not be associated with a reduction of cardiovascular disease risk and that the cardio-protective effects of exercise are the result of being "fit" (defined by maximal oxygen consumption) and not simply being "active". Consistent with this postulate, Blair et al. (1996) has reported that the relative risk of cardiovascular disease and all-cause mortality in men and women is associated with low aerobic fitness.

This thesis investigated one potential mechanism that may be mechanistically responsible for the cardio-protective effects attributed to aerobic fitness. Specifically, the influence of aerobic fitness on the sympathetic and cardiovascular response to physiological stress was investigated at rest and during exercise. An attenuated sympathetic nervous system and cardiovascular response to physiological stress in aerobically fit subjects would be interpreted as a physiological adaptation that protects fit individuals from the deleterious effects of stress.

Therefore, this thesis investigated a topic of fundamental importance to public health in Canada.

1.5 References

Anderson KM, Faber JE. Differential sensitivity of arteriolar alpha 1- and alpha 2-adrenoceptor constriction to metabolic inhibition during rat skeletal muscle contraction. *Circ Res*. 69(1): 174-84, 1991.

Blair SN, Kampert JB, Kowl HW 3rd, Barlow CE, Macera CA, Paffenbarger RS Jr, Gibbons LW. Influences of cardiorespiratory fitness and other precursors on cardiovascular disease and all-cause mortality in men and women. *JAMA*. 276 (3): 205-10, 1996.

Blair SN, Kohl HW 3rd, Paffenbarger RS Jr, Clark DG, Cooper KH, Gibbons LW. Physical fitness and all-cause mortality. A prospective study of healthy men and women. *JAMA.* 262: 17: 2395-2401, 1989.

Bradley E, Law A, Bell D, Johnson CD. Effects of varying impulse number on cotransmitter contributions to sympathetic vasoconstriction in rat tail artery. *Am J Physiol Heart Circ Physiol.* 284: 6: H2007-2014, 2003.

Buckwalter JB, Clifford PS. a-adrenergic vasoconstriction in active skeletal muscle during dynamic exercise. *Am J Physiol Heart Circ Physiol.* 277: H33-H39, 1999.

Buckwalter JB, Mueller PJ, Clifford PS. Autonomic control of skeletal muscle vasodilation during exercise. *J Appl Physiol.* 83: 2037-2042, 1997.

Buckwalter JB, Naik JS, Valic Z, Clifford PS. Exercise attenuates alpha-adrenergic-receptor responsiveness in skeletal muscle vasculature. *J Appl Physiol.* 90: 1: 172-178, 2001.

Brodal, P. The Central Nervous System: Structure and Function. *Oxford University Press*, 2003.

Chavoshan B, Sander M, Sybert TE, Hansen J, Victor RG, Thomas GD. Nitric oxide-dependent modulation of sympathetic neural control of oxygenation in exercising human skeletal muscle. *J Physiol (Lond)* 540: 1: 377-386, 2002.

Delp MD. Control of skeletal muscle perfusion at the onset of dynamic exercise. *Med Sci Sports Exerc.* 31: 7: 1011-1018, 1999.

DiCarlo SE, Chen CY, Collins HL. Onset of exercise increases lumbar sympathetic nerve activity in rats. *Med Sci Sports Exerc*. 28: 6: 677-684, 1996.

Dinenno FA. Hypoxic regulation of blood flow in humans. Alpha-adrenergic receptors and functional sympatholysis in skeletal muscle. *Adv Exp Med Biol.* 543: 237-48, 2003.

Dinenno FA, Joyner MJ. Blunted sympathetic vasoconstriction in contracting skeletal muscle of healthy humans: is nitric oxide obligatory? *J Physiol.* 553: 281–292, 2003.

Dinenno FA, Joyner MJ. Combined NO and PG inhibition augments alpha-adrenergic vasoconstriction in contracting human skeletal muscle. *Am J Physiol Heart Circ Physiol.* 287: 6: H2576-84, 2004.

Donato AJ, Lesniewski LA, Delp MD. Ageing and exercise training alter adrenergic vasomotor responses of rat skeletal muscle arterioles. *J Physiol.* 579: Pt 1: 115-125, 2007.

Elam M, Sverrisdottir YB, Rundqvist B, McKenzie D, Wallin BG, Macefield VG. Pathological sympathoexcitation: how is it achieved? *Acta Physiol Scand.* 177: 3: 405-411, 2003.

Evans RJ, Cunnane TC. Relative contributions of ATP and noradrenaline to the nerve evoked contraction of the rabbit jejunal artery. Dependence on stimulation parameters. *Naunyn Schmiedebergs Arch Pharmacol.* 345: 4: 424-430, 1992.

Forcier K, Stroud LR, Papandonatos GD, Hitsman B, Reiches M, Krishnamoorthy J, Niaura R. Links between physical fitness and cardiovascular reactivity and recovery to psychological stressors: A meta-analysis. *Health Psychol.* (6):723-39, 2006.

Grassi G, Seravalle G, Calhoun DA, Mancia G. Physical training and baroreceptor control of sympathetic nerve activity in humans. *Hypertension.* 23: 3: 294-301, 1994.

Green DJ, O'Driscoll G, Joyner MJ, Cable NT. Exercise and cardiovascular risk reduction: Time to update the rationale for exercise? *J Appl Physiol*. 105: 766-768, 2008.

Hagbarth KE, Vallbo AB. Pulse and respiratory grouping of sympathetic impulses in human muscle-nerves. *Acta Physiol Scand.* 74: 1: 96-108, 1968.

Hajduczok G, Hade JS, Mark AL, Williams JL, Felder RB. Central command increases sympathetic nerve activity during spontaneous locomotion in cats. *Circ Res.* 69: 1: 66-75, 1991.

Haniuda K, Nakane T, Chiba S. Different contributions of ATP and noradrenaline to neurotransmission in the isolated canine intermediate auricular artery. *Eur J Pharmacol.* 333: 2-3: 163-168, 1997.

Hansen J, Sander M, Thomas GD. Metabolic modulation of sympathetic vasoconstriction in exercising skeletal muscle. *Acta Physiol Scand*. 168(4): 489-503, 2000.

Hansen J, Thomas GD, Harris SA, Parsons WJ, Victor RG. Differential sympathetic neural control of oxygenation in resting and exercising human skeletal muscle. *J Clin Invest.* 98: 2: 584-596, 1996.

Hill JM, Adreani CM, Kaufman MP. Muscle reflex stimulates sympathetic postganglionic efferents innervating triceps surae muscles of cats. *Am J Physiol.* 271: 1 pt 2: H38-43, 1996.

Jendzjowsky NG, DeLorey DS. Short-term exercise training augments sympathetic vasoconstrictor responsiveness and endothelium-dependent vasodilation in resting skeletal muscle. *Am J Physiol Regul Integr Comp Physiol*. Jun [Epub ahead of print], 2012.

Johnson CD, Gilbey MP. On the dominant rhythm in the discharges of single postganglionic sympathetic neurones innervating the rat tail artery. *J Physiol.* 497 (Pt 1): Pt 1: 241-259, 1996.

Joyner MJ, Nauss LA, Warner MA, Warner DO. Sympathetic modulation of blood flow and O_2 uptake in rhythmically contracting human forearm muscles. *Am J Physiol Heart Circ Physiol.* 263: H1078-H1083, 1992.

Keller DM, Wasmund WL, Wray DW, Ogoh S, Fadel PJ, Smith ML, Raven PB. Carotid baroreflex control of leg vascular conductance at rest and during exercise. *J Appl Physiol.* 94: 542–548, 2003.

Koller A, Huang A, Sun D, Kaley G. Exercise training augments flow-dependent dilation in rat skeletal muscle arterioles. Role of endothelial nitric oxide and prostaglandins. *Circ Res.* 76: 4: 544-550, 1995.

Krantz DS, Manuck SB. Acute psychophysiological reactivity and risk of cardiovascular disease: Review and methodological critique. *Psychological Bulletin.* 96: 435-464, 1984.

Lakatta EG, Levy D. Arterial and Cardiac Aging: Major Shareholders in Cardiovascular Disease Enterprises: Part I: Aging Arteries: A "Set Up" for Vascular Disease. *Circulation.* 107: 1: 139-146, 2003.

Lash JM. Exercise training enhances adrenergic constriction and dilation in the rat spinotrapezius muscle. *J Appl Physiol.* 85: 1: 168-174, 1998.

Lash JM, Bohlen HG. Functional adaptations of rat skeletal muscle arterioles to aerobic exercise training. *J Appl Physiol.* 72: 6: 2052-2062, 1992.

Manuck SB. Cardiovascular reactivity in cardiovascular disease: "Once more unto the breach." *International Journal of Behavioral Medicine.* 1: 4-31, 1994.

Martin WH, Ogawa T, Kohrt WM, Malley MT, Korte, E, Kieffer PS, Schechtman KB. Effects of aging, gender, and physical training in peripheral vascular function. *Circulation.* 84: 2: 654-664, 1991.

McEwen B. Protective and damaging effects of stress mediators. *New England Journal of Medicine.* 388: 171-179, 1998.

Mora S, Cook N, Buring JE, Ridker PM, Lee IM. Physical activity and reduced risk of cardiovascular events: potential mediating mechanisms. *Circulation.* 116: 19: 2110-2118, 2007.

Nase GP, Boegehold MA. Nitric oxide modulates arteriolar responses to increased sympathetic nerve activity. *Am J Physiol.* 271: 3 Pt 2: H860-9, 1996.

Nase GP, Boegehold MA. Endothelium-derived nitric oxide limits sympathetic neurogenic constriction in intestinal microcirculation. *Am J Physiol.* 273: 1 Pt 2: H426-33, 1997.

Ng AV, Callister R, Johnson DG, Seals DR. Endurance exercise training is associated with elevated basal sympathetic nerve activity in healthy older humans. *J Appl Physiol.* 77: 3: 1366-1374, 1994.

O'Hagan KP, Bell LB, Mittelstadt SW, Clifford PS. Effect of dynamic exercise on renal sympathetic nerve activity in conscious rabbits. *J Appl Physiol.* 74: 5: 2099-2104, 1993.

O'Leary DS, Robinson ED, Butler JL. Is active skeletal muscle functionally vasocontricted during dynamic exercise in conscious dogs? *Am J Physiol Heart Circ Physiol.* 272: R386-R391, 1997.

O'Leary DS, Rowell LB, Scher AM. Baroreflex-induced vasocon-striction in active skeletal muscle of conscious dogs. *Am J Physiol Heart Circ Physiol.* 260: H37–H41, 1991.

O'Sullivan SE, Bell C. Training reduces autonomic cardiovascular responses to both exercise-dependent and –independent stimuli in humans. *Autonomic Neuroscience: Basal and Clinical.* 91: 76-84, 2001.

Paterson DH, Jones GR, Rice CL. Ageing and physical activity: evidence to develop exercise recommendations for older adults. *Appl Physiol Nutr Metab.* 32: S69-S108, 2007.

Pernow J, Schwieler J, Kahan T, Hjemdahl P, Oberle J, Wallin BG, Lundberg JM. Influence of sympathetic discharge pattern on norepinephrine and neuropeptide Y release. *Am J Physiol.* 257: 3 pt 2: H866-872, 1989.

Public Healthy Agency of Canada. Physical Activity for Health: The Evidence [Online]. http://www.phac-aspc.gc.ca/cd-mc/cvd-mcv/cvdmmrf-mmmcvfr-eng.php.

Ray CA. Sympathetic adaptations to one-legged training. *J Appl Physiol*. 86(5): 1583-7, 1999.

Ray CA, Carter JR. Effects of aerobic exercise training on sympathetic and renal responses to mental stress in humans. *Am J Physiol Heart Circ Physiol.* 298: H229-H234, 2010.

Remensnyder JP, Mitchell JH, Sarnoff SJ. Functional sympatholysis during muscular activity. Observations on influence of carotid sinus on oxygen uptake. *Circ Res.* 11: 370-380, 1962.

Ren LM, Burnstock G. Prominent sympathetic purinergic vasoconstriction in the rabbit splenic artery: potentiation by 2,2'-pyridylisatogen tosylate. *Br J Pharmacol.* 120: 3: 530-536, 1997.

Richardson RS, Kennedy B, Knight DR, Wagner PD. High muscle blood flows are not attenuated by recruitment of additional muscle mass. *Am J Physiol Heart Circ Physiol.* 269: H1545–H1552, 1995.

Rosenmeier JB, Dinenno FA, Fritzlar SJ, Joyner MJ. alpha1- and alpha2-adrenergic vasoconstriction is blunted in contracting human muscle. *J Physiol*. 547 (Pt 3): 971-6, 2003.

Rowell LB. *Human Cardiovascular Control.* New York: Oxford University Press, 1993.

Ruble SB, Valic Z, Buckwalter JB, Clifford PS. Dynamic exercise attenuates sympathetic responsiveness of canine vascular smooth muscle. *J Appl Physiol.* 89: 2294 –2299, 2000.

Ruble SB, Valic Z, Buckwalter JB, Tschakovsky ME, Clifford PS. Attenuated vascular responsiveness to noradrenaline release during dynamic exercise in dogs. *J Physiol.* 541 (Pt 2): 637-644, 2002.

Saltin B, Rådegran G, Koskolou MD, Roach RC. Skeletal muscle blood flow in humans and its regulation during exercise. *Acta Physiol Scand*. 162(3): 421-36, 1998.

Sander M, Chavoshan B, Harris SA, Iannaccone ST, Stull JT, Thomas GD, Victor RG. Functional muscle ischemia in neuronal nitric oxide synthase-deficient skeletal muscle of children with Duchenne muscular dystrophy. *PNAS.* 97: 25: 13818-13823, 2000.

Savard G, Strange S, Kiens B, Richter EA, Christensen NJ, Saltin B. Noradrenaline spillover during exercise in active versus resting skeletal muscle in man. *Acta Physiol Scand.* 131: 4: 507-515, 1987.

Savard GK, Richter EA, Strange S, Kiens B, Christensen NJ, Saltin B. Noradrenaline spillover from skeletal muscle during exercise in humans: role of muscle mass. *Am J Physiol.* 257: 6 Pt 2: H1812-8, 1989.

Seals DR. Sympathetic neural adjustments to stress in physically trained and untrained humans. *Hypertension.* 17: 36-43, 1991.

Shoemaker JK, Pandey P, Herr MD, Silber DH, Yang QX, Smith MB, Gray K, Sinoway LI. Augmented sympathetic tone alters muscle metabolism with exercise: lack of evidence for functional sympatholysis. *J Appl Physiol.* 82: 1932–1938, 1997.

Sinoway LI, Shenberger J, Leaman G, Zelis R, Gray K, Baily R, Leuenberger U. Forearm training attenuates sympathetic responses to prolonged rhythmic forearm exercise. *J Appl Physiol.* 81: 4: 1778-1784, 1996.

Smith EG, Voyles WF, Kirby BS, Markwald RR, Dinenno FA. Ageing and leg postjunctional alpha-adrenergic vasoconstrictor responsiveness in healthy men. *J Physiol*. 582(Pt 1): 63-71, 2007.

Somers VK, Leo KC, Shields R, Clary M, Mark AL. Forearm endurance training attenuates sympathetic nerve response to isometric handgrip in normal humans. *J Appl Physiol.* 72: 1039-1043, 1992.

Sothmann MS, Buckworth J, Claytor RP, Cox RH, White-Welkley JE, Dishman RK. Exercise training and the cross-stressor adaptation hypothesis. *Exerc Sports Sci Rev.* 24: 267-287, 1996.

Spier SA, Laughlin MH, Delp MD. Effects of acute and chronic exercise on vasoconstrictor responsiveness of rat abdominal aorta. *J Appl Physiol.* 87(5):1752-7, 1999.

Sun D, Huang A, Koller A, Kaley G. Short-term daily exercise activity enhances endothelial NO synthesis in skeletal muscle arterioles of rats. *J Appl Physiol.* 76: 5: 2241-2247, 1994.

Sun D, Huang A, Koller A, Kaley G. Enhanced NO-mediated dilations in skeletal muscle arterioles of chronically exercised rats. *Microvasc Res.* 64: 3: 491-496, 2002.

Sundlof G, Wallin BG. The variability of muscle nerve sympathetic activity in resting recumbent man. *J Physiol.* 272: 2: 383-397, 1977.

Svedenhag J, Wallin BG, Sundlof G, Henriksson J. Skeletal muscle sympathetic activity at rest in trained and untrained subjects. *Acta Physiol Scand.* 120: 4: 499-504, 1984.

Tanaka K, Yang XP, Chiba S. Purenergic and adrenergic cotransmission in canine isolated and perfused gastroepiploic arteries. *Clin Exp Pharmacol Physiol.* 30: 9: 678-683, 2003.

Tateishi J, Faber JE. ATP-sensitive K^+ channels mediate α_{2D}-adrenergic receptor contraction of arteriolar smooth muscle and reversal of contraction in hypoxia. *Circ Res.* 76: 53– 63, 1995.

Tesfamariam B, Cohen RA. Inhibition of adrenergic vasoconstriction by endothelial cell shear stress. *Circ Res.* 63: 4: 720-725, 1988.

Tesfamariam B, Weisbrod RM, Cohen RA. Endothelium inhibits responses of rabbit carotid artery to adrenergic nerve stimulation. *Am J Physiol.* 253: 4 Pt 2: H792-8, 1987.

Thomas GD. Neural control of the circulation. *Adv Physiol Educ.* 35: 28-32, 2011.

Thomas GD, Hansen J, Victor RG. ATP-sensitive potassium channels mediate contraction-induced attenuation of sympathetic vasoconstriction in rat skeletal muscle. *J Clin Invest*. 99 (11): 2602-9, 1997.

Thomas GD, Hansen J, Victor RG. Inhibition of α_2-adrenergic vasoconstriction during contraction of glycolytic, not oxidative, rat hindlimb muscle. *Am J Physiol Heart Circ Physiol.* 266: H920-929, 1994.

Thomas GD, Sander M, Lau KS, Huang PL, Stull JT, Victor RG. Impaired metabolic modulation of alpha -adrenergic vasoconstriction in dystrophin-deficient skeletal muscle. *PNAS.* 95: 25: 15090-15095, 1998.

Thomas GD, Segal SS. Neural control of muscle blood flow during exercise. *J Appl Physiol.* 97: 731-738, 2004.

Thomas GD, Victor RG. Nitric oxide mediates contraction-induced attenuation of sympathetic vasoconstriction in rat skeletal muscle. *J Physiol (Lond).* 506: 3: 817-826, 1998.

Thomas GD, Zhang W, Victor RG. Impaired modulation of sympathetic vasoconstriction in contracting skeletal muscle of rats with chronic myocardial infarctions: role of oxidative stress. *Circ Res.* 88: 8: 816-823, 2001.

Tschakovsky ME, Sujirattanawimol K, Ruble SB, Valic Z, Joyner MJ. Is sympathetic neural vasoconstriction blunted in the vascular bed of exercising human muscle? *J Physiol.* 541: 623– 635, 2002.

Wray DW, Donato AJ, Nishiyama SK, Richardson RS. Acute sympathetic vasoconstriction at rest and during dynamic exercise in cyclists and sedentary humans. *J Appl Physiol.* 102: 2: 704-712, 2007.

CHAPTER 2: The effect of aerobic fitness on the cardiovascular and sympathetic nervous system response to physiological stress at rest and during dynamic exercise

2.1 Introduction

Cardiovascular disease is the leading cause of morbidity and mortality in modern society (Pub Health Agency, 2004). The rapid aging of the Canadian population suggests that the prevalence of these diseases and their negative impact on the lives of Canadians, and the Canadian health care system will markedly increase over the next 20 years (Pub Health Agency, 2004). A sedentary, physically inactive lifestyle is a major modifiable risk factor for cardiovascular disease (Lakatta & Levy, 2003), whereas regular physical activity and aerobic fitness appear to have favorable effects on cardiovascular function, and have been linked to a reduced risk of cardiovascular disease (Blair et al., 1989). Surprisingly, ~ 60% of the decrease in cardiovascular disease risk associated with exercise-training cannot be attributed to a reduction of traditional cardiovascular disease risk-factors, such as weight loss and a decrease in blood pressure and blood lipids (Mora et al., 2007). This finding has prompted others to argue that future investigations should focus on the "direct impacts of exercise training on vascular function", specifically those effects related to endothelial and autonomic control of the vasculature, rather than on known risk-factor reduction (Green et al., 2008).

One potential mechanism that may be associated with the cardio-protective effects of regular exercise/physical fitness is an attenuated physiological response to stress. Repeated and/or chronic exposure to stress and an exaggerated physiological reactivity to stress have been linked to the development of cardiovascular disease (Krantz & Manuck, 1984; Manuck, 1994; McEwen, 1998). If being aerobically fit results in a diminished

physiological response to physiological/psychological stress it may decrease the patho-physiological effects of stress and be cardio-protective. The response to stress is characterized by an increase in sympathetic nervous system activity that elicits numerous physiological changes including an increase in heart rate (HR), vascular resistance and arterial blood pressure, the result in the peripheral vasculature being vasoconstriction and a reduction of tissue blood flow (Rowell, 1993).

During exercise there is substantial vasodilation in skeletal muscle to accommodate the requisite increase in oxygen delivery (Delp, 1999). Sympathetic nerve activity also increases to exercising and non-active tissues as a function of the exercise intensity (DiCarlo et al., 1996; Hill et al., 1996; O'Hagan et al., 1993; Savard et al., 1987; Savard et al., 1989). While an increase in muscle sympathetic nerve activity (MSNA) during active vasodilation appears counter-intuitive, tonic vasoconstriction in exercising skeletal muscle must balance the robust local vasodilation that occurs with exercise in order to maintain systemic arterial blood pressure.

Despite evidence of tonic sympathetic restraint of active skeletal muscle blood flow, it has also been demonstrated that the effectiveness of sympathetic outflow is attenuated in active compared to resting skeletal muscle (Buckwalter & Clifford, 1999; O'Leary et al., 1997). Remensnyder et al. (1962) first termed this attenuated vascular response to augmented sympathetic outflow during exercise "functional sympatholysis".

Many studies have shown that the responsiveness of post-synaptic adrenergic receptors may decline during exercise (Anderson et al., 1991; Buckwalter et al., 2001; Remensnyder et al., 1962; Rosenmeier et al., 2003; Ruble et al., 2002; Thomas et al., 1997).

Surprisingly little is known about the effect of aerobic fitness and regular physical activity on neurovascular control at rest and during exercise and whether aerobic fitness alters the vascular response to sympathetic stimulation during exercise has received little attention in the scientific literature.

Thus, the purpose of this study was to investigate the effect of aerobic fitness [defined by the subjects maximal oxygen consumption (VO_2max)] on the sympathetic nervous system and cardiovascular response to acute physiological stress at rest and during dynamic exercise in humans. It was hypothesized that the magnitude of the sympathetic and cardiovascular responses to acute physiological stress would decline as a function of aerobic fitness at rest and during exercise. Therefore, this study addressed a topic of fundamental and timely importance, and has direct and important implications for the health of Canadians. Moreover, this study was designed to advance our understanding of how aerobic fitness affects the basic physiological mechanisms involved in the regulation of the physiological response to stress at rest and during exercise.

2.2 Methods

2.2.1 Subjects

This study was approved by the University of Alberta Health Research Ethics Board. Twenty-three healthy, young (18-32 yr) males volunteered and gave written informed consent to participate in the study. Subjects were assigned to aerobic fitness groups according to their relative VO_2max as follows: Low ($VO_2max = \leq 35$ mL·kg^{-1}·min^{-1}, n=8), Mid (VO_2max = 45-55 mL·kg^{-1}·min^{-1}, n=8) and High ($VO_2max = \geq 65$ mL·kg^{-}

$^{1}\cdot min^{-1}$, n=7). This classification of aerobic fitness approximates the classification system of the American Heart Association (AHA). The AHA stratifies aerobic fitness levels based upon VO_2max for young adults (20-29 yr) as low (< 25 $mL\cdot kg^{-1}\cdot min^{-1}$), fair (25 - 33 $mL\cdot kg^{-1}\cdot min^{-1}$), average (34 - 42 $mL\cdot kg^{-1}\cdot min^{-1}$), good (43 - 52 $mL\cdot kg^{-1}\cdot min^{-1}$) and high (> 53 $mL\cdot kg^{-1}\cdot min^{-1}$).

2.2.2 Experimental Protocol

All testing was completed at the University of Alberta Integrative Human Exercise Physiology (IHEP) laboratory (E-439) in the Faculty of Physical Education & Recreation. Subjects reported to the IHEP lab on four separate occasions. Subjects were advised to refrain from caffeine, alcohol and ibuprofen for 12 hours prior to any testing day, and to arrive to the lab in a rested state (no vigorous exercise 24 hours prior) and have eaten a light meal ~2 hours before exercise testing. To minimize changes in body temperature during the experiments, laboratory temperature was maintained between 20°-22°C.

