LIMB AND INSPIRATORY METABOREFLEX ACTIVATION IN HEALTHY MALES AND FEMALES

by

Jenna Benbaruj

B.H.K., The University of British Columbia, 2020

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE AND POSTDOCTORAL STUDIES

(Kinesiology)

THE UNIVERSITY OF BRITISH COLUMBIA

(Vancouver)

August 2022

The following individuals certify that they have read, and recommend to the Faculty of Graduate and Postdoctoral Studies for acceptance, a thesis entitled:

Limb and Inspiratory Metaboreflex Activation in Healthy Males and Females

submitted by Jenna Benbaruj in partial fulfillment of the requirements for

the degree of Master of Science

in Kinesiology

Examining Committee:

Dr. William Sheel, Professor, Kinesiology, UBC (Vancouver)
Supervisor

Dr. Glen Foster, Associate Professor, Health and Exercise Sciences, UBC (Okanagan)
Supervisory Committee Member

Dr. Robert Boushel, Director and Professor, Kinesiology, UBC (Vancouver)
Supervisory Committee Member

Abstract

The muscle metaboreflex is a cardiovascular control mechanism that contributes to the reflex increase in mean arterial pressure (MAP) and blood flow redistribution during exercise, to meet metabolic demands. The MAP response to metaboreflex activation is attenuated in females compared to males, during both limb and respiratory muscle work. Whether this sex-based difference is similar in magnitude across muscle groups is currently not well-understood. **PURPOSE:** The purpose of this thesis was to compare sex-based differences in the pressure response to limb and inspiratory metaboreflex activation, during relative and absolute workloads. **METHODS:** Seventeen healthy participants (n=9 males, n=8 females) completed two experimental visits; the first visit included pulmonary function tests, forearm volume and circumference measurements, and two bouts of exercise. The second day mimicked the first, except with no pulmonary function measurements. The exercise performed on both days were acute bouts of intermittent handgrip exercise (IHE) and pressure threshold loading (PTL) to volitional exhaustion, performed in a randomized order, and separated by 30-min of rest. PTL is a resistive breathing task that requires participants to generate large inspiratory pressures to overcome a threshold load, and attain unimpeded breathing. Participants exercised at a predetermined relative (R) or absolute (A) workload, and cardiopulmonary measurements were recorded continuously throughout. **RESULTS:** A time-dependent rise in MAP was observed in all participants, regardless of sex, muscle, or workload ($p<0.001$). MAP was greater in males than females during all exercise bouts regardless of muscle group or workload ($p<0.001$). The change in MAP from baseline was also greater in males (R-IHE: Δ31±12 mmHg; R-PTL: Δ31±9; A-IHE: Δ35±6; A-PTL: Δ30±7) than females (R-IHE: Δ21±7 mmHg; R-PTL: Δ13±7; A-IHE: Δ21±7; A-PTL: Δ14±3) ($p<0.001$). **CONCLUSION:** The present study observed a time-

dependent increase in MAP in both males and females, in response to comparable amounts of limb and respiratory muscle work. The MAP response was greater in males than females, regardless of muscle group or workload. Findings from the present study suggest that the sex-based difference in the response to metaboreflex activation is similar between the limb and respiratory musculature.

Lay Summary

The reflex increase in mean arterial pressure (MAP) during exercise is attenuated in females compared to males during both limb exercise and resistive breathing tasks. This study compared the sex-based difference in the MAP response to similar amounts of limb and respiratory muscle work. It was hypothesized that the pressor response to exercise would be greater in males than females, but similar across muscle groups. 17 healthy adults (n=9 males, n=8 females) participated in this study and completed two bouts each of intermittent handgrip exercise and a resistive breathing task, all performed to task failure. Males exhibited a greater increase in MAP than females in all exercise bouts, regardless of muscle group or workload ($p < 0.001$). Findings from this study suggest that when the limb and respiratory muscle perform similar amounts of work, the pressor response and associated sex-based differences will be comparable regardless of workload.

Preface

This research study was designed by myself, Jenna Benbaruj, with the help of my supervisor and committee (Dr. Bill Sheel, Dr. Glen Foster, and Dr. Robert Boushel), and members of the Health and Integrative Physiology Lab at the University of British Columbia. The scheduling and testing were performed by myself, Michael Leahy, Thora Rae, and Rachel Jackman. Various other members of the lab assisted me greatly in the testing protocols. Analysis, interpretation, and writing was completed by myself with assistance from the Health and Integrative Physiology Lab students. All methods executed in this thesis were approved by The University of British Columbia's Research Ethics Board (H21-01579).

Table of Contents

List of Tables

List of Figures

List of Abbreviations

ANOVA	Analysis of Variance
f_B	Breathing Frequency
DLCO	Diffusion Capacity of the Lung
EELV	End Expiratory Lung Volume
EPR	Exercise Pressor Reflex
ETCO2	End-Tidal CO_2
FEV1.0	Fraction of Expired Volume in 1-second
F_IO_2	Fraction of Inspired Oxygen
FRC	Functional Residual Capacity
FTP	Force-Time Product
FTP_{BW}	Force-Time Product Normalized to Body Weight
FTP_{FV}	Force-Time Product Normalized to Forearm Volume
FVC	Full Vital Capacity
HR	Heart Rate
IHE	Intermittent Handgrip Exercise
IHE-A	Intermittent Handgrip Exercise, Performed at an Absolute Workload
IHE-R	Intermittent Handgrip Exercise, Performed at a Relative Workload
IMA	Inspiratory Metaboreflex Activation
LMA	Limb Metaboreflex Activation
MAP	Mean Arterial Pressure
MIP	Maximal Inspiratory Pressure

MIP_{mo}	Maximal Inspiratory Pressure at the Mouth
MVC	Maximal Voluntary Contraction
MVC_{PEAK}	Maximum Force Generated During Maximal Voluntary Contraction
P_{eso}	Esophageal Pressure
P_{di}	Diaphragm Pressure
$P_{di,MAX}$	Maximum Inspiratory Transdiaphragmatic Pressure
P_{ga}	Gastric Pressure
P_{mo}	Mouth Pressure
$P_{ET}CO_2$	Pressure of End-Tidal CO_2
PCO_2	Partial Pressure of CO_2
PNS	Peripheral Nervous System
PO_2	Partial Pressure of O_2
PTL	Pressure-Threshold Loading
PTL-A	Pressure-Threshold Loading, Performed at an Absolute Workload
PTL-R	Pressure-Threshold Loading, Performed at a Relative Workload
PTP	Pressure Time Product
PTP_{eso}	Pressure Time Product of the Esophagus
$PTP_{eso,BW}$	Pressure-Time Product of the Esophagus Normalized to Body Weight
PTP_{di}	Pressure Time Product of the Diaphragm
$PTP_{di,BW}$	Pressure-Time Product of the Diaphragm Normalized to Body Weight
PTP_{mo}	Pressure Time Product of the Mouth
$PTP_{mo,BW}$	Pressure-Time Product of the Mouth Normalized to Body Weight
PTP_{tot}	Pressure Time Product of the Total Respiratory System

SBP	Systolic Blood Pressure
T_C	Time Spent Contracting the Forearm Muscle
T_I	Time Spent Performing Inspiration
T_C/T_{TOT},	Ratio of Time Spent Contracting Muscle to Time of Total Contraction Cycle
T_I/T_{TOT}	Ratio of Time Spent Performing Inspiration to Time of Total Respiratory Cycle
TLC	Total Lung Capacity
TTF	Time to Task Failure
TTI	Tension-Time Index
TTI_{di}	Tension-Time Index of Diaphragm
TTI_{fm}	Tension-Time Index of Forearm Muscle
$\dot{V}_E$	Minute Ventilation
VT	Tidal Volume
W*b*	Work of Breathing

Acknowledgements

I would like to express my enduring gratitude to Dr. Sheel, Dr. Foster, Dr. Boushel, members of the Health and Integrative Physiology laboratory, and other students in the School of Kinesiology who have provided tremendous support throughout this thesis. I owe particular thanks to Dr. Sheel, whose guidance, encouragement, and support has been particularly impactful in the completion of this project, and who never ceases to amaze with thoughtful (and often humorous) words of wisdom. I would also like to thank Michael Leahy, Rachel Jackman, and Thora Rae for the many hours they spent assisting with data collection for this project. Importantly, I would also like to acknowledge that all the work for this thesis was conducted on the traditional territory of the Musqueam people.

Dedication

This work is dedicated to my parents, family, and loves ones; who have provided endless support throughout my academic career, and who are consistently outstanding role models and mentors for myself and others.

Chapter 1: Introduction

During dynamic exercise, metabolic turnover can increase up to 100-fold that of resting rates. Cardiorespiratory responses to dynamic exercise are fundamental in matching oxygen delivery to the increased metabolic demands. These responses rise from both central and peripheral signals; primarily central command, the exercise pressor reflex, and arterial baroreceptors. Of these three mechanisms, the exercise pressor reflex has been given abundant consideration given its role in inducing increases in both sympathetic outflow and arterial blood pressure during static and dynamic exercise (Alam & Smirk 1937; Mark *et al.*, 1985; Victor *et al*., 1988; Rowell & O'Leary, 1990; O'Leary, 1993). These responses occur due to the stimulation of muscle afferents, specifically myelinated group III and unmyelinated group IV nerve fibers, otherwise known as mechanoreceptors and metaboreceptors, respectfully (Alam & Smirk, 1937; McCloskey & Mitchell, 1972).

Alam and Smirk (1937) pioneered research on the exercise pressor reflex by using post-exercise circulatory occlusion (PECO) to trap metabolic by-products in the exercising limb, resulting in a sustained elevation in blood pressor following exercise. This technique isolates group IV afferent (or metaboreflex) activation from central command and group III (mechanoreflex) activation, and is still widely used as a model to investigate the metabolic component of the exercise pressor reflex (Kaufman *et al.*, 1984; Hayes *et al.*, 2006; Boushel, 2010; Teixeira *et al.,* 2019). The metaboreflex is accredited as a principal contributor to the exercise pressor response, as evidenced by the maintained elevation in systolic blood pressure (SBP) and mean arterial pressure (MAP) during PECO, despite minimized mechanical stimulation of group III afferents (Alam & Smirk, 1937; Kaufman *et al.,* 1984; Hayes *et al.*,

2006). As such, contemporary experimental models use PECO or ischemic exercise to isolate metaboreflex activation and infer to the exercise pressor response.

The importance of the metaboreflex is emphasized when considering the suggested role of the exercise pressor response in central and peripheral fatigability (Amann, 2012; Gandevia, 2001). The metaboreflex has a functional role in limiting the accumulation of fatigue-related metabolites by, 1) eliciting cardiorespiratory responses to increase exercising muscle perfusion and thus, oxygen delivery and metabolite clearance, and 2) providing sensory feedback to the central nervous system to inhibit central motor output (Amann, 2012; Broxterman *et al*., 2018). The latter function is evidenced by maintained deficits in voluntary activation and voluntary force output during PECO (Gandevia *et al*., 1996; Gandevia, 2001), and increased ATP consumption and metabolite accumulation when the metaboreflex is pharmacologically inhibited (Broxterman *et al*., 2018; Hureau *et al*., 2019). Therefore, the limb muscle metaboreflex demonstrates a role in both increasing exercising muscle perfusion and increasing central fatigue to prevent the development and progression of peripheral fatigue (Amann, 2012; Gandevia, 2001).

In an analogous manner, there is evidence to suggest that the metaboreflex is also present in the muscles of respiration. The inspiratory muscle metaboreflex is activated in cases of increased work of breathing (WOB) and diaphragmatic fatigue (DF), and is similar to the limb metaboreflex with respect to the evoked blood pressure responses (St. Croix *et al.* 2000; Derchak *et al.*, 2002; Sheel *et al.*, 2001). Limb and inspiratory muscle metaboreflex activation (LMA and IMA, respectively) both result in global sympathoexcitation and vasoconstriction of non-exercising muscle to maintain arterial pressure homeostasis at high exercise intensities (Sinoway *et al*., 1989; St Croix *et al.*, 2000; Sheel *et al*., 2001). However, IMA elicits vasoconstriction in

exercising limb skeletal muscle as well, resulting in an increase in respiratory muscle perfusion at the expense of the exercising limb (Harms *et al*., 1997; Harms *et al*., 1998; Hill, 2000; Sheel *et al*., 2001; Dominelli *et al*., 2017). Results from animal models suggest that this "stealing" of blood flow may result from differences in arteriole sensitivity to systemic vasoconstrictor outflow between the diaphragm and limb muscle (Laughlin *et al*., 1989; Wetter *et al*., 1999; Sheel *et al*., 2001). Therefore, the inspiratory and limb metaboreflexes can be considered to work in concert to modulate blood flow distribution between the limb and respiratory musculature during exercise.

There is growing evidence to suggest that sympathetic and pressor responses to both IMA and LMA are attenuated in females (Ettinger *et al.*, 1996; Jarvis *et al*., 2011; Smith *et al*., 2016; Katayama *et al*., 2018; Welch *et al*., 2018). Similarly, DF develops more slowly in females which is consistent with the interdependence of the onset of DF and IMA (Welch *et al*., 2018). Females are also more fatigue-resistant during isometric and rhythmic limb exercise (Hunter, 2014; Hunter, 2016). There are several proposed explanations for the observed differences in muscle fatigability between males and females (Hunter, 2014; Hunter, 2016), yet little is known about mechanisms underlying the sex-based differences in the limb and inspiratory metaboreflexes. Furthermore, differences in the pressor response to comparable levels of IMA and LMA are also not well researched. Recent evidence suggests that the limb and inspiratory metaboreflexes may interact in an additive manner, resulting in an augmented increase in blood pressure (BP) and systemic vascular resistance (SVR) (Smith *et al*., 2020). However, this study involved sub-maximal activation of both the limb and inspiratory metaboreflexes and did not investigate sex-based differences (Smith *et al.*, 2020). Additionally, the inspiratory and limb muscle work performed in this study was relative, despite evidence suggesting that the IMA

response is lower in females than males even when performing the same absolute level of diaphragmatic work (Geary *et al*., 2019). Lastly, evidence has shown that when the pressor response is adjusted to absolute handgrip strength (MVC; maximal voluntary contraction), sex-based differences are lost (Notay *et al*., 2018). Therefore, to better elucidate sex-based differences, it would be beneficial to also compare IMA- and LMA-induced cardiovascular responses to both relative and absolute workloads. This thesis examines sex-based differences in the blood pressure response to IMA and LMA during relative and absolute workloads.

1.1 Review of Literature

Cardiorespiratory responses to dynamic exercise are fundamentally important in the maintenance of homeostasis amidst increases in metabolic turnover of up to 100-fold that of resting rates. These responses include increases in heart rate (HR), arterial blood pressure (BP), muscle sympathetic nerve activity (MSNA), and minute ventilation ($\dot{V}_E$). The exercise pressor reflex is best defined as one of three main governing theories regarding these cardiovascular and ventilatory responses and postulates that they are, in part, elicited by feedback from muscle afferents that reside within the contracting muscle (Alam & Smirk, 1937). The exercise pressor reflex can be considered by its two component parts: the mechanoreflex and the metaboreflex (Kaufman *et al.,* 2002). The mechanoreflex and metaboreflex are stimulated primarily by mechanical stimuli and metabolic stimuli, respectively (Kaufman *et al.,* 1983), however the proportional contribution of each to the cardiovascular responses to exercise greatly differ. The role of the mechanoreflex is still controversial, with some evidence suggesting a prominent mechanoreflex-mediated tachycardic response (Hayes *et al.,* 2001; Gladwell & Coote, 2002; Gladwell *et al.,* 2005), and other studies observing minimal cardiovascular responses to isolated

mechanoreflex activation (Cui *et al.,* 2006; Matsukawa *et al.,* 2007). Meanwhile, the metaboreflex is suggested to have a larger role in the BP response to exercise, as evidenced by the maintained elevation of BP during post-exercise circulatory occlusion (PECO), an experimental condition wherein other feedforward and feedback cardiovascular control mechanisms are minimized (Alam & Smirk, 1937; Victor *et al.,* 1989).

Contemporary experimental models often use limb exercise and PECO as a model to investigate metaboreflex responses (Alam & Smirk, 1937; Kaufman *et al.,* 1984; Hayes *et al.,* 2006). It is generally well-accepted that pressor response to metaboreflex activation plays a functional role in the matching of limb blood flow to oxygen demand during exercise. However, there is evidence suggesting that the metaboreflex is also present in the muscles of respiration. This inspiratory muscle metaboreflex is activated in cases of increased work of breathing (WOB), and elicits a similar pressor response to the metaboreflex of the limb muscles (St. Croix *et al.,* 2000; Sheel *et al.,* 2001; Dercak *et al.,* 2002). Like the limb metaboreflex, the inspiratory metaboreflex is also suggested to play an important role in the blood flow response to exercise, particularly with respect to blood flow distribution between the exercising limb and respiratory musculature (Harms *et al.,* 1997; Sheel *et al.,* 2001; Dempsey *et al.,* 2006).

Evidence suggests that the pressor response to limb metaboreflex activation (LMA) exhibits differences based on biological sex, with females displaying an attenuated pressor response compared to males (Ettinger *et al.,* 1996; Jarvis *et al.,* 2011). This sex-based difference is also seen in response to inspiratory metaboreflex activation (IMA) (Smith *et al.,* 2016; Katayama *et al.,* 2018), even when generating the same absolute inspiratory work (Geary *et al.,* 2019). Sex differences in these responses are of significance when considering the implications to blood flow distribution and muscle fatigability. However, whether the same degree of limb and

inspiratory metaboreflex activation elicits comparable pressor responses is not well understood. It is also unclear if the sex-difference in the pressor response is of a similar magnitude between the limb and inspiratory muscles.

This review summarizes the current literature on the mechanisms giving rise to the cardiovascular and ventilatory responses to exercise, with a particular focus on the pressor response to metaboreflex activation. Recent research on blood flow distribution between the respiratory and limb muscles during exercise is also reviewed. Lastly, sex-based differences in limb muscle perfusion and implications to peripheral fatigability will also be discussed. It is important to note that in this review, and elsewhere in this document, gender (men/women) and sex (male/female) terms are discussed as reported in the original literature. While this thesis focuses on sex-based differences, gender terms are included if used in the original work.

1.1.1 Governing Theories for Cardiovascular Responses to Exercise

Three primary mechanisms have been proposed to explain the cardiorespiratory changes observed during exercise. There is plentiful evidence supporting all three mechanisms, which has led to the common belief that they are not mutually exclusive and likely work together to result in the observed cardiorespiratory responses (Kaufman *et al.,* 2002). These mechanisms are central command, the exercise pressor reflex, and the arterial baroreflex. Central command refers to the feedforward signals sent from higher brain centers during voluntary muscular contraction, which activates motor and cardiovascular control centers in parallel (Goodwin *et al.,* 1972; Williamson *et al.,* 2006). The exercise pressor reflex (EPR) refers to the sympathetically mediated increase in BP due to stimulation of limb muscle afferents (Alam & Smirk, 1937; McCloskey & Mitchell, 1972). The arterial baroreflex refers to the stimulation of arterial

baroreceptors with changes in arterial pressure and is important in making fine-tuning adjustments to BP during both rest and exercise (Fadel, 2008).

1.1.1.1 Central Command

The concept of central command is based largely on the work of Krogh & Lind-hard (1913), who found that cardiorespiratory measures, such as V_E and HR, increase almost instantaneously with the onset of exercise. These responses occur prior to the accumulation of metabolites in the exercising muscle or the significant alteration of blood gases (Krogh & Lind-hard, 1913; Eldridge *et al.,* 1985). Additionally, the increase in cardiorespiratory responses is also observed prior to exercise, in anticipation of increased metabolic demand (Krogh & Lind-hard, 1913). The temporal association between the onset of muscular work and cardiorespiratory responses led researchers to speculate the role of a neurological mechanism in activating motor and cardiovascular control centers in parallel (Krogh & Lind-hard, 1913; Goodwin *et al.,* 1972).

Goodwin *et al.* (1972) provided further evidence by investigating the cardiovascular and respiratory response to exercise with or without tendon vibration. Vibration of an agonist or antagonist muscle tendon activates muscle spindle primary afferents, to result in an excitatory or inhibitory reflex medullary response, respectively. Therefore, the input of central command required to maintain a given muscle tension is smaller when the agonist muscle tendon is vibrated due to the excitatory reflex response, and is greater during antagonist muscle tendon vibration due to the inhibitory reflex response. Goodwin *et al.* (1972) found that the increase in BP, HR, and V_E with isometric exercise is attenuated when central command input is reduced (i.e., with antagonist muscle tendon vibration), and is augmented when central command input is

increased (i.e., with agonist muscle tendon vibration). These results further support the concept of central command and the parallel activation of cardiovascular and motor control centers.

The specific neurocircuitry of central command has yet to be fully elucidated, however there appears to be a clear role of the periaqueductal grey (PAG) (Green *et al.*, 2007; Paterson *et al.*, 2014; Basnayake *et al.*, 2012). PAG activity increases upon anticipation of exercise and continues to increase during exercise (Green *et al.*, 2007). This increase in PAG activity is associated with corresponding increases in cardiorespiratory variables (Green *et al.*, 2007). Activity in the hypothalamic locomotor region, specifically the subthalamic nucleus (STN), has also been associated with central command due to the elicitation of motor and cardiovascular responses following electrical stimulation (Eldridge *et al.*, 1981; Eldridge *et al.*, 1985; Thornton *et al.*, 2002). However, the contribution of the STN to central command is still unclear, given that STN activity decreases during anticipation of exercise (Green *et al.*, 2007).

While the specific neurocircuitry of central command is still unclear, the findings mentioned above provide strong evidence and rationale for the concept of central command and suggests that this mechanism can function without input from the motor cortex (Nobrega *et al.*, 2014). It is important to acknowledge that feedback mechanisms from central and peripheral inputs can modulate the central command response, and maintain cardiovascular responses required to sustain metabolic needs despite withdrawn central command (Williamson *et al.*, 2001; Nobrega *et al.*, 2014). The integration of central command with such feedback mechanisms is discussed in more detail below.

1.1.1.2 Arterial Baroreflex

Arterial baroreceptors are stretch receptors that are stimulated by distortion of arterial vessel walls, and thus they respond to changes in BP. These baroreceptors are present in the carotid, aortic, and cardiopulmonary arteries, and are largely responsible for short-term BP regulation. Changes in arterial pressure are sensed by the baroreceptors, which reflexively alter parasympathetic and sympathetic nerve activity to arterioles and capacitance vessels (Pang, 2001; Fadel, 2008), as well as cardiac vagal activity (Fritsch *et al.*, 1991). The alteration in parasympathetic and sympathetic tone results in changes in total peripheral resistance and, thus, arterial pressure (Pang, 2001; Fadel, 2008). The modulation of cardiac vagal activity results in changes in HR (Fritsch *et al.*, 1991). In this manner, the arterial baroreflex provides feedback regulation of BP on a beat-by-beat basis.

During exercise, the carotid baroreflex resets to accommodate for the large upward shift in BP (Walgenbach & Donald, 1983; Scherrer *et al.*, 1990). This allows for baroreflex sensitivity to be maintained at a higher operating point (Melcher & Donald, 1981; Potts *et al.*, 1993). The resetting of the baroreflex and the maintained sensitivity has been shown to occur during both dynamic (Potts *et al.*, 1993; Papelier *et al.*, 1994; Norton *et al.*, 1999) and static exercise (Ebert, 1986). However, recent evidence suggests that baroreflex resetting during exercise may be contributed to by central command and the EPR (Raven *et al.*, 2006; Gallagher *et al.*, 2006).

1.1.1.3 Exercise Pressor Reflex

The EPR refers to exercise-induced cardiorespiratory responses such as elevated BP and total peripheral resistance, all of which occur in response to increased limb muscle afferent outflow (Alam & Smirk, 1937; McCloskey & Mitchell, 1972; Boushel, 2010). Myelinated group

III and unmyelinated group IV afferents, otherwise known as mechanoreceptors and metaboreceptors, respectively, comprise the afferent arm of the EPR (McCloskey & Mitchell, 1972; Kaufman *et al.,* 1983). These nerve fibers are stimulated primarily by mechanical and metabolic stimuli, respectively, during both static and dynamic exercise (McCloskey and Mitchell, 1972; Kaufman *et al.,* 1983; Adreani *et al.,* 1997). The afferent outflow from these nerve fibers is relayed to cardiovascular control centers in the brainstem (Mitchell *et al.,* 1983; Smith *et al.,* 2006), to evoke a sympathetically mediated increase in mean arterial pressure (MAP) (Mark *et al.,* 1985; Rowell & O'Leary, 1990).

The EPR was previously believed to modulate cardiovascular responses only in situations of reduced blood flow (i.e., ischemic exercise) (Rowell & O'Leary, 1990; O'Leary & Sheriff, 1995). In this manner, the EPR was thought to have a minor role in fine-tuning the cardiovascular responses to exercise, particularly to counter acute imbalances in metabolic demand and supply (O'Leary & Sheriff, 1995; Mitchell *et al.,* 1983). However, results from pharmacological blockade studies have shown that neural outflow from locomotor muscle afferents contributes to normal cardiovascular and ventilatory control during non-ischemic rhythmic exercise as well (Amann *et al.,* 2010; Amann *et al.,* 2011b). To date, it is generally well-accepted that the EPR has functional importance in the maintenance of cardiopulmonary homeostasis throughout exercise.

The metaboreflex is the metabolic arm of the EPR and refers to the reflex increase in MSNA and BP when group IV locomotor muscle afferents are stimulated (McCloskey & Mitchell, 1972; Mitchell *et al.,* 1983; Kaufman *et al.,* 1983). The mechanoreflex, or the mechanical arm of the EPR, refers to the stimulation of group III locomotor muscle afferents by mechanical stimuli (McCloskey & Mitchell, 1972; Mitchell *et al.,* 1983; Kaufman *et al.,* 1983).

The role of the mechanoreflex is still unclear, but research suggests a role in contributing to the HR response to exercise in conjunction with the feed-forward influence from central command (Gladwell & Coote, 2002). While the influence of the mechanoreflex on the BP response is unclear (Gallagher *et al.*, 2001; Victor & Seals, 1989; Rowell & O'Leary, 1990), the role of the metaboreflex is prominent and generally well-accepted. MAP is elevated during exercise, and remains elevated during PECO wherein mechanical stimulation of group III afferents and input from central command is minimized, thus suggesting a primary role of the metaboreflex in the BP response to exercise (Alam & Smirk, 1937; Kaufman *et al.*, 1984; Hayes *et al.*, 2006). As such, contemporary experimental models often use PECO or ischemic exercise to isolate the cardiovascular responses to metaboreflex activation and infer to the EPR.

1.1.1.4 Interactions Between Central Command, Arterial Baroreflex, and the EPR

As previously mentioned, it is well-accepted that central command, the EPR, and the arterial baroreflex work interactively to produce appropriate cardiovascular responses to exercise (Wilson & Hand, 1997; Kaufman *et al.*, 2002; Fadel, 2015). Previous work has suggested that the EPR and central command may contribute to baroreflex resetting during exercise (Coote & Dodds, 1976; Potts & Mitchell, 1998). With respect to baroreflex control of HR, research has shown that the cardiac vagal withdrawal induced by central command may be a primary mediator to the resetting of the carotid baroreflex, while the EPR may play a smaller modulatory role (Ogoh *et al.*, 2005; Gallagher *et al.*, 2006). However, when considering baroreflex control of BP during exercise (i.e., baroreflex-induced changes in sympathetic and parasympathetic outflow), the EPR and central command may independently contribute to baroreflex resetting to the same extent (Gallagher *et al.*, 2006; Michelini *et al.*, 2015). Interestingly, evidence is mixed

regarding the influence of muscle metaboreflex activation on spontaneous cardiac baroreflex sensitivity (Cui *et al.,* 2001; Sala-Mercado *et al.,* 2007; Ogoh *et al.,* 2005; Hartwich *et al.,* 2011).

The known cardiovascular and sympathetic responses to exercise evoked by central command, the EPR, and the arterial baroreflex, have functional importance in the increase of cardiac output during exercise and its redistribution to active muscles. This redistribution is mediated by the reflex changes in sympathetic and parasympathetic outflow (Mittelstadt *et al.,* 1994; Fisher *et al.,* 2015; Fadel 2015). Increases in systemic sympathetic nerve activity results in vasoconstriction in visceral organs, and both exercising and non-exercising skeletal muscle (Hansen *et al.,* 1994; Mittelstadt *et al.,* 1994; Mittelstadt *et al.,* 1996; O'Hagan *et al.,* 1997). The sympathetically mediated vasoconstriction in exercising muscles is offset by metabolite-induced local vasodilation (Remensnyder *et al.,* 1962; Stratton *et al.,* 1985; Thomas *et al.,* 1994; Kurjiaka & Segal, 1995; Hansen *et al.,* 2000). This phenomenon, called functional sympatholysis, is imperfectly balanced, with a net vasoconstrictor effect in active muscles (Pawelczyk *et al.,* 1992; Rowell, 1997; Thomas & Segal, 2004). Considering that blood flow to exercising muscle may increase up to 100-fold that of resting values during maximal exercise (Andersen & Saltin, 1985), the net vasoconstrictor effect in active muscles likely aids in maintaining systemic BP homeostasis during exercise (Thomas & Segal, 2004; Holwerda *et al.,* 2015).

The interactive nature between central command and the EPR, specifically the metabolic arm of the EPR, are reflected in the temporal differences in the cardiovascular and sympathetic responses to exercise. The metaboreflex-induced rise in BP and MSNA is not significant until approximately the second minute of exercise, due to the time-dependent accumulation of metabolites in the exercising muscle (Victor *et al.,* 1988; Seals *et al.,* 1989). The increase in blood flow observed during the first minute of exercise is largely attributed to the central

command-induced withdrawal of parasympathetic activity, and the resulting tachycardia at the onset of exercise (Victor *et al.,* 1987; Fisher *et al.,* 2015). The mechanoreflex likely supports the HR response evoked by central command, as evidenced by the decrease in HR back to resting levels during PECO wherein input from both central command and the mechanoreflex ceases (Gladwell & Coote, 2002). The influence of metaboreflex activation on the HR response to exercise is unclear, with differing responses observed between forearm and leg exercise. During forearm exercise, the metaboreflex likely has minimal influence on the HR response, as evidenced by the decrease in HR to resting levels during PECO despite the maintained elevation of BP and sympathetic activity (Alam & Smirk 1938; Mark *et al.,* 1985; Victor *et al.,* 1987). Yet some research has shown that HR maintains elevated during PECO when following two-leg exercise (Alam & Smirk, 1938; Bonde-Peterson *et al.,* 1978; Rowell *et al.,* 1991). However, the elevation in HR during leg exercise but not forearm exercise may be attributed to differences in muscle mass (Alam & Smirk, 1938), or to the mechanical effect of leg occlusion and subsequent changes in cardiac output (Asmussen & Nielsen 1964; Bonde-Peterson *et al.,* 1978; Pawelczyk *et al.,* 1997). Generally, it's well-accepted that central command is a primary contributor to the increase in HR, the metaboreflex stimulates a time-dependent rise in BP, and the arterial baroreflex serves to fine-tune the BP and HR responses throughout exercise.

1.1.2 *Limb and Respiratory Muscle Metaboreflex*

1.1.2.1 Limb Muscle Metaboreflex

As aforementioned, the metaboreflex is the metabolic arm of the EPR, and refers to the reflex increase in MSNA and BP when group IV muscle afferents are stimulated (McCloskey & Mitchell, 1972; Mitchell *et al*., 1983; Kaufman *et al*., 1983). Limb metaboreflex activation

(LMA) results in an intensity-dependent increase in sympathetic outflow to the systemic vasculature (Coote *et al.,* 1971; Mark *et al.,* 1985; Victor *et al.,* 1988; Seals *et al.,* 1989). The response to LMA also displays time-dependency, as evidenced by the delayed increase in MSNA until approximately the second minute of exercise which corresponds to the time-dependent accumulation of metabolites (Victor *et al.,* 1988; Seals *et al.,* 1989).

Outflow from group IV afferents synapses onto neurons in the superficial dorsal horn of the spinal cord (Mense & Craig, 1988; Wilson & Hand, 1997), and are relayed to areas in the brain stem to result in increased MSNA and parasympathetic withdrawal (Wilson & Hand, 1997; Murphy *et al.,* 2011). The basis of the resulting pressor response appears to shift with different exercise contraction patterns. During dynamic exercise, the increase in BP appears to be secondary to an increase in cardiac output, primarily by elevated HR (Sala-Mercado *et al.,* 2006; O'Leary & Augustyniak 1998; Crisafulli, 2003; Crisafulli, 2011; McNulty *et al.,* 2014). However, during static exercise, the increase in BP appears to be dependent on the increase in total peripheral resistance, by means of the sympathetically mediated vasoconstriction of skeletal muscles and visceral organs (Pawelczyk *et al.,* 1997; Bastos, 2000; McNulty *et al.,* 2014). It has also been suggested that total peripheral resistance and cardiac output have an inverse relationship with respect to the BP response during PECO, and there are large inter-individual variations in these responses (Watanabe *et al.*, 2014).

As mentioned above, functional sympatholysis refers to the balance between sympathetically-induced vasoconstriction and local metabolite-induced vasodilation, which results in the redistribution of cardiac output towards the exercising muscles (Remensnyder *et al.,* 1962; Stratton *et al.,* 1985; Thomas *et al.,* 1994; Kurjiaka & Segal, 1995; Hansen *et al.,* 2000). Functional sympatholysis is imperfectly balanced, and the increase in sympathetic

outflow to active muscles has a net restrictive effect on blood flow (Joyner *et al.,* 1992; Holwerda *et al.,* 2015). In considering the large capacity of skeletal muscle to increase blood flow during exercise (Andersen & Saltin, 1985), this restrictive effect is advantageous in limiting the amount of cardiac output delivered to the active muscles, in supporting BP homeostasis, and maintaining blood flow to vital organs (Thomas & Segal 2004; Holwerda, 2015).

1.1.2.2 Metaboreflex Feedback and Fatigue

The metaboreflex acts to minimize peripheral fatigability and maintain exercise performance in two ways. The first method refers to the metaboreflex-induced increase in MSNA and exercising muscle blood flow, to maintain adequate oxygen availability. Normally, in steady-state conditions of rest or low-intensity exercise, oxidative phosphorylation is the primary provider of resynthesized ATP. However, in situations where the demand for oxygen exceeds oxygen supply, such as during hypoxia and high-intensity exercise, flux through oxidative phosphorylation is limited by inadequate oxygen supply, and anaerobic energy systems produce more ATP to compensate and maintain the required work output (Haseler *et al.,* 1998; Hogan *et al.,* 1999; Hepple, 2002). The metabolic by-products produced by anaerobic glycolysis and the phosphocreatine energy system (such as P_i and H^+) are known to cause a decrease in force production, otherwise known as peripheral muscle fatigability (Fitts, 1994; Sahlin *et al.,* 1998; Hepple, 2002). This is reflected in the decrease in exercise performance in conditions of hypoxia (Fulco *et al.,* 1996; Hogan *et al.,* 1999). However, the metaboreflex-induced increase in exercising muscle perfusion supports ATP resynthesis by oxidative phosphorylation, thus minimizing the rate of metabolite production by anaerobic energy systems and enhancing metabolite washout (Barclay, 1986; O'Leary & Sheriff, 1995; Boushel, 2010; Broxterman *et al.,*

2018). Therefore, the continuous feedback provided by the metaboreflex contributes to the maintenance of appropriate cardiovascular responses, to decrease the rate of metabolic perturbation and, thus, delay peripheral muscle fatigability during exercise (Amann *et al.,* 2011a; Amann, 2012).

Secondly, LMA minimizes peripheral muscle fatigability by providing sensory information to the central nervous system and modulating central motor drive. During heavy-intensity exercise, motoneuron firing rate decreases and central motor drive increases to maintain a given force output (Fuglevand *et al.,* 1999; Gandevia, 2001). The impairment in motoneuron firing rate is maintained during recovery if blood flow to the muscle is occluded (i.e., PECO) (Bigland-Ritchie *et al.,* 1986; Woods *et al.,* 1987). This was found to be true following both voluntary and electrically-induced exercise (Bigland-Ritchie *et al.,* 1986; Woods *et al.,* 1987; Garland *et al.,* 1988). Impairments in motoneuron firing rate require an increase in central motor drive to maintain a given level of muscle work (Fuglevand *et al.,* 1999). However, when group III and IV muscle afferents are pharmacologically inhibited, the central motor drive is greater despite a faster rate of peripheral fatiguability (i.e., faster accumulation of fatigue-related metabolites) (Amann *et al.,* 2009). Overall, these findings suggest that the limb metaboreflex may contribute to the impairment in motoneuron firing rate, which leads to increased central muscle fatigue while minimizing peripheral muscle fatigability.

1.1.2.3 Respiratory Muscle Metaboreflex

Group III and IV nerve afferents are also present in the muscles involved with respiration, such as the diaphragm (Road, 1990). As such, there is evidence to suggest that the respiratory muscles also exhibit a metaboreflex response (Derchak *et al.,* 2002; St Croix *et al.,*

2000). Inspiratory and expiratory muscle metaboreflexes have both been investigated in humans, by means of increasing inspiratory or expiratory muscle work, respectively, independently of limb exercise. Imposing expiratory or inspiratory muscle work by means of resistive breathing in humans at rest elicits time-dependent increases in MSNA and blood pressure (St Croix *et al.,* 2000; Derchak *et al.,* 2002). Additionally, the MSNA and BP responses to resistive inspiratory breathing were found to be similar to that evoked by rhythmic handgrip exercise (St Croix *et al.,* 2000; Sheel *et al.,* 2001).

Importantly, the attainment and degree of inspiratory metaboreflex activation (IMA) appears to be partially dependent on the inspiratory duty cycle and contraction intensity. Pressor and sympathetic responses to IMA appear to only be significant when participants performed resistive inspiratory work at a prolonged inspiratory duty cycle (ratio of inspiratory contraction time to total respiratory cycle duration; T_I/T_{TOT}) and generated moderately high inspiratory pressures (St Croix *et al.,* 2000; Sheel *et al.,* 2001). This is supported by prior evidence in animal models suggesting that the generation of high inspiratory pressures translates to increased intrathoracic pressure during inspiration and, when sustained during prolonged duty cycles, compromises diaphragmatic blood flow (Bellemare *et al.,* 1983; Buchler *et al.,* 1985). This method of loaded inspiratory work provides inadequate time for complete metabolite washout during expiration (Bellemare *et al.,* 1983; Buchler *et al.,* 1985), and thus allows for increased stimulation of type IV muscle afferents without significantly altering cardiac output or stroke volume (Olgiati *et al.,* 1986; Coast *et al.,* 1993).

