Sex Differences in the Impact of a 12-Week High Intensity Interval Training Intervention on Sympathetic Transduction.

by

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Dalhousie University is located in Mi'kma'ki, the
ancestral and unceded territory of the Mi'kmaq.
We are all Treaty people.

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Abstract

Peak diastolic blood pressure (DBP) responses to resting bursts of muscle sympathetic nerve activity (MSNA; sympathetic transduction) are inversely associated with aerobic fitness in young males, but not females. I tested the hypothesis that 12-weeks of high-intensity interval training (HIIT) would improve aerobic fitness in young, healthy adults, but only attenuate sympathetic transduction in males. Aerobic fitness ($\dot{V}O_2$peak, via indirect calorimetry), as well as peroneal MSNA (microneurography), and DBP (finger photoplethysmography) were recorded for ~10-min during supine rest. HIIT improved absolute $\dot{V}O_2$peak in both sexes (both, $P \leq 0.004$), with greater increases observed in males (P=0.004). There was no change in sympathetic transduction following HIIT for either sex (both, $P \geq 0.523$). However, nadir DBP responses following cardiac cycles absent of MSNA bursts were enhanced (more negative) following HIIT in females (group P=0.019, females P=0.016, males P=0.345). These results indicate that HIIT-mediated increases in aerobic fitness did not alter sympathetic transduction in a group of younger males and females.

List of Abbreviations Used

BMI = Body mass index

cAMP = Cyclic adenosine monophosphate

CVD = Cardiovascular disease

CVLM = Caudal ventrolateral medulla

DAG = Diacylglycerol

DBP = Diastolic blood pressure

HIIT = High-intensity interval training

HR = Heart rate

IP_3 = Inositol triphosphate

LVC = Leg vascular conductance

LPA = Light-intensity physical activity

MAP = Mean arterial pressure

MET = Metabolic equivalent of task

MLC = Myosin light chain

MLCK = myosin light chain kinase

MLCP = myosin light chain phosphatase

MPA = Moderate-intensity physical activity

MVPA = Moderate-to-vigorous intensity physical activity

MSNA = Muscle sympathetic nerve activity

NE = Norepinephrine

NTS = Nucleus tractus solitarius

PAP = Peak aerobic power

PIP_2 = Phosphatidylinositol 4,5-bisphosphate

RER = Respiratory exchange ratio

RM-ANOVA = Repeated measures analysis of variance

RPE = Rating of perceived exertion

RVLM = Rostral ventrolateral medulla

SBP = Systolic blood pressure

SNS = Sympathetic nervous system

TPR = Total peripheral resistance

$\dot{V}CO_2$ = Volume rate of carbon dioxide production

VPA = Vigorous-intensity physical activity

$\dot{V}O_2$ = Volume rate of oxygen consumption

$\dot{V}O_2$peak = Peak volume rate of oxygen consumption

VT = Ventilatory threshold

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Chapter 1: Introduction

Cardiovascular diseases (CVDs) are the leading cause of death worldwide (111). Females (aged 20 years and older) tend to be less physically active (53), have lower aerobic fitness (39) and spend more time sedentary (22) compared to males (114), which all contribute to an increased risk for CVD (4). It is well established that aerobic fitness and training can reduce the risk of developing CVDs (101). However, there is emerging evidence that sex differences exist in the physiological adaptations to aerobic training, with females having attenuated cardiovascular adaptations (3, 38). This difference may influence the increased risk of cardiovascular disease observed in females as they age High-intensity interval training (HIIT) may be an alternative approach to addressing these sex differences as it has been documented that females may show greater improvements in aerobic fitness compared to males (142).

One mechanism that is associated with increased CVDs risk is elevated activity of the sympathetic nervous system (97). Sympathetic activity directed towards skeletal muscle arterioles (i.e., MSNA) causes vasoconstriction, which increases vascular resistance and blood pressure (85). In humans, integrated multi-unit MSNA can be measured directly using the microneurography technique (161). 'Bursts' of MSNA are identified from the mean neurogram, and quantified as burst frequency (bursts/min), burst incidence (bursts/100 heartbeats) and/or total activity (64). Although time-averaged MSNA outcomes provide important information regarding overall sympathetic activity levels, more dynamic regulatory information can be gleaned by concurrently assessing the corresponding vascular and/or hemodynamic responses to this activity (i.e., sympathetic transduction). Sympathetic transduction can be defined as the study of

vascular (e.g., peripheral vascular resistance) and/or systemic hemodynamic (e.g., blood pressure) responses to MSNA. Specifically, sympathetic transduction has been quantified as peak increases in mean arterial pressure (MAP) (34, 61, 120, 122, 125, 135, 163), or diastolic pressure (DBP) (18), as well as nadir decreases in vascular conductance (47, 48, 135) following spontaneous bursts of MSNA.

Research surrounding sex differences in sympathetic transduction is mixed. Briefly, some studies have observed no sex difference in peak MAP increases following spontaneous bursts of MSNA in young healthy adults (34, 163). Another study observed no sex differences in spontaneous sympathetic transduction when quantified as nadir leg vascular conductance (LVC) responses following MSNA bursts in young healthy adults (71). In addition, one study observed no sex differences in the peak MAP or nadir LVC response to spontaneous bursts of MSNA in young healthy adults (135). However, this study showed an inverse relationship between resting MSNA (burst incidence) with peak MAP and nadir LVC in males, but not females (135). Contrary to the studies listed above, a study conducted by Briant and colleagues (18) observed that young males had higher resting sympathetic transduction compared to young females. However, this was the only study (18) to use a regression-slope method of quantifying sympathetic transduction (versus the signal-averaged approach), which involves performing linear regression analysis on binned MSNA burst areas versus DBP. Overall, the lack of a sex difference observed using the signal-averaging approach versus the observation reported by Briant and colleagues (18) suggests that there may be a methodological difference involved with the interpretation of sex differences. However, a study previously conducted in our lab

observed no sex differences in sympathetic transduction when quantified using either the regression-slope or signal-averaging approach (123) in younger adults.

Sympathetic transduction can also differ with aerobic fitness level, which may be linked with the beneficial role that having greater cardiorespiratory fitness has on CVD risk. Specifically, lower aerobic fitness is associated with higher sympathetic transduction to blood pressure in older adults (122) and younger males (120). In addition, in a study that investigated the relationship between aerobic fitness and sympathetic transduction (assessed through the relationship between MSNA burst frequency and forearm vascular resistance responses to a graded lower body negative pressure protocol) found that a group of 'more aerobically fit' middle aged men had lower transduction compared to their 'unfit' counterparts (111). In contrast, a pilot study conducted in young females reported similar sympathetic transduction between habitual exercisers (i.e., higher aerobic fitness) compared to their non-exercising counterparts (152). These sex differences regarding the association between aerobic fitness and sympathetic transduction may be due to the beneficial impact of estrogen on vasodilation, potentially attenuating the impact of aerobic fitness on sympathetic transduction in females (30, 63, 152). The physiological mechanisms behind potential sex (49, 62, 94, 133)- and/or aerobic fitness (86) -based sympathetic transduction differences are potentially likely at the vascular level with increased β_2-adrenoreceptor-mediated vasodilation (49, 62, 86, 94, 133) and/or attenuated α-adrenoreceptor-mediated vasoconstriction (51, 86) reported in females. However, the cross-sectional nature of the above reports is a limitation. To date, the impact of an aerobic training intervention on sympathetic transduction in healthy young adults is unknown.

One mode of exercise that has been established to improve aerobic fitness is HIIT, which can be characterized by alternating short bursts of high-intensity exercise that typically reach ≥85% peak HR (heart rate), with recovery periods of rest or light exercise typically carried out at ≤70% peak HR (18). HIIT has been proven to increase cardiorespiratory (99) and skeletal muscle oxidative capacity (56). As well, short-term HIIT elicits improvements in aerobic fitness (117) and greater vascular adaptations compared to moderate-intensity continuous training (131) and resistance training (117). The high-volume HIIT protocol that was adapted in the current study increased aerobic fitness and improved vascular endothelial function after 6 weeks in older adults (117). In contrast, the same protocol did not improve aerobic fitness, but did increase peak aerobic power (PAP), in younger adults (102). Therefore, a greater training volume, including a longer intervention period, with progressive increases in duration and intensity may be required to improve aerobic fitness in a younger cohort.

The purpose of this study was to test the hypotheses that: 1) a 12-week HIIT intervention will improve aerobic fitness in young males and females, and 2) potential HIIT-mediated increases in aerobic fitness will decrease sympathetic transduction in young healthy males (120), but have no effect in young healthy females (152). Lastly, I tested the hypothesis that there would be no sex difference in sympathetic transduction regardless of the assessment method used [i.e., signal averaging (61) versus regression-based approach (18)] as our group has previously demonstrated.

Chapter 2: Literature Review

2.1: Aerobic Fitness and Training on Cardiovascular Health

2.1.1: Impact of Aerobic Fitness on Cardiovascular Health

It has been well established that having higher aerobic fitness reduces the risk of CVDs (109), the number one leading cause of death worldwide (111). As well, aerobic fitness is a strong predictor of CVD-specific mortality in healthy young adults (21, 50, 110, 124). As such, it is important to establish exercise interventions that can improve aerobic fitness to potentially reduce risk of CVD. Research has shown that having higher blood pressure as a younger adult (177) can greatly increase the risk of developing CVDs later in life. Therefore, developing training interventions to improve aerobic fitness as a young adult can help to mitigate this risk early.

Cardiovascular disease risk has been reported to be influenced by sex. Specifically, it has been shown that there are more females currently living with CVD than males (4). However, this may be due to a larger proportion of the older adult population consisting of females due to their longer life expectancy (107). Interestingly, premenopausal females tend to have a lower incidence of CVD compared to age-matched males (77, 175). This change in CVD risk may be due to the decline of estrogen (a known protectant against CVD) that come with menopause in females (77). To prevent the increase in CVD risk that comes with aging, especially in females, it is important to investigate other known factors to influence, such as aerobic fitness.

Sex differences in aerobic fitness may contribute to CVD susceptibility. In general, females have lower aerobic fitness compared to males, even when normalized for their smaller body size (39, 126). This may influence their CVD risk as it is well established that individuals with higher aerobic fitness have a lower risk of developing

CVD (90). To summarize, research should investigate the best methods to improve aerobic fitness to prevent the risk of developing CVDs.

Maximal exertion exercise tests that incorporate a large percentage of active muscle mass (e.g., treadmill and cycle ergometry) and collect respiratory gas exchange outcomes are considered the gold standard assessments of aerobic fitness (50, 110). The primary criterion variable is the maximum ($\dot{V}O_2max$) or peak volume rate of oxygen consumption ($\dot{V}O_2peak$), which is a reliable and reproducible outcome (55, 153). In addition, the ventilatory threshold (VT), defined as the point during a $\dot{V}O_2max$ test where minute ventilation increases non-linearly despite a linear increase in workload (54), provides another important index of cardiorespiratory capacity (55). As well, having a lower exercise capacity (i.e., peak metabolic equivalents of task, METs) was the stronger predictor of death among males compared to other established CVD risk factors (110). To further describe exercise capacity, 1 MET is defined as the basal rate of oxygen consumption (~3.5 mL/kg/min) (127). The reduction in mortality risk (per 1-MET increase in exercise capacity) can be ~15% in young healthy males (14) and ~25% females (106). All methods of quantifying aerobic fitness discussed above can be used to evaluate the relationship to CVD risk.

2.1.2: Impact of Aerobic Training on Cardiovascular Health

According to the Canadian 24-Hour Movement guidelines, adults should accumulate ≥150 minutes of moderate-to-vigorous physical activity (MVPA) per week (137). Increasing physical activity has been reported to reduce CVD risk (105). One way Canadians can achieve this recommendation is via aerobic exercise training, which can improve cardiovascular health through reductions in resting blood pressure (44, 83). A

meta-analysis examining randomized controlled aerobic exercise interventions (n=54) observed a negative association between aerobic exercise and systolic (SBP) and diastolic blood pressure (DBP) in healthy adults (mean age, 21 to 79 years), which may be related to improved resistance vessel (e.g., the arterioles) function (171).

Resistance vessels are important regulators of arterial pressure and blood flow to tissues and organs (85) and will be discussed in more detail later in the document. A recent study investigated the influence of 6-weeks of HIIT on resistance vessel health through measuring microvascular blood flow (68). This randomized controlled trial consisted of 25 healthy older adults (13 post-menopausal females). Skeletal muscle microvascular blood flow was recorded from the m. vastus lateralis using contrast enhanced ultrasound at rest and in response to a single bout of resistance (isometric leg extensions) exercise before and after a 6-week HIIT intervention or after 6-weeks of no-intervention activity (Control group). The HIIT intervention consisted of five, 1-min high-intensity intensity cycling bouts with 1.5-min active recovery periods, 3 days per week (68). This study concluded that engaging in HIIT elicited increases in microvascular blood flow responses to muscle contractions (68). Badrov and colleagues (8) assessed forearm resistance vessel endothelial function in 32 healthy young females following resistance training. Participants were divided into 3 groups, an isometric handgrip training group that trained 3 (group 1) or 5 (group 2) days per week, as well as in a non-trained control group (group 3) (8). The isometric handgrip exercise consisted of four, 2-min unilateral contractions using a programmed handgrip dynamometer at 30% of their maximum with a 4-min recovery period (8). Resistance vessel endothelial function was measured as the hyperemic response to a period of distal ischemia in the brachial

artery (8). This study reported that that isometric handgrip training increased upper limb resistance vessel function independent of training dose in normotensive females (8). Both studies highlighted above provided evidence regarding the impact of exercise training on resistance vessel function, an important component of blood pressure regulation, which will be discussed later in this document.

2.1.3: Vascular Adaptations to High-Intensity Interval Training

High-intensity interval training, which is characterized by alternating short bursts of high-intensity exercise (≥85% peak HR) with recovery periods of rest (passive recovery) or light-intensity exercise (≤70% peak HR), is an established method of improving aerobic fitness (19). Compared to moderate-intensity continuous training, HIIT elicits greater improvements in $\dot{V}O_2$peak and vascular function (i.e., brachial artery flow-mediated dilation) in adults (131). Specifically, HIIT has been reported to reduce peripheral arterial stiffness in hypertensive (n=65, 42♀) (59) and obese adults (n=18, 9♀) (141), as well as promote mitochondrial biogenesis in healthy young males (n=17) (95). This evidence supports a beneficial impact of HIIT for improving vascular function and aerobic fitness.

Many different HIIT protocols have been reported that vary with respect to bout duration, intensity, and intervention length, which may cause differential physiologic adaptations as highlighted by the studies listed below. A 12-week HIIT protocol that consisted of 10 × 1-min intervals at ~90% PAP (attained during pretraining exercise test) interspersed with 1-min of active recovery (10% of PAP), increased brachial artery endothelial function in coronary artery disease patients (n=22, 2♀) (37). Another protocol consisting of 8-s high cadence cycling bouts (120 revolutions per minute) with 12-s

slower paced recovery intervals (40 revolutions per minute), both at an intensity of 80-90% of their peak HR, decreased arterial stiffness, SBP and DBP after 12 weeks of training (n=38, all♂) (69). A third, high-volume HIIT protocol, involved 2 sets of forty, 15-s intervals that alternated between cycling at 100% PAP separated by 15-s passive recovery periods, with a 5-min passive recovery period between each set (n=88, 44 postmenopausal ♀) (60, 103). This HIIT protocol increased the duration of training from 40 to 45 minutes over the course of a 6-week intervention period to account for anticipated aerobic fitness improvements (103). This HIIT protocol improved both aerobic fitness and endothelial function in older adults. Importantly, this protocol was also used in a group of young adults that attained improvements in peak power output following 6-weeks of HIIT (n=25, 18♀) (102). For the current study, I will be adapting this protocol by increasing the intervention duration to 12-weeks from 6-weeks, and raising the intensity throughout the protocol (Section 3.3.1) with the goal of increasing $\dot{V}O_2$peak, as well as PAP following training. One of the potential mechanisms by which HIIT may improve peripheral vascular function is through attenuating sympathetic vasoconstrictor regulation of resistance vessels (i.e., reduced sympathetic vascular transduction), which will be discussed later in this literature review.

2.1.4: Sex Differences in the Adaptations to HIIT

Although HIIT is a well-established method of improving aerobic fitness in young healthy adults (170), it is unclear whether adaptations differ between sexes and/or vary depending on the specific protocol used. One study tested whether training performance adaptations (e.g., average running speed and speed decrement throughout a performance test) following 4-weeks of HIIT (4 × 30-s all-out running) with different rest durations

(30-s or 180-s between intervals), differed between moderately trained males and females (142). Schmitz and colleagues (142) observed that females had greater performance improvements following the 30-s versus 180-s rest intervals, whereas males did not improve with either protocol. The results of this study suggest that young females adapted better to HIIT than males and that females responded better when there were shorter rest intervals (142). In contrast, another study observed no sex differences in cycling performance (an incremental cycle trial to fatigue and a 40-km cycling trial) following 10 HIIT sessions in young endurance-trained adults (73). Specifically, participants completed 10 × 90-s cycling intervals at an intensity equivalent to the final output achieved during the incremental trial, separated by 60-s of low-intensity active recovery periods (73). Although no sex differences were observed in the post-HIIT 40-km time trial, both groups demonstrated improvements following the 10 training sessions (73). Contrary to both of these previous reports, a study conducted by Weber et al. (167) observed greater aerobic fitness improvements ($\dot{V}O_2$peak) in younger males following 8-weeks of HIIT. Specifically, healthy young adults performed 3 × 2-min of constant-load cycling intervals (83-100% PAP, with 6 minutes of recovery between intervals), 3 days per week (167). Interestingly, while $\dot{V}O_2$peak increased in males, there were no aerobic fitness improvement observed in females (167). Overall, research investigating the effect of sex in the adaptations to HIIT is mixed and there is a need to investigate potential sex differences in the adaptations to additional HIIT protocols. Whether HIIT-induced increases in aerobic fitness alter sympathetic regulation of blood pressure in young healthy adults is unknown and may uncover the mechanisms for the effect of sex.

2.2: Sympathetic Nervous System

2.2.1: Anatomy of Sympathetic Nervous System

The sympathetic nervous system (SNS) is one division of the autonomic nervous system responsible for effective cardiovascular regulation (127). The SNS is composed of pre-ganglionic neurons located in the thoracic and lumbar regions of the spinal cord and post-ganglionic neurons innervating the heart and the vasculature (Figure 2.1) (127). One important function of the SNS is to regulate DBP and MAP via constriction of resistance vessels, which will be discussed in more detail later in this chapter.

A key regulator of SNS activity is the arterial baroreflex. This negative feedback system is responsible for detecting changes in transmural pressure (i.e., the pressure difference between the inside and outside of a vessel) via baroreceptors located within the carotid artery sinuses and aortic arch via proportional changes in the amount of stretch that occurs in these arteries (168). With greater stretch/pressure, baroreceptors send more frequent action potentials via the carotid sinus nerves (a branch of the glossopharyngeal nerve) and aortic depressor nerve (a branch of the vagus nerve) to an integrating center in the brainstem. Specifically, the baroreceptor afferent signals provide an excitatory synapse on the nucleus tractus solitarius (NTS) in the medulla through the release of glutamate (168). The NTS serves as the key integrating site for baroreceptor input and relays this information to other autonomic regulatory nuclei to control sympathetic and parasympathetic efferent outflow to the heart and blood vessels (sympathetic only) (168). However, this project will be focused on the interaction of sympathetic nerve signals to resistance vessels located within skeletal muscle beds of the lower limb (see section 2.2.2

below for information on microneurographic recordings of sympathetic vasoconstrictor nerve activity). As such, autonomic control of the heart will not be discussed further.

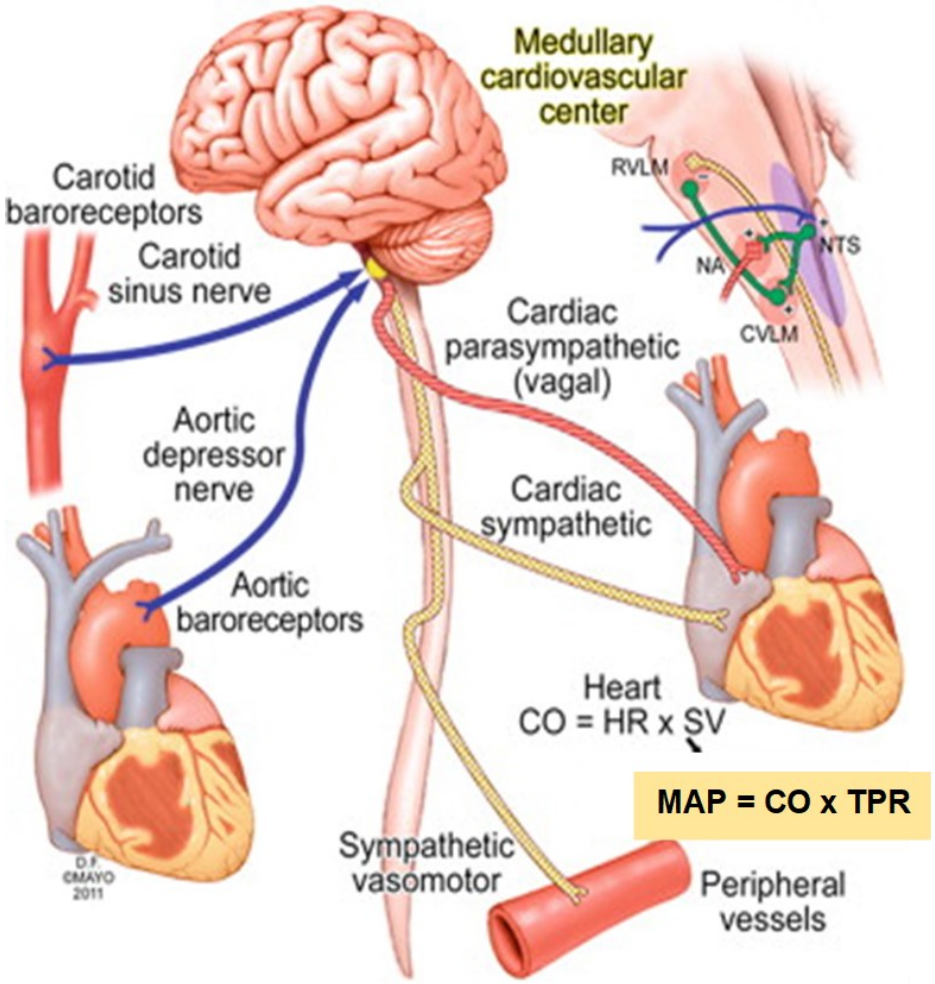

Figure 2.1 Overview of the functional anatomy and central neural integration of arterial baroreflex control of autonomic outflow to the cardiovascular system. Baroreceptors located in the aortic arch and carotid sinuses detect changes in transmural pressure via corresponding alterations in the magnitude of vessel wall stretch. Increased transmural pressure (and stretch) result in more frequent action potentials transmitted via the carotid sinus and aortic depressor afferent nerves to an integration center in the brainstem. Specifically, baroreceptor afferent nerves provide excitatory synapses with the nucleus tractus solitarius (NTS). The NTS then sends excitatory connections to the nucleus ambiguus (NA, ↑ parasympathetic activity to the heart) and the caudal ventrolateral medulla (CVLM). The CVLM inhibits the rostral ventrolateral medulla (RVLM), which represents the origin of efferent sympathetic activity. Efferent sympathetic outflow then travels to the intermediolateral cell column of the spinal cord (thoracic and lumber regions), through the sympathetic ganglia and onto the heart and blood vessels. Elevated sympathetic activity increases heart rate (HR), stroke volume (SV), cardiac output (CO), and total peripheral resistance (TPR), which promotes a corresponding increase in mean arterial pressure (MAP) (168).

Following the excitatory synapse to the NTS from baroreceptor afferent nerve fibres, the NTS activates neurons in the caudal ventrolateral medulla (CVLM), which directly inhibits neuron cell bodies in the rostral ventrolateral medulla (RVLM). This is important as the RVLM represents the origin of sympathetic efferent activity. As such, inhibition of sympathoexcitatory neurons in the RVLM reduces sympathetic vasoconstrictor nerve activity, peripheral vascular resistance and MAP (168). Once activated, the RVLM sends excitatory signals to the intermediolateral cell column in the thoracic and lumbar regions of the spinal cord. Sympathetic pre-ganglionic neurons from the intermediolateral cell column release acetylcholine to excite cell bodies in the sympathetic chain ganglia, which carry the post-ganglionic sympathetic vasoconstrictor signal to the blood vessels. The section of the vascular system with the greatest innervation density of sympathetic nerve fibres, and thus has the most significant impact on changes in total peripheral resistance (TPR) are the arterioles (85), which will represent the main sympathetic neurovascular regulatory focus of this project.

The efferent post-ganglionic sympathetic axons innervate blood vessels (i.e., resistance vessels) in the outermost layer (i.e., the *tunica adventitia* or *externa*) (85). At this neurovascular junction, neurotransmitters [mainly norepinephrine (NE)] are released from vesicles within the axon terminal through varicosities on the nerve fiber (85). Norepinephrine then binds to α_1- or α_2-adrenoreceptors on vascular smooth muscle cells, which causes them to contract (i.e., vasoconstriction) (85). Under conditions of heightened sympathetic vasoconstrictor activity (and NE accumulation in the neurovascular space), NE can bind to prejunctional α_2-adrenoreceptors on the sympathetic nerve terminals, which can inhibit additional NE release via a negative

feedback mechanism (85). In addition to NE, the co-transmitters adenosine triphosphate and neuropeptide Y may also be released from the post-ganglionic sympathetic nerves to cause further vasoconstriction via binding to purinergic- and Y_1-receptors, respectively (85).

Furthermore, circulating catecholamines as well as catecholamines released from the sympathetic neurons (NE and epinephrine) may also bind to luminal facing (i.e., towards the inside of the blood vessel) β_2-adrenoreceptors on the vascular smooth muscle cell (85). However, unlike the vasoconstricting effect of NE released onto adrenergic receptors from post-ganglionic sympathetic nerves, vascular smooth muscle cells relax (i.e., cause vasodilation) when plasma catecholamines (greater affinity for epinephrine than NE) bind to these β_2-adrenoreceptors (85). Sex differences in the relative impact of β_2-adrenoreceptor-mediated vasodilation have been observed (94) and will be expanded upon later in this section.

2.2.2: Microneurographic Recordings of Efferent Sympathetic Outflow in Humans

Quantifying sympathetic activity is a useful tool to help identify key neurovascular control mechanisms. Microneurography is a well-established technique that has been used to quantify the sympathetic signal that reaches vascular beds. In 1967, microneurography was established to record human peripheral sympathetic neural recordings to both muscle and skin vascular beds (23, 162). MSNA refers to post-ganglionic sympathetic vasoconstrictor activity directed towards the vascular smooth muscle cells located with the resistance vessels of skeletal muscles (172) and will be the focus of this project.

Microneurography involves the percutaneous insertion of tungsten microelectrodes into a post-ganglionic nerve bundle of a superficial nerve (e.g., common peroneal) (64). To localize placement of the recording microelectrode, mild surface electrical stimulation of the target nerve is performed until visual cues such as mild muscle 'twitches' are observed. Tungsten was the metal of choice for the microelectrodes because of its high conductance and thin, nonbrittle properties (64). In addition to the recording (or active) microelectrode, an uninsulated reference microelectrode is inserted percutaneously (i.e., beneath the skin) in close proximity (~2-3 cm) (Figure 2.2). The role of the reference electrode is to detect external sources of electrical noise from the participant and/or environmental surroundings (64).

The active electrode measures activity in the nerve fiber and has an epoxy coating along the shaft, prior to the microelectrode tip, which creates a high impedance to ensure the recording of a discrete population of individual sympathetic axons (64). The length of shaft covered with the epoxy coating can be altered to allow for a range of recording areas. Specifically, the further the coating extends along the shaft (i.e., smaller recording area at the tip), the higher the impedance and smaller the population of individual neurons that action potentials can be recorded from. Both active and reference microelectrodes are attached to a 1000× gain differential amplifier (Figure 2.2), which provides an initial method of removing external noise from the signal (i.e., removes signal recorded from the reference electrode). The signal from the differential amplifier is routed to a separate variable gain amplifier, which further amplifies the signal (another ~75 times for a total amplification of ~75 000×) (64). This raw signal is sampled at 20 kHz and sent through a band-pass filter (500-2000 Hz) before it is integrated (0.1s time

constant) and displayed as a mean voltage neurogram (Figure 2.3) (Nerve Traffic Analyzer; Univ. of Iowa Bioengineering, Iowa City, IA) (64). From the mean neurogram, the microneurographer can identify multi-unit (i.e., integrated activity of numerous individual sympathetic neurons) 'bursts' of MSNA (Figure 2.3) (64).

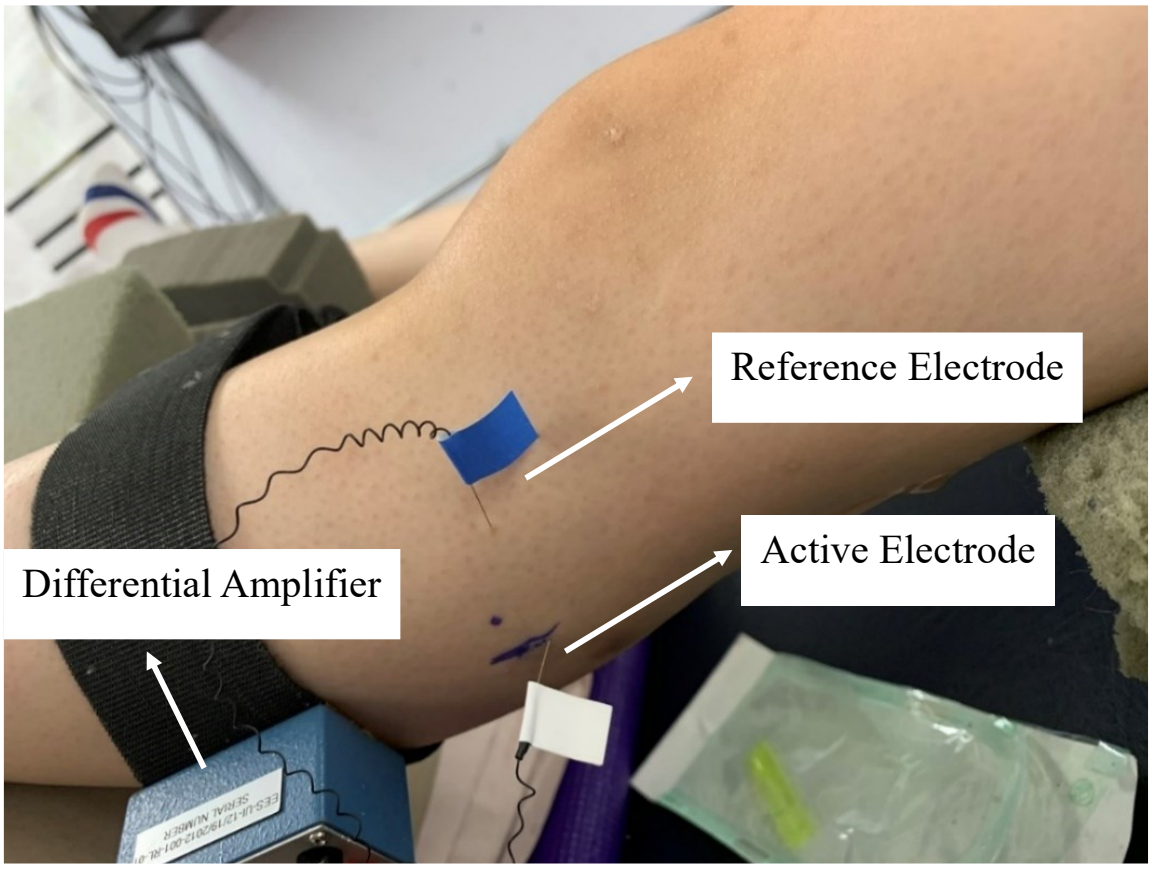

Figure 2.2 Microneurography setup for the left common peroneal nerve recording of muscle sympathetic nerve activity. This includes: the uninsulated reference electrode (blue flag), the epoxy-coated active electrode (white flag) and the differential amplifier secured below the knee. Pen marks at and above the active electrode indicate the head of the fibula (dot) and where the peroneal muscle contractions were elicited using mild surface electrical stimulation to accurately locate the common peroneal nerve (line).