Subjects completed an incremental exercise test to volitional exhaustion on a cycle ergometer (Ergoselect 200 K, Ergoline, Bitz, Germany) for determination of VO_2max on their first visit to the lab. Testing began with 2 minutes of resting baseline data collection, after which the work rate was progressively incremented in a ramp-like fashion at 30 $W\cdot min^{-1}$ to volitional fatigue. Criteria used to establish a maximal test included: a plateau in VO_2 despite an increase in work rate, a respiratory exchange ratio (RER) >1.10, achievement of >90% of age-predicted HRmax and volitional exhaustion. Subjects were subsequently assigned to one of the study groups based upon their

VO_2max. If based upon their VO_2max subjects did not "fit" into one of the aerobic fitness categories, they were excluded from the study (n=9).

Following familiarization to the knee-extensor device, subjects completed an incremental exercise test to volitional exhaustion on a custom-built, knee-extension (KE) ergometer as described previously (DeLorey et al., 2007). Subjects performed alternate-leg KE exercise at a cadence of 30 contractions per minute (cpm) and the work rate was incremented by 3 W every 2 minutes until volitional exhaustion. The maximum work rate achieved was used to calculate work rates for constant-load KE exercise tests on a subsequent day.

During the third day of testing, subjects rested quietly in a semi-recumbent position for measurement of the sympathetic nervous system and cardiovascular response to physiological stress under resting conditions. Subjects were exposed to two physiological stressors, a cold-pressor test (CPT) and isometric handgrip (IHG) exercise. The CPT and IHG exercise have both been shown to increase sympathetic nerve activity and produce a cardiovascular response at rest and during exercise (Seals, 1991). However, the afferent and efferent reflex pathways that mediate the sympathetic response may differ between the CPT and IHG (Victor et al., 1987; Victor et al., 1989). The CPT is an involuntary stimulus and the response to the CPT is mediated by type III and IV afferents (Rowell, 1993). In contrast, the IHG involves voluntary muscle contraction and the efferent sympathetic nervous system response to IHG includes activation of central command (Rowell, 1993). Subjects performed two maximal handgrip contractions to determine their maximal voluntary contractile force (MVC). Following 7 minutes of resting data collection, subjects were asked, in random order, to: 1) submerge their

hand in a -4°C ice-water slurry (CPT), and 2) perform isometric handgrip exercise (IHG) at 40% MVC. Each maneuver was performed for 3 minutes with 10 minutes of rest and recovery between stressors (Figure 2-1). IHG exercise was performed by squeezing a grip force transducer (ML T004; AD Instruments, Colorado Springs, CO, USA) connected to a data acquisition system (Power Lab 16/30; AD Instruments, Colorado Springs, CO, USA). Target and actual grip force were continuously displayed on a computer monitor and subjects were verbally encouraged to achieve the target force throughout the 3 minutes.

During the final day of testing, subjects performed two constant-load, alternate-leg KE exercise bouts at a work rate selected to elicit ~80 % of the VO_2 at ventilatory threshold (80% VT - moderate intensity) and two additional bouts at a work rate chosen to elicit a VO_2 corresponding to VT plus 50% of the difference between VT and VO_2max (Δ 50 - heavy intensity). Each exercise bout was 8 minutes in duration and was preceded by 2 minutes of rest and 1 minute of passive exercise, and followed by 1 minute of recovery. All exercise was performed at a cadence of 30 cpm. To facilitate passive exercise, each of the subjects' legs was secured to the lever arms of the KE ergometer while an assistant cycled on the ergometer at 30 cpm. Passive exercise was included in order to control the transition from rest to active exercise, as the mechanical inertia required to begin exercising without passive exercise would alter the ventilatory and cardiovascular responses to exercise. During the final 3 minutes of each exercise bout, subjects either performed IHG exercise at 40% MVC, or completed a CPT in random order (Figure 2-2).

2.2.3 Measurements

Pulmonary gas exchange (VO_2, VCO_2, RER) and minute expired ventilation (VE) were measured on a breath-by-breath basis by an "open-circuit" method (Vmax® 229d; Viasys™ Healthcare, Palm Springs, CA, USA) while subjects breathed through a mouthpiece and low-resistance mass-flow meter with their nose occluded by a nose clip. Before each test, the flow-meter was calibrated by pumping a 3-liter syringe at a range of flow rates expected during the exercise studies, while the O_2 and CO_2 analyzers were calibrated with gases of known concentration.

A three-lead electrocardiogram (ECG) was measured continuously and HR was derived from the ECG.

Beat-by-beat arterial blood pressure was measured by photo-plethysmography on the middle finger of the right hand (Finometer™, Finapres Medical Systems, Amsterdam, Netherlands). Mean arterial pressure (MAP) was calculated on a beat-by-beat basis. Stroke volume (SV) and cardiac output (Q) were derived from the pressure waveform with the Modelflow algorithm (Finapres Medical Systems, Amsterdam, Netherlands).

Femoral artery blood velocity (FBV) was measured using pulsed-Doppler ultrasonography (GE Vivid I, Waukesha, WI, USA). Data were acquired continuously with a 7.5 MHz probe with a 45° angle of insonation placed on the skin surface 2–3 cm distal to the inguinal ligament. The diameter of the common femoral artery was measured during diastole in triplicate at rest. Previous reports (DeLorey et al., 2004; MacPhee et al., 2005; Radegran & Saltin, 2000) have demonstrated that femoral artery diameter does not change from the resting value during exercise, therefore the three measures of vessel diameter at rest were averaged to obtain a femoral artery diameter for

each subject. Mean blood velocity (MBV, $cm \cdot s^{-1}$) was calculated on a beat-by beat basis. Limb blood flow (LBF) was calculated as LBF ($mL \cdot min^{-1}$) = $MBV \cdot \pi r^2 \cdot 60$, where r is the radius of the femoral artery averaged in triplicate during diastole at rest. Femoral vascular conductance (FVC = $LBF \cdot MAP^{-1}$, $L \cdot min^{-1} \cdot mmHg^{-1}$) was calculated.

Multiunit recordings of MSNA were obtained using the microneurography technique. A tungsten recording microelectrode (200-μm shaft, 1- to 3-μm tip, model UNA32F2S; FHC, Bowdoin, ME, USA) was inserted into the left peroneal nerve at the popliteal fossa posterior to the fibular head and a reference electrode was placed subcutaneously 2-3 cm from the recording electrode. The raw sympathetic neurogram was band-pass filtered with a low cut-off frequency of 700 Hz and high cut-off frequency of 2000 Hz, and full-wave integrated at a time constant of 0.1 seconds to acquire a mean voltage display of MSNA. If we were not able to acquire MSNA on a subject (n=3), their cardiovascular responses to the stressors were still included in the data.

Verification of adequate MSNA was achieved by observation of spontaneous, pulse-synchronous bursts of activity that were not affected by auditory arousal or touching of the skin, and increased in activity during a breath hold. MSNA could not be measured during dynamic exercise due to technical limitations associated with limb movement altering the position of the recording electrode.

2.3 Delimitations

This study recruited 23 healthy males between 18-32 years of age. This age range was selected to minimize confounding effects of aging on sympathetic nervous system

activity and peripheral vascular resistance (Iwase et al., 1991; Narkiewicz et al., 2005; Sundlof & Wallin, 1978).

A power analysis calculation performed with variance data from prior studies (O'Sullivan & Bell, 2001; Wray et al., 2007; Ray & Carter, 2010) and preliminary data collected in our laboratory, estimated that 8 subjects for each experimental group would permit detection of ~ 20% change / group difference in cardiovascular and sympathetic activity with a power of 0.90 at $\alpha = 0.05$.

Women were excluded, as cyclical hormone variations have been shown to alter vascular reactivity and women with very high VO_2max values may have altered levels of circulating hormones as a function of their training and nutritional status (Huang & Kaley, 2004; Kublickiene & Luksha, 2008).

All subjects were non-obese (BMI < 30). Obesity has been associated with a myriad of cardiovascular complications, including vascular dysfunction (Mensah et al., 2004), altered vascular reactivity (Van Guilder et al., 2006) and chronic inflammation that may compromise the cardiovascular response to physiological stress and exercise.

Individuals with previously diagnosed respiratory, cardiovascular, metabolic or musculoskeletal diseases, an abnormal ECG, and smokers were excluded as were individuals taking medications known to alter the cardiovascular response to exercise.

2.4 Data and Statistical Analysis

Data were recorded using a PowerLab 16/30 system and Chart 7^{TM} data acquisition software (AD Instruments, Colorado Springs, CO, USA). Arterial blood pressure and FBV were sampled at 100 Hz. The ECG was sampled at 4000 Hz and HR

was derived from the ECG waveform. Bursts of MSNA were identified from the integrated MSNA voltage display using a peak analysis threshold macro using Chart 7™. MSNA was assessed as burst frequency (bursts·min^{-1}) and burst incidence (bursts·100 heart beats^{-1}).

At rest, data were averaged over a 5-minute period to establish resting values. The cardiovascular response to physiological stressors was calculated by determining the magnitude of the increase or decrease in each variable during exposure to each stressor at rest and during contraction. The difference between the peak response (15 s average) and the preceding baseline value (at rest) or 1-minute steady state value (during exercise) was calculated for each variable and was expressed as an absolute and as a percent change. The magnitude of sympatholysis was calculated as the difference between the peak response (% change) to sympathetic stimulation at rest and during muscular contraction (Buckwalter & Clifford, 2001).

MSNA activity was assessed on a minute-by-minute basis. MSNA progressively increased through the exposure to each stressor. Therefore, the peak response was calculated with data from the final minute of the exposure and was expressed as a percent change from MSNA at rest.

Sympathetic vascular transduction (SVT) was calculated as the ratio of the % change in FVC and the % increase in burst frequency during each stressor.

All data were reported as mean ± standard deviation. The effect of aerobic fitness on MSNA was analyzed by one-way analysis of variance (ANOVA). Cardiovascular data (HR, MAP, LBF and FVC) were analyzed with two-way repeated-measures ANOVA (fitness group x exercise intensity). The effect of aerobic fitness and exercise

intensity on the magnitude of sympatholysis was also determined by two-way repeated-measures ANOVA. When significant F-ratios were found, Student Newman-Keuls post-hoc analysis was performed. A p-value < 0.05 was considered statistically significant. Relationships between variables were determined by Pearson product correlation. Statistics were performed using the analytical software SigmaPlot (SigmaPlot Version 11.0, Systat Software Inc., San Jose, CA, USA).

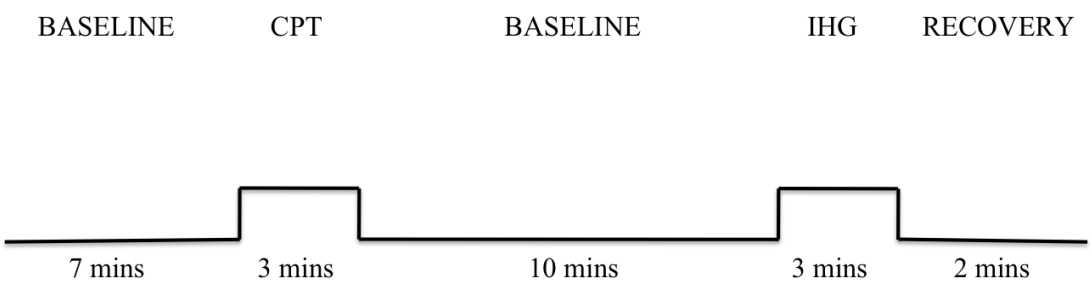

Figure 2-1. Experimental protocol at rest. (Cold-pressor test, CPT; isometric handgrip, IHG). CPT and IHG were completed in random order.

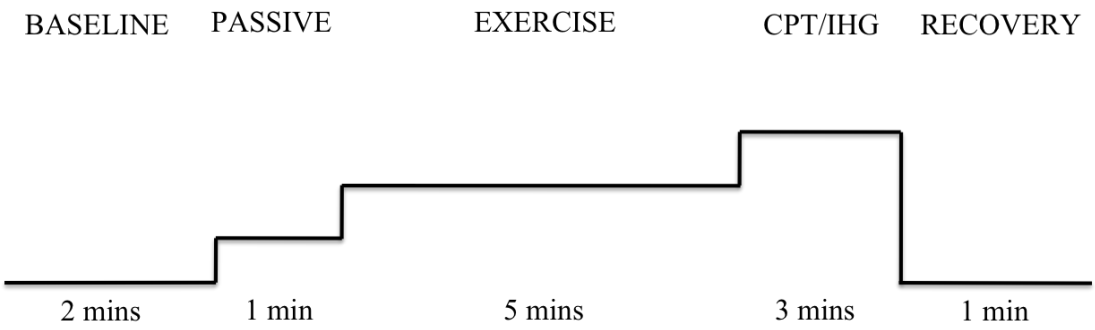

Figure 2-2. Experimental protocol during exercise. (Cold-pressor test, CPT; isometric handgrip, IHG). CPT and IHG were completed in random order.

2.5 Results

2.5.1 Subject Characteristics

Age, height, weight and body mass index (BMI) were not different ($p>0.05$) between groups (Table 2-1). Consistent with the design of the study, relative (Figure 2-3) and absolute (Table 2-1) VO_2max were different ($p<0.05$) between groups. In agreement with the findings from the maximal exercise test on the cycle ergometer, VO_2peak and the maximal work rate achieved during the incremental KE test were also different ($p<0.05$) between groups during the maximal alternate-leg KE test (Table 2-1).

Table 2-1. Subject characteristics for low, mid and high aerobic fitness groups.

	Low (n=8)	**Mid** (n=8)	**High** (n=7)
Age	26.3 ± 1.7	23.6 ± 3.7	24.9 ± 4.8
Height (cm)	180 ± 5.6	181 ± 6.0	181 ± 7.0
Weight (kg)	79.7 ± 8.6	74.1 ± 7.5	76.1 ± 11.7
BMI	24.6 ± 3.0	22.7 ± 1.6	23.0 ± 2.3
Resting HR (beats·min^{-1})	66.7 ± 9.6	59.0 ± 7.4	52.2 ± 8.8 $^{\#}$
Resting MAP (mmHg)	81.8 ± 12.1	76.3 ± 7.0	78.5 ± 11.0
Resting SV (mL)	111.3 ± 8.6	105.2 ± 14.4	98.3 ± 13.8
Resting Q (L·min^{-1})	7.5 ± 0.8 *	6.1 ± 1.1 *	5.1 ± 0.8 *
Resting LBF (L·min^{-1})	0.43 ± 0.1	0.57 ± 0.1	0.56 ± 0.2
Resting FVC (L·min^{-1}·mmHg^{-1})	0.006 ± 0.002	0.008 ± 0.002	0.007 ± 0.002
Resting TPR (mmHg·L^{-1}·min^{-1})	11.0 ± 1.8	12.7 ± 2.1	15.6 ± 1.8 $^{+}$
VO_2max (L·min^{-1})	2.62 ± 0.46 *	3.75 ± 0.49 *	5.14 ± 0.50 *
KEmax (W)	55.5 ± 15.3 *	78.8 ± 19.9 *	101.1 ± 25.3 *
Femoral Artery Diameter (mm)	8.9 ± 0.6	8.7 ± 1.1	10.3 ± 1.1 $^{+}$

Values are mean ± SD. Body mass index (BMI), heart rate (HR), mean arterial pressure (MAP), stroke volume (SV), cardiac output (Q), limb blood flow (LBF), femoral vascular conductance (FVC), total peripheral resistance (TPR), maximal knee-extension exercise work rate (KEmax) in Watts (W). * Significant difference between all aerobic fitness groups ($p<0.05$). $^{+}$ Significant difference between high aerobic fitness group and both mid and low aerobic fitness groups ($p<0.05$). $^{\#}$ Significant difference between high aerobic fitness group and low aerobic fitness group ($p<0.05$).

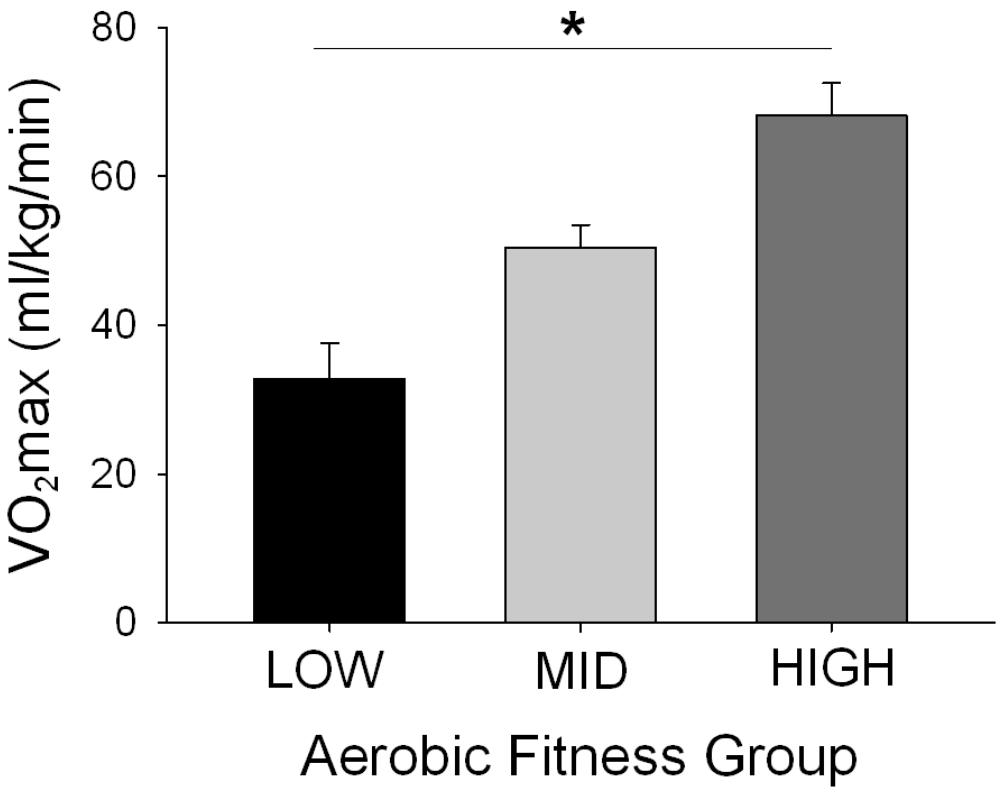

Figure 2-3. Relative VO_2max ($mL \cdot kg^{-1} \cdot min^{-1}$) in low (n=8), mid (n=8) and high (n=7) aerobic fitness groups. * Significant difference between groups ($p<0.05$). Values are mean ± SD.

2.5.2 Resting Muscle Sympathetic Nerve Activity and Hemodynamics

Resting HR was lower ($p<0.05$) in the high compared to low fitness group (Table 2-1), whereas stroke volume was not different ($p>0.05$) between groups (Table 2-1). As a result, cardiac output (Q) was different ($p<0.05$) between groups (Table 2-1), with resting Q decreasing as a function of aerobic fitness. All subjects were normotensive and MAP was not different ($p>0.05$) between groups (Table 2-1). Femoral artery diameter was larger ($p>0.05$) in the high compared to the mid and low fitness groups (Table 2-1). Resting LBF and FVC were not different between ($p>0.05$) aerobic fitness groups.

An original data tracing of resting MSNA from a representative subject is illustrated in Figure 2-4. At rest, MSNA burst frequency (Figure 2-5) and burst incidence (Figure 2-6) were lower ($p<0.05$) in high compared to mid and low fitness groups.

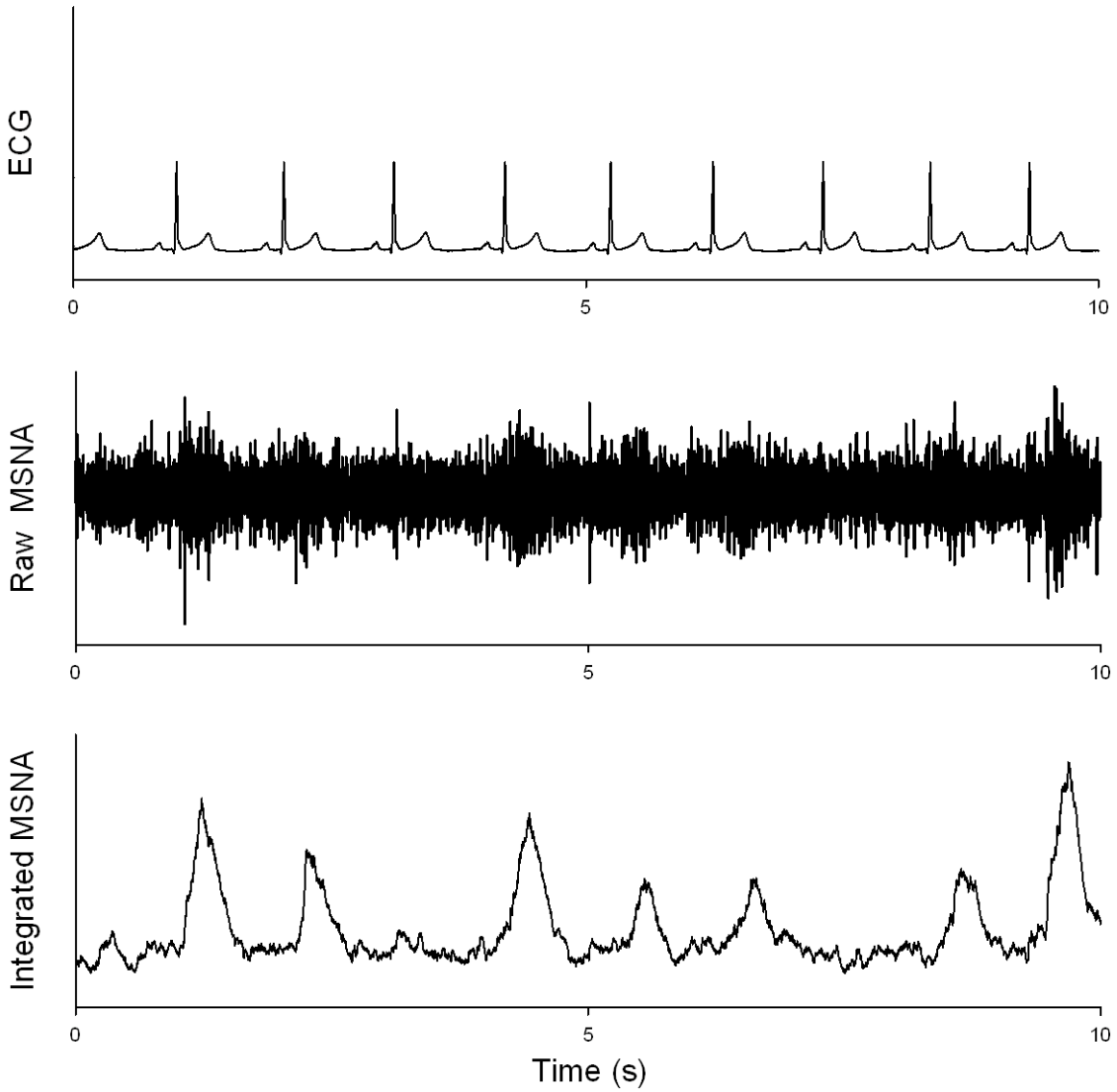

Figure 2-4. Original data tracing of raw and integrated muscle sympathetic nerve activity (MSNA) and electrocardiogram (ECG) in a representative subject.

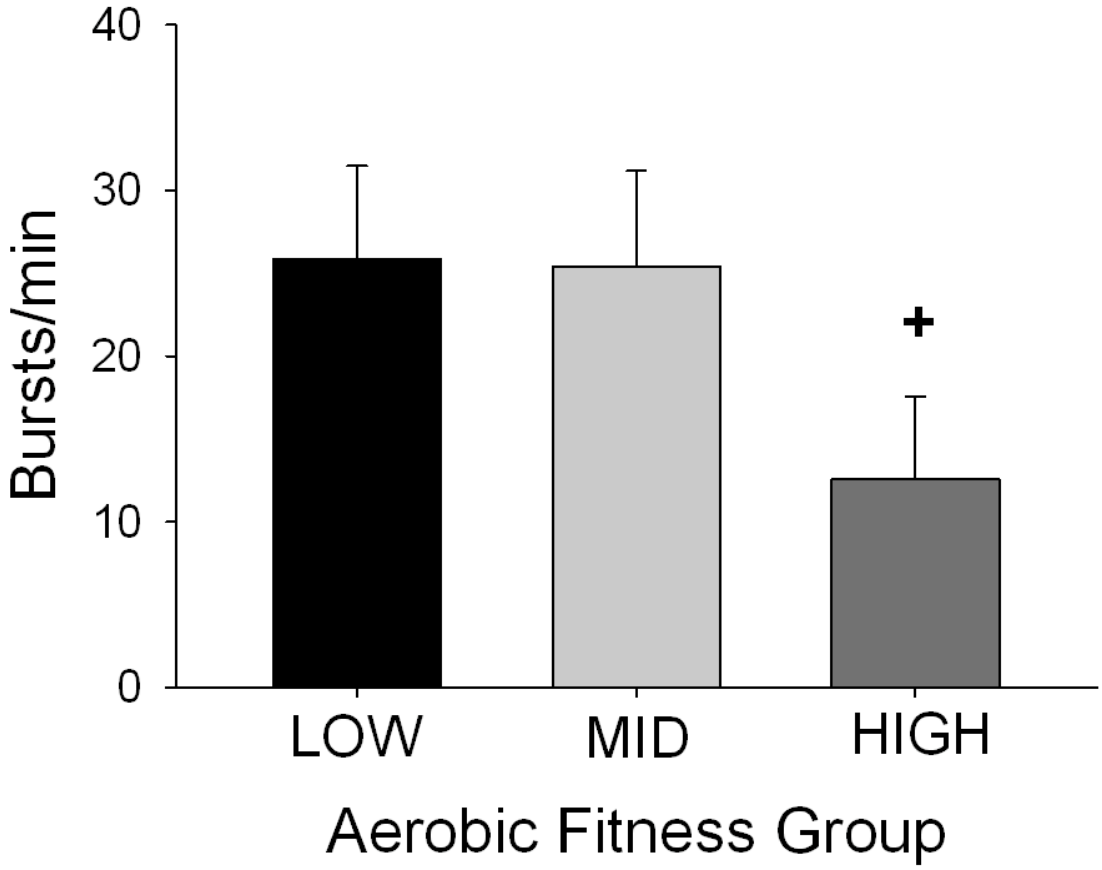

Figure 2-5. Muscle sympathetic nerve activity (MSNA) burst frequency at rest in low (n=6), mid (n=7) and high (n=7) aerobic fitness groups. $^{+}$ Significant difference from low and mid fitness groups ($p<0.05$). Values are mean ± SD.