Lastly, the contribution of central command to the sympathetic and pressor responses to inspiratory loading was likely minimal given that, 1) there was no temporal association observed between sympathetic burst outflow and central respiratory motor output (inferred from

diaphragmatic electromyographic activity), and 2) hyperpnea in the absence of inspiratory loading did not elicit a significant MSNA response (St Croix *et al.,* 2000; Sheel *et al.,* 2001). Therefore, these findings suggest that the pressor and sympathetic responses to the inspiratory loading protocol mentioned above results primarily from IMA, with minimal contribution from central command.

Expiratory loading protocols have been compared to Valsalva maneuvers with respect to the resulting effects on intrathoracic pressure and cardiac output (Iandelli *et al.,* 2002; Aliverti *et al.,* 2005; Aliverti *et al.,* 2007). The generation of high expiratory pressures results in large increases in abdominal and intrathoracic pressure, which reduces stroke volume and, thus, cardiac output during both rest and moderate exercise (Stark-Leyva *et al.,* 2004; Aliverti *et al.,* 2005). Although this change in stroke volume and cardiac output did not reach significance in all studies (Olgiati *et al.,* 1986). Considering the relationship between the metaboreflex-induced pressor and cardiac output during dynamic limb exercise (McNulty *et al.,* 2014), it is unclear if changes in stroke volume seen during expiratory loading protocols may confound the responses to expiratory metaboreflex activation. However, the increased abdominal muscle recruitment during forceful expiration, necessary to provide thoracic stability (De Troyer & Boriek, 2011), poses a challenge to expiratory metaboreflex isolation during resistive expiratory breathing tasks. Presently, the respiratory metaboreflex is commonly investigated using inspiratory loading protocols.

1.1.2.4 Inspiratory Muscle Metaboreflex and Blood Flow Redistribution

This inspiratory muscle metaboreflex is activated in cases of increased WOB and diaphragmatic fatigue (DF) (St. Croix *et al.,* 2000; Sheel *et al.,* 2001; Welch *et al.,* 2018). Like

LMA, IMA results in increased MSNA and subsequent systemic vasoconstriction (Sinoway *et al*., 1989; Harms *et al*., 1998; St Croix *et al.,* 2000). However, IMA also results in reductions in blood flow to the exercising limb muscle, but increased respiratory muscle perfusion (Harms *et al*., 1997; Harms *et al*., 1998; Hill, 2000; Sheel *et al*., 2001; Dominelli *et al*., 2017). Conversely, when WOB is reduced during exercise, limb muscle perfusion increases (Dominelli *et al.,* 2017). This phenomenon has been suggested to result from differences in arteriole sensitivity to systemic vasoconstrictor and vasodilator outflow between the diaphragm and limb muscle (Laughlin *et al*., 1989; Aaker & Laughlin, 2002; Dempsey, 2012). Specifically, evidence from rodent models suggest that phrenic artery feed vessels may be less sensitive to vasoconstrictor stimuli, such as noradrenaline, than vessels of other skeletal muscles (Aaker & Laughlin, 2002). The respiratory muscles also exhibit a larger response to vasodilatory stimuli than other skeletal muscle (Laughlin *et al.,* 1989). This suggests that there is a competitive relationship between the respiratory and limb muscles for cardiac output (Dempsey *et al.,* 2006; Sheel *et al.,* 2018; Sheel et al., 2020). Therefore, the regulation of blood flow distribution during increasing exercise intensity depends, at least in part, on the extent of LMA and IMA.

The effect of WOB on limb muscle perfusion during exercise has consequences to peripheral fatigability at the level of the exercising limb (Barclay, 1986; Hogan, 1999; Harms *et al.,* 2000; Romer *et al.,* 2006). Appropriate limb muscle perfusion is necessary to minimize the degree of metabolite accumulation during exercise and the associated peripheral fatigability (Barclay, 1986; Hepple, 2002). In considering the association between IMA and blood flow redistribution towards the respiratory musculature (Sheel *et al.,* 2001), it is reasonable to deduce that IMA may contribute to peripheral fatigability of the limb muscles. This is supported by the finding that increasing the WOB during exercise compromises limb muscle perfusion (Harms *et*

al., 1997) and exacerbates the degree of peripheral limb fatigue (Romer *et al.,* 2006). Conversely, decreasing WOB resulted in enhanced limb muscle perfusion (Harms *et al.,* 1997) and significantly reduced peripheral limb fatigability(Romer *et al.,* 2006). This effect of WOB on limb muscle blood flow appears to be dependent on the attainment of diaphragmatic fatigue (Sheel *et al.,* 2001), thus supporting the postulation that IMA may contribute to the rate of peripheral limb fatigability during exercise (Dempsey *et al.,* 2006; Dempsey, 2012).

1.1.3 *Sex-Based Differences in the Metaboreflex Response*

1.1.3.1 Sex-Based Differences to LMA

Research suggests that sympathetic and pressor responses to LMA are attenuated in females (Matthews & Stoney, 1988; Ettinger *et al.,* 1996; Jarvis *et al.,* 2011). Specifically, static handgrip exercise and PECO resulted in a smaller increase in BP and MSNA in women compared to men, independent of forearm muscle mass (Matthews & Stoney, 1988; Ettinger *et al.,* 1996; Jarvis *et al.,* 2011). However, this sex-based difference was not seen following ischemic, low-intensity rhythmic handgrip exercise (Ettinger *et al.,* 1996). Additionally, sex-differences in limb muscle perfusion during exercise have also been observed (Parker *et al.,* 2007; Hunter, 2014).

While the underlying mechanisms for the observed sex-based differences in metaboreflex responses are still unclear, there are several proposed ideas. Some groups suggest sex differences in muscle fiber type proportions and metabolite production may lead to different levels of metaboreflex activation. Depending on the muscle group, females tend to have a significantly higher proportion of type I fibers compared to males (Simoneau & Bouchard, 1989). While type II fibers are associated with a greater production of metabolites during exercise, type I fibers are

associated with greater metabolite clearance during exercise (Juel *et al.*, 1991) Additionally, individuals with a larger proportion of type II fibers elicited a larger BP response to LMA (Sadomoto *et al.*, 1992). If we assume that the sex-based difference in fiber type proportions is consistent across various muscle groups, females may have an increased capacity to buffer metabolites during exercise and, therefore, activate the metaboreflex to a lesser extent than males. This is supported by the significantly reduced relative concentrations of dihydrogen phosphate ($H_2PO_4^-$) in women during the last minute of static handgrip exercise and PECO, compared to men (Ettinger *et al.*, 1996). However, this observation occurred in the absence of peripheral fatigue, and therefore females and males were not compared at a common metabolic endpoint (Ettinger *et al.*, 1996). Additionally, sex-based differences in metabolite concentration were also seen following ischemic rhythmic handgrip exercise, despite no apparent sex-differences in MSNA (Ettinger *et al.*, 1996). Therefore, the role of fiber type proportions and metabolite accumulation, specifically H^+ and $H_2PO_4^-$, in sex-based differences to LMA is still unclear.

Similarly, some groups suggest a role of muscle mass in the sex-based differences in metaboreflex responses. This is based on the observation that isometric exercise involving more muscle mass is associated with a greater increase in MSNA (Seals, 1993; Saito, 1995), and female muscles tend to have a smaller mean cross-sectional area than males (Simoneau & Bouchard, 1989). However, evidence regarding this proposition is mixed. Some groups have found that the sex-based difference in the response to LMA is independent of muscle mass and absolute force output (Matthews & Stoney, 1988; Ettinger *et al.*, 1996; Jarvis *et al.*, 2011). Yet, a separate group found that sex-differences in the pressor response are lost when controlling for handgrip strength and absolute force output, but is maintained when controlling for forearm

circumference (Notay *et al.*, 2018; Lee *et al.*, 2021). Thus, the role of muscle mass in the observed sex-differences is also unclear, but emphasizes the importance in utilizing both absolute and relative workloads when comparing sex-based differences to metaboreflex activation.

Another common postulation relates to the cardioprotective effect of ovarian hormones. The cyclical variation of ovarian hormones throughout the menstrual cycle may be associated with altered metaboreflex responses, and therefore is proposed to contribute to the observed sex-based differences. LMA elicits a greater increase in MSNA, but not MAP, during the early follicular phase compared to the late follicular phase of the ovarian cycle (Ettinger *et al.*, 1998). However, a separate study found no significant differences between the early follicular phase and the midluteal phase (Jarvis *et al.*, 2011). Lastly, females taking oral contraceptives (i.e., exogenous estradiol and progestin) were found to have a similar MAP response to LMA as males, with both groups having a significantly greater response than females not taking oral contraceptives (Parmar *et al.*, 2017). The implications of these findings are unclear, but suggests a potential role of ovarian hormones in the observed sex differences.

1.1.3.2 Sex-Based Differences to IMA

Similar to the limb metaboreflex, sex-based differences in response to IMA have also been observed (Smith *et al.*, 2016; Welch *et al.*, 2018; Katayama *et al.*, 2018; Geary *et al.*, 2019). In females, IMA resulted in an attenuated increase in MAP, MSNA and total peripheral resistance, and less of a decrease in limb blood flow, compared to males (Smith *et al.*, 2016; Katayama *et al.*, 2018). Correspondingly, the development of DF occurs more slowly in females when performing a relative amount of inspiratory work, which is consistent with the

interdependence of the onset of DF and IMA (Welch *et al.,* 2018). This finding is of particular interest when considering the anatomical and physiological sex-differences in the respiratory system. Females have smaller lungs and a shorter diaphragm length than height-matched males (Bellemare *et al.,* 2003). In considering the greater work and cost of breathing for a given ventilation, it has been speculated that females may experience a greater degree of IMA to promote greater respiratory muscle blood flow for a given WOB, compared to males (Guenette *et al.,* 2007; Dominelli *et al.,* 2015). Yet, research has provided contradictory evidence; for the same amount of absolute inspiratory work, females have an attenuated MAP response compared to males despite attaining the same degree of diaphragmatic fatigue (Geary *et al.,* 2019).

Like the limb metaboreflex, underlying mechanisms for the observed sex-differences in inspiratory metaboreflex responses are still unclear. In addition to the mechanisms proposed for the limb metaboreflex, some suggest that sex-based differences in IMA may correspond to the greater proportional diaphragmatic contribution in females (Welch *et al.,* 2018). During pressure-threshold loading, the ratio of the pressure generated by the diaphragm and the total inspiratory pressure is higher in females than males (Welch *et al.,* 2018). It is possible that differences in the proportional contribution of the diaphragm and accessory inspiratory muscles may influence muscular efficiency, however this notion is speculative and remains under-investigated.

Generally, the underlying basis of the sex-differences in the response to limb and inspiratory muscle metaboreflex activation is not certain. It is also unknown if the mechanism(s) leading to the attenuated IMA response in females is the same mechanism underlying the sex-based differences to LMA. Lastly, whether the magnitude of the sex-difference to LMA is comparable to that of IMA is also unclear.

1.1.3.3 Sex Differences in Blood Flow Distribution and Implication to Peripheral Fatigue

Sex-based differences in the responses to LMA and IMA are of particular interest when considering the resulting blood flow responses and the implications to peripheral muscle fatiguability. The impairment in limb muscle blood flow during increased inspiratory work is greater in males than females (Smith *et al.,* 2016). This corresponds to the greater inspiratory metaboreflex-induced increase in MSNA and total peripheral resistance seen in males than females (Smith *et al.,* 2016; Katayama *et al.,* 2018). It is possible that metaboreflex-related differences in limb muscle blood flow may contribute to the observed sex-differences in peripheral muscle fatiguability.

Peripheral muscle fatigability is commonly defined as an impairment in the force generation capacity of a muscle, due to factors distal to the neuromuscular junction (Fitts, 1994). Peripheral muscle fatigability may be caused by circumstances such as altered cross-bridge dynamics, changes in sarcolemma excitability, or reduced substrate supply (Fitts, 1994; Kent-Braun *et al.,* 2012). Changes in metabolite concentration, especially H^+, inorganic phosphate, ADP, and calcium ions (Ca^{2+}), are commonly associated with peripheral muscle fatigability due to several processes, including the inhibition on actomyosin sliding velocity, decreased Ca^{2+} sensitivity, and impaired myofibrillar ATPase activity (Westerblad & Allen, 1991; Fitts, 2008; Greenberg *et al.,* 2010; Kent-Braun *et al.,* 2012).

In general, females tend to develop peripheral muscle fatigue at a slower rate than males during both dynamic and static exercise (Hunter, 2014; Hunter, 2016). There are several proposed mechanisms for the sex-based differences in peripheral fatiguability, one of them being differences in muscle blood flow (Russ & Kent-Braun, 2003; Clark *et al.,* 2005; Hunter, 2014). During isometric exercise, females tend to have greater limb muscle perfusion than males, which

is attributed to less arterial compression, differences in the vasodilatory response, and differences in sympathetic neural outflow (Hunter & Enoka, 2001; Ettinger *et al.,* 1996; Hogarth *et al.,* 2007; Parker *et al.,* 2007; Hunter, 2014). Elimination of sex-based differences in limb perfusion via ischemic exercise results in a similar time to task failure and degree of peripheral fatiguability between males and females (Russ & Kent-Braun, 2003; Clark *et al.,* 2005). Overall, this suggests that sex-differences in limb muscle perfusion contributes to the observed differences in the development of peripheral fatigability.

When considering the relationship between IMA and limb muscle perfusion, it is reasonable to deduce that sex-differences in IMA responses has significant implications to peripheral muscle fatigability and exercise tolerance. However, it is not yet known if the sex-difference in the pressor response to metaboreflex activation is constant across muscle groups. Specifically, it is unclear if the magnitude of the sex-difference observed during LMA is the same as that seen during IMA, when comparing responses to similar degrees of metaboreflex activation. This comparison is of importance when considering the role of LMA and IMA in blood flow distribution, and the ramification to peripheral muscle fatigability.

1.1.4 Conclusion

The limb and inspiratory muscle metaboreflexes have functional importance in contributing to blood flow distribution during exercise. Sex-based differences in the pressor response to limb and inspiratory muscle metaboreflex activation have been well-documented. Specifically, females exhibit less of an increase in MAP and MSNA during both LMA and IMA when compared to males performing the same relative or absolute work. However, it is unknown if the magnitude of this sex-based difference is constant across muscle groups, specifically

between the limb and respiratory muscles. This comparison may provide better insight to the disparity in limb muscle perfusion between females and males, and thus provide a better understanding of sex-based differences in peripheral fatigability and exercise tolerance.

1.2 Research Objectives

The objective of the proposed work was to compare sex-based differences in the blood pressure response to IMA and LMA during relative and absolute workloads. Specifically, the aim was to investigate whether the magnitude of the sex-based difference in the pressor response to exercise is consistent across muscle groups (i.e., the forearm and respiratory muscles), and at differing exercise intensities.

1.3 Hypotheses

It was hypothesized that: (1) the increase in blood pressure during IMA would be equal to that of LMA, and both responses would be attenuated in females; and (2) sex-based differences in the pressor response would be augmented when matched for absolute work during both IMA and LMA.

Chapter 2: Methods

2.1 Subjects

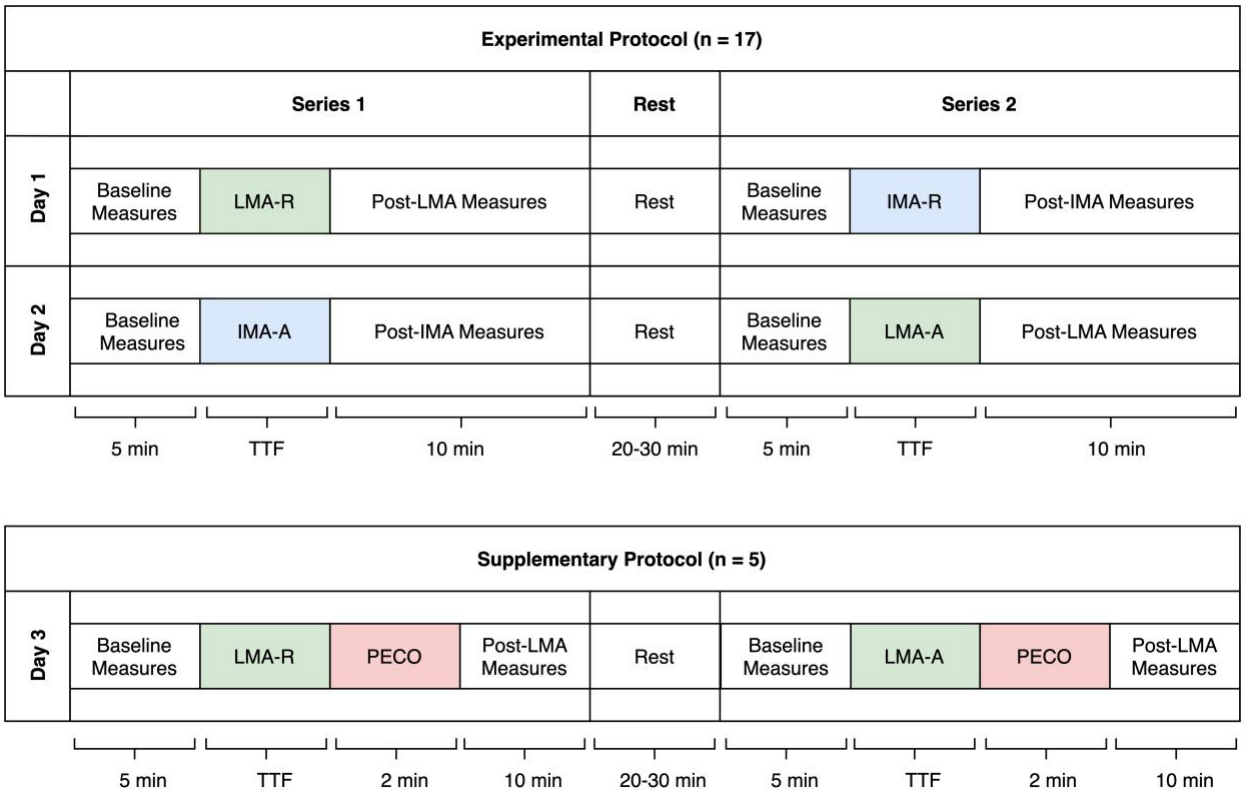
Twenty-one healthy young males (n=9) and females (n=8) between the ages of 18-35 were recruited from the University of British Columbia and Metro-Vancouver area, and provided both written and verbal informed consent for participation. Subjects were excluded from participation if they had a history of cardiorespiratory or other chronic diseases, a history of smoking or vaping, have a body mass index <18 or >30 $kg•m^2$, or were taking hormonal contraceptives (either oral or intrauterine). To understand the effects of ovarian hormones, female participants were tested during the midluteal (ML) phase of their menstrual cycle (days 19–22) as determined based on the participants' self-reported menstrual cycle history.

2.2 Experimental Overview

Participants visited the lab on two separate occasions, each separated by at least 24 hours. During the first visit (Day 1), participants underwent pulmonary function testing, forearm volume and circumference measures, and completed two bouts of exercise. The second visit (Day 2) mimicked the protocol of Day 1, except without pulmonary function testing. The exercise performed on both Days 1 and 2 involved one bout each of intermittent isometric handgrip exercise (IHE) and pressure-threshold loading (PTL). As shown in the schematic provided in Figure 1., the exercise bouts were performed to task failure, preceded by 5 minutes of seated baseline measures, and followed by 10-minutes of recovery. Cardiopulmonary parameters were continuously recorded pre-exercise, during exercise, and during post-exercise recovery. The target work rate performed on Days 1 and 2 were randomized for each participant, with one day involving exercise at a relative work rate, and the other at an absolute work rate.

A sub-set of participants (n=5) were invited to participate in a third day of testing (Day 3). Day 3 involved two bouts of IHE that mimicked those performed on Days 1 and 2, except with the addition of post-exercise circulatory occlusion (PECO). Immediately prior to the end of exercise, an occlusion cuff placed on the upper arm of the exercising limb was inflated to supra-systolic pressures (~200 mmHg) and maintained for 2 minutes. 10-minutes of post-exercise recovery measures were recorded, then participants rested for at least 30 minutes prior to beginning the second exercise bout. Cardiopulmonary parameters were continuously recorded. The exercise bouts of all three days were performed in a randomized order.

Figure 2.1 Experimental schematic.



Abbreviations: LMA: Limb Metaboreflex Activation; IMA: Inspiratory Metaboreflex Activation; TTF: Time to Task Failure; PECO: Post-Exercise Circulatory Occlusion.

2.3 Measurements

2.3.1 Pulmonary Function Tests

Measures of pulmonary function that were recorded include: total lung capacity (TLC), vital capacity (VC), functional residual capacity (FRC), residual volume (RV), forced vital capacity (FVC), forced expiratory volume in 1 second ($FEV_{1.0}$), $FEV_{1.0}$/FVC, peak expiratory flow (PEF), and forced expiratory flow between 25 and 75% of FVC (FEF_{25-75}) were determined using a body box plethysmograph (Vmax Autobox V62, CareFusion, USA). The single breath pulmonary diffusion capacity for carbon monoxide was measured and adjusted for hemoglobin concentration ($Hb201^{+}$, HemoCue, Sweden). All the above measures, including spirometry and single breath diffusion measures, follow the American Thoracic Society protocol, and the values obtained were compared to reference values (Crapo & Morris, 1981; Hankinson *et al.*, 1999; Graham *et al.,* 2017; Graham *et al.,* 2019).

The Vmax Autobox V62 pulmonary function testing system (otherwise known as the Body Box plethysmograph) is used to perform tests of lung function. The box is 185 cm high x 87 cm wide x 80 cm deep, with glass walls and a microphone and speaker to allow for communication between the participant and the researcher. There is a handle on both sides of the door which allowed either the participant or researcher to open the door at any time. Participants were informed that they were able to communicate with the researcher and terminate the test at any time if they were experiencing claustrophobia.

2.3.2 Esophageal, Gastric, and Diaphragm Pressures

Esophageal and gastric balloon-tipped catheters (no. 47-9005; Ackard Laboratory, Cranford, NJ) were used to obtain measures of esophageal and gastric pressures, respectively.

Prior to insertion of the catheters, a topical anesthetic (Viscous Lidocaine 2%) was applied to the nasal and pharyngeal passages of the participant via a syringe. The catheters were then inserted intranasally and placed in the stomach one at a time. The balloon catheters were attached to pressure transducers, and were used to measure esophageal pressure (P_{eso}) and gastric pressure (P_{ga}), respectively. Participants performed a Valsalva maneuver to empty the catheters of air, which were then filled with 1 mL and 2 mL of air, respectively. To retract the esophageal balloon catheter to the lower third of the esophagus, the catheter was retracted in increments until a sharp inspiration resulted in a negative P_{eso} inflection. The catheter was then retracted a further 10cm, and placement was confirmed by observation of end-expiratory P_{eso} approximately within -3 to -6 cmH_2O. The difference between P_{eso} and P_{ga} was calculated and used as an index of the pressure generated by the diaphragm (P_{di}). A custom port in the mouthpiece was connected to a separate pressure transducer, and used to sample mouth pressure (P_{mo}). All the previously mentioned pressures were measured using Validyne Pressure Transducers (model DP15-34, 31 Validyne Engineering; Northridge, CA, USA) and independently calibrated using a digital pressure manometer (2021P, Digitron; Torquay, UK).

2.3.3 Cardiopulmonary Parameters

Heart rate was measured using a lead-II electrocardiogram. Beat-by-beat blood pressure was measured continuously via a non-invasive hemodynamic device (Finometer PRO, Finopres Medical Systems, Enschede, The Netherlands). Manual blood pressure readings using a sphygmomanometer were also taken periodically during the protocol, except during IHE and PECO, and the succeeding five minutes. Participants breathed through a low resistance two-way non-rebreathing valve with a nose clip to account for all inspired and expired gases. The

breathing valve was attached to expiratory and inspiratory tubes, both connected to a pneumotachograph to measure inspired and expired flow. A customized metabolic cart was used to measure ventilatory and mixed-expired metabolic parameters, all of which were measured on a breath-by-breath basis and averaged over 20-second periods. The metabolic cart consisted of independently calibrated inspired and expired pneumotachographs (3818, Hans Rudolph) and O_2 and CO_2 analyzers (S-3-A/I and CD-3A, respectively, Applied Electrochemistry, Pittsburgh, PA, USA). Variables that were continuously measured include: minute ventilation ($\dot{V}_E$), partial pressure of end-tidal CO_2 ($P_{ET}CO_2$), tidal volume (V_T), breathing frequency (f_B), P_{mo}, P_{eso}, P_{ga}, heart rate (HR), and arterial pressure.

2.4 Procedures

2.4.1 Limb Metaboreflex Activation

While seated, participants breathed through the mouthpiece for 5 minutes to obtain baseline cardiopulmonary measures, as well as measures of P_{mo}, P_{eso}, and P_{ga}. Participants were then removed from the mouthpiece and performed at least three maximal voluntary contractions (MVC) of the forearm, while holding the handgrip dynamometer in the dominant arm in an upright, seated position with the elbow rested in a position of 90-degrees of flexion in the frontal plane. The first third of the best MVC was averaged and used as the participant's maximal grip strength (MVC_{PEAK}). Participants then performed a familiarization trial involving 2-5 practice handgrip bouts, each consisting of 5 intermittent isometric contractions at the target workload. Once the participant reported confidence in being able to adequately perform the task, they rested for another 5 minutes in a seated position to ensure that cardiopulmonary parameters were not elevated following the MVCs and practice bouts. Participants then performed the limb

metaboreflex activation (LMA) bout, which involved intermittent isometric handgrip exercise (IHE) to task failure. Participants performed the IHE at a duty cycle of 0.7 and a contraction frequency of 15 contractions per minute, in the same body position as the MVCs. A metronome was used to ensure correct contraction pattern during the trials (2.8 seconds contraction, 1.2 seconds relaxation), and verbal encouragement was provided throughout. Task failure was determined to be reached if any of the following conditions were met: 1) the participant felt unable to continue the exercise; 2) the participant was unable to reach the target force for three consecutive contractions; 3) the participant was unable to maintain the contraction for the duration at least 2 seconds; or 4) the researchers noticed that the participant was recruiting non-forearm muscles to achieve the target force (i.e., visible elbow flexion, shoulder movement, etc.). Condition #4 also included if participants were unable to maintain the appropriate body position despite verbal feedback and encouragement. On Day 1, the IHE bout was performed at a relative workload of 60% of the participants' MVC_{PEAK}, as guided by continuous visual feedback. On Day 2, the IHE bout was performed at an absolute workload of 167 N, also guided by continuous visual feedback. The order of Days 1 and 2 were randomized for each participant. Following exercise, the participants remained in a seated position for 10 minutes and cardiopulmonary measures were continuously recorded. The IHE bout, if performed first, was succeeded by a sufficient period of rest (≥30-min) to allow for cardiovascular variables to return to baseline prior to the PTL bout.

2.4.2 Inspiratory Metaboreflex Activation

Participants breathed through the mouthpiece for 5 minutes to obtain pre-exercise baseline measures. Participants were removed from the mouthpiece and performed a minimum

of three Mueller maneuvers from residual volume. The first third of the best maneuver was averaged and used as the participants' maximal inspiratory mouth pressure (MIP_{mo}) and maximal transdiaphragmatic pressure ($P_{di, MAX}$). Participants performed a brief familiarization trial to practice generating the target P_{di} consecutively for a maximum of five contractions in a row. Once the participant reported confidence in being able to adequately perform the task, they rested for another 5 minutes in a seated position to ensure that cardiopulmonary parameters were not elevated following the familiarization trial. Participants then performed the inspiratory metaboreflex activation (IMA) bout, which involved pressure-threshold loading (PTL) to task failure. Participants performed the PTL at a duty cycle of 0.7 and a breathing frequency of 15 contractions per minute. A metronome was used to ensure the correct breathing pattern during the trials (2.8 seconds inspiration, 1.2 seconds expiration), and verbal encouragement was provided throughout. Task failure was determined to be reached if any of the following conditions were met: 1) the participant felt unable to continue the exercise; 2) the participant was unable to reach the target P_{di} for three consecutive breaths; 3) the participant was unable to maintain an inspiration for the duration at least 2 seconds; or 4) the researchers observed any indication that the participant was unable to maintain adequate airflow (i.e., marked decrease in minute ventilation, sustained decrease in end-tidal partial pressure of CO_2 despite manual adjustments to inspired CO_2, etc.). On Day 1, the PTL bout was performed at a relative workload of 60% of the participants' $P_{di, MAX}$, as guided by continuous visual feedback. On Day 2, the PTL bout was performed at an absolute workload of 82 cmH_2O, also guided by continuous visual feedback.

To perform PTL, a bespoke PTL device was attached to a mouthpiece to enable participants to achieve the target P_{di}. This device has been previously described (Nickerson & Keens, 1982), and shown to induce changes in blood pressure (Geary *et al.,* 2019; Welch *et al.,* 2018). In brief, the apparatus requires participants to generate an inspiratory pressure sufficient to overcome a threshold load imposed by a weighted plunger connected to the inspiratory port of the mouthpiece. Expiration was unimpeded. The resistance added to the plunger was individualized to enable the participants to consistently generate the target P_{di}, and P_{di} was displayed on a computer screen to provide continuous visual feedback. Manual adjustments to the inspired fraction of CO_2 were made in the event of hypocapnia (i.e., PET_{CO2} < 30 mmHg) using a 100% CO_2 gas tank. After the exercise bouts, participants remained in the seated position for 10-min to obtain post-PTL cardiopulmonary measures. The PTL bout, if performed first, was succeeded by a sufficient period of rest (≥30-min) to allow for cardiovascular variables to return to baseline prior to the IHE bout.

2.4.3 Post-Exercise Circulatory Occlusion

A subset of male participants (n=5) returned to the laboratory for an additional day of testing (Day 3). The protocol for Day 3 was identical to Days 1 and 2, except with the PTL bout being replaced by an additional IHE bout, and both IHE bouts followed by post-exercise circulatory occlusion (PECO). The two bouts of IHE were performed at the same workloads as those performed on Days 1 and 2, and the bout performed at the relative workload was performed by the participant's non-dominant arm. Immediately prior to the end of handgrip exercise, an occlusion cuff placed on the upper arm was inflated to supra-systolic pressures (~200 mmHg) to attain PECO, and was maintained for two minutes. The occlusion cuff was then

released, and 10-minutes of post-exercise measures were recorded. The order of the handgrip bouts was randomized for each participant.

The purpose of Day 3 was to investigate whether the IHE protocol used on Days 1 and 2 sufficiently activated the limb metaboreflex. PECO creates a condition in which metabolites remain trapped in the limb and stimulate metaboreceptors, in the absence of input from central command or mechanoreceptors. Therefore, a maintained pressor response during PECO suggests a predominately metaboreflex-mediated pressor response. PECO was not incorporated into the protocol of Days 1 and 2, due to the inability to safely create an analogous condition with respect to the diaphragm and inspiratory metaboreflex.

Chapter 3: Data Analysis

3.1 Data Collection and Processing

3.1.1 Data Collection

All raw data were recorded continuously throughout the protocol at a sampling frequency of 1kHz using a 16-channel analog-to-digital converter (PowerLab 16SP model ML 795, AD Instruments; Colorado Springs, CO, USA) and were monitored and stored online using LabChart data acquisition software (v8.1, AD Instruments; Colorado Springs, CO, USA). Cardiorespiratory data were sampled and averaged over 20s immediately prior to specific time points before, during, and after PTL, IHE, and PECO. Time points selected include rest, minutes 1-5 of exercise, and 20, 40, 60, 80, and 100% time to task failure (TTF). Minutes 1 and 2 of PECO were also used as time points investigated. These values were also converted to relative values, expressed as the delta change from baseline (Δ).

3.1.2 Calculation of Force-Time and Pressure-Time Products

Pressure-time products (PTP) were calculated for each time point of the PTL bouts by selecting four breaths immediately prior to each time point, provided they are absent of any physiological artifact. The P_{di} during the breaths were averaged and integrated to calculate the pressure-time product of the diaphragm (PTP_{di}) according to the methods described by Dominelli & Sheel (2012). PTP_{di} is used as an index of the pressure generated by the diaphragm during inspiration. Pressure-time products were also calculated for mouth pressure (PTP_{mo}), esophageal pressure (PTP_{eso}), and gastric pressure (PTP_{ga}) using the same method. The ratio of PTP_{di}:PTP_{TOT} (where PTP_{TOT} is the sum of PTP_{eso} and PTP_{di}) is often used as an estimate of the proportional contribution of the diaphragm to total respiratory pressure generation. Similarly, the

force-time product of the forearm muscles (FTP) can be integrated in the same manner, and used as an index of the work of the forearm muscles during the contraction phase of the IHE bout. All PTPs are presented in absolute values, and as well as normalized to participant weight and FVC. FTPs are presented in absolute values, and normalized to participant weight and forearm volume. Cumulative PTPs and FTP were also calculated.

P_{di} during the inspiratory efforts of PTL, and handgrip force output during IHE, were both maintained in approximate square-wave fashion throughout the exercise bouts. Therefore, the mean P_{di} during the inspiratory phase of PTL was used as average P_{di} ($P_{di,av}$), and likewise for average handgrip force output ($F_{hg,av}$) during the contraction phase of IHE. Diaphragm tension-time index (TTI_{di}) was calculated for each time point of PTL as the product of $P_{di,av}/P_{di,max}$ and T_I/T_{TOT}. (where T_I is the time spent performing inspiration, and T_{TOT} is the time of the complete respiratory cycle). Tension-time index for the forearm muscles (TTI_{fm}) was also calculated for each time point of IHE, as the product of $F_{hg,av}/MVC_{PEAK}$ and $T_C/T_{TOT'}$ (where T_C is the time spent performing forearm contraction, and $T_{TOT'}$ is the time of the complete contraction cycle).

3.2 Statistical Analysis

Descriptive characteristics for independent samples were compared using Student's *t* test. A three-way mixed analysis of variance (ANOVA) was performed to compare differences in cardiorespiratory, PTP, and FTP variables due one between-subjects variable (biological sex: males vs females), and two within-subjects variables (muscle group: PTL vs IHE; target workload: absolute vs relative). Time points selected for analyses include absolute time (Baseline, Minutes 1-5) and normalized time (Baseline, 20-100% TTF). Based on the omnibus ANOVA output, post-hoc analysis with Tukey's adjustment was used to investigate main effects,

and a simple effects analysis was used to explicate significant interaction effects. For significant interactions involving time as a factor, individual two-way between-groups ANOVAs were performed for each time point of interest (i.e., Baseline, Mins 1-5, or %TTF). A two-way within-groups factorial ANOVA was performed to compare differences in cardiopulmonary variables and force-time products between the PECO and Control conditions, for both workloads (relative vs absolute). Post hoc analyses used for the PECO condition were identical to those described for Days 1 and 2. An analysis of covariance (ANCOVA) was also performed for the IHE bouts, to determine the interaction effects of forearm circumference and forearm volume on cardiopulmonary responses. These statistical tests allow for the investigation of the interaction between sex, muscle group, workload, and time. Significance was set at an alpha of $p=0.05$. All t-tests, ANOVAs, ANCOVAs, and post-hoc analyses were conducted using Jamovi (v2.2.5). All Simple effects analyses were conducted using JASP (v0.16.3).

Chapter 4: Results

4.1 Subject Characteristics and Exercise Parameters

4.1.1 Subject Demographics and Pulmonary Function Measures

Twenty-one healthy males (n=11) and females (n=10) were recruited for this study. Two participants voluntarily withdrew from the study due to minor discomfort from the esophageal and gastric balloon catheters. One participant was withdrawn from the study due to sustained hypertension at rest, likely resulting from the "White Coat effect" (Franklin *et al.*, 2013). One participant was unable to complete the absolute PTL exercise bout and was withdrawn from the study. A total of 17 healthy participants (n=9 male; n=8 female) completed the study and were included in data analysis. A subset (n=5) of males completed the third experimental visit (Day 3).

Participant anthropometric characteristics are presented in Table 4.1. Male participants were significantly taller ($p<0.001$) and had greater body mass ($p=0.009$) than female participants, while BMI was not statistically different ($p=0.604$). Forearm circumference and volume were larger in males than females (both $p<0.001$). Male participants also had a larger FVC ($p<0.001$) and TLC ($p<0.001$) than female participants.

4.1.2 Exercise Parameters

Exercise workload and intensity for Days 1, 2, and 3 are presented in Tables 4.2 and 4.3. Males had a significantly greater MVC than females ($p<0.001$), and a longer TTF during the absolute condition of IHE ($p=0.001$). No sex-based differences in TTF were observed during the relative condition of IHE ($p=0.576$), the relative condition of PTL ($p=0.387$), and the absolute condition of PTL ($p=0.688$). Males were also able to exercise for a longer duration during the absolute IHE condition compared to the relative IHE condition ($p = 0.002$), but no differences

were observed in females (p=0.600). TTF during PTL was similar between males and females during both the relative (p=0.387) and absolute (p = 0.688) conditions.

The relative IHE condition resulted in a higher target force output in males (265±39 N) than females (178±26 N; p<0.001). During the absolute IHE condition, the workload intensity was relatively greater in females (56±7% of MVC_{PEAK}) than males (37±5% of MVC_{PEAK}; p<0.001). No significant sex-based differences were observed in the workload of the relative PTL condition (males: 80±9 cmH_2O; females: 75±14 cmH_2O; p=0.326), or the relative intensity of the workload of the absolute PTL condition (males: 59±10% $P_{di,MAX}$; females: 64±9% $P_{di,MAX}$; p=0.264).

All participants experienced a decline in $P_{di,MAX}$ following PTL, regardless of workload (all p<0.05). However, only males experienced a decline in MVC_{PEAK} following IHE (males, both workloads: p<0.01; females, both workloads: p>0.1). No sex-based differences were observed in pre- or post- PTL $P_{di,MAX}$ (all p>0.05). MVC_{PEAK} was significantly lower in females pre- and post-IHE compared to males (all conditions p<0.01).