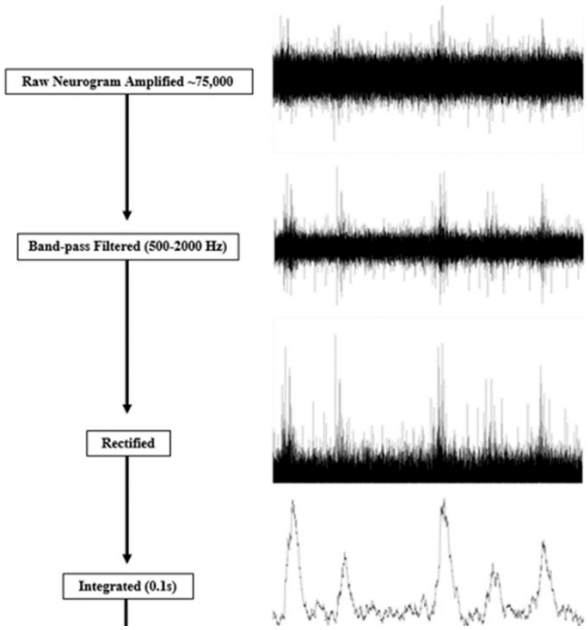

Figure 2.3 Overview of the signal processing steps involved with creation of the mean voltage neurogram from the raw muscle sympathetic nerve activity (MSNA) recordings. Top panel: Raw signal recorded from the active microelectrode amplified 75,000 times. Second panel: amplified signal is band-filtered (500-2000 Hz). Third panel: Full-wave rectified signal (eliminating data below baseline). Forth panel: Signal is integrated over a 0.1s period to produce the mean voltage neurogram. Bottom panel: Identified bursts of MSNA are shifted backwards to align with the cardiac cycle that it was generated.

To determine a successful MSNA recording, the mean neurogram is inspected for cardiac synchronicity (i.e., the burst peaks occur ~1.2-1.5 seconds following the R-wave on the electrocardiogram associated with the heartbeat it was generated) (46). This is because the MSNA bursts are entrained to the cardiac cycle (i.e., the via the baroreflex. Specifically, the initiation of an MSNA burst is linked to diastole (i.e., when the heart is relaxed and within beat blood pressure at a nadir as baroreceptor afferent innervation to the NTS is lowest during this phase of the cardiac cycle. This lessens the inhibitory effects of the CVLM on the RVLM, which increases sympathetic outflow. The subsequent systolic pulse increases baroreceptor afferent activity, resulting in a greater stimulation of the NTS-CVLM axis and subsequent inhibition of the RVLM. This corresponds to a decrease in sympathetic outflow and the downward slope of an MSNA

burst (Figure 2.3). Both burst strength (i.e., amplitude) and height of the individual being recorded from impact this baroreflex latency. Taller individuals have a longer transmission delay between the RVLM and the active recording electrode in the lower leg (46). Larger MSNA bursts have shorter latencies as they are associated with the recruitment of faster conducting post-ganglionic sympathetic neurons (139). Furthermore, increases in MSNA are elicited in response to a voluntary end-expiratory apnea (i.e., chemoreflex stimuli), and/or Valsalva's maneuver (i.e., baroreflex stimuli) (64). These tests are commonly performed to confirm that MSNA is being recorded. Another method to confirm that an MSNA signal is being recorded involves the use of a startle stimulus (such as yelling or clapping), which will cause an increase in skin sympathetic nerve activity only. After a successful MSNA recording (i.e., all the above criteria are met), bursts are identified from the mean voltage neurogram if they contain amplitudes that have a minimum 3:1 signal-to-noise ratio (i.e., the peak of the burst is at least 3 times higher than the average amplitude of the interburst noise) (64).

Time-averaged MSNA outcomes include burst frequency (bursts/min) and burst incidence (bursts/100 heart beats) (64). Burst frequency reflects the average MSNA that the downstream vascular smooth muscle cells are exposed to (64). Burst incidence accounts for how often the MSNA occurs in relation to the available number of heart beats (i.e., individuals with a faster HR have more 'opportunities' for a burst to occur due to the pulse synchronous nature of the signal) (64). The strength of a burst may also be quantified using the average amplitude (or area) of all recorded bursts (64). When analyzing an integrated neurogram, identifying a non-burst baseline is important for the quantification of bursts of MSNA (172). This can be done by finding a non-bursting

period, calculating its mean voltage, and resetting the voltage to 0 arbitrary units (172). By doing this, any activity that deviates from baseline will be represent a deviation from the background noise (172). To control for variations in burst amplitude between subjects or recordings sessions (angle, deepness, and location of the electrode in the nerve fascicle) bursts are normalized to the largest burst in the neurogram, which is assigned a value of 100% (31, 64). The rest of the bursts can then be normalized as a percentage of the peak burst height. Finally, total MSNA can be calculated as the product of burst frequency and the mean normalized (or relative) burst amplitude.

Muscle sympathetic nerve activity burst incidence and frequency may differ between the sexes, with MSNA burst frequency and incidence typically being higher in younger males (18, 63, 72, 82, 113, 135). The literature examining the relationship between aerobic fitness and MSNA is limited and therefore difficult to draw firm conclusions. However, the existing literature may point to potential sex differences. Specifically, in young males, there no relationship was observed between aerobic fitness and MSNA burst incidence or frequency (120). For young females, there is evidence of both an inverse relationship (10) and no relationship (152) between aerobic fitness and MSNA. Although resting MSNA provides us with an index regarding the amount of sympathetic activity reaching resistance vessels, the vascular response to MSNA may provide us with useful information concerning potential sex differences in blood pressure regulation.

2.2.3: Sympathetic Vascular Control

Muscle sympathetic nerve activity impacts blood pressure regulation primarily through vasoconstriction, or contraction of vascular smooth muscle cells in the arterioles.

Resistance vessels provide approximately 80% of total resistance to systemic blood flow, due to the relatively high percentage of vascular smooth muscle cells contained within, compared to other vessels in the vascular tree (e.g., conduit arteries, veins, venules) (98). Arteries and arterioles are composed of 3 distinct layers: the intima, media, and adventitia (Figure 2.4). The intima acts as a physical barrier between blood components and extravascular tissue, and predominantly consists of endothelial cells and an abluminal basement membrane (98). Endothelial cells are arranged longitudinally in the direction of flow with a thickness of 0.2 to 0.5 μm and are responsible for the production of vasoactive compounds that will be discussed later in this section (98). The function of the basement membrane under the endothelial cells is to provide anchoring support (98). The media layer consists primarily of vascular smooth muscle cells and an internal elastic lamina (Figure 2.4) (98). The adventitial layer consists of fibroblasts imbedded in an extracellular matrix made of thick bundles of collagen fibres (98) (Figure 2.4). This layer provides structural support for the vessel, as well as a scaffold for anchoring sympathetic nerve endings (98).

The vascular smooth muscle in an arteriole is typically 5 to 10μm in diameter and can vary from 50 to 300μm in length (85). When the vascular smooth muscle contracts, they create a cross-bridge where actin filaments overlap the thin filaments. When this occurs, the VSM cell diameter increases, decreasing the lumen diameter (85).

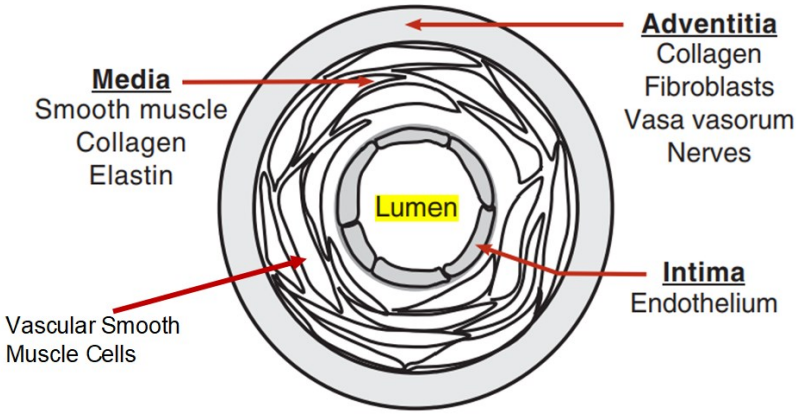

Figure 2.4. The 3 anatomical layers of an artery or arteriole surrounding the lumen: the adventitia (or externa), media, and intima (85). The adventitia consists of collagen fibroblasts, *vasa vasorum* (small vessels that provide blood to the arteriole), and sympathetic nerve endings. The media consists of vascular smooth muscle cells, collagen, and elastin. The most medial layer is the intima, which consists of a monolayer of endothelial cells. All layers surround the lumen, which is where blood flow occurs.

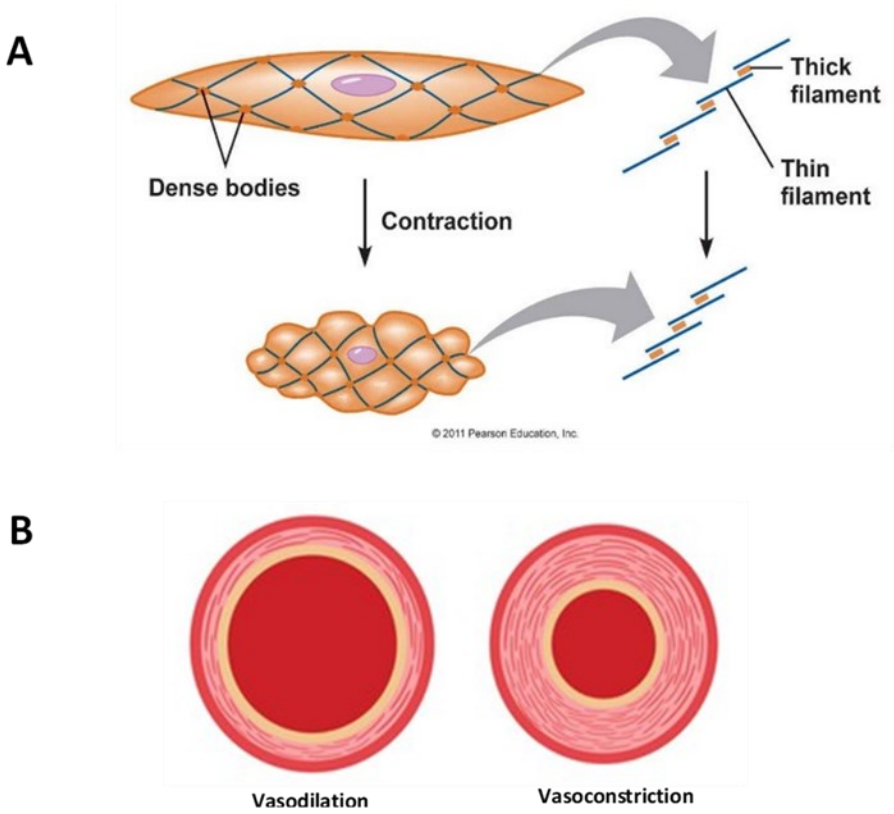

Figure 2.5 A) Contraction of a vascular smooth muscle cell. Shortening of cross-bridges cause "puffing" of the vascular smooth muscle, which encroaches into and reduces the diameter of the lumen, which increases the resistance to blood flow and blood pressure. B) Cross sectional representation of an artery or arteriole during vascular smooth muscle relaxation (vasodilation) and contraction (vasoconstriction) (85).

The sequential mechanisms by which MSNA elicits vasoconstriction of the vascular smooth muscle cells may provide insight into understanding sex differences (or the impact of HIIT) on sympathetic neurovascular control. The first step leading to vasoconstriction is an increase in the intracellular concentration of calcium of vascular smooth muscle cells. Depolarization of vascular smooth muscle cells causes opening of voltage-dependent calcium channels (L-type), which increase the influx of calcium from the extracellular space (Figure 2.6). Calcium can also be released from intracellular stores within the sarcoplasmic reticulum through either: 1) calcium-induced-calcium release or 2) the degradation of phosphatidylinositol 4,5-bisphosphate (PIP_2, a component of the phospholipid bilayer) into the second messengers diacylglycerol (DAG) and inositol triphosphate (IP_3), where IP_3 binds to the inositol triphosphate receptor on the sarcoplasmic reticulum (85).

Once intracellular calcium concentration is increased, free calcium binds to a calcium binding protein called calmodulin (85). The calcium-calmodulin complex then activates myosin light chain kinase (MLCK), which phosphorylates myosin light chains in the presence of adenosine triphosphate (85). Myosin light chain phosphorylation leads to cross-bridge formation between the myosin heads and actin filaments resulting in smooth muscle contraction (Figure 2.5). This contraction then "puffs up" the smooth muscle cell and decreases the lumen diameter (i.e., increases vascular resistance/decreases blood flow) (Figure 2.6).

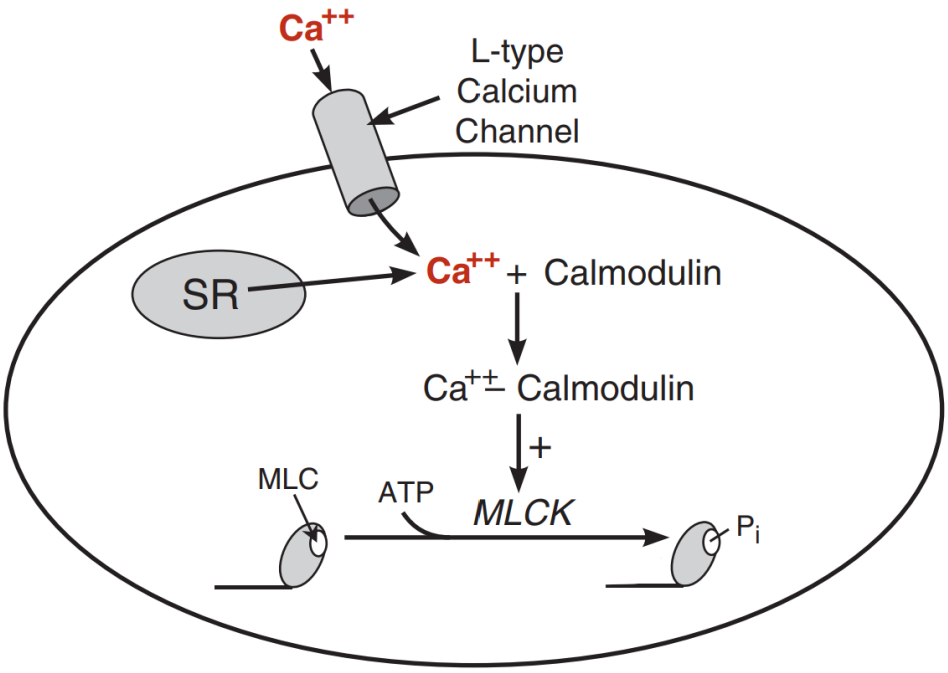

Figure 2.6 Regulation of vascular smooth muscle cell contraction. Increased intracellular calcium (Ca^{2+}) either by entry through voltage-gated L-type Ca^{2+} channels or release from the sarcoplasmic reticulum (SR) initiates contraction (vasoconstriction). Ca^{2+} then binds to calmodulin. This complex activates myosin light chain kinase (MLCK), which phosphorylates myosin light chains (MLC) causing contraction. ATP, adenosine triphosphate, P_i, inorganic phosphate (85).

There are multiple signal transduction pathways that modulate intracellular calcium concentration. These pathways can be impacted not only by the hormones that initiate the pathway (i.e., epinephrine, norepinephrine), but the receptors these hormones bind to. The inositol 1,4,5-triphosphate pathway begins with NE, released from post-ganglionic sympathetic nerves, binding to an α_1-adrenoreceptor (Figure 2.7). This receptor then activates phospholipase C through a G_q-coupled protein, causing the formation of DAG and IP_3 from PIP_2. Inositol triphosphate then releases calcium from the sarcoplasmic reticulum to increase intracellular calcium concentration. Diacylglycerol activates protein kinase C, which phosphorylates the myosin light chains and enhances vascular smooth muscle cell contraction through protein phosphorylation (84). Interestingly, it has been proven that α-adrenoreceptors are responsible for decreases in

vascular conductance (i.e., the inverse of vascular resistance or ease with which blood flows through a vascular bed at a given pressure difference) in young males (47).

The second pathway that regulates vascular smooth muscle contraction is the cyclic adenosine monophosphate (cAMP) pathway, which inhibits vascular smooth muscle contraction (Figure 2.7). This begins with NE or epinephrine binding to β_2-adrenoreceptors, which are associated with a Gs-couple protein. In the presence of adenosine triphosphate, the Gs-protein stimulates the production of cAMP through adenylyl cyclase. Cyclic adenosine monophosphate inhibits MLCK activity, thus decreasing cross-bridge formation and causing vascular smooth muscle relaxation.

The last pathway that controls sympathetic regulation of vascular tone (i.e., the degree of vasoconstriction/dilation occurring at the arteriole) works though the inhibition of the cAMP pathway to cause vasoconstriction (Figure 2.7). This starts with NE binding to α_2-adrenoreceptors, which is coupled with an inhibitory G-protein. This binding inhibits adenylyl cyclase activity, thereby decreasing intracellular cAMP levels. As such, there is less cAMP-mediated inhibition of MLCK, resulting in greater cross-bridge formation and vasoconstriction. It should also be highlighted that the three pathways listed above are not distinct and can occur simultaneously.

As highlighted above, it is important to note that there is also a pre-synaptic α_2-adrenoreceptor negative feedback loop responsible for minimizing vasoconstriction by inhibiting the release of NE from the sympathetic nerve terminals. This pathway becomes more important if the post-synaptic α-adrenoreceptors are fully saturated as can occur during heightened levels of MSNA (91).

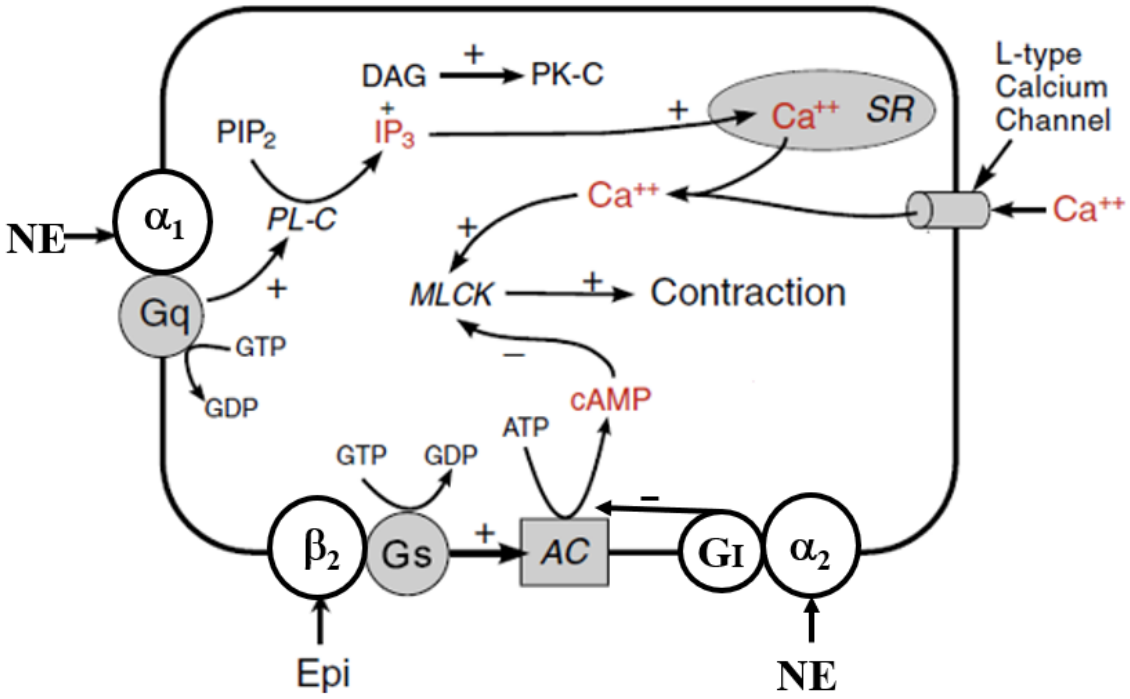

Figure 2.7. Adrenergic receptors and signal transduction pathways that regulate vascular smooth muscle contraction. α_1, alpha$_1$-adrenoreceptor; α_2, alpha$_2$-adrenoreceptor; β_2, beta$_2$-adrenoreceptor Gs, stimulatory G-protein; Gi, inhibitory G-protein; Gq, phospholipase C-coupled G-protein; AC, adenylyl cyclase; PL-C, phospholipase C; PIP_2, phosphatidylinositol 4,5-bisphosphate; IP3, inositol triphosphate; DAG, diacylglycerol; PK-C, protein kinase C; SR, sarcoplasmic reticulum; MLCK, myosin light chain kinase; NE, norepinephrine; GDP, guanosine diphosphate; GTP, guanosine triphosphate; ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate. α_2 pathway: NE binds to α_2-adrenoreceptors coupled to Gi which in the presence of ATP, will inhibit the production of cAMP through adenylyl cyclase stimulating MLCK activity and causing vasoconstriction. β_2 pathway: Epi binds to β_2-adrenoreceptors coupled to Gs which in the presence of ATP, will stimulate the production of cAMP through adenylyl, inhibiting MLCK activity which stimulates vasodilation. PIP_2 pathway: NE binds to an α_1-adrenoreceptor which activates phospholipase C through a G_q-coupled protein, causing the formation of DAG and IP_3 from PIP_2. IP_3 then releases calcium from the sarcoplasmic reticulum to increase intracellular calcium concentration, stimulating MLCK activity to increase vasoconstriction Diacylglycerol activates protein kinase C, which phosphorylates MLC and enhances vascular smooth muscle cell contraction (85)

These pathways are key in the regulation of vascular resistance and blood pressure, and the corresponding neurotransmitters and receptors contribute to sex differences in vascular health. For example, it has been established that the response to NE-induced vasoconstriction is attenuated in premenopausal females compared to males

(88, 89). Furthermore, neurotransmitter release is regulated by presynaptic α_2-adrenoreceptors. Studies have shown that females have a greater number of (30), and higher sensitivity to (134), these receptors potentially accounting for enhanced reuptake of sympathetic catecholamines. The mechanism behind this relationship may be due to sensitivity to adrenoreceptors. One study observed no differences in α_1-adrenoreceptor responsiveness to phenylephrine (α_1-adrenoreceptor agonist) during ganglionic blockade between young healthy females and males (15). This is contrary to another study that observed an attenuated vasoconstriction response to phenylephrine in females compared to males, which may explained by β_2-adrenoreceptors (51). Sex differences in β_2-adrenoreceptor sensitivity have also been examined, demonstrating controversial results (9, 62, 88, 93). Many studies observed a greater β_2-adrenoreceptor sensitivity in younger (62, 88), and postmenopausal females compared to males (62). The difference in sensitivity may have to do with estrogen, a relationship that has been previously observed in mice (49, 62), rats (133), and females using oral contraceptives (94). Estrogen may increase β_2-adrenoreceptor sensitivity through the increases in nitric-oxide (a potent vasodilator) bioavailability (87). Although there is conflicting evidence from a study that examined β-adrenoreceptor function in healthy adults and observed no differences between males and females in forearm blood flow when injecting isoproterenol (β-adrenergic agonist) (93). In summary, differences in adrenoreceptor sensitivity (mainly β_2-adrenoreceptor) may help to identify sex differences associated with the interaction between MSNA, resistance vessel smooth muscle vasoconstriction and dilatation and their impact on blood pressure.

2.3: Sympathetic Transduction

2.3.1: Methods of Quantifying Sympathetic Transduction

The original method of quantifying sympathetic transduction was developed by Wallin and colleagues (61) and used a signal-averaging approach. In this method, each burst of MSNA served as a "trigger" and corresponding beat-by-beat changes in MAP were followed for the subsequent 15 cardiac cycles (61). This process was then repeated for all cardiac cycles that contained an MSNA burst over a 3-min (minimum) resting period. The average peak MAP response for all recorded bursts was then calculated to represent sympathetic transduction (61) (Figure 2.8). Although blood pressure responses to resting MSNA bursts are commonly tracked for 15 cardiac cycles (34, 48, 163), research has also alternatively tracked blood pressure over 12 (120, 122, 125), or 10 (135) cardiac cycles as it is commonly seen that the peak response occurs 5-9 cardiac cycles following a burst (47). The signal averaging approach can also be used following cardiac cycles absent of an MSNA burst to document the nadir blood pressure responses (48). A study conducted in our lab compared shorter sampling durations (0.5, 1, 2, 5 minutes) of measuring sympathetic transduction relative to a 10-min recording period in younger, middle-aged, and older adults (119). This study concluded that a minimum of 5-minutes of sampling is needed to quantify both sympathetic transduction and the nadir blood pressure corresponding to cardiac cycles without bursts (119).

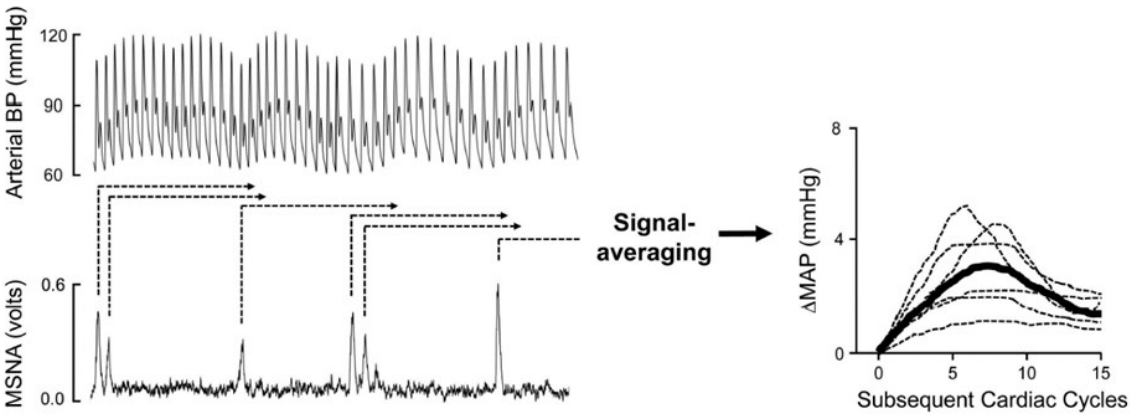

Figure 2.8. Signal-averaging approach of quantifying sympathetic transduction (61). During simultaneous recordings of common peroneal nerve muscle sympathetic nerve activity (MSNA) and arterial blood pressure (Arterial BP), the peak rise in mean arterial pressure (MAP) following each burst of MSNA was determined (see individual dashed lines on the right). The peak of the average MAP response (thick black line) was then calculated to quantify sympathetic transduction.

Another way of quantifying sympathetic transduction is by using a regression-based model (18). This method involved recording beat-by-beat DBP and MSNA during a 5-min supine resting period. For each DBP measurement, the summed MSNA burst area was calculated at a fixed 8-6 cardiac cycle lag prior to each burst (18). Normalized MSNA burst areas were binned into 1%-s bins and correlated against the corresponding DBP values (Figure 2.9). The slope of this relationship was used to assess sympathetic transduction (i.e., steeper slope represented a greater transduction response) (18).

A recent study conducted in our lab compared the two approaches for analyzing sympathetic transduction. The regression approach is beneficial in that it takes into consideration the size of the burst and has documented session-session repeatability (123). The signal-averaging approach is beneficial as it can be used to examine outcome responses to cardiac cycles absent of bursts, and uses the change in pressure as the outcome variable (123). Although both methods provide an index of sympathetic

transduction, they may not interchangeable as they do not exhibit the same units of measurement (123). For this study, we will primarily be using the signal averaging approach as it is widely used in the literature (34, 48, 61, 122, 125, 135, 163) and allows for inter-individual variability in the analysis compared to the regression approach that has only been used by one laboratory. The Briant et al. (18) method does not allow for flexibility regarding the number of cardiac cycles used to determine the peak DBP response following a burst because as it uses a fixed 6-8 cardiac cycle lag.

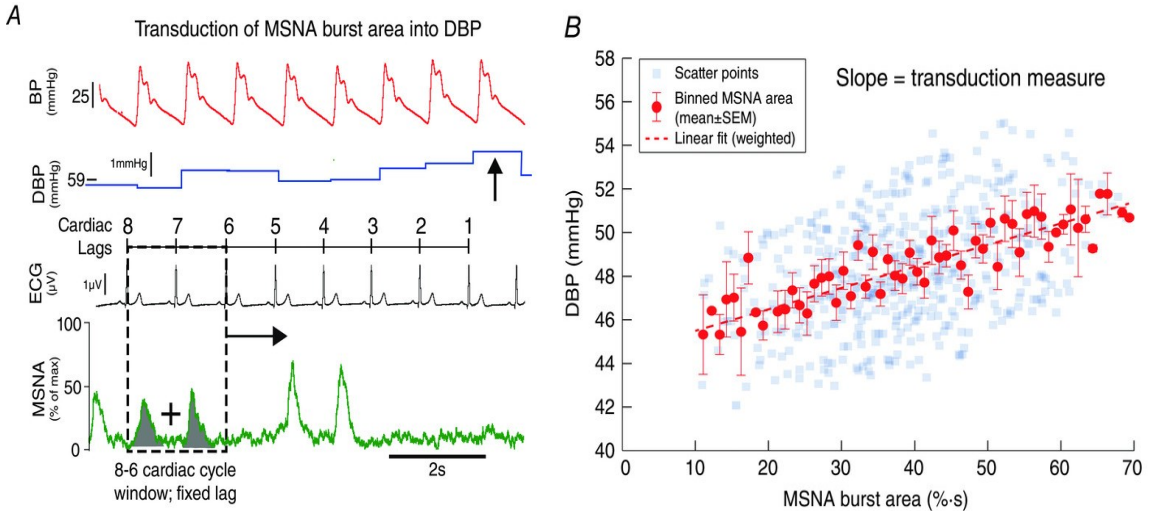

Figure 2.9. Depiction of the regression slope method developed by Briant et al. (18) for quantifying sympathetic transduction in humans. A) For each diastolic blood pressure (DBP) (arrow) value recorded, normalized muscle sympathetic nerve activity (MSNA) burst area (shaded) was summed over a 2 cardiac cycle window at a fixed lag of 8-6 cardiac cycles prior to the DBP measure (dashed lines). This 2 cardiac cycle window was shifted and used for each successive DBP value recorded during the entire recording period. B) Normalized MSNA burst areas (1%-s bins) were plotted versus the corresponding DBPs. A weighted linear regression model was applied to determine the slope of the relationship, which was used to quantify sympathetic transduction. BP, blood pressure; ECG, electrocardiogram.