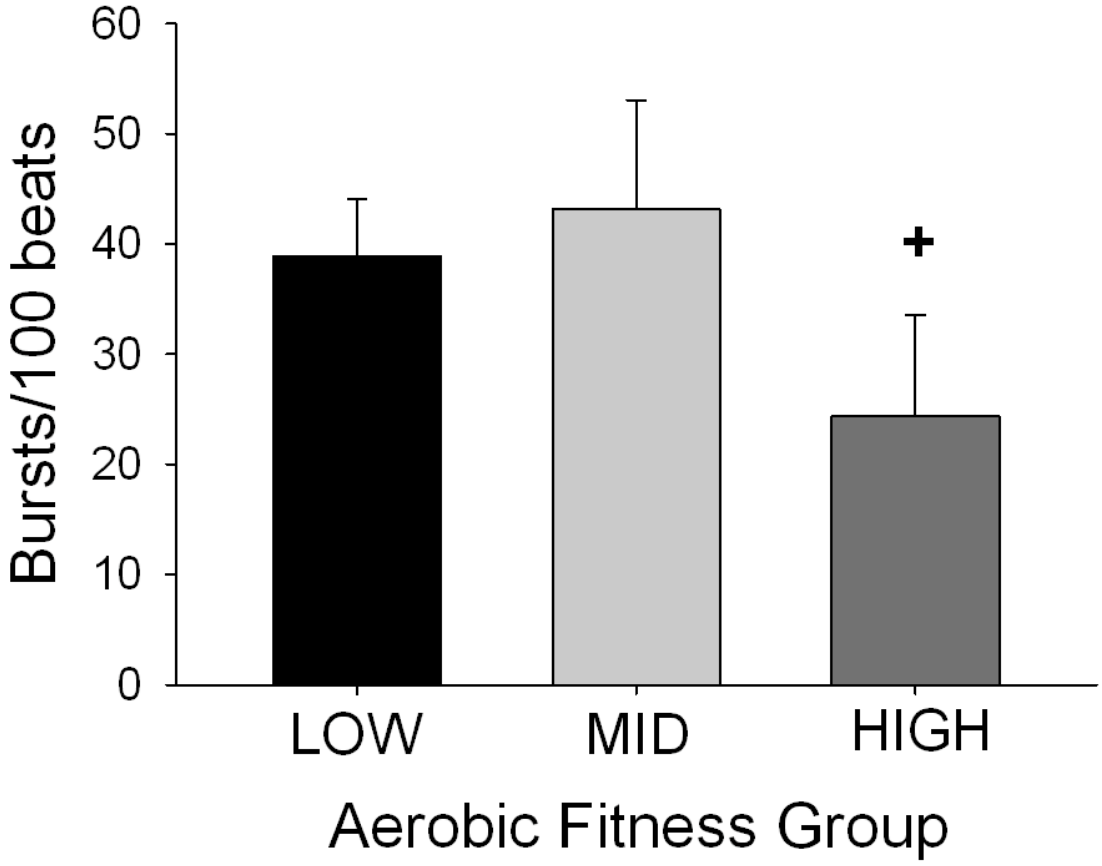

Figure 2-6. Muscle sympathetic nerve activity (MSNA) burst incidence at rest in low (n=6), mid (n=7) and high (n=7) aerobic fitness groups. $^{+}$ Significant difference from both low and mid fitness groups ($p < 0.05$). Values are mean ± SD.

2.5.3 Physiological response to cold-pressor test

Rest

A representative tracing of the cardiovascular response to the CPT during rest is illustrated in Figure 2-7.

MSNA increased during the CPT in all subjects. The increase in burst frequency (Figure 2-8) and incidence (Figure 2-9) during the CPT was greater ($p<0.05$) in the high compared to mid and low fitness groups.

In response to the CPT, SVT was significantly lower ($p<0.05$) in the high compared to the low and mid aerobic fitness groups (Figure 2-10).

The CPT caused a similar absolute increase in HR in high (8 ± 7 beats·min^{-1}), mid (4 ± 10 beats·min^{-1}) and low (5 ± 13 beats·min^{-1}) fitness groups. The percent change in HR from the pre-CPT baseline was also not different ($p>0.05$) between groups (Figure 2-12). The pressor response (increase in MAP) during the CPT was also not different between the high (27 ± 8 mmHg) mid (31 ± 6 mmHg) and low (29 ± 7 mmHg) fitness groups. The percent increase in MAP from the pre-CPT baseline was also not different ($p>0.05$) between groups (Figure 2-13).

LBF and FVC decreased in response to the CPT in all groups. LBF decreased ($p>0.05$) by a similar magnitude in the high (-0.09 ± 0.09 L·min^{-1}), mid (-0.1 ± 0.2 L·min^{-1}) and low (-0.1 ± 0.1 L·min^{-1}) aerobic fitness groups. The percent decrease in LBF was not different ($p>0.05$) in the high (-17 ± 20 %), mid (-16 ± 22 %) and low (-29 ± 15 %) fitness groups. The decrease in FVC in response to the CPT was not different ($p>0.05$) between the high (-0.003 ± 0.001 L·min^{-1}·mmHg^{-1}), mid (-0.003 ± 0.002 L·min^{-1}·mmHg^{-1}) and low fitness group (-0.003 ± 0.002 L·min^{-1}·mmHg^{-1}). The decrease in

FVC, expressed as a percent change from the pre-CPT baseline, was also not different ($p>0.05$) between groups (Figure 2-14). The time required to reach the nadir of the FVC response during the CPT was not different between high (98 ± 36 s), mid (108 ± 34 s) and low (120 ± 26 s) fitness groups.

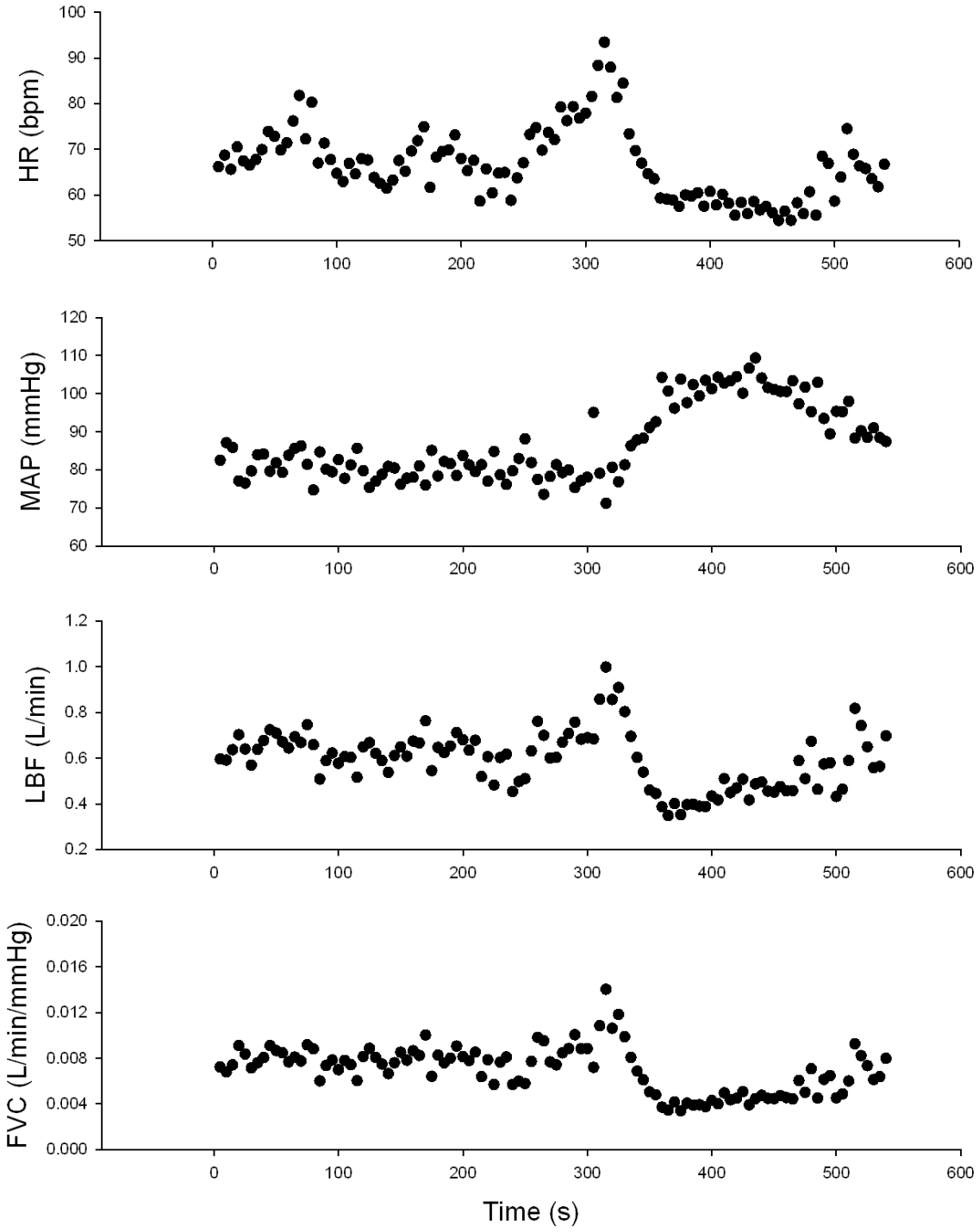

Figure 2-7. Individual representative tracing of the cardiovascular (heart rate, HR; mean arterial pressure, MAP; limb blood flow, LBF; femoral vascular conductance, FVC) response to the cold-pressor test (CPT) at rest. The CPT was initiated at 300 s, was 180 s in duration and was followed by 60 s of recovery. Each data point represents a 5 second average.

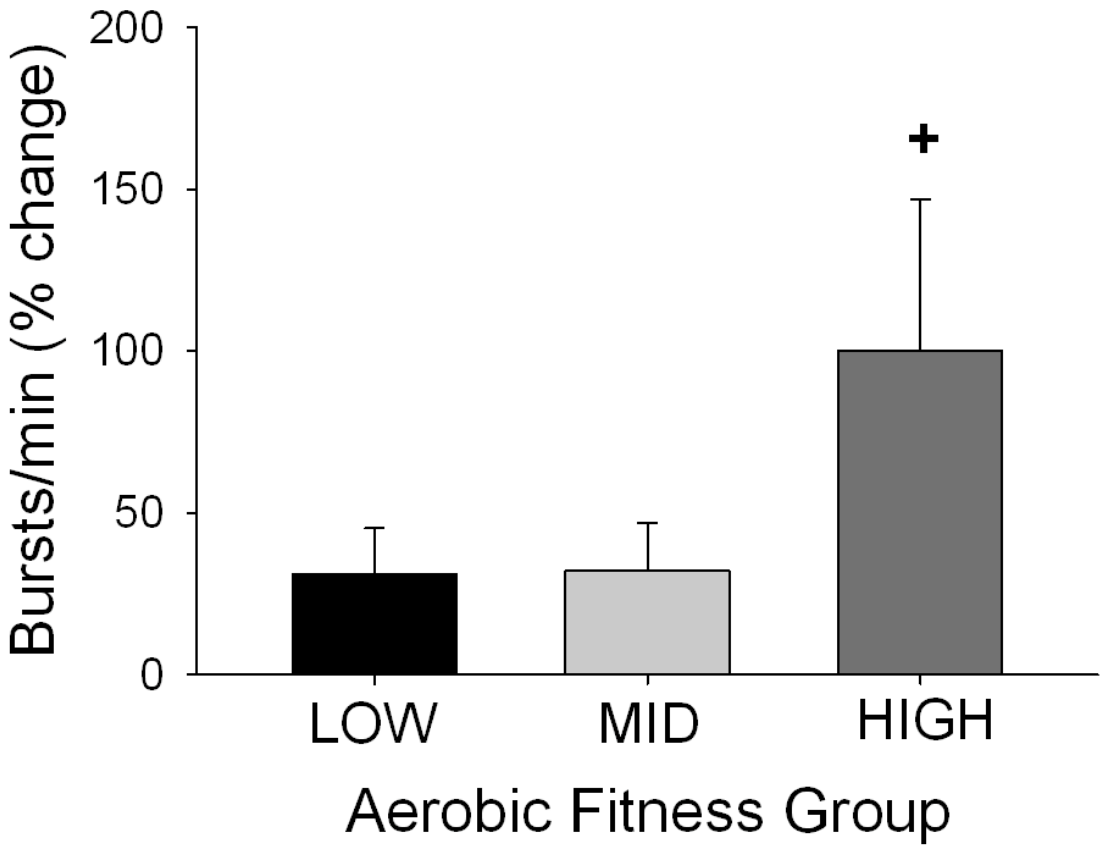

Figure 2-8. Percent change of muscle sympathetic nerve activity (MSNA) burst frequency in response to the cold-pressor test (CPT) at rest in low (n=5), mid (n=7) and high (n=7) aerobic fitness groups. $^{+}$ Significant difference from low and mid fitness groups ($p < 0.05$). Values are mean ± SD.

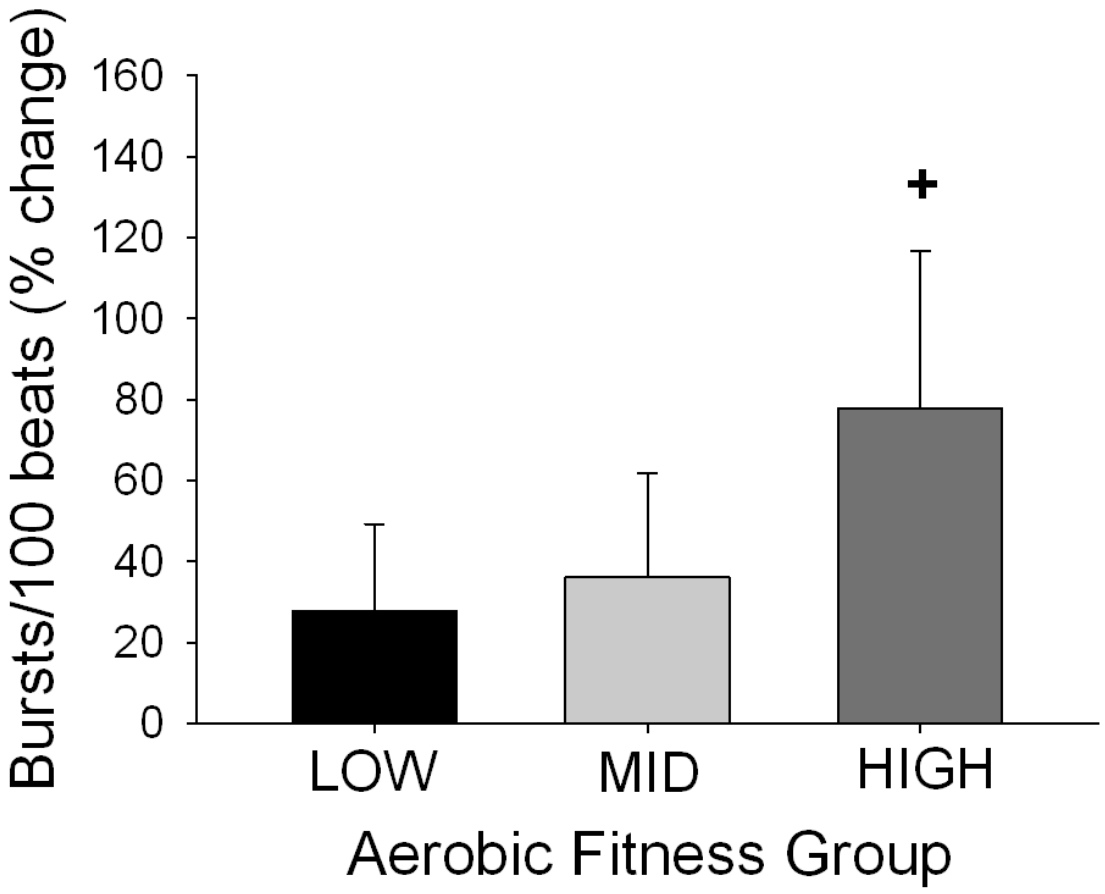

Figure 2-9. Percent change of muscle sympathetic nerve activity (MSNA) burst incidence in response to the cold-pressor test (CPT) at rest in low (n=5), mid (n=7) and high (n=7) aerobic fitness groups. $^{+}$ Significant difference from low and mid fitness groups ($p < 0.05$). Values are mean ± SD.

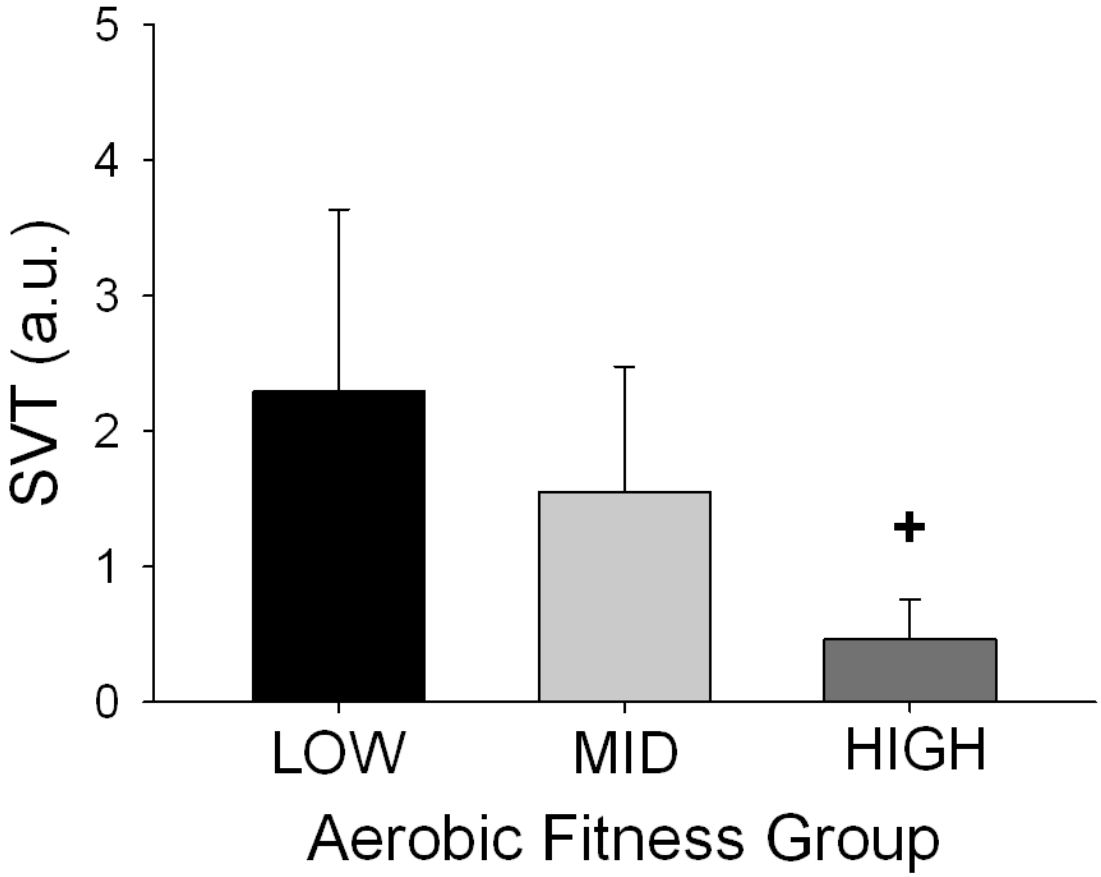

Figure 2-10. Sympathetic vascular transduction (SVT) during the cold-pressor test (CPT) at rest in low (n=5), mid (n=7) and high (n=7) aerobic fitness groups. $^{+}$ Significant difference from low and mid fitness groups ($p< 0.05$). Values are mean ± SD.

Moderate Exercise Intensity (80%VT)

A representative tracing of the cardiovascular response to alternate-leg KE exercise and to the CPT during exercise is illustrated in Figure 2-11. HR, MAP, LBF and FVC all increased in response to moderate-intensity KE exercise (Table 2-2).

Consistent with the differences in peak work rate achieved during the incremental KEmax test, the moderate-intensity work rates were also different ($p<0.05$) between groups, with work rate increasing as a function of VO_2max (Table 2-2). Despite the differences in the absolute work rates, HR was not different ($p>0.05$) between groups during moderate-intensity exercise (Table 2-2). MAP was also similar ($p>0.05$) in the three aerobic fitness groups during moderate-intensity exercise (Table 2-2).

LBF and FVC were different ($p>0.05$) between groups during moderate-intensity exercise and the increase in LBF and FVC were a function of the absolute work rate (Table 2-2).

In response to the CPT during alternate-leg KE exercise, HR increased by a similar ($p>0.05$) magnitude in high (2 ± 3 beats·min^{-1}), mid (1 ± 2 beats·min^{-1}) and low (5 ± 8 beats·min^{-1}) fitness groups. The percent change in HR in response to the CPT was not different ($p>0.05$) between groups (Figure 2-12), however a main effect of exercise was observed such that the increase in HR was blunted during exercise compared to rest. The CPT caused a similar increase ($p>0.05$) in MAP in high (22 ± 8 mmHg) mid (20 ± 5 mmHg) and low (22 ± 4 mmHg) fitness groups during exercise. The percent change in MAP was not different ($p>0.05$) between groups. However, the pressor response during the CPT was reduced compared to the response at rest in the high, mid and low fitness groups (Figure 2-13).

LBF decreased by a similar ($p>0.05$) magnitude in response to the CPT in the high (-0.57 ± 0.19 $L \cdot min^{-1}$), mid (-0.34 ± 0.20 $L \cdot min^{-1}$) and low (-0.45 ± 0.32 $L \cdot min^{-1}$) aerobic fitness groups. The percentage change in LBF was not different ($p>0.05$) between high (-17 ± 3 %), mid (-18 ± 15 %) and low (-29 ± 10 %) aerobic fitness groups and the change in LBF during moderate-intensity exercise was not different ($p>0.05$) from the percentage change in LBF in response to the CPT at rest. The decrease in FVC in response to the CPT was not different ($p>0.05$) between high (-0.013 ± 0.004 $L \cdot min^{-1} \cdot mmHg^{-1}$), mid (-0.008 ± 0.004 $L \cdot min^{-1} \cdot mmHg^{-1}$) and low (-0.007 ± 0.003 $L \cdot min^{-1} \cdot mmHg^{-1}$) fitness groups. The decrease in FVC, expressed as a percentage change, during the CPT was not different ($p>0.05$) between groups (Figure 2-14). The percent decrease in FVC in response to the CPT during moderate-intensity KE exercise was also not different ($p>0.05$) from the response to CPT at rest (Figure 2-14). The time taken to reach the nadir of the FVC response during the CPT was not different ($p>0.05$) between high (84 ± 45 s), mid (114 ± 29 s) and low (116 ± 42 s) fitness groups.

Heavy Intensity Exercise (Δ50%)

Absolute work rates corresponding to Δ*50%* were different between high, mid and low fitness groups, consistent with the differences in aerobic fitness between groups. HR was not different ($p>0.05$) between groups during heavy-intensity exercise. Heavy-intensity exercise was associated with a pressor response in all subjects, however MAP during exercise at Δ*50%* was not different ($p>0.05$) between groups (Table 2-2).

LBF and FVC were greater ($p<0.05$) in the high compared to the low and mid fitness groups. LBF and FVC were not different ($p<0.05$) between mid and low aerobic fitness groups (Table 2-2).