As shown in Table 4.3, The workload of the relative IHE condition during the PECO trial (244±35 N) was not statistically different to that of the control trial (248±15 N; p=0.777). The relative intensity of the absolute IHE condition was also not statistically different between the control (38±3% of MVC_{PEAK}) and PECO trials (40±6% of MVC_{PEAK}; p=0.453). TTF was similar between the control and PECO condition at both relative (p=0.291) and absolute workloads (p=0.061). However, participants were able to exercise for a longer duration during the relative condition compared to the absolute condition for both PECO (p=0.023) and control (p=0.016) trials.

4.2 Cardiovascular Measures

To fully explore the collected data and understand any potential sex-based differences, all results are presented in both relative and absolute terms. Data are presented as normalized time points (baseline, 20%, 40%, 60%, 80%, and 100% TTF) and iso-time points (minutes 1-5, post-exercise minutes 1-2). All statistical analyses were run using the normalized time points unless otherwise indicated.

4.2.1 Limb Metaboreflex Activation

As shown in Figure 4.1, a time-dependent rise in MAP was observed in all participants, regardless of workload ($p<0.001$). MAP was significantly greater in males than females at all normalized time points, regardless of workload (baseline: $p=0.021$: all exercise time points: $p<0.001$). The change in MAP from baseline to the end of exercise (ΔMAP) was significantly greater in males than females during both relative ($p=0.041$) and absolute ($p<0.001$) workloads, even when controlling for forearm circumference and volume (relative: $p=0.07$; absolute: $p=0.03$), as shown in Figure 4.2. A time-dependent rise was also observed in ΔMAP, regardless of sex or workload ($p<0.001$). The HR response to IHE was not statistically different between males and females ($p=0.319$), and increased in a time-dependent manner, regardless of workload ($p<0.001$).

4.2.2 Inspiratory Metaboreflex Activation

A time-dependent rise in MAP was observed in all participants, regardless of workload ($p<0.001$). MAP was significantly greater in males than females in all normalized time points except for baseline (baseline: $p=0.143$; all exercise time points: $p<0.001$). Males exhibited a

greater ΔMAP than females during both the relative ($p=0.004$) and absolute ($p<0.001$) workload conditions. The ΔMAP increased in a time-dependent manner in both males and females, regardless of workload ($p<0.001$). The HR response to PTL was statistically similar between males and females ($p=0.129$), and increased in a time-dependent manner in all participants regardless of workload ($p<0.001$).

4.3 Ventilatory Measures

4.3.1 Limb Metaboreflex Activation

Minute ventilation ($\dot{V}_E$) was significantly increased during exercise compared to baseline, regardless of workload or sex ($p<0.001$). $\dot{V}_E$ was also significantly greater in males than females, regardless of workload or time ($p=0.038$). Sex-based differences in $\dot{V}_E$ are lost when controlled for forearm volume ($p=0.320$) and circumference ($p=0.213$). $P_{ET}CO_2$ decreased towards the end of IHE, with significant differences found between 20% and 80% TTF (Δ3.7mmHg; $p=0.018$), 20% and 100% TTF (Δ4.8mmHg; $p=0.023$), 40% and 80% TTF (Δ3.4mmHg; $p=0.017$), 40% and 100% TTF (Δ4.5mmHg; $p=0.022$), 60% and 80% TTF (Δ2.1mmHg; $p=0.006$), and 60% and 80% TTF (Δ3.2mmHg; $p=0.040$). $P_{ET}CO_2$ was significantly lower in females than males during baseline for both the absolute ($p=0.005$) and relative workloads ($p<0.001$). $P_{ET}CO_2$ was significantly lower in females than males during the relative condition at 20%TTF ($p=0.022$), 60%TTF ($p=0.020$), and 80%TTF ($p=0.047$). Regarding breathing frequency, no significant differences were observed due to workload, sex, or muscle (all $p>0.05$).

4.3.2 Inspiratory Metaboreflex Activation

$\dot{V}_E$ significantly increased during exercise compared to baseline, regardless of workload or sex ($p<0.001$). $\dot{V}_E$ was also significantly greater in males than females, regardless of workload or time ($p=0.013$). No significant differences in $P_{ET}CO_2$ were found due to workload ($p=0.465$), time ($p=0.062$), or sex ($p=0.122$). No significant effect of muscle (IHE vs PTL) was found for $\dot{V}_E$ ($p=0.107$) or $P_{ET}CO_2$ ($p=0.105$). Regarding breathing frequency, no significant differences were observed due to workload, sex, or muscle (all $p>0.05$).

4.4 Pressure-Time and Force-Time Product Measures

4.4.1 Force-Time Product Measures

Force-time products (FTP) were calculated for each normalized time point of IHE (baseline, 20%, 40%, 60%, 80%, 100% TTF), and were normalized to body weight (FTP_{BW}) and forearm volume (FTP_{FV}). All FTP values are presented in Table 4.8. A main effect of workload ($p<0.001$), time ($p<0.001$), and sex ($p<0.001$) were observed for FTP. Males produced a greater FTP during the relative workload condition as compared to the absolute workload ($p<0.001$), as shown in Figure 4.4. The FTP produced by females was similar between the relative and absolute workloads ($p=0.631$). The FTP produced was not statistically different between males and females during the absolute condition ($p=0.482$), but was greater in males during the relative condition ($p<0.001$).

Sex-based differences are lost when FTP is normalized to body weight ($p=0.083$) and forearm volume ($p=0.163$). Males produced a greater FTP_{BW} and FTP_{FV} during the relative workload compared to the absolute workload (both $p<0.001$), but no differences were observed between workloads in females (both $p>0.1$). Cumulative FTP was calculated and exhibited a

significant workload (p=0.002), time (p<0.001), and sex effect (p<0.001). Cumulative FTP was similar between relative and absolute workloads for females (p=0.616), but was greater in the absolute condition for males (p = 0.004). Males produced a significantly greater cumulative FTP than females in all time points of the absolute condition (all p<0.001), but only at 100% TTF in the relative condition (20%TTF: p=0.132; 40%TTF: p=0.108; 60%TTF: p=0.074; 80%TTF: p=0.06; 100%TTF: p=0.049). At the end of exercise, males produced a greater cumulative FTP during the absolute workload than relative workload (p=0.004), however no differences between workloads were observed in females (p=0.725).

4.4.2 Pressure-Time Product Measures

Pressure-time products (PTP) were calculated for each normalized time point of PTL (baseline, 20%, 40%, 60%, 80%, 100% TTF), and were normalized to body weight (PTP_{BW}). All PTP values are presented in Table 4.9. PTP of the diaphragm (PTP_{di}) demonstrated an effect of time (p<0.001) and sex (p=0.021). Males produced a greater PTP_{di} than females at 20% (p=0.047), 60% (0.013), 80% (p=0.033), and 100% TTF (p=0.006), but were not statistically different at baseline (p=0.179) and 40% TTF (p=0.056). Sex-based differences are lost when PTP_{di} is normalized to body weight ($PTP_{di,BW}$; p=0.543).

PTP of the esophagus (PTP_{eso}) demonstrated an effect of time (p<0.001) and sex (p=0.019). Males produced a larger PTP_{eso} at 20% (p=0.004), 40% (p=0.021), 60% (p=0.026), and 100% TTF (p=0.032), but not at baseline (p=0.216) or 80% TTF (p=0.131). Sex based differences in PTP_{eso} are lost when normalized to body weight ($PTP_{eso,BW}$; p=0.164). No sex-based differences were observed with gastric PTP (PTP_{ga}; p=0.955), even when normalized to body weight ($PTP_{ga,BW}$; p=0.511).

A main effect of time ($p<0.001$) and sex ($p=0.005$) was found for PTP at the mouth (PTP_{mo}). Males produced a greater PTP_{mo} than females at every time point except for baseline (baseline: $p=0.511$; all exercise time points: $p<0.05$). The sex-based difference in PTP_{mo} is maintained when normalized to body weight ($PTP_{mo,BW}$; $p=0.014$). Males produced a larger $PTP_{mo,BW}$ than females at every time point except for baseline and 80% TTF (baseline: $p=0.243$; 20%TTF: $p=0.006$; 40%TTF $p=0.009$; 60%TTF: $p=0.05$; 80%TTF: $p=0.061$; 100%TTF: $p=0.02$). No effect of workload was observed for PTP_{di}, PTP_{eso}, PTP_{ga}, or PTP_{mo}, even when normalized to body weight (all $p>0.1$).

Cumulative PTPs were calculated, and no significant sex-based differences were observed for cumulative PTP_{di} ($p=0.444$), PTP_{eso} ($p=0.308$), PTP_{ga} ($p=0.754$), or PTP_{mo} ($p=0.226$), as shown in Figure 4.3. No significant effects were observed in the ratio of PTP_{di} to PTP produced by the total respiratory muscles (PTP_{di}/PTP_{tot}; all $p>0.1$).

4.5 Day 3: PECO Trial

4.5.1 Cardiovascular Measures

As shown in Table 4.10, baseline MAP, HR, $\dot{V}_E$, and $P_{ET}CO_2$ were not statistically different between all exercise conditions in the subset of participants that completed Day 3. Seen in Figure 4.6, a time-dependent rise in MAP was observed in all participants, with no significant difference between the PECO and control conditions ($p=0.698$). MAP was significantly higher during the first- and second-minute post-exercise (Post 1 and Post 2) in the PECO condition compared to the control condition ($p=0.021$ and $p<0.001$, respectively). MAP during Post 1 and Post 2 of the PECO condition was not statistically different to the final time point (100% TTF) of exercise, for both workloads (both $p>0.1$). The ΔMAP was statistically similar between control

and PECO conditions, regardless of workload (p=0.501). ΔMAP was significantly greater during Post 1 and Post 2 in the PECO condition compared to the control condition (p=0.016 and p<0.001, respectively). HR increased in a time-dependent manner, regardless of workload (p<0.001), and was not statistically different between the PECO and control conditions (p=0.434).

4.5.2 Ventilatory Measures

$\dot{V}_E$ significantly increased during exercise compared to baseline, regardless of workload or condition (p<0.001). $\dot{V}_E$ was not significantly different between conditions (p=0.136) or workloads (p=0.112). $P_{ET}CO_2$ was significantly lower during the absolute workload compared to the relative workloads (p=0.042). No significant effect of condition was observed for $P_{ET}CO_2$ (p=0.236).

4.5.3 Force-Time Product Measures

Force-time products (FTP) were calculated for each normalized time point of IHE (baseline, 20%, 40%, 60%, 80%, 100% TTF), as well as for the first- and second- minute following exercise (Post 1, Post 2). All FTPs were normalized to body weight (FTP_{BW}) and forearm volume (FTP_{FV}). All FTP values are presented in Table 4.12 and shown in Figure 4.7. Participants produced a greater FTP during the relative workload condition as compared to the absolute workload (p=0.004), as shown in Figure 4.7. The effect of workload is maintained when FTP is normalized to body weight (FTP_{BW}; p=0.003) and forearm volume (FTP_{FV}: p=0.01).

Cumulative FTP was calculated and exhibited a significant condition (p=0.023), workload (p=0.024), and time effect (p<0.001). Cumulative FTP was significantly greater during

the control condition than PECO for every time point (all $p<0.05$). Cumulative FTP was also significantly greater during the absolute workload than relative workload for every time point (all $p<0.05$).

Table 4.1 Participant characteristics and pulmonary function measures.

	Males (n=9)	Females (n=8)	Combined (n=17)	PECO (n=5)
Age (y)	23 ± 4	20 ± 2	21 ± 3	23 ± 4
Height (cm)	181.1 ± 5.4*	165.4 ± 7.8	174.0 ± 9.7	180.7 ± 6.9
Weight (kg)	76.6 ± 8.1*	63.8 ± 3.5	70.4 ± 8.8	75.5 ± 9.9
BMI (m^2/kg)	23.3 ± 2.1	23.4 ± 1.9	23.2 ± 1.9	23.1 ± 2.3
Forearm Volume (mL)	1280 ± 175*	880 ± 95	1090 ± 240	1280 ± 160
Forearm Circumference (cm)	27.3 ± 1.7*	24.1 ± 0.7	25.9 ± 1.7	27.0 ± 1.7
FVC (L)	5.9 ± 0.6 *	3.8 ± 0.6	4.9 ± 1.3	5.9 ± 0.8
FVC (% Predicted)	109 ± 12	94 ± 11	102 ± 13	109 ± 15
$FEV_{1.0}$ (L)	4.5 ± 0.4	3.2 ± 0.5	3.9 ± 0.8	4.4 ± 0.5
$FEV_{1.0}$ (% Predicted)	98 ± 8	93 ± 9	96 ± 9	96 ± 8
$FEV_{1.0}$/FVC (%)	76.7 ± 8.7	87.3 ± 7.4	81.7 ± 9.6	75.0 ± 8.3
TLC (L)	7.8 ± 1.0 *	5.0 ± 1.0	6.4 ± 1.8	8.1 ± 1.2
TLC (% Predicted)	106.6 ± 17.6	94.3 ± 11.3	100.8 ± 15.9	111.2 ± 20.6
DLCO (mL/mmHg/min)	34.2 ± 2.5	24.5 ± 5.0	29.0 ± 6.6	32.8 ± 4.9
Hemoglobin	13.9 ± 1.7 *	12.6 ± 0.8	13.4 ± 1.5	13.3 ± 1.5

Abbreviations: PECO = participants that completed the supplemental Day 3, y = years, BMI = body mass index, FVC = functional vital capacity, $FEV_{1.0}$ = forced expiratory volume ejected in one second, TLC = total lung capacity, DLCO = diffusion capacity of the lung for carbon monoxide, adjusted to hemoglobin. Values presented as Mean ± SD. * = significant difference compared to females.

Table 4.2 Exercise workload and intensity for Days 1 and 2.

	Males – R	Males – A	Females – R	Females – A
Pre-IHE MVC_{PEAK} (N)	441 ± 65 *	461 ± 68 *	296 ± 43	303 ± 35
Pre-PTL $P_{di,MAX}$ (cmH_2O)	134 ± 13	144 ± 23	125 ± 23	131 ± 18
Post-IHE MVC_{PEAK} (N)	402 ± 72 *⋄	392 ± 70 *⋄	274 ± 60	291 ± 47
Post-PTL $P_{di,MAX}$ (cmH_2O)	110 ± 11 ⋄	119 ± 11 ⋄	97 ± 28 ⋄	103 ± 22 ⋄
MVC_{PEAK} (% Change)	91 ± 7 †	85 ± 9	92 ± 14	94 ± 10
PTL $P_{di,MAX}$ (% Change)	82 ± 10	84 ± 10	78 ± 17	80 ± 15
IHE TTF (s)	202 ± 79 †‡	663 ± 297*	222 ± 64 ‡	208 ± 68 ‡
Target IHE Workload (N)	265 ± 39 *†	167	178 ± 26	167
Target IHE Workload (% MVC_{PEAK})	60 †	37 ± 5 *	60	56 ± 7
PTL TTF (s)	717 ± 350	728 ± 336	918 ± 567	662 ± 336
Target PTL Workload (cmH_2O)	80 ± 8	82	75 ± 14	82
Target PTL Workload (% $P_{di,MAX}$)	60	59 ± 10	60	64 ± 9

Abbreviations: R = relative workload, A = absolute workload, MVC_{PEAK} = peak maximum voluntary contraction of the forearm, $P_{di,MAX}$ = maximal pressure generated by the diaphragm, IHE = intermittent handgrip exercise, TTF = time to task failure, PTL = pressure-threshold loading. Values presented as Mean ± SD. * = significant difference compared to females; † = significant difference compared to absolute workload; ‡ = within-condition significant difference compared to PTL. ⋄ = significant difference from pre-exercise.

Table 4.3 Exercise workload and intensity for Day 3 (PECO) compared to control.

	Control – R	Control – A	PECO – R	PECO – A
MVC_{PEAK} (N)	413 ± 27	447 ± 37	407 ± 58	427 ± 53
IHE TTF (s)	235 ± 95 *	809 ± 274 *	200 ± 70	574 ± 193
Post-IHE MVC_{PEAK} (N)	372 ± 55	364 ± 42 ‡	388 ± 78	364 ± 32
MVC_{PEAK} (% Change)	91 ± 9	82 ± 11	95 ± 5	88 ± 7
Target IHE Workload (N)	248 ± 16	167	244 ± 35	167
Target IHE Workload (% MVC_{PEAK})	60 †	38 ± 3	60 †	40 ± 6

Abbreviations: R = relative workload, A = absolute workload, PECO = post-exercise circulatory occlusion, IHE = intermittent handgrip exercise, Control = IHE condition without PECO, MVC_{PEAK} = peak maximum voluntary contraction of the forearm, TTF = time to task failure. N=5. Values presented as Mean ± SD. * = significant difference compared to PECO. † = within-condition significant difference compared to absolute workload. ‡ = significant difference from pre-exercise.

Table 4.4 Cardiopulmonary measures during IHE, presented in time as a percent to task failure.

Variable	Sex	Baseline	20% TTF	40% TTF	60% TTF	80% TTF	100% TTF	p<0.05
Relative								
MAP (mmHg)	M	94 ± 5	105 ± 5	116 ± 8	120 ± 10	124 ± 10	124 ± 9	∗ †
	F	88 ± 5	99 ± 6	103 ± 5	107 ± 7	110 ± 6	110 ± 5	
ΔMAP (mmHg)	M		11 ± 8	21 ± 12	26 ± 12	30 ± 11	31 ± 12	∗ †
	F		9 ± 6	13 ± 5	18 ± 7	20 ± 6	21 ± 7	
HR (bpm)	M	65 ± 12	84 ± 11	88 ± 11	91 ± 11	96 ± 8	101 ± 9	†
	F	69 ± 9	90 ± 15	90 ± 12	93 ± 11	103 ± 11	105 ± 12	
$\dot{V}_E$ (L/min)	M	8.8 ± 1.3	14.7 ± 3.0	17.2 ± 4.9	15.1 ± 3.5	19.1 ± 9.6	22.8 ± 14.2	∗ †
	F	8.4 ± 1.2	11.4 ± 4.0	11.9 ± 4.2	14.7 ± 4.9	16.5 ± 4.5	16.2 ± 5.1	
$P_{ET}CO_2$ (mmHg)	M	39.8 ± 2.1	38.6 ± 2.7	38.4 ± 4.2	38.4 ± 5.0	36.9 ± 6.8	34.8 ± 7.6	†
	F	34.5 ± 2.9	35.5 ± 2.2	35.4 ± 3.0	33.1 ± 3.0	31.1 ± 3.5	31.8 ± 4.4	
Absolute								
MAP (mmHg)	M	93 ± 4	111 ± 5	119 ± 5	125 ± 8	128 ± 6	129 ± 7	* †
	F	89 ± 4	95 ± 6	101 ± 6	106 ± 5	109 ± 6	109 ± 5	
ΔMAP (mmHg)	M		17 ± 6	25 ± 6	31 ± 8	34 ± 6	35 ± 6	∗ †
	F		7 ± 6	13 ± 6	17 ± 6	20 ± 6	21 ± 7	
HR (bpm)	M	64 ± 10	76 ± 11	82 ± 9	90 ± 11	96 ± 12	101 ± 12	†
	F	70 ± 11	91 ± 11	90 ± 8	95 ± 11	97 ± 9	99 ± 16	
$\dot{V}_E$ (L/min)	M	9.4 ± 2.0	12.4 ± 2.2	13.4 ± 2.7	16.8 ± 7.8	24.7 ± 14.2	28.7 ± 15.4	∗ †
	F	7.7 ± 2.3	11.4 ± 4.0	11.1 ± 3.0	13.0 ± 5.3	14.8 ± 2.7	13.4 ± 3.0	
$P_{ET}CO_2$ (mmHg)	M	39.7 ± 2.0	38.9 ± 2.0	38.5 ± 2.9	36.8 ± 5.8	32.6 ± 7.5	29.7 ± 8.9	†
	F	36.7 ± 1.7	36.7 ± 3.2	36.2 ± 3.5	35.1 ± 3.9	34.3 ± 2.7	34.3 ± 0.9	

Abbreviations: IHE = intermittent handgrip exercise; TTF = time to task failure; M = males; F = females; MAP = mean arterial pressure; ΔMAP = change in MAP from baseline; HR = heart rate; bpm = beats per minute; $\dot{V}_E$ = minute ventilation; $P_{ET}CO_2$ = end-tidal CO_2. Values presented as Mean ± SD. * = within-condition main effect of sex; † = within-condition main effect of time.

Table 4.5 Cardiopulmonary measures during IHE, presented in absolute time.

Variable	Sex	Baseline	Min 1	Min 2	Min 3	Min 4	Min 5	p<0.05
Relative								
MAP (mmHg)	M	94 ± 5	112 ± 6	121 ± 10	128 ± 9	120	115	∗ †
	F	88 ± 5	102 ± 7	106 ± 7	109 ± 6	111 ± 7		
ΔMAP (mmHg)	M		18 ± 8	28 ± 11	36 ± 10	31	26	∗ †
	F		13 ± 7	16 ± 8	19 ± 8	20 ± 7		
HR (bpm)	M	65 ± 12	87 ± 13	92 ± 16	96 ± 14	72	82	∗ †
	F	69 ± 9	89 ± 13	95 ± 13	95 ± 9	99 ± 14		
$\dot{V}_E$ (L/min)	M	8.8 ± 1.3	14.3 ± 2.7	16.7 ± 3.8	16.7 ± 6.8	19.0	37.0	†
	F	8.4 ± 1.2	11.8 ± 3.7	14.4 ± 6.2	16.0 ± 7.6	15.7 ± 4.1		
$P_{ET}CO_2$ (mmHg)	M	39.8 ± 2.1	39.9 ± 3.0	37.4 ± 3.9	36.9 ± 5.2	30.1	22.5	‡
	F	34.5 ± 2.9	35.7 ± 2.7	33.8 ± 3.5	31.6 ± 3.7	31.2±3.2		
Absolute								
MAP (mmHg)	M	93 ± 4	105 ± 7	110 ± 8	114 ± 7	118 ± 7	121 ± 6	∗ †
	F	89 ± 4	100 ± 7	105 ± 6	108 ± 7	105	105	
ΔMAP (mmHg)	M		12 ± 6	16 ± 9	21 ± 9	25±9	27 ± 8	∗ †
	F		11 ± 6	16 ± 6	18 ± 6	19	19	
HR (bpm)	M	64 ± 10	76 ± 13	76 ± 11	78 ± 12	82 ± 13	85 ± 13	∗ †
	F	70 ± 11	90 ± 9	93 ± 9	101 ± 11	113	116	
$\dot{V}_E$ (L/min)	M	9.4 ± 2.0	12.5 ± 2.1	12.3 ± 1.5	12.5 ± 2.6	15.1 ± 3.7	17.9 ± 8.9	†
	F	7.7 ± 2.3	11.7 ± 4.3	12.2 ± 3.5	13.2 ± 1.8	14.5	13.8	
$P_{ET}CO_2$ (mmHg)	M	39.7 ± 2.0	38.8 ± 1.3	38.7 ± 1.8	37.9 ± 3.3	35.9 ± 5	39.7 ± 2.0	‡
	F	36.7 ± 1.7	35.8 ± 3.2	35.4 ± 3.1	34.8 ± 2.2	34.2	33.6	

Abbreviations: IHE = intermittent handgrip exercise; TTF = time to task failure; M = males; F = females; MAP = mean arterial pressure; ΔMAP = change in MAP from baseline; HR = heart rate; bpm = beats per minute; $\dot{V}_E$ = minute ventilation; $P_{ET}CO_2$ = end-tidal CO_2. Values presented as Mean ± SD. Statistical analyses do not include min 4 and 5 time points, due to only one participant reaching said time points in some conditions. * = within-condition main effect of sex; † = within-condition main effect of time. ‡ = main effect of workload.

Table 4.6 Cardiopulmonary measures during PTL, presented in time as a percent to task failure.

Variable	Sex	Baseline	20% TTF	40% TTF	60% TTF	80% TTF	100% TTF	p<0.05
Relative								
MAP	M	92 ± 7	109 ± 9	110 ± 11	117 ± 7	123 ± 6	123 ± 7	∗ †
(mmHg)	F	87 ± 4	96 ± 4	99 ± 2	100 ± 4	102 ± 5	102 ± 4	
ΔMAP	M		16 ± 10	18 ± 11	25 ± 10	31 ± 7	31 ± 9	∗ †
(mmHg)	F		9 ± 5	11 ± 4	13 ± 7	15 ± 6	15 ± 6	
HR (bpm)	M	65 ± 10	88 ± 9	90 ± 12	92 ± 11	92 ± 9	94 ± 10	†
	F	70 ± 9	93 ± 12	96 ± 12	99 ± 13	101 ± 14	100 ± 14	
$\dot{V}_E$ (L/min)	M	8.7 ± 1.5	21.9 ± 6.0	22.5 ± 5.6	21.6 ± 5.3	18.9 ± 7.0	19.4 ± 7.8	∗ †
	F	6.9 ± 1.5	12.8 ± 5.0	13.2 ± 5.1	13.1 ± 4.5	14.4 ± 5.3	15.0 ± 5.4	
$P_{ET}CO_2$	M	39.7 ± 2.9	37.0 ± 3.6	38.8 ± 4.1	38.5 ± 2.9	40.0 ± 1.6	38.5 ± 3.2	
(mmHg)	F	36.2 ± 3.7	37.1 ± 2.4	37.4 ± 2.3	39.2 ± 2.4	38.6 ± 1.6	39.3 ± 1.3	
Absolute								
MAP	M	93 ± 6	107 ± 6	114 ± 6	118 ± 5	121 ± 5	123 ± 6	∗ †
(mmHg)	F	88 ± 6	98 ± 5	98 ± 5	97 ± 6	100 ± 6	102 ± 7	
ΔMAP	M		14 ± 8	21 ± 8	24 ± 8	28 ± 7	30 ± 7	∗ †
(mmHg)	F		10 ± 5	10 ± 4	9 ± 5	12 ± 4	14 ± 3	
HR (bpm)	M	67 ± 11	90 ± 10	93 ± 12	93 ± 12	93 ± 14	94 ± 13	†
	F	69 ± 10	97 ± 11	101 ± 13	105 ± 16	107 ± 15	107 ± 18	
$\dot{V}_E$ (L/min)	M	8.4 ± 1.1	21.2 ± 4.2	21.9 ± 4.1	21.1 ± 4.3	20.2 ± 5.4	20.5 ± 6.6	∗ †
	F	8.5 ± 1.7	15.9 ± 2.9	16.5 ± 4.3	18.6 ± 4.7	18.2 ± 4.5	16.8 ± 3.9	
$P_{ET}CO_2$	M	38.9 ± 2.8	39.1 ± 3.2	40.8 ± 2.3	39.5 ± 1.6	39.9 ± 2.6	39.3 ± 2.2	
(mmHg)	F	36.1 ± 2.5	38.1 ± 4.2	38.7 ± 2.5	37.0 ± 3.6	38.1 ± 3.1	38.9 ± 2.7	

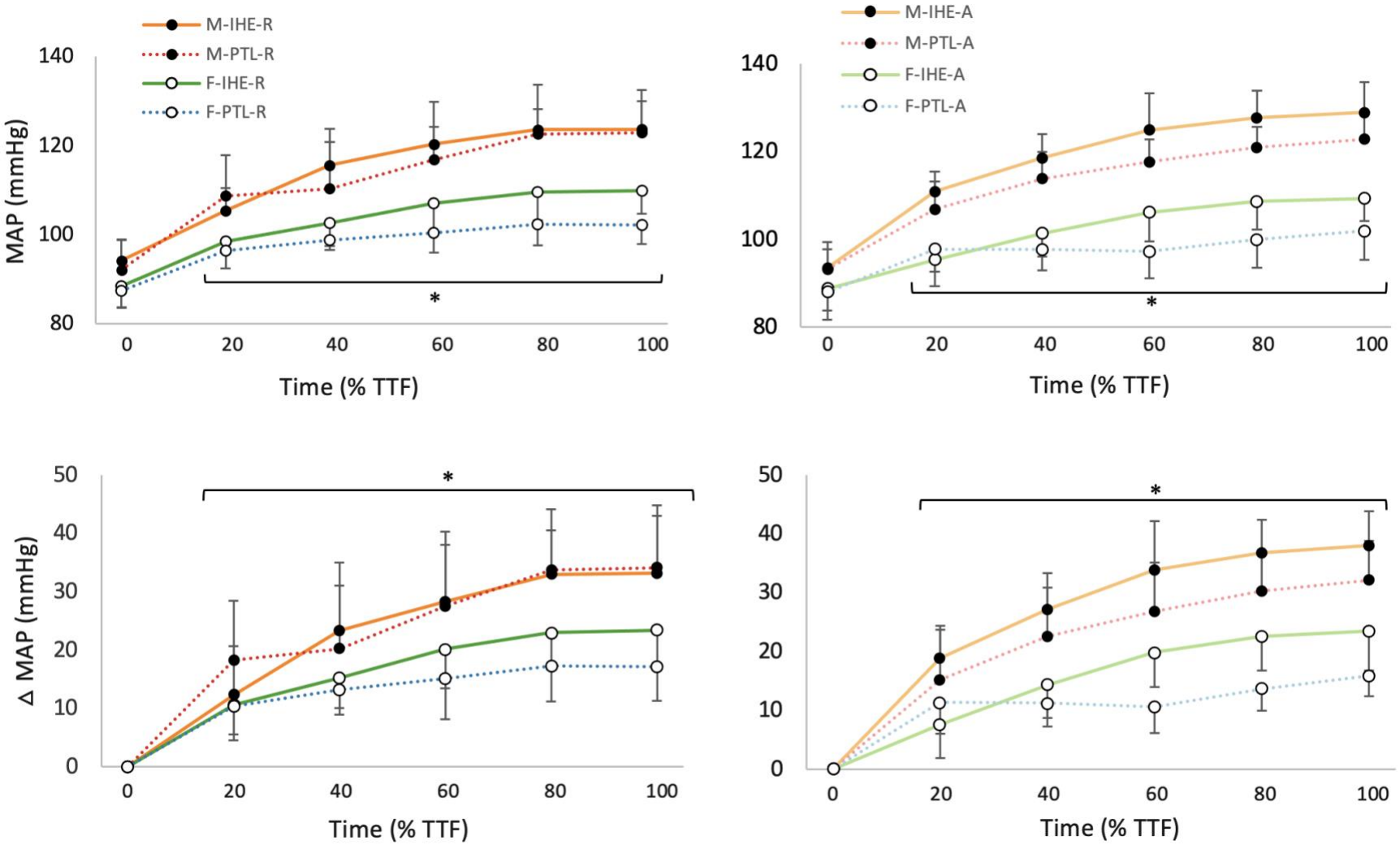
Abbreviations: PTL = pressure-threshold loading; TTF = time to task failure; M = males; F = females; MAP = mean arterial pressure; ΔMAP = change in MAP from baseline; HR = heart rate; bpm = beats per minute; $\dot{V}_E$ = minute ventilation; $P_{ET}CO_2$ = end-tidal CO_2. Values presented as Mean ± SD. * = within-condition main effect of sex; † = within-condition main effect of time.

Table 4.7 Cardiopulmonary measures during PTL, presented in absolute time.

Variable	Sex	Baseline	Min 1	Min 2	Min 3	Min 4	Min 5	p<0.05
Relative								
MAP	M	92 ± 7	103 ± 13	105 ± 12	111 ± 12	115 ± 12	116 ± 11	∗ †
(mmHg)	F	87 ± 4	98 ± 6	98 ± 6	99 ± 4	100 ± 7	102 ± 8	
ΔMAP	M		10 ± 12	15 ± 13	19 ± 12	23 ± 13	24 ± 11	∗ †
(mmHg)	F		11 ± 6	10 ± 6	11 ± 4	12 ± 6	15 ± 6	
HR (bpm)	M	65 ± 10	87 ± 10	88 ± 11	89 ± 11	91 ± 10	93 ± 10	†
	F	70 ± 9	93 ± 10	93 ± 14	95 ± 15	98 ± 17	91 ± 11	
$\dot{V}_E$ (L/min)	M	8.7 ± 1.5	21.4 ± 5.5	23.7 ± 5.4	22.3 ± 5.8	21.1 ± 5.1	20.6 ± 6.3	∗ †
	F	6.9 ± 1.5	12.5 ± 6.2	12.8 ± 5.0	13.8 ± 5.9	13.7 ± 5.7	13.2 ± 5.2	
$P_{ET}CO_2$	M	39.7 ± 2.9	35.2 ± 4.6	35.3 ± 3.6	37.8 ± 3.5	39.5 ± 3.3	39.7 ± 2.4	†
(mmHg)	F	36.2 ± 3.7	37.0 ± 10.2	37.1 ± 2.9	36.7 ± 2.7	38.6 ± 2.6	38.1 ± 2.7	
Absolute								
MAP	M	93 ± 6	95 ± 6	106 ± 5	112 ± 4	117 ± 4	119 ± 5	∗ †
(mmHg)	F	88 ± 6	95 ± 7	99 ± 6	101 ± 6	101 ± 6	102 ± 6	
ΔMAP	M		2 ± 10	13 ± 8	19 ± 7	24 ± 7	25 ± 7	∗ †
(mmHg)	F		7 ± 7	11 ± 4	13 ± 4	13 ± 4	12 ± 5	
HR (bpm)	M	67 ± 11	88 ± 11	89 ± 11	91 ± 11	91 ± 11	92 ± 12	†
	F	69 ± 10	98 ± 13	98 ± 13	99 ± 10	100 ± 9	104 ± 14	
$\dot{V}_E$ (L/min)	M	8.5 ± 1.7	23.3 ± 5.1	21.1 ± 3.6	20.6 ± 2.9	20.6 ± 4.2	21.5 ± 3.8	∗ †
	F	8.5 ± 1.7	16.6 ± 5.3	16.9 ± 5.1	16.2 ± 2.8	16.2 ± 2.5	17.7 ± 4.8	
$P_{ET}CO_2$	M	38.9 ± 2.8	35.0 ± 3.5	40.1 ± 3.4	40.5 ± 1.9	39.9 ± 2.2	18.9 ± 2.3	†
(mmHg)	F	36.1 ± 2.5	35.1 ± 4.4	37.6 ± 3.7	38.6 ± 2.9	38.7 ± 1.7	37.1 ± 3.0	

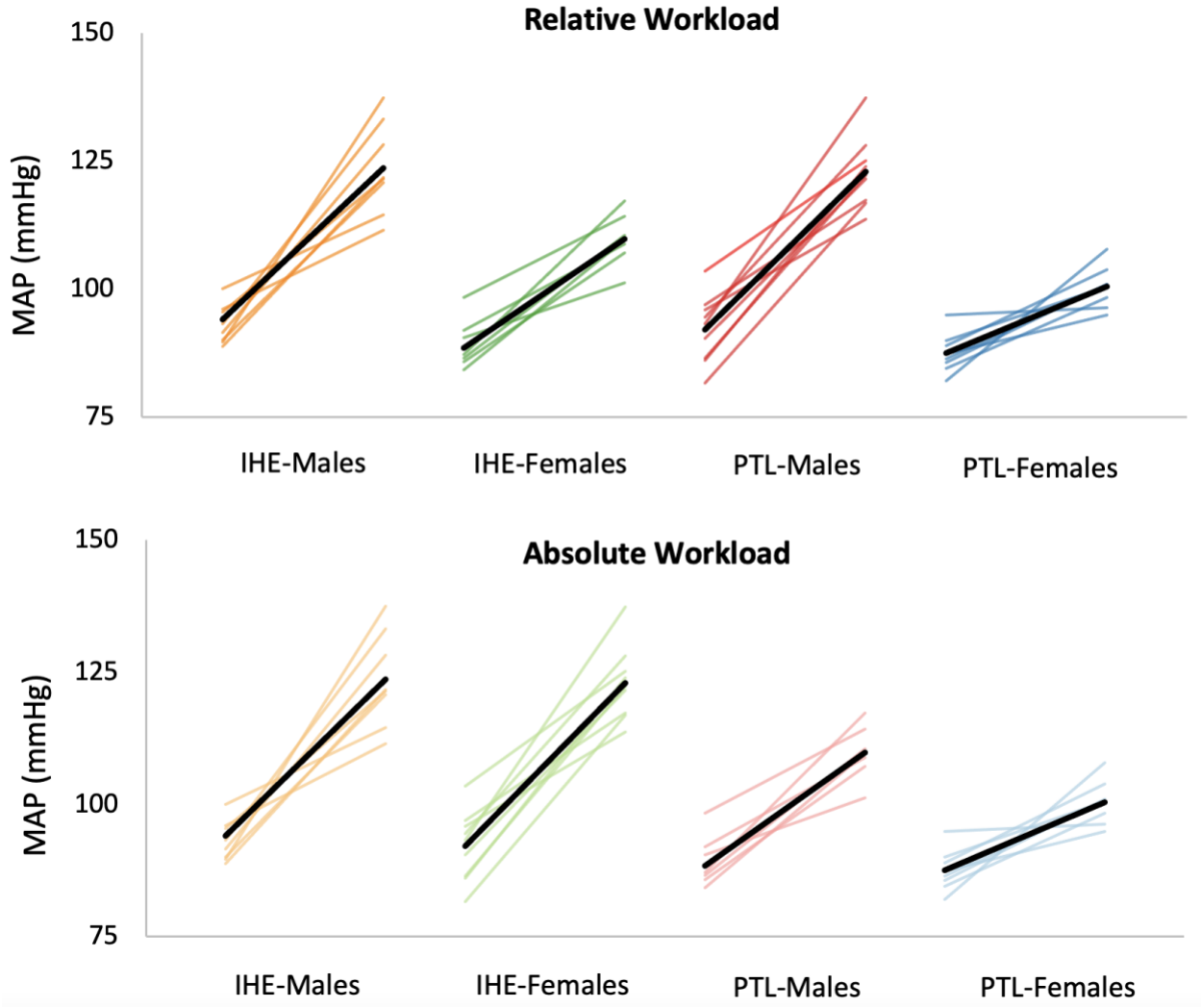
Abbreviations: PTL = pressure-threshold loading; TTF = time to task failure; M = males; F = females; MAP = mean arterial pressure; ΔMAP = change in MAP from baseline; HR = heart rate; bpm = beats per minute; $\dot{V}_E$ = minute ventilation; $P_{ET}CO_2$ = end-tidal CO_2. Values presented as Mean ± SD. * = within-condition main effect of sex; † = within-condition main effect of time.