2.3.2: Impact of Sex on Sympathetic Transduction

The impact of sex on sympathetic transduction has well been documented, although the results are controversial (72, 178). To start, inverse relationships have been

observed between sympathetic transduction versus both baroreflex sensitivity (71) and resting MSNA (135) in young males, but not in females (71, 135). As well, premenopausal women have been observed to have less effective baroreflex buffering of BP compared to males of similar age (27). Baroreflex sensitivity is important in that the higher the sensitivity of the arterial baroreflex to changes in blood pressure, the more efficient the buffering of higher blood pressure values due to MSNA, thereby influencing sympathetic transduction (155). The dissimilarity in the relationship between sympathetic transduction and baroreflex sensitivity may be a contributor to sex differences in the pressor responses to MSNA.

There is contradicting evidence concerning sex differences in sympathetic transduction depending on the quantification method (71). The study highlighted above by Briant et al. (18) (Chapter 2.4.1) observed lower sympathetic transduction in young females compared to males (18). Opposing this, two separate studies observed no sex difference between peak increases in MAP following resting MSNA bursts in young healthy adults using the signal averaging approach (87, 163). Interestingly, females demonstrated a longer lasting increase in blood pressure following a burst compared to males (34). Similarly, no difference in sympathetic transduction between young males and females were observed when quantified using peak MAP or nadir LVC responses (135) following MSNA bursts. To determine the impact of quantification method on sympathetic transduction, our lab compared peak DBP responses to spontaneous bursts of MSNA between the signal-averaging and regression-slope approaches (123). Although we observed that the sympathetic transduction outcomes were unrelated between the two methods, there were no sex differences observed using either method (123). As such, it

can be hypothesized that there are no sex differences in spontaneous sympathetic transduction, regardless of the quantification technique used.

Contrary to all studies listed above, a sympathetic transduction quantification method using MSNA along with beat-by-beat blood pressure (signal averaging) and popliteal blood flow reported higher sympathetic transduction values in young females compared to males during an isometric handgrip exercise to exhaustion (156). Another study investigated sex differences in MSNA and blood pressure during a cold pressor test and a static handgrip to fatigue test in young adults (79). Although this study did not directly measure the corresponding changes in blood pressure following stress-induced MSNA, they reported higher burst frequency and incidence in males and no differences in blood pressure between sexes during the stress tests. These studies emphasizes the importance of the condition (rested vs. stress) and method in determining sex differences in sympathetic transduction (156). This evidence demonstrates the lack of understanding concerning the sex differences in sympathetic transduction and whether differences in MSNA act upon blood pressure regulation or the vasculature.

2.3.3: Impact of Aerobic Fitness on Sympathetic Transduction

It has been reported that higher levels of aerobic fitness are negatively associated with sympathetic transduction in young healthy males (120) and older males (122). These studies measured relative $\dot{V}O_2$peak along with resting beat-to-beat blood pressure and common peroneal nerve MSNA (120, 122). Peak increases in MAP following spontaneous bursts of MSNA were tracked for 12 cardiac cycles following each burst and used to quantify sympathetic transduction. Both studies observed a inverse relationship between relative $\dot{V}O_2$peak and peak MAP increases ($R = -0.69$, $P<0.001$ in young males

(120) (Figure 2.10). This highlights that higher aerobic fitness may be associated with reduced sympathetic transduction, potentially leading to a lowered risk of developing CVD.

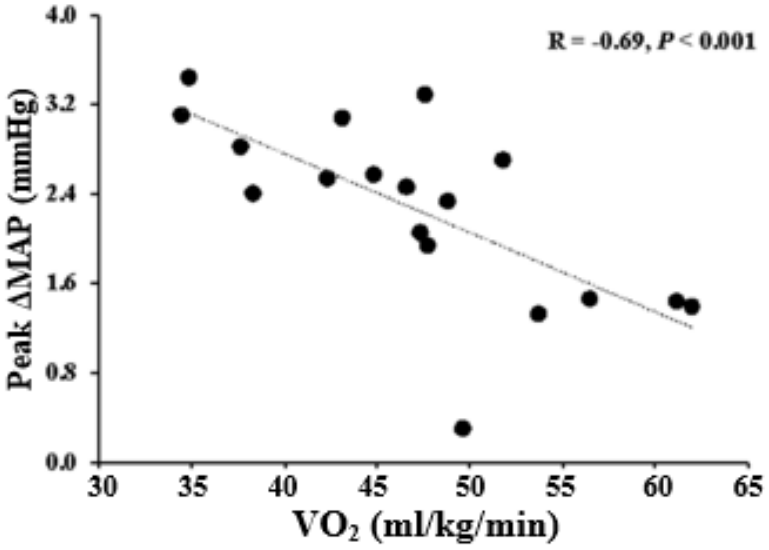

Figure 2.10 Inverse relationship between peak increases in mean arterial pressure (ΔMAP) following spontaneous bursts of common peroneal nerve muscle sympathetic nerve activity and aerobic fitness (relative $\dot{V}O_2$ peak, volume rate of oxygen consumption) reported in younger males. These results highlight reduced sympathetic transduction in younger males with higher aerobic fitness (120).

A study by Notarius et al. (115) examined a similar hypothesis in middle-aged 'unfit' versus aerobically 'fit' males. This study assessed $\dot{V}O_2$peak (Fit group=129% ±4%, 'Unfit' group=85% ± 3% of predicted peak oxygen uptake) and determined the relationship between graded lower body negative pressure-induced increases in forearm vascular resistance (i.e., MAP/forearm blood flow) and MSNA burst frequency as their metric of sympathetic transduction. This study observed a positive relationship in the 'Unfit' group, but no relationship in the 'Fit' group, which the authors interpreted as greater sympathetic transduction in the 'Unfit' participants (Figure 2.11) (115).

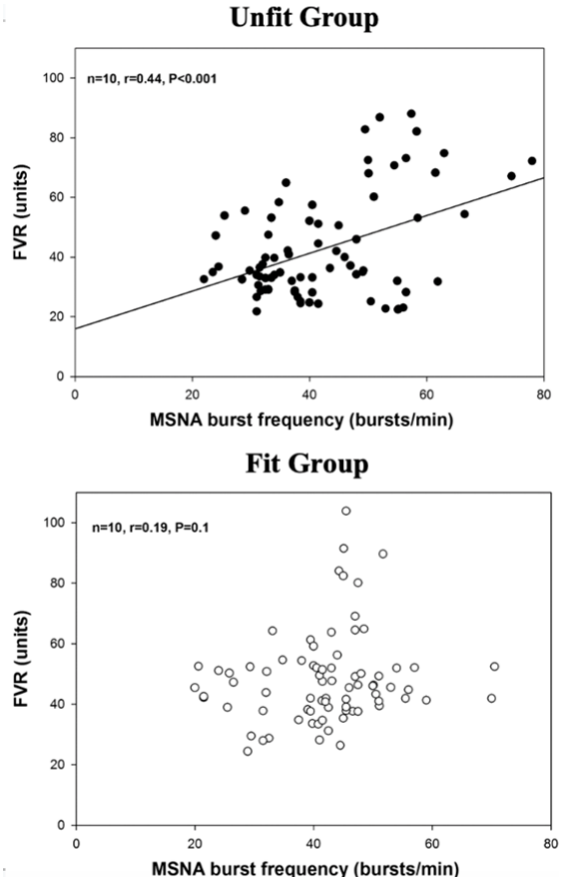

Figure 2.11 Relationships between forearm vascular resistance (FVR) and common peroneal muscle sympathetic nerve activity (MSNA) burst frequency in Sedentary and Fit middle-aged males during a lower-body negative pressure protocol. A significant positive relationship occurred in the Sedentary group, while no relationship was observed in the Fit group (115)

Another study examined differences in sympathetic transduction in exercising females (relative $\dot{V}O_2$peak = 44 ± 3 mL/kg/min) versus non-exercising (relative $\dot{V}O_2$peak = 30 ± 8 mL/kg/min) (152). Sympathetic transduction was measured using the signal averaging approach, using peak increases in MAP as the response variable (61). This study concluded that sympathetic transduction was similar between groups, indicating no relationship between aerobic fitness and sympathetic transduction in healthy, pre-menopausal females (Figure 2.12) (152).

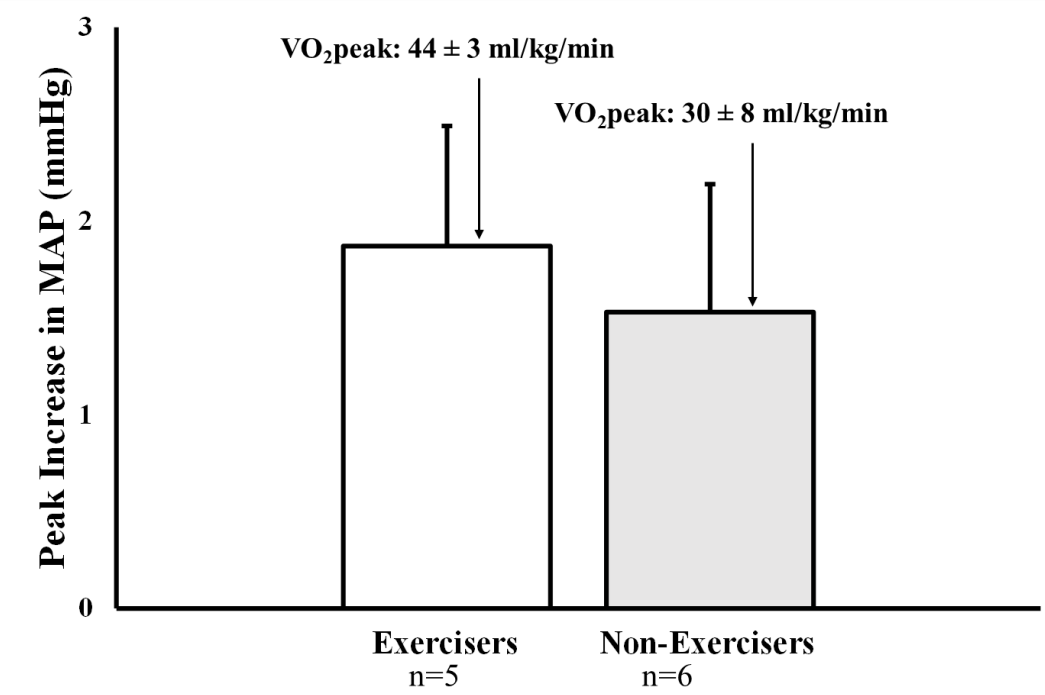

Figure 2.12 Differences in the peak increase in mean arterial pressure (MAP) following spontaneous bursts of common peroneal muscle sympathetic nerve activity in a group of young healthy female Exercisers (white bar) and Non-Exercisers (grey bar) (152).

Interestingly, another study in rats examined the impact of exercise training (moderate-intensity continuous training) on arteriolar adrenergic vasoreactivity (40). Young (4-6 month) male Fischer rats were randomly divided into a sedentary group and an exercise-trained group. The exercise-trained rats performed treadmill running at 15m/min up to a 15° incline, 1h/day, 5 days/week for 10-12 weeks (40). Norepinephrine-mediated vasoconstriction was measured in isolated soleus and gastrocnemius muscle arterioles *in vitro*. The results of this study showed that exercise training attenuated noradrenaline-mediated α_2-adrenoreceptor vasoconstriction in resistance arterioles from the training group. Lastly, one study investigated the influence of 11-16 weeks (3-4 days/week) of training at 50-70% of HR reserve (using various modalities of exercise) on sympathetic transduction (signal averaging approach) in pregnant younger females (147). The results of this study showed no change in sympathetic transduction with training

however, they observed blunted transduction in the Control group (147). Overall, these findings suggest that aerobic fitness and/or training can reduce sympathetic transduction in males, but not in females. The literature is limited in that no study has examined these relationships or sex differences in transduction using an aerobic training intervention in healthy young adults.

Purposes & Hypotheses:

The purpose of this study was to test the hypotheses that: 1) a 12-week HIIT intervention will improve aerobic fitness in young males and females, and 2) potential HIIT-mediated increases in aerobic fitness will decrease sympathetic transduction in young healthy males (120), but have no effect in young healthy females (152). Lastly, I tested the hypothesis that there would be no sex difference in sympathetic transduction regardless of the assessment method used [i.e., signal averaging (61) versus regression-based approach (18)] as our group has previously demonstrated.

Chapter 3: Methodology

3.1: Participants

Healthy younger adults (i.e., non-smoking, normotensive, and free of chronic disease, aged 18-59 years old) were recruited via word of mouth, recruitment posters, and classroom presentations. The exclusion criteria included individuals who: 1) were hypertensive [i.e., seated resting SBP ≥140 mmHg and/or DBP >90 mmHg, as indicated in the Canadian Hypertension Guidelines (128)], 2) were prescribed medications known to affect sympathetic activity and/or vascular function (e.g., calcium channel blockers, renin-angiotensin acting agents, lipid-lowering drugs), 3) smoked or vaporized nicotine or marijuana-containing products most days of the week (≥4 days/week) within the past 6 months, 4) were obese [i.e., body mass index (BMI) >30 kg/m^2], 5) had a known allergy to the clear medical adhesive used to secure the physical activity monitors, 6) were on hormone replacement therapy (females using oral contraceptives were included in the study), 7) were pregnant, breastfeeding or planning on becoming pregnant within 3-months upon entry into the study, or 8) were afraid of needles (e.g., microelectrodes). Interested candidates completed a health history questionnaire (Appendix A) and the Get Active Readiness Questionnaire (Appendix B). Participants received an information package, including a consent form, prior to the first assessment day and were informed of the experimental protocols in writing and verbally. All protocols conformed to the Declaration of Helsinki, except registration in a public database, and were approved by the Dalhousie University Health Sciences Research Ethics Board (REB# 2021-5555; Appendix C).

3.2: Experimental Procedures and Analyses

Participants were randomly assigned to either the HIIT group (n=18) or a Control group (n=15). Participant availability for the training sessions was also considered following assignment of group. Complete descriptive characteristics for each group are presented in the Results section. Participants initially assigned to the Control group were asked to maintain their habitual activity patterns for 12-weeks. They were then provided the opportunity to enter the HIIT group after completing their Follow-up assessments (see below for assessment details).

3.2.1: Anthropometrics & Aerobic Fitness Assessments

Height (to the nearest 0.1-cm) and weight (to the nearest 0.1-kg) were measured using a calibrated stadiometer (Health-O-Meter, McCook IL, USA), and BMI was calculated (kg/m^2).

Aerobic fitness was assessed using a graded maximal exertion test on an electromagnetically braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands) (Figure 3.1). The ergometer seat was adjusted to a comfortable position for the participant while ensuring that a slight knee bend (~10-20 degrees) was achieved at the lowest pedal position. Participants were equipped with a chest strap-based heart rate (HR) monitor (Polar H9, Kempele, FI) and a full-face mask, or mouthpiece and nose clip set-up (52). Peak oxygen consumption ($\dot{V}O_2peak$), peak metabolic equivalents of task (peak METs), the volume rate of carbon dioxide production ($\dot{V}CO_2$), and respiratory exchange ratio (RER, $\dot{V}CO_2/\dot{V}O_2$) were determined via a commercial, mixing chamber-based metabolic system (TrueOne 2400, Parvo Medics Inc., Sandy, UT, USA). The

ventilatory threshold (VT) was estimated automatically by the metabolic software using the V-slope method (54) and expressed as a percentage of $\dot{V}O_2$peak (12).

Participants started with a 5-minute warm up of light-intensity cycling (50 W), the workload was then step increased to 70 W, with a progressive 20 watts/minute rise in power output (i.e., 1 watt every 3 seconds) until voluntary exhaustion. Participants were asked for their Ratings of Perceived Exertion (RPE) on the Borg 6-20 Scale (6 = no effort and 20 = maximal effort) (17) every 2 minutes initially, then more frequently near the end of the test. Strong verbal encouragement was provided throughout the testing period. The test was considered finished when: 1) The participant no longer continued cycling at a pedalling rate of 40 revolutions per minute for longer than 15 seconds, 2) their HR suddenly decreased >30 beats/minute, or 3) the participant decided to stop for any reason. Upon test completion, the intensity was reduced to 50W for a ≥5-minute cool-down period. All HR and metabolic data were averaged every 15-s and maximum/peak values determined from the greatest consecutive 30-s average. The final workload achieved was considered PAP), which was used to determine exercise intensity for the HIIT protocol (see below).

To be considered a maximal effort (i.e., a $\dot{V}O_2$max), the participant must have reached a plateau in oxygen consumption (i.e., <1.5 ml/kg/min increase in relative $\dot{V}O_2$ between successive 15-s averaged timepoints). Otherwise, a $\dot{V}O_2$peak was considered if participants achieved ≥2 of the following criteria: 1) maximal HR ≥ 90% of age-predicted maximum (208 - 0.7 × age) (157), 2) a peak RER ≥ 1.10, or 3) a maximal RPE >18. All HIIT and Control participants achieved $\dot{V}O_2$peak (≥2 of the criteria) at Baseline, 6-weeks, and Follow-up timepoints. Since not all participants achieved the criterion for $\dot{V}O_2$max,

the term $\dot{V}O_2$peak will be used to qualify aerobic fitness outcomes in this project. Sex- and age-specific relative $\dot{V}O_2$peak fitness categories were determined for each participant at Baseline and Follow-up (35).

3.2.2: Habitual Activity Monitoring

Approximately 7 days (6.9 ± 0.6 days) of continuous (i.e., 24-h/day) habitual activity was monitored using the activPAL® triaxial accelerometer and inclinometer (41). Habitual activity was measured the week prior to training, and the week following training. The activPAL was waterproofed in a nitrile finger coat and secured using transparent medical dressing (Tegaderm™, 3M, London, ON, Canada) to the midline of their right thigh, one-third of the way between the hip and knee (41). Participants self-reported their waking hours (for sedentary time determination) and any activities that were not stride-based (e.g., biking, swimming) to accommodate activPAL analysis to ensure the device does not incorrectly identify habitual activity. For example, the activPAL analysis cannot identify if an individual is asleep (not sedentary) versus lying down awake (sedentary). If the participant engaged in non-stride based physical activity (swimming or biking) the participant indicated the time spent in this behaviour and their perceived intensity and this information was manually added to their recorded habitual activities. Stride-based intensity-related physical activity levels (light, moderate, and vigorous) were determined using step rate thresholds determined from a cross-validated, height-adjusted curvilinear cadence-intensity equation (80). Specifically, stepping cadence thresholds that corresponded to light physical activity (LPA), moderate physical activity (MPA), and vigorous physical activity (VPA) were calculated, and time spent stepping within each intensity category determined and the curvilinear cadence-intensity

model was cross-validated (116) and improved the validity in measuring activity intensity in laboratory and free-living condition from the default activPAL method. Equivalence testing demonstrated that activPAL outcomes using this individualized, curvilinear equation estimated MET values that were statistically equivalent to indirect calorimetry (116). The activPAL data were analyzed using a customized LabVIEW program (LabVIEW 2020, National Instruments, Austin, TX, USA) that confirmed waking hours and summarized daily averages of time spent standing, stepping, and sitting/lying down.

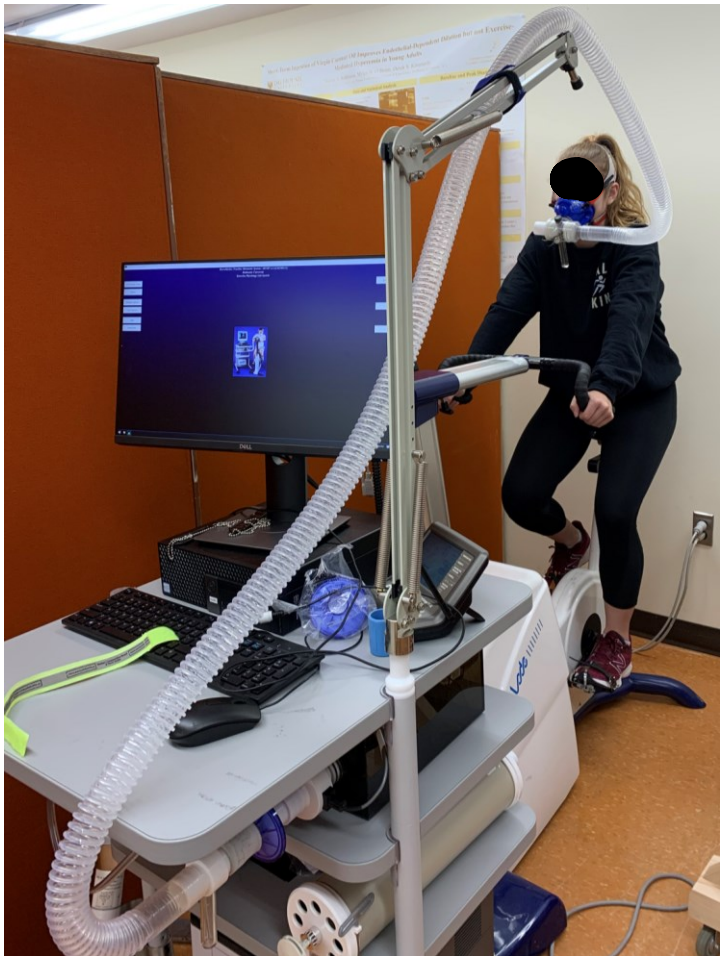

Figure 3. 1.Electromagnetically braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands) and mixing chamber-based metabolic cart (TrueOne 2400, Parvo Medics Inc., Sandy, UT, USA) used for aerobic fitness testing.

3.2.3: Systemic Hemodynamics

Heart rate was calculated from successive R-R cardiac intervals obtained from lead II of a standard bipolar electrocardiogram set-up. Beat-by-beat measurements of SBP and DBP were recorded using a non-invasive finger photoplethysmography (Portapres or Finometer Model 2; Finapres Medical Systems, Amsterdam, The Netherlands). The electrocardiogram (1000 Hz) and Portapres (200 Hz) recordings were sampled continuously using a PowerLab (PL3508 PowerLab 8/53, ADInstruments, Sydney, Australia) data acquisition system and analyzed offline using LabChart software (Version 8, ADInstruments, Sydney, Australia). A 'physiological calibration' of the finger blood pressure waveform was performed using the average of 2-3 consistent brachial SBP and DBP values collected from semi-automated vital signs monitor (Carescape v100, General Electric Healthcare, Mississauga, ON, Canada). Finger SBP and DBP values were determined as the maximum and minimum within beat values, respectively. MAP was calculated as ⅓SBP + ⅔ DBP. Baseline beat-by-beat hemodynamic data were averaged over a 10-minute supine rest period.

3.2.4: Microneurographic Recordings of Muscle Sympathetic Nerve Activity

All microneurography sessions were performed >6-hours post-prandial in a thermoneutral environment (~21°C) (96). Participants voided their bladder before testing to minimize the impact of bladder distension on increases in MAP and MSNA (45). Furthermore, participants refrained from strenuous physical activity 24-hours prior, and the consumption of products known to acutely influence vascular function (e.g., caffeine, alcohol, chocolate, citrus fruits, saturated fats, folic acid, antioxidants, and multivitamin

supplements) for 12-hours before these visits (158). This was confirmed verbally by the participant and the researcher prior to each assessment.

Multi-unit post-ganglionic MSNA was recorded from the lower leg in the common peroneal nerve using standardized microneurography techniques (96). In brief, the nerve was first mapped using external isolated stimulation to determine the optimal location for insertion of the active microelectrode. The location of the peroneal nerve was estimated by palpating the proximal head of the fibula and mapping under the bone. A non-insulated reference electrode was positioned under the skin approximately 2-3 cm from the desired recording location. The purpose of this reference electrode was to record "background/external noise". A 2-mΩ unipolar tungsten microelectrode (FHC, Bowdoin, MA) was inserted percutaneously into the nerve, just under the head of the fibula. The microelectrode was slowly manipulated until an appropriate recording site was found. The microelectrodes were secured to an isolated differential pre-amplifier (×1000 constant gain) connected to a dedicated nerve traffic analysis system (662C-4 Nerve Traffic Analysis System, University of Iowa Bioengineering, Iowa City, IA). The raw MSNA signal was sampled at 20kHz, further amplified (~75,000 × total), band-pass filtered (500-2000 Hz), full-wave rectified, and integrated (0.1 second time constant) to obtain a mean voltage neurogram from which individual MSNA 'bursts' were identified for subsequent analysis. All MSNA recordings were confirmed via burst morphology, the presence of cardiac synchronicity, responsiveness to an expiratory breath hold, and a Valsalva's maneuver, as well as lack of responsiveness to skin stimulation or mental arousal (e.g., loud clap). Only MSNA recordings with a 3:1 signal-to-inter burst noise

ratio were included for subsequent analysis. Once a stable recording was obtained, participants remained rested in the supine position for at least 10-min of data collection. Time-averaged integrated MSNA was quantified as burst frequency (bursts/minute) and burst incidence (bursts/100 heart beats). The absolute amplitude (mV) of each burst was determined and normalized as a percentage of the tallest burst measured during the recording period. Normalization of burst height is important for comparing between time points over separate recording sessions (e.g., pre- versus post-intervention) since the population of neurons differs between recordings, which impacts the absolute burst height (i.e., more single units recorded from produces larger absolute burst amplitudes). Total normalized MSNA activity was calculated as the product of the mean relative burst amplitude and burst frequency (% x bursts/minute). Only MSNA recordings absent of shifts in baseline activity were used for analysis. A shift in the baseline is indicative of microelectrode movement and may indicate a different population of neurons being recorded from.

3.2.5: Sympathetic Transduction Quantification

3.2.5.1: Signal-Averaging Approach

A signal-averaging approach to quantify sympathetic transduction was achieved using an open-source Microsoft Excel-based program developed by our lab (118). In LabChart (AD Instruments, Sydney, Australia), separate semi-automatic macros were used to insert 'Beat' comments in the middle of each cardiac cycle (i.e., between successive R-waves in the electrocardiogram) and 'Burst' comments at the peak of each MSNA burst (Figure 3.2). Beat-by-beat cardiac intervals, DBP and the integrated MSNA signal were time-aligned so that MSNA bursts were situated within the cardiac cycle that

they were generated (Figure 3.2). This was completed by ensuring the peaks of the MSNA bursts were aligned in the middle of the cardiac cycle from which they were generated (Appendix D). Both burst size (i.e., amplitudes) and the height of an individual can impact the time delay between the R-wave from the cardiac cycle that the MSNA burst was generated and the peak of the burst itself (i.e., the baroreflex latency). Specifically, taller individuals have a longer transmission delay from the origin of the sympathetic signal in the brainstem to the active microelectrode in the lower leg (i.e., increased latency) (46) and larger bursts having shorter latencies (i.e., decreased latency) because they are associated with the recruitment of faster conducting post-ganglionic sympathetic neurons (139). The range of this baroreflex latency across participants was ~1.2-1.5 seconds. Time-aligned hemodynamic and MSNA data were extracted on a beat-by-beat basis and exported to a customized Excel spreadsheet (Microsoft, Washington, USA) such that each row of data corresponded to one cardiac cycle, which was achieved by extracting data between successive "Beat" comments.

The primary hemodynamic outcome variable used to quantify sympathetic transduction was DBP, as it provides the best pressor variable to estimate the sympathetic vasoconstrictor-mediated response on the peripheral vasculature with minimal influence from cardiac-related factors (e.g., left ventricular contractility and stroke volume) (85). The Excel spreadsheet contains built-in functions that track absolute changes in DBP (from the cardiac cycle the burst was generated, cardiac cycle 0) for 12 cardiac cycles following each burst (118). Twelve cardiac cycles were used based on previous literature that noted peak DBP response typically occur between 5-8 cardiac cycles following a, MSNA burst (48). Further, the 12 cardiac cycle incorporates the mean ± 2 standard

deviations of when the peak DBP response to a burst may occur. The absolute changes in DBP across cardiac cycles 1 through 12 for all bursts were then averaged for each participant. The average peak increase in DBP was extracted to represent sympathetic transduction regardless of the cardiac cycle number that it occurred (122). The average number of cardiac cycles to the peak DBP response was also recorded for each participant.

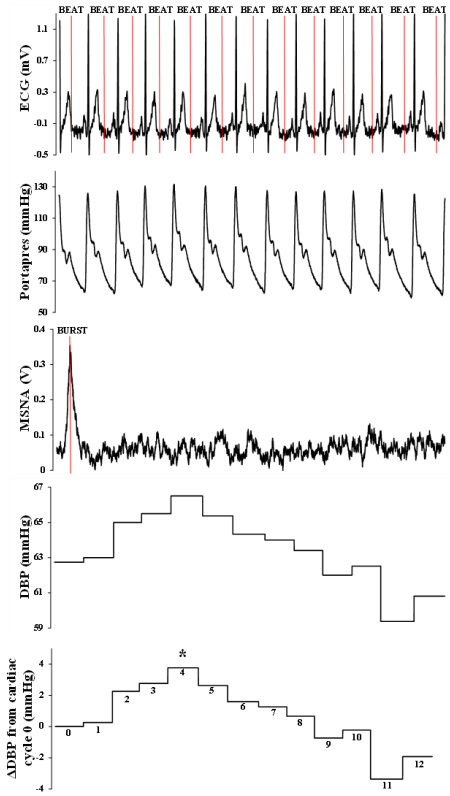

Figure 3.2 Example of time-aligned electrocardiogram, finger photoplethysmography, and integrated muscle sympathetic nerve activity (MSNA) recordings, as well as calculated beat-by-beat absolute diastolic blood pressure (DBP), and changes in DBP (from the cardiac cycle that the MSNA burst was generated in). Each cardiac cycle was identified by a comment labelled 'Beat', while the peak of each MSNA burst was labelled with a 'Burst' comment. The integrated MSNA channel was manually shifted backwards in time to align each 'Burst' comment with the corresponding 'Beat' associated with the cardiac cycle it was generated (i.e., cardiac cycle 0). This allowed for extraction of the MSNA data based on each cardiac cycle. As shown in the ΔDBP channel (bottom), the numbers (0-12) represent the cardiac cycles from the first burst in the MSNA channel and the asterisk corresponds to the peak increase in DBP, which in this example occurred 4 cardiac cycles following the burst. The ΔDBP is based on the calculated DBP channel as the difference from cardiac cycle 0 (e.g., ~3.5 mmHg between cardiac cycles 0 to 4).

In addition, the nadir DBP responses following cardiac cycles absent of MSNA bursts were similarly analyzed (48, 122) as this may provide information concerning hemodynamic regulation during brief periods of sympathetic quiescence. The number of cardiac cycles to the nadir DBP response was also determined for each participant.

3.2.5.2: Regression Slope-Based Sympathetic Transduction Approach

Sympathetic transduction was also quantified using the regression slope-based approach developed by Briant and colleagues (18) (Figure 2.9). Summed Relative burst heights (% of tallest burst) were placed into 1% bins and a cross-correlation conducted between the corresponding DBP values to determine the optimal 2 cardiac-cycle window time delay. In the absence of a clear optimal window, the default 6–8 cardiac cycle window was used. The summed relative MSNA burst height data in the time delay 2 cardiac-cycle window 6-8 cardiac cycles prior to every DBP value was used. The slope of this relationship was used to assess sympathetic transduction (i.e., steeper slope representing a greater transduction response). These data were collected for both HIIT and Control participants at Baseline to assist with the determination of whether the sympathetic transduction analysis method (i.e., signal-average vs regression slope method) impacted the determination of sex difference in the sympathetic transduction of blood pressure.