In response to the CPT during heavy-intensity alternate-leg KE exercise, HR increased by a similar ($p>0.05$) magnitude in high (6 ± 5 beats·min^{-1}), mid (5 ± 8 beats·min^{-1}) and low (5 ± 11 beats·min^{-1}) fitness groups. The percent change in HR in response to the CPT was not different ($p>0.05$) between groups (Figure 2-12). The CPT caused a similar ($p>0.05$) increase in MAP in high (17 ± 10 mmHg), mid (11 ± 8 mmHg) and low (20 ± 10 mmHg) fitness groups during exercise (Figure 2-13). The percent change in MAP was not different ($p>0.05$) between groups, however the increase in MAP in response to the CPT during heavy-intensity KE exercise was reduced ($p<0.05$) compared to the response at rest and during moderate-intensity exercise in the mid and high fitness groups. The pressor response in the low fitness group was reduced ($p<0.05$) compared to rest, but not moderate-intensity exercise.

LBF decreased by a similar ($p>0.05$) magnitude in response to the CPT in the high (-0.43 ± 0.13 L·min^{-1}), mid (-0.33 ± 0.41 L·min^{-1}) and low (-0.20 ± 0.49 L·min^{-1}) aerobic fitness groups. The percentage change in LBF was not different ($p>0.05$) between high (-12 ± 6 %), mid (14 ± 18 %) and low (-8 ± 11 %) fitness groups. The percent change in LBF in response to the CPT during heavy-intensity exercise was not different from rest or moderate intensity exercise in all groups. The decrease in FVC in response to the CPT was not different ($p>0.05$) between the high (-0.010 ± 0.004 L·min^{-1}·mmHg^{-1}), mid (-0.005 ± 0.003 L·min^{-1}·mmHg^{-1}) and low (-0.005 ± 0.004 L·min^{-1}·mmHg^{-1}) fitness groups. The percentage change in FVC in response to the CPT during

heavy-intensity exercise was not different ($p>0.05$) between groups (Figure 2-14). However, the percent decrease in FVC in response to the CPT during heavy-intensity KE exercise was smaller ($p<0.05$) than the response to CPT at rest and during moderate-intensity exercise (Figure 2-14). The time to the nadir of the FVC response during the CPT was not different ($p>0.05$) in high (86 ± 44 s), mid (111 ± 38 s) and low (113 ± 36 s) fitness groups.

Magnitude of Sympatholysis

The magnitude of sympathetic vasoconstriction (% change in FVC) evoked by the CPT was not different ($p>0.05$) from rest during moderate-intensity exercise, however the constrictor response was reduced ($p<0.05$) during heavy-intensity exercise compared to rest (i.e. sympatholysis) in all groups. Thus, the magnitude of sympatholysis was greater ($p<0.05$) during heavy-intensity exercise compared to moderate-intensity exercise. However, the magnitude of sympatholysis was similar ($p>0.05$) between high, mid and low aerobic fitness groups during moderate and heavy-intensity KE exercise (Figure 2-15).

Table 2-2. Hemodynamic responses to moderate- and heavy-intensity alternate-leg knee-extension exercise (CPT trials).

	Low Fitness	Mid Fitness	High Fitness
HR (beats·min^{-1})			
Rest	77 ± 13	70 ± 9	67 ± 14
Passive Exercise	86 ± 13 $^{+}$	80 ± 14 $^{+}$	74 ± 15 $^{+}$
Moderate Exercise	104 ± 17 $^{\#}$	103 ± 14 $^{\#}$	98 ± 13 $^{\#}$
Heavy Exercise	121 ± 19 *	119 ± 19 *	113 ± 19 *
MAP (mmHg)			
Rest	85 ± 16	78 ± 8	78 ± 12
Passive Exercise	85 ± 12	79 ± 10	80 ± 15
Moderate Exercise	96 ± 16 $^{\#}$	93 ± 13 $^{\#}$	88 ± 13 $^{\#}$
Heavy Exercise	104 ± 13 *	113 ± 17 *	98 ± 15 *
LBF (L·min^{-1})			
Rest	0.5 ± 0.1	0.5 ± 0.1	0.7 ± 0.2
Passive Exercise	0.8 ± 03	1.0 ± 0.3	1.2 ± 0.3
Moderate Exercise †	1.7 ± 0.5 $^{\#}$	2.4 ± 1.0 $^{\#}$	3.3 ± 0.9 $^{\#}$
Heavy Exercise $^{£}$	2.6 ± 1.0 *	2.9 ± 0.9 ^{x}	4.1 ± 1.3 *
FVC (L·min^{-1}·mmHg^{-1})			
Rest	0.006 ± 0.002	0.007 ± 0.002	0.009 ± 0.002
Passive Exercise	0.01 ± 0.003	0.01 ± 0.003 $^{+}$	0.02 ± 0.003 $^{+}$
Moderate Exercise †	0.02 ± 0.005 $^{\#}$	0.03 ± 0.01 $^{\#}$	0.04 ± 0.009 $^{\#}$
Heavy Exercise $^{£}$	0.03 ± 0.008 *	0.03 ± 0.008 ^{x}	0.04 ± 0.01 ^{x}
Work Rate (W)			
Moderate Exercise	23 ± 7	33 ± 9	47 ± 11
Heavy Exercise	42 ± 10 **	60 ± 16 **	77 ± 17 **

Values are mean ± SD. Heart rate (HR); mean arterial pressure (MAP); limb blood flow (LBF); femoral vascular conductance (FVC). * Indicates a significant difference between heavy-intensity exercise and moderate-intensity exercise, passive exercise and rest. $^{\#}$ Indicates a significant difference between moderate-intensity exercise and heavy-intensity exercise, passive exercise and rest. $^{+}$ Indicates a significant difference between passive exercise and heavy-, moderate-intensity exercise and rest. ^{x} Indicates a significant difference between heavy-intensity exercise and both passive exercise and rest. ** Indicates a significant difference between all aerobic fitness groups and from moderate-intensity. † Indicates a significant difference between all aerobic fitness groups. $^{£}$ Indicates a significant difference between the high aerobic fitness group and both mid and low aerobic fitness groups.

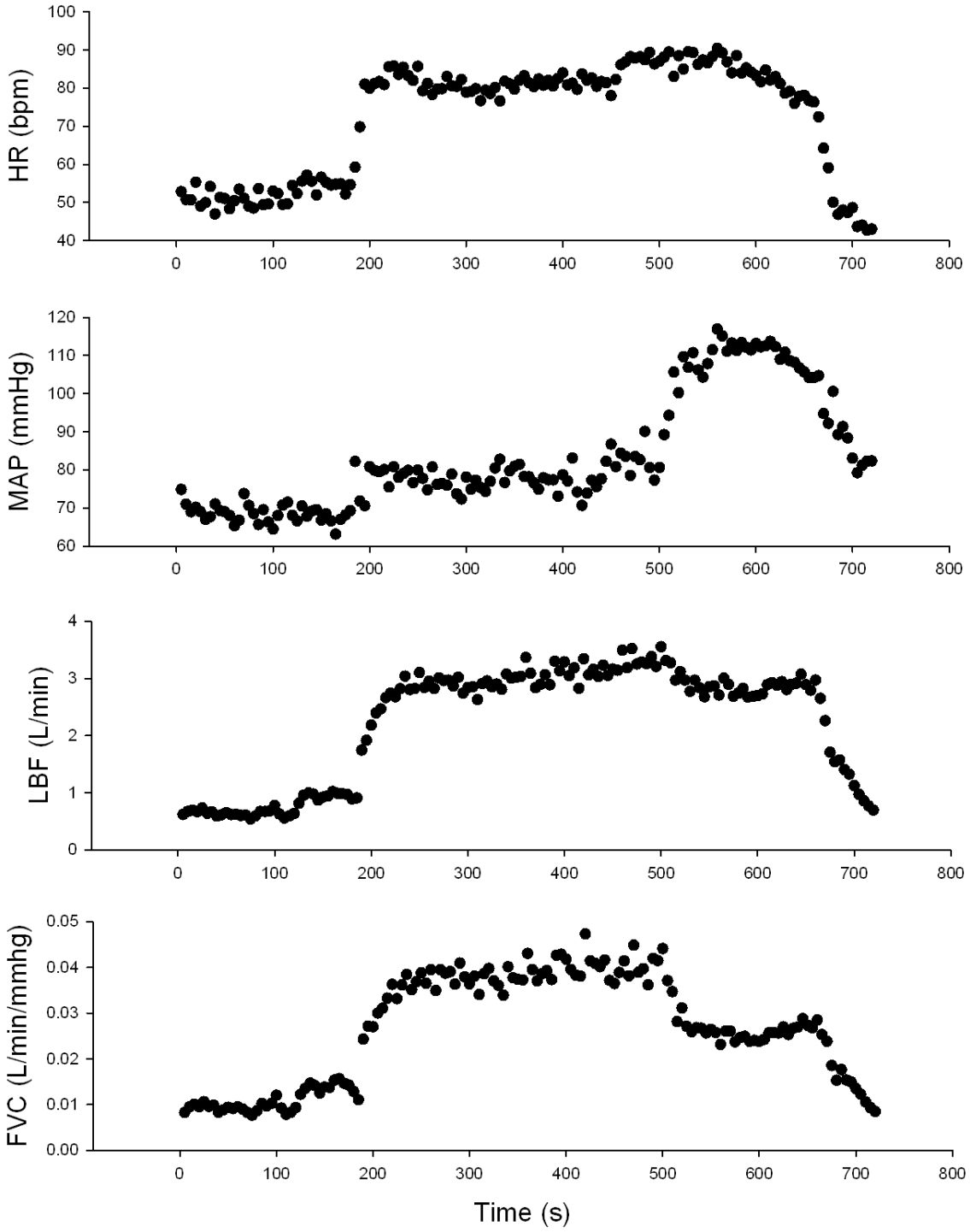

Figure 2-11. Individual representative tracing of the cardiovascular (heart rate, HR; mean arterial pressure, MAP; limb blood flow, LBF; femoral vascular conductance, FVC) response to the cold-pressor test (CPT) during knee-extension exercise. The CPT was initiated at 480 s, was 180 s in duration and was followed by 60 s of recovery. Each data point represents a 5 second average.

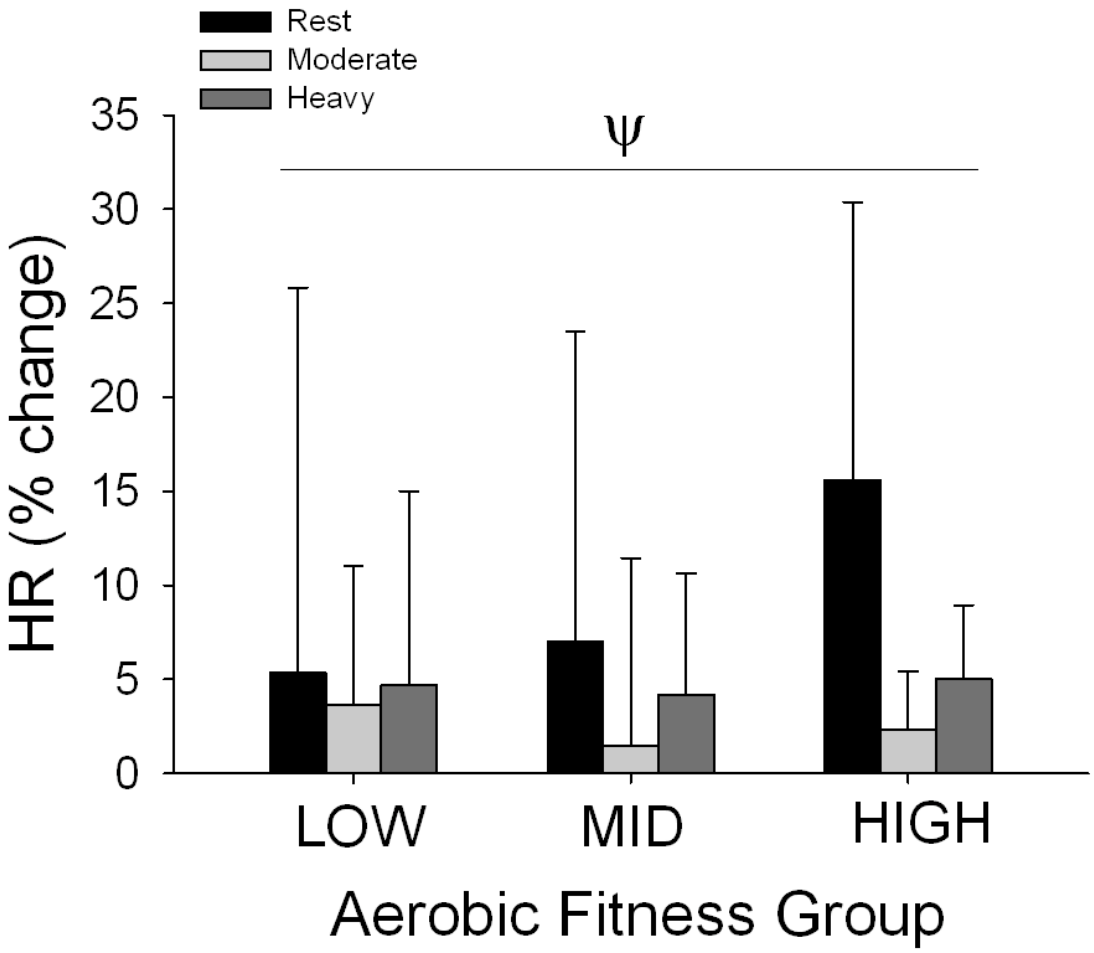

Figure 2-12. Percent change of heart rate (HR) in response to the cold-pressor test (CPT) at rest, and during moderate- and heavy-intensity knee-extension exercise in low (n=7), mid (n=8) and high (n=7) aerobic fitness groups. ψ Indicates a significant difference between rest and exercise. Values are mean ± SD.

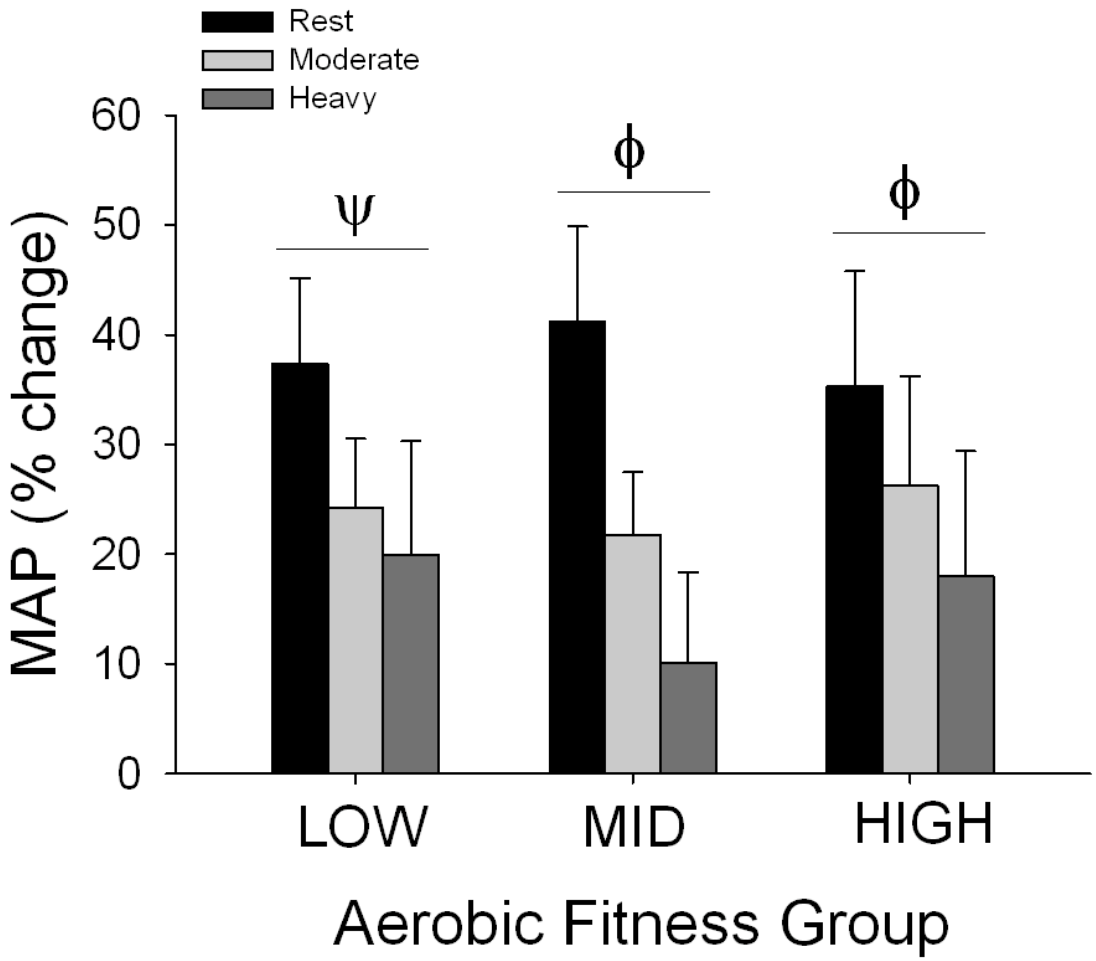

Figure 2-13. Percent change of mean arterial pressure (MAP) in response to the cold-pressor test (CPT) at rest, and during moderate- and heavy-intensity knee-extension exercise in low (n=7), mid (n=8) and high (n=7) aerobic fitness groups. ψ Indicates a significant difference between rest and exercise. ϕ Indicates a significant difference between all exercise intensities. Values are mean ± SD.

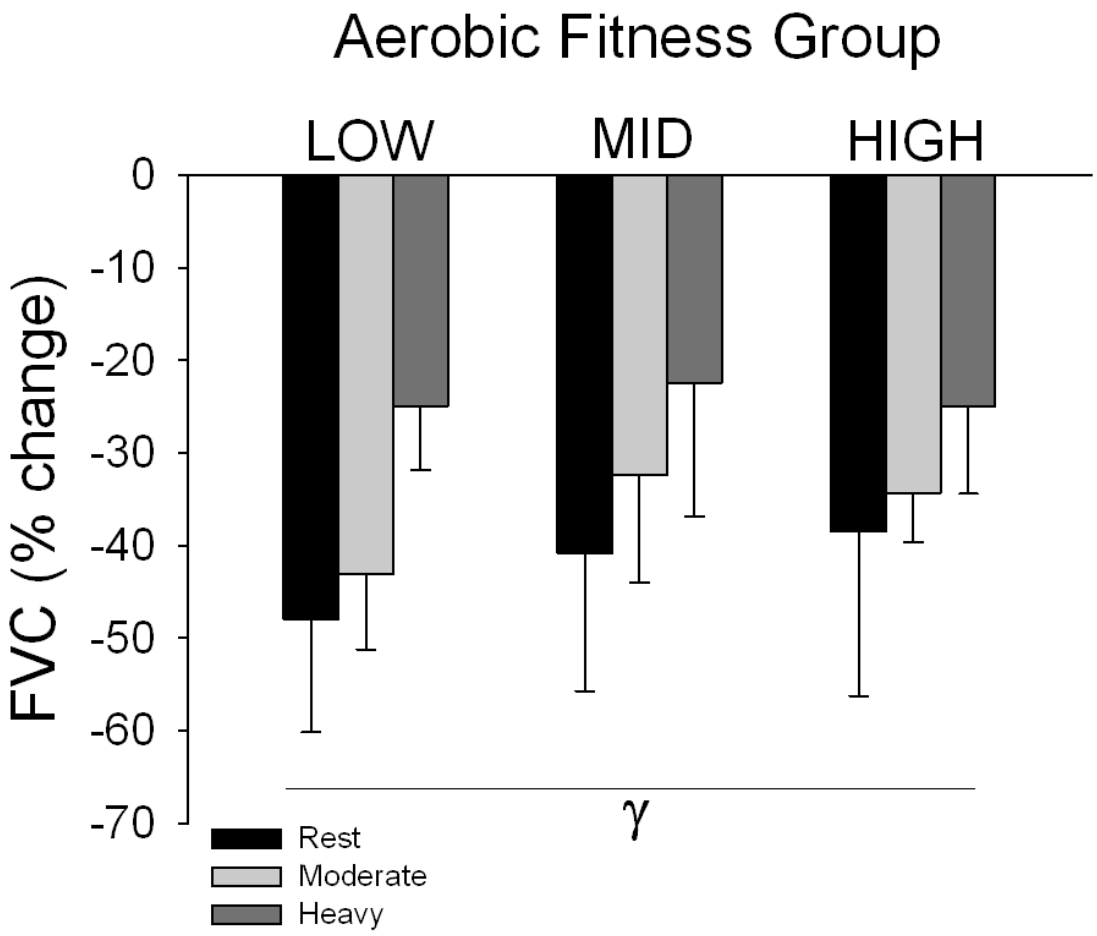

Figure 2-14. Percent change of femoral vascular conductance (FVC) in response to the cold-pressor test (CPT) at rest, and during moderate- and heavy-intensity knee-extension exercise in low (n=7), mid (n=8) and high (n=7) aerobic fitness groups. γ Indicates a significant difference between heavy-intensity exercise, and rest and moderate-intensity exercise. Values are mean ± SD.

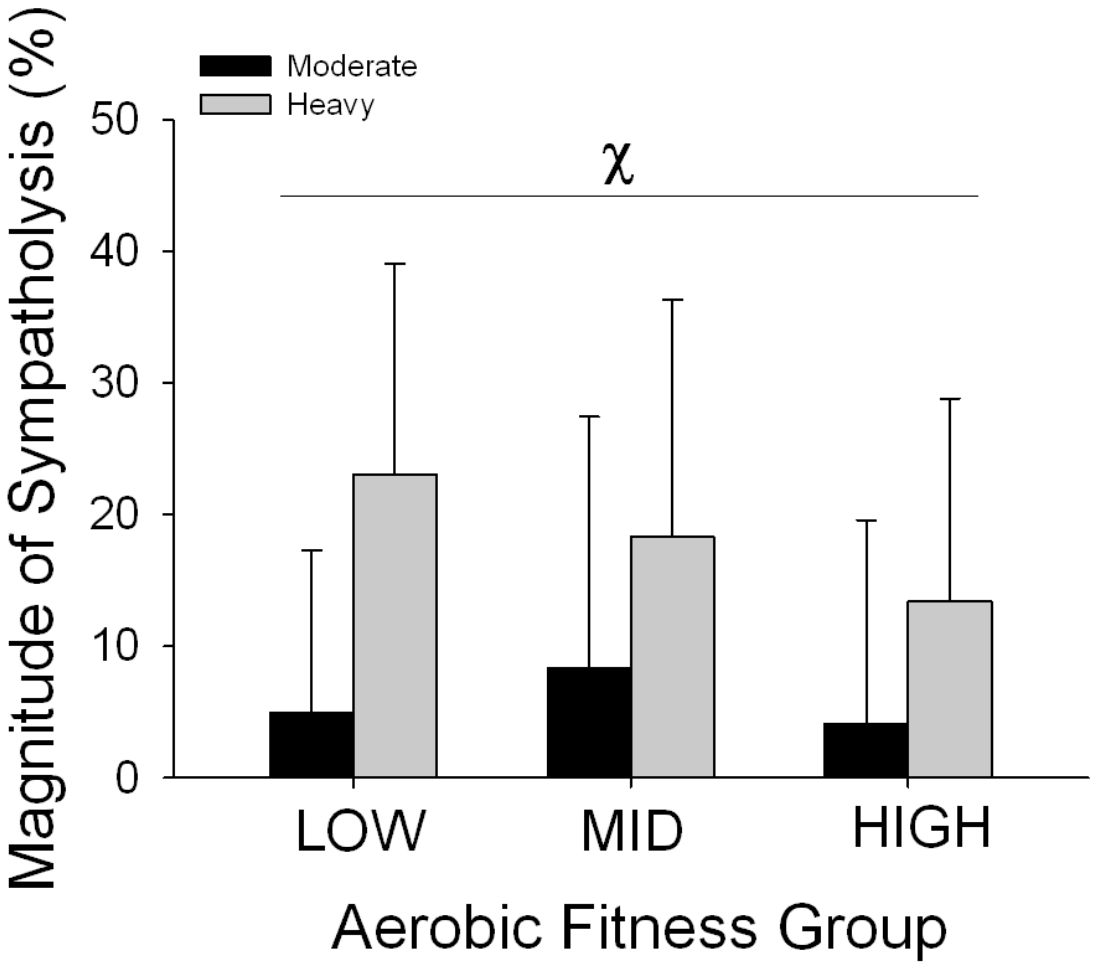

Figure 2-15. Magnitude of sympatholysis (expressed as the difference in %ΔFVC in response to CPT during exercise, from the %ΔFVC during CPT at rest) in response to the cold-pressor test (CPT) during moderate- and heavy-intensity knee-extension (KE) exercise in low (n=7), mid (n=8) and high (n=7) aerobic fitness groups. χ Indicates a significant difference between moderate- and heavy-intensity KE exercise. Values are mean ± SD.