Figure 4.1 Mean arterial pressure during IHE and PTL, presented in time as a percent to task failure.



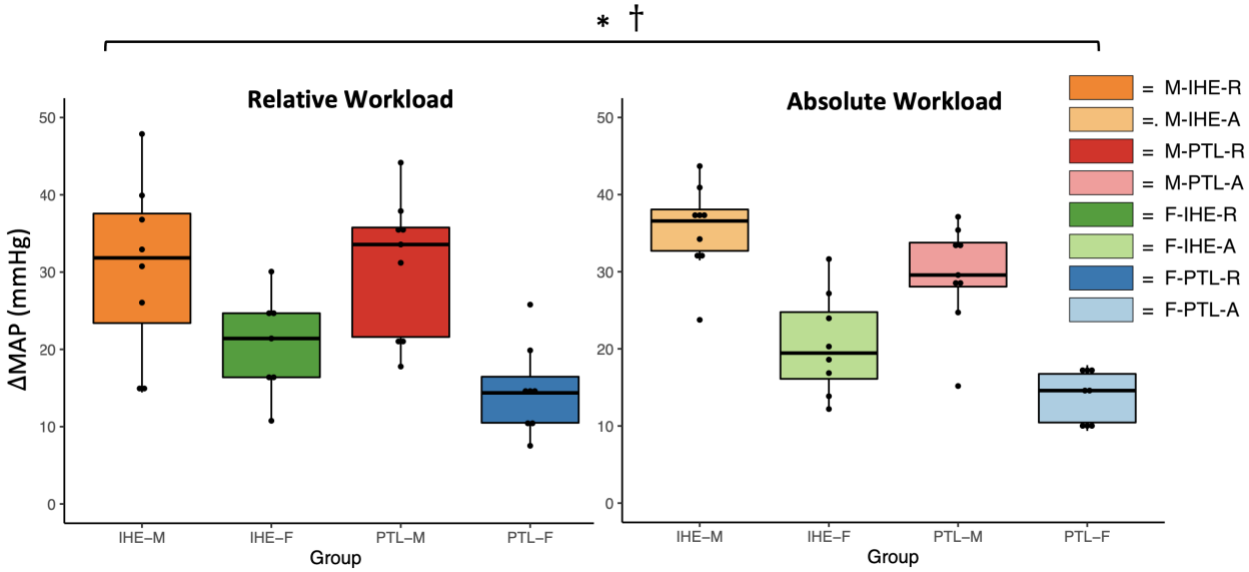
Abbreviations: IHE = intermittent handgrip exercise; PTL = pressure-threshold loading; TTF = time to task failure; M = males; F = females; LMA = limb metaboreflex activation; IMA = inspiratory metaboreflex activation; MAP = mean arterial pressure; ΔMAP = change in MAP from baseline. * = significant difference between males and females, for both LMA and IMA.

Figure 4.2 Mean arterial pressure during IHE and PTL, presented as the change from 0% to 100% time to task failure.



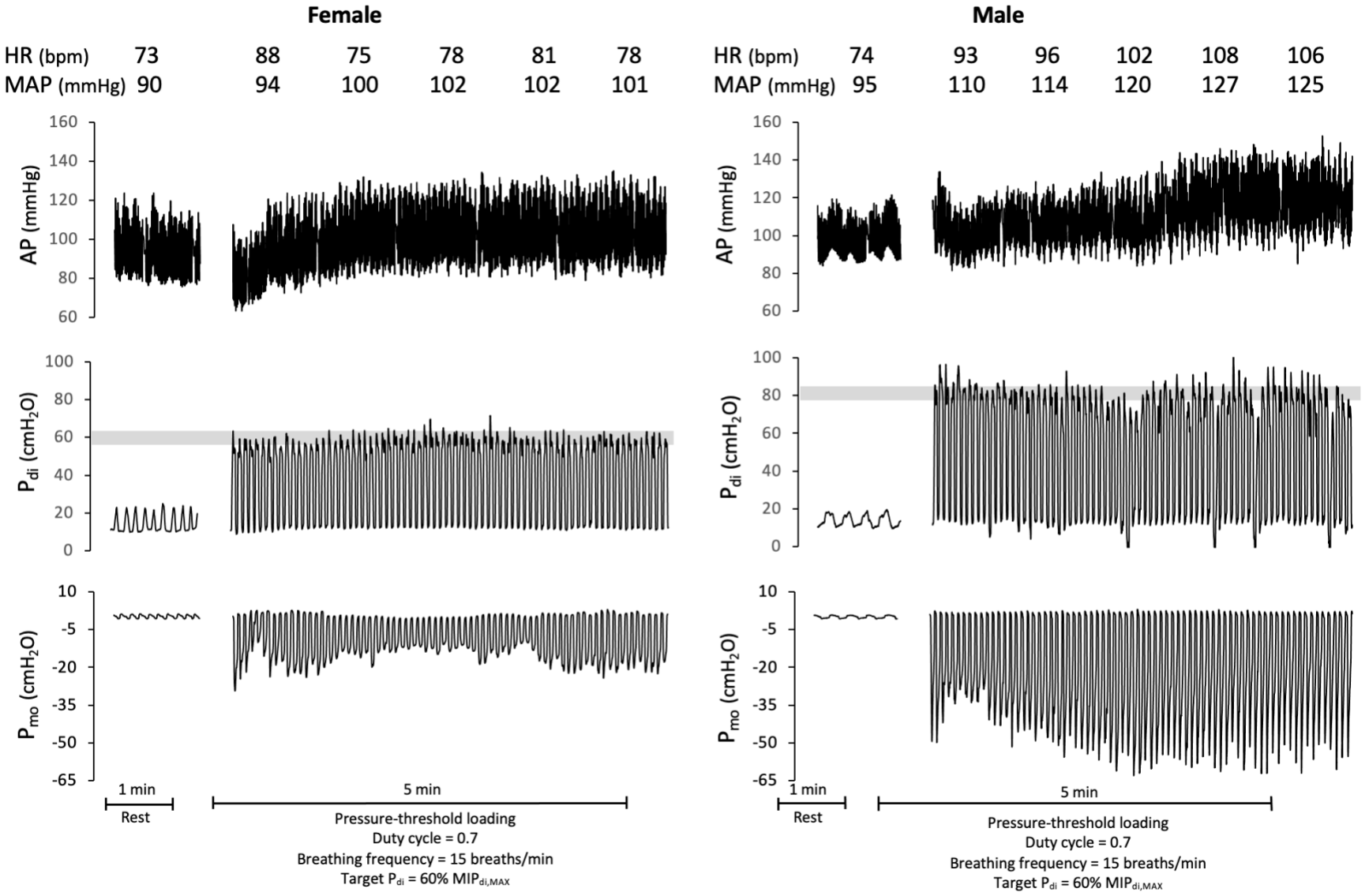
Abbreviations: IHE = intermittent handgrip exercise; PTL = pressure-threshold loading; M = males; F = females; MAP = mean arterial pressure.

Figure 4.3 Change in mean arterial pressure from baseline to task failure during IHE and PTL.



Abbreviations: IHE = intermittent handgrip exercise; PTL = pressure-threshold loading; M = males; F = females; R = relative workload; A = Absolute workload; ΔMAP = change in mean arterial pressure from baseline. * = main effect of sex; †= main effect of muscle.

Figure 4.4 Example of study protocol. Raw traces of cardiorespiratory responses to PTL performed at a relative workload, from one representative female and male participant.



Abbreviations: PTL = pressure-threshold loading; HR = heart rate; MAP = mean arterial pressure; AP = arterial pressure; P_{di} = diaphragmatic pressure; P_{mo} = mouth pressure; $MIP_{di,MAX}$ = maximal inspiratory pressure of the diaphragm. Shaded bar = target P_{di}.

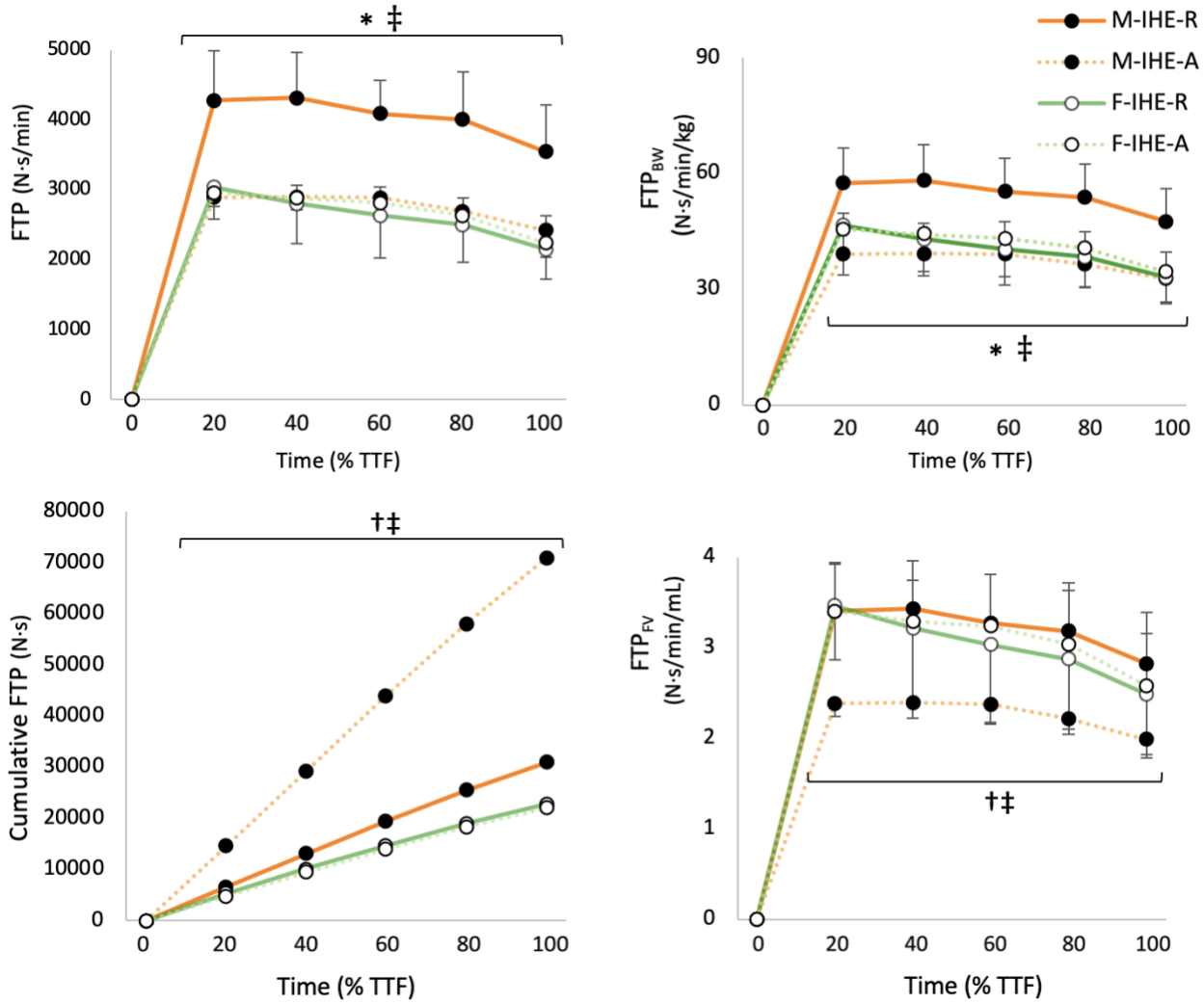
Table 4.8. Force-time products during IHE, presented in time as a percent to task failure.

Variable	Sex	20% TTF	40% TTF	60% TTF	80% TTF	100% TTF	p<0.05
Relative							
T_C/T_{TOT}	M	0.69 ± 0.02	0.70 ± 0.02	0.69 ± 0.03	0.70 ± 0.02	0.68 ± 0.03	†
	F	0.72 ± 0.04	0.71 ± 0.04	0.71 ± 0.04	0.71 ± 0.06	0.70 ± 0.05	
HG/MVC_{PEAK} (%)	M	58 ± 4	57 ± 4	56 ± 4	53 ± 5	49 ± 5	† ‡
	F	60 ± 3	58 ± 2	57 ± 4	53 ± 5	48 ± 5	
TTI	M	0.40 ± 0.03	0.40 ± 0.03	0.39 ± 0.04	0.37 ± 0.04	0.33 ± 0.04	† ‡
	F	0.44 ± 0.02	0.41 ± 0.03	0.40 ± 0.04	0.38 ± 0.05	0.34 ± 0.04	
FTP (N x s/min)	M	4284 ± 707	4312 ± 657	4092 ± 467	4006 ± 682	3546 ± 655	∗ † ‡
	F	3035 ± 450	2803 ± 569	2631 ± 608	2500 ± 534	2155 ± 431	
FTP_{BW} (N x s/min/kg)	M	58 ± 9	58 ± 9	55 ± 9	54 ± 9	48 ± 8	∗ † ‡
	F	47 ± 7	43 ± 8	40 ± 9	38 ± 8	33 ± 7	
FTP_{FV} (N x s/min/mL)	M	3.4 ± 0.5	3.4 ± 0.5	3.3 ± 0.5	3.2 ± 0.5	2.8 ± 0.6	† ‡
	F	3.5 ± 0.6	3.2 ± 0.8	3.0 ± 0.9	2.9 ± 0.8	2.5 ± 0.7	
Absolute							
Tcont/Ttot	M	0.65 ± 0.15	0.65 ± 0.15	0.66 ± 0.15	0.65 ± 0.14	0.63 ± 0.15	†
	F	0.73 ± 0.02	0.72 ± 0.03	0.73 ± 0.02	0.73 ± 0.02	0.72 ± 0.02	
HG/MVC_{PEAK} (%)	M	37 ± 5	37 ± 5	36 ± 5	35 ± 5	32 ± 4	∗ † ‡
	F	57 ± 8	57 ± 8	55 ± 6	53 ± 6	49 ± 7	
TTI	M	0.26 ± 0.03	0.26 ± 0.03	0.26 ± 0.04	0.24 ± 0.04	0.22 ± 0.03	∗ † ‡
	F	0.42 ± 0.05	0.41 ± 0.05	0.40 ± 0.04	0.39 ± 0.05	0.35 ± 0.06	
FTP (N x s/min)	M	2891 ± 87	2899 ± 166	2884 ± 154	1691 ± 195	2414 ± 213	† ‡
	F	2967 ± 200	2879 ± 167	2818 ± 223	2633 ± 181	2237 ± 205	
FTP_{BW} (N x s/min/kg)	M	39 ± 5	39 ± 6	39 ± 6	37 ± 6	33 ± 6	† ‡
	F	46 ± 4	44 ± 3	43 ± 4	41 ± 4	35 ± 5	
FTP_{FV} (N x s/min/mL)	M	2.4 ± 0.1	2.4 ± 0.2	2.4 ± 0.2	2.2 ± 0.2	2.0 ± 0.2	∗ † ‡
	F	3.4 ± 0.5	3.3 ± 0.5	3.2 ± 0.6	3.0 ± 0.6	2.6 ± 0.6	

Abbreviations: IHE = intermittent handgrip exercise; TTF = time to task failure; M = males; F = females; T_C/T_{TOT} = time spent performing forearm contraction to total duty cycle; FTP = force time product; FTP_{BW} = force time product normalized to body weight; FTP_{FV} = force time

product normalized to forearm volume. Values presented as Mean ± SD. * = within-condition main effect of sex; † = within-condition main effect of time; ‡ = main effect of workload.

Figure 4.5. Force-time products during IHE, presented in time as a percent to task failure.



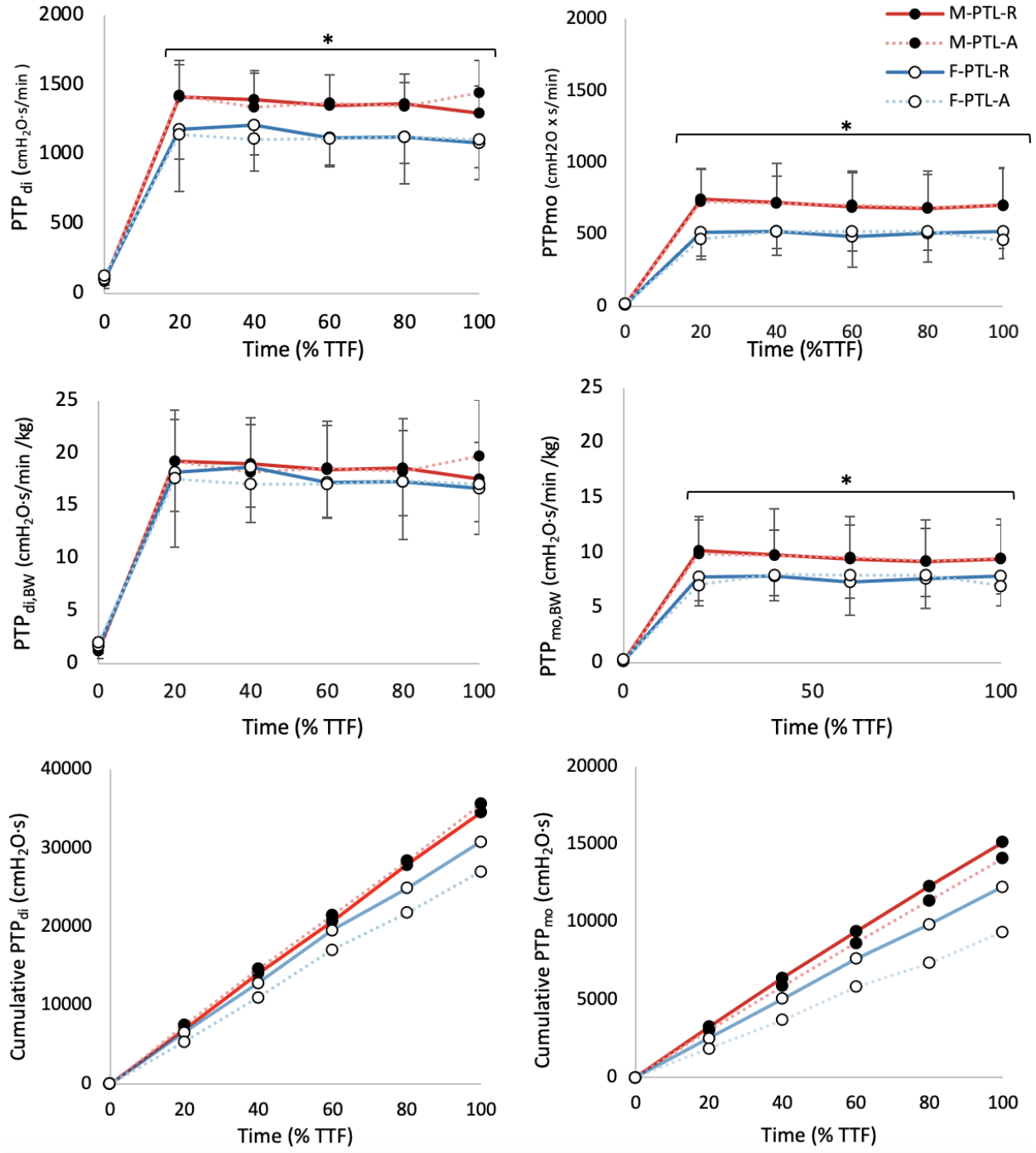
Abbreviations: TTF = time to task failure; FTP = force-time product; M = males; F = females; LMA-R = limb metaboreflex activation at a relative workload; LMA-A = limb metaboreflex activation at an absolute workload. * = significant difference between males and females during the relative workload; † = significant difference between males and females during the absolute workload; ‡ = significant difference between workloads in males.

Table 4.9. Pressure-time products during PTL, presented in time as a percent to task failure.

Variable	Sex	Baseline	20% TTF	40% TTF	60% TTF	80% TTF	100% TTF	p<0.05
Relative								
T_I/T_{TOT}	M	0.36 ± 0.06	0.73 ± 0.02	0.72 ± 0.02	0.73 ± 0.03	0.74 ± 0.03	0.74 ± 0.05	†
	F	0.40 ± 0.11	0.73 ± 0.05	0.72 ± 0.08	0.71 ± 0.08	0.70 ± 0.04	0.71 ± 0.05	
$P_{di}/P_{di,MAX}$ (%)	M	9 ± 1	60 ± 2	60 ± 2	59 ± 4	59 ± 3	58 ± 8	∗ †
	F	13 ± 3	56 ± 4	56 ± 4	54 ± 3	54 ± 3	50 ± 3	
TTI_{di}	M	0.03 ± 0.01	0.44 ± 0.02	0.43 ± 0.02	0.43 ± 0.03	0.44 ± 0.02	0.43 ± 0.04	*
	F	0.05 ± 0.02	0.41 ± 0.05	0.40 ± 0.06	0.39 ± 0.06	0.38 ± 0.04	0.35 ± 0.04	
PTP_{di}	M	86 ± 30	1414 ± 229	1393 ± 191	1352 ± 219	1361 ± 219	1295 ± 193	∗ †
(cm·s/min)	F	106 ± 43	1180 ± 214	1211 ± 215	1119 ± 196	1123 ± 190	1081 ± 264	
$PTP_{di,BW}$	M	1.2 ± 0.5	19.3 ± 4.8	19.0 ± 4.4	18.5 ± 4.6	18.6 ± 4.7	17.5 ± 3.5	*
(cm·s/min/kg)	F	1.6 ± 0.7	18.2 ± 3.8	18.7 ± 3.8	17.2 ± 3.3	17.3 ± 3.2	16.7 ± 4.4	
PTP_{mo}	M	10 ± 4	747 ± 211	720 ± 184	690 ± 240	679 ± 240	702 ± 266	∗ †
(cm·s/min)	F	11 ± 4	516 ± 170	518 ± 166	486 ± 212	507 ± 197	518 ± 187	
$PTP_{mo,BW}$	M	0.1 ± 0.1	10.2 ± 2.8	9.8 ± 2.2	9.4 ± 3.1	9.2 ± 3.0	9.5 ± 3.1	∗ †
(cm·s/min/kg)	F	0.2 ± 0.1	7.8 ± 2.2	7.9 ± 2.2	7.4 ± 3.0	7.7 ± 2.7	7.9 ± 2.7	
Absolute								
T_I/T_{TOT}	M	0.39 ± 0.05	0.71 ± 0.03	0.71 ± 0.03	0.71 ± 0.04	0.72 ± 0.03	0.70 ± 0.04	†
	F	0.40 ± 0.08	0.72 ± 0.04	0.69 ± 0.05	0.70 ± 0.06	0.69 ± 0.06	0.68 ± 0.07	
$P_{di}/P_{di,MAX}$ (%)	M	10 ± 2	55 ± 10	56 ± 9	55 ± 8	55 ± 8	55 ± 9	†
	F	14 ± 4	61 ± 7	61 ± 8	61 ± 8	61 ± 7	58 ± 7	
TTI_{di}	M	0.04 ± 0.01	0.39 ± 0.07	0.40 ± 0.07	0.39 ± 0.06	0.40 ± 0.07	0.39 ± 0.08	*
	F	0.06 ± 0.03	0.44 ± 0.06	0.42 ± 0.06	0.42 ± 0.07	0.42 ± 0.07	0.39 ± 0.06	
PTP_{di}	M	90 ± 30	1426 ± 248	1338 ± 261	1370 ± 201	1346 ± 169	1441 ± 231	∗ †
(cm·s/min)	F	120 ± 95	1143 ± 408	1109 ± 228	1111 ± 201	1126 ± 339	1108 ± 205	
$PTP_{di,BW}$	M	1.2 ± 0.5	19.2 ± 4.0	18.2 ± 4.5	18.6 ± 4.0	18.3 ± 3.9	19.8 ± 5.3	*
(cm·s/min/kg)	F	2.0 ± 1.5	17.6 ± 6.5	17.0 ± 3.6	17.1 ± 3.3	17.3 ± 5.6	17.1 ± 3.6	
PTP_{mo}	M	14 ± 4	731 ± 221	715 ± 279	705 ± 239	687 ± 255	705 ± 254	∗ †
(cm·s/min)	F	17 ± 9	465 ± 139	522 ± 121	520 ± 135	520 ± 131	485 ± 59	
$PTP_{mo,BW}$	M	0.2 ± 0.1	9.9 ± 3.4	9.8 ± 3.4	9.6 ± 3.7	9.3 ± 3.7	9.5 ± 3.5	∗ †
(cm·s/min/kg)	F	0.3 ± 0.2	7.1 ± 1.9	8.0 ± 1.9	8.0 ± 2.1	8.0 ± 1.9	7.0 ± 0.8	

Abbreviations: TTF = time to task failure; M = males; F = females; T_I/T_{TOT} = time spent performing inspiration to time of total respiratory cycle; $P_{di}/P_{di,MAX}$ = average diaphragm pressure during inspiration as a percent of maximum; TTI_{di} = tension time index of the diaphragm; PTP_{di} = pressure-time product of the diaphragm; $PTP_{di,BW}$ = PTP_{di} normalized to body weight; PTP_{mo} = pressure-time product of the mouth; $PTP_{mo,BW}$ = PTP_{mo} normalized to body weight. Values presented as Mean ± SD. * = within-condition main effect of sex; † = within-condition main effect of time.

Figure 4.6. Pressure-time products during PTL, presented in time as a percent to task failure.



Abbreviations: PTL = pressure-threshold loading; TTF = time to task failure; PTP_{di} = pressure-time product of the diaphragm; PTP_{mo} = pressure-time product of the mouth; M = males; F = females; R = relative workload; A = absolute workload. * = significant difference between males and females, regardless of workload.

Table 4.10. Cardiopulmonary measures during PECO and control conditions, presented in time as a percent to task failure (n=5).

Variable	Condition	Baseline	20% TTF	40% TTF	60% TTF	80% TTF	100% TTF	p<0.05
Relative								
MAP (mmHg)	C	93 ± 5	107 ± 3	119 ± 8	126 ± 8	128 ± 11	126 ± 9	†
	P	93 ± 6	101 ± 9	110 ± 10	114 ± 10	122 ± 7	129 ± 12	
ΔMAP (mmHg)	C		13 ± 7	26 ± 12	33 ± 10	34 ± 12	32 ± 13	†
	P		8 ± 9	17 ± 10	21 ± 8	29 ± 4	36 ± 9	
HR (bpm)	C	60 ± 9	80 ± 15	85 ± 14	89 ± 14	97 ± 7	102 ± 10	†
	P	65 ± 14	89 ± 20	92 ± 19	97 ± 15	105 ± 16	104 ± 24	
$\dot{V}_E$ (L/min)	C	9.2 ± 1.5	15.3 ± 3.2	19.4 ± 5.3	15.9 ± 4.6	20.1 ± 13.2	25.8 ± 19.2	†
	P	10.0 ± 1.0	18.1 ± 6.6	17.0 ± 5.9	20.8 ± 8.4	21.1 ± 7.1	26.9 ± 17.3	
$P_{ET}CO_2$ (mmHg)	C	39.4 ± 0.8	38.6 ± 1.8	37.2 ± 4.1	36.7 ± 4.9	36.0 ± 8.6	35.2 ± 10.5	*†
	P	37.6 ± 3.4	36.9 ± 4.7	36.2 ± 4.6	35.9 ± 5.3	34.5 ± 7.1	33.5 ± 8.6	
Absolute								
MAP (mmHg)	C	94 ± 3	111 ± 5	120 ± 7	125 ± 11	129 ± 8	130 ± 8	†
	P	93 ± 5	110 ± 8	120 ± 14	120 ± 7	123 ± 8	124 ± 6	
ΔMAP (mmHg)	C		17 ± 6	26 ± 7	31 ± 11	35 ± 6	36 ± 5	†
	P		17 ± 3	27 ± 10	27 ± 3	30 ± 4	31 ± 4	
HR (bpm)	C	60 ± 11	74 ± 15	82 ± 12	92 ± 15	93 ± 18	101 ± 14	†
	P	64 ± 11	77 ± 18	80 ± 14	85 ± 14	90 ± 13	98 ± 21	
$\dot{V}_E$ (L/min)	C	9.6 ± 1.5	12.3 ± 2.4	14.0 ± 2.9	14.2 ± 2.5	21.9 ± 8.5	21.5 ± 10.7	†
	P	9.0 ± 3.2	17.9 ± 8.8	17.7 ± 8.1	21.5 ± 6.1	23.6 ± 8.3	30.6 ± 9.0	
$P_{ET}CO_2$ (mmHg)	C	39.5 ± 1.2	38.5 ± 2.2	38.7 ± 3.4	38.6 ± 4.6	34.5 ± 5.6	32.9 ± 8.8	*†
	P	38.5 ± 3.3	35.4 ± 5.7	33.5 ± 7.6	31.1 ± 7.4	29.2 ± 7.4	26.1 ± 9.8	

Abbreviations: TTF = time to task failure; C = control condition; P = PECO condition; MAP = mean arterial pressure; ΔMAP = change in MAP from baseline; HR = heart rate; bpm = beats per minute; $\dot{V}_E$ = minute ventilation; $P_{ET}CO_2$ = end-tidal CO_2. Values presented as Mean ± SD. * = within-condition main effect of condition; † = within-condition main effect of time.

Table 4.11 Cardiopulmonary measures during PECO and control conditions, presented in absolute time (n=5).

Variable	Condition	Baseline	Min 1	Min 2	Final	Post 1	Post 2	p<0.05
Relative								
MAP (mmHg)	C	93 ± 5	112 ± 7	124 ± 11	126 ± 9	107 ± 11*	102 ± 6*	† ‡
	P	93 ± 6	105 ± 11	121 ± 12	129 ± 12	118 ± 5*	122 ± 5*	
ΔMAP (mmHg)	C		18 ± 8	31 ± 12	32 ± 13	14 ± 13	9 ± 5*	† ‡
	P		12 ± 10	27 ± 8	36 ± 9	24 ± 5	28 ± 6*	
HR (bpm)	C	60 ± 9	82 ± 16	87 ± 18	102 ± 10	63 ± 7	58 ± 3	† ‡
	P	65 ± 14	89 ± 20	102 ± 25	104 ± 24	66 ± 12	68 ± 16	
$\dot{V}_E$ (L/min)	C	9.2 ± 1.5	14.4 ± 3.1	16.5 ± 4.2	25.8 ± 19.2	18.1 ± 3.9	11.3 ± 3.9	‡
	P	10.0 ± 1.0	16.9 ± 6.5	19.6 ± 8.8	26.9 ± 17.3	21.1 ± 8.5	20.1 ± 15.3	
$P_{ET}CO_2$ (mmHg)	C	39.4 ± 0.8	39.6 ± 1.5	37.0 ± 4.4	35.2 ± 10.5	36.5 ± 8.2	36.3 ± 6.8	
	P	37.6 ± 3.4	36.3 ± 5.1	35.7 ± 6.7	33.5 ± 8.6	34.2 ± 9.3	32.3 ± 9.9	
Absolute								
MAP (mmHg)	C	94 ± 3	103 ± 5	106 ± 8	130 ± 8	102 ± 7*	96 ± 7*	† ‡
	P	93 ± 5	103 ± 5	111 ± 7	124 ± 6	116 ± 3*	118 ± 3*	
ΔMAP (mmHg)	C		9 ± 7	12 ± 10	36 ± 5	8 ± 5	2 ± 4*	† ‡
	P		10 ± 5	18 ± 4	31 ± 4	23 ± 6	25 ± 5*	
HR (bpm)	C	60 ± 11	72 ± 17	74 ± 14	101 ± 14	63 ± 10	62 ± 9	† ‡
	P	64 ± 11	74 ± 17	77 ± 19	98 ± 21	63 ± 10	63 ± 14	
$\dot{V}_E$ (L/min)	C	9.6 ± 1.5	13.0 ± 2.0	12.5 ± 2.0	21.5 ± 10.7	10.4 ± 3.8	8.9 ± 1.6	‡
	P	9.0 ± 3.2	16.1 ± 5.5	17.5 ± 9.6	30.6 ± 9.0	16.7 ± 11.6	17.0 ± 13.7	
$P_{ET}CO_2$ (mmHg)	C	39.5 ± 1.2	38.6 ± 1.1	38.6 ± 2.4	32.9 ± 8.8	37.1 ± 4.4	37.4 ± 3.9	
	P	38.5 ± 3.3	36.5 ± 4.4	34.7 ± 6.4	26.1 ± 9.8	28.6 ± 10.6	29.0 ± 9.8	

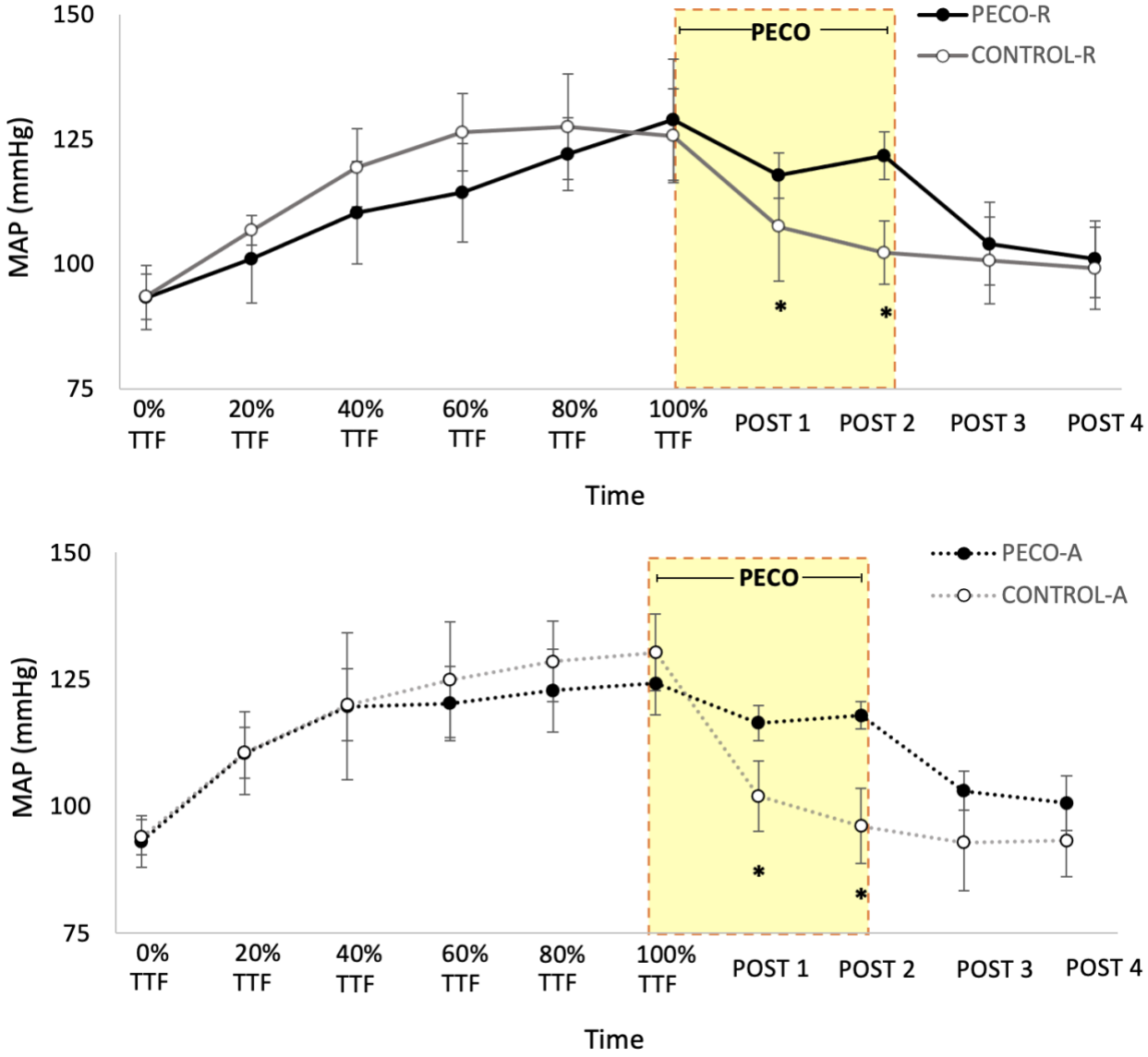
Abbreviations: C = control condition; P = PECO condition; MAP = mean arterial pressure; ΔMAP = change in MAP from baseline; HR = heart rate; bpm = beats per minute; $\dot{V}_E$ = minute ventilation; $P_{ET}CO_2$ = end-tidal CO_2; Post = Post-exercise time in minutes. Values presented as Mean ± SD. * = significant effect of condition; † = main effect of time.‡ = significant effect of workload.

Table 4.12. Force-time products during PECO and control, presented in time as a percent to task failure (n=5).