3.3: Experimental Design

Figure 3.3 displays a schematic of the study design involved with the 13-week HIIT intervention (i.e., 12-weeks of training and 1-week of no HIIT when the mid-training aerobic fitness assessments were performed following 6-weeks). Participants in the HIIT group visited the lab 5 times (excluding training days), while those in the

Control group visited the lab 4 times (i.e., no Week 7 aerobic fitness assessment as no changes in aerobic fitness were observed using this HIIT protocol following 6-weeks of training (102)). Specifically, anthropometrics, and aerobic fitness were assessed at 3 time points (Baseline, following 6-weeks, and Follow-up/post-training) for the HIIT group and 2 time points (Baseline and Follow-up) for the Control group. In addition, habitual physical and sedentary activities were assessed at Baseline and Follow-up for both groups.

The Baseline and Follow-up sessions involved 2 separate assessment days, the first dedicated to the microneurography and hemodynamic measurements, and the second for the assessment of aerobic fitness. At least 48-hours separated each pair of assessments. As indicated above, only aerobic fitness was assessed following 6-weeks for the HIIT group. This test was designed to document anticipated improvements in PAP to help maintain the training intensity stimulus for the second half of the HIIT protocol.

3.3.1: High-Intensity Interval Training Protocol

This training protocol was based on a 6-week version reported by Mekari et al. (102), with modification made to the progressions in intensity and set duration to account for the longer intervention in the current study (i.e., 12 versus 6 weeks). This protocol followed the typically recommended progressions for an exercise program wherein the volume of training is initially increased via longer durations, followed by a higher exercise intensity (60). All HIIT sessions were supervised and conducted in groups of 2-3 participants, 3 days per week (Mondays, Wednesdays, and Fridays) for a total of 12-weeks. There was a 1-week cessation of training during Week 7 to accommodate scheduling of the mid-training aerobic fitness assessment. Session attendance was

documented to determine adherence. To ensure an adequate training stimulus was achieved, participants were removed from the study if they missed 3 consecutive training days or attended <80% of all sessions (i.e., <29 of 36 sessions).

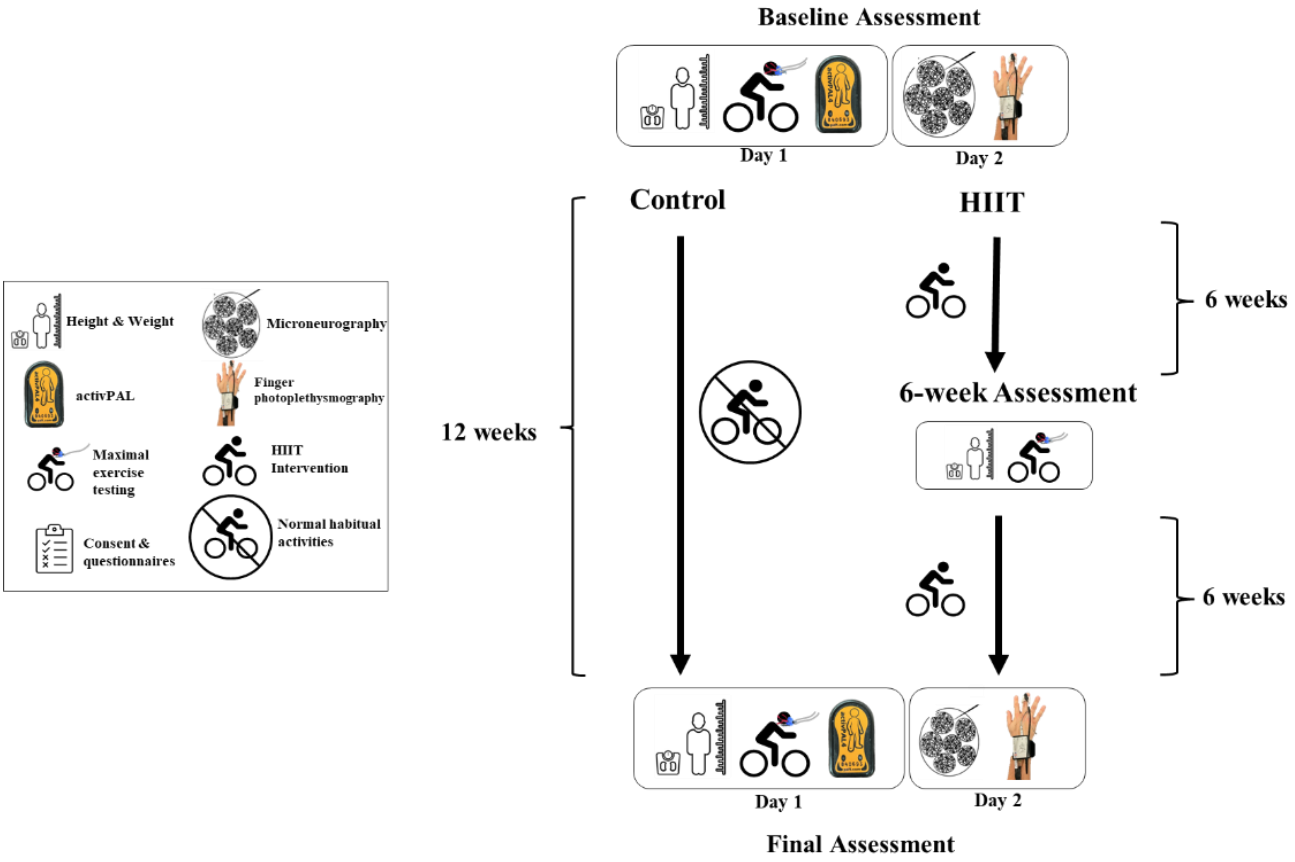

Figure 3.3. The 12-week high-intensity interval training (HIIT) research protocol schematic. Both the Baseline and Follow-up assessment periods included two separate days of testing. Day 1: Measurement of height and weight, placement of an activPAL monitor to measure habitual activity for 7 days, and an aerobic fitness test. Day 2: Resting hemodynamics and microneurography recordings. The order of each day changed depending on availability of the participant but there was always ~48-h between measurement days. The Control group continued with their regular habitual activities for 12 weeks. The HIIT group participated in 12 weeks of training. After the first 6-weeks of training, aerobic fitness was assessed during Week 7. The highest peak aerobic power output obtained between the Baseline and Week 7 aerobic fitness test was used to set the training intensity for the second half of training. There were no HIIT sessions completed during Week 7.

Warm-up and cool-down periods involved ~5-minutes of light-intensity cycling (25% PAP). For the first 3-weeks of training (i.e., 9 sessions), the protocol consisted of

forty, 15-second intervals of cycling at 100% PAP interspersed with 15-seconds of passive recovery (i.e., seated on ergometer without pedalling). This 20-minute set was repeated following a 5-minute passive recovery period (i.e., 40-minutes of exercise in total). To accommodate anticipated improvements in aerobic fitness, PAP and exercise tolerance, the duration of each HIIT session increased to 46-minutes (i.e., 2 × 23-minute sets or 2 sets of 46 intervals) for weeks 4 and 5. However, training volume was decreased back to 40-minutes (2 ×20-minute sets or 2 sets of 40 intervals) for week 6 as a tapering period to ensure appropriate recovery prior to the mid-training assessment of aerobic fitness during week 7.

The highest PAP recorded between the Baseline and Week 7 assessments was used to determine the cycling intensity for the second half of training. During weeks 8 and 9, the session duration increased to 52-minutes of exercise (2 × 26-minute sets or 2 sets of 52 intervals) at 100% PAP. The intensity increased to 110% PAP for week 9, and then to 115% PAP for week 11, with the total session duration maintained at 52-minutes. The set durations were again decreased to 20-minutes (i.e., 40-minutes of total exercise) and the intensity reduced to 100% PAP for the final week of training (i.e., week 13) in preparation for the Follow-up aerobic fitness assessment.

3.4: Statistical Analysis

All data were assessed for normality using a Shapiro-Wilk test, and non-normalized data analyzed via non-parametric tests (Mann-Whitney U for between-participant assessments and Wilcoxon signed-rank for within-participant assessments). Non-parametric tests were required for VPA, Peak RPE, Number of cardiac cycles to peak DBP and nadir DBP.

To test the hypothesis that 12-weeks of HIIT improved aerobic fitness, a Group (Control, HIIT) × Time (Baseline, Follow-up) repeated measures analysis of variance (RM-ANOVA) was conducted on all aerobic fitness outcomes. Group × Time RM-ANOVAs were also conducted on participant descriptive characteristics, daily habitual activity, resting hemodynamic, MSNA, and sympathetic transduction outcome variables. In addition, differences in training intensity between Weeks 1 and 6, and Weeks 8 and 13 were evaluated using paired t-tests (data were normally distributed).

To test the hypothesis that HIIT would decrease sympathetic transduction in young healthy males (120), but have no effect in young females (152), a separate Sex × Time RM-ANOVA was conducted on the HIIT group data only to determine whether there were any sex differences in the response to training. Effect sizes were calculated for main effects (small = 0.1, medium = 0.3, large = 0.5) and *post hoc* analyses using Partial Eta Squared (140). For all ANOVAs, the variance of differences was assessed using Mauchly's test of sphericity and the Greenhouse-Geisser correction factor to the degrees of freedom used if assumptions of sphericity was violated. Bonferroni *post-hoc* testing was used for pairwise comparisons if significant interactions were identified. To further investigate the sex differences in their response to training, independent sample t-tests were performed between the % difference between Baseline and Follow-up absolute $\dot{V}O_2$peak between sexes.

To investigate potential relationships between aerobic fitness and sympathetic transduction, Pearson correlations (all variables were normally distributed) were conducted between aerobic fitness (separately using both absolute and relative $\dot{V}O_2$peak as metrics for aerobic fitness) and measurements of sympathetic transduction (separately

for both the regression slope and signal averaging methods). Baseline data from both the HIIT and Control groups were compiled for these analyses.

To investigate the potential differences in the relationship between sympathetic transduction and sex between the two methods of quantification (signal averaging and regression slope), independent-sample t-tests using the Baseline data from both the HIIT and Control groups was assessed (all data was normally distributed). All statistical analyses were performed using SPSS software (version 28) with significance set *a priori* at $P<0.05$. Data are presented as means ± SD.

Chapter 4: Results.

4.1: Participant Demographic Characteristics and Habitual Activity

Thirty-two individual participants were recruited for this study [15 Control (9♀) and 18 HIIT (10♀)]. Three participants (2♀) from the Control group were removed from the study due to an inability to obtain an MSNA signal (n=1), a change in living location (n=1) and COVID-19 illness that prevented them from completing the Follow-up aerobic fitness assessment (n=1). Two HIIT participants (1♀) were excused from the study due to a knee injury (n=1) and prolonged illness that caused them to be absent for >6 training sessions (n=1). Five participants (2♀) were enrolled in the Control group ≥6 months after completing the HIIT intervention, while 1 male completed HIIT ~1 month after participating in the Control group. This resulted in a final sample of 15 (25 ± 9 years, 9♀) in the Control group and 18 (26 ± 11 years, 10♀) who completed the HIIT intervention, with 27 unique participants. For the statistical analysis with hemodynamic and MSNA measures, due to the inability to measure either beat-by-beat blood pressure (n=5, 4♀), or obtain a MSNA signal (n=8, 5♀) this resulted in a sample of 10 participants (6♀) in the Control group and 14 (8♀) in the HIIT group.

In the Control group, 3 females were naturally menstruating, 1 used oral contraceptive pills (second generation), 4 had intrauterine devices, and 1 utilized a birth control patch. In the HIIT group, 5 females were naturally menstruating, 4 were prescribed oral contraceptive pills (second generation), and 1 used an intrauterine device.

All participant characteristics are presented in Table 4.1. There were no Group × Time interaction effects for any participant characteristics or habitual activity variables. The Control and HIIT groups had similar body mass (kg) and BMI (kg/m^2) at Baseline

and Follow-up (all, $P \geq 0.228$). As well, males had a higher body mass compared to females at Baseline (61 ± 7 kg versus 86 ± 15 kg) and Follow-up (61 ± 6 kg versus 81± 12 kg) in the HIIT group (both, $P<0.001$).

Habitual activity data are also presented in Table 4.1. There were no differences in daily step counts, sedentary times, standing times, as well as habitual light-, moderate-, vigorous-, and moderate-to-vigorous physical activity between or within the groups (all, $P \geq 0.259$). In the HIIT group, both sexes had similar daily habitual activity at Baseline and Follow-up (all, $P \geq 0.505$).

4.2: Impact of HIIT on Aerobic Fitness Related Outcomes

Adherence to the 36 HIIT sessions was 93.6 ± 4.5% (34 ± 2 sessions). The average HR response of all training sessions attended was 157 ± 11 beats/min, which represented 84 ± 6% of pre-training peak HR. The mean weekly exercising HR (i.e., average of all 3 training sessions) was reduced following Week 6 versus Week 1, as well as following Week 13 versus Week 8 (i.e., when comparing training weeks with equivalent session durations and intensities, both $P<0.007$) (Figure 4.1).

All outcomes recorded during the aerobic fitness assessments are presented in Table 4.2. In summary, 11/18 (6 females♀) and 12/15 (7♀) participants at Baseline, 13/18 (7♀) participants following 6 weeks (HIIT group only), and 10/18 (5♀) and 13/15 (8♀) participants at Follow-up achieved a plateau in oxygen consumption in the HIIT and Control groups, respectively. Furthermore, 13/18 (7♀) and 11/15 (7♀) participants at Baseline, 16/18 (10♀) (HIIT only) participants following 6-weeks, and 18/18 (10♀) and 13/15 (8♀) participants at Follow-up, achieved all 3 criteria in the HIIT and Control groups, respectively. With respect to sex- and age-specific normative aerobic fitness

categories (35) for the 18 HIIT participants at Baseline (based on relative $\dot{V}O_2$peak): 7 (4♀) were classified as: 'Poor', 1 male was 'Fair' , 9 (5♀) were 'Good', and 1 female was 'Very Good'. For the 15 Control group participants: 7 (4♀) were 'Poor', 2 (1♀) were 'Fair', 3 females were Good', and 4 (1♀) were 'Very Good'. Following HIIT, 3 participants (1♀) improved fitness categories from 'Poor' to 'Fair', 2 females improved from 'Poor' to 'Good', 1 male improved from 'Poor' to 'Very Good', 1 male improved from 'Fair' to 'Very good', 2 female participants improved from 'Good' to 'Very good', and 3 participants (1♀) improved from 'Good' to 'Excellent'. For the Control group, there were no differences in PAP, absolute $\dot{V}O_2$peak, relative $\dot{V}O_2$peak, peak METs, peak HR, peak RER, peak RPE, VT (%$\dot{V}O_2$peak) or HR at the VT between Baseline and Follow-up.

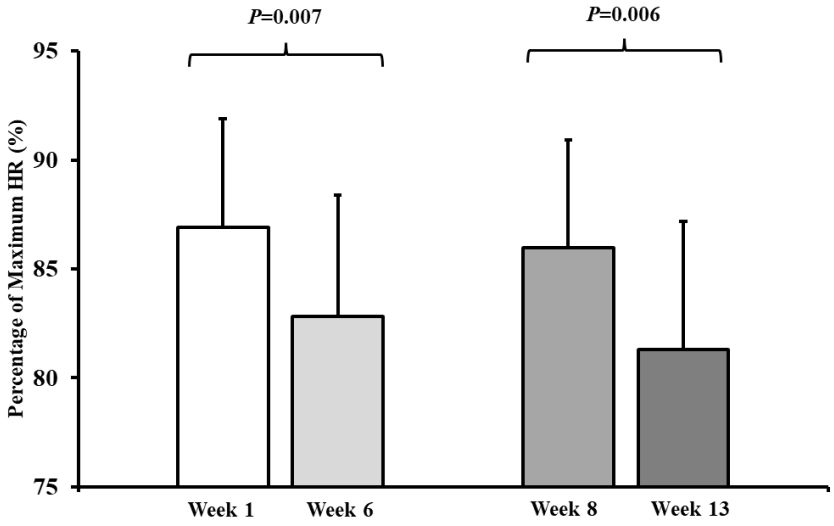

Figure 4.1. Average weekly training heart rates (i.e., average of all 3 weekly sessions), expressed as percentages of Baseline peak heart rate (HR), recorded following Weeks 1 and 6 (i.e., first half of training), as well as after Weeks 8 and 13 (i.e., second half of training) when participants were cycling at equivalent durations (2 × 20-minute sets) and intensities (100% PAP). Differences between Weeks 1 and 6, and Weeks 8 and 13 were evaluated using paired t-tests. Data presented as means ± standard deviations.

Table 4.1 High-intensity interval training (HIIT) and Control participant characteristics and habitual activity

	HIIT		Sex Effect *P*-Value (Effect Size)	Control		Group Effect *P*-Value (Effect Size)
	Baseline	**Follow-up**		**Baseline**	**Follow-up**	
Participant characteristics						
Body Mass (kg)						
Females	61 ± 7*	61 ± 6*	0.084 (0.175)	66 ± 8	65 ± 7	0.244 (0.047)
Males	86 ± 15	81±12		80 ± 12	79 ± 13	
Group	72 ± 17	70±14		71 ± 12	70 ± 12	
Body Mass Index (kg/m^2)						
Females	22.4 ± 1.9	22.6 ± 1.7	0.062 (0.201)	23.4 ± 2.8	22.8 ± 2.4	0.212 (0.053)
Males	26.3 ± 3.3	25.1 ± 3.4		24.2 ± 2.5	23.4 ± 2.8	
Group	24.1 ± 3.3	23.7 ± 2.8		23.5 ± 2.5	23.3 ± 2.5	
Habitual Activity						
Step Count (steps/day)						
Females	12586 ± 4174	11407 ± 4118	0.110 (0.172)	10770 ± 2337	9883 ± 1782	0.686 (0.006)
Males	11277 ± 1835	13063 ± 5142		12467 ± 4250	13263 ± 4122	
Group	12004 ± 3325	12089 ± 4490		11512 ± 3301	11332 ± 3355	
Sedentary Time (mins/day)						
Females	496 ± 83	508 ± 67	0.628 (0.017)	526 ± 91	501 ± 77	0.136 (0.078)
Males	513 ± 127	500 ± 156		551 ± 94	521 ± 75	
Group	504 ± 102	505 ±1 08		537 ± 90	509 ± 74	
Standing Time (mins/day)						
Females	390 ± 80	347 ± 83	0.164 (0.134)	339 ± 76	338 ± 60	0.558 (0.012)
Males	401 ± 116	398 ± 101		352 ± 63	351 ± 109	
Group	395 ± 94	366 ± 91		345 ± 69	344 ± 81	
LPA (mins/day)						
Females	88 ± 17	77 ± 22	0.174 (0.138)	78 ± 21	73 ± 17	0.396 (0.027)
Males	87 ± 24	103 ± 50		82 ± 27	94 ± 27	
Group	88 ± 20	87 ± 36		80 ± 23	82 ± 23	

	HIIT		Sex Effect *P*-Value (Effect Size)	Control		Group Effect *P*-Value (Effect Size)
	Baseline	**Follow-up**		**Baseline**	**Follow-up**	
MPA (mins/day)						
Females	60 ± 22	50 ± 27	0.614 (0.020)	42 ± 9	34 ± 11	0.898 (0.001)
Males	42 ± 17	52 ± 34		45 ± 27	47 ± 23	
Group	53 ± 22	51 ± 29		43 ± 18	40 ± 18	
VPA (mins/day)						
Females	4 ± 4	5 ± 6	**a**	4 ± 6	2 ± 1	**a**
Males	7 ± 7	4 ± 4		8 ± 7	11 ± 7	
Group	5 ± 5	5 ± 5		6 ± 7	6 ± 7	
MVPA (mins/day)						
Females	62 ± 25	55 ± 31	0.292 (0.085)	46 ± 13	38 ± 15	0.915 (0.000)
Males	46 ± 20	55 ± 33		53 ± 33	56 ± 28	
Group	55 ± 32	55 ± 31		49 ± 24	46 ± 22	

Sample size in each group: HIIT (Group: n=18, 10♀) and Control (Group: n=15, 9♀). Data are presented as Means ± Standard Deviations. LPA, light physical activity; MPA, moderate physical activity; VPA, vigorous physical activity; MVPA, moderate-to-vigorous physical activity. Group × Time interaction effects were assessed using a 2-way repeated measures analysis of variance with Bonferroni *post hoc* pairwise comparisons to determine within- and between-group differences. Time × Sex interaction effects were assessed using a 2-way repeated measures analysis of variance with Bonferroni *post hoc* pairwise comparisons to determine within- and between-sex differences in the HIIT group only. [a], between- (Mann-Whitney U) and within-participants (Wilcoxon signed-rank) non-parametric tests were used to assess vigorous physical activity. *, P<0.05 versus Baseline within the same group and/or sex. Effect sizes reported as Partial Eta Squared.

Furthermore, there were no differences in any aerobic fitness outcomes between the HIIT and Control groups and between sexes at Baseline ($P \geq 0.126$). In contrast, the HIIT group increased PAP (231 ± 62 to 267 ± 69 watts), absolute $\dot{V}O_2$peak (2.77 ± 0.84 to 3.10 ± 0.94 L/min, Figure 4.2A), relative $\dot{V}O_2$peak (38.1 ± 6.5 mL to 44.7 ± 8.0 mL/kg/min, Figure 4.2B), peak METs (11.1 ± 1.9 to 12.8 ± 2.3), and peak RER (1.19 ± 0.07 to 1.26 ± 0.11) from Baseline to Follow-up (all, $P \leq 0.029$). Additionally, the HIIT group demonstrated an increase in absolute $\dot{V}O_2$peak from Baseline and following 6-weeks, as well as following 6-weeks and Follow-up timepoints (Baseline: 2.77 ± 0.84, 6-week: 3.01 ± 0.89, Follow-up: 3.10 ± 0.94 L/min, all, $P \leq 0.006$) (Figure 4.2A). At Follow-up, the HIIT group also attained higher absolute $\dot{V}O_2$peak and peak METs compared to the Control group (both $P \leq 0.015$), with no other between-group effects observed (all, $P \geq 0.126$).

Males attained a higher absolute $\dot{V}O_2$peak at Baseline, Following 6-weeks, and Follow-up (all, $P \leq 0.001$). Both males (3.46 ± 0.55 to 3.99 ± 0.66 L/min) and females (2.21 ± 0.58 to 2.48 ± 0.50 L/min) in the HIIT group increased their absolute $\dot{V}O_2$peak from Baseline to Follow-up (both, $P \leq 0.004$). However, only males increased their absolute $\dot{V}O_2$peak between Baseline and 6-week measurements, as well as from 6-weeks to the Follow-up timepoints (Males, both P=0.040; Females, both $P \geq 0.064$). Although both sexes had higher absolute $\dot{V}O_2$peak following HIIT, the rise was greater in males (+0.52 ± 0.21 versus +0.18 ± 0.17 L/min, P=0.004). Additionally, there was an increase in peak RPE between Baseline and 6-week for males (P=0.010), with no other differences in RPE between- or within-sexes or time points (all, $P \geq 0.060$). There were no other Sex × Group interaction effects in the HIIT group (all $P \geq 0.061$).

Table 4.2 High-intensity interval training (HIIT) and Control aerobic fitness outcomes

	HIIT			Sex Effect *P*-Value (Effect Size)	Control		Group Effect *P*-Value (Effect Size)
	Baseline	**6-week**	**Follow-up**		**Baseline**	**Follow-up**	
Peak Aerobic Power (watts)							
Females	192 ± 48	210 ± 41	220 ± 43	0.347 (0.132)	218 ± 23	210 ± 28	**<0.001 (0.448)**
Males	288 ± 45	312 ± 45	325 ± 46		270 ± 43	270 ± 33	
Group	231± 62	256 ± 67	267 ± 69*		240 ± 42	234 ± 42	
Absolute $\dot{V}O_2$peak (L/min)							
Females	2.21 ± 0.58#	2.41 ± 0.49#	2.48 ± 0.50#*	**0.045 (0.357)**	2.39 ± 0.34	2.38 ± 0.32	**0.003 (0.279)**
Males	3.46 ± 0.55	3.75 ± 0.70*	3.99 ± 0.66*		3.18 ± 0.50	3.36 ± 0.39	
Group	2.77 ± 0.84	3.01 ± 0.89*	3.10 ± 0.94*		2.74 ± 0.57	2.77 ± 0.60	
Relative $\dot{V}O_2$peak (ml/kg/min)							
Females	36.0 ± 6.5	39.5 ± 5.5	40.4 ±6.0	0.061 (0.330)	37.3 ± 4.2	36.7 ± 4.2	**0.002 (0.288)**
Males	41.6 ± 5.6	45.9 ± 5.9	50.8 ± 6.3		40.2 ± 6.2	43.1 ± 5.8	
Group	38.1 ± 6.5	42.3 ± 6.4	44.7 ± 8.0*		38.3 ± 5.3	39.3 ± 5.7	
Peak METs							
Females	10.6 ± 2.0	11.4 ± 1.5	11.5 ± 1.7	0.064 (0.324)	10.6 ± 1.1	10.5 ± 12	**0.006 (0.247)**
Males	11.9 ± 1.6	13.1 ± 1.6	14.5 ± 1.8		12.2 ± 2.6	11.9 ± 1.4	
Group	11.1 ± 1.9	12.2 ± 2.0	12.8 ± 2.3*		11.3 ± 1.9	11.0 ± 1.4	
Peak HR (beats/min)							
Females	187 ± 7	187 ± 8	187 ± 8	0.293 (0.151)	189 ± 5	189 ± 6	0.335 (0.032)
Males	184 ± 16	184 ± 13	188 ± 14		189 ± 16	185 ± 14	
Group	186 ± 9	186 ± 12	188 ± 11		189 ± 11	187 ± 10	
Peak RER ($\dot{V}CO_2/\dot{V}O_2$)							
Females	1.20 ± 0.07	1.21 ± 0.07	1.26 ± 0.11	0.981 (0.003)	1.18 ± 0.10	1.18 ± 0.09	**0.028 (0.155)**
Males	1.18 ± 0.06	1.21 ± 0.09	1.26 ± 0.10		1.22 ± 0.11	1.16 ± 0.07	
Group	1.19 ± 0.07	1.21 ± 0.08	1.26 ± 0.11*		1.20 ± 0.10	1.17 ± 0.08	
Peak RPE							
Females	19.3 ± 1.3	19.6 ± 0.7	19.3 ± 1.1	**a**	19.4 ± 0.7	18.9 ± 0.9	**a**
Males	18.9 ± 1.1	19.9 ± 0.4*	19.9 ± 0.4		19.0 ± 1.2	18.8 ± 1.0	
Group	19.1 ± 1.2	19.7 ± 0.6	19.6 ± 0.9		19.3 ± 1.1	18.9 ± 0.9	

	HIIT		Sex Effect *P*-Value (Effect Size)	Control		Group Effect *P*-Value (Effect Size)
	Baseline	Follow-up		Baseline	Follow-up	
VT (%$\dot{V}O_2$peak)						
Females	75 ± 10	67 ± 7	76 ± 7		71 ± 9	70 ± 10	
Males	73 ± 8	71 ± 6	75 ± 8	0.565 (0.078)	78 ± 9	69 ± 11	0.133 (0.079)
Group	74 ± 9	69 ± 7	75 ± 7		74 ± 10	70 ± 10	
HR at VT (beats/min)						
Females	168 ± 11	161 ± 14	165 ± 14		161 ± 13	159 ± 11	
Males	163 ± 16	159 ± 18	171± 12	0.697 (0.050)	163 ± 21	161± 14	0.970 (0.000)
Group	165 ± 13	160 ± 15	168 ± 13		162 ± 16	160± 12	

Sample size in each group: HIIT (Group: n=18, 10♀) and Control (Group: n=15, 9♀). Data are presented as Means ± Standard Deviations. $\dot{V}O_2$peak, peak volume rate of oxygen consumption; $\dot{V}CO_2$, volume rate of carbon dioxide production; METs, metabolic equivalents of task; HR, heart rate; RER, respiratory exchange ratio; RPE, rating of perceived exertion; VT, Ventilatory Threshold. Group × Time interaction effects were assessed using a 2-way repeated measures analysis of variance with Bonferroni *post hoc* pairwise comparisons to determine within- and between-group differences. Time × Sex interaction effects were assessed using a 2-way repeated measures analysis of variance with Bonferroni *post hoc* pairwise comparisons to determine within- and between-sex differences in the HIIT group only. [a], between- (Mann-Whitney U) and within-participant (Wilcoxon signed-rank) non-parametric tests were used to assess peak RPE. There was an increase in peak RPE between Baseline and 6-weeks in males (P=0.010). There were no other significant within- or between-participant differences (all, $P \geq 0.060$). [#], P<0.05 versus males within the same group and timepoint. [*], P<0.05 versus Baseline within the same group and/or sex. Effect sizes reported as Partial Eta Squared.

4.3: Resting Hemodynamics and MSNA Outcomes.

Beat-by-beat blood pressure measurements were collected in 100% (18/18) and 93% (14/15) of participants at Baseline, as well as 94% (17/18) and 80% (12/15) of individuals at Follow-up for the Control and HIIT groups, respectively. As such, SBP, DBP, and MAP are only presented for the 16 participants (9♀) in the HIIT group and 12 participants (7♀) in the Control group that provided both Baseline and Follow-up data (Table 4.3). The HIIT and Control groups had similar Baseline resting blood pressure and HR (all, $P \geq 0.288$). There was a Group × Time interaction effect for resting HR ($P=0.039$). Specifically, there was a reduction in HR (61 ± 6 to 57 ± 7 beats/min) between Baseline and Follow-up in the HIIT group ($P=0.002$). The HIIT and Control groups had similar resting blood pressures at Follow-up (all, $P \geq 0.128$). There were no Sex × Time interaction effects within the HIIT group (all, $P \geq 0.132$).

The microneurography success rate was 80% (12/15) and 94% (17/18) at Baseline, as well as 77% (14/18) and 76% (10/15) at Follow-up for the Control and HIIT groups, respectively. Only complete MSNA data sets from the 14 participants (8♀) in the HIIT group and 10 participants in the Control group (6♀) are presented (Table 4.3). The HIIT and Control groups had similar MSNA burst frequency, MSNA burst incidence, normalized burst amplitude, and total normalized MSNA that were unchanged at Follow-up (all, $P \geq 0.158$).