2.5.4 Physiological response to isometric handgrip

Rest

A representative tracing of the cardiovascular response to the IHG during rest is illustrated in Figure 2-16.

MSNA increased during IHG in all subjects. The increase in burst frequency, expressed as a percent change, was greater ($p<0.05$) in the high compared to mid and low fitness groups (Figure 2-17). The percent increase in burst incidence during IHG was greater in the high compared to mid fitness group, but not different from the increase in burst incidence in the low fitness group (Figure 2-18).

In response to IHG, SVT was greater ($p< 0.05$) in mid compared to low and high aerobic fitness groups (Figure 2-19).

IHG exercise caused a similar absolute increase in HR in high (25 ± 13 beats·min^{-1}), mid (20 ± 12 beats·min^{-1}) and low (24 ± 17 beats·min^{-1}) fitness groups. The percent change in HR from the pre-IHG baseline was also not different ($p>0.05$) between groups (Figure 2-21). The pressor response during IHG was also not different between the high (37 ± 6 mmHg), mid (38 ± 7 mmHg) and low (33 ± 8 mmHg) fitness groups. The percent increase in MAP from the pre-IHG baseline was also not different ($p>0.05$) between groups (Figure 2-22).

In response to IHG, the change in LBF was not different ($p>0.05$) in high (0.02 ± 0.18 L·min^{-1}), mid (-0.02 ± 0.19 L·min^{-1}) and low (-0.04 ± 0.07 L·min^{-1}) aerobic fitness groups. The percent change in LBF from the pre-IHG baseline was also not different ($p>0.05$) between the high (4 ± 42 %), mid (-13 ± 13 %) and low (-8 ± 16 %) fitness groups. The decrease in FVC in response to IHG was not different ($p>0.05$) between the

high (-0.002 ± 0.002 $L \cdot min^{-1} \cdot mmHg^{-1}$), mid (-0.003 ± 0.002 $L \cdot min^{-1} \cdot mmHg^{-1}$) and low fitness group (-0.002 ± 0.001 $L \cdot min^{-1} \cdot mmHg^{-1}$). As illustrated in Figure 2-23, the decrease in FVC expressed as a percent change from the pre-IHG baseline, was also not different ($p>0.05$) between groups. The time required to reach the nadir of the FVC response during IHG was not different ($p>0.05$) between high (149 ± 19 s), mid (111 ± 32 s) and low (121 ± 44 s) fitness groups.

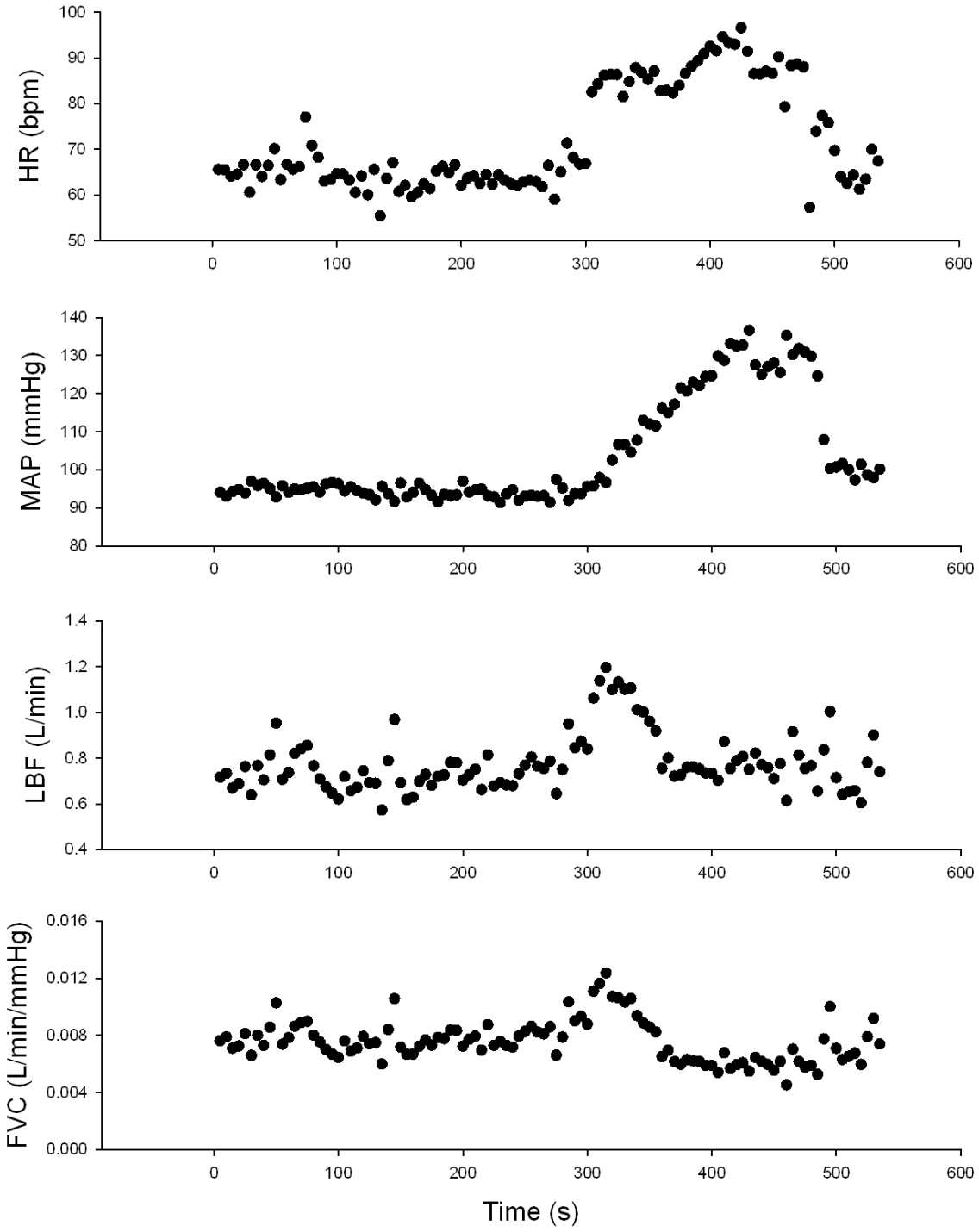

Figure 2-16. Individual representative tracing of the cardiovascular (heart rate, HR; mean arterial pressure, MAP; limb blood flow, LBF; femoral vascular conductance, FVC) response to the IHG at rest. The IHG was initiated at 300 s, was 180 s in duration and was followed by 60 s of recovery. Each data point represents a 5 second average.

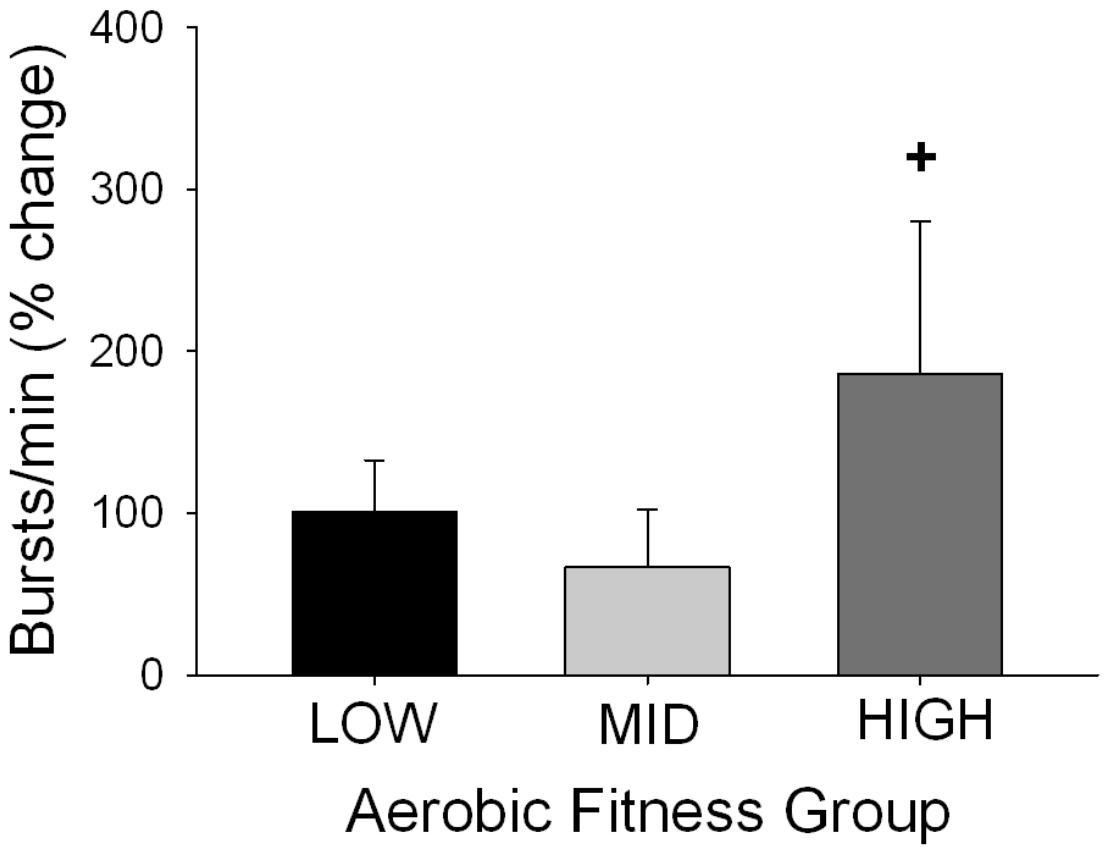

Figure 2-17. Percent change of muscle sympathetic nerve activity (MSNA) burst frequency in response to isometric handgrip (IHG) at rest in low (n=5), mid (n=6) and high (n=6) aerobic fitness groups. $^{+}$ Significant difference from both low and mid fitness groups ($p < 0.05$). Values are mean ± SD.

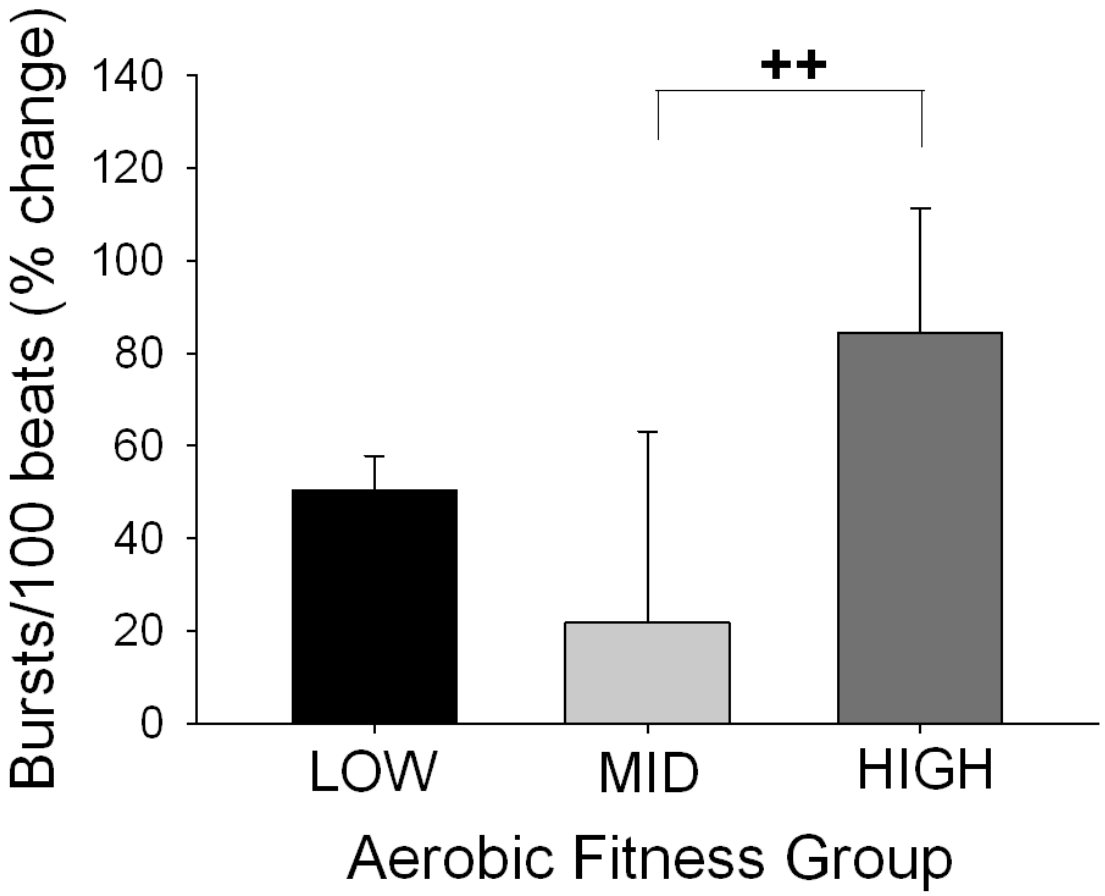

Figure 2-18. Percent change of muscle sympathetic nerve activity (MSNA) burst incidence in response to isometric handgrip (IHG) at rest in low (n=5), mid (n=6) and high (n=6) aerobic fitness groups. $^{++}$ Significant difference between high aerobic fitness and mid aerobic fitness group ($p < 0.05$). Values are mean ± SD.

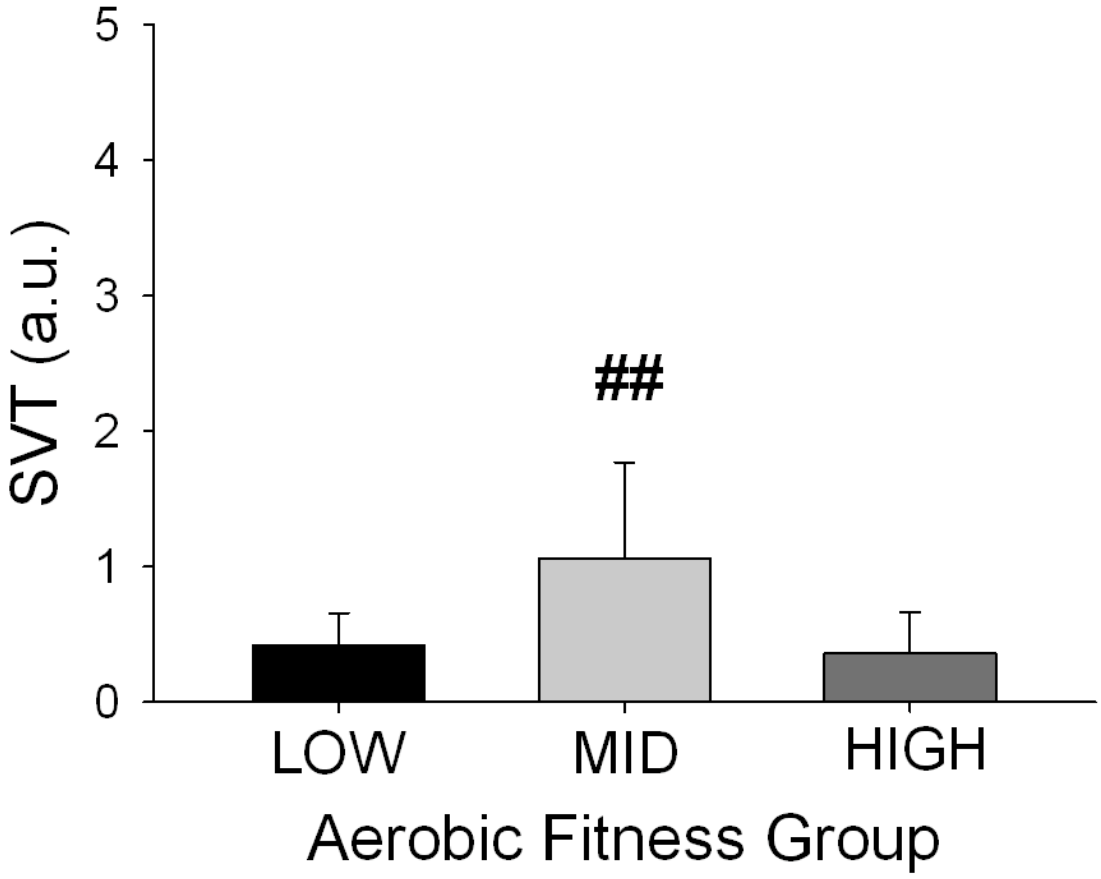

Figure 2-19. Sympathetic vascular transduction (SVT) during isometric handgrip (IHG) at rest in low (n=5), mid (n=6) and high (n=6) aerobic fitness groups. ## Significant difference from high and low aerobic fitness groups ($p < 0.05$). Values are mean ± SD.

Moderate Exercise Intensity (80%VT)

A representative tracing of the cardiovascular response to alternate-leg KE exercise and to IHG during exercise is illustrated in Figure 2-20. HR, MAP, LBF and FVC all increased in response to moderate-intensity KE exercise (Table 2-3).

Consistent with the differences in peak work rate achieved during the incremental KE max test, the moderate-intensity work rates were also different ($p<0.05$) between groups, with work rate increasing as a function of VO_2max (Table 2-3). Despite the differences in the absolute work rates, HR was not different ($p>0.05$) between groups during moderate-intensity exercise (Table 2-3). MAP was also similar in the three aerobic fitness groups during moderate-intensity exercise (Table 2-3).

LBF and FVC were different ($p>0.05$) between groups during moderate-intensity exercise and the increase in LBF and FVC were a function of the absolute work rate (Table 2-3).

In response to IHG during alternate-leg KE exercise, HR increased by a similar ($p>0.05$) magnitude in high (20 ± 7 beats·min^{-1}), mid (13 ± 11 beats·min^{-1}) and low (15 ± 5 beats·min^{-1}) fitness groups. The percent change in HR in response to IHG was not different ($p>0.05$) between groups (Figure 2-21). IHG caused a similar ($p>0.05$) increase in MAP in high (30 ± 13 mmHg), mid (29 ± 6 mmHg) and low (26 ± 8 mmHg) fitness groups during exercise (Figure 2-22). The percent change in MAP was not different ($p>0.05$) between groups. However, the pressor response during IHG was reduced compared to the response at rest in the high, mid and low fitness groups.

LBF decreased by a similar magnitude in response to IHG in the high (-0.29 ± 0.34 L·min^{-1}), mid (-0.41 ± 0.44 L·min^{-1}) and low (-0.18 ± 0.17 L·min^{-1}) aerobic fitness

groups. The percentage change in LBF was not different ($p>0.05$) between high (-10 ± 10 %), mid (-17 ± 18 %) and low (-11 ± 12 %) fitness group values, and the change in LBF during moderate-intensity exercise was not different ($p>0.05$) from the percentage change in LBF in response to IHG at rest. The decrease in FVC in response to IHG was not different ($p>0.05$) between high (-0.011 ± 0.006 $L \cdot min^{-1} \cdot mmHg^{-1}$), mid (-0.010 ± 0.005 $L \cdot min^{-1} \cdot mmHg^{-1}$) and low (-0.005 ± 0.002 $L \cdot min^{-1} \cdot mmHg^{-1}$) fitness groups. The decrease in FVC, expressed as a percentage change, during IHG was not different ($p>0.05$) between groups (Figure 2-23). The percent decrease in FVC in response to IHG during moderate-intensity KE exercise was also not different ($p>0.05$) from the response to IHG at rest (Figure 2-23). The time taken to reach the nadir of the FVC response during IHG was not different ($p>0.05$) between high (128 ± 52 s), mid (160 ± 16 s) and low (159 ± 22 s) fitness groups.

Heavy Intensity Exercise (Δ50%)

Absolute work rates corresponding to *Δ50%* were different ($p<0.05$) between high, mid and low fitness groups, consistent with the differences in aerobic fitness between groups. HR was not different ($p>0.05$) between groups during heavy-intensity exercise. Heavy-intensity exercise was associated with a pressor response in all subjects, however MAP during exercise at *Δ50%* was not different between groups (Table 2-3).

LBF and FVC were greater ($p<0.05$) in the high compared to the low and mid fitness groups. LBF and FVC were not different ($p<0.05$) between mid and low aerobic fitness groups (Table 2-3).

In response to IHG during heavy-intensity alternate-leg KE exercise, HR increased by a similar ($p>0.05$) magnitude in high (17 ± 8 beats·min^{-1}), mid (14 ± 8 beats·min^{-1}) and low (17 ± 7 beats·min^{-1}) fitness groups. The percent change in HR in response to IHG was not different ($p>0.05$) between groups (Figure 2-21). IHG caused a similar ($p>0.05$) increase in MAP in high (16 ± 10 mmHg), mid (19 ± 8 mmHg) and low (17 ± 9 mmHg) groups during exercise (Figure 2-22). The percent change in MAP was not different ($p>0.05$) between groups, however the increase in MAP in response to IHG during heavy-intensity KE exercise was reduced ($p<0.05$) compared to the response at rest and during moderate-intensity exercise.

LBF decreased by a similar ($p>0.05$) magnitude in response to IHG in the high (-0.39 ± 0.75 L·min^{-1}), mid (-0.25 ± 0.28 L·min^{-1}) and low (-0.36 ± 0.42 L·min^{-1}) aerobic fitness groups. The percentage change in LBF was not different ($p>0.05$) between high (-7 ± 11 %), mid (-8 ± 8%) and low (-11 ± 13 %) aerobic fitness group values. The percent change in LBF in response to IHG during heavy-intensity exercise was not different from rest or moderate intensity exercise in all groups. The decrease in FVC in response to IHG was not different ($p>0.05$) between the high (-0.011 ± 0.011 L·min^{-1}·mmHg^{-1}), mid (-0.007 ± 0.002 L·min^{-1}·mmHg^{-1}) and low (-0.007 ± 0.005 L·min^{-1}·mmHg^{-1}) fitness groups. The percentage change in FVC in response to IHG during heavy-intensity exercise was not different between groups (Figure 2-23). However, the percent decrease in FVC in response to IHG during heavy-intensity KE exercise was smaller ($p<0.05$) than the response to IHG at rest and during moderate-intensity exercise (Figure 2-23). The time to the nadir FVC response during IHG was not different in high (122 ± 30 s), mid (141 ± 35 s) and low (140 ± 38 s) fitness groups.

Magnitude of Sympatholysis

The magnitude of sympathetic vasoconstriction (% change in FVC) evoked by IHG exercise was not different ($p>0.05$) from rest during moderate-intensity exercise, however the constrictor response was reduced ($p<0.05$) during heavy-intensity exercise compared to rest (i.e. sympatholysis) in all groups. Thus, the magnitude of sympatholysis was greater ($p<0.05$) during heavy-intensity exercise compared to moderate-intensity exercise. However, the magnitude of sympatholysis was similar ($p>0.05$) between high, mid and low aerobic fitness groups during moderate and heavy-intensity KE exercise (Figure 2-24).

Table 2-3. Hemodynamic responses to moderate- and heavy-intensity alternate-leg knee-extension exercise (IHG trials).

	Low Fitness	Mid Fitness	High Fitness
HR (beats·min^{-1})			
Rest	77 ± 13	72 ± 9	66 ± 13
Passive Exercise	85 ± 14 $^{+}$	82 ± 11 $^{+}$	74 ± 17 $^{+}$
Moderate Exercise	103 ± 15 $^{\#}$	105 ± 13 $^{\#}$	97 ± 15 $^{\#}$
Heavy Exercise	123 ± 14 *	116 ± 19 *	112 ± 15 *
MAP (mmHg)			
Rest	84 ± 11	77 ± 12	77 ± 11
Passive Exercise	88 ± 10	77 ± 11	79 ± 10
Moderate Exercise	96 ± 13 $^{\#}$	93 ± 10 $^{\#}$	91 ± 14 $^{\#}$
Heavy Exercise	106 ± 15 *	106 ± 12 *	100 ± 13 *
LBF (L·min^{-1})			
Rest	0.5 ± 0.1	0.7 ± 0.2	0.7 ± 0.2
Passive Exercise	0.8 ± 0.3	1.1 ± 0.5	1.1 ± 0.3
Moderate Exercise †	1.6 ± 0.3 $^{\#}$	2.5 ± 0.9 $^{\#}$	3.2 ± 0.9 $^{\#}$
Heavy Exercise $^{£}$	2.7 ± 1.0 *	3.2 ± 0.9 ^{x}	4.4 ± 1.5 *
FVC (L·min^{-1}·mmHg^{-1})			
Rest	0.006 ± 0.001	0.009 ± 0.002	0.009 ± 0.002
Passive Exercise	0.009 ± 0.002	0.01 ± 0.005	0.01 ± 0.004
Moderate Exercise †	0.02 ± 0.003 $^{\#}$	0.03 ± 0.01 $^{\#}$	0.03 ± 0.008 $^{\#}$
Heavy Exercise $^{£}$	0.03 ± 0.009 *	0.03 ± 0.008 ^{x}	0.04 ± 0.02 ^{x}
Work Rate (W)			
Moderate Exercise	23 ± 7	33 ± 9	47 ± 11
Heavy Exercise	42 ± 10 **	60 ± 16 **	77 ± 17 **

Values are mean ± SD. Heart rate (HR); mean arterial pressure (MAP); limb blood flow (LBF); femoral vascular conductance (FVC). * Indicates a significant difference between heavy-intensity exercise and moderate-intensity exercise, passive exercise and rest. $^{\#}$ Indicates a significant difference between moderate-intensity exercise and heavy-intensity exercise, passive exercise and rest. $^{+}$ Indicates a significant difference between passive exercise and heavy-, moderate-intensity exercise and rest. ^{x} Indicates a significant difference between heavy-intensity exercise and both passive exercise and rest. ** Indicates a significant difference between all aerobic fitness groups and from moderate-intensity. † Indicates a significant difference between all aerobic fitness groups. $^{£}$ Indicates a significant difference between the high aerobic fitness group and both mid and low aerobic fitness groups.