Variable	Condition	20% TTF	40% TTF	60% TTF	80% TTF	100% TTF	p<0.05
Relative							
Tcont/Ttot	C	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	*
	P	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	
HG/MVC_{PEAK} (%)	C	59 ± 3	58 ± 3	58 ± 3	55 ± 4	51 ± 6	† ‡
	P	58 ± 5	58 ± 4	55 ± 6	53 ± 7	47 ± 5	
TTI	C	0.41 ± 0.02	0.41 ± 0.03	0.41 ± 0.03	0.39 ± 0.03	0.35 ± 0.05	† ‡
	P	0.43 ± 0.04	0.42 ± 0.03	0.41 ± 0.03	0.39 ± 0.04	0.34 ± 0.04	
FTP (N x s/min)	C	4157 ± 338	4151 ± 462	4122 ± 475	3912 ± 504	3521 ± 589	† ‡
	P	4317 ± 602	4238 ± 737	4048 ± 599	3898 ± 525	3356 ± 599	
FTP_{BW} (N x s/min/kg)	C	56 ± 10	56 ± 10	55 ± 10	52 ± 8	47 ± 10	† ‡
	P	59 ± 10	57 ± 12	54 ± 9	52 ± 8	45 ± 10	
FTP_{FV} (N x s/min/mL)	C	3.3 ± 0.6	3.3 ± 0.7	3.3 ± 0.6	3.1 ± 0.5	2.8 ± 0.7	† ‡
	P	3.4 ± 0.6	3.4 ± 0.7	3.2 ± 0.5	3.1 ± 0.5	2.7 ± 0.6	
Absolute							
Tcont/Ttot	C	0.7 ± 0.02	0.7 ± 0.03	0.7 ± 0.02	0.7 ± 0.01	0.7 ± 0.04	*
	P	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.1	
HG/MVC_{PEAK} (%)	C	38 ± 3	38 ± 3	37 ± 3	36 ± 3	33 ± 3	† ‡
	P	38 ± 5	38 ± 5	37 ± 6	37 ± 6	33 ± 7	
TTI	C	0.26 ± 0.02	0.26 ± 0.02	0.26 ± 0.02	0.25 ± 0.02	0.23 ± 0.02	† ‡
	P	0.27 ± 0.03	0.27 ± 0.03	0.26 ± 0.04	0.27 ± 0.04	025 ± 0.05	
FTP (N x s/min)	C	2858 ± 68	2839 ± 89	2832 ± 78	2751 ± 122	2472 ± 160	† ‡
	P	2971 ±199	2962 ± 86	2791 ± 147	2819 ± 142	2591 ± 338	
FTP_{BW} (N x s/min/kg)	C	38 ± 5	38 ± 5	38 ± 5	37 ± 6	33 ± 7	† ‡
	P	40 ± 5	40 ± 6	38 ± 6	38 ± 5	35 ± 8	
FTP_{FV} (N x s/min/mL)	C	2.3 ± 0.2	2.3 ± 0.1	2.3 ± 0.2	2.2 ± 0.2	2.0 ± 0.2	† ‡
	P	2.3 ± 0.2	2.3 ± 0.3	2.2 ± 0.4	2.2 ± 0.2	2.1 ± 0.4	

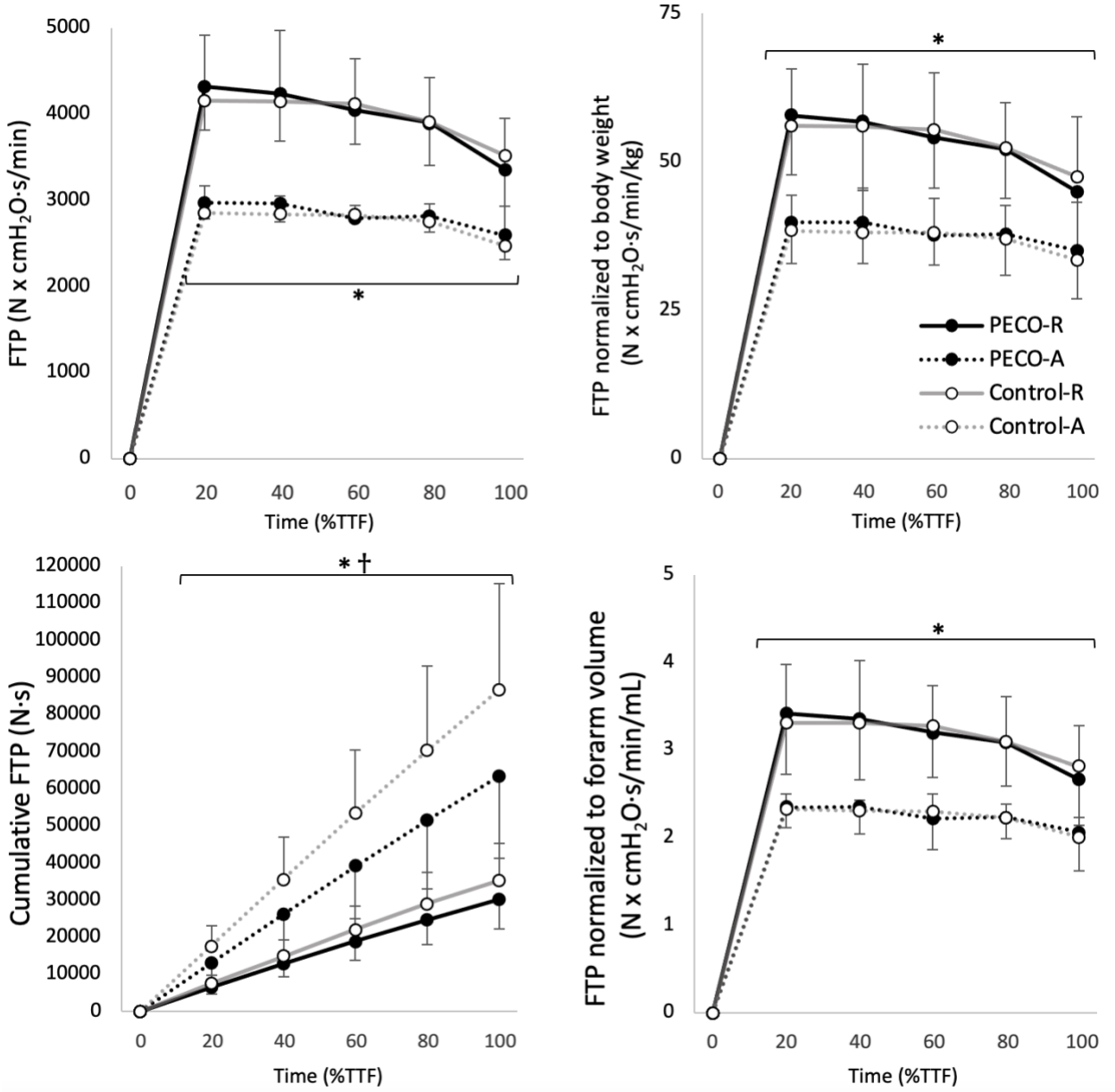
Abbreviations: TTF = time to task failure; M = males; F = females; FTP = force time product; FTP_{BW} = force time product normalized to body weight; FTP_{FV} = force time product normalized to forearm volume. Values presented as Mean ± SD. * =significant effect of condition; †=main effect of time. ‡ = significant effect of workload.

Figure 4.7 Mean arterial pressure during and post- IHE, with and without PECO (n=5).



Abbreviations: PECO = post-exercise circulatory occlusion; IHE = intermittent handgrip exercise; R = relative workload; A = Absolute workload; MAP = mean arterial pressure; TTF = time to task failure; Post = minutes post-exercise. * = significant difference between PECO and control.

Figure 4.8 Force-time products during control and PECO IHE, presented in time as a percent to task failure (n=5; male subset).



Abbreviations: TTF = time to task failure; FTP = force-time product; M = males; F = females; PECO = post-exercise circulatory occlusion; R = relative workload; A = absolute workload. * = significant difference between workloads; † = significant difference between PECO and control.

Chapter 5: Discussion

5.1 Major Findings

The purpose of this thesis was to compare sex-based differences in the blood pressure response to IHE and PTL performed at relative and absolute workloads. This thesis was designed to investigate whether the magnitude of the sex-based difference in the MAP response to metaboreflex activation is consistent between the forearm and respiratory muscles at both exercise intensities. It was hypothesized that the increase in MAP would be similar between IHE and PTL, but that both responses would be attenuated in females compared to males. It was also hypothesized that sex-based differences in MAP would be augmented when matched for absolute work, regardless of muscle group. The main findings of the study are threefold. First, the time-dependent rise in MAP was significantly greater in males than females, regardless of muscle group or workload. Second, it was found that the sex-based differences in the MAP response was not significantly different between relative or absolute workloads. This suggests that exercise intensity or absolute work performed by the muscle does not contribute significantly to the attenuated metaboreflex response observed in females. Lastly, the pressor response to IHE was maintained during PECO, indicating that rhythmic handgrip exercise performed at a high relative workload and a prolonged duty cycle elicits a predominantly metaboreflex-mediated pressor response. Collectively, the findings of the present study suggest that the sex-based difference in the pressor response to metaboreflex activation is consistent across muscle groups, regardless of exercise intensity.

5.1.1 Justification for IHE Protocol

Sustained isometric handgrip exercise and PECO is widely used as a model to investigate the limb metaboreflex (Alam & Smirk, 1937; Gladwell & Coote, 2002; Teixeira *et al.,* 2019). In the present study, rhythmic handgrip exercise was utilized to allow for more suitable comparisons between muscle groups. The protocol selected enabled participants to perform limb and respiratory muscle work with the same contraction pattern, duty cycle, frequency, and intensity; thus, making comparisons between the two muscle groups more equitable. To ascertain whether the selected IHE protocol adequately activated the limb metaboreflex, a subset of males (n=5) completed an additional experimental visit wherein they performed the relative and absolute IHE bouts followed by 2 minutes of PECO. PECO is commonly used to create an environment in which the metaboreflex remains activated in the absence of input from central command and the mechanoreflex (Alam & Smirk, 1937; Gladwell & Coote, 2002). In the present study, MAP at the end of exercise was similar between PECO and control conditions; however, MAP was significantly greater during the first two minutes post-exercise (Post 1 and Post 2) during PECO as compared to control. MAP during Post 1 and Post 2 was also statistically similar from the final time point of exercise (100%TTF) during the PECO condition but not the control condition; thereby indicating a maintained pressor response despite the withdrawal of feedforward cardiovascular control mechanisms and the cessation of mechanoreceptor stimulation. This suggests that the IHE protocol of the present study sufficiently activated the limb muscle metaboreflex.

Rhythmic handgrip exercise is not often used as a model to investigate metaboreflex responses due to the potential confounding input from central command and mechanoreceptors. To date, studies that have investigated the cardiovascular responses to rhythmic handgrip

exercise have utilized a combination of brief contraction durations (Victor *et al.,* 1987; Victor *et al.,* 1988), moderate duty cycles (T_C/T_{TOT}, of 0.5) (Taylor *et al.,* 1988; McNulty *et al.,* 2014; Doherty *et al.,* 2019), and a range of exercise intensities (5-60% MVC_{PEAK}). Whether the cardiovascular responses to such protocols are largely due to central command, the mechanoreflex, the metaboreflex, or a combination of the three is not entirely clear. For example, Taylor *et al.* (1988) suggest a larger role of central command, due to the observed correlation between increases in arterial pressure and heart rate, with changes in electromyogram activity of the forearm. Conversely, Doherty *et al.* (2019) observed significant increases in MSNA burst frequency in response to rhythmic handgrip exercise, suggesting a potential role of the muscle metaboreflex, however MSNA burst amplitude and total MSNA remained unchanged. Meanwhile, Victor *et al,* (1987) propose that central command plays a primary role in initiating the increase in HR and withdrawal of parasympathetic activity, whereas the metaboreflex is largely responsible for the sympathetic response to rhythmic handgrip exercise. In a subsequent study, Victor *et al.* (1988) reported an apparent coupling of muscle cell pH to sympathetic outflow, providing further evidence for a role of the metaboreflex in contributing to the sympathetic response to rhythmic handgrip exercise.

The LMA protocol of the present study was designed: 1) due to the mechanical effect of sustained muscle contraction in compromising muscle perfusion; 2) in acknowledging the successful activation of the inspiratory metaboreflex via select PTL protocols; and 3) in effort to standardize the task performed by both the limb and respiratory muscles. As mentioned in Section 1.1.2.3, research in animal models indicates that a prolonged respiratory duty cycle paired with the generation of high inspiratory pressures results in compromised diaphragmatic blood flow (Bellemare *et al.,* 1983; Buchler *et al.,* 1985). Similarly, reducing muscle relaxation

time during handgrip exercise with mild forearm occlusion (to mimic a duty cycle of 0.66) resulted in impaired forearm blood flow at exercise intensities >30% MVC_{PEAK} (Bentley *et al.* 2017). Likewise, McNulty *et al.* (2014) used an occlusion cuff inflated during rhythmic handgrip exercise, performed at a duty cycle of 0.5 and 25% MVC_{PEAK}, to "enhance" metaboreflex activation during exercise. Rhythmic handgrip exercise performed with the occlusion cuff significantly increased MAP, total peripheral resistance, and HR; however, the free-flowing control condition found no significant differences in the same variables from baseline (McNulty *et al.*, 2014). These findings suggest that the impaired muscle perfusion that occurs during prolonged duty cycles and ischemic handgrip exercise results in greater activation of the limb metaboreflex. Therefore, the rationale for selecting the LMA protocol can be summarized in two parts: 1) to align with current research suggesting that rhythmic forearm exercise performed at prolonged duty cycles and high relative workloads would impair forearm blood flow in an intermittent manner; and 2) to provide an analogous muscle contraction model for the selected PTL protocol, which has been validated as a method to activate the inspiratory metaboreflex (Sheel *et al.*, 2001; Sheel *et al.*, 2002). The results from the present study showed a maintained MAP response during PECO, suggesting that the pressor response to rhythmic handgrip exercise performed at a high relative exercise intensity (60% MVC_{PEAK}) and a prolonged duty cycle of 0.7, is largely mediated by activation of the limb muscle metaboreflex.

5.1.2 Effect of Muscle Group and Workload

Cardiovascular responses to metaboreflex activation were similar between the limb and respiratory musculature, regardless of sex or workload, as shown in Figure 4.1. This suggests that when a relative or absolute amount of muscle work is performed, the corresponding

metaboreflex-induced increases in arterial pressure, HR, and $\dot{V}_E$ is similar regardless of muscle group. Comparisons of cardiovascular responses to exercise across muscle groups is not novel to this study. Several groups have investigated cardiovascular responses to isometric exercise across muscle groups (e.g., finger and forearm flexors, knee and hip extensors, back extensors) (Mitchell *et al.,* 1980; Seals *et al.,* 1983; Nagle *et al.,* 1988; McCloskey & Streatfeild, 1975; Hunter, 2014); however, research on rhythmic exercise and comparisons inclusive of the respiratory musculature is lacking.

In isometric models, there is evidence to suggest that regardless of muscle group, the cardiovascular response to exercise at a given $\%MVC_{PEAK}$ is proportional to muscle mass (Mitchell *et al.,* 1980; Seals *et al.,* 1983; McCloskey & Streatfeild, 1975). The same findings have been observed in a dynamic exercise model (Gonzales et al., 2007). Given that muscle groups with greater mass tend to have a greater absolute force output for a given $\%MVC_{PEAK}$, some suggest that cardiovascular responses may correlate to the absolute force produced during the exercise (Nagle *et al.,* 1988; Gonzales *et al.,* 2007; Notay *et al.,* 2018). However, in the present study no significant effect of workload was observed for either MAP, HR, or $\dot{V}_E$, despite a large difference between the absolute and relative IHE workloads in males (Δ23% MVC_{PEAK}). It is relevant to note that target pressures during the relative and absolute PTL bouts were similar for both males and females, and thus any potential influence of absolute pressure generation on cardiovascular responses between relative and absolute PTL bouts is negated. It is also unclear whether muscle mass between the forearm flexors and diaphragm substantially differ. Previous work suggests that muscle mass between the two groups may be relatively comparable; post-mortem diaphragm mass in non-obese adults averages 262g (Arora & Rochester, 1982), while the mass of flexor digitorum superficialis, flexor digitorum profundus, and flexor pollicis longus

sum to 224g (Kerkof *et al.*,2018). Correspondingly, the present study found no significant differences in MAP, HR, or $\dot{V}_E$ responses between the limb and respiratory musculature. Therefore, results from the present study suggest that when the forearm flexors and diaphragm produce similar relative and absolute amounts of muscle work, performed using a similar contraction pattern and duty cycle, the resulting pressor responses are comparable. The non-significant difference observed between the absolute and relative IHE condition in males suggests that the role of absolute force output on the pressor response to rhythmic isometric exercise may be different to that observed during static isometric (Mitchell *et al.,* 1980; Seals *et al.,* 1983; McCloskey & Streatfeild, 1975) or dynamic exercise (Gonzales *et al.,* 2007).

5.1.3 Effect of Sex

In the present study, males demonstrated a greater MAP response to both limb and inspiratory metaboreflex activation than females, regardless of workload. The sex-based difference in the MAP response to metaboreflex activation has previously been shown in both isometric handgrip models (Ettinger *et al.,* 1996; Jarvis *et al.,* 2011; Joshi *et al.,* 2019), and resistive breathing tasks (Smith *et al.,* 2016; Welch *et al.,* 2018; Geary *et al.,* 2019). The present study is the first to demonstrate a maintained sex-based difference in a rhythmic handgrip model of metaboreflex activation. The sex-based difference observed in the present study is specific to arterial pressure and is not also observed in the HR response to exercise, which is supported by previous findings in isometric handgrip models (Ettinger *et al.,* 1996; Jarvis *et al.,* 2011; Joshi *et al.,* 2019), as well as PTL and resistive breathing tasks (Smith *et al.,* 2016; Geary *et al.,* 2019). Additionally, the absolute MAP and HR values of the present study are comparable to that shown by previous work from our lab and colleagues (St Croix *et al.,* 2000; Geary *et al.,* 2019).

Mechanisms for the sex-based difference in responses to metaboreflex activation may be present anywhere along the metaboreflex arc, such as differences in: 1) local metabolite concentration (i.e., metabolite production, muscle mass, and metabolite clearance); 2) afferent nerve input from metaboreceptors; 3) efferent sympathetic outflow; or 4) vascular responsiveness to vasoconstrictor or vasodilatory stimuli. Each possibility is discussed below.

5.1.3.1 Effect of Sex – Local Metabolite Production

It is possible that the sex-based differences observed in the present study may result from differences in muscle fiber type proportions, metabolite production and accumulation, and muscle mass. Compared to males, females generally have a significantly higher proportion of type I fibers in several (Simoneau *et al.,* 1985; Simoneau & Bouchard, 1989; Haizlip *et al.,* 2015; Fornier *et al.,* 2022), but not all muscles (Miller *et al.,* 1993; Porter *et al.,* 2002). Despite males generally having a larger muscle cross-sectional area, the proportional percentage of type I fiber cross-sectional area is greater in females (Simoneau & Bouchard 1989; Carter *et al.,* 2001; Porter *et al.,* 2002; Fournier *et al.,* 2022). Differences in fiber type distribution between males and females may result in inequal metabolite production and clearance during heavy exercise. During exercise, type II muscle fibers are associated with a greater production of metabolites (i.e., lactate, inosine monophosphate, H^+, inorganic phosphate, etc.) while type I fibers are associated with greater metabolite clearance (Juel *et al.,* 1991; Greenhaff *et al.,* 1994; Esbjornsson-Lijedahl *et al.,* 1999; Esbjornsson-Lijedahl *et al.,* 2002). This is due to the greater reliance on glycolytic metabolism by type II fibers, compared to greater reliance on oxidative phosphorylation by type I fibers (Essen *et al.,* 1975; Greenhaff *et al.,* 1994). Correspondingly, some metabolites (circulating blood lactate, intramuscular lactate, IMP, inosine, and H^+), have been shown to be

lower in females than males following repeated sprints, or heavy-intensity exercise (Esbjornsson-Lijedahl *et al.,* 1999; Esbjornsson-Lijedahl *et al.,* 2002; Russ *et al.,* 2005). Metabolite production and reduced muscle cell pH are coupled to muscle sympathetic outflow (Victor *et al.,* 1988), therefore sex-based differences in fiber type distribution and metabolite accumulation likely have downstream effects on sympathetic outflow, vasoconstriction, and pressor responses to exercise. This concept is further supported by the larger pressor response to isometric handgrip exercise observed in individuals with a larger proportion of type II fibers (Sadamoto *et al.,* 1992).

The present study utilized forearm and respiratory exercise models to investigate metaboreflex responses, however indices of metabolic changes were not recorded. While sex-based differences in fiber type distribution in human forearm flexors is unknown, some evidence suggests that women exhibit a lower concentration of dihydrogen phosphate ($H_2PO_4^-$) compared to men when compared at iso-time points during isometric handgrip exercise, and at task failure following ischemic rhythmic handgrip exercise (Ettinger *et al.,* 1996). No such metabolic outcomes have been investigated during PTL or resistive breathing tasks, and it is also unknown whether sex-based differences in diaphragm fiber type proportions are present in humans. The human male diaphragm consists of ~50-55% type I fibers, ~45-50% type II fibers (Lieberman *et al.,* 1973; Mizuno, 1991). While no data on human female diaphragm fiber type proportions is currently available, the presence of sex-based differences in fiber type distribution in other skeletal muscles may suggest differences between the male and female diaphragm as well. Additionally, the presence of such sex-based differences may correspond to the greater fatigue resistance in the female diaphragm during resistive breathing tasks compared to males (Gonzales & Sheuermann, 2006; Guenette *et al.,* 2010; Welch *et al.,* 2018). Future research is required to determine whether fiber type-mediated differences in metabolite production between males and

females have separate, additive, or antagonistic effects to that of muscle mass and absolute strength with respect to the metaboreflex.

For most muscle groups, males generally have a larger muscle mass and greater absolute muscle strength than females, even when normalized to body weight and regardless of training status (Maughan *et al.,* 1983; Landen *et al.,* 2021). Muscle mass has been suggested to influence the sex-based differences in metaboreflex responses, as evidenced by the greater MSNA response observed following isometric exercise involving larger muscle mass (Seals, 1993; Saito, 1995). The effect of muscle mass on the metaboreflex is still under debate, with some groups observing metaboreflex responses to be independent of muscle mass and absolute force output (Matthews & Stoney, 1988; Ettinger *et al.,* 1996; Jarvis *et al.,* 2011). Meanwhile, others have found that sex-differences in the pressor response are proportional to muscle mass (Mitchell *et al.,* 1980; Seals *et al.,* 1983; McCloskey & Streatfeild, 1975). A separate research group observed that sex-based differences are maintained when normalized to forearm circumference, but disappear when normalized to handgrip strength (Notay *et al.,* 2018).

In the present study, forearm volume, forearm circumference, and MVC_{PEAK} were significantly larger in males than females. However, sex-based differences in the change in MAP from baseline to the end of exercise (ΔMAP) was maintained when controlling for forearm volume and circumference. This suggests that while the males of the present study had a significantly greater forearm muscle volume and circumference than the females, it likely did not contribute significantly to the sex-based difference in ΔMAP during the IHE bouts. No index of diaphragm mass or thickness were measured in this study. Therefore, it is unknown if sex-differences in diaphragm muscle mass are present. Maximal inspiratory pressure generated by the diaphragm ($MIP_{di,MAX}$) was not statistically different between males and females, suggesting

no sex-based strength differences of the diaphragm. The MAP response was greater in males than females during both IHE and PTL, despite absolute strength sex-differences only being observed with respect to the forearm and not the diaphragm. Additionally, the sex-based differences in MAP were maintained when participants performed IHE and PTL at an absolute workload, regardless of MVC_{PEAK} or $MIP_{di,MAX}$. Findings from this study therefore suggest two possible explanations: 1) the role of muscle mass in contributing to the sex-based differences in the metaboreflex differs between isometric, rhythmic, and dynamic contraction patterns; or 2) muscle mass contributes minimally to the observed sex-based differences to the metaboreflex.

5.1.3.2 Effect of Sex – Muscle Perfusion

There is evidence to suggest that the sex-based differences to metaboreflex activation, observed in the present study and elsewhere, may correspond to differences in exercising muscle perfusion between males and females. Research suggests that exercising limb blood flow is greater in women than men, even when blood flow is normalized to muscle mass (Parker *et al.*, 2007). Women also demonstrated greater exercise-induced local vasodilation compared to men (Parker *et al.*, 2007; Just & DeLorey, 2017). Additionally, separate studies have demonstrated a smaller sympathetically mediated vasoconstrictor response in women compared to men, which was correlated to greater non-exercising limb blood flow in women (Ettinger *et al.*, 1996; Hogarth *et al.*, 2007; Just & DeLorey, 2017). These findings suggest that exercising limb muscle perfusion is decreased in males compared to females and may result in a greater local accumulation of metabolites at the level of the exercising muscle. As such, Ettinger *et al.* (1996) attributed the observed augmentation of MSNA in men to a greater metaboreflex response, given the observation of a higher local metabolite concentration in men compared to women. Lastly,

the observed sex-based differences in MSNA and peripheral muscle fatiguability are abolished during ischemic exercise, wherein the limitation in muscle perfusion is similar across all participants (Ettinger *et al.,* 1996; Russ & Kent-Braun, 2003; Clark *et al.,* 2005). In sum, these findings suggest a significant sex-based difference in exercising muscle perfusion, which may augment metabolite accumulation and contribute to the greater metaboreflex pressor response observed in males.

5.1.3.3 Effect of Sex – Role of Circulating Sex Hormones

The current study went to great lengths to control for sex hormone status in female participants. Additionally, to maximize the difference between circulating hormone environments between males and females, females were tested in the midluteal phase of their menstrual cycle. Ovarian sex hormones, specifically estrogen and progesterone, are known to have a protective effect on the cardiovascular system (Godsland *et al.,* 1987; Sullivan *et al.,* 1988; Rosano *et al.,* 1996). Evidence suggests that estrogen reduces arterial blood pressure in both normotensive and hypertensive postmenopausal women (Mercuro *et al.,* 1997; Mercuro *et al.,* 1998; Zacharieva *et al.,* 2002; Yoon *et al.,* 2021). Additionally, estrogen reduces peripheral vascular resistance and extends time to task failure during forearm exercise in postmenopausal women (Volterrani *et al.,* 1995). Beneficial effects have also been seen with progesterone, in working in concert with estrogen to regulate vascular tone (Simoncini *et al.,* 2004; Thomas & Peng, 2013). However, the reduction in ambulatory blood pressure was similar between administration of estrogen and progesterone together, compared to estrogen alone, thus suggesting that estrogen has a larger role in reducing blood pressure (Yoon et al., 2021).

Females exhibit a smaller response to norepinephrine, a potent vasoconstrictor which acts on both α- and β-adrenergic receptors, when compared to males (Riedel *et al.,* 2019). Differences in responses to vasoconstrictor stimuli may result from either decreased sensitivity, or decreased expression, of α- and β-adrenergic receptors. Studies in both animal and human models have shown that female α-adrenergic receptors are less sensitive to phenylephrine, a potent vasoconstrictor (Freednamdn et al., 1987), and female β-adrenergic receptors are more sensitive to vasodilatory stimuli (isoprenaline, nitroprusside, and verapamil) (Freedman *et al.,* 1987; Kneale *et al.,* 2000; Al-Gburi *et al.,* 2017). Additionally, there is evidence to suggest that female arteries contain more β_1- and β_2-adrenergic receptors than males (Al-Gburi *et al.,* 2017; Riedel *et al.,* 2019). Riedel *et al.* (2019) found that estrogen regulates the expression of β-adrenergic receptors in both small and large arterial vessels in an animal model; it was found that increased circulating estrogen leads to greater β_1- and β_3-adrenergic receptor expression. It was also found that sensitivity to vasoconstrictor stimuli was more than doubled following ovariectomy (Riedel *et al.,* 2019). Progesterone, testosterone, and orchiectomization were not found to have the same regulatory effect on β-adrenergic receptor expression or sensitivity (Riedel *et al.,* 2019). Therefore, it is likely that estrogen contributes to a decreased metaboreflex response, by means of decreasing vascular responses to sympathetic outflow.

The role of estrogen on mediating sex-based differences to metaboreflex activation is supported by investigations observing metaboreflex responses post-menopause, wherein circulating estrogen concentrations are similar between males and females (Greenblatt *et al.,* 1976). The pressor response to an inspiratory resistive breathing task was not statistically different between older males and females post-menopause, while a sex-based difference was observed in their young counterparts (Smith *et al.,* 2016). Similar observations were observed

following limb exercise, with postmenopausal women having a significantly greater MAP than their young counterparts, and no sex-based difference observed post-menopause (Choi *et al.,* 2012; Parker *et al.,* 2008; Trinity *et al.,* 2018; Van Iterson *et al.,* 2016). The role of circulating sex hormones in modulating the metaboreflex is further supported by investigations on exogenous hormone supplementation. Parmar *et al.* (2017) observed that women taking oral hormonal contraceptives consisting of combined estradiol and progestin had a statistically similar pressor response compared to men, during isometric handgrip exercise and PECO (Parmar *et al.,* 2017).

It is currently unclear whether the cyclical variation in ovarian hormones across the menstrual cycle modulates sex-based differences to metaboreflex activation. Significant changes in the MSNA, but not MAP, response to handgrip exercise have been observed between early and late follicular phases (Ettinger *et al.,* 1998), however a separate study found no significant effect between early follicular and midluteal phases (Jarvis *et al.,* 2011). It is possible that the discrepancy in findings correlates to the greater difference between hormone environments in the latter study, as compared to the former. The lowest concentration of circulating estrogen and progesterone are observed during the early follicular phase, with an increase in estrogen occurring towards ovulation, and high concentrations in both hormones observed during the midluteal phase. All the females of the present study were naturally cycling, were not currently using hormonal contraceptives, and were tested in the midluteal phase of their menstrual cycle. It is relevant to note that while there is evidence suggesting variations in circulating sex hormones may influence sympathetic outflow from arterial baroreceptors, this difference is not mirrored in signal transduction or subsequent changes in vascular resistance (Minson *et al.,* 2000).

5.1.3.4 Effect of Sex – Summary

Previous work suggests that differences in metabolite accumulation, muscle perfusion, sympathetic outflow, and responsiveness to vasoconstrictor stimuli might all contribute to the observed sex-based differences in the pressor response to metaboreflex activation. Findings from this study support those from previous work suggesting a role of circulating sex hormones, specifically estrogen, in mediating the attenuated pressor response in females. It is likely that changes in β-adrenergic receptor sensitivity resulting from circulating estrogen results in decreased sympathetically mediated vasoconstriction in females. Results from the present study do not support a significant role of muscle fiber type, muscle mass, or absolute muscle strength in contributing to the observed sex-based differences. However, it is important to note that measures of muscle fiber type and muscle mass were not recorded in this study, and it is possible differences by sex and by muscle group are present. Further research is required to confirm whether β-adrenergic receptor sensitivity is a primary casual factor, or whether other factors along the metaboreflex arc also contribute significantly to the differences observed between males and females.

5.2 Implications to Exercise

5.2.1 Muscle Fatigability

Historically, research in exercise physiology has largely considered exercise tolerance and volitional exhaustion primarily within the context of central and/or peripheral muscle fatigability (Merton, 1954; Asmussen, 1979; Walsh, 2000; Burnley & Jones, 2007). Local metabolite accumulation, peripheral fatigability, and limb metaboreflex activation are known to contribute to the attainment of volitional exhaustion (Amann *et al.,* 2011; Hunter, 2014).

However, it is not uncommon for investigators to overlook other factors such as participant motivation, perception of effort, and feedback from respiratory or non-exercising muscles. Recently, researchers have proposed the existence of a "sensory tolerance limit", wherein the sum of all sensory feedback and feedforward inputs contribute to the realization of volitional exhaustion or task failure (Gandevia, 2001; Amann *et al.*, 2013; Hureau *et al.*, 2018). Additionally, Marcora *et al.*, (2010) found that exercise tolerance is highly correlated to perception of effort, rather than the attainment of central/peripheral fatigue. Therefore, for the purposes of this thesis, volitional exhaustion and muscle fatigability are considered as separate phenomena.

5.2.1.1 Muscle Fatigability – Role of Muscle Strength and Neuromuscular Activation

In the present study, both males and females experienced a decline in $P_{di,MAX}$ following PTL, while only males experienced a decline in MVC_{PEAK} following IHE. This suggests the presence of a sex-based difference in muscle fatigability at the level of the forearm. A summary of the physiological basis of peripheral fatigability, as well as sex-based differences in peripheral fatigability, is provided in Section 1.1.2.2. In brief, females are generally more resistant to peripheral muscle fatigability than males during both dynamic and static exercise (Hicks *et al.*, 2001; Hunter, 2014; Hunter, 2016). Several mechanisms have been proposed to contribute to the observed sex-based differences in peripheral muscle fatigability, including differences in muscle mass, neuromuscular activation, substrate utilization, and muscle blood flow (Hicks *et al.*, 2001; Clark *et al.*, 2005). If considering the change in MVC_{PEAK} pre- to post-IHE as an index of impaired force generating capacity, the present study suggests that the sex-based difference in peripheral muscle fatigability is maintained, even when males and females produce the same

absolute force output, which is supported by previous work (Fulco *et al.*, 1999; Ditor & Hicks, 2000). Hunter (2014) suggests that the sex-based difference in peripheral fatigability is strength-dependent; however, this assertion arose from evidence that time to task failure is similar between males and females when matched for absolute force output (Hunter *et al.*, 2004; Hunter *et al.*, 2006). Given that time to task failure and peripheral fatigability may not be direct correlates (Marcora *et al.*, 2010; Hureau *et al.*, 2018), further research is needed to determine the role of muscle mass or absolute muscle strength on the observed sex-based differences. In the present study, males had significantly greater forearm volume, forearm circumference, and MVC_{PEAK} than females. It is possible that muscle mass contributed to the greater reduction in post-IHE MVC_{PEAK} observed in males compared to females, however this sex-based difference was maintained when both groups were performing the same absolute muscle work. In the absolute workload condition, the target force output was a significantly lower relative intensity for males than females ($37\pm5\%$ vs $56\pm7\%$ of MVC_{PEAK}). Therefore, results from the present study suggest that absolute muscle strength and absolute force output do not significantly contribute to differences in muscle fatigability between males and females.

It is important to note that the present study did not measure electromyogram activity in either the forearm or respiratory musculature, and therefore influencing inputs, such as central motor drive or voluntary activation, cannot be accounted for. Previous research suggests that electromyogram activity does not increase with the progression of sustained isometric forearm contractions in females, as was observed in males (Semmler *et al.*, 1999), however this finding is not unanimous (Hunter & Enoka, 2001). Females also demonstrated less of a decline in electromyogram activity following maximal leg extension exercise compared to males, despite exhibiting a similar reduction in maximum force production (Hakkinen, 1993). Hicks *et al.*,

(2001) interpret these findings to suggest that there is a disparity in neuromuscular system adaption between males and females than contributes to differences in fatigue resistance; however, more evidence is needed to determine causation, over correlation or coincidence.

5.2.1.2 Muscle Fatigability – Role of Muscle Perfusion, Metabolism, and the Metaboreflex

Current research supports the role of muscle fiber type, substrate utilization, and muscle blood flow as contributors to differences in peripheral muscle fatigability between males and females. Changes in the aforementioned factors may result in greater local metabolite accumulation, and faster development of peripheral fatigability during heavy exercise. There is evidence suggesting females have a greater proportion of type I muscle fibers and greater reliance on oxidative phosphorylation compared to males (Simoneau *et al.,* 1985; Simoneau & Bouchard, 1989; Haizlip *et al.,* 2015; Fornier *et al.,* 2022), as well as greater Ca^{2+}-ATPase activity during intense exercise (Harmer *et al.,* 2014). Differences in the reliance on aerobic or anaerobic metabolism, as well as Ca^{2+}-ATPase activity may result in a quicker accumulation of metabolites in the muscle (Harmer *et al.,* 2014; Hunter, 2014). Additionally, there is considerable evidence for a decrease in exercising skeletal muscle perfusion in males than females, which may further exacerbate the accumulation of metabolites in males. There is some evidence to suggest that exercising limb blood flow is greater in women than males, even when blood flow is normalized to muscle mass (Parker *et al.,* 2007). Women demonstrated greater exercise-induced vasodilation compared to men (Parker *et al.,* 2007). Additionally, separate studies have demonstrated a smaller sympathetically mediated vasoconstrictor response in women compared to men, which was correlated to greater non-exercising limb blood flow in women (Ettinger *et al.,* 1996; Hogarth *et al.,* 2007). Ettinger et al. (1996) attributed the observed augmentation of

MSNA to a greater metaboreflex response in men, given the greater accumulation of metabolites also observed in men compared to women. Lastly, the observed sex-based differences in MSNA and peripheral muscle fatiguability are abolished during ischemic exercise, wherein the limitation in muscle perfusion is similar across all participants (Ettinger *et al.,* 1996; Russ & Kent-Braun, 2003; Clark *et al.,* 2005). In summary, these findings suggest a significant role of muscle perfusion and the muscle metaboreflex in contributing to the development of peripheral muscle fatiguability, and differences between males and females.

5.2.1.3 Muscle Fatigability – Summary

The present study observed a marked sex-based difference in the pressor response to both limb and respiratory muscle work, however no direct measure of peripheral or central muscle fatigability was recorded. As shown in Table 4.2, the average time to task failure was significantly shorter during IHE than PTL, except in the relative workload condition in males. Yet, as previously stated, evidence suggests that the determinants of volitional exhaustion or task failure are multifactorial, and may not directly correspond to the development of peripheral muscle fatigability (Marcora *et al.,* 2010; Hureau *et al.,* 2018). All the participants of the present study demonstrated a decrease in $MIP_{di,MAX}$ following PTL, however only males demonstrated a decrease in MVC_{PEAK} following IHE. While the greater decrease in MVC_{PEAK} may appear to suggest greater limb muscle fatigability in males, it is important to note that the change in MVC_{PEAK} does not provide a complete picture of a muscle's force generating capacity, and factors proximal to the neuromuscular junction (i.e., central fatigue, voluntary activation) may also contribute (Taylor *et al.,* 2016). Future research is needed to determine whether the observed differences in the metaboreflex pressor response are associated with the known

differences in muscle fatigability between males and females; specifically, whether increased peripheral fatigability in males is correlated with the greater metaboreflex response as compared to females.

5.2.2 Muscle Perfusion and Functional Sympatholysis

As discussed in Section in 1.1.2, the metaboreflex contributes to exercise-related functional sympatholysis; the redistribution of blood flow away from resting skeletal muscle and towards the exercising muscles (Hansen *et al.,* 2000). There is evidence to suggest that metaboreflex activation may result in the "stealing" of blood flow away from other exercising muscles as well. Previous work has shown that superimposing arm exercise onto ongoing leg exercise impairs leg blood flow, and vice versa; superimposing leg exercise onto ongoing arm exercise impairs arm blood flow (Secher *et al.,* 1977). Blood flow redistribution between muscle groups during exercise allows for the maximization of oxygen extraction while maintaining arterial pressure, particularly in instances where cardiac output is maximal or near-maximal (Thomas & Segal, 2004; Holwerda *et al.,* 2015; Joyner & Casey, 2015).