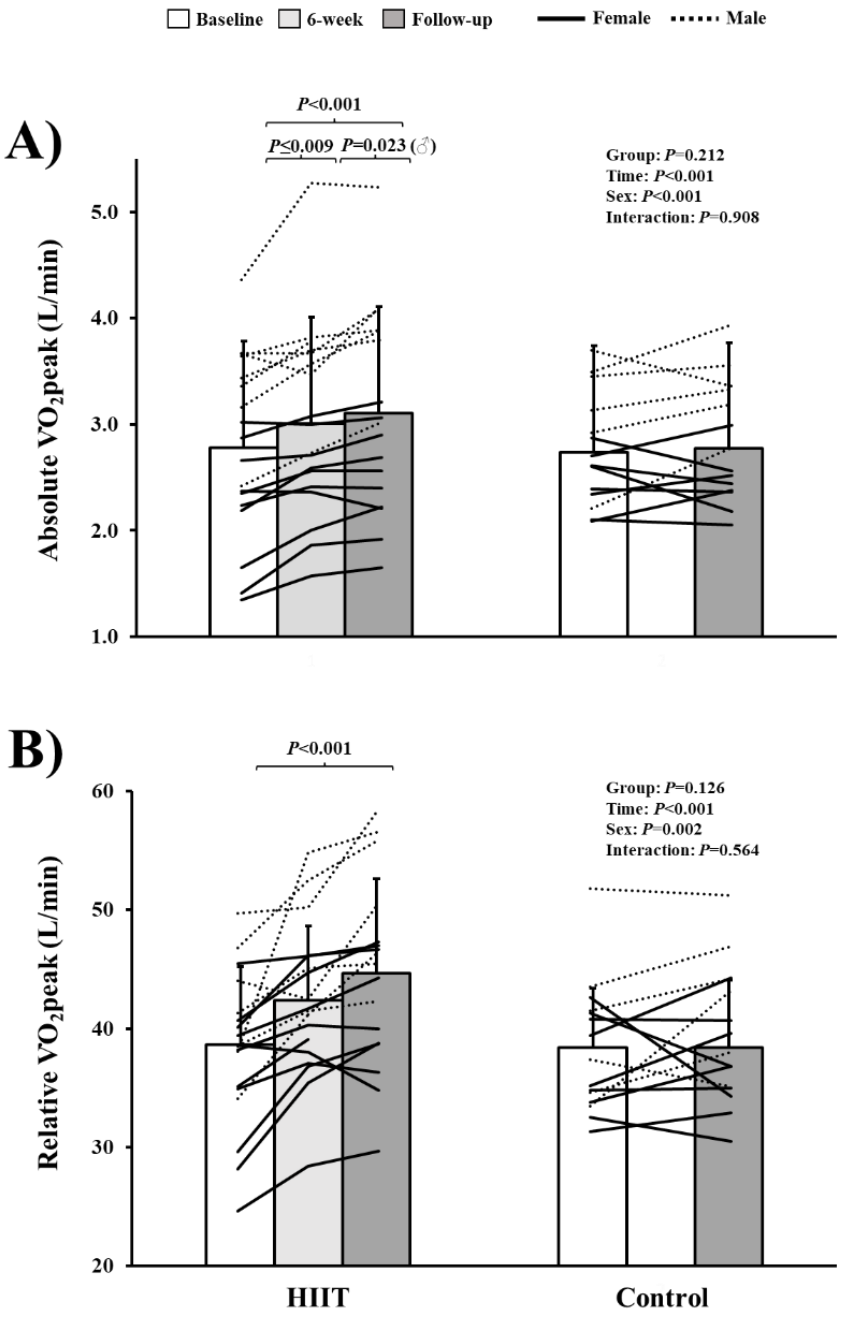

Figure 4.2. Absolute $\dot{V}O_2$peak (A) and relative $\dot{V}O_2$peak (B) outcomes for the high-intensity interval training (HIIT) group (left) and Control group (right) at Baseline (white bars), 6-weeks (light grey bars, HIIT only) and Follow-up (grey bars) timepoints. Solid and dashed lines represent individual female and male participants, respectively. Group × Time interactions were assessed using a 2-way repeated measures analysis of variance with Bonferroni *post hoc* pairwise comparisons. Sex × Time interactions were assessed using a 2-way repeated measures analysis of variance with Bonferroni *post hoc* pairwise comparisons within the HIIT group only. All group data are presented as Means ± Standard Deviations

4.4: Sympathetic Transduction

4.4.1: Baseline Sex Differences in Sympathetic Transduction.

The peak DBP responses to spontaneous bursts of MSNA (i.e., the signal averaging approach) were not different between sexes at Baseline (Males: 1.34 ± 1.24 mmHg versus Females: 1.79 ± 0.83 mmHg, P=0.31) (Figure 4.3A). Similarly, there were no sex differences observed when Baseline sympathetic transduction was analyzed via the regression slope method (Males: 0.012 ± 0.016 mmHg $(\%s)^{-1}$ versus Females: 0.006 ± 0.016 mmHg $(\%s)^{-1}$, P =0.47) (Figure 4.3.B). Data for this comparison was utilized from participants with complete Baseline data. Therefore, these numbers differ from the participants where we investigated Baseline versus Follow-up (n= 26, 15♀ versus n=24, 14♀).

There were no sex differences in the nadir response to cardiac cycles without bursts of MSNA at Baseline or Follow-up (both $P \geq 0.093$). As well, there were no sex differences in the number of cardiac cycles to peak DBP responses to spontaneous bursts of MSNA or nadir responses to heart beats without bursts of MSNA (both, $P \geq 0.211$).

Table 4.3 High-intensity interval training (HIIT) and Control resting hemodynamics and muscle sympathetic nerve activity outcomes.

	HIIT		Sex Effect *P*-Value (Effect Size)	Control		Group Effect *P*-Value (Effect Size)
	Baseline	**Follow-up**		**Baseline**	**Follow-up**	
Resting Hemodynamics						
Heart Rate (beats/min)						
Females	62 ± 5	59 ± 4	0.654 (0.007)	61 ± 6	60 ± 9	**0.039 (0.139)**
Males	60 ± 7	56 ± 9		58 ± 6	59 ± 2	
Group	61 ± 6	57 ± 7*		60 ± 6	59 ± 7	
Systolic Blood Pressure (mmHg)						
Females	111 ± 11	112 ± 9	0.301 (0.034)	113 ± 10	112 ± 8	0.399 (0.025)
Males	118 ± 7	113 ± 7		113 ± 10	113 ± 6	
Group	114 ± 10	112 ± 9		113 ± 9	112 ± 7	
Diastolic Blood Pressure (mmHg)						
Females	63 ± 9	64 ± 6	0.437 (0.020)	64 ± 7	64 ± 8	0.611 (0.009)
Males	60 ± 4	59 ± 7		64 ± 9	61 ± 5	
Group	61 ± 7	62 ± 7		64 ± 7	63 ± 6	
Mean Arterial Blood Pressure (mmHg)						
Females	79 ± 9	80 ± 7	0.132 (0.072)	78 ± 9	80 ± 6	0.628 (0.008)
Males	79 ± 4	76 ± 7		81 ± 5	79 ± 5	
Group	79 ± 7	78 ± 7		79 ± 7	80 ± 6	
MSNA Burst Frequency (bursts/min)						
Females	11 ± 5	13 ± 6	0.473 (0.023)	13 ± 8	13 ± 5	0.984 (0.000)
Males	20 ± 9	19 ± 10		16 ± 3	16 ± 5	
Group	15 ± 8	16 ± 8		14 ± 7	14 ± 5	
Muscle Sympathetic Nerve Activity						
MSNA Burst Incidence (bursts/100 heartbeats)						
Females	19 ± 8	23 ± 11	0.511 (0.019)	17 ± 9	21 ± 7	0.588 (0.014)
Males	31 ± 14	33 ± 13		29 ± 9	27 ± 8	
Group	24 ± 13	27 ± 13		21± 10	24 ± 7	

	HIIT		Sex Effect *P*-Value (Effect Size)	Control		Group Effect *P*-Value (Effect Size)
	Baseline	**Follow-up**		**Baseline**	**Follow-up**	
Resting Hemodynamics						
Normalized Burst Amplitude (% maximum)						
Females	45 ± 9	48 ± 7	0.751 (0.004)	54 ± 11	48 ± 10	0.815 (0.003)
Males	46 ± 9	41± 8		46 ± 5	48 ± 5	
Group	45 ± 9	45 ± 8		51 ± 10	48 ± 8	
Total MSNA (% maximum · bursts/min)						
Females	520 ± 250	663 ± 348	0.331 (0.043)	540 ± 263	635 ± 266	0.918 (0.001)
Males	840 ± 391	732 ± 270		745 ± 120	772 ± 290	
Group	661 ±3 49	693 ± 307		615 ± 238	690 ± 269	

Sample size for SBP, DBP, and MAP: HIIT (Group: n=18, 10♀) and Control (Group: n=15, 9♀). Sample size for MSNA outcomes: HIIT (Group: n=14, 8♀) and Control (Group: n=10, 6♀). Data are presented as Means ± Standard Deviations. MSNA, Muscle Sympathetic Nerve Activity. Group × Time interaction effects were assessed using a 2-way repeated measures analysis of variance with Bonferroni *post hoc* pairwise comparisons to determine within- and between-group differences. Time × Sex interaction effects were assessed using a 2-way repeated measures analysis of variance with Bonferroni *post hoc* pairwise comparisons to determine within- and between-sex differences in the HIIT group only. Between- (Mann-Whitney U) and within-participants (Wilcoxon signed-rank) non-parametric tests were used to assess diastolic blood pressure variability. No other significant differences were observed between- or within-participants (all, $P \geq 0.132$). *, $P < 0.05$ versus Baseline-within the same group and/or sex. Effect sizes reported as Partial Eta Squared.

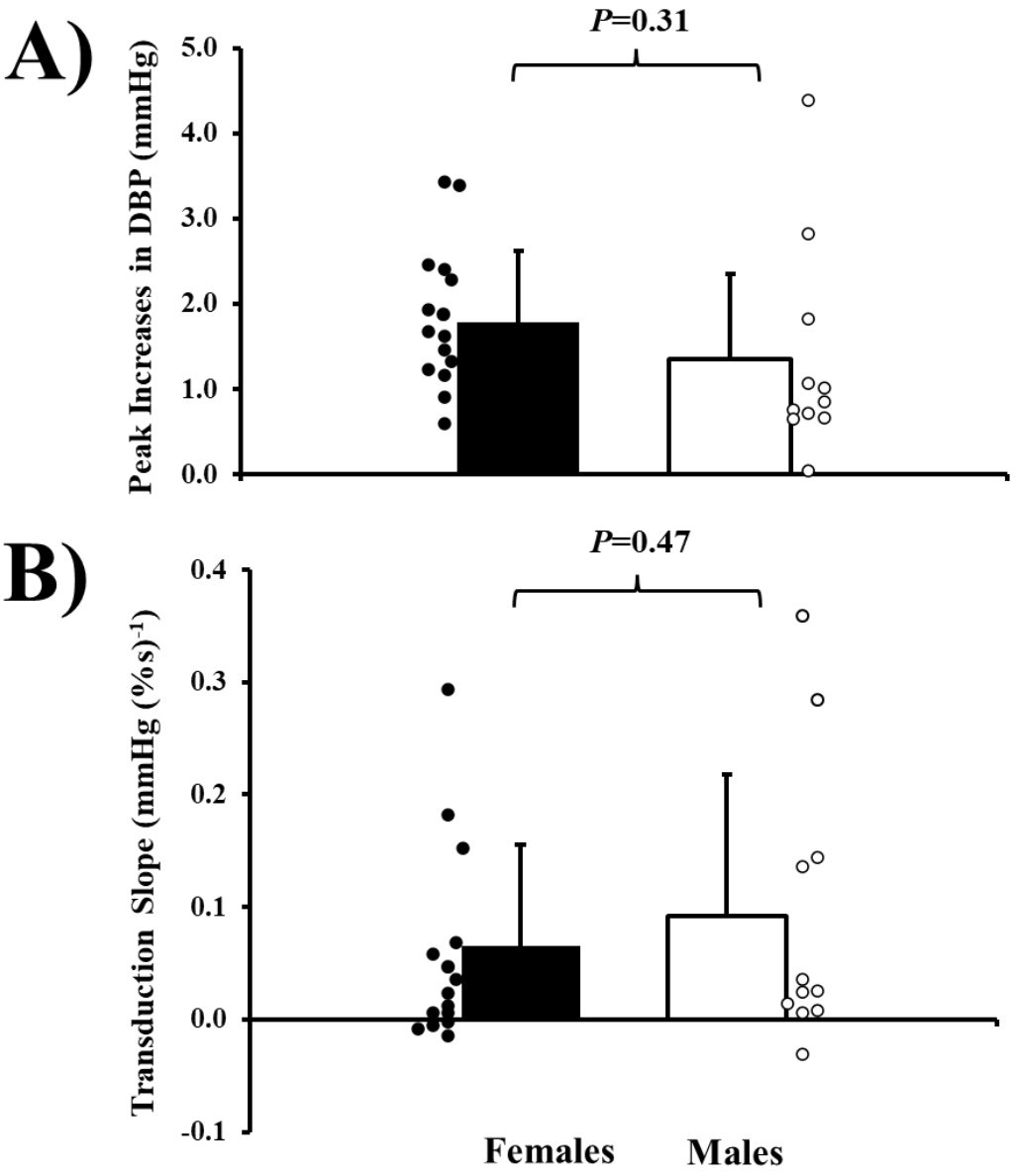

Figure 4.3. Comparison of sympathetic transduction between sexes using (A) the peak increases in diastolic blood pressure (DBP) following spontaneous bursts of muscle sympathetic nerve activity (MSNA) (i.e., the signal averaging approach) and (B) the slope calculated for the relationship between DBP and %MSNA normalized burst amplitude (i.e., the regression slope method). Baseline data from both the High-Intensity Interval Training and Control groups were used for these comparisons. Female data are presented in black and male data are presented in white. Individual data for females (n=15) and males (n=11) are presented by white circles. Differences between sexes were assessed using independent sample t-tests.

4.4.2: Impact of HIIT on Sympathetic Transduction

All sympathetic transduction data are presented in Table 4.4. No Group × Time interaction effect was observed for sympathetic transduction between Baseline and Follow-up ($P \geq 0.090$) (Figure 4.4). No Sex × Time interaction effect was observed for sympathetic transduction between Baseline and Follow-up using the signal averaging method ($P = 0.385$). However, there was a greater nadir DBP response (i.e., a larger reduction in DBP) to cardiac cycles without bursts of MSNA at Follow-up versus Baseline in the HIIT group ($P = 0.019$) (Figure 4.4). However, this group-level response was primarily driven by females ($P = 0.016$) compared to males ($P = 0.345$).

4.4.3 Relationships between Aerobic Fitness and Sympathetic Transduction

In the entire cohort (i.e., both sexes and groups combined), there were no correlations between Baseline aerobic fitness (relative or absolute $\dot{V}O_2$peak) and sympathetic transduction when assessed using either the signal averaging (both, $R \leq 0.045$ and $P \geq 0.509$, for relative and absolute $\dot{V}O_2$peak, respectively) or regression slope method of sympathetic transduction (both, $R \leq 0.287$ and 0.205 $P \geq 0.122$, for relative and absolute $\dot{V}O_2$peak, respectively).

Furthermore, there were no associations using either aerobic fitness outcomes when assessed separately for males using the signal averaging (both, $R \leq -0.140$ and $P \geq 0.200$) or regression slope method (both, $R \leq 0.305$ and $P \geq 0.289$) for relative and absolute $\dot{V}O_2$peak, respectively. Similarly, aerobic fitness was not related to transduction in females when investigated using the signal averaging (both, $R \leq 0.276$ and $P \geq 0.267$) or regression slope approaches (both, $R \leq 0.023$ and $P \geq 0.941$), for relative and absolute $\dot{V}O_2$peak, respectively.

Table 4.4. High-intensity interval training (HIIT) and Control sympathetic transduction outcomes.

	HIIT		Sex Effect *P*-Value (Effect Size)	Control		Group Effect *P*-Value (Effect Size)
	Baseline	Follow-up		Baseline	Follow-up	
Peak DBP (mmHg)						
Females	1.5 ± 0.5	2.1 ± 1.6	0.385 (0.036)	1.8 ± 1.2	1.3 ± 0.5	0.090 (0.144)
Males	1.5 ± 1.3	1.5 ± 0.7		1.7 ± 1.0	1.2 ± 0.6	
Group	1.5 ± 0.9	1.8 ± 1.3		1.8 ± 1.1	1.3 ± 0.5	
Cardiac Cycles to Peak DBP						
Females	5 ± 1	6 ± 2	**a**	5 ± 3	6 ± 2	**a**
Males	5 ± 1	7 ± 3		5 ± 1	6 ± 2	
Group	5 ± 1	6 ± 2*		6 ± 4	6 ± 2	
Nadir DBP (mmHg)						
Females	-0.38 ± 0.17	-0.60 ± 0.21*	**b**	-0.49 ± 0.55	-0.33 ± 0.23	**b**
Males	-0.61 ± 0.26	-0.58 ± 0.32		-0.94 ± 0.69	-0.44 ± 0.13	
Group	-0.50 ± 0.25	-0.59 ± 0.25*		-0.66 ± 0.64	-0.38± 0.20	
Cardiac Cycles to Nadir DBP						
Females	6 ± 3	6 ± 2	**c**	7 ± 4	6 ± 3	**c**
Males	6 ± 2	6 ± 2		10 ± 3	7 ± 2	
Group	6 ± 2	6 ± 2		8 ± 4	6 ± 3	

Sample size in each group: HIIT (Group: n=14, 8♀) and Control (Group: n=10, ♀6). Data are presented as Means ± Standard Deviations. DBP, diastolic blood pressure; MSNA, Muscle Sympathetic Nerve Activity. Group × Time interaction effects were assessed using a 2-way repeated measures analysis of variance with Bonferroni *post hoc* pairwise comparisons to determine within- and between-group differences. Time × Sex interaction effects were assessed using a 2-way repeated measures analysis of variance with Bonferroni *post hoc* pairwise comparisons to determine within- and between-sex differences in the HIIT group only. Between- (Mann-Whitney U) and within-participants (Wilcoxon signed-rank) non-parametric tests were used to assess the number of cardiac cycles to peak DBP, nadir DBP, the number of cardiac cycles to nadir DBP. [a], an increase in the number of cardiac cycles to peak DBP was observed within the HIIT group (P=0.012). No other significant differences were observed between- or within-participant (all, $P \geq 0.112$). [b], A reduction in the nadir responses to cardiac cycles without MSNA bursts was observed in the HIIT group (P=0.019), which was driven by females (P=0.016). No other differences were observed between- or within-participants (all, $P \geq 0.345$). [c], no differences were observed between- or within-participants (all, $P \geq 0.092$). *, $P<0.05$ versus Baseline-within the same group and/or sex. Effect sizes reported as Partial Eta Squared.

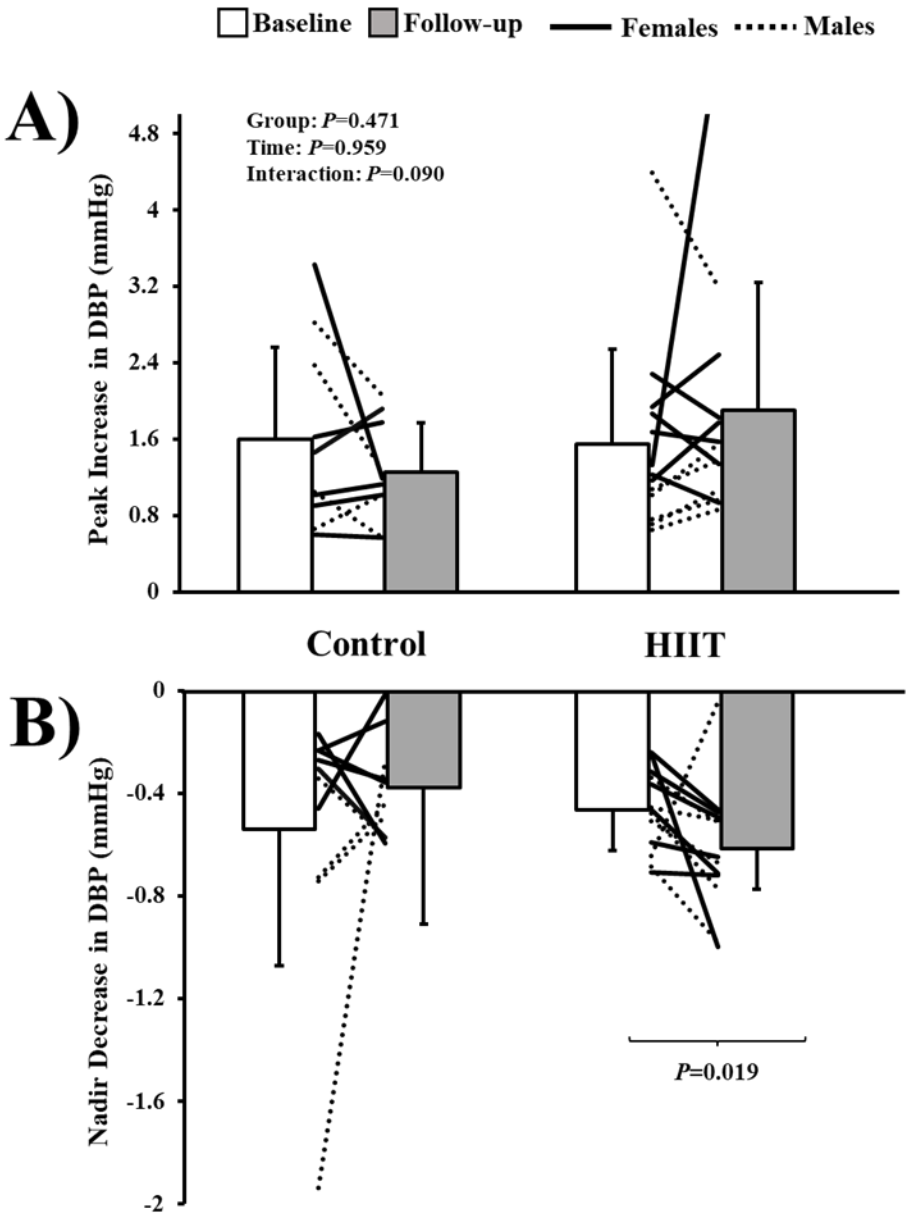

Figure 4.4. A) Peak increases in diastolic blood pressure (DBP) following spontaneous bursts of muscle sympathetic nerve activity (MSNA). B) Nadir decreases in DBP following cardiac cycles without MSNA bursts. Baseline data are presented in white and Follow-up data in grey. Individual data for Control (n=10, 6♀) and HIIT (n=14, 8♀) are presented between the group means. Females are presented as solid lines and males as dotted lines. Group × Time interactions were assessed using a 2-way repeated measures analysis of variance with Bonferroni *post hoc* pairwise comparisons. Sex × Time interactions were assessed using a 2-way repeated measures analysis of variance with Bonferroni *post hoc* pairwise comparisons within the HIIT group only. Between- (Mann-Whitney U) and within-participant (Wilcoxon signed-rank) non-parametric tests were used to assess nadir non-burst decreases in DBP.

Chapter 5: Discussion

The primary purpose of this study was to examine potential sex differences regarding the impact of a 12-week HIIT intervention on sympathetic transduction in younger, healthy adults. Based on previous HIIT-based studies of similar duration, (6, 66, 150, 159, 165), it was hypothesized that aerobic fitness would increase following our 12-week HIIT protocol. Considering the inverse relationship previously observed between aerobic fitness and sympathetic transduction in younger males (120), and the lack of relationship reported in younger females (152), it was hypothesized that HIIT-induced improvements in aerobic fitness would result in decreased sympathetic transduction in males only. Lastly, based on a study previously conducted in our lab (123), I hypothesized that there would be no sex difference in sympathetic transduction regardless of the assessment method used [i.e., signal averaging (61) versus regression-based approach (18)]. In agreement with the first hypothesis, aerobic fitness improved in the HIIT group with no corresponding changes observed in the Control group (Figure 4.2). However, these HIIT-mediated improvements in aerobic fitness did not alter sympathetic transduction in either of the sexes (Figure 4.4). In agreeance with the last hypothesis, it was observed that both methods of quantification revealed no sex differences in sympathetic transduction. The results of this study provide the first longitudinal investigation into the impact of HIIT on sympathetic transduction as well as the first to explore associated sex differences.

5.1: Impact of HIIT on Aerobic Fitness

As indicated above, the HIIT group attained improvements in aerobic fitness following the 12-week HIIT intervention (Figure 4.2). The independent impact of HIIT

on aerobic fitness was strengthened by the consistency of habitual LPA (89±20 versus 86±36 mins/day), MPA (51±22 versus 51±28 mins/day), and VPA (5±5 versus 5±5 mins/day) between the Baseline and Follow-up timepoints, respectively (Table 4.1). In addition to having similar relative $\dot{V}O_2$peak values at Baseline (HIIT: 38.1 ± 6.5 versus Control: 38.3 ± 5.3 ml/kg/min), there was a similar number of participants in each aerobic fitness category (HIIT: 7 'Poor', 1 'Fair', 9 'Good', and 2 'Very Good', Control: 7 'Poor', 2 'Fair', 3 'Good', 4 'Very Good'). The HIIT group revealed 15±11% and 17±15% improvements in relative and absolute $\dot{V}O_2$peak, respectively. Interestingly, most of these improvements (10±12% in absolute $\dot{V}O_2$peak) were attained during the first 6 weeks of training, indicative that a plateau occurred during the first half of training. While the HIIT protocol contained increases in both intensity and duration throughout the 12-weeks, these progressions were insufficient to produce further enhancements in aerobic fitness during the second half of training. The reason for this plateau may be due to the Baseline fitness levels of the participants. Specifically, when divided into individual Baseline aerobic fitness categories, HIIT participants originally rated as 'Poor' and 'Fair' observed greater improvements in relative $\dot{V}O_2$peak than those who began training in the 'Good' and 'Very Good' categories after the first [n=9, 4♀, 18 ± 14 ('Poor/'Fair') versus 6 ± 6% ('Good'/'Very Good')], and second [n=9, 6♀, 7 ± 7 ('Poor/'Fair') versus 3 ± 7% ('Good'/'Very Good')] half of training. This suggests that there may have been a ceiling effect for aerobic fitness improvements in those starting with higher baseline values. Future research should investigate optimal strategies for increasing modifiable training variables (i.e., intensity and/or duration) in similar HIIT

protocol to maximize aerobic fitness improvements in those starting with higher starting levels.

There are both central and peripheral physiological factors that may have contributed to the observed HIIT-induced increases in $\dot{V}O_2$peak. These include: enhancement to peak stroke volume and cardiac output (central), hemoglobin mass (i.e., O_2 carrying capacity) (central), peripheral vascular adaptations and/or skeletal muscle mitochondrial biogenesis (11, 136). Considering central adaptations, peak HR was unchanged following HIIT, which disagrees with previous literature that exercise training (MICT) may reduce peak HR in healthy young adults (70, 151, 169). In addition, previous HIIT interventions have demonstrated the capacity to improve stroke volume (43, 76, 100, 146, 148, 165, 174) through increases in end-diastolic volume (43, 146), plasma volume (174), blood volume (165), left ventricle mass (100, 148), as well as left ventricular contractility (76) following ≥ 2 weeks of HIIT training consisting of cycling in sedentary young females (148), sedentary young males (100), and untrained healthy young males (43, 174). Although not directly measured, this research may help to identify potential central adaptions responsible for the increase in $\dot{V}O_2$peak following HIIT.

Peripheral vascular and/or skeletal muscle adaptations may also have contributed to the HIIT-induced rise in aerobic fitness. Specifically, increases in mitochondrial content (78) and function (skeletal muscle oxidative capacity) (20) have been observed following ≥2 weeks of HIIT (78) and low-volume sprint interval training (20) in active but untrained healthy young (78) and older (28) adults. These studies show that increased mitochondrial activity may facilitate enhancements in oxygen extraction (↑ peak arterial-

venous O_2 difference). In addition, peripheral vascular improvements may contribute to the increases in aerobic fitness through enhanced oxygen transport via increased capillarization and angiogenesis in skeletal muscles. Specifically, 6-weeks of interval training has been shown to enhance angiogenesis in healthy young males (16). As well, increases in muscle capillary density were observed following 6-weeks of low volume interval training in recreationally active (143) and sedentary (29) young males. This provides evidence that peripheral adaptations to HIIT training may be responsible for the increase in aerobic fitness (via ↑ peak arterial-venous O_2 difference) in our sample. Overall, it can be said that the improvements in aerobic fitness attained following HIIT may have resulted from a combination of both central and peripheral adaptations.

5.2: Impact of Sex on Aerobic Fitness and HIIT-mediated Improvements

It has been established that males have greater absolute and relative aerobic fitness compared to females (38). In agreeance, the current study demonstrated that males had higher absolute $\dot{V}O_2$peak values at all timepoints compared to females (Table 4.2). Interestingly, both sexes exhibited improvements in absolute $\dot{V}O_2$peak between Baseline and Follow-up (Figure 4.2). However, males observed a larger increase in absolute $\dot{V}O_2$peak following HIIT (9±5%) compared to females (4±4%). When dividing participants by sex and into the less fit ('Poor' and 'Fair' categories) and more fit ('Good' and 'Very Good') groups, a similar relationship to the whole group was observed such that males and females in the 'less fit' category exhibited greater increases in aerobic fitness versus those who are the 'more fit' category. It should also be noted that in the HIIT group, there were more females classified with 'Good' and 'Very Good' (females: n=6 vs males: n=3) fitness versus 'Fair' and 'Poor' fitness (females: n=4 vs males: n=5).

These sex differences in aerobic fitness improvements align with an 8-week cycling-based HIIT study by Weber et al. (167) in a similarly aged and aerobically fit group to the current cohort. However, the study conducted by Weber and colleagues (167) only observed an increase in aerobic fitness in males, which is inconsistent with the findings from this study. The reason for these sex differences may be due to a variety factors. Firstly, females have lower absolute hemoglobin concentrations compared to males (1, 36, 39, 74, 108, 164), which may attenuate the magnitude of arterial oxygen saturation and delivery to the active skeletal muscles. In addition, females have lower absolute left ventricular end diastolic volume and blood volume (13, 39) which may contribute to a smaller absolute stroke volume and cardiac output.

Although literature is sparse, one study investigated the differences in cardiovascular adaptions to 1 year of endurance training in 'sedentary' healthy adults (75). Howden and colleagues measured $\dot{V}O_2$max and left ventricular mass, following 1-year of endurance training (Protocol details can be found here: (5)). Interestingly, the attained $\dot{V}O_2$max improvement in females plateaued after 3 months of training compared to males who had continual improvements after 3 and 9 months (75). A similar trend occurred with increases in LV mass throughout the training protocol such that males had greater improvements compared to females. The results of this study agree with our findings that females observed a plateau in improvements compared to males who had continuous fitness growth. As well, the results of the Howden study may provide a potential explanation for greater improvements in males versus females through higher LV mass adaptations to training, although this study implemented a 1-year endurance intervention in young-middle-aged adults. Future research should further investigate the

specific physiological differences between sexes that account for differences in aerobic fitness improvements following HIIT.