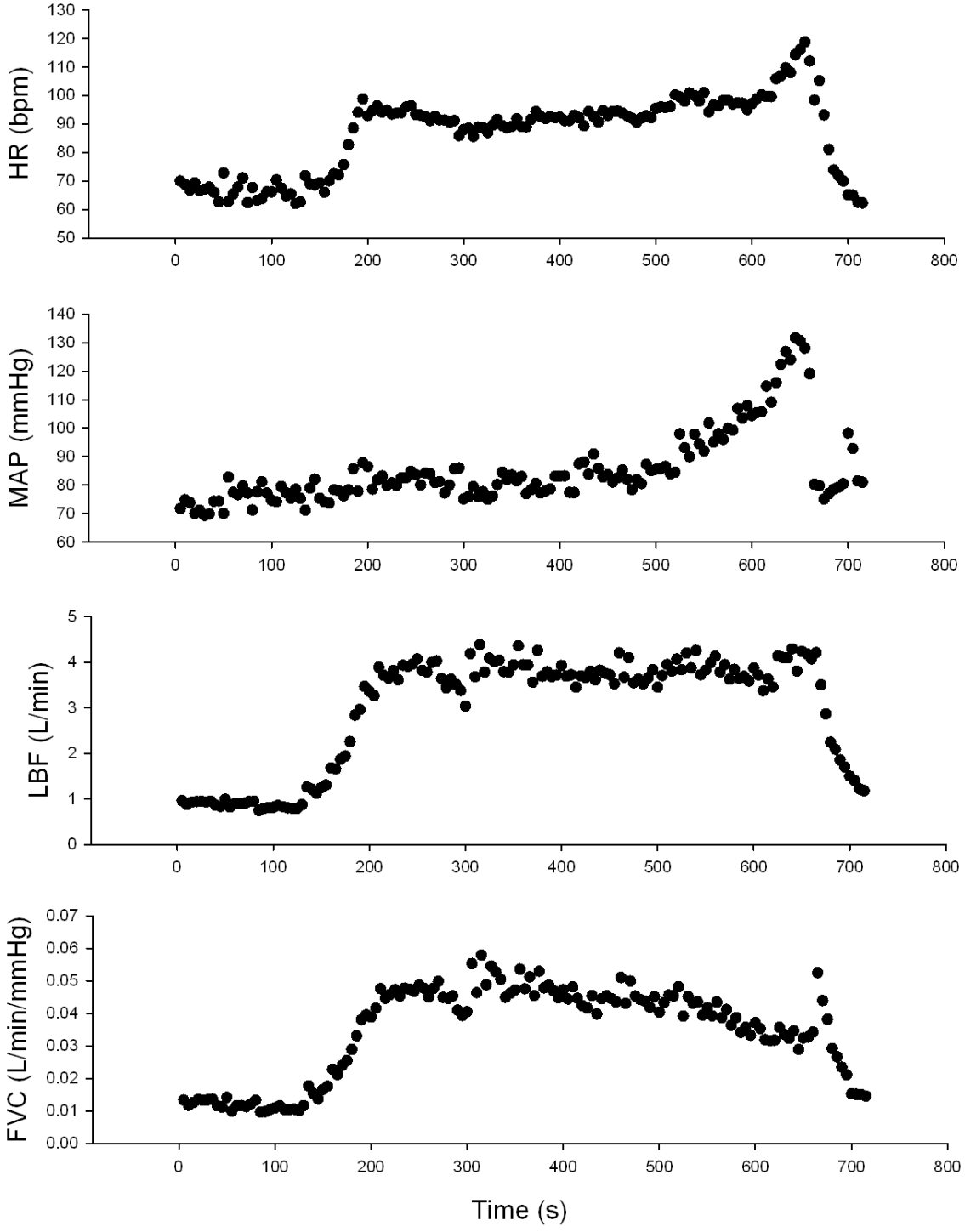

Figure 2-20. Individual representative tracing of the cardiovascular (heart rate, HR; mean arterial pressure, MAP; limb blood flow, LBF; femoral vascular conductance, FVC) response to isometric handgrip (IHG) during knee-extension exercise. The IHG was initiated at 480 s, was 180 s in duration and was followed by 60 s of recovery. Each data point represents a 5 second average.

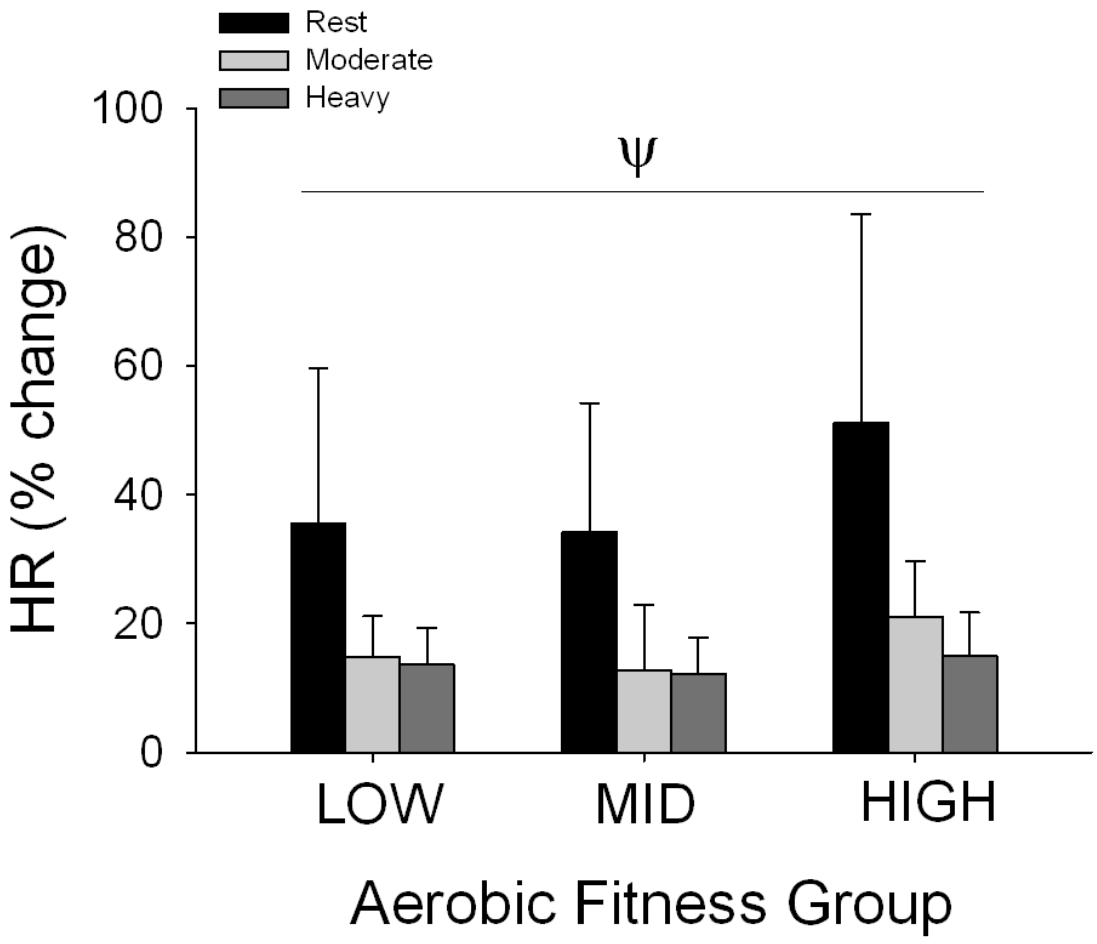

Figure 2-21. Percent change of heart rate (HR) in response to isometric handgrip (IHG) at rest, and during moderate- and heavy-intensity knee-extension exercise in low (n=8), mid (n=7) and high (n=7) aerobic fitness groups. ψ Indicates a significant difference between rest and exercise. Values are mean ± SD.

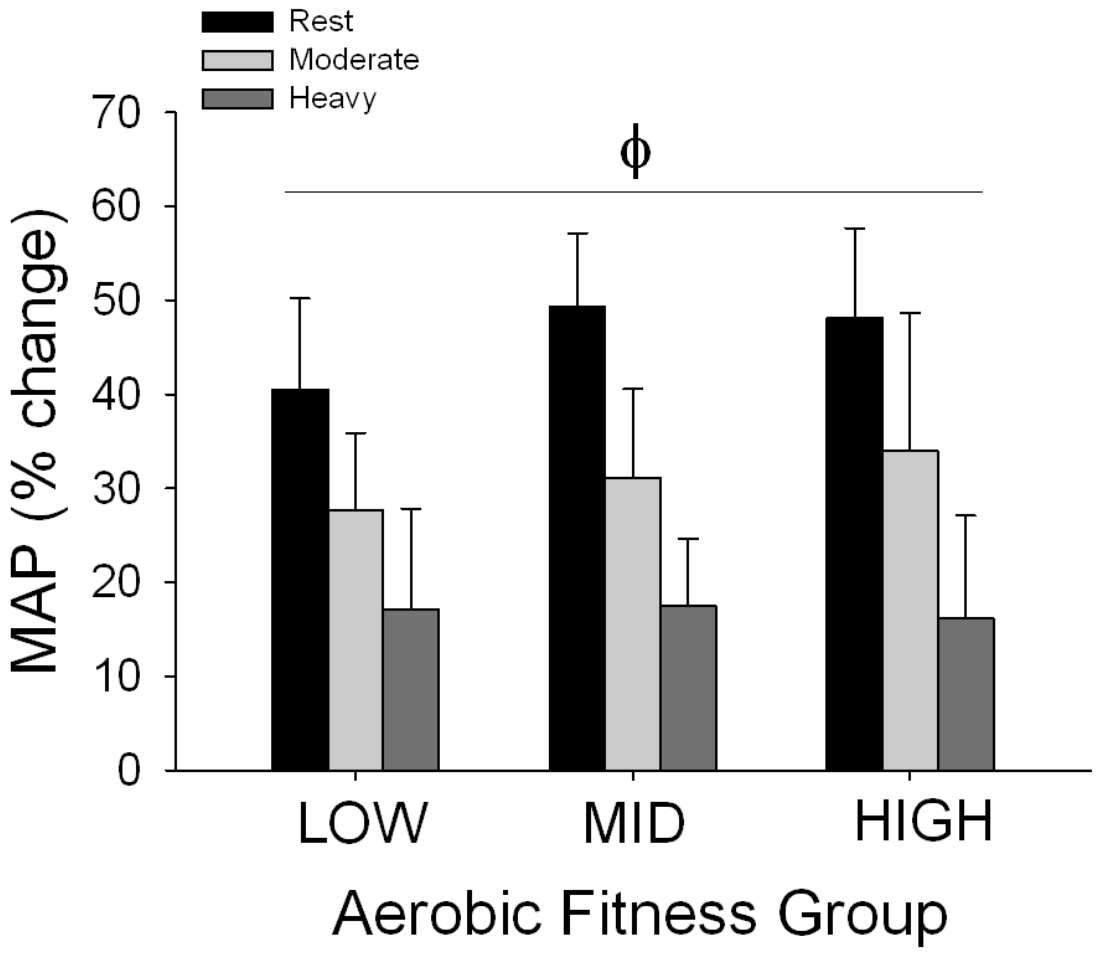

Figure 2-22. Percent change of mean arterial pressure (MAP) in response to isometric handgrip (IHG) at rest, and during moderate- and heavy-intensity knee-extension exercise in low (n=8), mid (n=7) and high (n=7) aerobic fitness groups. ϕ Indicates a significant difference between all exercise intensities. Values are mean ± SD.

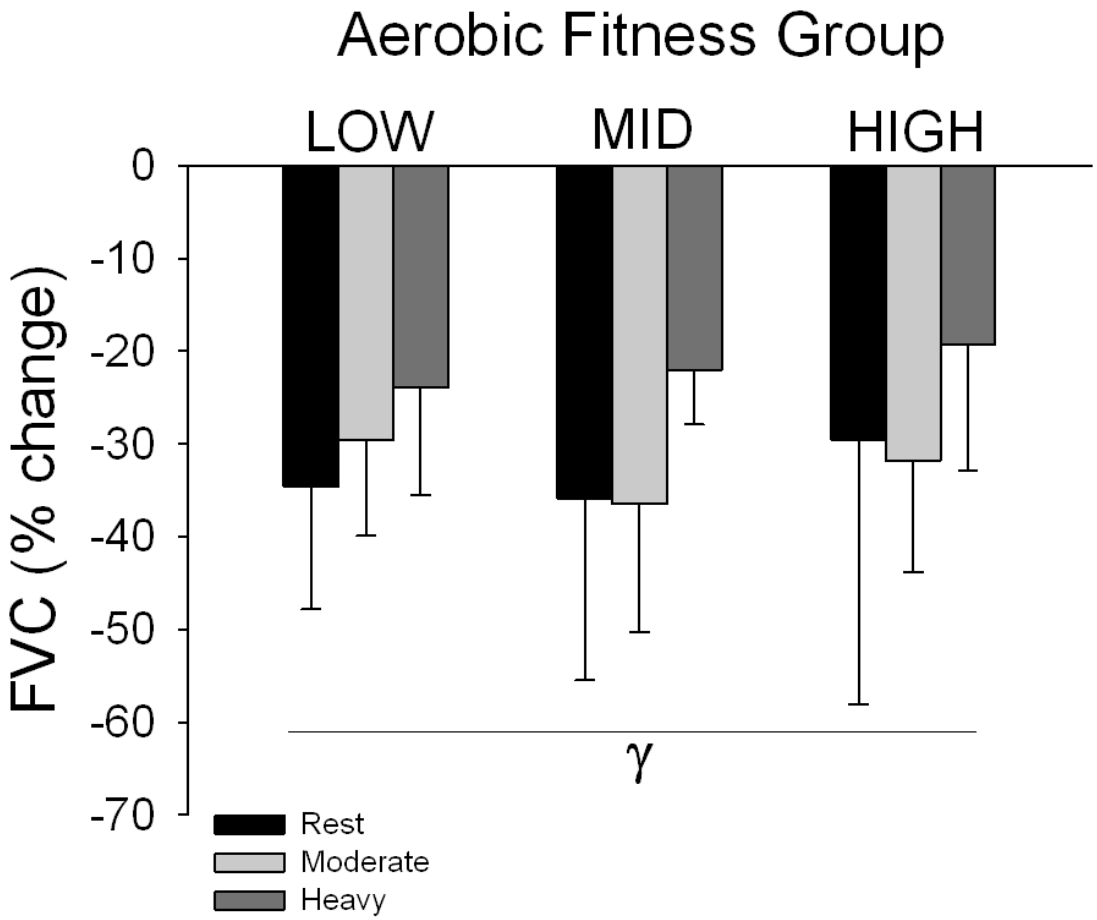

Figure 2-23. Percent change of femoral vascular conductance (FVC) in response to isometric handgrip (IHG) at rest, and during moderate- and heavy-intensity knee-extension exercise in low (n=8), mid (n=7) and high (n=7) aerobic fitness groups. γ Indicates a significant difference between heavy-intensity exercise, and rest and moderate-intensity exercise. Values are mean ± SD.

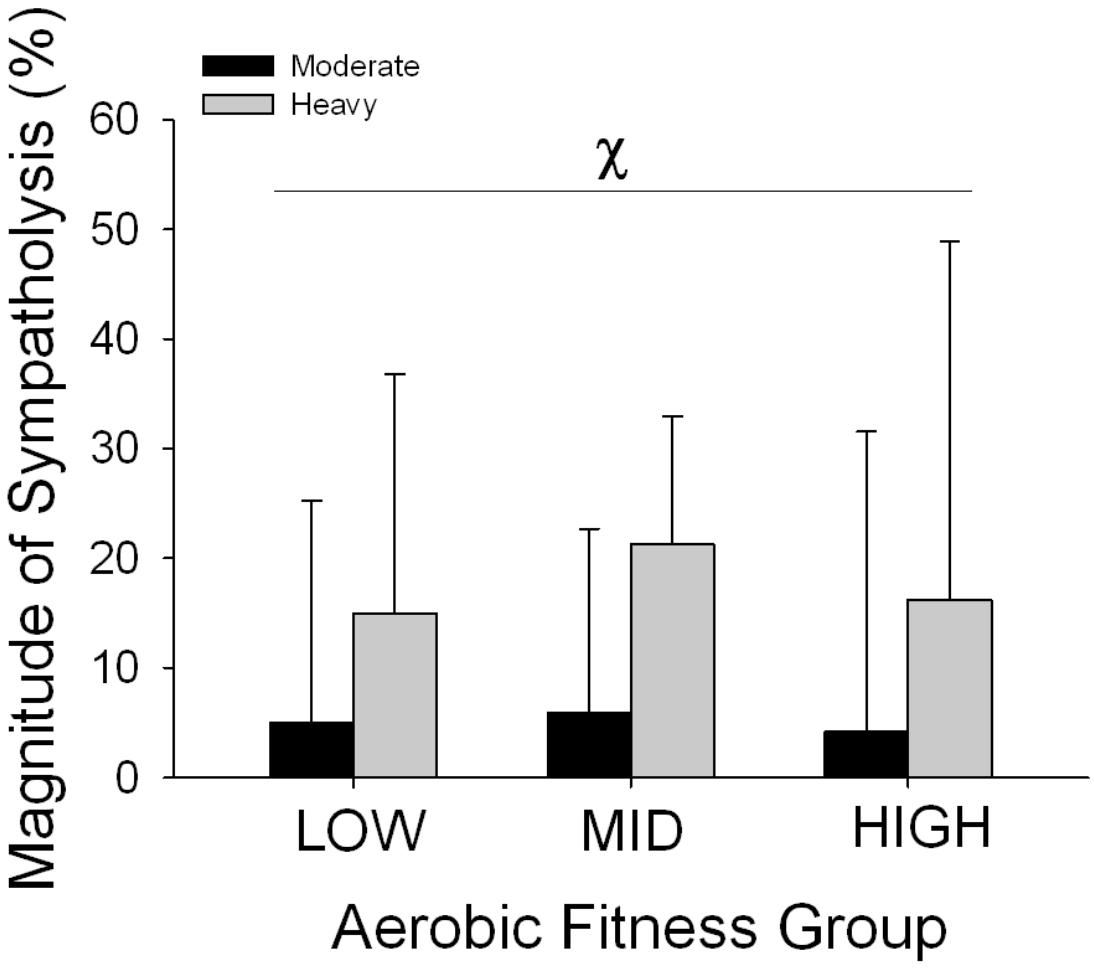

Figure 2-24. Magnitude of sympatholysis (expressed as the difference in %ΔFVC in response to IHG during exercise, from the %ΔFVC during IHG at rest) in response to isometric handgrip (IHG) during moderate- and heavy-intensity knee-extension (KE) exercise in low (n=8), mid (n=7) and high (n=7) aerobic fitness groups. χ Indicates a significant difference between moderate- and heavy-intensity KE exercise. Values are mean ± SD.

2.5.5 Relationships between variables

Relative VO_2max ($mL \cdot kg^{-1} \cdot min^{-1}$) was negatively correlated with resting HR ($r = -0.637$, $p<0.05$), resting SV ($r = -0.469$, $p<0.05$) and resting Q ($r = -0.807$, $p<0.05$). Relative VO_2max ($mL \cdot kg^{-1} \cdot min^{-1}$) was also negatively correlated with MSNA burst frequency ($r = -0.684$, $p<0.05$) and burst incidence ($r = -0.500$, $p<0.05$) at rest. Resting HR was correlated with resting burst frequency ($r = 0.618$, $p<0.05$). Q was positively correlated with both resting burst frequency ($r = 0.705$, $p<0.05$) and resting burst incidence ($r = 0.403$, $p<0.05$). In response to the CPT at rest, the percentage increases in MSNA burst frequency ($r = 0.677$, $p<0.05$) and burst incidence ($r = 0.551$, $p<0.05$) were associated with relative VO_2max ($mL \cdot kg^{-1} \cdot min^{-1}$). SVT was also positively correlated ($r = 0.600$, $p<0.05$) with relative VO_2max ($mL \cdot kg^{-1} \cdot min^{-1}$) in response to the CPT.

2.6 Discussion of Results

2.6.1 Main Findings

The purpose of this study was to investigate the effect of aerobic fitness on the sympathetic nervous system and cardiovascular response to physiological stress at rest and during dynamic exercise in young, healthy males. It was hypothesized that the magnitude of the sympathetic and cardiovascular responses to acute physiological stress at rest and during moderate- and heavy-intensity exercise would decline as a function of aerobic fitness. An attenuated physiological response to stress may be mechanistically linked to the lower risk and incidence of cardiovascular disease associated with aerobic fitness (Krantz & Manuck, 1984; Manuck, 1994; McEwen, 1998).

In the present study, resting MSNA burst frequency and incidence were lower in the high, compared to the low and mid aerobic fitness groups, indicating that increased aerobic fitness decreased resting sympathetic outflow. In response to physiological stress at rest, the high aerobic fitness group had a larger increase (expressed as a % change from rest) in MSNA burst frequency and incidence, compared to the low and mid-fitness groups, suggesting that highly fit subjects had an amplified sympathetic nervous system response to physiological stress, in contrast to our hypothesis. However, the augmented increase in MSNA in the highly fit did not result in a larger cardiovascular response to physiological stress, as IHG exercise and the CPT produced similar cardiovascular responses in the low, mid and high fitness groups. The dissociation of the cardiovascular and MSNA responses to stress suggests that aerobic fitness may alter neuro-vascular coupling. Indeed, SVT was markedly reduced in the high, compared to low and mid fitness groups in response to the CPT.

Consistent with the resting data, the cardiovascular response to physiological stress was not different between aerobic fitness groups during moderate- or heavy-intensity exercise. During moderate-intensity exercise, the cardiovascular response to physiological stress was also not different from the response at rest within each group. However, during heavy-intensity exercise, the cardiovascular response to both the CPT and IHG exercise was reduced compared to the response at rest and during moderate-intensity exercise, and the magnitude of sympatholysis was not different between groups.

Collectively, the findings from this study demonstrate that aerobic fitness alters the basic physiological regulation of sympathetic vasoconstriction under resting conditions. During moderate- and heavy-intensity exercise the cardiovascular response to physiological stress, including the magnitude of sympatholysis, was not altered by aerobic fitness. The lack of an effect of aerobic fitness on the cardiovascular response to physiological stress suggests that the cardio-protective benefits associated with aerobic fitness are not due to an attenuated physiological response to stress.

2.6.2 Resting Muscle Sympathetic Nerve Activity and Hemodynamics

In the present study, the high aerobic fitness group had lower resting MSNA burst frequency and incidence compared to the low and mid fitness groups. Consistent with the group differences in resting MSNA, VO_2max was negatively correlated with resting MSNA burst frequency and incidence. Nitric oxide (NO) has been shown to inhibit activity in brain stem regions responsible for the regulation of sympathetic outflow (DiCarlo et al., 2002, Krukoff, 1999; Mueller, 2007; Patel et al., 2001; Zanzinger, 1999). In animals, pharmacological inhibition of NO synthase (N^G-monomethyl-L-arginine; L-

NMMA) has been shown to increase renal sympathetic activity (Sakuma et al., 1992). Intra-cerebroventricular injection of L-NMMA has also been shown to increase sympathetic activity and blood pressure in rats (Sakima et al., 1998; Shankar et al., 1998). An increase in NO bioavailability has been reported following exercise-training (Sindler et al., 2009). NO bioavailability was not measured in the present study, however if NO bioavailabilty was increased in the high fit subjects, NO-mediated inhibition of central sympathetic outflow may have contributed to the lower resting MSNA in the high fit subjects. Further investigation will be required to determine the role of NO bioavailability in the regulation of MSNA in young, healthy males.