The competition for blood flow is not unique to limb skeletal muscle. Increased respiratory muscle work results in a sympathetically mediated increase in systemic vasoconstriction, and subsequent increases in respiratory muscle perfusion at the expense of the exercising limb (Sinoway *et al*., 1989; Harms *et al.,* 1997; Harms *et al*., 1998; Hill, 2000; St Croix *et al.,* 2000; Sheel *et al*., 2001; Olson, *et al.,* 2010; Dominelli *et al*., 2017). In a reciprocal manner, Vogiatzis *et al.* (2009) found that cycling exercise reduced maximal intercostal muscle blood flow, as compared to resting isocapnic hyperpnea matched for tidal volume and breathing frequency. This finding suggests that changes in blood flow demands of the limb may also

impair respiratory muscle perfusion. However, other work suggests that blood flow redistribution is solely evoked by changes in respiratory muscle work (Dominelli *et al.,* 2017; Sheel *et al.,* 2018). Changing the work of breathing by mechanically loading or unloading the respiratory muscles during exercise results in inverse changes in limb muscle perfusion (Dominelli *et al.,* 2017). Increasing the work of breathing resulted in increased respiratory muscle perfusion but impaired limb muscle blood flow (Dominelli *et al.,* 2017). Vice versa, decreasing the work of breathing resulted in decreased respiratory muscle perfusion but enhanced limb muscle blood flow (Dominelli *et al.,* 2017). The contrasting evidence observed between Dominelli *et al.,* (2017) and Vogiatzis *et al.,* (2009) is acknowledged in a review by Sheel *et al.,* (2018). Reviewers address methodological considerations of the earlier study, including the potential role of lung tissue in interfering with the measurements of intercostal blood flow during high lung volumes, such as during the high-intensity cycling protocol used in the study (Vogiatzis *et al.,* 2009; Sheel *et al.,* 2018). Additionally, the vasoconstriction required to impair intercostal muscle blood flow during exercise would elicit significant changes in MAP, which was not also observed by others (Harms *et al.,* 1997; Harms *et al.,* 1998; Sheel *et al.,* 2018). Lastly, results from animal models suggest that phrenic artery feed vessels are less sensitive to vasoconstrictor stimuli, and exhibit larger vasodilatory responses as compared to other skeletal muscle (Laughlin *et al.,* 1989; Aaker & Laughlin, 2002). Thus, for a given vasoconstrictor and/or vasodilatory stimulus, respiratory muscle perfusion will be less impaired or display a greater increase, respectively, compared to limb skeletal muscle. Therefore, while the reciprocal nature of blood flow competition during exercise is not fully elucidated, evidence is pointing to a preferential redistribution of blood flow towards the respiratory musculature.

The "stealing" of blood flow away from the limb muscles and towards the respiratory muscles is, at least in part, intensity dependent. The sympathetically mediated increase in arterial pressure and corresponding changes in limb blood flow, elicited by the respiratory metaboreflex, is observed with respiratory loading during both maximal and submaximal cycling exercise in healthy individuals (Olson *et al.,* 2010; Katayama *et al.,* 2012; Dominelli *et al.,* 2017; Smith *et al.,* 2020). The same observation was not observed during submaximal cycling exercise superimposed with respiratory loading at a mild breathing frequency in trained cyclists (Wetter *et al.,* 1999). These findings suggest that respiratory metaboreflex-induced blood flow redistribution can occur at both submaximal and maximal exercise intensities when respiratory loading is sufficient and may be influenced by training status. It is also likely that blood flow redistribution during exercise is largely moderated by both the inspiratory and limb metaboreflexes (Dempsey *et al.,* 2006; Sheel *et al.,* 2018; Sheel *et al.,* 2020).

Evidence suggests that blood flow redistribution during exercise is largely mediated by the metaboreflex, and there is a preferential redistribution towards the respiratory musculature. Results from the present study suggest that the pressor response to comparable amounts of muscle work are similar between the limb and respiratory musculature. However, it is unclear if the similar pressor responses correspond to comparable changes in blood flow redistribution. It is also unclear if comparable levels of afferent metaboreflex outflow between the limb and respiratory musculature would elicit equally reciprocal changes in blood flow redistribution. Future research is required to infer the relationship of the observed findings to blood flow redistribution, and the subsequent development of both limb and respiratory peripheral muscle fatigability during exercise.

5.3 Methodological Considerations

5.3.1 Hypocapnia during Absolute IHE

The absolute workload of the IHE condition was a significantly lower relative intensity for males (males: 37±5 % MVC_{PEAK}), as compared to the relative workload of 60% MVC_{PEAK}. This lower relative intensity in males contributed to a significantly longer time to task failure as compared to the relative workload condition. Male participants had a tendency towards synchronizing their breathing duty cycle to the handgrip duty cycle during IHE. This effect was most prominent during longer exercise durations (i.e., the absolute IHE condition), and was persistent despite verbal feedback to breathe independently of the handgrip metronome. Synchronization of breathing duty cycle to handgrip contraction pattern resulted in hyperventilation and subsequent reductions in $P_{ET}CO_2$ in affected males. In the most severe case, $P_{ET}CO_2$ decreased from 38 mmHg at baseline to 14 mmHg at the end of exercise. In accordance with the sensory tolerance limit of exercise performance (Marcora *et al.,* 2010; Hureau *et al.,* 2018), hypocapnia may have contributed to an increase perception of effort, and thus a shorter time to task failure in some participants. Additionally, hyperventilation-induced hypocapnia decreases peripheral resistance and blood pressure, and increases cardiac output and forearm blood flow (Burnum *et al.,* 1954; Richardson, *et al.,* 1961; Little & Smith, 1964; Marshall, 1994). In the present study, males still demonstrated a greater MAP response to IHE than females, despite the cardiovascular effects of voluntary hyperventilation and hypocapnia.

5.3.2 Cardiac Output and the Observed Pressor Response

It is unclear if the increase in cardiac output significantly differs between rhythmic handgrip exercise and pressure threshold loading protocols. It is generally accepted that the

metaboreflex causes a sympathetically mediated increase in systemic vasoconstriction, total peripheral resistance, and blood pressure, while central command plays a primary role in elevating heart rate and cardiac output with exercise (Mark *et al.,* 1985; Rowell & O'Leary, 1990; Boushel, 2010). However, some suggest that the increase in blood pressure during rhythmic handgrip exercise and PECO occurs secondary to the increase in stoke volume and cardiac output (Crisafulli *et al.,* 2003; Crisafulli *et al.,* 2011). McNulty *et al.* (2014) propose that the pressor response to rhythmic handgrip exercise is dependent on this increase in cardiac output, while the pressor response to static handgrip exercise is mediated by changes in total peripheral resistance. More research is needed to investigate the potential dependency of the pressor response on cardiac output, particularly considering differences in the cardiac output response to difference exercise modalities.

The increase in cardiac output with exercise is correlated to oxygen consumption, regardless of exercise modality or muscle mass (Lewis *et al.,* 1983; Coast *et al.,* 1993; Joyner & Casey, 2015). However, some groups suggests that increase in cardiac output is larger during rhythmic handgrip exercise and single-leg exercise, compared to static, isometric handgrip exercise (Lewis *et al.,* 1985; McNulty *et al.,* 2014; Doherty *et al.,* 2019). Additionally, there is some evidence that there is a minimal cardiac output response to resistive breathing tasks (Olgiati *et al.,* 1986; Coast *et al.,* 1993), although the resistive breathing protocols are considerably different from the present study. In the present study, it is unclear whether the cardiac output response differs between the IHE and PTL protocols, or between males and females during either condition. While there is evidence that cardiac output is greater during exercise involving larger muscle mass (Lewis *et al.,* 1983), the present study did not include a measure of diaphragm or forearm muscle mass. It is also possible that the large swings in

intrathoracic pressure during PTL influenced venous return, and thus the cardiac output and MAP response to PTL. It is therefore unknown if there are differences in cardiac output between muscle groups or by sex, and if such differences would influence the observed pressor responses.

5.3.3 Valsalva Maneuvers During PTL

Valsalva maneuvers significantly increase intra-abdominal and intrathoracic pressure, which results in reduced stroke volume and cardiac output (Startk-Leyva *et al.,* 2004; Aliverti *et al.,* 2005). During expiratory loading protocols, participants increase abdominal muscle recruitment during forceful expiration to provide thoracic stability (De Troyer & Boriek, 2011). In a similar manner, it is possible that participants of the present study also increased abdominal muscle recruitment during the forceful inspiration phase of PTL, in efforts to generate and maintain the high target P_{di} (60% of $MIP_{di,MAX}$). In requiring participants to reach a target P_{di} rather than target P_{mo}, it is possible that participants performed small Valsalva maneuvers during each inspiration, to help increase intra-abdominal pressure and maintain the target P_{di} for the entire inspiration (2.8 seconds). If this was the case, the Valsalva maneuvers may have influenced the pressor response to PTL by intermittently attenuating venous return and cardiac output.

5.4 Methodological Improvements

As mentioned in Section 5.3.3, it is possible that participants performed small Valsalva maneuvers during the PTL protocol, in efforts to maintain the target P_{di}. For future studies, selecting a target mouth pressure may minimize additional abdominal muscle recruitment during inspiration. Previous work from our lab has successfully used relative P_{mo} targets during PTL, to

investigate the inspiratory metaboreflex (St Croix *et al.,* 2000; Sheel *et al.,* 2001; Welch *et al.,* 2018).

Additionally, future studies would benefit from including a measure of muscle fatiguability following exercise protocols to volition exhaustion. For example, cervical magnetic stimulation of the phrenic nerve and the corresponding diaphragm electromyogram activity is occasionally used to investigate changes in force generating capacity of the diaphragm following exercise or resistive breathing tasks (Welch *et al.,* 2017; Ramsook *et al.,* 2021). Inclusion of CMS would provide an index of diaphragm fatigability and could therefore be used to investigate whether the observed sex-based difference in the pressor response to PTL is correlated to differences in diaphragm fatigability. Likewise comparisons of the forearm can be made utilizing electrically or magnetically induced handgrip contractions, and measures of forearm electromyogram activity.

5.5 Unresolved Questions and Future Direction

To extend the findings of the present study, future studies could measure limb and respiratory muscle blood flow using doppler ultrasound to provide insight on the implications of the sex-based difference in metaboreflex response, to peripheral muscle fatigability. Additionally, utilizing measures of diaphragm thickness as an index of diaphragm muscle mass may be insightful in considering the proposed relationship of muscle mass to metaboreflex responses. Lastly, future work could also investigate the responses to PTL superimposed onto limb exercise and vice versa, to further investigate the competition for blood flow between the limb and respiratory musculature; specifically, if the limb metaboreflex has the capability to "steal" blood flow from the respiratory muscles.

Chapter 6: Conclusion

Limb and inspiratory metaboreflex activation results in a time-dependent increase in mean arterial pressure in both males and females, albeit the magnitude of this increase is attenuated in females regardless of muscle group or workload. This study also observed that the pressor response to comparable amounts of limb and inspiratory muscle work were similar, regardless of workload or sex. Therefore, the mechanisms contributing to the sex-based differences in the metaboreflex pressor response appear to be similar between the limb and inspiratory musculature. Lastly, this study found that rhythmic handgrip exercise performed at a high relative exercise intensity and prolonged duty cycle is sufficient to activate the limb muscle metaboreflex, as evidenced by a maintained pressor response during PECO. This suggests that rhythmic handgrip exercise is an effective model to use when comparing metaboreflex responses between muscle groups performing rhythmic or dynamic exercise protocols. Future research is needed to relate the findings of the present study to muscle perfusion and peripheral fatigability, as well as the generalizability to other populations or exercise modalities.

References

Aaker, A., & Laughlin, M. H. (2002). Differential adenosine sensitivity of diaphragm and skeletal muscle arterioles. *Journal of Applied Physiology, 93*(3), 848-856. Doi:10.1152/japplphysiol.00032.2002

Adreani, C. M., Hill, J. M., & Kaufman, M. P. (1997). Responses of group III and IV afferents to dynamic exercise. *Journal of Applied Physiology, 82*(6), 1811-1817. Doi:10.1152/jappl.1997.82.6.1811

Alam, M., & Smirk, F. H. (1937). Observations in man upon a blood pressure raising reflex arising from the voluntary muscles. *Journal of Physiology-London, 89*(4), 372-383. Doi:10.1113/jphysiol.1937.sp003485

Alam, M., & Smirk, F. H. (1938). Observations in man on a pulse-accelerating reflex from the voluntary muscles of the legs. *Journal of Physiology-London, 92*(2), 167-177. Doi:10.1113/jphysiol.1938.sp003592

Aliverti, A., Dellaca, R. L., Lotti, P., Bertini, S., Duranti, R., Scano, G., Heyman, J., Lo Mauro A., Pedotti, A., & Macklem, P. T. (2005). Influence of expiratory flow limitation during exercise on systemic oxygen delivery in humans. *European Journal of Applied Physiology, 95*(2-3), 229-242. Doi:10.1007/s00421-005-1386-4

Aliverti, A., Kayser, B., & Macklem, P. T. (2007). A human model of the pathophysiology of chronic obstructive pulmonary disease. *Respirology, 12*(4), 478-485. Doi:10.1111/j.1440-1843.2007.01106.x

Al-Gburi, S., Deussen, A., Zatschler, B., Weber, S., Kunzel, S., El-Armouche, A., Lorenz, K., Cybularz, M., Morawietz, H., & Kopaliani, I. (2017). Sex-difference in expression and function of beta-adrenoceptors in macrovessels: role of the endothelium. *Basic Research in Cardiology, 112*(3), 29. Doi:10.1007/s00395-017-0617-2

Amann, M. (2011). Central and peripheral fatigue: interaction during cycling exercise in humans. *Medicine and Science in Sports and Exercise, 43*(11), 2039-2045. Doi:10.1249/MSS.0b013e31821f59ab

Amann, M. (2012). Significance of group III and IV muscle afferents for the endurance exercising human. *Clinical and Experimental Pharmacology and Physiology, 39*(9), 831-835. Doi:10.1111/j.1440-1681.2012.05681.x

Amann, M., Blain, G. M., Proctor, L. T., Sebranek, J. J., Pegelow, D. F., & Dempsey, J. A. (2010). Group III and IV muscle afferents contribute to ventilatory and cardiovascular response to rhythmic exercise in humans. *Journal of Applied Physiology, 109*(4), 966-976. Doi:10.1152/japplphysiol.00462.2010

Amann, M., Blain, G. M., Proctor, L. T., Sebranek, J. J., Pegelow, D. F., & Dempsey, J. A. (2011). Implications of group III and IV muscle afferents for high-intensity endurance

exercise performance in humans. *Journal of Physiology-London, 589*(21), 5299-5309. Doi:10.1113/jphysiol.2011.213769

Amann, M., Proctor, L. T., Sebranek, J. J., Pegelow, D. F., & Dempsey, J. A. (2009). Opioid-mediated muscle afferents inhibit central motor drive and limit peripheral muscle fatigue development in humans. *Journal of Physiology-London, 587*(1), 271-283. Doi:10.1113/jphysiol.2008.163303

Amann, M., Runnels, S., Morgan, D. E., Trinity, J. D., Fjeldstad, A. S., Wray, D. W., Reese, V. R., & Richardson, R. S. (2011). On the contribution of group III and IV muscle afferents to the circulatory response to rhythmic exercise in humans. *Journal of Physiology-London, 589*(15), 3855-3866. Doi:10.1113/jphysiol.2011.209353

Amann, M., Venturelli, M., Ives, S. J., McDaniel, J., Layec, G., Rossman, M. J., & Richardson, R. S. (2013). Peripheral fatigue limits endurance exercise via a sensory feedback-mediated reduction in spinal motoneuronal output. *Journal of Applied Physiology, 115*(3), 355-364. Doi:10.1152/japplphysiol.00049.2013

Andersen, P., & Saltin, B. (1985). Maximal perfusion of skeletal muscle in man. *Journal of Physiology-London, 366*, 233-249. Doi:10.1113/jphysiol.1985.sp015794

Archiza, B., Reinhard, P. A., Welch, J. F., & Sheel, A. W. (2020). Sex differences in diaphragmatic fatigue: Effects of hypoxia during inspiratory loading. *Journal of Physiology-London, 599*(4), 1319-1333. Doi:10.1113/JP280704

Archiza, B., Welch, J. F., Geary, C. M., Allen, G. P., Borghi-Silva, A., & Sheel, A. W. (2018). Temporal characteristics of exercise-induced diaphragmatic fatigue. *Journal of Applied Physiology, 124*(4), 906-914. Doi:10.1152/japplphysiol.00942.2017

Arora, N. S., & Rochester, D. F. (2018). Effect of body weight and muscularity on human diaphragm muscle mass, thickness, and area. *Journal of Applied Physiology, 52*(1), 64-70. Doi:10.1152/jappl.1982.52.1.64

Asmussen, E. (1979). Muscle fatigue. *Medicine and Science in Sports and Exercise, 11*(4), 313-321.

Asmussen, E., & Nielsen, M. (1964). Experiments on nervous factors controlling respiration and circulation during exercise employing blocking of blood flow. *Acta Physiologica Scandinavica, 60*(1-2), 103-11. Doi:10.1111/j.1748-1716.1964.tb02873.x

Barclay, J. K. (1986). A delivery-independent blood flow effect on skeletal muscle fatigue. *Journal of Applied Physiology, 61*(3), 1084-1090. Doi:10.1152/jappl.1986.61.3.1084

Basnayake, S. D., Green, A. L., & Paterson, D. J. (2012). Mapping the central neurocircuitry that integrates the cardiovascular response to exercise in humans. *Experimental Physiology, 97*(1), 29-38. Doi:10.1113/expphysiol.2011.060848

Bastos, B. G., Williamson, J. W., Harrelson, T., & Da Nobrega, A. C. L. (2000). Left ventricular volumes and hemodynamic responses to postexercise ischemia in healthy humans. *Medicine and Science in Sports and Exercise, 32*(6), 1114-1118. Doi:10.1097/00005768-200006000-00012

Bellemare, F., Jeanneret, A., & Couture, J. (2003). Sex differences in thoracic dimensions and configuration. *American Journal of Respiratory and Critical Care Medicine, 168*(3), 305-312. Doi:10.1164/rccm.200208-876OC

Bellemare, F., Wight, D., Lavigne, C. M., & Grassino, A. (1983). Effect of tension and timing of contraction on the blood flow of the diaphragm. *Journal of Applied Physiology, 54*(6), 1597-1606. Doi:10.1152/jappl.1983.54.6.1597

Bentley, R. F., Poitras, V. J., Hong, T., & Tschakovsky, M. E. (2017). Characteristics and effectiveness of vasodilatory and pressor compensation for reduced relaxation time during rhythmic forearm contractions. *Experimental Physiology, 102*(6), 621-634. Doi:10.1113/EP086069

Bigland-Ritchie, B. R., Dawson, N. J., Johansson, R. S., & Lippold, O. C. J. (1986). Reflex origin for the slowing of motoneuron firing rates in fatigue of human voluntary contractions. *Journal of Physiology-London, 379*, 451-459. Doi:100.1113/jphysiol.1986.sp016263

Bonde-Petersen, F., Rowell, L. B., Murray, R. G., Blomqvist, G. G., White, R., Karlsson, E., Campbell, W., & Mitchell, J. H. (1978). Role of cardiac output in the pressor responses to graded muscle ischemia in man. *Journal of Applied Physiology, 45*(4), 574-580. Doi:10.1152/jappl.1978.45.4.574

Boushel, R. (2010). Muscle metaboreflex control of the circulation during exercise. *Acta Physiologica, 199*(4), 367-383. Doi:10.1111/j.1748-1716.2010.02133.x

Boutellier, U., & Farhi, L. E. (1986). Influence of breathing frequency and tidal volume on cardiac output. *Respiration Physiology, 66*(2), 123-133. Doi:10.1016/0034-5687(86)90066-6

Broxterman, R. M., Hureau, T. J., Layec, G., Morgan, D. E., Bledsoe, A. D., Jessop, J. E., Amann, M., & Richardson, R. S. (2018). Influence of group III/IV muscle afferents on small muscle mass exercise performance: a bioenergetics perspective. *Journal of Physiology-London, 596*(12), 2301-2314. Doi:10.1113/JP275817

Buchler, B., Magder, S., Katsardis, H., Jammes, Y., & Roussos, C. (1985). Effects of pleural pressure and abdominal pressure on diaphragmatic blood flow. *Journal of Applied Physiology, 58*(3), 691-697. Doi:10.1152/jappl.1985.58.3.691

Burnley, M., & Jones, A. M. (2007). Oxygen uptake kinetics as a determinant of sports performance. *European Journal of Sport Science, 7*(2), 63-79. Doi:10.1080/17461390701456148

Burnum, J. F., Hickam, J. B., & Mcintosh, H. D. (1954). The effect of hypocapnia on arterial blood pressure. *Circulation, 9*, 89-95. Doi:10.1161/01.CIR.9.1.89

Carter, S. L., Rennie, C. D., Hamilton, S. J., & Tarnopolsky, M. A. (2001). Changes in skeletal muscle in males and females following endurance training. *Canadian Journal of Physiology and Pharmacology, 79*(5), 386-392. Doi:10.1139/cjpp-79-5-386

Choi, H. M., Stebbins, C. L., Nho, H., Kim, K. A., Kim, C., & Kim, J. K. (2012). Skeletal muscle metaboreflex is enhanced in postmenopausal women. *European Journal of Applied Physiology, 112*(7), 2671-2678. Doi:10.1007/s00421-011-2245-0

Clark, B. C., Collier, S. R., Manini, T. M., & Ploutz-Snyder, L. L. (2005). Sex differences in muscle fatigability and activation patterns of the human quadriceps femoris. *European Journal of Applied Physiology, 94*(1-2), 196-206. Doi:10.1007/s00421-004-1293-0

Coast, J. R., & Krause, K. M. (1993). Relationship of oxygen-consumption and cardiac output to work of breathing. *Medicine and Science in Sports and Exercise, 25*(3), 335-340.

Coote, J. H., & Dodds, W. N. (1976). The baroreceptor reflex and the cardiovascular changes associated with sustained muscular contraction in the cat. *Pflugers Archiv-European Journal of Physiology, 363*(2), 167-173. Doi:10.1007/BF01062286

Coote, J. H., Hilton, S. M., & Perezgon, J. F. (1971). The reflex nature of the pressor response to muscular exercise. *Journal of Physiology-London, 215*(3), 789-804. Doi:10.1113/jphysiol.1971.sp009498

Crapo, R. O., & Morris, A. H. (1981). Standardized single breath normal values for carbon monoxide diffusing capacity. *The American Review of Respiratory Disease, 123*(2) ,185.

Crisafulli, A., Piras, F., Filippi, M., Piredda, C., Chiappori, P., Melis, F., Milia, R., Tocco, F., & Concu, A. (2011). Role of heart rate and stroke volume during muscle metaboreflex-induced cardiac output increase: differences between activation during and after exercise. *Journal of Physiological Sciences, 61*(5), 385-394. Doi:10.1007/s12576-011-0163-x

Crisafulli, A., Scott, A. C., Wensel, R., Davos, C. H., Francis, D. P., Pagliaro, P., Coats, A. J. S., Concu, A., & Piepoli, M. F. (2003). Muscle metaboreflex-induced increases in stroke volume. *Medicine and Science in Sports and Exercise, 35*(2), 221-228. Doi:10.1249/01.MSS.0000048639.02548.24

Cui, J., Blaha, C., Moradkhan, R., Gray, K. S., & Sinoway, L. I. (2006). Muscle sympathetic nerve activity responses to dynamic passive muscle stretch in humans. *Journal of Physiology-London, 576*(2), 625-634. Doi:10.1113/jphysiol.2006.116640

Cui, J., Wilson, T. E., Shibasaki, M., Hodges, N. A., & Crandall, C. G. (2001). Baroreflex modulation of muscle sympathetic nerve activity during posthandgrip muscle ischemia in humans. *Journal of Applied Physiology, 91*(4), 1679-1686. Doi:10.1152/jappl.2001.91.4.1679

De Troyer, A., & Boriek, A. M. (2011). Mechanics of the respiratory muscles. *Comprehensive Physiology, 1*(3), 1273-1300. Doi:10.1002/cphy.c100009

Dempsey, J. A. (2012). New perspectives concerning feedback influences on cardiorespiratory control during rhythmic exercise and on exercise performance. *Journal of Physiology-London, 590*(17), 4129-4144. Doi:10.1113/jphysiol.2012.233908

Dempsey, J. A., Romer, L., Rodman, J., Miller, J., & Smith, C. (2006). Consequences of exercise-induced respiratory muscle work. *Respiratory Physiology & Neurobiology, 151*(2-3), 242-250. Doi:10.1016/j.resp.2005.12.015

Derchak, P. A., Sheel, A. W., Morgan, B. J., & Dempsey, J. A. (2002). Effects of expiratory muscle work on muscle sympathetic nerve activity. *Journal of Applied Physiology, 92*(4), 1539-1552. Doi:10.1152/japplphysiol.00790.2001

Ditor, D. S., & Hicks, A. L. (2000). The effect of age and gender on the relative fatigability of the human adductor pollicis muscle. *Canadian Journal of Physiology and Pharmacology, 78*(10), 781-790. Doi:10.1139/cjpp-78-10-781

Doherty, C. J., King, T. J., Incognito, A. V., Lee, J. B., Shepherd, A. D., Cacoilo, J. A., Slysz, J. T., Burr, J. F., & Miller, P. J. (2019). Effects of dynamic arm and leg exercise on muscle sympathetic nerve activity and vascular conductance in the inactive leg. *Journal of Applied Physiology, 127*(2), 464-472. Doi:10.1152/japplphysiol.00997.2018

Dominelli, P. B., Archiza, B., Ramsook, A. H., Mitchell, R. A., Peters, C. M., Molgat-Seon, Y., Henderson, W. R., Koehle, M. S., Boushel, R., & Sheel, A. W. (2017). Effects of respiratory muscle work on respiratory and locomotor blood flow during exercise. *Experimental Physiology, 102*(11), 1535-1547. Doi:10.1113/EP086566

Dominelli, P. B., Render, J. N., Molgat-Seon, Y., Foster, G. E., Romer, L. M., & Sheel, A. W. (2015). Oxygen cost of exercise hyperpnoea is greater in women compared with men. *Journal of Physiology-London, 593*(8), 1965-1979. Doi:10.1113/jphysiol.2014.285965

Dominelli, P. B., & Sheel, A. W. (2012). Experimental approaches to the study of the mechanics of breathing during exercise. *Respiratory Physiology & Neurobiology, 180*(2-3), 147-161. Doi:10.1016/j.resp.2011.10.005

Ebert, T. J. (1986). Baroreflex responsiveness is maintained during isometric exercise in humans. *Journal of Applied Physiology, 61*(2), 797-803. Doi:10.1152/jappl.1986.61.2.797

Eldridge, F. L., Millhorn, D. E., Kiley, J. P., & Waldrop, T. G. (1985). Stimulation by central command of locomotion, respiration and circulation during exercise. *Respiratory Physiology, 59*(3), 313-337. Doi:10.1016/0034-5687(85)90136-7

Eldridge, F. L., Millhorn, D. E., & Waldrop, T. G. (1981). Exercise hyperpnea and locomotion – parallel activation from the hypothalamus. Science, 211(4484), 844-846. Doi:10.1126/science.7466362

Enoka, R. M., & Duchateau, J. (2008). Muscle fatigue: what, why and how it influences muscle function. *Journal of Physiology-London, 586*(1), 11-23. Doi:10.1113/jphysiol.2007.139477

Esbjornsson-Lijedahl, M., Bodin, K., & Jansson, E. (2002). Smaller muscle ATP reduction in women than in men by repeated bouts of sprint exercise. *Journal of Applied Physiology, 93*(3), 1075-1083. Doi:10.1152/japplphysiol.00732.1999

Esbjornsson-Lijedahl, M., Sundberg, C. J., Norman, B., & Jansson, E. (1999). Metabolic response in type I and type II muscle fibers during a 30-s cycle sprint in men and women. *Journal of Applied Physiology, 87*(4), 1326-1332. Doi:10.1152/jappl.1999.87.4.1326

Essen, B., Jansson, E., Henriksson, J., Taylor, A. W., & Saltin, B. (1975). Metabolic characteristics of fiber types in human skeletal muscle. *Acta Physiologica Scandinavica, 95*(2), 153-165. Doi:10.1111/j.1748-1716.1975.tb10038.x

Ettinger, S. M., Silber, D. H., Collins, B. G., Gray, K. S., Sutliff, G., Whisler, S. K., McClain, J. M., Smith, M. B., Yang, Q. X., & Sinoway, L. I. (1996). Influences of gender on sympathetic nerve responses to static exercise. *Journal of Applied Physiology, 80*(1), 245-251. Doi:10.1152/jappl.1996.80.1.245

Ettinger, S. M., Silber, D. H., Gray, K. S., Smith, M. B., Yang, Q. X., Kunselman, A. R., & Sinoway, L. I. (1998). Effects of the ovarian cycle on sympathetic neural outflow during static exercise. *Journal of Applied Physiology, 85*(6), 2075-2081. Doi:10.1152.jappl.1998.85.6.2075

Fadel, P. J., Ogoh, S., Watenpaugh, D. E., Wasmund, W., Olivencia-Yurvati, A., Smith, M. L., & Raven, P. B. (2001). Carotid baroreflex regulation of sympathetic nerve activity during dynamic exercise in humans. *American Journal of Physiology – Heart and Circulatory Physiology, 280*(3), H1383-H1390. Doi:10.1152/ajpheart.2001.280.3.H1383

Fadel, P. J. (2008). Arterial baroreflex control of the peripheral vasculature in humans: rest and exercise. *Medicine and Science in Sports and Exercise, 40*(12), 2055-2062. Doi:10.1249/MSS.0b013e318180bc80

Fadel, P. J. (2015). Reflex control of the circulation during exercise. *Scandinavian Journal of Medicine & Science in Sports, 25*, 74-82. Doi:10.1111/sms.12600

Fisher, J. P., Young, C. N., & Fadel, P. J. (2015). Autonomic adjustments to exercise in humans. *Comprehensive Physiology, 5*(2), 475-512. Doi:10.1002/cphy.c140022

Fitts, R. H. (1994). Cellular mechanisms of muscle fatigue. *Physiological Reviews, 74*(1), 49-94. Doi:10.1152/physrev.1994.74.1.49

Fitts, R. H. (2008). The cross-bridge cycle and skeletal muscle fatigue. *Journal of Applied Physiology, 104*(2), 551-558. Doi:10.1152/japplphysiol.01200.2007

Fournier, G., Bernard, C., Cievet-Bonfils, M., Kenney, R., Pingon, M., Sappey-Marinier, E., Chazaud, B., Gondin, J., & Servien, E. (2022). Sex differences in semitendinosus muscle fiber-type composition. *Scandinavian Journal of Medicine & Science in Sports, 32*(4), 720-727. Doi:10.1111/sms.14127

Franklin, S. S., Thijs, L., Hansen, T. W., O'Brien, E., & Staessen, J. A. (2013). White-coat hypertension: New insights from recent studies. *Hypertension, 62*(6), 982-987. Doi:10.1161/HYPERTENSIONAHA.113.01275

Freedman, R. R., Sabharwal, S. C., & Desai, N. (1987). Sex-differences in peripheral vascular adrenergic-receptors. *Circulation Research, 61*(4), 581-585. Doi:10.1161/01.RES.61.4.581

Fritsch, J. M., Smith, M. L., Simmons, D. T. F., & Eckberg, D. L. (1991). Differential baroreflex modulation of human vagal and sympathetic activity. *American Journal of Physiology, 260*(3), R635-R641. Doi:10.1152/ajpregu.1991.260.3.R635

Fuglevand, A. J., Macefield, V. G., & Bigland-Ritchie, B. (1999). Force-frequency and fatigue properties of motor units in muscles that control digits of the human hand. *Journal of Neurophysiology, 81*(4), 1718-1729. Doi:10.1152/jn.1999.81.4.1718

Fulco, C. S., Lewis, S. F., Frykman, P. N., Boushel, R., Smith, S., Harman, E. A., Cymerman, A., & Pandolf, K. B. (1996). Muscle fatigue and exhaustion during dynamic leg exercise in normoxia and hypobaric hypoxia. *Journal of Applied Physiology, 81*(5), 1891-1900. Doi:10.1152/jappl.1996.81.5.1891

Fulco, C. S., Rock, P. B., Muza, S. R., Lammi, E., Cymerman, A., Butterfield, G., Moore, L. G., Braun, B., & Lewis, S. F. (1999). Slower fatigue and faster recovery of the adductor pollicis muscle in women matched for strength with men. *Acta Physiologica Scandinavica, 167*(3), 233-239. Doi:10.1046/j.1365-201x.1999.00613.x

Gallagher, K. M., Fadel, P. J., Smith, S. A., Norton, K. H., Querry, R. G., Olivencia-Yurvati, A., & Raven, P. B. (2001). Increases in intramuscular pressure raise arterial blood pressure during dynamic exercise. *Journal of Applied Physiology, 91*(5), 2351-2358. Doi:10.1152/jappl.2001.91.5.2351

Gallagher, K. M., Fadel, P. J., Smith, S. A., Stromstrad, M., Ide, K., Secher, N. H., & Raven, P. B. (2006). The interaction of central command and the exercise pressor reflex in mediating baroreflex resetting during exercise in humans. *Experimental Physiology, 91*(1), 79-87. Doi:10.1113/expphysiol.2005.032110

Gandevia, S. C. (2001). Spinal and supraspinal factors in human muscle fatigue. *Physiological Reviews, 81*(4), 1725-1789. Doi:10.1152/physrev.2001.81.4.1725

Gandevia, S. C., Allen, G. M., Butler, J. E., & Taylor, J. L. (1996). Supraspinal factors in human muscle fatigue: Evidence for suboptimal output from the motor cortex. *Journal of Physiology-London, 490*(2), 529-536. Doi:10.1113/jphysiol.1996.sp021164

Garland, S. J., Garner, S. H., & McComas, A. J. (1988). Reduced voluntary electromyographic activity after fatiguing stimulation of human muscle. *Journal of Physiology-London, 401*, 547-556. Doi:10.1113/jphysiol.1988.sp017178

Geary, C. M., Welch, J. F., McDonald, M. R., Peters, C. M., Leahy, M. G., Reinhard, P. A., & Sheel, A. W. (2019). Diaphragm fatigue and inspiratory muscle metaboreflex in men and women matched for absolute diaphragmatic work during pressure-threshold loading. *Journal of Physiology, 597*(18), 4797-4808. Doi:10.1113/JP278380

Gladwell, V. F., & Coote, J. H. (2002). Heart rate at the onset of muscle contraction and during passive muscle stretch in humans: a role for mechanoreceptors. *Journal of Physiology-London, 540*(3), 1095-1102. Doi:10.1113/jphysiol.2001.013486

Gladwell, V. F., Fletcher, J., Patel, N., Elvidge, L. J., Lloyd, D., Chowdhary, S., & Coote, J. H. (2005). The influence of small fibre muscle mechanoreceptors on the cardiac vagus in humans. *Journal of Physiology-London, 567*(2), 713-721. Doi:10.1113/jphysiol.2005.089243

Godsland, I. F., Wynn, V., Crook, D., & Miller, N. E. (1987). Sex, plasma lipoproteins, and atherosclerosis: Prevailing assumptions and outstanding questions. *American Heart Journal, 114*(6), 1467-1503. Doi:10.1016/0002-8703(87)90552-7

Gonzales, J. U., & Scheuermann, B. W. (2006). Gender differences in the fatigability of the inspiratory muscles. *Medicine & Science in Sports & Exercise, 38*(3), 472-479. Doi:10.1249/01.mss.0000189318.80061

Gonzales, J. U., Thompson, B. C., Thistlethwaite, J. R., Harper, A. J., & Scheuermann, B. W. (2007). Forearm blood flow follows work rate during submaximal dynamic forearm exercise independent of sex. *Journal of Applied Physiology, 103*(6), 1950-1957. Doi:10.1152/japplphysiol.00452.2007

Goodwin, G. M., McCloskey, D. I., & Mitchell, J. H. (1972). Cardiovascular and respiratory responses to changes in central command during isometric exercise at constant muscle tension. *Journal of Physiology-London, 226*(1), 173-190. Doi:10.1113/jphysiol.1972.sp009979

Green, A. L., Wang, S. Y., Purvis, S., Owen, S. L. F., Bain, P. G., Stein, J. F., Guz, A., Aziz, T. Z., & Paterson, D. J. (2007). Identifying cardiorespiratory neurocircuitry involved in central command during exercise in humans. *Journal of Physiology – London, 578*(2), 605-612. Doi:10.1113/jphysiol.2006.122549

Greenberg, M. J., Mealy, T. R., Jones, M., Szczesna-Cordary, D., & Moore, J. R. (2010). The direct molecular effects of fatigue and myosin regulatory light chain phosphorylation on the actomyosin contractile apparatus. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology, 298*(4), R989-R996. Doi:10.1152/ajpregu.00566.2009

Greenblatt, R. B., Oettinger, M., & Bohler, C. S. S. (1976). Estrogen-androgen levels in aging men and women – therapeutic considerations. *Journal of the American Geriatrics Society, 24*(4), 173-178. Doi:10.1111/j.1532-5415.1976.tb04294.x

Greenhaff, P. L., Nevill, M. E., Soderlund, K., Bodin, K., Boobis, L. H., Williams, C., & Hultman, E. (1994). The metabolic responses of human type I and type II muscle fibers during maximal treadmill sprinting. *Journal of Physiology-London, 478*(1), 149-155. Doi:10.1113/jphysiol.1994.sp020238

Guenette, J. A., Romer, L. M., Querido, J. S., Chua, R., Eves, N. D., Road, J. D., McKenzie, D. C., & Sheel, W. A. (2010). Sex difference in exercise-induced diaphragmatic fatigue in endurance-trained athletes. *Journal of Applied Physiology, 109*, 35-46. Doi:10.1152/jappphysiol.01341.2009

Guenette, J. A., Witt, J. D., McKenzie, D. C., Road, J. D., & Sheel, A. W. (2007). Respiratory mechanics during exercise in endurance-trained men and women. *Journal of Physiology-London, 581*(3), 1309-1322. Doi:10.1113/jphysiol.2006.126466

Haizlip, K. M., Harrison, B. C., & Leinwand, L. A. (2015). Sex-based differences in skeletal muscle kinetics and fiber-type composition. *Physiology, 30*(1), 30-39. Doi:10.1152/physiol.00024.2014

Hakkinen, K. (1993). Neuromuscular fatigue and recovery in male and female athletes during heavy resistance exercise. *International Journal of Sports Medicine, 14*(2), 53-59. Doi:10.1055/s-2007-1021146