5.3: Impact of HIIT on Resting Hemodynamics

In addition to investigating aerobic fitness, the impact of HIIT on resting hemodynamics was also explored. There were no changes in resting SBP, DBP or MAP in either sex following HIIT. This is consistent with studies that observed no changes in resting blood pressure following 8 weeks (5 days/week) of high-intensity interval cycle training in healthy young males (100), 6 weeks (≤4 days/week) of vigorous-intensity aerobic cycling training (57) and sprint interval cycling training in (129) in young healthy adults. Interestingly, there was a reduction in resting HR following HIIT. This finding is inconsistent with previous literature that has observed no effect of 2-3 weeks of HIIT on resting HR in young healthy adults (7) and 6 weeks of low volume sprint interval training in healthy untrained adults (129). However, the improvements attained in this study may be due to increases in stroke volume following training (29, 67, 76, 138, 146). Specifically, a study that investigated the impact of 8-weeks (5 days/week) of low volume cycling HIIT in healthy sedentary males observed a reduction in resting HR, as well as increases in stroke volume with no improvements in other resting hemodynamics (100). Another potential mechanism for the HIIT-induced bradycardia is enhanced parasympathetic regulation that had been observed to increase following endurance training in healthy young males (145, 149, 176) and middle aged healthy adults (58). In addition, our study demonstrated a reduction in training HR following Week 6 versus Week 1, as well as following Week 13 versus Week 8 (i.e., when comparing training weeks with equivalent session durations and intensities (Figure 4.1). This shows that for a

given intensity of exercise, participants worked at a lower exertion level following training, suggesting a cardiorespiratory adaption to training.

5.4: Impact of Sex on Resting MSNA and Sympathetic Transduction

The literature surrounding whether sex differences exist in resting MSNA and sympathetic transduction is controversial (72). In this study, there were no sex differences in MSNA burst frequency, burst incidence, relative burst amplitude, or total MSNA (Table 4.3). These similar findings between younger males and females corresponds with previous literature that observed no sex differences in MSNA burst frequency or burst incidence in young healthy adults (13, 34, 82, 112, 163). On the contrary, the lack of a sex difference in this study contradicts other reports that observed higher MSNA burst frequency and incidence in young healthy males compared to females (18, 63, 71, 113, 135). Overall, it is still unclear whether there is a sex difference in resting MSNA although, the vascular influence of this activity may differ between sexes.

Including Baseline MSNA and DBP data from participants in both groups (n=26, 15♀), no sex differences in sympathetic transduction were observed when assessed using either the regression slope method (18) or the signal-averaging approach (61). This agrees with the study previously done in our lab that observed no sex differences in sympathetic transduction using both approach in both younger (n=32, 12♀) and older (n=20, 7♀) adults (123).The lack of difference using the regression slope method contrasts with Briant and colleagues (18) that observed larger transduction in younger males than females. This inconsistency may be due to corresponding differences in resting MSNA levels. Specifically, the present study included males and females with similar MSNA burst frequencies, whereas Briant and colleagues (18) reported higher

levels in their young males. It has been suggested that an inverse relationship exists between resting MSNA burst frequency and sympathetic transduction in males, but not females (135). If our study demonstrated a sex difference in burst frequency, the likelihood of observing a corresponding sex difference in sympathetic transduction using the regression slope method may be plausible. The contradiction between literature and our results when using the regression slope method differs from the comparison to research using the signal-averaging method. With respect to the lack of sex difference reported using the signal-averaging method, the current study supports findings of studies that observed no sex differences in the peak MAP response (34, 135, 163), or the nadir LVC response (i.e. inverse of vascular resistance) following bursts of MSNA (71), as well as nadir MAP responses to cardiac cycles absent of MSNA bursts in healthy young adults (163). Furthermore, divergent findings regarding sex differences in sympathetic transduction when stress-induced methods have been observed. For instance, Coovadia and colleagues (33) reported that young males had higher MAP responses to cold pressor test-induced increases in MSNA compared to young females (33), whereas no sex differences were observed when sympathetic transduction was assessed via a fatiguing isometric handgrip task (quantified as the slope of the ΔDBP/Δtotal MSNA activity relationship) (79). To summarize, most of the available evidence supports the current study that no sex differences exist in spontaneous sympathetic transduction in young healthy adults, regardless of the assessment method used.

5.5: Impact of Sex on the Relationship Between Aerobic Fitness and Transduction

To directly compare against previous studies that assessed the relationship between aerobic fitness and signal-averaged sympathetic transduction in younger males

(120) and females (152), these same associations were compared using the compiled Baseline data from both groups. In the current study, there were no relationships between aerobic fitness and sympathetic transduction, at the group level or when explored within each of the sexes. Furthermore, it did not matter whether aerobic fitness was quantified using absolute or relative $\dot{V}O_2$peak, nor if sympathetic transduction was assessed via the regression slope or signal averaging approaches. In agreement with our hypothesis, the lack of a relationship between aerobic fitness and sympathetic transduction in females is supported by Stickford et al. (152), whose participants had similar mean and ranges of relative $\dot{V}O_2$peak to the current study. Overall, more research needs to be done in female populations (both younger and peri/post menopausal) to investigate the relationship between aerobic fitness and sympathetic transduction and how it changes with age.

Contrary to our hypothesis, the lack of a relationship between aerobic fitness and sympathetic transduction in younger males contradicts previous research that observed an inverse relationship in younger males (120) and older males (122). The discrepancy with O'Brien et al. (120) may be due to the higher average and/or larger range of relative $\dot{V}O_2$peak values in their study [47.1±8.1 ml/kg/min (Range: 35-65 ml/kg/min) (120)] versus the current study [41.6±5.6 (Range: 34-50 ml/kg/min)]. Lastly, the lack of a relationship between aerobic fitness and sympathetic transduction reported in the present subset of males does not support the lower sympathetic transduction observed by Notarius et al. (115) in their 'more aerobically fit' cohort of middle-aged males assessed via the relationship between MSNA burst frequency and forearm vascular resistance responses to a graded lower body negative pressure protocol. This may be due to the notion that stress-induced versus spontaneous transduction may not provide the same

information. Specifically, the increase in blood pressure associated with sympathetic stressors (e.g., lower body negative pressure, cold pressor tests, and/or fatiguing handgrip exercise) may result in increases in blood pressure inducing baroreflex-mediated changes in MSNA thereby influencing the measurement of sympathetic transduction (160). To summarize, aerobic fitness does not appear to influence spontaneous sympathetic transduction in young females. However, the relationship in males is inconsistent and requires future investigations with a larger range of aerobic fitness levels to confirm, or refute, the current findings (i.e., no association between aerobic fitness and sympathetic transduction).

5.6: Impact of HIIT on Resting MSNA and Sympathetic Transduction

The impact of HIIT on resting MSNA and sympathetic transduction may further highlight the relative importance of aerobic fitness (or lack thereof) on sympathetic control of the vasculature. The 12-week HIIT intervention did not impact resting MSNA burst frequency or incidence (Table 4.3). This disagrees with a recent study that investigated the effect of a 6-week (2 days/week) HIIT program on MSNA in middle aged males with essential hypertension and age-matched normotensive controls (42). This HIIT protocol consisted of 2-3 × 5 min (weeks 1 and 2) of 30 s of low-intensity cycling (30–80 W), 20 s of moderate-intensity cycling (50–120 W), and 10 s of maximal sprint efforts (>300 W) (42). Specifically, this study observed post-HIIT reductions in MSNA burst frequency (both groups) and burst incidence (only normotensive) (42). However, the results of our study agree with the following studies. One study observed no change in resting MSNA (burst frequency and incidence) following 8-weeks (4 days/week) of moderate-intensity continuous training (40 minutes a day at 60%-75%

(increased throughout intervention) of pretraining $\dot{V}O_2max$) in young healthy males (154). Another study observed no change in resting MSNA following 4-weeks (3 days/week) of moderate-intensity continuous training (30 minutes at ≥70% of peak HR) in young healthy males (32). Lastly, one study observed no changes in resting MSNA (burst frequency) following 8 weeks (4 days/week) of aerobic exercise (either cycling or running for 20-60 (increased throughout intervention) minutes/day at 80% of peak HR) in young healthy adults (132).

Overall, it has been shown that exercise training does not impact resting sympathetic vasoconstrictor outflow in young healthy adults, although most of this research has been conducted only in males and future research should document whether sex differences exist. In our HIIT group, the peak DBP responses to spontaneous MSNA bursts were unaltered following training (Figure 4.4A). This is in contrast with previous research in male Fischer rats that observed a reduction in noradrenaline-mediated α_2-adrenoreceptor vasoconstriction in resistance arterioles (measure of sympathetic transduction) following moderate-intensity continuous training (40). Their training protocol consisted of treadmill running at 15 m/min up a 15° incline, 1 h/day, 5 days/week for 10–12 weeks. It should be noted that although this study had a similar intervention duration to our current project, relative to the lifespan of a rat [~3 years (144)], a much larger percentage of their life span (i.e., ~8% of a rats life versus ~0.25% of a humans'). As such, adaptations to training as observed in this study may take longer to attain in humans. In addition, the results of the present study agreed with a previous report that investigated the influence of 11-16 weeks (3-4 days/week) of training at 50-70% of HR reserve (using various modalities of exercise) on sympathetic transduction

(signal averaging approach) in pregnant younger females (147). The Skow et al. (147) study observed no differences in sympathetic transduction with training. However, they observed blunted transduction in the Control group. This may indicate that training increased sympathetic transduction, thereby reducing the changes in sympathetic transduction associated with entering the third trimester of pregnancy. Although both the studies (40, 147) investigated the influence of aerobic training on sympathetic transduction, the physiological differences between pregnant females, Fischer male rats, and healthy young adults makes direct comparisons challenging. Overall, although the HIIT protocol used in this project increased aerobic fitness, perhaps greater improvements and/or a longer durations of training are required to observe an impact on factors known to decrease sympathetic transduction such as reduced α-adrenergic receptor sensitivity (40) or enhanced β_2-adrenergic receptors or sensitivity (increased vasodilation) (92).

Interestingly, nadir decreases in DBP following cardiac cycles absent of MSNA bursts were greater following HIIT, but unchanged in the Control group (Figure 4.4B). This may be due to aerobic fitness-mediated enhancements in vasodilatory signalling mechanisms (131). As well, this relationship was primarily driven by females in the HIIT group. The potential mechanism for this could be enhanced vasodilatory function (via flow mediated dilation), which have been shown to improve with HIIT in young healthy females (26) and males (130, 173) although research is limited.

5.7: Study Strengths and Limitations

This study was the first longitudinal investigation into aerobic training-induced changes in sympathetic transduction in healthy adults and to explore potential sex

differences. Although previous studies have explored the impact of aerobic training in rats (40) and prenatal during pregnancy (147), the results of this study can act as a basis for future studies to investigate these relationships in additional populations (e.g., older adults, patients with hypertension, etc.). As well, the inclusion of a Control group increased the strength of this study by further demonstrating that any relationships reported following HIIT were due to the intervention *per se* rather than other potential confounding factors [i.e., increases in habitual physical activity (121, 152, 166) or differences in starting aerobic fitness levels]. The Control group also provided the opportunity to explore the day-to-day reproducibility of sympathetic transduction, which based on qualitative observations, appears to exhibit large intra-individual variability (Figure 4.4). This study is also strengthened by investigating sex differences in both sympathetic transduction and aerobic fitness following HIIT.

This study was limited by an ability to standardize resting conditions while recording MSNA and beat-by-beat systemic hemodynamics. While we attempted to control for exercise, hydration, sleep, digestion, and avoiding consumption of foods that are known to influence vascular function, not all factors influencing MSNA, and blood pressure could be standardized. For instance, mental stress (i.e., discomfort from the microelectrode or impatience from prolonged lying) (25) or higher breathing rate (short fast breathing versus long and slow breathing) (34) have been shown to increase resting MSNA and blood pressure. Additionally, during periods of slow, paced breathing, females demonstrated reduced nadir DBP responses to cardiac cycles without bursts, which may account for potential sex differences (34). Although accounting for these variables would help to standardize the resting period, having these confounding

variables can provide better implications for daily living as stress levels and breathing rate do not remain constant in daily life.

The current study also did not standardize testing sessions in females for menstrual or oral contraceptive pill phases. This was not standardized as the experimental design testing periods would not correspond to the standard 28-day menstrual cycle. Our protocol consists of three testing sessions, 6-weeks apart. If we were to change the protocol to ensure that females were within the same menstrual phase (28 days instead of 6-weeks apart), it would alter the established training protocol. Phases of the menstrual cycle have been shown to influence MSNA such that the ratio of oestrogen to progesterone has an inverse relationship with baseline levels of MSNA (24). Specifically, when females are in the early follicular phase, estrogen is low, leading to lower resting MSNA (104). When females are in the luteal phase, and estrogen is high, resting MSNA is higher (104). Although menstrual phase and hormone levels influence MSNA, this study observed no differences in neurovascular transduction (derived from the relationship between calf vascular resistance and MSNA during a 2-minute resting period) (104). Of the females in the current study that were naturally menstruating, 57% (4/7) were in the early follicular phase and 43% (3/7) were in the luteal phase at Baseline, and 71% (5/7) were in the early follicular phase and 29% (2/7) were in the luteal phase at Follow-up, thereby providing this study with a variety of sex hormone discrepancies between timepoints. In addition to MSNA, endothelium-dependent flow-mediated dilation is higher during the follicular and luteal phases (high oestrogen) of the menstrual cycle potentially reducing resting sympathetic transduction results via increased vasodilation (65), although this speculative as it did not directly measure transduction.

Furthermore, it has been observed that there are no major MSNA differences between females who naturally menstruate versus those who take oral contraceptive pills (81) although, females using oral contraceptives have higher forearm vascular conductance compared to naturally menstruating females in the early follicular stage (94). This suggests that the lack of standardization of contraceptives would not have a major influence on MSNA but may have influenced neurovascular transduction, which may have influenced the findings of this study.

Lastly, the result of the cycle ergometry aerobic fitness test may have been impacted by practice effects. Specifically, previous exposure to the cycle ergometry in the Baseline testing may have contributed to improvement in the Control group at Follow-up as the participants would be more familiar with the test. The Intervention group may have been influenced by practice effects as the mode of the training protocol was identical to that of the aerobic fitness test, potentially leading to a plateau after being exposed for 12 weeks.

5.8: Implications and Future Directions

The results of this study provide an effective method for improving aerobic fitness in healthy males and females, which is known to reduce the risk of developing CVDs (2). Further, the results highlight that aerobic fitness improvements following HIIT are larger in males compared to females, potentially indicating that HIIT is more effective in males. This may lead researchers to future studies that investigate sex differences in the specific adaptations to training (i.e., to peak stroke volume/cardiac output and/or peak arterial-venous O_2 difference). This study demonstrated that improvements in aerobic fitness following HIIT do not impact sympathetic transduction. Further, the greater reduction in

nadir DBP responses to cardiac cycles without MSNA bursts, primarily in females, provides evidence for a potential beneficial vascular adaptation to HIIT that should be further investigated by uncover the physiological mechanisms associated with these nadir responses, increasing the duration of training, and investigating training studies in other populations with elevated MSNA levels (older and hypertensive adults).

In conclusion, 12-weeks of HIIT improved aerobic fitness in both males and females with larger improvements observed in males. Short term improvements in aerobic fitness did not alter sympathetic transduction in males or females although, decreases in the nadir DBP to periods of sympathetic quiescence were observed primarily in females. No sex differences were observed in sympathetic transduction using both the signal averaging and regression slope method. This study provides a starting point to further investigate sex differences in the adaptations to HIIT interventions and the specific mechanisms responsible for these adaptations.

References

1. **Aåstrand P-O**. Human physical fitness with special reference to sex and age. *Physiol Rev* 36: 307–335, 1956. doi: 10.1152/physrev.1956.36.3.307.

2. **Al-Mallah MH**, **Sakr S**, **Al-Qunaibet A**. Cardiorespiratory fitness and cardiovascular disease prevention: an update. *Curr Atheroscler Rep* 20: 1, 2018. doi: 10.1007/s11883-018-0711-4.

3. **Ansdell P**, **Thomas K**, **Hicks KM**, **Hunter SK**, **Howatson G**, **Goodall S**. Physiological sex differences affect the integrative response to exercise: acute and chronic implications. *Exp Physiol* 105: 2007–2021, 2020. doi: https://doi.org/10.1113/EP088548.

4. **Appelman Y**, **van Rijn BB**, **ten Haaf ME**, **Boersma E**, **Peters SAE**. Sex differences in cardiovascular risk factors and disease prevention. *Atherosclerosis* 241: 211–218, 2015. doi: https://doi.org/10.1016/j.atherosclerosis.2015.01.027.

5. **Arbab-Zadeh A**, **Perhonen M**, **Howden E**, **Peshock RM**, **Zhang R**, **Adams-Huet B**, **Haykowsky MJ**, **Levine BD**. Cardiac remodeling in response to 1 year of intensive endurance training. *Circulation* 130: 2152–2161, 2014.

6. **Arboleda-Serna VH**, **Feito Y**, **Patiño-Villada FA**, **Vargas-Romero AV**, **Arango-Vélez EF**. Effects of high-intensity interval training compared to moderate-intensity continuous training on maximal oxygen consumption and blood pressure in healthy men: A randomized controlled trial. *Biomedica* 39: 524–536, 2019. doi: 10.7705/biomedica.4451.

7. **Astorino TA**, **Allen RP**, **Roberson DW**, **Jurancich M**. Effect of high-intensity interval training on cardiovascular function, vo2max, and muscular force. *J Strength Cond Res* 26, 2012.

8. **Badrov MB**, **Bartol CL**, **DiBartolomeo MA**, **Millar PJ**, **McNevin NH**, **McGowan CL**. Effects of isometric handgrip training dose on resting blood pressure and resistance vessel endothelial function in normotensive women. *Eur J Appl Physiol* 113: 2091–2100, 2013. doi: 10.1007/s00421-013-2644-5.

9. **Baker SE**, **Limberg JK**, **Ranadive SM**, **Joyner MJ**. Neurovascular control of blood pressure is influenced by aging, sex, and sex hormones. *Am J Physiol Integr Comp Physiol* 311: R1271–R1275, 2016. doi: 10.1152/ajpregu.00288.2016.

10. **Baker SE**, **Limberg JK**, **Scruggs ZM**, **Curry TB**, **Nicholson WT**, **Barnes JN**, **Joyner MJ**. Greater influence of aerobic fitness on autonomic support of blood pressure in young women than in older women. *Hypertens (Dallas, Tex 1979)* 75: 1497–1504, 2020. doi: 10.1161/HYPERTENSIONAHA.119.14042.

11. **Bassett DRJ**, **Howley ET**. Limiting factors for maximum oxygen uptake and determinants of endurance performance. *Med Sci Sport Exerc* 32, 2000.

12. **Beaver WL**, **Wasserman K**, **Whipp BJ**. A new method for detecting anaerobic threshold by gas exchange. *J Appl Physiol* 60: 2020–2027, 1986. doi: 10.1152/jappl.1986.60.6.2020.

13. **Best SA**, **Okada Y**, **Galbreath MM**, **Jarvis SS**, **Bivens TB**, **Adams-Huet B**, **Fu Q**. Age and sex differences in muscle sympathetic nerve activity in relation to haemodynamics, blood volume and left ventricular size. *Exp Physiol* 99: 839–848, 2014. doi: https://doi.org/10.1113/expphysiol.2013.077248.

14. **Blair SN**, **Kohl HW**, **Barlow CE**, **Paffenbarger RS**, **Gibbons LW**, **Macera CA**. Changes in physical fitness and all-cause mortality: a prospective study of healthy and unhealthy men. *Jama* 273: 1093–1098, 1995.

15. **Boeno FP**, **Ramis TR**, **Munhoz S V**, **Farinha JB**, **Moritz CEJ**, **Leal-Menezes R**, **Ribeiro JL**, **Christou DD**, **Reischak-Oliveira A**. Effect of aerobic and resistance exercise training on inflammation, endothelial function and ambulatory blood pressure in middle-aged hypertensive patients. *J Hypertens* 38, 2020.

16. **Bonafiglia JT**, **Edgett BA**, **Baechler BL**, **Nelms MW**, **Simpson CA**, **Quadrilatero J**, **Gurd BJ**. Acute upregulation of PGC-1α mRNA correlates with training-induced increases in SDH activity in human skeletal muscle. *Appl Physiol Nutr Metab* 42: 656–666, 2017. doi: 10.1139/apnm-2016-0463.

17. **Borg GA V**. Psychophysical bases of perceived exertion. .

18. **Briant LJB**, **Burchell AE**, **Ratcliffe LEK**, **Charkoudian N**, **Nightingale AK**, **Paton JFR**, **Joyner MJ**, **Hart EC**. Quantifying sympathetic neuro-haemodynamic transduction at rest in humans: insights into sex, ageing and blood pressure control. *J Physiol* 594: 4753–4768, 2016. doi: https://doi.org/10.1113/JP272167.

19. **Buchheit M**, **Laursen PB**. High-intensity interval training, solutions to the programming puzzle. *Sport Med* 43: 313–338, 2013. doi: 10.1007/s40279-013-0029-x.

20. **Burgomaster KA**, **Howarth KR**, **Phillips SM**, **Rakobowchuk M**, **Macdonald MJ**, **McGee SL**, **Gibala MJ**. Similar metabolic adaptations during exercise after low volume sprint interval and traditional endurance training in humans. *J Physiol* 586: 151–160, 2008. doi: 10.1113/jphysiol.2007.142109.

21. **Carnethon MR**, **Gidding SS**, **Nehgme R**, **Sidney S**, **Jacobs DRJ**, **Liu K**. Cardiorespiratory fitness in young adulthood and the development of cardiovascular disease risk factors. *JAMA* 290: 3092–3100, 2003. doi: 10.1001/jama.290.23.3092.

22. **Carson V**, **Wong SL**, **Winkler E**, **Healy GN**, **Colley RC**, **Tremblay MS**. Patterns of sedentary time and cardiometabolic risk among Canadian adults. *Prev Med (Baltim)* 65: 23–27, 2014. doi: https://doi.org/10.1016/j.ypmed.2014.04.005.

23. **Carter JR**. Microneurography and sympathetic nerve activity: a decade-by-decade journey across 50 years. *J Neurophysiol* 121: 1183–1194, 2019. doi: 10.1152/jn.00570.2018.

24. **Carter JR**, **Fu Q**, **Minson CT**, **Joyner MJ**. Ovarian cycle and sympathoexcitation in premenopausal women. *Hypertens (Dallas, Tex 1979)* 61: 395–399, 2013. doi: 10.1161/HYPERTENSIONAHA.112.202598.

25. **Carter JR**, **Ray CA**. Sympathetic neural responses to mental stress: responders, nonresponders and sex differences. *Am J Physiol Heart Circ Physiol* 296: H847-53, 2009. doi: 10.1152/ajpheart.01234.2008.

26. **Chidnok W**, **Wadthaisong M**, **Iamsongkham P**, **Mheonprayoon W**, **Wirajalarbha W**, **Thitiwuthikiat P**, **Siriwittayawan D**, **Vachirasrisirikul S**, **Nuamchit T**. Effects of high-intensity interval training on vascular function and maximum oxygen uptake in young sedentary females. *Int J Health Sci (Qassim)* 14: 3–8, 2020.

27. **Christou DD**, **Jones PP**, **Jordan J**, **Diedrich A**, **Robertson D**, **Seals DR**. Women have lower tonic autonomic support of arterial blood pressure and less effective baroreflex buffering than men. *Circulation* 111: 494–498, 2005. doi: 10.1161/01.CIR.0000153864.24034.A6.

28. **Chrøis KM**, **Dohlmann TL**, **Søgaard D**, **Hansen CV**, **Dela F**, **Helge JW**, **Larsen S**. Mitochondrial adaptations to high intensity interval training in older females and males. *Eur J Sport Sci* 20: 135–145, 2020. doi: 10.1080/17461391.2019.1615556.

29. **Cocks M**, **Shaw CS**, **Shepherd SO**, **Fisher JP**, **Ranasinghe AM**, **Barker TA**, **Tipton KD**, **Wagenmakers AJM**. Sprint interval and endurance training are equally effective in increasing muscle microvascular density and eNOS content in sedentary males. *J Physiol* 591: 641–656, 2013. doi: 10.1113/jphysiol.2012.239566.

30. **Colucci WS**, **Gimbrone Jr MA**, **McLaughlin MK**, **Halpern W**, **Alexander RW**. Increased vascular catecholamine sensitivity and alpha-adrenergic receptor affinity in female and estrogen-treated male rats. *Circ Res* 50: 805–811, 1982.

31. **Cooke WH**, **Carter JR**, **Kuusela TA**. Human cerebrovascular and autonomic rhythms during vestibular activation. *Am J Physiol Integr Comp Physiol* 286: R838–R843, 2004. doi: 10.1152/ajpregu.00562.2003.

32. **Cooke WH**, **Reynolds B V**, **Yandl MG**, **Carter JR**, **Tahvanainen KUO**, **Kuusela TA**. Effects of exercise training on cardiovagal and sympathetic responses to Valsalva's maneuver. *Med Sci Sports Exerc* 34: 928–935, 2002. doi: 10.1097/00005768-200206000-00004.

33. **Coovadia Y**, **Adler TE**, **Martin-Arrowsmith PW**, **Usselman CW**. Sex differences in sympathetic neurovascular and neurohemodynamic relationships during cold pressor test. *Am J Physiol Regul Integr Comp Physiol* 322: R411–R420, 2022. doi: 10.1152/ajpregu.00223.2021.

34. **Coovadia Y**, **Adler TE**, **Steinback CD**, **Fraser GM**, **Usselman CW**. Sex differences in dynamic blood pressure regulation: beat-by-beat responses to muscle sympathetic nerve activity. *Am J Physiol Circ Physiol* 319: H531–H538, 2020. doi: 10.1152/ajpheart.00245.2020.

35. **CSEP**. Health benefit ratings for adults. In: *CSEP-PATH - Toolkit*. 2019.

36. **Cureton K**, **Bishop P**, **Hutchinson P**, **Newland H**, **Vickery S**, **Zwiren L**. Sex difference in maximal oxygen uptake: effect of equating haemoglobin concentration. *Eur J Appl Physiol Occup Physiol* 54: 656–660, 1986.

37. **Currie KD**, **Dubberley JB**, **McKelvie RS**, **MacDonald MJ**. Low-volume, high-intensity interval training in patients with CAD. *Med Sci Sports Exerc* 45: 1436–1442, 2013. doi: 10.1249/MSS.0b013e31828bbbd4.

38. **Diaz-Canestro C**, **Montero D**. Sex dimorphism of VO2max trainability: A systematic review and meta-analysis. *Sport Med* 49: 1949–1956, 2019. doi: 10.1007/s40279-019-01180-z.

39. **Diaz-Canestro C**, **Pentz B**, **Sehgal A**, **Montero D**. Sex differences in cardiorespiratory fitness are explained by blood volume and oxygen carrying capacity. *Cardiovasc Res* 118: 334–343, 2022. doi: 10.1093/cvr/cvab028.

40. **Donato AJ**, **Lesniewski LA**, **Delp MD**. Ageing and exercise training alter adrenergic vasomotor responses of rat skeletal muscle arterioles. *J Physiol* 579: 115–125, 2007. doi: 10.1113/jphysiol.2006.120055.

41. **Edwardson CL**, **Winkler EAH**, **Bodicoat DH**, **Yates T**, **Davies MJ**, **Dunstan DW**, **Healy GN**. Considerations when using the activPAL monitor in field-based research with adult populations. *J Sport Heal Sci* 6: 162–178, 2017. doi: https://doi.org/10.1016/j.jshs.2016.02.002.

42. **Ehlers TS**, **Sverrisdottir Y**, **Bangsbo J**, **Gunnarsson TP**. High-intensity interval training decreases muscle sympathetic nerve activity in men with essential hypertension and in normotensive controls. *Front. Neurosci.* 14: 2020.

43. **Esfandiari S**, **Sasson Z**, **Goodman JM**. Short-term high-intensity interval and continuous moderate-intensity training improve maximal aerobic power and diastolic filling during exercise. *Eur J Appl Physiol* 114: 331–343, 2014. doi: 10.1007/s00421-013-2773-x.

44. **Fagard RH**. Exercise is good for your blood pressure: effects of endurance training and resistance training. *Clin Exp Pharmacol Physiol* 33: 853–856, 2006. doi: https://doi.org/10.1111/j.1440-1681.2006.04453.x.

45. **Fagius J**, **Karhuvaara S**. Sympathetic activity and blood pressure increases with bladder distension in humans. *Hypertension* 14: 511–517, 1989. doi: 10.1161/01.HYP.14.5.511.

46. **Fagius J**, **Wallin BG**. Sympathetic reflex latencies and conduction velocities in normal man. *J Neurol Sci* 47: 433–448, 1980. doi: 10.1016/0022-510x(80)90098-2.

47. **Fairfax ST**, **Holwerda SW**, **Credeur DP**, **Zuidema MY**, **Medley JH**, **Dyke PC 2nd**, **Wray DW**, **Davis MJ**, **Fadel PJ**. The role of α-adrenergic receptors in mediating beat-by-beat sympathetic vascular transduction in the forearm of resting man. *J Physiol* 591: 3637–3649, 2013. doi: 10.1113/jphysiol.2013.250894.

48. **Fairfax ST**, **Padilla J**, **Vianna LC**, **Davis MJ**, **Fadel PJ**. Spontaneous bursts of muscle sympathetic nerve activity decrease leg vascular conductance in resting humans. *Am J Physiol Heart Circ Physiol* 304: H759-66, 2013. doi: 10.1152/ajpheart.00842.2012.

49. **Ferrer M**, **Meyer M**, **Osol G**. Estrogen replacement increases β-adrenoceptor-mediated relaxation of rat mesenteric arteries. *J Vasc Res* 33: 124–131, 1996.

50. **Franklin BA**, **McCullough PA**. Cardiorespiratory fitness: an independent and additive marker of risk stratification and health outcomes. In: *Mayo Clinic Proceedings*. Elsevier, 2009, p. 776–779.

51. **Freedman RR**, **Sabharwal SC**, **Desai N**. Sex differences in peripheral vascular adrenergic receptors. *Circ Res* 61: 581–585, 1987. doi: 10.1161/01.RES.61.4.581.

52. **Freemas JA**, **Wilhite DP**, **Greenshields JT**, **Adamic EM**, **Mickleborough TD**. Comparison between a facemask and mouthpiece on breathing mechanics and gas exchange variables during high-intensity exercise. *Eur J Sport Sci* 20: 211–218, 2020. doi: 10.1080/17461391.2019.1628309.

53. **Garriguet D**, **Colley RC**. Daily patterns of physical activity among Canadians. *Heal reports* 23: B1, 2012.

54. **Gaskill SE**, **Ruby BC**, **Walker AJ**, **Sanchez OA**, **Serfass RC**, **Leon AS**. Validity and reliability of combining three methods to determine ventilatory threshold. *Med Sci Sports Exerc* 33: 1841–1848, 2001.

55. **Gibbons RJ**, **Balady GJ**, **Beasley JW**, **Bricker JT**, **Duvernoy WF**, **Froelicher VF**, **Mark DB**, **Marwick TH**, **McCallister BD**, **Thompson PDJ**, **Winters WL**, **Yanowitz FG**, **Ritchie JL**, **Gibbons RJ**, **Cheitlin MD**, **Eagle KA**, **Gardner TJ**, **Garson AJ**, **Lewis RP**, **O'Rourke RA**, **Ryan TJ**. ACC/AHA guidelines for

exercise testing. A report of the American college of cardiology/American heart association task force practice guidelines (committee on exercise testing). *J Am Coll Cardiol* 30: 260–311, 1997. doi: 10.1016/s0735-1097(97)00150-2.

56. **Gillen JB**, **Gibala MJ**. Is high-intensity interval training a time-efficient exercise strategy to improve health and fitness? *Appl Physiol Nutr Metab* 39: 409–412, 2013. doi: 10.1139/apnm-2013-0187.