In contrast to the present findings, other studies have reported that aerobic fitness does not alter resting MSNA (Ray, 1999; Ray & Carter, 2010; Seals, 1991; Svedenhag et al., 1984). Svedenhag et al. (1984) reported that resting MSNA burst frequency and incidence were not different between endurance-trained cyclists (VO_2max = 63.5 ± 1.2 mL·kg^{-1}·min^{-1}) and untrained, age-matched controls (VO_2max = 40.1 ± 1.5 mL·kg^{-1}·min^{-1}) and that individual differences in resting MSNA were independent of aerobic fitness. Seals (1991) also reported that resting burst frequency and incidence were not different between highly-trained endurance athletes (VO_2max range = 60-75 mL·kg^{-1}·min^{-1}) and untrained subjects (had not performed regular aerobic exercise for at least 2 years). An explanation for the conflicting findings between studies is not readily apparent. However, in the present study, MSNA was measured over a wide range of aerobic fitness levels (26 mL·kg^{-1}·min^{-1} to 72 mL·kg^{-1}·min^{-1}), including a group with a mean VO_2max of 68 mL·kg^{-1}·min^{-1}. It is conceivable that aerobic fitness only alters resting MSNA at very high levels of aerobic fitness and previous studies were unable to detect differences in

resting MSNA due to the range of VO_2max values studied. To our knowledge, the present study includes the only measurements of MSNA in a group of young healthy males with a mean VO_2max above 65 mL·kg^{-1}·min^{-1}.

Previous studies that have prospectively investigated the effects of endurance exercise-training on resting MSNA have also produced conflicting results. Sinoway et al. (1996) have shown that 4 weeks of unilateral forearm exercise training increased resting burst frequency. In contrast, Grassi et al. (1994) reported that resting burst frequency and incidence were significantly reduced following ten weeks of exercise training (long-distance running, individualized intensity, 2 hours·day^{-1}, 5 days·week^{-1}). Resting MSNA has also been shown to not change following 6 weeks of one-legged cycle exercise training (Ray, 1999). Similarly, Ray and Carter (2010) recently reported that 8 weeks of aerobic exercise training did not alter resting MSNA burst frequency.

In summary, the available evidence related to the effects of aerobic fitness and exercise training on the regulation of resting sympathetic outflow is inconclusive. However, the present data suggest that young men with a very high level of aerobic fitness have reduced resting MSNA.

With respect to regulation of the cardiovascular system, resting HR and Q were lower in highly fit, young males, whereas resting SV, MAP, LBF, and leg vascular conductance were not different between groups. A decrease in resting HR with increasing aerobic fitness has been well documented (Chen & DiCarlo, 1997; Goldsmith et al., 2000; Shi et al., 1995). However, the similar SV between groups and the lower Q in the high, compared to the low and mid fitness groups, is in contrast to several previous cross-sectional studies that have reported a larger SV and similar Q in endurance-trained

and sedentary individuals (Levine et al., 1991; Nelson et al., 2010; Senitko et al., 2002; Stickland et al., 2006) and is puzzling. The Modelflow method of estimating SV and Q from the arterial pressure waveform was used in the present study (Wesseling et al., 1993). The Modelflow estimates of stroke volume are based upon a three-component model that includes the characteristic impedance of the aorta, total arterial compliance and systemic vascular resistance. Aortic impedance and total arterial compliance parameters are based upon published aortic pressure - area relationship data (Langewouters et al., 1984) and systemic vascular resistance is calculated on a beat-by beat basis. If any of the model components are altered by aerobic fitness, it seems possible that estimates of SV and Q could be inaccurate in those subjects. However, the Modelflow method has been validated against other measurement techniques and has been shown to provide a reliable estimate of SV and Q at rest and in response to a variety of perturbations in several subject populations (Dyson et al., 2010; Harms et al., 1999; Jansen et al., 2001; Matsukawa et al., 2004; Rang et al., 2007; Sugawara et al., 2003) and we are not aware of any published studies that indicate that aerobic fitness alters the accuracy of Modelflow estimates.

The similar resting MAP in all groups, despite a reduced resting MSNA and Q in high fit subjects, suggests that highly fit young adults maintain resting MAP at lower levels of sympathetic outflow and Q compared to individuals with lower levels of aerobic fitness. Charkoudian et al. (2005) reported that MSNA burst incidence was inversely related to Q and SV at rest, and argued that inputs from Q and SV influence baroreceptor regulation of sympathetic outflow. SV was not different between groups and was positively correlated ($r=0.705$; $p<0.01$) to resting MSNA burst frequency in the present

study. Whether aerobic fitness alters the relationship between MSNA and SV reported by Charkoudian et al. (2005) has not been established. Nonetheless, the decreased resting sympathetic outflow in the present study appears to be related to factors other than the magnitude of resting Q and SV. These data also suggest that the maintenance of resting MAP at lower levels of sympathetic outflow and Q may be related to differences in vascular responsiveness to sympathetic activity at rest. Consistent with that interpretation, total peripheral resistance (TPR) was significantly greater in the high, compared to the low and mid, fitness groups and was positively correlated with VO_2max in the present study. However, in contrast to TPR, femoral vascular resistance was not different between groups.

2.6.3 Sympathetic and cardiovascular response to physiological stress at rest

In the present study, the percent increase in MSNA burst frequency in response to IHG exercise and the CPT at rest was greater in the high compared to the low and mid fitness groups. The percent increase in burst incidence was greater in the high compared to low and mid fit groups during the CPT, and greater than the mid fit group, but similar to the low fit group during IHG.

Previous studies of the effect of aerobic fitness on the sympathetic nervous system response to physiological stress have reported conflicting results. A smaller increase in MSNA burst frequency in response to physiological stress has been reported in physically active or exercise-trained compared to sedentary subjects (Sinoway et al., 1996; Sinoway et al., 1992; Somers et al., 1992). In contrast, other studies have shown that the increase in burst frequency (Ray & Carter, 2010; Seals, 1991) or burst incidence (Seals, 1991) in

response to physiological or mental stress was not different between exercise-trained and sedentary subjects.

Resting MSNA burst frequency and incidence were significantly lower in the high, compared to the low and mid aerobic fitness groups in the present study, suggesting that aerobic fitness may have altered the regulation of basal sympathetic outflow. Therefore, to account for differences in resting sympathetic outflow, the response to stress was expressed as a percentage increase from resting MSNA. Conventionally, the MSNA response to stress has been expressed as an absolute change in burst frequency and/or incidence (Ray & Carter, 2010, Seals, 1991, Sinoway et al., 1996; Victor et al., 1987), however previous studies of aerobic fitness and MSNA have not reported fitness related difference in resting MSNA (Ray & Carter, 2010; Seals, 1991, Svedenhag et al., 1984).

The cardiovascular response to the CPT and IHG exercise at rest was not affected by aerobic fitness in the present study. Previous studies that have investigated the effects of aerobic fitness and/or exercise-training on the physiological response to physiological or psychological stressors have produced equivocal findings (Salmon, 2001; Sothmann et al., 1996). Consistent with the present findings, Wray et al. (2007) reported similar changes in HR, MAP and leg and arm vascular conductance in response to the CPT at rest in aerobically fit cyclists (VO_2max = 64 ± 2 $mL \cdot kg^{-1} \cdot min^{-1}$) and sedentary ($VO_2max$ = 40 ± 2 $mL \cdot kg^{-1} \cdot min^{-1}$) subjects. Wimer and Baldi (2012) also recently reported that the CPT evoked similar changes in HR, blood pressure and forearm vascular conductance in runners (at least 60 miles per week), sport rock climbers (at least 10 hours per week) and recreationally active controls (recreationally active, less than 2 hours per week).

Several previous studies have reported a blunted HR and blood pressure response to psychological or physiological stress (Bond et al., 2000; Georgiades et al., 2000; Hull et al., 1984; O'Sullivan & Bell, 2001; Sherwood et al., 1989; Throne et al., 2000; Turner et al., 1988) in aerobically fit subjects compared to sedentary controls. For example, O'Sullivan & Bell (2001) reported that HR and blood pressure responses to sustained handgrip exercise, mental stress and a CPT were attenuated in an aerobically trained group ($VO_2max = 68 \pm 3$ mL·kg^{-1}·min^{-1}) compared to subjects they characterized as "unfit" ($VO_2max = 54 \pm 2$ mL·kg^{-1}·min^{-1}).

Studies of the effect of exercise training on the cardiovascular response to sympatho-excitation have also produced conflicting results. Five weeks of moderate-intensity aerobic exercise training, which significantly increased maximal aerobic capacity, caused a blunting of the HR, blood pressure and forearm vascular conductance response to mental stress and a CPT in untrained young men (O'Sullivan & Bell, 2001). In agreement, Anshel (1996) demonstrated that 10 weeks of exercise training (30 min·day^{-1}, 3 days·week^{-1}, cycling at 170 beats·min^{-1}) attenuated both the HR and blood pressure response to a motor task (visually tracking a rotating light) when compared to controls. In contrast, Wimer and Baldi (2012) recently reported that 6-weeks of handgrip training did not alter the HR, MAP or forearm vascular conductance response to the CPT at rest.

A novel component of the present study was the simultaneous measurement of both sympathetic nervous system and cardiovascular responses to physiological stress, which allowed us to investigate the effect of aerobic fitness on the coupling between sympathetic activity and vascular responses. Sympathetic vascular transduction was

lower in the high, compared to the low and mid, aerobic fitness groups and SVT was inversely correlated with VO_2max during the CPT, suggesting that a high level of aerobic fitness was associated with a diminished vascular response to sympathetic nerve traffic. In response to IHG exercise, SVT was greater in the mid compared to the low and high fitness groups. SVT during IHG was not different from the response during CPT in the high and mid fit subjects, suggesting that neuro-vascular coupling was similar during both stressors in these subjects. In contrast, SVT was lower in the low fitness group compared to their response during the CPT. These data suggest that in the low fit subjects, neurovascular transduction may be different during IHG exercise compared to the CPT. The increase in MSNA in low fit subjects in response to IHG was markedly larger than compared to the CPT. The larger increase in MSNA during IHG in the low fit subject may be attributable to greater contributions of the muscle metaboreflex or central command to sympathetic activation. All subjects were unable to maintain the target force during the 3 minutes of IHG, indicating that effort and therefore contributions from central command may have increased throughout IHG. The low fit subjects may have been less familiar with sustained physical effort, and may have therefore had a greater increase in central command. Their lack of fitness may have also contributed to a greater local accumulation of muscle metabolites and disturbances in local acid base balance that may have activated the muscle metaboreflex to a larger degree.

Notarius et al. (2012) recently investigated the effect of fitness on neurovascular coupling in sedentary (VO_2peak = 25.8 ± 1.2 mL·kg^{-1}·min^{-1}; 49 ± 3 years) and fit (42.7 ± 2.8 mL·kg^{-1}·min^{-1}; 55 ± 4 years) middle-aged men. Despite similar resting MSNA burst frequency and femoral vascular resistance (FVR) in sedentary and fit groups,

neurovascular transduction (calculated as the slope between the increase in FVR and MSNA burst frequency) in response to graded lower body negative pressure, was reduced in fit compared to sedentary subjects. Notarius et al. (2012) suggested that the reduced neurovascular transduction may be a cardio-protective adaptation that may minimize the effects of an age-associated increase in resting MSNA on systemic vascular resistance and blood pressure in fit middle-aged men. Based upon the CPT data, the present study extends these findings to young, adult males.

Previous studies that have prospectively investigated the effects of exercise training on vascular responsiveness have reported conflicting results. A decreased responsiveness of skeletal muscle blood vessels to selective α-adrenergic receptor agonists in exercise-trained, compared to sedentary rats has been documented in several studies (Delp et al., 1993; Donato et al., 2007; Spier et al., 1999; Wiegman et al., 1981). In contrast, other studies have reported that sympathetic vascular responsiveness was increased (Jendzjowsky & DeLorey, 2012; Lash, 1998) or not different (Jasperse & Laughlin, 1999; Sun et al., 1994) in exercise-trained compared to sedentary rats. The reduced SVT in the high fit group in the present study suggests that sympathetic vascular responsiveness may decline as a function of aerobic fitness in young, healthy males. However, additional studies will be required to confirm this finding and to identify the mechanism responsible for the diminished vascular response in humans.

2.6.4 Cardiovascular response to physiological stress during exercise

Consistent with the response at rest, the cardiovascular response to physiological stress was not different between aerobic fitness groups during moderate- and heavy-

intensity exercise. Due to inherent limitations with the microneurography technique, we were unable to measure MSNA during exercise. Therefore, whether the sympathetic nervous system response to exercise, and to the CPT and IHG during exercise, was altered by aerobic fitness could not be determined, nor could sympathetic vascular transduction during exercise. The decrease in femoral vascular conductance in response to IHG and the CPT during moderate-intensity KE exercise was not different from the response at rest (i.e. no sympatholysis) in the high, mid and low fitness groups. In contrast, the decrease in FVC in response to IHG and CPT during heavy-intensity KE exercise was significantly reduced compared to the response both at rest, and during moderate-intensity exercise in the high, mid and low fitness groups. However, the magnitude of sympatholysis, calculated as the difference between the change in femoral vascular conductance in response to the CPT and IHG at rest and during exercise, was not different between aerobic fitness groups during heavy-intensity KE exercise. These data indicate that aerobic fitness does not alter the magnitude of sympatholysis in young men and that heavy-intensity exercise was required to inhibit the vascular response to sympathetic stimulation in young males.

Consistent with the present findings, Wimer and Baldi (2012) recently reported that the response of HR, MAP and forearm vascular conductance to the CPT was not different between rock climbers, runners and controls during handgrip exercise. In 2007, Wray and colleagues also reported that the CPT caused similar HR, MAP and leg vascular conductance responses in sedentary and trained subjects during KE exercise.

The magnitude of sympathetic vasoconstriction is determined by the level of efferent sympathetic nerve activity and the responsiveness of post-synaptic receptors

(Piascik et al., 1993; Ruffolo et al., 1991). We did not investigate the effect of aerobic fitness on specific post-synaptic receptor responsiveness in the present study. However, previous investigations have indicated that the relationship between exercise intensity and responsiveness may differ between α_1- and α-adrenergic and non-adrenergic receptors (Anderson & Faber, 1991; Buckwalter et al., 2001; Dinenno & Joyner, 2006; Rosenmeier et al., 2003; Thomas et al., 1994; Wray et al., 2004). Studies of isolated blood vessels from animals have shown that α_1-receptors are typically located on larger blood vessels that lie outside of the muscle, while α_2-receptors are located more distally on smaller arterioles within the muscle (Anderson & Faber, 1991; McGillivray-Anderson & Faber, 1990; Ohyanagi et al., 1990). It has been argued that this distribution of receptors may place the α_2-receptors in closer proximity to the local interstitial environment of active muscle fibers, and may make them more susceptible to inhibition (Dinenno & Joyner, 2006) at low and moderate exercise-intensities; while heavy-intensity muscle contraction may be required to inhibit α_1-receptors located outside the muscle (Anderson & Faber, 1991; Buckwalter et al., 2001). Consistent with this notion, Wray and colleagues (2004) reported that the vascular response to a selective α_2-adrenergic receptor agonist (BHT-933) was attenuated during mild, moderate- and heavy-intensity exercise in the human thigh muscle. In contrast, the vascular response to the selective α_1-adrenergic receptor agonist phenylephrine (PE) was only reduced during heavy-intensity exercise. In agreement, Buckwalter et al. (2001) demonstrated α_1-receptor responsiveness was only reduced during heavy-intensity exercise, whereas α_2-adrenegic receptor responsiveness was diminished during mild- and heavy-intensity exercise in chronically instrumented dogs.

In contrast, others have reported that moderate-intensity forearm exercise decreased α_1-receptor responsiveness to the infusion of selective agonists (Dinenno & Joyner, 2003; Rosenmeier et al., 2003).

Whether aerobic fitness alters the distribution, expression and/or responsiveness of individual post-synaptic receptors is presently unknown and will require further investigation. The similar vascular response to sympatho-excitation in all groups in the present study suggests that aerobic fitness may not alter post-synaptic receptor responsiveness in young, healthy males during exercise.

2.6.5 Conclusions

In summary, this study investigated the effect of aerobic fitness on the sympathetic nervous system and cardiovascular response to acute physiological stress at rest and during dynamic exercise in young, healthy males. At rest, the high fitness group had lower resting sympathetic outflow, an augmented sympathetic response to physiological stress and a reduced sympathetic vascular transduction compared to the low and mid fitness groups. Despite these altered sympathetic and vascular responses to stress in highly fit subjects, the overall cardiovascular response to physiological stress was not different between groups. During exercise, the cardiovascular response to physiological stress was not different between groups and a similar magnitude of sympatholysis was observed in all groups during heavy-intensity exercise. Overall, these findings indicate that aerobic fitness alters the sympathetic and vascular components of the physiological response to stress at rest, whereas the integrated cardiovascular response (i.e. the change in blood pressure and femoral vascular conductance) to stress at

rest and during exercise does not appear to be effected by aerobic fitness. Thus, these findings in young, healthy males suggest that the cardio-protective benefits associated with aerobic fitness are not mediated by an attenuated physiological response to stress.

2.7 References

Anderson KM, Faber JE. Differential sensitivity of arteriolar alpha 1- and alpha 2-adrenoceptor constriction to metabolic inhibition during rat skeletal muscle contraction. *Circ Res*. 69(1): 174-84, 1991.

Anshel MH. Effect of chronic aerobic exercise and progressive relaxation on motor performance and affect following acute stress. *Behavioral Medicine.* 21: 186-197, 1996.

Blair SN, Kohl HW 3rd, Paffenbarger RS Jr, Clark DG, Cooper KH, Gibbons LW. Physical fitness and all-cause mortality. A prospective study of healthy men and women. *JAMA.* 262: 17: 2395-2401, 1989.

Bond V Jr., Adams RG, Vaccaro P, Blakely R, Franks BD, Williams D, Obisesan TO, Millis R. Physical activity and blood pressure responsiveness to cold pressor test in normotensive young adults African-American males. *Ethnic Diseases*. 11: 217-223, 2001.

Buckwalter JB, Clifford PS. a-adrenergic vasoconstriction in active skeletal muscle during dynamic exercise. *Am J Physiol Heart Circ Physiol.* 277: H33-H39, 1999.

Buckwalter JB, Clifford PS. The paradox of sympathetic vasoconstriction in exercising skeletal muscle. *Exerc Sport Sci Rev.* 4: 159-63, 2001.

Buckwalter JB, Naik JS, Valic Z, Clifford PS. Exercise attenuates alpha-adrenergic-receptor responsiveness in skeletal muscle vasculature. *J Appl Physiol.* 90: 1: 172-178, 2001.

Charkoudian N, Joyner MJ, Johnson CP, Eisenach JH, Dietz HM, Wallin BG. Balance between cardiac output and sympathetic nerve activity in resting humans: role in arterial pressure regulation. *J Physiol*. 568.1: 315-321, 2005.

Chen C, DiCarlo SE. Endurance exercise training-induces resting bradycardia: a brief review. *Sports Med Training Rehab*. 8 (1): 37-77, 1997.

DeLorey DS, Kowalchuk JP, Heenan AP, Dumanoir GR, Paterson DH. Prior exercise speeds pulmonary O2 uptake kinetics by increases in both local muscle O2 availability and O2 utilization. *J Appl Physiol*. 103(3): 771-8, 2007.

DeLorey DS, Shaw CN, Shoemaker JK, Kowalchuk JM, Paterson DH. The effect of hypoxia on pulmonary O_2 uptake, leg blood flow and muscle deoxygenation during single-leg knee-extension exercise. *Exp Physiol.* 89: 293–302, 2004.

Delp MD. Control of skeletal muscle perfusion at the onset of dynamic exercise. *Med Sci Sports Exerc.* 31: 7: 1011-1018, 1999.

Delp MD, McAllister RM, Laughlin MH. Exercise training alters endothelium-dependent vasoreactivity of rat abdominal aorta. *J Appl Physiol* 75: 1354-1363, 1993.

DiCarlo SE, Chen CY, Collins HL. Onset of exercise increases lumbar sympathetic nerve activity in rats. *Med Sci Sports Exerc*. 28: 6: 677-684, 1996.

DiCarlo SE, Zheng H, Collins HL, Rodenbaugh DW, Patel KP. Daily exercise normalizes the number of diaphorase (NOS) positive neurons in the hypothalamus of hypertensive rats. *Brain Res*. 955: 153–60, 2002.

Dinenno FA, Joyner MJ. a-adrenergic control of skeletal muscle circulation at rest and during exercise in aging humans. *Microcirc.* 13: 329-341, 2006.

Dinenno FA, Joyner MJ. Blunted sympathetic vasoconstriction in contracting skeletal muscle of healthy humans: is nitric oxide obligatory? *J Physiol.* 533.1: 281-292, 2003.

Donato AJ, Lesniewski LA, Delp MD. Ageing and exercise training alter adrenergic vasomotor responses of rat skeletal muscle arterioles. *J Physiol.* 579: Pt 1: 115-125, 2007.

Dyson KS, Shoemaker JK, Arbeille P, Hughson RL. Modelflow estimates of cardiac output compared with Doppler ultrasound during acute changes in vascular resistance in women. *Exp Physiol.* 95: 561–568, 2010.

Georgiades A, Sherwood A, Gullette ECD, Babyak MA, Hinderliter A, Waugh R, Tweedy D, Craighead L, Bloomer R, Blumenthal JA. Effects of exercise and weight loss on mental stress-induced cardiovascular responses in individuals with high blood pressure. *Hypertension.* 35: 171-176, 2000.

Goldsmith RL, Bloomfeld DM, Rosenwinkel ET. Exercise and autonomic function. *Coronary Artery Dis*. 11: 129-35, 2000.

Grassi G, Seravalle G, Calhoun DA, Mancia G. Physical training and baroreceptor control of sympathetic nerve activity in humans. *Hypertension.* 23: 3: 294-301, 1994.

Green DJ, O'Driscoll G, Joyner MJ, Cable NT. Exercise and cardiovascular risk reduction: Time to update the rationale for exercise? *J Appl Physiol*. 105: 766-768, 2008.

Harms MP, Wesseling KH, Pott F, Jenstrup M, Van Goudoever J, Secher NH, Van Lieshout JJ. Continuous stroke volume monitoring by modelling flow from non-invasive measurement of arterial pressure in humans under orthostatic stress. *Clin Sci (Lond).* 97: 291–301, 1999.

Hill JM, Adreani CM, Kaufman MP. Muscle reflex stimulates sympathetic postganglionic efferents innervating triceps surae muscles of cats. *Am J Physiol.* 271: 1 pt 2: H38-43, 1996.

Huang A, Kaley G. Gender-specific regulation of cardiovascular function: estrogen as key player. *Microcirculation.* 11(1): 9-38, 2004.

Hull EM, Young SH, Ziegler MG. Aerobic fitness affects cardiovascular and catecholamine responses to stressors. *Psychophysiology.* 21: 353-360, 1984.

Iwase S, Mano T, Watanabe T, Saito M, Kobayashi F. Age-related changes of sympathetic outflow to muscles in humans. *J Gerontol*. 46: M1–M5, 1991.

Jansen JR, Schreuder JJ, Mulier JP, Smith NT, Settels JJ, Wesseling KH. A comparison of cardiac output derived from the arterial pressure wave against thermodilution in cardiac surgery patients. *Br J Anaesth.* 87: 212–222, 2001.

Jasperse JL, Laughlin MH. Vasomotor responses of soleus feed arteries from sedentary and exercise-trained rats. *J Appl Physiol.* 86: 441-449, 1999.

Jendzjowsky NG, DeLorey DS. Short-term exercise training augments sympathetic vasoconstrictor responsiveness and endothelium-dependent vasodilation in resting skeletal muscle. *Am J Physiol Regul Integr Comp Physiol*. Jun [Epub ahead of print], 2012.

Krantz DS, Manuck SB. Acute psychophysiological reactivity and risk of cardiovascular disease: Review and methodological critique. *Psychological Bulletin.* 96: 435-464, 1984.

Krukoff TL. Central actions of nitric oxide in regulation of autonomic functions. *Brain Res Rev.* 30: 52–65, 1999.

Kublickiene K, Luksha L. Gender and the endothelium. *Pharmacol Rep*. 60(1): 49-60, 2008.

Lakatta EG, Levy D. Arterial and Cardiac Aging: Major Shareholders in Cardiovascular Disease Enterprises: Part I: Aging Arteries: A "Set Up" for Vascular Disease. *Circulation.* 107: 1: 139-146, 2003.

Langewouters GJ, Wesseling KH, Goedhard WJ. The static elastic properties of 45 human thoracic and 20 abdominal aortas in vitro and the parameters of a new model. *J Biomech*. 17(6): 425-35, 1984.

Lash JM. Exercise training enhances adrenergic constriction and dilation in the rat spinotrapezius muscle. *J Appl Physiol.* 85: 1: 168-174, 1998.

Levine BD, Lane LD, Buckey JC, Friedman DB, Blomqvist CG. Left ventricular pressure-volume and Frank-Starling relations in endurance athletes. Implications for orthostatic tolerance and exercise performance. *Circulation.* 84 (3):1016-23, 1991.