Hankinson, J. L., Odencrantz, J. R., & Fedan, K. B. (1999). Spirometric reference values from a sample of the general U.S. population. *American Journal of Respiratory Critical Care Medicine, 159*(1), 179-187. Doi:10.1164/ajrccm.159.1.9712108

Hansen, J., Sander, M., & Thomas, G. D. (2000). Metabolic modulation of sympathetic vasoconstriction in exercising skeletal muscle. *Acta Physiologica Scandinavica, 168*(4), 489-503. Doi:10.1046/j.1365-201x.2000.00701.x

Hansen, J., Thomas, G. D., Jacobsen, T. N., & Victor, R. G. (1994). Muscle metaboreflex triggers parallel sympathetic activation in exercising and resting human skeletal muscle. *American Journal of Physiology – Heart and Circulatory Physiology, 226*(6), H2508-H2514. Doi:10.1152/ajpheart.1994.266.6.H2508

Harms, C. A., Babcock, M. A., McClaran, S. R., Pegelow, D. F., Nickele, G. A., Nelson, W. B., & Dempsey, J. A. (1997). Respiratory muscle work compromises leg blood flow during maximal exercise. *Journal of Applied Physiology, 82*(5), 1573-1583. Doi:10.1152/jappl.1997.82.5.1573

Harms, C. A., Wetter, T. J., McClaran, S. R., Pegelow, D. F., Nickele, G. A., Nelson, W. B., Hanson, P., & Dempsey, J. A. (1998). Effects of respiratory muscle work on cardiac output and its distribution during maximal exercise. *Journal of Applied Physiology, 85*(2), 609-618. Doi:10.1152/jappl.1998.95.2.609

Harms, C. A., Wetter, T. J., St Croix, C. M., Pegelow, D. F., & Dempsey, J. A. (2000). Effects of respiratory muscle work on exercise performance. *Journal of Applied Physiology, 89*(1), 131-138. Doi:10.1152/jappl.2000.89.1.131

Hartwich, D., Dear, W. E., Waterfall, J. L., & Fisher, J. P. (2011). Effect of muscle metaboreflex activation on spontaneous cardiac baroreflex sensitivity during exercise in humans. *Journal of Physiology-London, 589*(24), 6157-6171. Doi:10.1113/jphysiol.2011.219964

Haseler, L. J., Richardson, R. S., Videen, J. S., & Hogan, M. C. (1998). Phosphocreatine hydrolysis during submaximal exercise: the effect of FIO2. *Journal of Applied Physiology, 85*(4), 1457-1463. Doi:10.1152/jappl.1998.85.4.1457

Hayes, S. G., & Kaufman, M. P. (2001). Gadolinium attenuates exercise pressor reflex in cats. *American Journal of Physiology-Heart and Circulatory Physiology, 280*(5), H2153-2161. Doi:10.1152/ajpheart.2001.280.5.H2153

Hayes, S. G., Kindig, A. E., & Kaufman, M. P. (2006). Cyclooxygenase blockade attenuates responses of group III and IV afferents to dynamic exercise in cats. *American Journal of Physiology-Heart and Circulatory Physiology, 290*(6), H2239-H2246. Doi:10.1152/ajpheart.01274.2005

Hepple, R. T. (2002). The role of O2 supply in muscle fatigue. *Canadian Journal of Applied Physiology, 27*(1), 56-69. Doi:10.1139/h02-004

Hicks, A. L., Kent-Braun, J., & Ditor, D. S. (2001). Sex differences in human skeletal muscle fatigue. *Exercise and Sport Science Reviews, 29*(3), 109-112. Doi:10.1097/00003677-200107000-00004

Hill, J. M. (2000). Discharge of group IV phrenic afferent fibers increases during diaphragmatic fatigue. *Brain Research, 856*(1-2), 240-244. Doi:10.1016/S0006-8993(99)02366-5

Hogan, M. C., Richardson, R. S., & Haseler, L. J. (1999). Human muscle performance and PCr hydrolysis with varied inspired oxygen fractions: a P-31-MRS study. *Journal of Applied Physiology, 86*(4), 1367-1373. Doi:10.1152/jappl.1999.86.4.1367

Hogarth, A. J., Mackintosh, A. F., & Mary, D. A. S. G. (2007). Gender-related differences in the sympathetic vasoconstrictor drive of normal subjects. *Clinical Science, 112*(5-6), 353-361. Doi:10.1042/CS20060288

Holwerda, S. W., Restaino, R. M., & Fadel, P. J. (2015). Adrenergic and non-adrenergic control of active skeletal muscle blood flow: Implications for blood pressure regulation during exercise. *Autonomic Neuroscience-Basic & Clinical, 188*, 24-31. Doi:10.1016/j.autneu.2014.10.010

Hunter, S. K. (2014). Sex differences in human fatigability: mechanisms and insight to physiological responses. *Acta Physiologica, 210*(4), 768-689. Doi:10.1111/apha.12234

Hunter, S. K. (2016). Sex differences in fatigability of dynamic contractions. *Experimental Physiology, 101*(2), 250-255. Doi:10.1113/EP085370

Hunter, S. K., Critchlow, A., Shin, I. S., Enoka, R. M. (2004). Fatigability of the elbow flexor muscles for a sustained submaximal contraction is similar in men and women matched for strength. *Journal of Applied Physiology, 96*(1), 195-202. Doi:10.1152/japplphysiol.00893.2003

Hunter, S. K., & Enoka, R. M. (2001). Sex differences in the fatigability of arm muscles depends on absolute force during isometric contractions. *Journal of Applied Physiology, 91*(6), 2686-2694. Doi:10.1152/jappl.2001.91.6.2686

Hunter, S. K., Schletty, J. M., Schlachter, K. M., Griffith, E. E., Polichnowski, A. J., & Ng, A. V. (2006). Active hyperemia and vascular conductance differ between men and women for an isometric fatiguing contraction. *Journal of Applied Physiology, 101*(1), 140-150. Doi:10.1152/japplphysiol.01567.2005

Hureau, T. J., Romer, L. M., & Amann, M. (2018). The "sensory tolerance limit": a hypothetical construct determining exercise performance? *European Journal of Sport Science, 18*(1), 13-24. Doi:10.1080/17461391.2016.1252428

Hureau, T. J., Weavil, J. C., Thurston, T. S., Wan, H. Y., Gifford, J. R., Jessop. J. E., Buys, M. J., Richardson, R. S., & Amann, M. (2019). Pharmacological attenuation of group III/IV afferents improves endurance performance when oxygen delivery to locomotor muscles is preserved. *Journal of Applied Physiology, 127*(5), 1257-1266. Doi:10.1152/japplphysiol.00490.2019

Hurewitz, A. N., Sidhu, U., Bergofsky, E. H., & Chanana, A. D. (1984). How alterations in pleural pressure influence esophageal pressure. *Journal of Applied Physiology, 56*(5), 1162-1169. Doi:10.1152/jappl.1984.56.5.1162

Iandelli, I., Aliverti, A., Kayser, B., Dellaca, R., Cala, S. J., Duranti, R., Kelly, S., Scano, G., Sliwinski, P., Yan, S., Macklem, P. T., & Pedotti, A. (2002). Determinants of exercise performance in normal men with externally imposed expiratory flow limitation. *Journal of Applied Physiology, 92*(5), 1943-1952. Doi:10.1152/japplphysiol.00393.2000

Jarvis, S. S., VanGundy, T. B., Galbreath, M. M., Shibata, S., Okazaki, K., Reelick, M. F., Levine, B. D., & Fu, Q. (2011). Sex differences in the modulation of vasomotor sympathetic outflow during static handgrip exercise in healthy young humans. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology, 301*(1), R193-R200. Doi:10.1152/ajpregu.00562.2010

Joshi, H., & Edgell, H. (2019). Sex differences in the ventilatory and cardiovascular response to supine and tilted metaboreflex activation. *Physiological Reports, 7*(6), e14041. Doi:10.14814/phy2.14041

Joyner, M. J. (1992). Muscle chemoreflexes and exercise in humans. *Clinical Autonomic Research, 2*(3), 201-208. Doi:10.1007/BF01818963

Joyner, M. J., & Casey, D. P. (2015). Regulation of increased blood flow (hyperemia) to muscles during exercise: a hierarchy of competing physiological needs. *Physiological Reviews, 95*(2), 549-601. Doi:10.1152/physrev.00035.2013

Joyner, M. J., Nauss, L. A., Warner, M. A., & Warner, D. O. (1992). Sympathetic modulation of blood flow and O2 uptake in rhythmically contracting human forearm muscles. *American Journal of Physiology – Heart and Circulatory Physiology, 263*(4), H1078-H1083. Doi:10.1152/ajpheart.1992.263.4.H1078

Juel, C., Honig, A., & Pilegaard, H. (1991). Muscle lactate transport studied in sarcolemmal giant vesicles – dependence on fiber type and age. *Acta Physiologica Scandinavica, 143*(4), 361-365. Doi:10.1111/j.1748-1716.1991.tb09246.x

Just, T. P., & DeLorey, D. S. (2017). Sex differences in sympathetic vasoconstrictor responsiveness and sympatholysis. *Journal of Applied Physiology, 123*(1), 128-135. Doi:10.1152/japplphysiol.00139.2017

Katayama, K., Iwamoto, E., Ishida, K., Koike, T., & Saito, M. (2012). Inspiratory muscle fatigue increases sympathetic vasomotor outflow and blood pressure during submaximal exercise. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology, 302*(10), R1167-R1175. Doi:10.1152/ajpregu.00006.2012

Katayama, K., Smith, J. R., Goto, K., Shimizu, K., Saito, M., Ishida, K., Koike, T., Iwase, S., & Harms, C. A. (2018). Elevated sympathetic vasomotor outflow in response to increased inspiratory muscle activity during exercise is less in young women compared with men. *Experimental Physiology, 103*(4), 570-580. Doi:10.1113/EP086817

Kaufman, M. P., & Hayes, S. G. (2002). The exercise pressor reflex. *Clinical Autonomic Research, 12*(6), 429-439. Doi:10.1007/s10286-002-0059-1

Kaufman, M. P., Longhurst, J. C., Rybicki, K. J., Wallach, J. H., & Mitchell, J. H. (1983). Effects of static muscular contraction on impulse activity of group III and group IV afferents in cats. *Journal of Applied Physiology, 55*(1), 105-112. Doi:10.1152/jappl.1983.55.1.105

Kaufman, M. P., Rybicki, K. J., Waldrop, T. G., & Ordway, G. A. (1984). Effect of ischemia on responses of group III and group IV afferents to contraction. *Journal of Applied Physiology, 57*(3), 644-650. Doi:10.1152/jappl.1984.57.3.644

Kent-Braun, J. A., Fitts, R. H., & Christie, A. (2012). Skeletal muscle fatigue. *Comprehensive Physiology, 2*(2), 997-1044. Doi:10.1002/cphy.c110029

Kerkof, F. D., van Leeuwen, T., & Vereecke, E. E. (2018). The digital human forearm and hand. *Journal of Anatomy, 233*(5), 557-566. Doi:10.1111/joa.12877

Kneale, B. J., Chowienczyk, P. J., Brett, S. E., Coltart, D. J., & Ritter, J. M. (2000). Gender differences in sensitivity to adrenergic agonists of forearm resistance vasculature. *Journal*

of the American College of Cardiology, 36(4), 1233-1238. Doi:10.1016/S0735-1097(00)00849-4

Krogh, A., & Lind-hard, J. (1913). The regulation of respiration and circulation during the initial stages of muscular work. *Journal of Physiology-London, 47*(1-2), 112-136. Doi:10.1113/jphysiol.1913.sp001616

Landen, S., Hiam, D., Voisin, S., Jacques, M., Lamon, S., & Eynon, N. (2021). Physiological and molecular sex differences in human skeletal muscle in response to exercise training. *Journal of Physiology-London*. Doi:10.1113/JP279499

Laughlin, M. H., Klabunde, R. E., Delp, M. D., & Armstrong, R. B. (1989). Effects of dipyridamole on muscle blood flow in exercising miniature swine. *American Journal of Physiology-Heart and Circulatory Physiology, 257*(5), H1507-H1515. Doi:10.1152/ajpheart.1989.257.5.H1507

Lee, J. B., Notay, K., Seed, J. D., Nardone, M., Omazic, L. J., & Millar, P. J. (2021). Sex differences in muscle metaboreflex activation after static handgrip exercise. *Medicine & Science in Sports & Exercise, 53*(12), 2596-2604. Doi:10.1249/MSS0000000000002747

Lewis, S. F., Snell, P. G., Taylor, W. F., Hamra, M., Graham, R. M., Pettinger, W. A., & Blomqvist, C. G. (1985). Role of muscle mass and mode of contraction in circulatory responses to exercise. *Journal of Applied Physiology, 58*(1), 146-151. Doi:10.1152/jappl.1985.58.1.146

Lewis, S. F., Taylor, W. F., Graham, R. M., Pettinger, W. A., Schutte, J. E., & Blomqvist, C. G. (1983). Cardiovascular responses to exercise as functions of absolute and relative work load. *Journal of Applied Physiology, 54*(5), 1314-1323. Doi:10.1152/jappl.1983.54.5.1314

Lieberman, D. A., Faulkner, J. A., Craig, A. B., & Maxwell, L. C. (1973). Performance and histochemical composition of guinea pig and human diaphragm. *Journal of Applied Physiology, 34*(2), 233-237. Doi:10.1152/jappl.1973.34.2.233

Little, R. C., & Smith, C. W. (1964). Cardiovascular response to acute hypocapnia due to overbreathing. *American Journal of Physiology, 206*(5), 1025-1030. Doi:10.1152/ajplegacy.1964.206.5.1025

Marcora, S. M., & Staiano, W. (2010). The limit to exercise tolerance in humans: mind over muscle?. *European Journal of Applied Physiology, 109*(4), 763-770. Doi:10.1007/s00421-010-1418-6

Mark, A. L., Victor, R. G., Nerhed, C., & Wallin, B. G. (1985). Microneurographic studies of the mechanisms of sympathetic nerve responses to static exercise in humans. *Circulation Research, 57*(3), 461-469. Doi:10.1162/01.RES.57.3.461

Marshall, J. M. (1994). Peripheral chemoreceptors and cardiovascular regulation. *Physiological Reviews, 74*(3), 543-594. Doi:10.1152/physrev.1994.74.3.543

Matsukawa, K., Makamoto, T., & Inomoto, A. (2007). Gadolinium does not blunt the cardiovascular responses at the onset of voluntary static exercise in cats: a predominant role of central command. *American Journal of Physiology-Heart and Circulatory Physiology, 292*(1), H121-H129. Doi:10.1152/ajpheart.00028-2006

Matthews, K. A., & Stoney, C. M. (1988). Influences of sex and age on cardiovascular responses during stress. *Psychosomatic Medicine, 50*(1), 46-56. Doi:10.1097/00006842-198801000-00006

Maughan, R. J., Watson, J. S., & Weir, J. (1983). Strength and cross-sectional area of human skeletal muscle. *Journal of Physiology-London, 338*, 37-49. Doi:10.1113/jphysiol.1983.sp014658

McCloskey, D. I., & Mitchell, J. H. (1972). Reflex cardiovascular and respiratory responses originating in exercising muscle. *Journal of Physiology-London, 224*(1), 173-186. Doi:10.1113/jphysiol.1972.sp009887

McCloskey, D. I., & Streatfeild, K. A. (1975). Muscular reflex stimuli to cardiovascular system during isometric contractions of muscle groups of different mass. *Journal of Physiology-London, 250*(2), 431-441. Doi:10.1113/jphysiol.1975.sp011063

McNulty, C. L., Moody, W. E., Wagenmakers, A. J. M., & Fisher, J. P. (2014). Effect of muscle metaboreflex activation on central hemodynamics and cardiac function in humans. *Applied Physiology Nutrition and Metabolism, 39*(8), 861-870. Doi:10.1139/apnm-2013-0414

Melcher, A., & Donald, D. E. (1981). Maintained ability of carotid baroreflex to regulate arterial pressure during exercise. *American Journal of Physiology – Heart and Circulatory Physiology, 241*(6), H838-H849. Doi:10.1152/ajpheart.1981.241.6.H838

Mense, S., & Craig, A. D. (1988). Spinal and supraspinal terminations of primary afferent-fibers from the gastrocnemius-soleus muscle in the cat. *Neuroscience, 26*(3), 1023-1035. Doi:10.1016/0306-4522(88)90117-0

Mercuro, G., Zoncu, D., Piano, D., Pilia, I., Lao, A., Melis, G. B., & Cherchi, A. (1998). Estradiol-17beta reduces blood pressure and restores the normal amplitude of the circadian blood pressure rhythm in postmenopausal hypertension. *American Journal of Hypertension, 11*(8), 909-913. doi:10.1016/s0895-7061(98)00096-x.

Mercuro, G., Zoncu, S., Pilia, I., Lao, A., Melis, G. B., & Cherchi, A. (1997). Effects of acute administration of transdermal estrogen on postmenopausal women with systemic hypertension. American *Journal of Cardiology, 80*(5), 652-655. doi:10.1016/s0002-9149(97)00444-x.

Merton, P. A. (1954). Voluntary strength and fatigue. *Journal of Physiology-London, 123*(3), 553-564. Doi:10.1113/jphysiol.1954.sp005070

Michelini, L. C., O'Leary, D. S., Raven, P. B., & Nobrega, A. C. L. (2015). Neural control of circulation and exercise: a translational approach disclosing interactions between central command, arterial baroreflex, and muscle metaboreflex. *American Journal of Physiology-Heart and Circulatory Physiology, 309*(3), H381-H392. Doi:10.1152/ajpheart.00077.2015

Miller, A. E. J., Macdougall, J. D., Tarnopolsky, M. A., & Sale, D. G. (1993). Gender differences in strength and muscle-fiber characteristics. *European Journal of Applied Physiology and Occupational Physiology, 66*(3), 254-262. Doi:10.1007/BF00235103

Minson, C. T., Halliwill, J. R., Young, T. M., & Joyner, M. J. (2000). Influence of the menstrual cycle on sympathetic activity, baroreflex sensitivity, and vascular transduction in young women. *Circulation, 101*(8), 862-868. Doi:10.1161/01.CIR.101.8.862

Mitchell, J. H., Kaufman, M. P., & Iwamoto, G. A. (1983). The exercise pressor reflex – its cardiovascular effects, afferent mechanisms, and central pathways. *Annual Review of Physiology, 45,* 229-242. Doi:10.1146/annurev.ph.45.030183.001305

Mitchell, J. H., Payne, F. C., Saltin, B., & Schibye, B. (1980). The role of muscle mass in the cardiovascular response to static contractions. *Journal of Physiology-London, 309,* 45-54. Doi:10.1113/jphysiol.1980.sp013492

Mittelstadt, S. W., Bell, L. B., O'Hagan, K. P., & Clifford, P. S. (1994). Muscle chemoreflex alters vascular conductance in nonischemic exercising skeletal muscle. *Journal of Applied Physiology, 77*(6), 2761-2766. Doi:10.1152/jappl.1994.77.6.2761

Mittelstadt, S. W., Bell, L. B., O'Hagan, K. P., Sulentic, J. E., & Clifford, P. S. (1996). Muscle chemoreflex causes renal vascular constriction. *American Journal of Physiology-Heart and Circulatory Physiology, 270*(3), H951-H956. Doi:10.1152/ajpheart.1996.270.3.H951

Mizuno, M. (1991). Human respiratory muscles – fiber morphology and capillary supply. European Respiratory Journal, 4(5), 587-601.

Murphy, M. N., Mizuno, M., Mitchell, J. H., & Smith, S. A. (2011). Cardiovascular regulation by skeletal muscle reflexes in health and disease. *American Journal of Physiology – Heart and Circulatory Physiology, 301*(4), 1191-1204. Doi:10.1152/ajpheart.00208.2011

Nagle, F. J., Seals, D. R., & Hanson, P. (1988). Time to fatigue during isometric exercise using different muscle masses. *International Journal of Sports Medicine, 9*(5), 313-315. Doi:10.1055/s-2007-1025030

Nickerson, B. G., & Keens, T. G. (1982). Measuring ventilatory muscle endurance in humans as sustainable inspiratory pressure. *Journal of Applied Physiology, 52*(3), 768-772. Doi:10.1152/jappl.1982.52.3.768

Nobrega, A. C. L., O'Leary, D., Silva, B. M., Marongiu, E., Piepoli, M. F., & Crisafulli, A. (2014). Neural regulation of cardiovascular response to exercise: role of central command

and peripheral afferents. *Biomed Research International, 2014*, 478965. Doi:10.1155/2014/478965

Norton, K. H., Boushel, R., Strange, S., Saltin, B., & Raven, P. B. (1999). Resetting of the carotid arterial baroreflex during dynamic exercise in humans. *Journal of Applied Physiology, 87*(1), 332-338. Doi:10.1152/jappl.199.87.1.332

Notay, K., Lee, J. B., Incognito, A. V., Seed, J. D., Arthurs, A. A., & Millar, P. J. (2018). Muscle strength influences pressor responses to static handgrip in men and women. *Medicine and Science in Sports and Exercise, 50*(4), 778-784. Doi:10.1249/MSS.0000000000001485

O'Hagan, K. P., Casey, S. M., & Clifford, P. S. (1997). Muscle chemoreflex increases renal sympathetic nerve activity during exercise. *Journal of Applied Physiology, 82*(6), 181-1825. Doi:10.1152/jappl.1997.82.6.1818

O'Leary, D. S. (1993). Autonomic mechanisms of muscle metaboreflex control of heart rate. *Journal of Applied Physiology, 74*(4), 1748-1754. Doi:10.1152/jappl.1993.74.4.1748

O'Leary, D. S., & Augustyniak, R. A. (1998). Muscle metaboreflex increases ventricular performance in conscious dogs. *American Journal of Physiology-Heart and Circulatory Physiology, 275*(1), H220-H224. Doi:10.1152/ajpheart.1998.275.1.H220

O'Leary, D. S., & Sheriff, D. D. (1995). Is the muscle metaboreflex important in control of blood flow to ischemic active skeletal muscle in dogs? *American Journal of Physiology-Heart and Circulatory Physiology, 268*(3), H980-H986. Doi:10.1152/ajpheart.1995.268.3.H980

Ogoh, S., Fisher, J. P., Dawson, E. A., White, M. J., Secher, N. H., & Raven, R. B. (2005). Autonomic nervous system influence on arterial baroreflex control of heart rate during exercise in humans. *Journal of Physiology-London, 566*(2), 599-611. Doi:10.1113/jphysiol.2005.084541

Olgiati, R., Atchou, G., & Cerretelli, P. (1986). Hemodynamic effects of resistive breathing. *Journal of Applied Physiology, 60*(3), 846-853. Doi:10.1152/jappl.1986.60.3.846

Olson, T. P., Joyner, M. J., Dietz, N. M., Eisenach, J. H., Curry, T. B., & Johnson, B. D. (2010). Effects of respiratory muscle work on blood flow redistribution during exercise in heart failure. *Journal of Physiology-London, 588*(13), 2487-2501. Doi:10.1113/jphysiol.2009.186056

Pang, C. C. Y. (2001). Autonomic control of the venous system in health and disease – effect of drugs. *Pharmacology & Therapeutics, 90*(2-3), 179-230. Doi:10.1016/S0163-7258(01)00138-3

Papelier, Y., Escourrou, P., Gauthier, J. P., & Rowell, L. B. (1994). Carotid baroreflex control of blood pressure and heart rate in men during dynamic exercise. *Journal of Applied Physiology, 77*(2), 502-506. Doi:10.1152/jappl.1994.77.2.502

Parmar, H. R., Sears, J., Molgat-Seon, Y., McCulloch, C. L., McCracken, L. A., Brown, C. V., Sheel, A. W., & Dominelli, P. B. (2017). Oral contraceptives modulate the muscle metaboreflex in healthy young women. *Applied Physiology Nutrition and Metabolism, 43*(5), 460-466. Doi:10.1139/apnm-2017-0482

Parker, B. A., Smithmyer, S. L., Pelberg, J. A., Mishkin, A. D., Herr, M. D., & Proctor, D. N. (2007). Sex differences in leg vasodilation during graded knee extensor exercise in young adults. *Journal of Applied Physiology, 103*(5), 1583-1591. Doi:10.1152/japplphysiol.00662.2007

Parker, B. A., Smithmyer, S. L., Pelberg, J. A., Mishkin, A. D., & Proctor, D. N. (2008). Sex-specific influence of aging one exercising leg blood flow. *Journal of Applied Physiology, 104*(3), 655-664. Doi:10.1152/japplphysiol.01150-2007

Paterson, D. J. (2014). Defining the neurocircuitry of exercise hyperpnoea. *Journal of Physiology-London, 592*(3), 433-444. Doi:10.1113/jphysiol.2013.261586

Pawelcyzk, J. A., Hanel, B., Pawelcyzk, R. A., Warberg, J., & Secher, N. H. (1992). Leg vasoconstriction during dynamic exercise with reduced cardiac output. *Journal of Applied Physiology, 73*(5), 1838-1846. Doi:10.1152/jappl.1992.73.5.1838

Pawelczyk, J. A., Pawelczyk, R. A., Warberg, J., Mitchell, J. H., & Secher, N. H. (1997). Cardiovascular and catecholamine responses to static exercise in partially curarized humans. *Acta Physiologica Scandinavica, 160*(1), 23-28. Doi:10.1046/j.1365-201X.1997.00115.x

Polla, B., D'Antona, G., Bottinelli, R., & Reggiani, C. (2004). Respiratory muscle fibres: specialisation and plasticity. *Thorax, 59*(9), 808-817. Doi:10.1136/thx.2003.009894

Porter, M. M., Stuart, S., Boij, M., & Lexell, J. (2002). Capillary supply of the tibialis anterior muscle in young, healthy, and moderately active men and women. *Journal of Applied Physiology, 92*(4), 1451-1457. Doi:10.1152/japplphysiol.00744.2001

Potts, J. T., & Mitchell, J. H. (1998). Rapid resetting of carotid baroreceptor reflex by afferent input from skeletal muscle receptors. *American Journal of Physiology-Heart and Circulatory Physiology, 275*(6), H2000-H2008. Doi:10.1152/ajpheart.1998.275.6.H2000

Potts, J. T., Shi, X. R., & Raven, P. B. (1993). Carotid baroreflex responsiveness during dynamic exercise in humans. *American Journal of Physiology-Heart and Circulatory Physiology, 265*(6), H1928-1938. Doi:10.1152/ajpheart.1993.265.6.H1928

Ramsook, A. H., Molgat-Seon, Y., Boyle, K. G., Mitchell, R. A., Puyat, J. H., Koehle, M. S., Sheel, A. W., & Guenette, J. A. (2021). Reliability of diaphragm voluntary activation measurements in healthy adults. *Applied Physiology Nutrition and Metabolism, 46*(3), 247-256. Doi:10.1139/apnm-2020-0221

Raven, P. B., Fadel, P. J., & Ogoh, S. (2006). Arterial baroreflex resetting during exercise: a current perspective. *Experimental Physiology, 91*(1), 37-49. Doi:10.1113/expphysiol.2005.032250

Remensnyder, J. P., Mitchell, J. H., & Sarnoff, S. J. (1962). Functional sympatholysis during muscular activity – observations on influence of carotid sinus on oxygen uptake. *Circulation Research, 11*(3), 370-380. Doi:10.1161/01.RES.11.3.370

Richardson, D., Wasserman, A. J., & Patterson, J. L. (1961). General and regional circulatory responses to change in blood pH and carbon dioxide tension. *Journal of Clinical Investigation, 40*(1), 31-43. Doi:10.1172/JCI104234

Riedel, K., Duessen, A. J., Tolkmitt, J., Weber, S., Schlinkert, P., Zatschler, B., Friebel, C., Muller, B., El-Armouche, A., Morawietz, H., Matschke, K., Kopaliani, I. (2019). Estrogen determines sex differences in adrenergic vessel tone by regulation of endothelial β-adrenoceptor expression. *American Journal of Physiology – Heart and Circulatory Physiology, 317*(2), H234-H254. Doi:10.1152/ajpheart.00456.2018

Road, J. D. (1990). Phrenic afferents and ventilatory control. *Lung, 168*(3), 137-149. Doi:10.1007/BF02719685

Romer, L. M., Lovering, A. T., Haverkamp, H. C., Pegelow, D. F., & Dempsey, J. A. (2006). Effect of inspiratory muscle work on peripheral fatigue of locomotor muscles in healthy humans. *Journal of Physiology-London, 571*(2), 425-439. Doi:10.1113/jphysiol.2005.099697

Rosano, G. M. C., Chierchia, S. L., Leonardo, F., Beale, C. M., & Collins, P. (1996). Cardioprotective effects of ovarian hormones. *European Heart Journal, 17,* 15-19. Doi:10.1093/eurheart/17.suppl_D.15

Rowell, L. B. (1997). Neural control of muscle blood flow: importance during dynamic exercise. *Clinical and Experimental Pharmacology and Physiology, 24*(2), 117-125. Doi:10.1111/j.1440-1681.1997.tb01793.x

Rowell, L. B., & O'Leary, D. S. (1990). Reflex control of the circulation during exercise – chemoreflexes and mechanoreflexes. *Journal of Applied Physiology, 69*(2), 407-418. Doi:10.1152/jappl.1990.69.2.407

Rowell, L. B., Savage, M. V., Chambers, J., & Blackmon, J. R. (1991). Cardiovascular responses to graded reductions in leg perfusion in exercising humans. *American Journal of Physiology – Heart and Circulatory Physiology, 261*(5), H1545-H1553. Doi:10.1152/ajpheart.1991.261.5.H1545

Russ, D. W., & Kent-Braun, J. A. (2003). Sex differences in human skeletal muscle fatigue are eliminated under ischemic conditions. Journal of Applied Physiology, 94(6), 2414-2422. Doi:10.1152/japplphysiol.01145.2002

Russ, D. W., Lanza, I. R., Rothman, D., & Kent-Braun, J. A. (2005). Sex differences in glycolysis during brief, intense isometric contractions. *Muscle & Nerve, 32*(5), 647-655. Doi:10.1002/mus.20396

Sahlin, K., Tonkonogi, M., & Soderlund, K. (1998). Energy supply and muscle fatigue in humans. *Acta Physiologica Scandinavica, 162*(3), 261-266. Doi:10.1046/j.1365-201X.1998.0298f.x

Saito, M. (1995). Differences in muscle sympathetic nerve responses to isometric exercise in different muscle groups. *European Journal of Applied Physiology and Occupational Physiology, 70*(1), 26-35. Doi:10.1007/BF00601805

Sala-Mercado, J. A., Hammond, R. L., Kim, J. K., Rossi, N. F., Stephenson, L. W., & O'Leary, D. S. (2006). Muscle metaboreflex control of ventricular contractility during dynamic exercise. *American Journal of Physiology-Heart and Circulatory Physiology, 290*(2), H751-H757. Doi:10.1152/ajpheart.00869.2005

Sala-Mercado, J. A., Ichinose, M., Hammond, R. L., Ichinose, T., Pallante, M., Stephenson, L. W., O'Leary, D. S., & Iellamo, F. (2007). Muscle metaboreflex attenuate spontaneous heart rate baroreflex sensitivity during dynamic exercise. *American Journal of Physiology-Heart and Circulatory Physiology, 292*(6), H2867-H2873. Doi:10.1152/ajpheart.00043.2007

Seals, D. R. (1993). Influence of active muscle size on sympathetic nerve discharge during isometric contractions in humans. *Journal of Applied Physiology, 75*(3), 1426-1431. Doi:10.1152/jappl.1993.75.3.1426

Seals, D. R., & Reiling, M. J. (1989). Sympathetic neural discharge and vascular resistance during exercise in humans. *Journal of Applied Physiology, 66*(5), 2472-2478. Doi:10.1152/jappl.1989.66.5.2472

Seals, D. R., Washburn, R. A., Hanson, P. G., Painter, P. L., & Nagle, F. J. (1983). Increased cardiovascular response to static contraction of larger muscle groups. *Journal of Applied Physiology, 54*(2), 434-437. Doi:10.1152/jappl.1983.54.2.434

Secher, N. H., Clausen, J. P., Klausen, K., Noer, I., & Trap-Jensen, J. (1977). Central and regional circulatory effects of adding arm exercise to leg exercise. *Acta Physiologica Scandinavica, 100*(3), 288-297. Doi:10.1111/j.1748-1716.1977.tb05952.x

Semmler, J. G., Kutzscher, D. V., & Enoka, R. M. (1999). Gender differences in the fatigability of human skeletal muscle. *Journal of Neurophysiology, 82*(6), 3590-3593. Doi:10.1152/jn.1999.82.6.3590

Sheel, A. W., Boushel, R., & Dempsey, J. A. (2018). Competition for blood flow distribution between respiratory and locomotor muscles: implications for muscle fatigue. *Journal of Applied Physiology, 125*(3), 820-831. Doi:10.1152/japplphysiol.00189.2018

Sheel, A. W., Derchak, P. A., Morgan, B. J., Pegelow, D. F., Jacques, A. J., & Dempsey, J. A. (2001). Fatiguing inspiratory muscle work causes reflex reduction in resting leg blood flow in humans. *Journal of Physiology-London, 537*(1), 277-289. Doi:10.1111/j.1469-7793.2001.0277k.x

Sheel, A. W., Derchak, P. A., Pegelow, D. F., & Dempsey, J. A. (2002). Threshold effects of respiratory muscle work on limb vascular resistance. *American Journal of Physiology – Heart and Circulatory Physiology, 282*(5), H1732-H1738. Doi:10.1152/ajpheart.00798.2001

Sheel, A. W., Taylor, J. L., & Katayama, K. (2020). The hyperpnoea of exercise in health: Respiratory influences on neurovascular control. *Experimental Physiology, 105*(12), 1984-1989. Doi:10.1113/EP088103

Simoncini, T., Mannella, P., Fornari, L., Caruso, A., Willis, M. Y., Garibaldi, S., Baldacci, C., & Genazzani, A. R. (2004). Differential signal transduction of progesterone and medroxyprogesterone acetate in human endothelial cells. *Endocrinology, 145*(12), 5745-5756. Doi:10.1210/en.2004-0510

Simoneau, J. A., & Bouchard, C. (1989). Human variation in skeletal muscle fiber-type proportion and enzyme activities. *American Journal of Physiology-Endocrinology and Metabolism, 257*(4), E567-E572. Doi:10.1152/ajpendo.1989.257.4.E567

Simoneau, J. A., Lortie, G., Boulay, M. R., Thibault, M. C., Theriault, G., & Bouchard, C. (1985). Skeletal muscle histochemical and biochemical characteristics in sedentary male and female subjects. *Canadian Journal of Physiology and Pharmacology, 63*(1), 30-35. Doi:10.1139/y85-005

Sinoway, L., Prophet, S., Gorman, I., Mosher, T., Shenberger, J., Bolecki, M., Briggs, R., & Zelis, R. (1989). Muscle acidosis during static exercise is associated with calf vasoconstriction. *Journal of Applied Physiology, 66*(1), 429-436. Doi:10.1152/jappl.1989.66.1.429

Smith, J. R., Broxterman, R. M., Hammer, S. M., Alexander, A. M., Didier, K. D., Kurti, S. P., Barstow, T. J., & Harms, C. A. (2016). Sex differences in the cardiovascular consequences of the inspiratory muscle metaboreflex. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology, 311*(3), R574-R581. Doi:10.1152/ajpregu.00187.2016

Smith, J. R., Bruhn, E. J., Berg, J. D., Nur, A. A, Villarraga, N., & Olsen, T. P. (2020). Combined influence of inspiratory loading and locomotor subsystolic cuff inflation on cardiovascular responses during submaximal exercise. Journal of Applied Physiology, 128(5), 1338-1345. Doi:10.1152/japplphysiol.00781.2019

Smith, J. R., Mitchell, J. H., & Garry, M. G. (2006). The mammalian exercise pressor reflex in health and disease. *Experimental Physiology, 91*(1), 89-102. Doi:10.1113/expphysiol.2005.032367

St Croix, C. M., Morgan, B. J., Wetter, T. J., & Dempsey, J. A. (2000). Fatiguing inspiratory muscle work causes reflex sympathetic activation in humans. *Journal of Physiology-London, 529*(2), 493-504. Doi:10.1111/j.1469-7793.2000.00493.x

Stark-Leyva, K. N., Beck, K. C., & Johnson, B. D. (2004). Influence of expiratory loading and hyperinflation on cardiac output during exercise. *Journal of Applied Physiology, 96*(5), 1920-1927. Doi:10.1152/japplphysiol.00756.2003

Stratton, J. R., Pfeifer, M. A., Ritchie, J. L., & Halter, J. B. (1985). Hemodynamic effects of epinephrine – concentration effect study in humans. *Journal of Applied Physiology, 58*(4), 1199-1206. Doi:10.1152/jappl.1985.58.4.1199

Sullivan, J. M., Vanderzwaag, R., Lemp, G. F., Hughes, J. P., Maddock, V., Kroetz, F. W., Ramanathan, K. B., & Mirvis, D. M. (1988). Postmenopausal estrogen use and coronary atherosclerosis. *Annals of Internal Medicine, 108*(3), 358-363. Doi:100.7326/0003-4819-108-3-358

Taylor, J. A., Chase, P. B., Enoka, R. M., & Seals, D. R. (1988). Cardiovascular adjustments to rhythmic handgrip exercise – relationship to electromyographic activity and post-exercise hyperemia. *European Journal of Applied Physiology, 58*(1-2), 32-38. Doi:10.1007/BF00636600

Taylor, J. L., Amann, M., Duchateau, J., Meeusen, R., & Rice, C. L. (2016). Neural contributions to muscle fatigue: from the brain to the muscle and back again. *Medicine and Science in Sports and Exercise, 48*(11), 2294-2306. Doi:10.1249/MSS.0000000000000923

Teixeira, A. L., Samora, M., & Vianna, L. C. (2019). Muscle metaboreflex activation via postexercise ischemia as a tool for teaching cardiovascular physiology for undergraduate students. *Advances in Physiology Education, 43*(1), 34-41. Doi:10.1152/advan.00174.2018

Thomas, G. D., Hansen, J., & Victor, R. G. (1994). Inhibition of alpha(2)-adrenergic vasoconstriction during contraction of glycolytic, not oxidative, rat hindlimb muscle. *American Journal of Physiology-Heart and Circulatory Physiology, 266*(3), H920-H929. Doi:10.1152/ajpheart.1994.266.3.H920