57. **Gormley SE**, **Swain DP**, **High R**, **Spina RJ**, **Dowling EA**, **Kotipalli US**, **Gandrakota R**. Effect of intensity of aerobic training on VO2max. *Med Sci Sports Exerc* 40: 1336–1343, 2008. doi: 10.1249/MSS.0b013e31816c4839.

58. **Gregoire J**, **Tuck S**, **Yamamoto Y**, **Hughson RL**. Heart rate variability at rest and exercise: influence of age, gender, and physical training. *Can J Appl Physiol = Rev Can Physiol Appl* 21: 455–470, 1996. doi: 10.1139/h96-040.

59. **Guimarães GV**, **Ciolac EG**, **Carvalho VO**, **D'Avila VM**, **Bortolotto LA**, **Bocchi EA**. Effects of continuous vs. interval exercise training on blood pressure and arterial stiffness in treated hypertension. *Hypertens Res* 33: 627–632, 2010. doi: 10.1038/hr.2010.42.

60. **Guiraud T**, **Juneau M**, **Nigam A**, **Gayda M**, **Meyer P**, **Mekary S**, **Paillard F**, **Bosquet L**. Optimization of high intensity interval exercise in coronary heart disease. *Eur J Appl Physiol* 108: 733–740, 2010. doi: 10.1007/s00421-009-1287-z.

61. **Gunnar Wallin B**, **Nerhed C**. Relationship between spontaneous variations of muscle sympathetic activity and succeeding changes of blood pressure in man. *J Auton Nerv Syst* 6: 293–302, 1982. doi: https://doi.org/10.1016/0165-1838(82)90002-9.

62. **Hart EC**, **Charkoudian N**, **Wallin BG**, **Curry TB**, **Eisenach J**, **Joyner MJ**. Sex and ageing differences in resting arterial pressure regulation: the role of the β-adrenergic receptors. *J Physiol* 589: 5285–5297, 2011. doi: 10.1113/jphysiol.2011.212753.

63. **Hart EC**, **Charkoudian N**, **Wallin BG**, **Curry TB**, **Eisenach JH**, **Joyner MJ**. Sex differences in sympathetic neural-hemodynamic balance: implications for human blood pressure regulation. *Hypertension* 53: 571–576, 2009.

64. **Hart EC**, **Head GA**, **Carter JR**, **Wallin BG**, **May CN**, **Hamza SM**, **Hall JE**, **Charkoudian N**, **Osborn JW**. Recording sympathetic nerve activity in conscious humans and other mammals: guidelines and the road to standardization. *Am J Physiol Circ Physiol* 312: H1031–H1051, 2017. doi: 10.1152/ajpheart.00703.2016.

65. **Hashimoto M**, **Akishita M**, **Eto M**, **Ishikawa M**, **Kozaki K**, **Toba K**, **Sagara Y**, **Taketani Y**, **Orimo H**, **Ouchi Y**. Modulation of endothelium-dependent flow-mediated dilatation of the brachial artery by sex and menstrual cycle. *Circulation* 92: 3431–3435, 1995. doi: 10.1161/01.CIR.92.12.3431.

66. **Helgerud J**, **Hoydal K**, **Wang E**, **Karlen T**, **Berg P**, **Bjerkaas M**, **Simonsen T**, **Helgesen C**, **Hjorth N**, **Bach R**, **Hoff J**. Aerobic high-intensity intervals improve V˙O2max more than moderate training. *Med Sci Sport Exerc* 39, 2007.

67. **Helgerud J**, **Høydal K**, **Wang E**, **Karlsen T**, **Berg P**, **Bjerkaas M**, **Simonsen T**, **Helgesen C**, **Hjorth N**, **Bach R**, **Hoff J**. Aerobic high-intensity intervals improve VO2max more than moderate training. *Med Sci Sports Exerc* 39: 665–671, 2007. doi: 10.1249/mss.0b013e3180304570.

68. **Herrod PJJ**, **Atherton PJ**, **Smith K**, **Williams JP**, **Lund JN**, **Phillips BE**. Six weeks of high-intensity interval training enhances contractile activity induced vascular reactivity and skeletal muscle perfusion in older adults. *GeroScience* 43: 2667–2678, 2021. doi: 10.1007/s11357-021-00463-6.

69. **Heydari M**, **Boutcher YN**, **Boutcher SH**. High-intensity intermittent exercise and cardiovascular and autonomic function. *Clin Auton Res Off J Clin Auton Res Soc* 23: 57–65, 2013. doi: 10.1007/s10286-012-0179-1.

70. **Hickson RC**, **Hagberg JM**, **Ehsani AA**, **Holloszy JO**. Time course of the adaptive responses of aerobic power and heart rate to training. *Med Sci Sports Exerc* 13: 17–20, 1981.

71. **Hissen SL**, **Macefield VG**, **Brown R**, **Taylor CE**. Sympathetic baroreflex sensitivity is inversely related to vascular transduction in men but not women. *Am J Physiol Circ Physiol* 317: H1203–H1209, 2019. doi: 10.1152/ajpheart.00501.2019.

72. **Hissen SL**, **Taylor CE**. Sex differences in vascular transduction of sympathetic nerve activity. *Clin Auton Res* 30: 381–392, 2020. doi: 10.1007/s10286-020-00722-0.

73. **Hoffmann S**, **Skinner T**, **van Rosendal S**, **Osborne M**, **Emmerton L**, **Jenkins D**. Sex differences in adaptations to high intensity interval training. *J Sci Med Sport* 20: e18, 2017. doi: https://doi.org/10.1016/j.jsams.2016.12.044.

74. **Holmgren A**. Cardiorespiratory determinants of cardiovascular fitness. *Can Med Assoc J* 96: 697–705, 1967.

75. **Howden EJ**, **Perhonen M**, **Peshock RM**, **Zhang R**, **Arbab-Zadeh A**, **Adams-Huet B**, **Levine BD**. Females have a blunted cardiovascular response to one year of intensive supervised endurance training. *J Appl Physiol* 119: 37–46, 2015. doi: 10.1152/japplphysiol.00092.2015.

76. **Huang Y-C**, **Tsai H-H**, **Fu T-C**, **Hsu C-C**, **Wang J-S**. High-intensity interval training improves left ventricular contractile function. *Med Sci Sport Exerc* 51: 1420–1428, 2019.

77. **Iorga A**, **Cunningham CM**, **Moazeni S**, **Ruffenach G**, **Umar S**, **Eghbali M**. The protective role of estrogen and estrogen receptors in cardiovascular disease and the controversial use of estrogen therapy. *Biol Sex Differ* 8: 33, 2017. doi: 10.1186/s13293-017-0152-8.

78. **Jacobs RA**, **Flück D**, **Bonne TC**, **Bürgi S**, **Christensen PM**, **Toigo M**, **Lundby C**. Improvements in exercise performance with high-intensity interval training coincide with an increase in skeletal muscle mitochondrial content and function. *J Appl Physiol* 115: 785–793, 2013. doi: 10.1152/japplphysiol.00445.2013.

79. **Jarvis SS**, **VanGundy TB**, **Galbreath MM**, **Shibata S**, **Okazaki K**, **Reelick MF**, **Levine BD**, **Fu Q**. Sex differences in the modulation of vasomotor sympathetic outflow during static handgrip exercise in healthy young humans. *Am J Physiol Integr Comp Physiol* 301: R193–R200, 2011. doi: 10.1152/ajpregu.00562.2010.

80. **Johns JA**, **Frayne RJ**, **Goreham JA**, **Kimmerly DS**, **O'Brien MW**. The bout cadence method improves the quantification of stepping cadence in free-living conditions. *Gait Posture* 79: 96–101, 2020. doi: https://doi.org/10.1016/j.gaitpost.2020.04.014.

81. **Joyner MJ**, **Wallin BG**, **Charkoudian N**. Sex differences and blood pressure regulation in humans. *Exp Physiol* 101: 349–355, 2016. doi: https://doi.org/10.1113/EP085146.

82. **Keir DA**, **Badrov MB**, **Tomlinson G**, **Notarius CF**, **Kimmerly DS**, **Millar PJ**, **Shoemaker JK**, **Floras JS**. Influence of sex and age on muscle sympathetic nerve activity of healthy normotensive adults. *Hypertension* 76: 997–1005, 2020. doi: 10.1161/HYPERTENSIONAHA.120.15208.

83. **Kelley GA**, **Kelley KA**, **Tran Z V**. Aerobic exercise and resting blood pressure: a meta-analytic review of randomized, controlled trials. *Prev Cardiol* 4: 73–80, 2001. doi: 10.1111/j.1520-037x.2001.00529.x.

84. **Khalil R**. Chapter 6, Protein kinase C. In: *Regulation of Vascular Smooth Muscle Function*. Morgan & CLaypool Life Sciences, 2010.

85. **Klabunde RE**. *Cardiovascular Physiology Concepts*. Lippincott Williams & Wilkins, 2nd ed. 2012.

86. **Klassen SA**, **Joyner MJ**, **Baker SE**. The impact of ageing and sex on sympathetic neurocirculatory regulation. *Semin Cell Dev Biol* 116: 72–81, 2021. doi: https://doi.org/10.1016/j.semcdb.2021.01.001.

87. **Kleinert H**, **Wallerath T**, **Euchenhofer C**, **Ihrig-Biedert I**, **Li H**, **Förstermann U**. Estrogens increase transcription of the human endothelial NO synthase gene: analysis of the transcription factors involved. *Hypertension* 31: 582–588, 1998.

88. **Kneale BJ**, **Chowienczyk PJ**, **Brett SE**, **Coltart DJ**, **Ritter JM**. Gender differences in sensitivity to adrenergic agonists of forearm resistance vasculature. *J Am Coll Cardiol* 36: 1233–1238, 2000. doi: https://doi.org/10.1016/S0735-1097(00)00849-4.

89. **Kneale BJ**, **Chowienczyk PJ**, **Cockcroft JR**, **Coltart DJ**, **Ritter JM**. Vasoconstrictor sensitivity to noradrenaline and ng-monomethyl-l-arginine in men and women. *Clin Sci* 93: 513–518, 1997. doi: 10.1042/cs0930513.

90. **Kodama S**, **Saito K**, **Tanaka S**, **Maki M**, **Yachi Y**, **Asumi M**, **Sugawara A**, **Totsuka K**, **Shimano H**, **Ohashi Y**, **Yamada N**, **Sone H**. Cardiorespiratory fitness as a quantitative predictor of all-cause mortality and cardiovascular events in healthy men and women: a meta-analysis. *JAMA* 301: 2024–2035, 2009. doi: 10.1001/jama.2009.681.

91. **Lenders JW**, **Golczynska A**, **Goldstein DS**. Glucocorticoids, sympathetic activity, and presynaptic alpha 2-adrenoceptor function in humans. *J Clin Endocrinol Metab* 80: 1804–1808, 1995. doi: 10.1210/jcem.80.6.7775627.

92. **Leosco D**, **Iaccarino G**, **Cipolletta E**, **De Santis D**, **Pisani E**, **Trimarco V**, **Ferrara N**, **Abete P**, **Sorriento D**, **Rengo F**, **Trimarco B**. Exercise restores β-adrenergic vasorelaxation in aged rat carotid arteries. *Am J Physiol Circ Physiol* 285: H369–H374, 2003. doi: 10.1152/ajpheart.00019.2003.

93. **Limberg JK**, **Johansson RE**, **Peltonen GL**, **Harrell JW**, **Kellawan JM**, **Eldridge MW**, **Sebranek JJ**, **Schrage WG**. β-Adrenergic-mediated vasodilation in young men and women: cyclooxygenase restrains nitric oxide synthase. *Am J Physiol Heart Circ Physiol* 310: H756-64, 2016. doi: 10.1152/ajpheart.00886.2015.

94. **Limberg JK**, **Peltonen GL**, **Johansson RE**, **Harrell JW**, **Kellawan JM**, **Eldridge MW**, **Sebranek JJ**, **Walker BJ**, **Schrage WG**. Greater beta-adrenergic receptor mediated vasodilation in women using oral contraceptives. *Front Physiol* 7: 215, 2016. doi: 10.3389/fphys.2016.00215.

95. **Little JP**, **Safdar A**, **Wilkin GP**, **Tarnopolsky MA**, **Gibala MJ**. A practical model of low-volume high-intensity interval training induces mitochondrial biogenesis in human skeletal muscle: potential mechanisms. *J Physiol* 588: 1011–1022, 2010.

96. **Macefield VG**. Recording and quantifying sympathetic outflow to muscle and skin in humans: methods, caveats and challenges. *Clin Auton Res* 31: 59–75, 2021. doi: 10.1007/s10286-020-00700-6.

97. **Malpas SC**. Sympathetic Nervous System Overactivity and Its Role in the Development of Cardiovascular Disease. *Physiol Rev* 90: 513–557, 2010. doi: 10.1152/physrev.00007.2009.

98. **Martinez-Lemus LA**. The dynamic structure of arterioles. *Basic Clin Pharmacol Toxicol* 110: 5–11, 2012. doi: 10.1111/j.1742-7843.2011.00813.x.

99. **Martland R**, **Mondelli V**, **Gaughran F**, **Stubbs B**. Can high-intensity interval training improve physical and mental health outcomes? A meta-review of 33 systematic reviews across the lifespan. *J Sports Sci* 38: 430–469, 2020. doi: 10.1080/02640414.2019.1706829.

100. **Matsuo T**, **Saotome K**, **Seino S**, **Shimojo N**, **Matsushita A**, **Iemitsu M**, **Ohshima H**, **Tanaka K**, **Mukai C**. Effects of a low-volume aerobic-type interval exercise on V˙O2max and cardiac mass. *Med Sci Sport Exerc* 46, 2014.

101. **McKinney J**, **Lithwick DJ**, **Morrison BN**, **Nazzari H**, **Isserow SH**, **Heilbron B**, **Krahn AD**. The health benefits of physical activity and cardiorespiratory fitness. *B C Med J* 58: 131–137, 2016.

102. **Mekari S**, **Earle M**, **Martins R**, **Drisdelle S**, **Killen M**, **Bouffard-Levasseur V**, **Dupuy O**. Effect of high intensity interval training compared to continuous training on cognitive performance in young healthy adults: A pilot study. *Brain Sci* 10: 1-13, 2020. doi: 10.3390/brainsci10020081.

103. **Mekari S**, **Neyedli HF**, **Fraser S**, **O'Brien MW**, **Martins R**, **Evans K**, **Earle M**, **Aucoin R**, **Chiekwe J**, **Hollohan Q**, **Kimmerly DS**, **Dupuy O**. High-intensity interval training improves cognitive flexibility in older adults. *Brain Sci.* 10(11): 796. 2020.

104. **Minson CT**, **Halliwill JR**, **Young TM**, **Joyner MJ**. Influence of the menstrual cycle on sympathetic activity, baroreflex sensitivity, and vascular transduction in young women. *Circulation* 101: 862–868, 2000. doi: 10.1161/01.cir.101.8.862.

105. **Mora S**, **Cook N**, **Buring JE**, **Ridker PM**, **Lee I-M**. Physical Activity and Reduced Risk of Cardiovascular Events. *Circulation* 116: 2110–2118, 2007. doi: 10.1161/CIRCULATIONAHA.107.729939.

106. **Mora S**, **Redberg RF**, **Cui Y**, **Whiteman MK**, **Flaws JA**, **Sharrett AR**, **Blumenthal RS**. Ability of exercise testing to predict cardiovascular and all-cause death in asymptomatic women: A 20-year follow-up of the lipid research clinics prevalence study. *JAMA* 290: 1600–1607, 2003. doi: 10.1001/jama.290.12.1600.

107. **Mosca L**, **Barrett-Connor E**, **Wenger NK**. Sex/gender differences in cardiovascular disease prevention: what a difference a decade makes. *Circulation* 124: 2145–2154, 2011. doi: 10.1161/CIRCULATIONAHA.110.968792.

108. **Murphy WG**. The sex difference in haemoglobin levels in adults - mechanisms, causes, and consequences. *Blood Rev* 28: 41–47, 2014. doi: 10.1016/j.blre.2013.12.003.

109. **Myers J**, **McAuley P**, **Lavie CJ**, **Despres J-P**, **Arena R**, **Kokkinos P**. Physical activity and cardiorespiratory fitness as major markers of cardiovascular risk: their independent and interwoven importance to health status. *Prog Cardiovasc Dis* 57: 306–314, 2015. doi: https://doi.org/10.1016/j.pcad.2014.09.011.

110. **Myers J**, **Prakash M**, **Froelicher V**, **Do D**, **Partington S**, **Atwood JE**. Exercise capacity and mortality among men referred for exercise testing. *N Engl J Med* 346: 793–801, 2002. doi: 10.1056/NEJMoa011858.

111. **Naghavi M**, **Al. E**. Global, regional, and national age-sex specific mortality for 264 causes of death, 1980-2013;2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet* 390: 1151–1210, 2017. doi: 10.1016/S0140-6736(17)32152-9.

112. **Narkiewicz K**, **Phillips BG**, **Kato M**, **Hering D**, **Bieniaszewski L**, **Somers VK**. Gender-selective interaction between aging, blood pressure, and sympathetic nerve activity. *Hypertension* 45: 522–525, 2005. doi: 10.1161/01.HYP.0000160318.46725.46.

113. **Ng A V**, **Callister R**, **Johnson DG**, **Seals DR**. Age and gender influence muscle sympathetic nerve activity at rest in healthy humans. *Hypertension* 21: 498–503, 1993. doi: 10.1161/01.HYP.21.4.498.

114. **Norris CM**, **Yip CYY**, **Nerenberg KA**, **Clavel M**, **Pacheco C**, **Foulds HJA**, **Hardy M**, **Gonsalves CA**, **Jaffer S**, **Parry M**, **Colella TJF**, **Dhukai A**, **Grewal J**, **Price JAD**, **Levinsson ALE**, **Hart D**, **Harvey PJ**, **Van Spall HGC**, **Sarfi H**, **Sedlak TL**, **Ahmed SB**, **Baer C**, **Coutinho T**, **Edwards JD**, **Green CR**, **Kirkham AA**, **Srivaratharajah K**, **Dumanski S**, **Keeping-Burke L**, **Lappa N**, **Reid RD**, **Robert H**, **Smith G**, **Martin-Rhee M**, **Mulvagh SL**. State of the science in women's cardiovascular disease: A canadian perspective on the influence of sex and gender. *J Am Heart Assoc* 9: e015634, 2020. doi: 10.1161/JAHA.119.015634.

115. **Notarius CF**, **Murai H**, **Morris BL**, **Floras JS**. Effect of fitness on reflex sympathetic neurovascular transduction in middle-age men. *Med Sci Sport Exerc* 44, 2012.

116. **O'Brien MW**, **Johns JA**, **Fowles JR**, **Kimmerly DS**. Validity of the activPAL and height-adjusted curvilinear cadence-METs equations in healthy adults. *Meas Phys Educ Exerc Sci* 24: 147–156, 2020.

117. **O'Brien MW**, **Johns JA**, **Robinson SA**, **Bungay A**, **Mekary S**, **Kimmerly DS**. Impact of high-intensity interval training, moderate-intensity continuous training, and resistance training on endothelial function in older adults. *Med Sci Sports Exerc* 52: 1057–1067, 2020. doi: 10.1249/MSS.0000000000002226.

118. **O'Brien MW**, **Petterson JL**, **Kimmerly DS**. An open-source program to analyze spontaneous sympathetic neurohemodynamic transduction. *J Neurophysiol* 125:

972–976, 2021. doi: 10.1152/jn.00002.2021.

119. **O'Brien MW**, **Petterson JL**, **Kimmerly DS**. Impact of sampling duration on spontaneous sympathetic transduction. *Clin Auton Res* 32: 155–158, 2022. doi: 10.1007/s10286-022-00861-6.

120. **O'Brien MW**, **Ramsay D**, **Johnston W**, **Kimmerly DS**. Aerobic fitness and sympathetic responses to spontaneous muscle sympathetic nerve activity in young males. *Clin Auton Res* 31: 253–261, 2021. doi: 10.1007/s10286-020-00734-w.

121. **O'Brien MW**, **Ramsay D**, **Johnston W**, **Kimmerly DS**. The association between habitual posture and intensity-related physical activity with sympathetic neurohemodynamic transduction in young males. *Clin. Auton. Res. Off. J. Clin. Auton. Res. Soc.* 31: 339–341, 2021.

122. **O'Brien MW**, **Ramsay DJ**, **O'Neill CD**, **Petterson JL**, **Dogra S**, **Mekary S**, **Kimmerly DS**. Aerobic fitness is inversely associated with neurohemodynamic transduction and blood pressure variability in older adults. *GeroScience* 43: 2737–2748, 2021. doi: 10.1007/s11357-021-00389-z.

123. **O'Brien MW**, **Schwartz BD**, **Petterson JL**, **Kimmerly DS**. Comparison of signal-averaging and regression approaches to analyzing sympathetic transduction. *Clin Auton Res* 32: 299-302. https://doi.org/10.1007/s10286-022-00874-1.

124. **Parker ED**, **Schmitz KH**, **Jacobs DR**, **Dengel DR**, **Schreiner PJ**. Physical activity in young adults and incident hypertension over 15 years of follow-up: The CARDIA study. *Am J Public Health* 97: 703–709, 2007. doi: 10.2105/AJPH.2004.055889.

125. **Petterson JL**, **O'Brien MW**, **Ramsay DJ**, **Johnston W**, **O'Neill CD**, **Dogra S**, **Mekari S**, **Floras JS**, **Kimmerly DS**. Sympathetic neurohemodynamic transduction is attenuated in older males independent of aerobic fitness. *Clin Auton Res* 32: 73–76, 2022. doi: 10.1007/s10286-021-00845-y.

126. **Pfaffenberger S**, **Bartko P**, **Graf A**, **Pernicka E**, **Babayev J**, **Lolic E**, **Bonderman D**, **Baumgartner H**, **Maurer G**, **Mascherbauer J**. Size matters! Impact of age, sex, height, and weight on the normal heart size. *Circ Cardiovasc Imaging* 6: 1073–1079, 2013. doi: 10.1161/CIRCIMAGING.113.000690.

127. **Powers SK**, **Howley ET**, **Quindry J**. *Exercise physiology: Theory and application to fitness and performance*. McGraw-Hill New York, NY, 2007.

128. **Rabi DM**, **McBrien KA**, **Sapir-Pichhadze R**, **Nakhla M**, **Ahmed SB**, **Dumanski SM**, **Butalia S**, **Leung AA**, **Harris KC**, **Cloutier L**, **Zarnke KB**, **Ruzicka M**, **Hiremath S**, **Feldman RD**, **Tobe SW**, **Campbell TS**, **Bacon SL**, **Nerenberg KA**, **Dresser GK**, **Fournier A**, **Burgess E**, **Lindsay P**, **Rabkin SW**, **Prebtani APH**, **Grover S**, **Honos G**, **Alfonsi JE**, **Arcand J**, **Audibert F**, **Benoit G**, **Bittman J**, **Bolli P**, **Côté A-M**, **Dionne J**, **Don-Wauchope A**, **Edwards C**, **Firoz T**, **Gabor JY**, **Gilbert RE**, **Grégoire JC**, **Gryn SE**, **Gupta M**, **Hannah-Shmouni F**, **Hegele RA**, **Herman RJ**, **Hill MD**, **Howlett JG**, **Hundemer GL**, **Jones C**, **Kaczorowski J**, **Khan NA**, **Kuyper LM**, **Lamarre-Cliche M**, **Lavoie KL**, **Leiter LA**, **Lewanczuk R**, **Logan AG**, **Magee LA**, **Mangat BK**, **McFarlane PA**, **McLean D**, **Michaud A**, **Milot A**, **Moe GW**, **Penner SB**, **Pipe A**, **Poppe AY**, **Rey E**, **Roerecke M**, **Schiffrin EL**, **Selby P**, **Sharma M**, **Shoamanesh A**, **Sivapalan P**, **Townsend RR**, **Tran K**, **Trudeau L**, **Tsuyuki RT**, **Vallée M**, **Woo V**, **Bell AD**, **Daskalopoulou SS**. Hypertension Canada's 2020 comprehensive guidelines for the prevention, diagnosis, risk assessment, and treatment of hypertension in adults and children. *Can J Cardiol* 36: 596–624, 2020. doi: https://doi.org/10.1016/j.cjca.2020.02.086.

129. **Rakobowchuk M**, **Tanguay S**, **Burgomaster KA**, **Howarth KR**, **Gibala MJ**, **MacDonald MJ**. Sprint interval and traditional endurance training induce similar improvements in peripheral arterial stiffness and flow-mediated dilation in healthy humans. *Am J Physiol Regul Integr Comp Physiol.* 295(1): R236-R242. https://doi.org/10.1152/ajpregu.00069.2008

130. **Ramírez-Vélez R**, **Hernández-Quiñones PA**, **Tordecilla-Sanders A**, **Álvarez C**, **Ramírez-Campillo R**, **Izquierdo M**, **Correa-Bautista JE**, **Garcia-Hermoso A**, **Garcia RG**. Effectiveness of HIIT compared to moderate continuous training in improving vascular parameters in inactive adults. *Lipids Health Dis* 18: 42, 2019. doi: 10.1186/s12944-019-0981-z.

131. **Ramos JS**, **Dalleck LC**, **Tjonna AE**, **Beetham KS**, **Coombes JS**. The impact of high-intensity interval training versus moderate-intensity continuous training on vascular function: a systematic review and meta-analysis. *Sport Med* 45: 679–692, 2015. doi: 10.1007/s40279-015-0321-z.

132. **Ray CA**, **Carter JR**. Effects of aerobic exercise training on sympathetic and renal responses to mental stress in humans. *Am J Physiol Circ Physiol* 298: H229–H234, 2009. doi: 10.1152/ajpheart.00880.2009.

133. **Riedel K**, **Deussen AJ**, **Tolkmitt J**, **Weber S**, **Schlinkert P**, **Zatschler B**, **Friebel C**, **Müller B**, **El-Armouche A**, **Morawietz H**, **Matschke K**, **Kopaliani I**. Estrogen determines sex differences in adrenergic vessel tone by regulation of endothelial β-adrenoceptor expression. *Am J Physiol Circ Physiol* 317: H243–H254, 2019. doi: 10.1152/ajpheart.00456.2018.

134. **Roberts JM**, **Insel PA**, **Goldfien A**. Regulation of myometrial adrenoreceptors and adrenergic response by sex steroids. *Mol Pharmacol* 20: 52–58, 1981.

135. **Robinson AT**, **Babcock MC**, **Watso JC**, **Brian MS**, **Migdal KU**, **Wenner MM**, **Farquhar WB**. Relation between resting sympathetic outflow and vasoconstrictor responses to sympathetic nerve bursts: sex differences in healthy young adults. *Am J Physiol Integr Comp Physiol* 316: R463–R471, 2019. doi: 10.1152/ajpregu.00305.2018.

136. **Rosenblat MA**, **Granata C**, **Thomas SG**. Effect of interval training on the factors influencing maximal oxygen consumption: A systematic review and meta-analysis. *Sports Med.* 52: 1329–1352, 2022.

137. **Ross R**, **Chaput J-P**, **Giangregorio LM**, **Janssen I**, **Saunders TJ**, **Kho ME**, **Poitras VJ**, **Tomasone JR**, **El-Kotob R**, **McLaughlin EC**, **Duggan M**, **Carrier J**, **Carson V**, **Chastin SF**, **Latimer-Cheung AE**, **Chulak-Bozzer T**, **Faulkner G**, **Flood SM**, **Gazendam MK**, **Healy GN**, **Katzmarzyk PT**, **Kennedy W**, **Lane KN**, **Lorbergs A**, **Maclaren K**, **Marr S**, **Powell KE**, **Rhodes RE**, **Ross-White A**, **Welsh F**, **Willumsen J**, **Tremblay MS**. Canadian 24-Hour Movement Guidelines for Adults aged 18–64 years and Adults aged 65 years or older: an integration of physical activity, sedentary behaviour, and sleep. *Appl Physiol Nutr Metab* 45: S57–S102, 2020. doi: 10.1139/apnm-2020-0467.

138. **Saadatnia A**, **Ebrahim K**, **Rashidlamir A**. Echocardiographic evaluation of the effects of high-intensity interval training on cardiac morphology and function. *Arch Cardiovasc Imaging* Inpress: e36007. 2016. doi: 10.5812/acvi.36007.

139. **Salmanpour A**, **Brown LJ**, **Steinback CD**, **Usselman CW**, **Goswami R**, **Shoemaker JK**. Relationship between size and latency of action potentials in human muscle sympathetic nerve activity. *J Neurophysiol* 105: 2830–2842, 2011. doi: 10.1152/jn.00814.2010.

140. **Sawilowsky S**. New effect size rules of thumb. *J Mod Appl Stat Methods* 8: 597–599, 2009. doi: 10.22237/jmasm/1257035100.

141. **Sawyer BJ**, **Tucker WJ**, **Bhammar DM**, **Ryder JR**, **Sweazea KL**, **Gaesser GA**. Effects of high-intensity interval training and moderate-intensity continuous training on endothelial function and cardiometabolic risk markers in obese adults. *J Appl Physiol* 121: 279–288, 2016. doi: 10.1152/japplphysiol.00024.2016.

142. **Schmitz B**, **Niehues H**, **Thorwesten L**, **Klose A**, **Krüger M**, **Brand S-M**. Sex differences in high-intensity interval training–Are HIIT protocols interchangeable between females and males? *Front Physiol* 11, 2020. doi: 10.3389/fphys.2020.00038.

143. **Scribbans TD**, **Edgett BA**, **Vorobej K**, **Mitchell AS**, **Joanisse SD**, **Matusiak JBL**, **Parise G**, **Quadrilatero J**, **Gurd BJ**. Fibre-specific responses to endurance and low volume high intensity interval training: striking similarities in acute and chronic adaptation. *PLoS One* 9: e98119, 2014. doi: 10.1371/journal.pone.0098119.

144. **Sengupta P**. The laboratory rat: relating its age with human's. *Int J Prev Med* 4: 624–630, 2013.

145. **Shi X**, **Stevens GHJ**, **Foresman BH**, **Stern SA**, **Raven PB**. Autonomic nervous system control of the heart: endurance exercise training [Online]. *Med Sci Sport Exerc* 27, 1995. https://journals.lww.com/acsm-msse/Fulltext/1995/10000/Autonomic_nervous_system_control_of_the_heart_.9.aspx.

146. **Shirvani H**, **Farahani MM**, **Ahmadzadeh M**, **Dinca AR**. Comparison of three-month HIIT and CMT effects on left ventricle echocardiography observations in male employees. *J. Clin. Med.* 11: 2022.

147. **Skow RJ**, **Fraser GM**, **Steinback CD**, **Davenport MH**. Prenatal exercise and cardiovascular health (PEACH) study: impact on muscle sympathetic nerve (re)activity. *Med Sci Sports Exerc* 53: 1101–1113, 2021. doi: 10.1249/MSS.0000000000002583.

148. **Slordahl SA**, **Madslien VOE**, **Stoylen A**, **Kjos A**, **Helgerud J**, **Wisloff U**. Atrioventricular plane displacement in untrained and trained females. *Med Sci Sport Exerc* 36, 2004.

149. **Smith ML**, **Hudson DL**, **Graitzer HM**, **Raven PB**. Exercise training bradycardia: the role of autonomic balance. *Med Sci Sport Exerc* 21, 1989.

150. **Soylu Y**, **Arslan E**, **Kilit B**, **Clemente F**. Effects of self-paced high-intensity training and moderate-intensity continous training on the physical performance and psychophsiological responses in recreationally active young adults. *Biol Sport* 38: 555–562, 2021. doi: 10.5114/biolsport.2021.100359.

151. **Spina RJ**, **Ogawa T**, **Martin WH 3rd**, **Coggan AR**, **Holloszy JO**, **Ehsani AA**. Exercise training prevents decline in stroke volume during exercise in young healthy subjects. *J Appl Physiol* 72: 2458–2462, 1992. doi: 10.1152/jappl.1992.72.6.2458.

152. **Stickford ASL**, **Stute NL**, **Luck JC**, **Goodman T**, **Stickford JL**. Neural cardiovascular control is similar in female habitual exercisers and non-exercisers: a pilot study. *Clin Auton Res* 31: 775–778, 2021. doi: 10.1007/s10286-021-00828-z.

153. **Sullivan M**, **Genter F**, **Savvides M**, **Roberts M**, **Myers J**, **Froelicher V**. The reproducibility of hemodynamic, electrocardiographic, and gas exchange data during treadmill exercise in patients with stable angina pectoris. *Chest* 86: 375–382, 1984. doi: 10.1378/chest.86.3.375.

154. **Svedenhag J**, **Wallin BG**, **Sundlof G**, **Henriksson J**. Skeletal muscle sympathetic activity at rest in trained and untrained subjects. *Acta Physiol Scand* 120: 499–504, 1984. doi: https://doi.org/10.1111/j.1748-1716.1984.tb07413.x.

155. **Swenne CA**. Baroreflex sensitivity: mechanisms and measurement. *Netherlands Hear J* 21: 58–60, 2013. doi: 10.1007/s12471-012-0346-y.

156. **Tan CO**, **Tzeng YC**, **Taylor JA**. Sex differences in sympathetic neurovascular transduction in humans. *Auton Neurosci Basic Clin* 163: 72, 2011. doi: 10.1016/j.autneu.2011.05.098.

157. **Tanaka H**, **Monahan KD**, **Seals DR**. Age-predicted maximal heart rate revisited. *J Am Coll Cardiol* 37: 153–156, 2001. doi: 10.1016/S0735-1097(00)01054-8.

158. **Thijssen DHJ**, **Bruno RM**, **van Mil ACCM**, **Holder SM**, **Faita F**, **Greyling A**, **Zock PL**, **Taddei S**, **Deanfield JE**, **Luscher T**, **Green DJ**, **Ghiadoni L**. Expert consensus and evidence-based recommendations for the assessment of flow-mediated dilation in humans. *Eur Heart J* 40: 2534–2547, 2019. doi: 10.1093/eurheartj/ehz350.

159. **Trapp EG**, **Chisholm DJ**, **Freund J**, **Boutcher SH**. The effects of high-intensity intermittent exercise training on fat loss and fasting insulin levels of young women. *Int J Obes* 32: 684–691, 2008. doi: 10.1038/sj.ijo.0803781.

160. **Tymko MM**, **Berthelsen LF**, **Skow RJ**, **Steele AR**, **Fraser GM**, **Steinback CD**. Assessing static and dynamic sympathetic transduction using microneurography. *J Appl Physiol* 130: 1626–1634, 2021. doi: 10.1152/japplphysiol.00032.2021.

161. **Vallbo ÅB**, **Hagbarth K-E**, **Wallin BG**. Microneurography: how the technique developed and its role in the investigation of the sympathetic nervous system. *J Appl Physiol* 96: 1262–1269, 2004. doi: 10.1152/japplphysiol.00470.2003.

162. **Vallbo AB**, **Hagbarth KE**. Impulses recorded with micro-electrodes in human muscle nerves during stimulation of mechanoreceptors and voluntary contractions. *Electroencephalogr Clin Neurophysiol* 23: 392, 1967.

163. **Vianna LC**, **Hart EC**, **Fairfax ST**, **Charkoudian N**, **Joyner MJ**, **Fadel PJ**. Influence of age and sex on the pressor response following a spontaneous burst of muscle sympathetic nerve activity. *Am J Physiol Circ Physiol* 302: H2419–H2427, 2012. doi: 10.1152/ajpheart.01105.2011.

164. **Von Dobeln W**. Human standard and maximal metabolic rate in relation to fat-free body mass. *Acta Physiol Scand Suppl* 37: 1–79, 1956.

165. **Warburton DE**, **Haykowsky MJ**, **Quinney HA**, **Blackmore D**, **Teo KK**, **Taylor DA**, **McGavock J**, **Humen DP**. Blood volume expansion and cardiorespiratory function: effects of training modality. *Med Sci Sport Exerc* 36, 2004.

166. **Watso JC**, **Babcock MC**, **Migdal KU**, **Farquhar WB**, **Robinson AT**. The relation between habitual physical activity and sympathetic vascular transduction in healthy young adults. *Clin Auton Res* 31: 335–337, 2021. doi: 10.1007/s10286-021-00770-0.

167. **Weber CL**, **Schneider DA**. Increases in maximal accumulated oxygen deficit after high-intensity interval training are not gender dependent. *J Appl Physiol* 92: 1795–1801, 2002. doi: 10.1152/japplphysiol.00546.2001.

168. **Wehrwein EA**, **Joyner MJ**. Chapter 8 - Regulation of blood pressure by the arterial baroreflex and autonomic nervous system. In: *Autonomic Nervous System*, edited by Buijs RM, Swaab DFBT-H of CN. Elsevier, p. 89–102.

169. **Weir LL**, **Weir JP**, **Housh TJ**, **Johnson GO**. Effect of an aerobic training program on physical working capacity at heart rate threshold. *Eur J Appl Physiol Occup Physiol* 75: 351–356, 1997. doi: 10.1007/s004210050171.

170. **Wen D**, **Utesch T**, **Wu J**, **Robertson S**, **Liu J**, **Hu G**, **Chen H**. Effects of different protocols of high intensity interval training for VO2max improvements in adults: A meta-analysis of randomised controlled trials. *J Sci Med Sport* 22: 941–947, 2019. doi: https://doi.org/10.1016/j.jsams.2019.01.013.

171. **Whelton SP**, **Chin A**, **Xin X**, **He J**. Effect of aerobic exercise on blood pressure. *Ann Intern Med* 136: 493–503, 2002. doi: 10.7326/0003-4819-136-7-200204020-00006.

172. **White DW**, **Shoemaker JK**, **Raven PB**. Methods and considerations for the analysis and standardization of assessing muscle sympathetic nerve activity in humans. *Auton Neurosci* 193: 12–21, 2015.

173. **Williams JS**, **Del Giudice M**, **Gurd BJ**, **Pyke KE**. Reproducible improvement in endothelial function following two separate periods of high-intensity interval training in young men. *J Appl Physiol* 129: 725–731, 2020. doi: 10.1152/japplphysiol.00054.2020.

174. **Wright S**, **Esfandiari S**, **Elmayergi N**, **Sasson Z**, **Goodman JM**. Left atrial functional changes following short-term exercise training. *Eur J Appl Physiol* 114: 2667–2675, 2014. doi: 10.1007/s00421-014-2989-4.

175. **Xue B**, **Singh M**, **Guo F**, **Hay M**, **Johnson AK**. Protective actions of estrogen on angiotensin II-induced hypertension: role of central nitric oxide. *Am J Physiol Heart Circ Physiol* 297: H1638-46, 2009. doi: 10.1152/ajpheart.00502.2009.

176. **Yamamoto K**, **Miyachi M**, **Saitoh T**, **Yoshioka A**, **Onodera S**. Effects of endurance training on resting and post-exercise cardiac autonomic control. *Med Sci Sport Exerc* 33, 2001.

177. **Yano Y**. Blood pressure in young adults and cardiovascular disease later in life. *Am J Hypertens* 34: 250–257, 2021. doi: 10.1093/ajh/hpab005.

178. **Young BE**, **Greaney JL**, **Keller DM**, **Fadel PJ**. Sympathetic transduction in humans: recent advances and methodological considerations. *Am J Physiol Circ Physiol* 320: H942–H953, 2021. doi: 10.1152/ajpheart.00926.2020.

Appendix A: Health History Questionnaire

Age: _____ years
Dominant Hand: _____

PARTICIPANT I.D. (Completed by Research Team): _____
Determination of body mass index (BMI):

i. What is your approximate weight in kilograms? _____
To convert from pounds to kilograms, multiply by 0.454

ii. What is your approximate height in meters? _____
To convert from inches to meters, multiple by 0.0254

iii. To calculate BMI, please click this link:
http://www.nhlbi.nih.gov/health/educational/lose_wt/BMI/bmicalc.htm

Calculated BMI: _____kg/m^2

The following questions will determine your eligibility for the study. If you answer 'Yes to any question you will <u>not</u> be able to participate in the study.

1. Was your calculated body mass index above <u>greater than</u> 30 kg/m^2? ☐YES ☐ NO
2. Are you younger than 18 years old? ☐YES ☐ NO
3. Have you smoked or consumed any nicotine/marijuana-containing products daily within the past 6 months? ☐YES ☐ NO
4. Are you allergic to Tegaderm™ (3M) medical adhesive dressing? ☐YES ☐ NO
5. Have you been prescribed medications for high blood pressure? ☐YES ☐ NO
6. Do you have a cardiovascular, neural (e.g., Raynaud's disease), respiratory or metabolic disorder (e.g., diabetes)? ☐YES ☐ NO
7. Are you afraid of needles? ☐YES ☐ NO

<u>For females only:</u>

7. Are you pregnant, breastfeeding or intending to become pregnant in the next 3 months? ☐YES ☐ NO
8. Are you currently on, or planning on starting, hormone replacement therapy? ☐YES ☐ NO
9. If you are <u>55 years or older</u>: Have you *had* a menstrual period in the last 12 months? ☐ not applicable ☐YES ☐ NO
10. If you are <u>younger than</u> 55 years: Have you been *without* a menstrual period for the last 12 months? ☐ not applicable ☐YES ☐ NO

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Get Active Questionnaire

CANADIAN SOCIETY FOR EXERCISE PHYSIOLOGY –
PHYSICAL ACTIVITY TRAINING FOR HEALTH (CSEP-PATH®)

Physical activity improves your physical and mental health. Even small amounts of physical activity are good, and more is better.

For almost everyone, the benefits of physical activity far outweigh any risks. For some individuals, specific advice from a Qualified Exercise Professional (QEP – has post-secondary education in exercise sciences and an advanced certification in the area – see csep.ca/certifications) or health care provider is advisable. **This questionnaire is intended for all ages – to help move you along the path to becoming more physically active.**

☐ I am completing this questionnaire for myself.

☐ I am completing this questionnaire for my child/dependent as parent/guardian.

PREPARE TO BECOME MORE ACTIVE

The following questions will help to ensure that you have a safe physical activity experience. Please answer **YES** or **NO** to each question before you become more physically active. If you are unsure about any question, answer **YES**.

YES	NO	
○	○	1 Have you experienced **ANY** of the following (A to F) **within the past six months**?
○	○	**A** A diagnosis of/treatment for heart disease or stroke, or pain/discomfort/pressure in your chest during activities of daily living or during physical activity?
○	○	**B** A diagnosis of/treatment for high blood pressure (BP), or a resting BP of 160/90 mmHg or higher?
○	○	**C** Dizziness or lightheadedness during physical activity?
○	○	**D** Shortness of breath at rest?
○	○	**E** Loss of consciousness/fainting for any reason?
○	○	**F** Concussion?
○	○	2 Do you currently have pain or swelling in any part of your body (such as from an injury, acute flare-up of arthritis, or back pain) that affects your ability to be physically active?
○	○	3 Has a health care provider told you that you should avoid or modify certain types of physical activity?
○	○	4 Do you have any other medical or physical condition (such as diabetes, cancer, osteoporosis, asthma, spinal cord injury) that may affect your ability to be physically active?

NO to all questions: go to Page 2 – ASSESS YOUR CURRENT PHYSICAL ACTIVITY

YES to any question: go to Reference Document – ADVICE ON WHAT TO DO IF YOU HAVE A YES RESPONSE

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Get Active Questionnaire

ASSESS YOUR CURRENT PHYSICAL ACTIVITY

Answer the following questions to assess how active you are now.

1 During a typical week, on how many days do you do moderate- to vigorous-intensity aerobic physical activity (such as brisk walking, cycling or jogging)? ☐ DAYS/WEEK

2 On days that you do at least moderate-intensity aerobic physical activity (e.g., brisk walking), for how many minutes do you do this activity? ☐ MINUTES/DAY

For **adults**, please multiply your average number of days/week by the average number of minutes/day: ☐ MINUTES/WEEK

Canadian Physical Activity Guidelines recommend that adults accumulate at least 150 minutes of moderate- to vigorous-intensity physical activity per week. For children and youth, at least 60 minutes daily is recommended. Strengthening muscles and bones at least two times per week for adults, and three times per week for children and youth, is also recommended (see csep.ca/guidelines).

GENERAL ADVICE FOR BECOMING MORE ACTIVE

Increase your physical activity gradually so that you have a positive experience. Build physical activities that you enjoy into your day (e.g., take a walk with a friend, ride your bike to school or work) and reduce your sedentary behaviour (e.g., prolonged sitting).

If you want to do **vigorous-intensity physical activity** (i.e., physical activity at an intensity that makes it hard to carry on a conversation), and you do not meet minimum physical activity recommendations noted above, consult a Qualified Exercise Professional (QEP) beforehand. This can help ensure that your physical activity is safe and suitable for your circumstances.

Physical activity is also an important part of a healthy pregnancy.

Delay becoming more active if you are not feeling well because of a temporary illness.

DECLARATION

To the best of my knowledge, all of the information I have supplied on this questionnaire is correct.
If my health changes, I will complete this questionnaire again.

I answered NO to all questions on Page 1

Sign and date the Declaration below

I answered YES to any question on Page 1

Check the box below that applies to you:

☐ I have consulted a health care provider or Qualified Exercise Professional (QEP) who has recommended that I become more physically active.

☐ I am comfortable with becoming more physically active on my own without consulting a health care provider or QEP.

Name (+ Name of Parent/Guardian if applicable) [Please print] | Signature (or Signature of Parent/Guardian if applicable) | Date of Birth

Date | Email (optional) | Telephone (optional)

With planning and support you can enjoy the benefits of becoming more physically active. A QEP can help.

☐ Check this box if you would like to consult a QEP about becoming more physically active.
(This completed questionnaire will help the QEP get to know you and understand your needs.)

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Get Active Questionnaire – Reference Document

ADVICE ON WHAT TO DO IF YOU HAVE A **YES** RESPONSE

Use this reference document if you answered **YES** to any question and you have not consulted a health care provider or Qualified Exercise Professional (QEP) about becoming more physically active.

2 Do you currently have pain or swelling in any part of your body (such as from an injury, acute flare-up of arthritis, or back pain) that affects your ability to be physically active? ☐ YES

If this swelling or pain is new, consult a health care provider. Otherwise, keep joints healthy and reduce pain by moving your joints slowly and gently through the entire pain-free range of motion. If you have hip, knee or ankle pain, choose low-impact activities such as swimming or cycling. As the pain subsides, gradually resume your normal physical activities starting at a level lower than before the flare-up. Consult a Qualified Exercise Professional (QEP) in follow-up to help you become more active and prevent or minimize future pain.

3 Has a health care provider told you that you should avoid or modify certain types of physical activity? ☐ YES

Listen to the advice of your health care provider. A Qualified Exercise Professional (QEP) will ask you about any considerations and provide specific advice for physical activity that is safe and that takes your lifestyle and health care provider's advice into account.

4 Do you have any other medical or physical condition (such as diabetes, cancer, osteoporosis, asthma, spinal cord injury) that may affect your ability to be physically active? ☐ YES

Some people may worry if they have a medical or physical condition that physical activity might be unsafe. In fact, regular physical activity can help to manage and improve many conditions. Physical activity can also reduce the risk of complications. A Qualified Exercise Professional (QEP) can help with specific advice for physical activity that is safe and that takes your medical history and lifestyle into account.

After reading the ADVICE for your YES response, go to Page 2 of the *Get Active Questionnaire* – ASSESS YOUR CURRENT PHYSICAL ACTIVITY

WANT ADDITIONAL INFORMATION ON BECOMING MORE PHYSICALLY ACTIVE?

- **csep.ca/certifications**
  CSEP Certified members can help you with your physical activity goals.
- **csep.ca/guidelines**
  Canadian Physical Activity Guidelines for all ages.

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Get Active Questionnaire – Reference Document

ADVICE ON WHAT TO DO IF YOU HAVE A **YES** RESPONSE

Use this reference document if you answered YES to any question and you have not consulted a health care provider or Qualified Exercise Professional (QEP) about becoming more physically active.

1 Have you experienced ANY of the following (A to F) within the past six months?	
A A diagnosis of/treatment for heart disease or stroke, or pain/discomfort/pressure in your chest during activities of daily living or during physical activity? ☐ YES	Physical activity is likely to be beneficial. If you have been treated for heart disease but have not completed a cardiac rehabilitation program within the past 6 months, consult a doctor – a supervised cardiac rehabilitation program is strongly recommended. If you are resuming physical activity after more than 6 months of inactivity, begin slowly with light- to moderate-intensity physical activity. If you have pain/discomfort/pressure in your chest and it is new for you, talk to a doctor. Describe the symptom and what activities bring it on.
B A diagnosis of/treatment for high blood pressure (BP), or a resting BP of 160/90 mmHg or higher? ☐ YES	Physical activity is likely to be beneficial if you have been diagnosed and treated for high blood pressure (BP). If you are unsure of your resting BP, consult a health care provider or a Qualified Exercise Professional (QEP) to have it measured. If you are taking BP medication and your BP is under good control, regular physical activity is recommended as it may help to lower your BP. Your doctor should be aware of your physical activity level so your medication needs can be monitored. If your BP is 160/90 or higher, you should receive medical clearance and consult a QEP about safe and appropriate physical activity.
C Dizziness or lightheadedness during physical activity ☐ YES	There are several possible reasons for feeling this way and many are not worrisome. Before becoming more active, consult a health care provider to identify reasons and minimize risk. Until then, refrain from increasing the intensity of your physical activity.
D Shortness of breath at rest ☐ YES	If you have asthma and this is relieved with medication, light to moderate physical activity is safe. If your shortness of breath is not relieved with medication, consult a doctor.
E Loss of consciousness/fainting for any reason ☐ YES	Before becoming more active, consult a doctor to identify reasons and minimize risk. Once you are medically cleared, consult a Qualified Exercise Professional (QEP) about types of physical activity suitable for your condition.
F Concussion ☐ YES	A concussion is an injury to the brain that requires time to recover. Increasing physical activity while still experiencing symptoms may worsen your symptoms, lengthen your recovery, and increase your risk for another concussion. A health care provider will let you know when you can start becoming more physically active, and a Qualified Exercise Professional (QEP) can help get you started.

After reading the ADVICE for your YES response, go to Page 2 of the *Get Active Questionnaire* – ASSESS YOUR CURRENT PHYSICAL ACTIVITY

Appendix C: Letter of REB Approval

DALHOUSIE UNIVERSITY
Research Services

Health Sciences Research Ethics Board
Letter of Approval

May 25, 2021

Derek Kimmerly
Health\School of Health and Human Performance

Dear Derek,

REB #: 2021-5555
Project Title: The impact of a 12-week high-intensity interval training program on arterial health

Effective Date: May 25, 2021
Expiry Date: May 25, 2022

The Health Sciences Research Ethics Board has reviewed your application for research involving humans and found the proposed research to be in accordance with the Tri-Council Policy Statement on *Ethical Conduct for Research Involving Humans*. This approval will be in effect for 12 months as indicated above. This approval is subject to the conditions listed below which constitute your on-going responsibilities with respect to the ethical conduct of this research.

Effective March 16, 2020: Notwithstanding this approval, any research conducted during the COVID-19 public health emergency must comply with federal and provincial public health advice as well as directives from Dalhousie University (and/or other facilities or jurisdictions where the research will occur) regarding preventing the spread of COVID-19.

Sincerely,

[redacted]
Dr. Lori Weeks, Chair

Appendix D: LabChart Analysis Instruction

1. Calibrate the Portapres signal using the static automated blood pressure measurements using units conversion.
2. Derive heart rate, stroke volume, systolic blood pressure, and diastolic blood pressure, into their own channels using cyclic measurements or the non-invasive cardiac output add-on (stroke volume only).
    a. Setup → Channel Settings → Use number of channels along bottom of pop-up box to add or remove channels → Create new channels for HR, SBP, DBP, SV
    b. Heart rate → Calculation → Cyclic Measurements → Source → ECG → Measurement → Rate → Change preset detection setting to "ECG - Human" → adjust detection adjustment if it isn't picking up the peak r-waves
    c. Systolic blood pressure → Calculation → Cyclic Measurements → Source → Portapres → Measurement → Maximum → Preset detection to "Cardiovascular - Arterial pressure"
    d. Diastolic blood pressure → Calculation → Cyclic Measurements → Source → Portapres → Measurement → Minimum → Preset detection to "Cardiovascular - Arterial pressure"
    e. Stroke Volume → Calculation → Non-invasive cardiac output → Pressure signal → Portapres (must already be calibrated to mmHg) → Method→ Windkessel Model → Output → Stroke Volume → Adjust subject age and sex
3. Time align the Portapres signal to the ECG signal using the marker ('M' in bottom left-hand corner).
    a. Zoom in on a small section of data → Drag "M" to the peak of an r-wave in the ECG channel → place cursor at the closest peak in the Portapres channel → a time difference between the "M" and where the cursor is will show in the top right hand corner of the screen above where the channels are listed → this time difference is the shift in the data → Click on the Portapres menu → Shift → insert time difference (negative numbers will shift backwards, positive shifts forwards)
    b. The MSNA signal will need to be shifted to the middle of the portapres and ECG signal so that each burst occurs in the middle of the ECGs R-R interval to ensure each MSNA burst corresponds to a cardiac cycle. This can be done using the same method as above, but should be done after completing the "beat macro" in section 2 (i.e. shift bursts to align with "beat comment").
4. Set up datapad. In datapad, each channel will have its own column automatically. Create the following new channels (in any order):
    a. Int MSNA Maximum – Minimum (Statistics → Source Int MSNA)
    b. Int MSNA Comment Number (Comment → Source Int MSNA)
    c. Int MSNA Integral Relative to Baseline (V*s) (Integral → Source Int MSNA)
    d. Time (Selection & Active Point)

e. Int MSNA Comment Time (Comment → Source Int MSNA)
f. ECG Full Comment Text (Comment → Source ECG)
g. ECG Comment Number (Comment → Source ECG)
h. Mean Heart Rate (Statistics → Source Heart Rate)
i. Mean SBP (Statistics → Source SBP)
j. Mean DBP (Statistics → Source DBP)
k. Mean SV (Statistics → Source SV)

5. If you have the macros already on your computer (they can be imported), play them or create them following instructions below.
    a. Two macros are needed for this. One to identify each MSNA burst and one to identify each cardiac cycle. Click anywhere in the corresponding channel to begin (e.g., integrated MSNA recording in row 3).
        i. <u>MSNA Burst Macro:</u> Start Recording Macro → Recording Commands → Repeat while in Block → Commands → Find → Search in MSNA Channel → Find data → Local Maxima → Threshold =1% → Single point selection → Hit 'Enter' → Add Comment 'Burst' in MSNA Channel
            1. Repeat using 'Commands' → 'find next'. After adding a second comment to the next burst, press 'Macro' → Stop Recording (Do not close any other windows that have popped up before stopping recording).
        ii. <u>ECG Beat Macro:</u> Identical to above except for searching in ECG channel, naming the comment 'Beat' and using 'select to previous point' under the find data heading.
    b. Go to the beginning of your resting period with stable MSNA data and run both macros.
6. Go through the integrated MSNA channel and ensure all valid bursts are selected. Add or delete 'Burst' comments when applicable.
7. Delete 'BEAT' comments that are inaccurately detected (could change macro threshold if necessary).
8. Highlight area of interest. Click 'Multiple add to DataPad'. Find using comment from ECG channel containing 'Beat'. Select 0.9s around comment. Current selection.
    a. 0.9s may not be applicable for everyone, go through the file and use the marker to get a sense of how wide most of your bursts are (record this value). Adjust if bursts are larger than 0.45s to the left and 0.45s to the right of the "burst" comment.
    b. Copy and paste entire Datapad into the first sheet on the excel template provided
    c. (Control-A, control-C, and Control-V to select all data, copy the data and paste the data).

Appendix E: Curriculum Vitae

ACADEMIC BACKGROUND

MSc Kinesiology Candidate, Dalhousie University, Canada

Supervisor: Dr. Derek Kimmerly

Defence Date: 06/2023

BSc Honours Kinesiology, Dalhousie University, Canada

Supervisor: Dr. Ryan Frayne

Thesis Title: "Validation of activPAL Determined Knee-Bent Sitting Angles"

WORK EXPERIENCE

Personal Trainer, Dalplex, Dalhousie University **03/2022 – Present**

- Creates personalized exercise and health plans for clients and runs individual exercise sessions based on the health requirements and goals set by both the trainer and client
- Creates exercise programs that can be done on independently of the training sessions with the trainer

Research Assistant, Nova Scotia Health, Halifax, NS. **12/2021– 05/2023**

- Runs sedentary behaviour intervention in Geriatrics Clinic at QEII Health Sciences Center involving the monitoring of activity devices, creating activity goals, and promoting an active lifestyle in geriatric patients

Teaching Assistant, Dalhousie University, Halifax **09/2021– 05/2023**

- Instruct exercise physiology, motor control, and statistics/research labs and grade lab assignments

Women's Basketball Athletic Trainer, St. Mary's University **09/2021– 05/2022**

- Assessment, treatment, and therapy for all injuries that occurred during games/practices
- Evaluation and handling of emergency injuries on court

Cardiology Research Assistant, Toronto General Hospital, Toronto **06/2019– 09/2019**

- Data extraction and compilation for research study regarding *improving neural network patient prediction of heart failure through breath-by-breath measurement*
- Observed rounds and procedures with **Dr. Phyllis Billia,** cardiologist, heart failure specialist, and Director of Research at the Peter Munk Cardiac Centre and Co-Director of Peter Munk Cardiac Centre biobank

Cardiology Research Assistant, Toronto General Hospital, Toronto **06/2019– 09/2019**

- Data extraction and compilation for research study regarding *improving neural network patient prediction of heart failure through breath-by-breath measurement*
- Observed rounds and procedures with **Dr. Phyllis Billia,** cardiologist, heart failure specialist, and Director of Research at the Peter Munk Cardiac Centre and Co-Director of Peter Munk Cardiac Centre biobank

Private Swim Instructor, **05/2019 – Present**

- Lead swim classes involving teaching toddles fundamental swim skills
- Demonstrated and explained swim safety precautions involving toddlers to their parents

DISTINCTIONS/AWARDS

Top Graduate Student Presentation, APES+ Conference (2023)

Conference distinction and monetary prize ($300)

Frederick Banting and Charles Best Canada Graduate Scholarship-Master's, CIHR

Monetary allotment: $17,500 over 1 year

Research Nova Scota Graduate Scholarship

Monetary allotment: $10,000 over 1 year

Dalhousie University Undergraduate Entrance and In-Course and Scholarships

Monetary allotment: $2,150 over 4 years

2nd Place Undergraduate Student Presentation, APES+ Conference (2021)

Physiology Undergraduate Summer Research Award, University of Toronto

Monetary allotment: $4,000 over 3 months

CERTIFICATIONS

Canadian Society of Exercise Physiology Certified Personal Trainer

Lifesaving Society Bronze Medallion Examiner

Lifesaving Society Bronze National Lifeguard

Lifesaving Society Life Saving Instructor

PRESENTATIONS:

- American College of Sports Medicine (June 2023). Title: Impact of High-Intensity Interval Training on Spontaneous Sympathetic Transduction in Healthy Younger Adults
- Atlantic Province Exercise Scientists and Socio-Culturalists (March 2023). Title: Impact of High-Intensity Interval Training on Spontaneous Sympathetic Transduction in Healthy Younger Adults
- Canadian Society for Exercise Physiology (October 2022): Impact of Habitual Sedentary Time and Physical Activity on Beat-by-Beat Blood Pressure Variability in Healthy Adults
- Atlantic Province Exercise Scientists and Socio-Culturalists (March 2021). Title: Validation of ActivPAL Determined Knee-Bent Sitting Angles

PUBLICATIONS

Abstracts:

- Yanlin Wu, Myles W O'Brien, Alex Peddle, W Seth Daley, **Beverly Schwartz**, Derek S Kimmerly, Ryan J Frayne. (2022). Criterion Validity Of Tri-monitor Activpal Configuration In Determining Knee-flexion Angles During Sitting In Laboratory Setting. *Medicine & Science in Sports & Science*, Sppl.9, S54

Peer Reviewed Publications:

- **Schwartz BD,** Pellerine LP, Bray NW, Fowles, JR, Furlano JA, Morava A, Nagpal TS, O'Brien MW. Binge drinking and smoking are associated with worse academic performance in Canadian undergraduate students, *J Am Coll Health,* doi: 10.1080/07448481.2023.2232871
- **Schwartz BD,** Shivgulam ME, Petterson JL, Wu Y, Frayne RJ, Kimmerly DS, O'Brien MW. More moderate-intensity physical activity and less prolonged sedentary time are associated with better very short-term systolic blood pressure

variability in healthy adults**.** *Journal of Human Hypertension,* https://doi.org/10.1038/s41371-023-00832-y

- O'Brien MW, **Schwartz BD,** Shivgulam ME, Daley WS, Frayne RJ, Kimmerly DS. Higher habitual lying time is inversely associated with vagal-related heart rate variability outcomes in younger adults. *Appl Physiol Nutr Metab.* doi: https://doi.org/10.1139/apnm-2023-0167
- Shivgulam ME, Frayne RJ, **Schwartz BD**, Wu Y, Daley SW, Kimmerly DS, O'Brien MW. Validity of activPAL CREA software detection of sitting and lying during free-living conditions. *Physiol Meas.* doi: 0.1088/1361-6579/ace14e
- Shivgulam ME, Liu H**, Schwartz BD**, Langley JE, Bray NW, Kimmerly DS, O'Brien MW. Impact of Exercise Training Interventions on Flow-Mediated Dilation in Adults: An Umbrella Review. *Sport Med,* doi: 10.1007/s40279-023-01837-w.
- O'Brien MW, Daley WS, **Schwartz BD**, Shivgulam ME, Wu Y, Kimmerly DS, Frayne RJ. Characterization of Detailed Sedentary Postures Using a Tri-Monitor ActivPAL Configuration in Free-Living Conditions. *Sensors,* https://doi.org/10.3390/s23020587
- O'Brien MW, **Schwartz BD**, Petterson JL, Kimmerly DS. Comparison of signal-averaging and regression approaches to analyzing sympathetic transduction. *Clin Auton Res,* https://doi.org/10.1007/s10286-022-00874-1

Appendix F: Demographics Table

Table 1S. Demographics of participants.

	Control (n=15)	**HIIT (n=18)**
Age (years) (Mean±SD)	25 ± 9 (19 – 59)	26 ± 11 (19 – 59)
Sex		
Male (n)	6	8
Female (n)	9	10
Race		
White (n)	12	14
Asian (n)	2	2
Multi-Racial (n)	1	2