MacPhee SL, Shoemaker JK, Paterson DH, Kowalchuk JM. Kinetics of O_2 uptake, leg blood flow, and muscle deoxygenation are slowed in the upper compared with lower region of the moderate-intensity exercise domain. *J Appl Physiol.* 99: 1822–1834, 2005.

Manuck SB. Cardiovascular reactivity in cardiovascular disease: "Once more unto the breach." *International Journal of Behavioral Medicine.* 1: 4-31, 1994.

Matsukawa K, Kobayashi T, Nakamoto T, Murata J, Komine H, Noso M. Noninvasive evaluation of cardiac output during postural change and exercise in humans: comparison between the modelflow and pulse dye-densitometry. *Jpn J Physiol*. 54: 153-160, 2004.

McEwen B. Protective and damaging effects of stress mediators. *New England Journal of Medicine.* 388: 171-179, 1998.

McGillivray-Anderson KM, Faber JE. Effects of acidosis on contraction of microvascular smooth muscle by alpha 1- and alpha 2-adrenoceptors: implications for neural and metabolic regulation. *Circ Res.* 66: 1643–1657, 1990.

Mensah GA, Mokdad AH, Ford E, Narayan KM, Giles WH, Vinicor F, Deedwania PC. Obesity, metabolic syndrome, and type 2 diabetes: emerging epidemics and their cardiovascular implications. *Cardiol Clin.* 22: 485-504, 2004.

Mora S, Cook N, Buring JE, Ridker PM, Lee IM. Physical activity and reduced risk of cardiovascular events: potential mediating mechanisms. *Circulation.* 116: 19: 2110-2118, 2007.

Mueller PJ. Exercise training and sympathetic nervous system activity: evidence for physical activity dependent neural plasticity. *Clin Exp Pharmacol Physiol*. 2007 34(4): 377-84, 2007.

Narkiewicz K, Phillips BG, Kato M, Haring D, Bieniaszewski L, Somers VK. Gender selective interaction between aging, blood pressure, and sympathetic nerve activity. *Hypertension*. 45: 522-525, 2005.

Nelson MD, Haykowsky MJ, Petersen SR, DeLorey DS, Stickland MK, Cheng-Baron J, Thompson RB. Aerobic fitness does not influence the biventricular response to whole body passive heat stress. *J Appl Physiol.* 109: 1545-1551, 2010.

Notarius CF, Murai H, Morris BL, Floras JS. Effect of fitness on reflex sympathetic neurovascular transduction in middle-aged men. *Med Sci Sports Exerc.* 44(2): 232-7, 2012.

Ohyanagi M, Faber JE, Nishigaki K. Differential activation of alpha 1- and alpha 2-adrenoceptors on microvascular smooth muscle during sympathetic nerve stimulation. *Circ Res.* 68: 232–244, 1991.

O'Hagan KP, Bell LB, Mittelstadt SW, Clifford PS. Effect of dynamic exercise on renal sympathetic nerve activity in conscious rabbits. *J Appl Physiol.* 74: 5: 2099-2104, 1993.

O'Leary DS, Robinson ED, Butler JL. Is active skeletal muscle functionally vasocontricted during dynamic exercise in conscious dogs? *Am J Physiol Heart Circ Physiol.* 272: R386-R391, 1997.

O'Sullivan SE, Bell C. Training reduces autonomic cardiovascular responses to both exercise-dependent and –independent stimuli in humans. *Autonomic Neuroscience: Basal and Clinical.* 91: 76-84, 2001.

Patel KP, Li YF, Hirooka Y. Role of nitric oxide in central sympathetic outflow. *Exp Biol Med (Maywood)*. 226(9): 814-24, 2001.

Piascik MT, Soltis EE, Piascik MM, Macmillan LB. Alpha-adrenoceptors and vascular regulation: molecular, pharmacologic and clinical correlates. *Pharmacol Ther*. 72: 215–241, 1996.

Public Healthy Agency of Canada. Physical Activity for Health: The Evidence [Online]. http://www.phac-aspc.gc.ca/cd-mc/cvd-mcv/cvdmmrf-mmmcvfr-eng.php.

Radegran G, Saltin B. Human femoral artery diameter in relation to knee extensor muscle mass, peak blood flow, and oxygen uptake. *Am J Physiol Heart Circ Physiol* 278: H162–H167, 2000.

Rang S, de Pablo Lapiedra B, van Montfrans GA, Bouma BJ, Wesseling KH, Wolf H. Modelflow: a new method for noninvasive assessment of cardiac output in pregnant women. *Am J Obstet Gynecol.* 196: e231–e238, 2007.

Ray CA. Sympathetic adaptations to one-legged training. *J Appl Physiol.* 86(5): 1583-7, 1999.

Ray CA, Carter JR. Effects of aerobic exercise training on sympathetic and renal responses to mental stress in humans. *Am J Physiol Heart Circ Physiol.* 298: H229-H234, 2010.

Remensnyder JP, Mitchell JH, Sarnoff SJ. Functional sympatholysis during muscular activity. Observations on influence of carotid sinus on oxygen uptake. *Circ Res.* 11: 370-380, 1962.

Rosenmeier JB, Dinenno FA, Fritzlar SJ, Joyner MJ. alpha1- and alpha2-adrenergic vasoconstriction is blunted in contracting human muscle. *J Physiol*. 547 (Pt 3): 971-6, 2003.

Rowell LB. *Human Cardiovascular Control.* New York: Oxford University Press, 1993.

Ruble SB, Valic Z, Buckwalter JB, Tschakovsky ME, Clifford PS. Attenuated vascular responsiveness to noradrenaline release during dynamic exercise in dogs. *J Physiol.* 541 (Pt 2): 637-644, 2002.

Ruffolo RR, Nichols AJ, Stadel JM, Hieble JP. Structure and function of alpha-adrenoceptors. *Pharmacol Rev*. 43: 475–501, 1991.

Sakima A, Teruya H, Yamazato M, Matayoshi R, Muratani H, Fukiyama K. Prolonged NOS inhibition in the brain elevates blood pressure in normotensive rats. *Am J Physiol*. 275: R410-R417, 1998.

Sakuma I, Togashi H, Yoshioka M, Saito H, Yanagida M, Tamura M, Kobayashi T, Yasuda H, Gross SS, Levi R. N G-methyl-l-arginine, an inhibitor of l-arginine-derived nitric oxide synthesis, stimulates renal sympathetic nerve activity in vivo. *Circ Res*. 70: 607-611, 1992.

Salmon P. Effects of physical exercise on anxiety, depression, and sensitivity to stress: A unifying theory. *Clin Of Psychology Review*. 21: 33-61, 2001.

Savard G, Strange S, Kiens B, Richter EA, Christensen NJ, Saltin B. Noradrenaline spillover during exercise in active versus resting skeletal muscle in man. *Acta Physiol Scand.* 131: 4: 507-515, 1987.

Savard GK, Richter EA, Strange S, Kiens B, Christensen NJ, Saltin B. Noradrenaline spillover from skeletal muscle during exercise in humans: role of muscle mass. *Am J Physiol.* 257: 6 Pt 2: H1812-8, 1989.

Seals DR. Sympathetic neural adjustments to stress in physically trained and untrained humans. *Hypertension.* 17: 36-43, 1991.

Senitko AN, Charkoudian N, Halliwill JR. Influence of endurance exercise training status and gender on postexercise hypotension. *J Appl Physiol.* 92(6): 2368-74, 2002.

Shankar R, Zhu JS, Ladd B, Henry D, Shen HQ, Baron AD. Central nervous system nitric oxide synthase activity regulates insulin secretion and insulin action. *J Clin Invest*. 102: 1403-1412, 1998.

Sherwood A, Light KC, Blumenthal JA. Effects of aerobic exercise training on hemodynamic responses during psychosocial stress in normotensive and borderline hypertensive Type A men: A preliminary report. *Psychomatic Medicine.* 51: 123-136, 1989.

Shi X, Stevens GH, Foresman BH, Stern SA, Raven PB. Autonomic nervous system control of the heart: endurance exercise training. *Med Sci Sports Exerc.* 27 (10): 1406-13, 1995.

Sindler AL, Delp MD, Reyes R, Wu G, Muller-Delp JM. Effects of ageing and exercise training on eNOS uncoupling in skeletal muscle resistance arterioles. *J Physiol*. 587(Pt 15): 3885-97, 2009.

Sinoway LI, Rea RF, Mosher TJ, Smith MB, Mark AL. Hydrogen ion concentration is not the sole determinant of muscle metaboreceptor responses in humans. *J Clin Invest.* 89: 1875-1874, 1992.

Sinoway LI, Shenberger J, Leaman G, Zelis R, Gray K, Baily R, Leuenberger U. Forearm training attenuates sympathetic responses to prolonged rhythmic forearm exercise. *J Appl Physiol.* 81: 4: 1778-1784, 1996.

Somers VK, Leo KC, Shields R, Clary M, Mark AL. Forearm endurance training attenuates sympathetic nerve response to isometric handgrip in normal humans. *J Appl Physiol.* 72: 1039-1043, 1992.

Sothmann MS, Buckworth J, Claytor RP, Cox RH, White-Welkley JE, Dishman RK. Exercise training and the cross-stressor adaptation hypothesis. *Exerc Sports Sci Rev.* 24: 267-287, 1996.

Spier SA, Laughlin MH, Delp MD. Effects of acute and chronic exercise on vasoconstrictor responsiveness of rat abdominal aorta. *J Appl Physiol.* 87(5):1752-7, 1999.

Stickland MK, Welsh RC, Petersen SR, Tyberg JV, Anderson WD, Jones RL, Taylor DA, Bouffard M, Haykowsky MJ. Does fitness level modulate the cardiovascular hemodynamic response to exercise? *J Appl Physiol.* 100: 1895-1901, 2006.

Sugawara J, Tanabe T, Miyachi M, Yamamoto K, Takahashi K, Iemitsu M, Otsuki T, Homma S, Maeda S, Ajisaka R, Matsuda M. Non-invasive assessment of cardiac output during exercise in healthy young humans: comparison between Modelflow method and Doppler echocardiography method. *Acta Physiol Scand*. 179: 361–366, 2003.

Sun D, Huang A, Koller A, Kaley G. Short-term daily exercise activity enhances endothelial NO synthesis in skeletal muscle arterioles of rats. *J Appl Physiol.* 76: 2241-2247, 1994.

Sundlof G, Wallin BG. Human muscle nerve sympathetic activity at rest: relationship to blood pressure and age. *J Physiol*. 274: 621–637, 1978.

Svedenhag J, Wallin BG, Sundlof G, Henriksson J. Skeletal muscle sympathetic activity at rest in trained and untrained subjects. *Acta Physiol Scand.* 120: 4: 499-504, 1984.

Thomas GD, Hansen J, Victor RG. ATP-sensitive potassium channels mediate contraction-induced attenuation of sympathetic vasoconstriction in rat skeletal muscle. *J Clin Invest*. 99 (11): 2602-9, 1997.

Thomas GD, Hansen J, Victor RG. Inhibition of α_2-adrenergic vasoconstriction during contraction of glycolytic, not oxidative, rat hindlimb muscle. *Am J Physiol Heart Circ Physiol.* 266: H920-929, 1994.

Throne LC, Bartholomew JB, Craig J, Farrar RP. Stress reactivity in fire fighters: An exercise intervention. *International Journal of Stress Management.*7: 235-246, 2000.

Turner JR, Carroll D, Costello M, Sims J. The effects of aerobic fitness on additional heart rates during active psychological challenge. *Journal of Psychophysiology.* 2: 91-97, 1988.

Van Guilder GP, Hoetzer GL, Dengel DR, Stauffer BL, Desouza CA. Impaired endothelial-dependent vasodilation in normotensive and normoglycemic obese adult humans. *J Cardiovasc Pharmacol.* 47: 310-313, 2006.

Victor RG, Leimbach WN Jr, Seals DR, Wallin BG, Mark AL. Effects of the cold pressor test on muscle sympathetic nerve activity in humans. *Hypertension*. 9(5): 429-36, 1987.

Victor RG, Pryor SL, Secher NH, Mitchell JH. Effects of partial neuromuscular blockade on sympathetic nerve responses to static exercise in humans. *Circ Res.* 65: 468-476, 1989.

Wesseling KH, Jansen JR, Settels JJ, Schreuder JJ. Computation of aortic flow from pressure in humans using a nonlinear, three-element model. *J Appl Physiol*. 74 (5): 2566-73, 1993.

Wiegman DL, Harris PD, Joshua IG, Miller FN. Decreased vascular sensitivity to norepinephrine following exercise training. *J Appl Physiol* 51: 282-287, 1981.

Wimer GS, Baldi JC. Limb-specific training affects exercise hyperemia but not sympathetic vasoconstriction. *Eur J Appl Physiol*. [Epub ahead of print], 2012.

Wray DW, Donato AJ, Nishiyama SK, Richardson RS. Acute sympathetic vasoconstriction at rest and during dynamic exercise in cyclists and sedentary humans. *J Appl Physiol.* 102: 2: 704-712, 2007.

Wray DW, Fadel PJ, Smith ML, Raven P, Sander M. Inhibition of alpha-adrenergic vasoconstriction in exercising human thigh muscles. *J Physiol.* 555 (Pt 2): 545-563, 2004.

Zanzinger J. Role of nitric oxide in the neural control of cardiovascular function. *Cardiovasc Res.* 43: 639–649, 1999.

CHAPTER 3: General Discussion

3.1 Main Findings

This thesis produced a number of novel findings. First, resting sympathetic nerve activity was reduced in the high fitness group, suggesting that aerobic fitness alters the fundamental control of sympathetic outflow at rest. Second, in contrast to the hypothesis, aerobic fitness did not attenuate the sympathetic nervous system response to physiological stress at rest. Instead there was a larger increase in sympathetic nerve activity in response to stress in the high, compared to low and mid fitness groups. A larger sympathetic nervous system response to stress in highly fit subjects could be interpreted as a negative physiological adaptation to aerobic fitness, however it may be a necessary consequence of an apparent down-regulation of peripheral vascular responsiveness, evidenced by the decreased sympathetic vascular transduction (SVT), in highly fit subjects. Finally, the integrated cardiovascular response to physiological stress at rest and during moderate- and heavy-intensity exercise was not different in the high, mid and low aerobic fitness groups.

In summary, this thesis demonstrated that aerobic fitness alters the sympathetic and vascular components of the physiological response to stress at rest, whereas the integrated response of these systems that produces measureable changes in blood pressure and femoral vascular conductance does not appear to be effected by aerobic fitness. Thus, these findings in young, healthy males suggest that the cardio-protective benefits associated with aerobic fitness are not mediated by an attenuated physiological response to stress.

3.2 Experimental considerations and limitations

A major strength of the present experimental approach was the inclusion of subjects with 3 different levels of aerobic fitness, including a low (33 ± 5 $mL \cdot kg^{-1} \cdot min^{-1}$), mid (51 ± 3 $mL \cdot kg^{-1} \cdot min^{-1}$) and high (68 ± 4 $mL \cdot kg^{-1} \cdot min^{-1}$) fitness group. To our knowledge the effect of aerobic fitness on the sympathetic nervous system and cardiovascular response to physiological stress has not been studied over this range of aerobic powers in previous investigations. We were also able to simultaneously investigate the sympathetic and cardiovascular response to physiological stress at rest and determine the effect of moderate- and heavy-intensity exercise on the cardiovascular response to sympathetic stimulation.

Due to technical limitations associated with the microneurography technique, it was not possible to measure muscle sympathetic nerve activity (MSNA) during dynamic knee-extension (KE) exercise, therefore MSNA measurements were only made under resting conditions. Given the fitness related differences in the MSNA response to stress under resting conditions, knowledge of the sympathetic nervous system response to stress and sympathetic vascular transduction during exercise would advance our understanding of how aerobic fitness affects the basic physiological regulation of sympathetic vasoconstriction during exercise.

The analysis of MSNA is complex and while we utilized commonly accepted criteria for burst determination, such as pulse synchronous bursts and burst voltage threshold criteria, burst selection still required confirmation by an investigator. In order to minimize variability in burst identification between subjects, all bursts were identified by the same investigator (DAR) while blinded to the subjects' identity and level of

fitness. Confirmation of burst identification was confirmed by random "spot-checking" of subject files by an investigator with several years of experience in the analysis of MSNA.

The respiratory cycle has been shown to cyclically affect MSNA due to pulmonary stretch reflexes that cause MSNA to decrease during inspiration and increase during expiration (Dempsey et al., 2002; Seals et al., 1990). Thus, the rate and depth of breathing are often controlled to minimize respiratory effects on MSNA. However, recent evidence indicates that resting MSNA burst frequency and incidence and the MSNA response to isometric handgrip (IHG) exercise or the cold-pressor test (CPT) were not different between controlled and spontaneous breathing conditions in young males (DeBeck et al. 2010). Thus, subjects were allowed to breathe spontaneously at rest and during exercise in the present study.

During the constant load exercise bouts, work was set at a relative, not absolute intensity. MSNA increases as a function of the relative, not absolute intensity of exercise and the magnitude of sympatholysis has also been shown to be sensitive to the relative intensity of exercise (Rowell, 1993). Thus, in order to investigate sympathetic vasoconstriction in subjects with markedly different aerobic capacities, it was necessary to perform exercise at the same relative intensities.

With respect to the analysis of the sympathetic and cardiovascular responses to stress at rest and during exercise, responses were analyzed as both an absolute and percentage change from baseline of each variable prior to exposure to stress. To account for potentially confounding effects of different baseline values between fitness groups at rest, and particularly during exercise, we believe that the percentage change data best

represents the physiological response. The magnitude of sympathetic vasoconstriction is commonly expressed as a percentage change in vascular conductance, as a given percentage change in the radius of a blood vessel will result in a corresponding percentage change in vascular conductance (Buckwalter & Clifford, 2001).

Finally, two distinct types of physiological stress were utilized in the present study. The CPT and IHG exercise have both been shown to produce robust, and directionally similar, sympathetic and cardiovascular responses at rest and during exercise and can be performed by subjects with little additional training or instruction (Seals, 1991). However, the afferent and efferent reflex pathways that mediate the sympathetic response differ between the CPT and IHG (Victor et al., 1987; Victor et al., 1989). The response to the CPT is mediated by type III and IV afferents that primarily respond to painful stimuli (Rowell, 1993). The IHG involves voluntary muscle contraction and the recruitment of skeletal and an increased metabolic demand. IHG exercise is associated with changes in local muscle acid base status that stimulate the muscle metaboreflex and contribute to the magnitude of the sympathetic response (Rowell, 1993). Activation of central command during the voluntary contraction may also contribute to the sympathetic nervous system response during IHG. Previous studies (Ettinger et al., 1991; Mark et al., 1985; Pryor et al., 1990; Sinoway et al., 1988; Victor et al., 1988; Victor et al., 1989) have reported that central command makes a relatively small contribution to the sympathetic response to IHG at contraction intensities of 30% MVC or lower. However, Victor and colleagues (1995) have shown that central command makes a progressively larger contribution to the increase in MSNA at contraction intensities above 30 % MVC. IHG exercise was performed at 40% MVC in

the present study. It has been argued that the contribution of central command to MSNA may be proportional to the effort associated with contraction (Mark et al., 1985; Victor et al., 1989). All subjects were unable to maintain the target force during the 3 minutes of IHG, indicating that effort and therefore contributions from central command may have increased throughout IHG. The low fit subjects may have been less familiar with sustained physical effort, and may have therefore had a greater increase in central command. Their lack of fitness may have also contributed to a greater local accumulation of muscle metabolites and disturbances in local acid base balance that may have activated the muscle metaboreflex to a larger degree.

3.3 Future directions

The present study utilized a cross-sectional study design in order to investigate the effects of aerobic fitness on the sympathetic and cardiovascular response to physiological stress. A cross-sectional design allowed us to study a wide range of aerobic capacities, however it did not allow us to control the training history or genetic makeup of the subject population. In the future, an aerobic exercise-training study, designed to improve VO_2max, would allow precise control of the training regimen and determination of the dose-response relationship between improvements in aerobic fitness and the sympathetic nervous system and cardiovascular response to stress within the same group of subjects.

3.4 Conclusion

This thesis investigated the effect of aerobic fitness on the sympathetic nervous system and cardiovascular response to acute sympatho-excitation at rest and during

dynamic exercise in young, healthy males. The present data demonstrate that resting sympathetic vascular regulation is altered as a function of aerobic fitness, with resting sympathetic outflow being lower and the sympathetic response to physiological stress being larger in high, compared to low and mid fitness young, healthy males. This study also demonstrated that aerobic fitness does not affect the cardiovascular response to stress at rest and during exercise and suggests that a reduced physiological response to stress may not be one of the mediators of the cardio-protective benefits associated with aerobic fitness.

The lack of evidence to indicate that aerobic fitness was cardio-protective in the present study was unexpected given the strong epidemiological evidence linking aerobic fitness with a decreased risk and incidence of cardiovascular disease (Blair & Jackson, 2001). However, the development of cardiovascular disease is a chronic progressive process (Lakatta & Levy, 2003) and therefore, it is possible that changes in the physiological response to stress begin to develop with advancing age and being aerobically fit may inhibit or delay the progression. It was equally surprising that the low fitness group did not have an amplified physiological response to stress or present with evidence of elevated cardiovascular disease risk as a sedentary lifestyle is a modifiable risk factor for cardiovascular disease (Lakatta & Levy, 2003). Based strictly upon the findings of this thesis it would appear that having a low level of aerobic fitness at a relatively young age was associated with very few cardiovascular consequences.

It is conceivable that the functional physiological benefit of being aerobically fit at a young age is the development of a "physiological reserve" that delays the progression of chronic disease and/or allows attainment of an older age before the progressive decline

in physiological function that accompanies aging begins to limit functional capacity. A longitudinal study that follows the subjects who participated in the present study would be fascinating and may help to further elucidate the mechanisms responsible for cardio-protective benefits associated with aerobic fitness.

3.5 References

Blair SN, Jackson AS. Physical fitness and activity as separate as heart disease risk factors: a meta-analysis. *Med Sci Sports Exerc*. 33: 5: 762-764, 2001.

Buckwalter JB, Clifford PS. The paradox of sympathetic vasoconstriction in exercising skeletal muscle. *Exerc Sport Sci Rev.* 4: 159-63, 2001.

DeBeck LD, Petersen SR, Jones KE, Stickland MK. Heart rate variability and muscle sympathetic nerve activity response to acute stress: the effect of breathing. *Am J Physiol Regul Integr Comp Physiol*. 299: R80-R91, 2010.

Dempsey JA, Sheel AW, St Croix CM, Morgan BJ. Respiratory influences on sympathetic vasomotor outflow in humans. *Respir Physiol Neurobiol.* 130: 3–20, 2002.

Ettinger E, Gray K, Whisler S, Sinoway L. Dichloroacetate reduces sympathetic nerve responses to static exercise. *Am J Physiol.* 261: H1653-H1658, 1991.

Lakatta EG, Levy D. Arterial and Cardiac Aging: Major Shareholders in Cardiovascular Disease Enterprises: Part I: Aging Arteries: A "Set Up" for Vascular Disease. *Circulation.* 107: 1: 139-146, 2003.

Mark AR, Victor R, Nerhed C, Wallin B. Microneurographic studies of the mechanisms of sympathetic nerve responses to static exercise in humans. *Circ Res.* 57: 461-469, 1985.

Pryor SL, Lewis SF, Haller RG, Bertocci LA, Victor RG. Impairment of sympathetic activation during static exercise in patients with muscle phosphorylase deficiency (McArdle's Disease). *J Clin Invest*. 85: 1444-1449, 1990.

Rowell LB. *Human Cardiovascular Control.* New York: Oxford University Press, 1993.

Seals DR. Sympathetic neural adjustments to stress in physically trained and untrained humans. *Hypertension.* 17: 36-43, 1991.

Seals DR, Suwarno NO, Dempsey JA. Influence of lung volume on sympathetic nerve discharge in normal humans. *Circ Res.* 67: 130 –141, 1990.

Sinoway LI, Prophet S, Gorman I, Moser T, Shenberger J, Dolecki M, Briggs R, Zelis R. Muscle acidosis during static exercise is associated with calf vasoconstriction. *J Appl Physiol.* 66: 429-436, 1988.

Victor RG, Bertocci LA, Pryor SL, Nunnally RL. Sympathetic nerve discharge is coupled to muscle cell pH during exercise in humans. *J Clin Invest.* 82: 1301-1305, 1988.

Victor RG, Leimbach WN Jr, Seals DR, Wallin BG, Mark AL. Effects of the cold pressor test on muscle sympathetic nerve activity in humans. *Hypertension*. 9(5): 429-36, 1987.

Victor RG, Pryor SL, Secher NH, Mitchell JH. Effects of partial neuromuscular blockade on sympathetic nerve responses to static exercise in humans. *Circ Res.* 65: 468-476, 1989.

Victor RG, Secher NH, Lyson T, Mitchell JH. Central Command Increases Muscle Sympathetic Nerve Activity During Intense Intermittent Isometric Exercise in Humans. *Circ Res.* 76: 127-131, 1995.