Thomas, G. D., & Segal, S. S. (2004). Neural control of muscle blood flow during exercise. *Journal of Applied Physiology, 97*(2), 731-738. Doi:10.1152/japplphysiol.00076.2004

Thomas, P., & Peng, Y. (2013). Protective actions of progesterone in the cardiovascular system: Potential role of membrane progesterone receptors (mPRs) in mediating rapid effects. *Steroids, 78*(6), 583-588. Doi:10.1016/j.steroids.2013.01.003

Thornton, J. M., Aziz, T., Schlugman, D., & Paterson, D. J. (2002). Electrical stimulation of the midbrain increases heart rate and arterial blood pressure in awake humans. *Journal of Physiology-London, 539*(2), 615-621. Doi:10.1113/jphysiol.2001.014621

Trinity, J. D., Layec, G., Hart, C. R., & Richardson, R. S. (2018). Sex-specific impact of aging on the blood pressure response to exercise. *American Journal of Physiology – Heart and Circulatory Physiology, 314*(1), H95-H104. Doi:10.1152/ajpheart.00505.2017

Van Iterson, E. H., Gramm, C., Randall, N. R., & Olson, T. P. (2016). Influence of menopause status and age on integrated central and peripheral hemodynamic responses to subsystolic cuffing during submaximal exercise. *American Journal of Physiology – Heart and Circulatory Physiology, 311*(6), H1382-H1291. Doi:10.1152/ajpheart.00310.2016

Victor, R. G., Bertocci, L. A., Pryor, S. L., & Nunnally, R. L. (1988). Sympathetic nerve discharge is coupled to muscle cell pH during exercise in humans. *Journal of Clinical Investigation, 82*(4), 1301-1305. Doi:10.1172/JCI113730

Victor, R. G., & Seals, D. R. (1989). Reflex stimulation of sympathetic outflow during rhythmic exercise in humans. *American Journal of Physiology*, *257*(6), H2017-H2024. Doi:10.1152/ajpheart.1989.257.6.H2017

Victor, R. G., Seals, D. R., & Mark, A. L. (1987). Differential control of heart rate and sympathetic nerve activity during dynamic exercise – insight from intraneural recordings in humans. *Journal of Clinical Investigation, 79*(2), 508-516. Doi:10.1172/JCI112841

Vogiatzis, I., Athanasopoulos, D., Habazettl, H., Kuebler, W. M., Wagner, H., Roussos, C., Wagner, P. D., & Zakynthinos, S. (2009). Intercostal muscle blood flow limitation in athletes during maximal exercise. *Journal of Physiology-London, 587*(14), 3665-3677. Doi:10.1113/jphysiol.2009.171694

Volterrani, M., Rosano, G., Coats, A., Beale, C., & Collins, P. (1995). Estrogen acutely increases peripheral blood flow in postmenopausal women. *The American Journal of Medicine, 99*(2), 119-122. Doi:10.1016/S0002-9343(99)80130-2

Walgenbach, S. C., & Donald, D. E. (1983). Inhibition by carotid baroreflex of exercise-induced increases in arterial-pressure. *Circulation Research, 52*(3), 253-262. Doi:10.1161/01.RES.52.3.253

Walsh, M. L. (2000). Whole body fatigue and critical power – a physiological interpretation. *Sports Medicine, 29*(3), 153-166. Doi:10.2165/00007256-200029030-00002

Watanabe, K., Ichinose, M., Tahara, R., & Nishiyasu, T. (2014). Individual differences in cardiac and vascular components of the pressor response to isometric handgrip exercise in humans, *American Journal of Physiology-Heart and Circulatory Physiology, 306*(2), H251-H260. Doi:10.1152/ajpheart.00699.2013

Welch, J. F., Archiza, B., Guenette, J. A., West, C. R., & Sheel, A. W. (2018). Effect of diaphragm fatigue on subsequent exercise tolerance in healthy men and women. *Journal of Applied Physiology, 125(*6), 1987-1996. Doi:10.1152/japplphysiol.00630.2018

Welch, J. F., Archiza, B., Guenette, J. A., West, C. R., & Sheel, A. W. (2018). Sex differences in diaphragmatic fatigue: the cardiovascular response to inspiratory exercise. *Journal of Physiology-London, 596*(17), 4017-4032. Doi:10.1113/JP275794

Welch, J. F., Mildren, R. L., Zaback, M., Archiza, B., Allen, G. P., & Sheel, A. W. (2017). Reliability of the diaphragmatic compound muscle action potential evoked by cervical magnetic stimulation and recorded via chest wall surface EMG. *Respiratory Physiology & Neurobiology, 243*, 101-106. Doi:10.1016/j.resp.2017.05.011

Westerblad, H., & Allen, D. G. (1991). Changes in myoplasmic calcium-concentration during fatigue in single-mouse muscle fibers. *Journal of General Physiology, 98*(3), 615-635. Doi:10.1085/jgp.98.3.615

Williamson, J. W., Fadel, P. J., & Mitchell, J. H. (2006). New insights into central cardiovascular control during exercise in humans: a central command update. *Experimental Physiology, 91*(1), 51-58. Doi:10.1113/expphysiol.2005.032037

Williamson, J. W., McColl, R., Mathews, D., Mitchell, J. H., Raven, P. B., & Morgan, W. P. (2001). Hypnotic manipulation of effort sense during dynamic exercise: cardiovascular responses to brain activation. *Journal of Applied Physiology, 90*(4), 1392-1399. Doi:10.1152/jappl.2001.90.4.1392

Wilson, L. B., & Hand, G. A. (1997). The pressor reflex evoked by static contraction: neurochemistry at the site of the first synapse. *Brain Research Reviews, 23*(3), 196-209. Doi:10.1016/S0165-0173(96)00019-7

Woods, J. J., Furbush, F., & Bigland-Ritchie, B. (1987). Evidence for a fatigue-induced reflex inhibition of motoneuron firing rates. *Journal of Neurophysiology, 58*(1), 125-137. Doi:10.1152/jn.1987.58.1.125

Yoon, B-K., Sung, J., Song, Y-M., K, S-M., Son, K-A., Yoo, J. H., Park, S-J., & Kim, D-K. (2021). Effects of menopausal hormone therapy on ambulatory blood pressure and arterial stiffness in postmenopausal Korean women with grade 1 hypertension: a randomized, placebo-controlled trial. *Clinical Hypertension, 27*.

Zacherieva, S., Atanassova, I., Kirilov, G., Kalinov, K., Shigarminova, R., Nachev, E., & Aslanova, N. (2002). Effect of transdermal estrogen therapy on some vasoactive humoral factors and 24-h ambulatory blood pressure in normotensive postmenopausal women. *Climacteric, 5*(3), 293-299.

Appendices

Appendix A Forms and Questionnaires

A.1 Consent Form

THE UNIVERSITY OF BRITISH COLUMBIA

School of Kinesiology

210, War Memorial Gym

6081 University Boulevard

Vancouver, B.C., Canada V6T

Participant Information and Consent Form

(Please read through carefully and sign the last page of the document if you would like to consent to the study)

Limb and inspiratory muscle metaboreflex activation in healthy males and females

Principal Investigator: William Sheel, Ph.D.[1]
School of Kinesiology
The University of British Columbia
Office: [redacted]

Co-Investigators: Jenna Benbaruj, B.H.K.[1]
Michael Leahy, MSc[1]
Thora Rae, BKin Student[1]

[1] School of Kinesiology, The University of British Columbia

Contact Person: Jenna Benbaruj, B.H.K.
School of Kinesiology
The University of British Columbia
[redacted]

Study Contact Number: [redacted]
24 hours: [redacted]

Sponsor: The Natural Science and Engineering Research Council of Canada

1. INVITATION

You are being invited to take part in this research study because you are a healthy male or female between the ages of 18-35 with no history of cardiopulmonary (i.e., heart and/or lung) ailments.

2. YOUR PARTICIPATION IS VOLUNTARY

Your participation in this study is completely voluntary. You have the right to refuse participation in this study. Should you choose to participate, you may opt to withdraw from the study at any time without penalty. Before you decide, it is important for you to understand what the research involves. This consent form will tell you about the study, why the research is being done, what will happen to you during the study and the possible benefits, risks, and discomforts.

3. WHO IS CONDUCTING THE STUDY?

The study is being conducted by Dr. William Sheel, Jenna Benbaruj, and the study team members, Michael Leahy and Thora Rae of the Health and Integrative Physiology Laboratory at the University of British Columbia. The study is funded by the Natural Science and Engineering Research Council (NSERC) of Canada. Details of source of funding can be provided upon request.

4. BACKGROUND

During exercise, blood pressure reflexively increases to promote increased blood flow to the exercising muscle. This is functionally important to support the energetic requirements of exercise. During heavy exercise when ventilatory rates are higher, the muscles responsible for breathing (respiratory muscles) also have a greater energetic requirement and, thus, also require an increase in blood flow. Similarly to the limb muscles, increasing the work performed by the respiratory muscles also stimulates an increase in blood pressure. The increase in blood pressure with limb exercise or respiratory muscle work is largely mediated by a reflex known as the metaboreflex. In brief, the metaboreflex is activated when metabolites accumulate within exercising skeletal muscle, and evokes a reflex increase in blood pressure and exercising muscle perfusion. Interestingly, the blood pressure response to metaboreflex activation is lower in females than males, and this has been shown during both limb exercise and increased respiratory muscle work. However, it is unknown if the magnitude of this sex-difference is constant across muscle groups, specifically between the limb and respiratory muscles.

By isolating limb and inspiratory muscle work, we can compare the resulting blood pressure responses to limb and inspiratory metaboreflex activation. To do this, participants can perform handgrip exercise and a type of resistive breathing task known pressure-threshold loading (PTL). PTL involves breathing through a loaded apparatus designed such that participants will be required to generate a large inspiratory pressure during each breath to allow for unimpeded inspiration. By performing handgrip exercise and PTL we can activate the limb and inspiratory metaboreflexes, respectively, and investigate the resulting responses. We can also investigate the responses to exercise performed at both absolute (set exercise intensity) and relative (exercise intensity set at a percent of maximal effort) workloads, in order to account for the strength differences between males and females.

5. WHAT IS THE PURPOSE OF THE STUDY?

The primary purpose of the study is to observe the blood pressure response to two types of exercise: handgrip exercise and increased respiratory work, in healthy young males and females. We are also interested in observing the effect of workload (either absolute or relative workload) on any sex-differences seen in the blood pressure response.

6. WHO CAN PARTICIPATE IN THIS STUDY?

You may be able to participate in this study if:

- You are a male or female between the ages of 18-35 years

- You are fully proficient in communicating in the English language
- Have a body mass index between 18 and 30 kg•m^2
- You have normal lung function
- You do not smoke or vape, or have a history of smoking or vaping
- You have no symptoms of cardiopulmonary disease (symptoms include but are not limited to: high blood pressure, chest tightness, feelings of dizziness or light-headedness, breathlessness and/or wheezing at rest or during light-intensity exercise)

7. WHO SHOULD NOT PARTICIPATE IN THE STUDY?

You cannot participate in the study if:

- You have a history, or current symptoms of cardiopulmonary disease (symptoms include but are not limited to: high blood pressure, chest tightness, feelings of dizziness or light-headedness, breathlessness and/or wheezing at rest or during light-intensity exercise)
- You have a history of smoking or vaping
- Have a body mass index below 18 kg•m^2 or above 30 kg•m^2
- You are currently taking hormonal contraceptive medication (either oral or intrauterine)
- You are pregnant
- Have an ulcer or tumor of the esophagus or have had recent nasopharyngeal surgery
- Are allergic to latex or lidocaine

8. WHAT DOES THE STUDY INVOLVE?

Overview of the Study

You are being invited to participate in a study that involves three days of testing, and participation in the study is entirely voluntary. The total time to participate will be approximately 8 hours: 2 hours on Day 0, and 3 hours each on Days 1 and 2. The three visits will be at least 24 hours apart. Laboratory testing will characterize the blood pressure response to exercise with an inflatable blood pressure cuff. We will also measure your beathing patterns from air volume measures using a mouthpiece and nose clip. Participants may be invited to return for a fourth visit (Day 3), which involves a similar time commitment and testing protocol as Days 1 and 2 (Day 3: 3 hours; Total time commitment: 11 hours). The sessions will take place at the Health and Integrative Physiology Laboratory at the Chan Gunn Pavilion, Rm 230 at the University of British Columbia, Vancouver Campus. After providing informed written consent, we will ask you to complete some questionnaires regarding your medical history (PAR-Q+), physical activity levels, and your menstrual cycle history if applicable. You are not required to answer any questions that make you feel uncomfortable.

If You Decide to Join This Study:

Specific Procedures – Day 0

Firstly, your height and weight will be measured. Females will be asked to complete a menstrual cycle history questionnaire. We will then have you perform several lung function tests, as per American Thoracic Society guidelines. These tests will be conducted using a body box (185 cm high x 87 cm wide x 80 cm deep), as shown in the photo on the right. You will sit on a chair in the box and breathe through a mouthpiece while wearing a nose clip. While in the body box, you will be able to communicate with the researcher and exit the box at any time if you need. You will perform two different lung function tests in the box detailed below:

- *Pulmonary Diffusion Capacity*: We will measure the transfer of gas (carbon monoxide) from air in the lung to red blood cells.
- *Plethysmography*: We will be measuring the volume of air in the lungs upon the maximum effort of inspiration.

Secondly, resting conditions of your breathing, heart rate, and blood pressure will be measured. You will then become familiarized with the PTL apparatus. This device is designed to add a resistance upon inspiration, such that you will be required to generate a larger inspiratory pressure during each breath. During this protocol you will find it more difficult to get air into your lungs. The resistance added to the PTL device will be individualized to help you achieve a specific inspiratory pressure. When familiar, you will complete a practice exercise bout by performing the same inspiratory exercise for 5 minutes.

Specific Procedures – Days 1 and 2

For female participants, the pulmonary diffusion capacity test performed on Day 0 will be performed again on Day 1 prior to instrumentation. For all participants, on both experimental days (Days 1 and 2) a trained member of the study team will place two thin, flexible tubes through your nose and into your esophagus and stomach after applying a local anesthetic to your nose and throat to minimize any discomfort. These will allow us to measure the pressure in your ribcage and abdomen. You will also be fitted with heart rate, finger blood pressure monitors, and an electrocardiogram. The electrocardiogram will entail 3-lead electrode sensors adhered to your chest to monitor heart rate. Once instrumented, you will perform two 5-min bouts of exercise, separated by 30-min of rest. One bout of exercise will involve sustained or intermittent handgrip exercise, and you will be asked to reach a target force output as guided by visual feedback. This will be followed by a 10-min period of rest where we will be continuously recording cardiorespiratory measures. The second bout of exercise will involve PTL which will mimic the familiarization procedure from Day 0. You will breathe through the PTL apparatus and be asked to reach a target inspiratory pressure as guided by visual feedback.

Specific Procedures – Day 3

Participants may be invited to return to the laboratory for an additional day of testing. Participants will again be instrumented with heart rate, finger blood pressure monitors, and an electrocardiogram. Once instrumented, participants will perform two 5-min bouts of exercise, separated by 30-min of rest. Both exercise bouts will involve sustained or intermittent handgrip

exercise identical to those performed on Days 1 and 2, with the exception of an occlusion cuff placed on the upper portion of the dominant arm. The occlusion cuff is a type of blood pressure cuff specified to impair limb blood flow. Immediately prior to the end of the exercise bouts, the occlusion cuff will be rapidly inflated to impair blood flow to the forearm. Inflation of the occlusion cuff and will be maintained for two minutes, and will be followed by a 10-min period of rest where we will be continuously recording cardiorespiratory measures.

9. WHAT ARE MY RESPONSIBILITIES?

You will be expected to participate in three (or four) testing sessions and to avoid alcohol for 24 hours, food and caffeine for 2 hours, and exercise for at least 12 hours prior to each session. Female participants will be asked to be tested in the mid-luteal phase of their menstrual (18-22 days after the onset of menstruation) for Days 1 and 2, in order to control for the potential effect of ovarian hormones on the blood pressure response to exercise.

10. WHAT ARE THE POSSIBLE HARMS AND DISCOMFORTS?

It must be noted that individual responses to the experimental procedures exist and you are encouraged to report any unusual sensations or symptoms to the investigator. You are permitted to end testing at any time for any reason. All procedures used to collect physiological data will pose no risk to your continued health and well-being. Below are the known possible harms for the specific methods we will be using.

Spirometry Tests: When completing pulmonary function, you could potentially experience mild light-headedness or breathlessness. You can also cough or wheeze at the end of some of the breathing tests. There are no harms or discomforts associated with pulmonary function maneuvers, and discomforts are temporary and will subside once the test is complete. However, you may feel claustrophobic when in the box. When in the box, you will be able to communicate with the researcher and terminate the test if you are experiencing extreme claustrophobia. When completing a diffusion capacity assessment, there are small traces of carbon monoxide (CO) which might make you feel dizzy or light-headed. Minimal trials will be conducted with a minimum of 3 minutes between each trial to ensure your safety.

Balloon Catheters: You may feel mild discomfort or soreness in the nose and upper airway during the placement of the tubes in your esophagus and stomach. It is possible that you may experience a nosebleed as a result of tube placement (less than 5% of people). You may also experience slight discomfort as a result of 'gagging' while swallowing the tube and during the removal of the tube (less than 5% of people). This may cause some people to vomit (less than 1% of people). A numbing gel called lidocaine will be used to minimize the discomfort, as well as to minimize the likelihood of laryngospasm (a temporary spasm of the vocal cords which may make breathing difficult). Adverse reactions to lidocaine are extremely rare but include light-headedness, blurred/double vision, euphoria, confusion, dizziness, convulsions, sensations of heat, cold or numbness (all of these happen in less than 1% of people). We are unaware of any laboratory that has experienced any of the aforementioned adverse reactions to such a small amount of lidocaine. There is a small risk that the thin flexible tubes may be placed in the wrong position. In some extremely rare cases the catheter may enter your trachea (wind pipe). This happens in less than 0.5% of people. If this occurs, you may experience mild discomfort in the back of your throat and you may cough. The tube will then be removed and repositioned.

Perforation of the esophagus (a small hole being poked through the esophageal wall), esophageal injury, and laryngospasm are very rare.

You will not be allowed to participate in the study if you are known to be sensitive to local anesthetics or if you have allergies to latex. In the event of vomiting, esophageal injury, laryngospasm, or other medical disturbance participants will be provided with immediate medical assistance. The severity of the illness and willingness of the participant to continue will dictate if the experiment is carried out any further.

Limb Muscle Metaboreflex Activation: Inflating a cuff around the upper arm following a bout of handgrip exercise serves to trap muscle metabolites within the muscle. The metabolites can activate pain receptors and lead to some discomfort. The discomfort is alleviated shortly after the cuff is released and circulation is restored.

Pressure Threshold Loading: High intensity inspiratory pressure loading can be difficult. You can potentially experience discomfort in having to repeatedly generate high inspiratory pressures. Much like any exercise, respiratory muscles (e.g., ribcage) can feel sore the day following testing. Potential risks from pressure threshold loading include: vomiting (5%), abnormal blood pressure (less than 1%), fainting, (less than 1%), disorders of heartbeat (less than 0.1%), and very rare instances of heart attack (less than 0.001%). You will be asked to report immediately any unusual symptoms during the test. You can stop the test if you feel tired or uncomfortable.

In addition, you will not be allowed to participate in the study if you are pregnant. Reported risks associated with participating in strenuous exercise during pregnancy are: dizziness, chest pain, preterm labour and decreased fetus movement. If you expect you may be pregnant, you are encouraged to take a pregnancy test before participating in the study (the research team will not provide pregnancy tests).

11. WHAT ARE THE POTENTIAL BENEFITS OF PARTICIPATING?

As a result of your participation in this study, you will receive detailed pulmonary function assessment. Beyond this, you may not benefit directly from participating in this study.

12. WHAT HAPPENS IF I DECIDE TO WITHDRAW MY CONSENT TO PARTICIPATE?

You may withdraw from this study at any time without giving reasons. If you choose to enter the study and then decide to withdraw at a later time, you have the right to request the withdrawal of your information collected during the study. This request will be respected to the extent possible. Please note however that there may be exceptions where the data will not be able to be withdrawn for example where the data is no longer identifiable (meaning it cannot be linked in any way back to your identity) or where the data has been merged with other data. If you would like to request the withdrawal of your data, please let your study doctor know. If your participation in this study includes enrolling in any optional studies, or long-term follow-up, you will be asked whether you wish to withdraw from these as well.

13. WILL TAKING PART IN THIS STUDY BE KEPT CONFIDENTIAL?

Your confidentiality will be respected. However, research records and health or other source records identifying you may be inspected in the presence of the investigator or his designate, and

by NSERC, or the UBC Clinical Research Ethics Board for the purpose of monitoring the research. No information or records that disclose your identity will be published without your consent, nor will any information or records that disclose your identity be removed or released without your consent unless required by law. You will be assigned a unique study number as a participant in this study. This number will not include any personal information that could identify you (e.g., it will not include your Personal Health Number, SIN, or your initials, etc.). Only this number will be used on any research-related information collected about you during the course of this study, so that your identity will be kept confidential. Information that contains your identity will remain only with the Principal Investigator and/or designate. The list that matches your name to the unique study number that is used on your research-related information will not be removed or released without your consent unless required by law.

Your de-identified research data may be published or deposited into a publicly accessible location at the time of publication. This data could include sex, age, height, and weight. At no time will identifying information, such as your name, birth date or street address be included in such data. This means that other researchers may analyze the data for different reasons other than those described in this consent form. Once the data is made publicly available, you will not be able to withdraw your data. The extent of the risk of you being identified through public data is unknown, but currently appears to be low.

Your rights to privacy are legally protected by federal and provincial laws that require safeguards to ensure that your privacy is respected, and also give you the right of access to the information about you that has been provided to the sponsor and, if need be, an opportunity to correct any errors in this information. Further details about these laws are available on request to your study doctor.

14. WHAT HAPPENS IF SOMETHING GOES WRONG?

By signing this form, you do not give up any of your legal rights and you do not release the study doctor, participating institutions, or anyone else from their legal and professional duties. If you become ill or physically injured as a result of participation in this study, medical treatment will be provided at no additional cost to you. The costs of your medical treatment will be paid by your provincial medical plan.

In the unlikely event of a medical emergency during the study, immediate care will be provided by researchers with valid CPR and AED certification (Primary Contact: Jenna Benbaruj and co-investigators: Michael Leahy and Thora Rae) who will be present in the study area at all times. There is an automated emergency defibrillator and first aid supplies (including airway management material) in the study area and the distance to the nearest hospital emergency room is less than 1 km.

15. WHAT WILL THIS STUDY COST ME?

This study does not impose a cost on the participant. You will be reimbursed for parking (upon provision of a parking receipt).

16. WHO DO I CONTACT IF I HAVE QUESTIONS ABOUT THE STUDY DURING MY PARTICIPATION?

If you have any questions or desire further information about this study before or during participation, or if you experience any adverse effects, you can contact Jenna Benbaruj (phone: [redacted]; email: [redacted]) or Dr. William Sheel (phone: [redacted]).

17. WHO DO I CONTACT IF I HAVE ANY QUESTIONS OR CONCERNS ABOUT MY RIGHTS AS A SUBJECT?

If you have any concerns or complaints about your rights as a research subject and/or your experiences while participating in this study, contact the *Research Participant Complaint Line* in the University of British Columbia Office of Research Services by e-mail at *RSIL@ors.ubc.ca* or by phone at *604-822-8598* (Toll Free: 1-877-822-8598). Please reference the study number [H21-01579] when calling so the Complaint Line staff can better assist you.

Limb and inspiratory muscle metaboreflex activation in healthy males and females.

CONSENT FORM

My signature on this consent form means:

- I have read and understood the subject information and consent form.
- I have had sufficient time to consider the information provided and to ask for advice if necessary.
- I have had the opportunity to ask questions and have had satisfactory responses to my questions.
- I understand that all of the information collected will be kept confidential and that the results will only be used for scientific objectives.
- I understand that my participation in this study is voluntary and that I am completely free to refuse to participate or to withdraw from this study at any time.
- I understand that I am not waiving any of my legal rights as a result of signing this consent form.
- I understand that there is no guarantee that this study will provide any benefits to me.

I will receive a signed copy of this consent form for my own records.

I consent to participate in this study.

Subject's Signature	Printed Name	Date

Person Obtaining Consent	Printed Name and Study Role	Date

A.2 PAR-Q+ Form

CSEP approved Sept 12 2011 version

PAR-Q+

The Physical Activity Readiness Questionnaire for Everyone

Regular physical activity is fun and healthy, and more people should become more physically active every day of the week. Being more physically active is very safe for MOST people. This questionnaire will tell you whether it is necessary for you to seek further advice from your doctor OR a qualified exercise professional before becoming more physically active.

SECTION 1 - GENERAL HEALTH

	Please read the 7 questions below carefully and answer each one honestly: check YES or NO.	YES	NO
1.	Has your doctor ever said that you have a heart condition OR high blood pressure?	☐	☐
2.	Do you feel pain in your chest at rest, during your daily activities of living, OR when you do physical activity?	☐	☐
3.	Do you lose balance because of dizziness OR have you lost consciousness in the last 12 months? Please answer NO if your dizziness was associated with over-breathing (including during vigorous exercise).	☐	☐
4.	Have you ever been diagnosed with another chronic medical condition (other than heart disease or high blood pressure)?	☐	☐
5.	Are you currently taking prescribed medications for a chronic medical condition?	☐	☐
6.	Do you have a bone or joint problem that could be made worse by becoming more physically active? Please answer NO if you had a joint problem in the past, but it does not limit your current ability to be physically active. For example, knee, ankle, shoulder or other.	☐	☐
7.	Has your doctor ever said that you should only do medically supervised physical activity?	☐	☐

If you answered NO to all of the questions above, you are cleared for physical activity.

Go to Section 3 to sign the form. You do not need to complete Section 2.

- Start becoming much more physically active – start slowly and build up gradually.
- Follow the Canadian Physical Activity Guidelines for your age (www.csep.ca/guidelines).
- You may take part in a health and fitness appraisal.
- If you have any further questions, contact a qualified exercise professional such as a CSEP Certified Exercise Physiologist® (CSEP-CEP) or CSEP Certified Personal Trainer® (CSEP-CPT).
- If you are over the age of 45 yrs. and NOT accustomed to regular vigorous physical activity, please consult a qualified exercise professional (CSEP-CEP) before engaging in maximal effort exercise.

If you answered YES to one or more of the questions above, please GO TO SECTION 2.

Delay becoming more active if:

- You are not feeling well because of a temporary illness such as a cold or fever – wait until you feel better
- You are pregnant – talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the PARmed-X for Pregnancy before becoming more physically active OR
- Your health changes – please answer the questions on Section 2 of this document and/or talk to your doctor or qualified exercise professional (CSEP-CEP or CSEP-CPT) before continuing with any physical activity programme.

CSEP | SCPE
THE GOLD STANDARD IN EXERCISE SCIENCE AND PERSONAL TRAINING

SECTION 2 - CHRONIC MEDICAL CONDITIONS

Please read the questions below carefully and answer each one honestly: check YES or NO.			YES	NO
1.	Do you have Arthritis, Osteoporosis, or Back Problems?		☐ If yes, answer questions 1a-1c	☐ If no, go to question 2
	1a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	☐	☐
	1b.	Do you have joint problems causing pain, a recent fracture or fracture caused by osteoporosis or cancer, displaced vertebra (e.g., spondylolisthesis), and/ or spondylolysis/pars defect (a crack in the bony ring on the back of the spinal column)?	☐	☐
	1c.	Have you had steroid injections or taken steroid tablets regularly for more than 3 months?	☐	☐
2.	Do you have Cancer of any kind?		☐ If yes, answer questions 2a-2b	☐ If no, go to question 3
	2a.	Does your cancer diagnosis include any of the following types: lung/bronchogenic, multiple myeloma (cancer of plasma cells), head, and neck?	☐	☐
	2b.	Are you currently receiving cancer therapy (such as chemotherapy or radiotherapy)?	☐	☐
3.	Do you have Heart Disease or Cardiovascular Disease? This includes Coronary Artery Disease, High Blood Pressure, Heart Failure, Diagnosed Abnormality of Heart Rhythm		☐ If yes, answer questions 3a-3e	☐ If no, go to question 4
	3a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	☐	☐
	3b.	Do you have an irregular heart beat that requires medical management? (e.g. atrial fibrillation, premature ventricular contraction)	☐	☐
	3c.	Do you have chronic heart failure?	☐	☐
	3d.	Do you have a resting blood pressure equal to or greater than 160/90 mmHg with or without medication? (Answer YES if you do not know your resting blood pressure)	☐	☐
	3e.	Do you have diagnosed coronary artery (cardiovascular) disease and have not participated in regular physical activity in the last 2 months?	☐	☐
4.	Do you have any Metabolic Conditions? This includes Type 1 Diabetes, Type 2 Diabetes, Pre-Diabetes		☐ If yes, answer questions 4a-4c	☐ If no, go to question 5
	4a.	Is your blood sugar often above 13.0 mmol/L? (Answer YES if you are not sure)	☐	☐
	4b.	Do you have any signs or symptoms of diabetes complications such as heart or vascular disease and/or complications affecting your eyes, kidneys, and the sensation in your toes and feet?	☐	☐
	4c.	Do you have other metabolic conditions (such as thyroid disorders, pregnancy-related diabetes, chronic kidney disease, liver problems)?	☐	☐
5.	Do you have any Mental Health Problems or Learning Difficulties? This includes Alzheimer's, Dementia, Depression, Anxiety Disorder, Eating Disorder, Psychotic Disorder, Intellectual Disability, Down Syndrome)		☐ If yes, answer questions 5a-5b	☐ If no, go to question 6
	5a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	☐	☐
	5b.	Do you also have back problems affecting nerves or muscles?	☐	☐

Please read the questions below carefully and answer each one honestly: check YES or NO.			YES	NO
6.	Do you have a Respiratory Disease? This includes Chronic Obstructive Pulmonary Disease, Asthma, Pulmonary High Blood Pressure		☐ If yes, answer questions 6a-6d	☐ If no, go to question 7
	6a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	☐	☐
	6b.	Has your doctor ever said your blood oxygen level is low at rest or during exercise and/or that you require supplemental oxygen therapy?	☐	☐
	6c.	If asthmatic, do you currently have symptoms of chest tightness, wheezing, laboured breathing, consistent cough (more than 2 days/week), or have you used your rescue medication more than twice in the last week?	☐	☐
	6d.	Has your doctor ever said you have high blood pressure in the blood vessels of your lungs?	☐	☐
7.	Do you have a Spinal Cord Injury? This includes Tetraplegia and Paraplegia		☐ If yes, answer questions 7a-7c	☐ If no, go to question 8
	7a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	☐	☐
	7b.	Do you commonly exhibit low resting blood pressure significant enough to cause dizziness, light-headedness, and/or fainting?	☐	☐
	7c.	Has your physician indicated that you exhibit sudden bouts of high blood pressure (known as Autonomic Dysreflexia)?	☐	☐
8.	Have you had a Stroke? This includes Transient Ischemic Attack (TIA) or Cerebrovascular Event		☐ If yes, answer questions 8a-c	☐ If no, go to question 9
	8a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	☐	☐
	8b.	Do you have any impairment in walking or mobility?	☐	☐
	8c.	Have you experienced a stroke or impairment in nerves or muscles in the past 6 months?	☐	☐
9.	Do you have any other medical condition not listed above or do you live with two chronic conditions?		☐ If yes, answer questions 9a-c	☐ If no, read the advice on page 4
	9a.	Have you experienced a blackout, fainted, or lost consciousness as a result of a head injury within the last 12 months OR have you had a diagnosed concussion within the last 12 months?	☐	☐
	9b.	Do you have a medical condition that is not listed (such as epilepsy, neurological conditions, kidney problems)?	☐	☐
	9c.	Do you currently live with two chronic conditions?	☐	☐

Please proceed to Page 4 for recommendations for your current medical condition and sign this document.

PAR-Q+

If you answered NO to all of the follow-up questions about your medical condition, you are ready to become more physically active:

- It is advised that you consult a qualified exercise professional (e.g., a CSEP-CEP or CSEP-CPT) to help you develop a safe and effective physical activity plan to meet your health needs.
- You are encouraged to start slowly and build up gradually – 20-60 min. of low- to moderate-intensity exercise, 3-5 days per week including aerobic and muscle strengthening exercises.
- As you progress, you should aim to accumulate 150 minutes or more of moderate-intensity physical activity per week.
- If you are over the age of 45 yrs. and NOT accustomed to regular vigorous physical activity, please consult a qualified exercise professional (CSEP-CEP) before engaging in maximal effort exercise.

If you answered YES to one or more of the follow-up questions about your medical condition:

- You should seek further information from a licensed health care professional before becoming more physically active or engaging in a fitness appraisal and/or visit a or qualified exercise professional (CSEP-CEP) for further information.

Delay becoming more active if:

- You are not feeling well because of a temporary illness such as a cold or fever – wait until you feel better
- You are pregnant - talk to your health care practitioner, your physician, a qualified exercise profesional, and/or complete the PARmed-X for Pregnancy before becoming more physically active OR
- Your health changes - please talk to your doctor or qualified exercise professional (CSEP-CEP) before continuing with any physical activity programme.

SECTION 3 - DECLARATION

- You are encouraged to photocopy the PAR-Q+. You must use the entire questionnaire and NO changes are permitted.
- The Canadian Society for Exercise Physiology, the PAR-Q+ Collaboration, and their agents assume no liability for persons who undertake physical activity. If in doubt after completing the questionnaire, consult your doctor prior to physical activity.
- If you are less than the legal age required for consent or require the assent of a care provider, your parent, guardian or care provider must also sign this form.
- Please read and sign the declaration below:

I, the undersigned, have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that a Trustee (such as my employer, community/fitness centre, health care provider, or other designate) may retain a copy of this form for their records. In these instances, the Trustee will be required to adhere to local, national, and international guidelines regarding the storage of personal health information ensuring that they maintain the privacy of the information and do not misuse or wrongfully disclose such information.

NAME _____ DATE _____

SIGNATURE _____ WITNESS _____

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER _____

For more information, please contact:
Canadian Society for Exercise Physiology
www.csep.ca

KEY REFERENCES
1. Jamnik VJ, Warburton DER, Makarski J, McKenzie DC, Shephard RJ, Stone J, and Gledhill N. Enhancing the eectiveness of clearance for physical activity participation; background and overall process. APNM 36(S1):S3-S13, 2011.
2. Warburton DER, Gledhill N, Jamnik VK, Bredin SSD, McKenzie DC, Stone J, Charlesworth S, and Shephard RJ. Evidence-based risk assessment and recommendations for physical activity clearance; Consensus Document. APNM 36(S1):S266-s298, 2011.

The PAR-Q+ was created using the evidence-based AGREE process (1) by the PAR-Q+Collaboration chaired by Dr. Darren E. R. Warburton with Dr. Norman Gledhill, Dr. Veronica Jamnik, and Dr. Donald C. McKenzie (2). Production of this document has been made possible through financial contributions from the Public Health Agency of Canada and the BC Ministry of Health Services. The views expressed herein do not necessarily represent the views of the Public Health Agency of Canada or BC Ministry of Health Services.

CSEP | SCPE
THE GOLD STANDARD IN EXERCISE SCIENCE AND PERSONAL TRAINING

CSEP approved Sept 12 2011 version

A.3 Menstrual Cycle History Questionnaire

Menstrual History Questionnaire
Limb and inspiratory muscle metaboreflex activation in healthy males and females

Study ID # : _____

Age: _____

Height _____ Weight _____

1. Have your menstrual periods stopped permanently?
Please circle one.

a. NO – I am still menstruating.

b. YES - No menstrual periods, I have had menopause.

c. YES – I had menopause, but now I have periods
because I am taking medication (hormones).

d. NOT SURE

IF YES to either b or c:

2a. At what age did your menstrual periods stop?
Age = _____(years)

2b. Have you ever used estrogen replacement therapy (such as Premarin, Estraderm, Ogen, Estrace, etc.)? Please circle: yes or no

IF YES to a:

2b. At present which statement best describes your menstrual cycle?
___I'm still having regular periods: The date of my last period was: ___/___/_____
___My periods are irregular: The date of my last period was: ___/___/_____

3. Are you currently using any oral contraceptive (birth control pills) for any reason (birth control, acne, menstrual irregularity, etc.)?
Please circle: yes or no

If yes, please specify: _____

_____

A.4 Data Collection Forms

Limb and inspiratory muscle metaboreflex activation

in healthy males and females

Day #0 – Screening and Familiarization

Subject ID:_____

Sex:_____

Height:_____

Weight:_____

Age:_____

Day #1 – Relative Exercise Intensity

100% MIP =

60% MIP =

100% MVC =

_____% MVC =

Exercise bout order:

Blood Pressure Measurements (Systolic/Diastolic):

	Measurement 1	Measurement 2	Additional Measurement
Rest (pre-PTL)			
During PTL			
Rest (post-PTL)			
Rest (pre-HG)			
During HG	****No BP During Handgrip Exercise****		
Rest (post-HG)			

Additional Notes:

Limb and inspiratory muscle metaboreflex activation

in healthy males and females

Day #2 – Absolute Exercise Intensity

Subject ID: _____

100% MIP =

Exercise intensity =

100% MVC =

Exercise intensity =

Exercise bout order:

Blood Pressure Measurements (Systolic/Diastolic):

	Measurement 1	**Measurement 2**	**Additional Measurement**
Rest (pre-PTL)			
During PTL			
Rest (post-PTL)			
Rest (pre-HG)			
During HG	****No BP During Handgrip Exercise****		
Rest (post-HG)			

Additional Notes:

Limb and inspiratory muscle metaboreflex activation

in healthy males and females

Day #3 – Supplemental Day

Subject ID: _____

100% MVC =

Absolute exercise intensity =

Relative: _____ % MVC =

Exercise bout order:

Blood Pressure Measurements (Systolic/Diastolic):

	Measurement 1	**Measurement 2**	**Additional Measurement**
Rest (pre-HG bout #1)			
Rest (post-PECO bout #1)			
Rest (pre-HG bout #2)			
Rest (post-PECO bout #2)			

Additional Notes: