The effect of diaphragm fatigue on the multidimensional components of dyspnea and diaphragm EMG during exercise

by

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B.Sc., The University of British Columbia, 2016

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE AND POSTDOCTORAL STUDIES

(Rehabilitation Sciences)

THE UNIVERSITY OF BRITISH COLUMBIA

(Vancouver)

July 2019

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

Effects of diaphragm fatigue on the multidimensional components of dyspnea and diaphragm EMG during exercise.

submitted by Kyle Geoffrey P.J.M. Boyle in partial fulfillment of the requirements for

the degree of Master of Science

in Rehabilitation Sciences

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Abstract

Purpose: To determine the effect diaphragm fatigue (DF) has on the multidimensional components of dyspnea and diaphragm EMG (EMG_{di}) during exercise.

Methods: Sixteen healthy males (age=27, $\dot{V}O_{2Max}$=45.8 ± 9.8) underwent three study visits. Visit 1 comprised of an incremental cycle exercise test to determine maximal work rate. The following two visits involved a constant work rate (CWR) exercise test at an intensity equal to their gas exchange threshold (GET) plus 60% of the delta between GET and peak. One of the two CWR exercise tests was performed following pressure threshold loading (PTL) to induce DF, while the other served as a control. PTL involved inspiring to 60% of maximum transdiaphragmatic pressure (P_{di}) to overcome a weighted load in order to initiate inspiration. DF was assessed by measuring transdiaphragmatic pressure in response to cervical magnetic stimulation of the phrenic nerves. P_{di} and EMG_{di} were both assessed by the same esophageal balloon catheter. Breathing intensity, unpleasantness and leg discomfort ratings were assessed with the modified 0-10 category ratio Borg scale. Participants were also asked to select applicable breathing sensations during and after exercise. Peak dyspnea responses were assed via the Multidimensional Dyspnea Profile (MDP).

Results: Exercise performance decreased by 1.7 minutes in the pre-fatigue condition compared to control *(*p=0.04). There were no changes in breathing intensity and leg discomfort (p>0.05) throughout exercise. Breathing unpleasantness increased in the pre-fatigue condition by 0.2 (p=0.09), 0.6 (p=0.04), and 0.6 (p=0.04) units at all three of the dyspnea measurement points achieved by every participant during exercise. One additional time point achieved by fifteen of the sixteen participants increased by 0.9 units (p=0.03). There were no differences in EMG_{di}. EMG_{di} significantly correlated with intensity and unpleasantness ratings in both conditions (all p<0.001). There was a significant increase in the immediate perception domain of the MDP (p=0.04) during the pre-fatigue exercise test and feelings of anxiety and frustration trended higher in the pre-fatigue exercise test (p=0.08, p=0.06, respectfully) compared to the control condition.

Conclusion: DF in isolation may not be sufficient enough to alter EMG_{di}, but does appear to limit exercise by increasing the sensation of breathing unpleasantness.

Lay Summary

Like all muscles, the primary muscle we use to breathe can become fatigued during exercise. However, little is known about the sensory consequences of this fatigue, specifically how it relates to breathing sensations. The purpose of this thesis was to explore what effect fatiguing the primary breathing muscle has on the intensity and unpleasantness of one's breathing, as well as the specific breathing sensations and emotions experienced during exercise (i.e. "work and effort" or "unsatisfied inspiration"). This thesis also explored if these sensations could be attributed to an increase in muscle activation following fatigue. The results of this thesis show that breathing muscle fatigue may not impact the intensity of breathing, but does increase the feeling of unpleasantness during exercise.

Preface

This document is the work of MSc. Candidate Kyle Geoffrey P.J.M. Boyle, under the supervision of Dr. Jordan A. Guenette. Experimental design and setup were a joint effort of Dr. Jordan A. Guenette and Kyle Geoffrey P.J.M. Boyle. Kyle Geoffrey P.J.M. Boyle collected and analyzed the data at the Cardiopulmonary Exercise Physiology Laboratory at St. Paul's Hospital in Vancouver, British Columbia, Canada.

Experiments obtained ethical approval from the University of British Columbia and Providence Health Care Research Institute Ethics Board (UBC-PHC REB Number: H17-00696).

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List of Abbreviations

ANOVA	Analysis of variance
CMAP	Compound muscle action potential
CMS	Cervical magnetic stimulation
CWR	Constant work rate
DF	Diaphragm fatigue
EELV	End-expiratory lung volume
EILV	End-inspiratory lung volume
EMG	Electromyography
EMG_{di}	Electromyography of the diaphragm
EMG_{sca}	Electromyography of the scalene
EMG_{scm}	Electromyography of the sternocleidomastoid
f_b	Breathing frequency
FEV_1	Forced expiratory volume in one second
FRC	Functional residual capacity
GET	Gas exchange threshold
HR	Heart rate
IC	Inspiratory capacity
MSNA	Muscle sympathetic nerve activity
MDP	Multidimensional Dyspnea Profile
P_{di}	Transdiaphragmatic pressure
$P_{di,tw}$	Transdiaphragmatic twitch pressure
PCO_2	Partial pressure of carbon dioxide

$P_{ET}CO_2$	End-tidal partial pressure of carbon dioxide
P_e	Esophageal pressure
P_{ga}	Gastric pressure
PTL	Pressure threshold loading
PTP	Pressure-time product
PTP_{di}	Pressure-time product of the diaphragm
rpm	Revolutions per minute
SD	Standard deviation
S_pO_2	Peripheral oxygen saturation
T_I/T_{TOT}	Inspiratory duty cycle
TTE	Time to exhaustion
TTI	Tension time index
$\dot{V}CO_2$	Carbon dioxide production
$\dot{V}_E$	Minute ventilation
$\dot{V}_E/VCO_2$	Ventilatory equivalent for carbon dioxide
$\dot{V}_E/VO_2$	Ventilatory equivalent for oxygen
$\dot{V}O_2$	Oxygen consumption
$\dot{V}O_{2max}$	Maximal oxygen consumption
V_t	Tidal volume
W	Watt

Acknowledgements

First and foremost, I would like to thank my supervisor, Dr. Jordan Guenette, for his encouragement, enthusiasm and assistance throughout this project. The Cardiopulmonary Exercise Physiology Laboratory provides a second to none training environment that promotes collaboration and scientific exploration. Dr. Guenette displays continuous mentorship and commitment to his students, which is reflected in the high level and standard of work from all members of the lab.

I would like to thank my thesis committee members, Dr. Bill Sheel and Dr. Michael Koehle, who have both put in time to assist me not only with this project, but securing an opportunity following my time as a student. It has been a great pleasure to spend time with both individuals and it is easy to see why current and former students speak so highly of both individuals.

Thank you to the members of the Cardiopulmonary Exercise Physiology Laboratory for creating a positive and hardworking culture within the laboratory. Each member has contributed dramatically to my learning within the last few years and has become a friend for life.

I would like to acknowledge the participants of my thesis. Thank you for your commitment to the project and your positive attitudes throughout.

Lastly, I would like to thank my family and friends for their support throughout my time as a graduate student. You have all been a great source of encouragement and inspiration. I love you all.

Dedication

For John McPhee.

Chapter 1: Literature Review

Dyspnea is defined as "a subjective experience of breathing discomfort that consists of qualitatively distinct sensations that vary in intensity" (Parshall *et al.*, 2012). Breathing discomfort or breathlessness is a major source of hospitalization (Ong *et al.*, 2005) and thus, is not only a health concern, but also a large economic burden (Ehteshami-Afshar *et al.*, 2016).

The physiological mechanisms of dyspnea are complex and multifactorial. Accumulating evidence shows that exertional dyspnea can largely be explained by an increased conscious awareness of neural respiratory drive (NRD), which may be indirectly measured using diaphragmatic electromyography (EMG_{di}) (Luo *et al.*, 1999; Luo *et al.*, 2001; Sinderby *et al.*, 2001; Luo *et al.*, 2011; Schaeffer *et al.*, 2014; Faisal *et al.*, 2016). When the demand for ventilation is not matched by the respiratory muscles, which occurs during high intensity exercise, dyspnea sensations increase (Parshall *et al.*, 2012). In the 1960's, this paradigm was coined "length-tension inappropriateness" (Campbell & Howell, 1963), and has since been referred to by others as "neuromechanical uncoupling" (Lougheed *et al.*, 1993; O'Donnell *et al.*, 2007; Jensen *et al.*, 2009). Neuromechanical uncoupling represents a state of imbalance between higher cortical efferent output of the brain and afferent neuromechanical feedback of the respiratory system (O'Donnell & Webb, 2008; Parshall *et al.*, 2012).

One afferent pathway to be further explored is the neuromechanical afferent feedback from the diaphragm. It is known that diaphragmatic fatigue (DF) occurs in the majority of healthy humans following high-intensity constant work rate exercise at ≥85% of maximum (Johnson *et al.*, 1993; Babcock *et al.*, 1996; Guenette *et al.*, 2010). This provides indirect evidence to the possibility that afferent feedback from DF could be contributing, at least in part, to the increased levels of EMG_{di} and dyspnea during exercise. Indeed, previous research has

shown that pre-fatiguing the diaphragm increases dyspnea intensity ratings during loaded breathing (Gandevia *et al.*, 1981; McConnell & Romer, 2004) and whole body exercise (Mador & Acevedo, 1991b; Sliwinski *et al.*, 1996; McConnell & Romer, 2004). However, these studies did not measure EMG_{di} and dyspnea was not evaluated as a multidimensional experience.

1.1 The Diaphragm

1.1.1 Anatomy and Morphology

The diaphragm is a striated skeletal muscle that separates the thoracic cavity from the abdominal cavity (De Troyer & Loring, 1986; De Troyer & Estenne, 1988). In healthy individuals, the diaphragm takes on the shape of an elliptical cylinder capped by a dome (De Troyer & Estenne, 1988), giving it the ability to increase the volume of the chest and inflate the lungs as it contracts (Poole *et al.*, 1997). The diaphragm is considered to be composed of three components rather than just a single muscle (De Troyer *et al.*, 1981). Two muscular components collectively called the hemi-diaphragms are composed of a crural and costal portion (De Troyer & Estenne, 1988; Poole *et al.*, 1997). Each hemi-diaphragm inserts into the third component, a non-contractile central tendon (De Troyer & Estenne, 1988; Poole *et al.*, 1997). The crural muscular fibres arise from the lumbar vertebrae L_1-L_3 and the arcuate ligament (De Troyer & Estenne, 1988; Poole *et al.*, 1997). Meanwhile, the costal fibres arise from ribs 7-12 (De Troyer & Estenne, 1988). The costal fibres run cranially to directly appose the inner aspect of the lower ribcage creating the "zone of apposition" (Mead, 1979).

1.1.2 Function and Mechanics

In ancient Greece the function of the diaphragm interested both physicians and philosophers (Fritts, 1976). Many believed the diaphragm was a metaphysical structure, while others believed the diaphragm served a physiological function (Fritts, 1976; De Troyer & Loring, 1986). It was the work of Galen in which the diaphragm became known as the primary muscle for inspiration (Fritts, 1976; De Troyer & Loring, 1986; Otis, 1986). Specifically, the diaphragm works rhythmically with muscles of the ribcage to displace the chest and pump air into the body (De Troyer & Loring, 1986; Poole *et al.*, 1997).

During inspiration the muscle fibres of the diaphragm shorten and develop tension (Poole *et al.*, 1997). As a result, the axial length of the apposed diaphragm decreases, displacing the dome caudally in a "piston-like" mechanism (De Troyer & Loring, 1986; Poole *et al.*, 1997). The descent of the diaphragm dome has three primary results. First, the thoracic cavity expands causing a reduction in pleural pressure, allowing air to flow into the alveoli and increase lung volume. Second, the pressure of the abdominal cavity increases pushing the abdominal wall outward. Third, the ribcage becomes displaced upwards and outwards (De Troyer & Loring, 1986; Poole *et al.*, 1997).

The tension and force a muscle can generate is dependent on its initial length. The optimal force generating length of the diaphragm is between functional residual capacity (FRC) and residual volume (RV). During exercise there is an increase in expiratory muscle activation, reducing the end-expiratory lung volume (EELV) (Henke *et al.*, 1988). A consequence of reduced EELV is that the diaphragm lengthens to its optimal length (Road *et al.*, 1986; Smith & Bellemare, 1987), thus increasing its force output during exercise.

1.1.3 Neural Innervation

The early work of Galen showed that severing the phrenic nerves in pigs left the diaphragm paralyzed and the intercostal muscles unaffected, indicating that the motor control of the diaphragm was controlled by the phrenic nerves (Fritts, 1976; De Troyer & Loring, 1986). Research today has shown that each hemi-diaphragm is innervated exclusively by a single phrenic nerve (Sant'Ambrogio *et al.*, 1963; Frazier & Revelette, 1991). The two phrenic nerves exit the spinal cord between cervical roots C_3-C_5 and pass down between the lungs to innervate the left or right hemi-diaphragm (Muller Botha, 1957; Frazier & Revelette, 1991).

The diaphragm contains few proprioceptive afferents including group I and II fibres that sense changes in the length and stretch of the muscle. The majority of the proprioceptive afferents arise from Golgi tendon organs opposed to muscle spindles (Road, 1990). Alternatively, the diaphragm is rich in small diameter myelinated (group III) and unmyelinated (group IV) afferents (Frazier & Revelette, 1991) that are activated by mechanical and chemical stimuli. When diaphragm afferents are stimulated there are various sensory and physiological effects. For example, Campbell and Howell (1963) proposed that when sensory afferent receptors detect a disruption in the usual length-tension relationship as a result of ventilatory loading, the resulting afferent feedback plays a sufficient role in producing the sensations associated with the increased loads. Additionally, when small diameter afferents of the respiratory muscles are stimulated, there is an increase in efferent sympathetic nerve activity (Dempsey *et al.*, 2002). The increase in efferent activity is capable of producing widespread vasomotor outflow, contributing to the respiratory muscle metaboreflex (Dempsey *et al.*, 2002).

1.1.4 Blood Flow

Blood flow to the diaphragm is provided by the phrenic arteries. Inability to adequately perfuse the diaphragm would ultimately result in diaphragmatic fatigue and ventilatory failure. As such, diaphragm blood flow is tightly controlled by vasomotor tone, influenced by both central and local vascular mechanisms (Laughlin *et al.*, 1996). It has been shown that when inspiratory resistance increases, blood flow to the diaphragm also increases (Rochester & Bettini, 1976). However, upon reaching a critical pressure-time threshold, blood flow to the diaphragm is inhibited (Bellemare *et al.*, 1983; Buchler *et al.*, 1985a; Buchler *et al.*, 1985b).

1.1.5 Histochemical Composition

The diaphragm is the only skeletal muscle in the body thought to be essential to sustain life (Poole *et al.*, 1997). Throughout the lifespan of an individual, the diaphragm must remain active to pump air into the body (De Troyer & Loring, 1986; Poole *et al.*, 1997). Failure to continuously contract and relax would result in alveolar hypoventilation, hypercapnia and acidosis (Macklem, 1980). Thus, the diaphragm must have a large endurance capacity. Fortunately, unique to the diaphragm is its muscle fibre's ability to spend 45% of the day contracting (Sieck, 1994). This feat is accomplished in part due to the histochemical composition of the diaphragm.

Based on early work by Dubowittz (1960), Brooke and Kaiser (1970) classified three primary muscle types based on histochemical and morphological properties. Due to these histochemical properties, the fibre types can also be categorized by the rate each fatigues (Burke *et al.*, 1971). The three fibre types include 1) slow-twitch oxidative or *type I*; 2) fast-twitch oxidative glycolytic or *type IIa*; and 3) fast-twitch glycolytic *type IIb* (Brooke & Kaiser, 1970).

The fibre type most resistant to fatigue is slow-twitch oxidative fibres due to their vast supply of capillaries, mitochondria and myoglobin. Slow-twitch oxidative fibres constitute ~45% of the diaphragm fibre composition (Levine *et al.*, 1997). Fast-twitch oxidative fibres are also resistive to fatigue and contribute ~39% of the diaphragm histochemical composition (Levine *et al.*, 1997). The least fatigue resistant fibre type is fast-twitch glycolytic fibres because of their high glycolytic content, fast shortening velocity and low oxidative capacity. Fast-twitch glycolytic fibres comprise ~16% of the diaphragm composition (Levine *et al.*, 1997). The high oxidative to glycolytic fibre composition ratio gives the diaphragm the incredible endurance capacity to perform its lifelong function of inspiration.

1.1.6 Definition of Fatigue

Skeletal muscle fatigue is defined as a "reduction in muscle force or pressure generation resulting from activity under load that is reversible by rest" (NHLBI, 1990). The two types of fatigue include central and peripheral fatigue. Central fatigue is a "reduction in voluntary force output as a result of a reduction in motor output from the central nervous system" (Carroll *et al.*, 2017). Peripheral fatigue is a reduction in force due to "processes distal to the neuromuscular junction" (Carroll *et al.*, 2017). Peripheral fatigue can be further categorized as high and low-frequency peripheral fatigue. High-frequency fatigue is characterized by loss of force during high-frequency stimulation (usually 50-100 Hz) and rapid recovery (Jones, 1996). Low-frequency peripheral fatigue is characterized by a reduction in force during low-frequency stimulation (1-30 Hz) and prolonged recovery time (Jones, 1996). High and low-frequency peripheral fatigue do not necessarily occur in isolation, especially when considering the work required to sustain exercise or loaded breathing.

1.1.7 Overview of Diaphragm Fatigue

As previously discussed, the diaphragm is anatomically and physiologically designed to sustain prolonged bouts of aerobic work. Gandevia *et al.* (1983) compared the endurance properties of the respiratory muscles with the flexors and extensors of the elbows in healthy male volunteers. The authors found that during multiple short sustained contractions, all tested muscles displayed a decrease in force production. However, the inspiratory muscles displayed the ability to completely recover during 1-minute of rest compared to the muscles of the elbow and the expiratory muscles. Additionally, the authors found that during a series of repeated maximal contractions, the inspiratory muscles showed less fatigue than the muscles of the elbow.

Despite being highly resistant to fatigue, the diaphragm is not immune. Roussos and Macklem (1977) found that a target transdiaphragmatic pressure (P_{di}) during inspiration of 40% of maximum P_{di} could not be sustained longer than one hour. In addition, any P_{di} below this critical pressure ($P_{di,crit}$) could be sustained for much longer without evidence of fatigue. Further work by Bellemare and Grassino (1982a) proposed that the diaphragm would fatigue more rapidly with an increase in the ratio of inspiratory time (T_I) to total breathing cycle duration (T_{tot}), and that there may be a range of $P_{di,crit}$ depending on the adopted T_I/T_{tot} (duty cycle). This relationship is called the Pressure-Time index of the diaphragm and is defined by the following equation:

$$\mathbf{PT_{di} = (P_{di}/P_{di,max})(T_I/T_{tot})}$$

The hypothesis that the diaphragm would fatigue more rapidly with a decreased T_I/T_{tot} is based on the idea that the diaphragm benefits from prolonged recovery time (expiration), similar to that seen in the muscles of the hand during hand grip maneuvers (Park & Rodbard, 1962;

Rodbard & Pragay, 1968). Bellemare and Grassino (1982a) found that when individuals primarily breathe using their diaphragm, the critical PT_{di} is 0.15-0.18. When individuals breathe to a PT_{di} higher than the critical value, task failure occurs rapidly. Fatigue is believed to occur with increased P_{di} due to a reduction of blood flow to the diaphragm. A prolonged duty cycle decreases the time of muscle relaxation and therefore perfusion time, causing diaphragm ischemia. In sum, combining prolonged duty cycles with high pressures, the rising abdominal pressures compress the phrenic arties, resulting in decreased diaphragm blood flow and ischemia as shown in anesthetized dogs (Bellemare *et al.*, 1983; Buchler *et al.*, 1985a; Buchler *et al.*, 1985b).

1.1.8 Evaluation of Diaphragm Fatigue

"Pre-fatigue" studies are experiments in which the respiratory muscles are systematically fatigued as an intervention, giving researchers the ability to explore the effect of fatigue on various dependent variables. Accurately measuring the occurrence of DF is critical in pre-fatigue studies. The ATS/ERS statement on respiratory muscle testing (2002) outlines important considerations when testing for DF in humans. Briefly, a single measurement of force is not adequate to detect fatigue. Instead, muscle force generating capability must be shown to fall over time by serial measurements. Additionally, for fatigue to be detected, it must be demonstrated that force output returns to pre-intervention levels following a rest period. The aforementioned distinguishes fatigue from muscle weakness, in which force is only shown to decrease at a single point in time, and muscle injury, in which force reduction does not improve following a rest period. There are various ways to measure the occurrence of DF, each with its own advantages, disadvantages and limitations. Techniques to measure DF include 1) breathing

pattern; 2) volitional maneuvers and maximal pressures; 3) electromyography (EMG); and 4) response to external stimulation.

1.1.8.1 Breathing Pattern

The technique of using breathing pattern to assess DF is based on the rationale that high breathing frequency and low tidal volume are common occurrences during respiratory failure, which may potentially be associated with respiratory muscle fatigue (American Thoracic Society/European Respiratory, 2002). The technique is advantageous in that measuring breathing frequency and tidal volume is non-invasive and a common technique in routine spirometry. However, rapid shallow breathing is more likely a consequence of increasing respiratory muscle workload (Tobin *et al.*, 1986) and not directly fatigue (Mador & Tobin, 1992).

1.1.8.2 Volitional Maneuvers

Volitional maneuvers producing maximal static inspiratory or expiratory, sniff, and transdiaphragmatic pressures is another technique used to measure fatigue. It has been shown that there is a decrease in volitional pressure generation during loaded breathing (Aldrich, 1988) and exercise (Loke *et al.*, 1982; Chevrolet *et al.*, 1993), which may be a result of respiratory muscle fatigue. The advantage of the volitional maneuver technique is that it is non-invasive (excluding measuring transdiaphragmatic pressure); however, the technique greatly depends on the motivation of the participant, and a maximal maneuver is difficult to obtain in clinical populations (American Thoracic Society/European Respiratory, 2002). Additionally, maximal

maneuvers are associated with high neuronal firing frequency, and thus may not reflect low-frequency fatigue (American Thoracic Society/European Respiratory, 2002).

1.1.8.3 Electromyography

The power spectrum of recorded EMG signals have been shown to shift to lower frequencies during fatigue (Gross *et al.*, 1979). Diaphragm EMG is recorded either by surface electrodes or using a multi-pair esophageal electrode catheter. Despite shifts in the EMG power spectrum having been shown during both exercise (Pardy & Bye, 1985) and loaded breathing (Gandevia *et al.*, 1981; Bower *et al.*, 1984), there are a number of limitations to this technique. First, the cause of EMG power spectral shifts can be caused by a number of mechanisms more associated with neural or sarcolemmal events rather than at the level of the sacromeres (Sieck & Fournier, 1990), including a decrease in motor unit discharge rate, synchronization of motor units firing and slowing of muscle fibre conduction velocity (De Luca, 1984). Second, Moxham *et al.* (1982) has shown that EMG power spectrum shifts do not correlate with mechanical measures of fatigue. Lastly, signal to noise ratio, crosstalk and position can affect the EMG power spectrum (Aldrich *et al.*, 2002; Luo *et al.*, 2008).

1.1.8.4 Response to External Stimulation

With fatigue being defined as a reduction in muscle force or pressure generation (NHLBI, 1990), the pressure-frequency relationship of the diaphragm has been used to assess DF following response of the diaphragm to stimulation. The pressure the diaphragm can develop in response to stimulation is assessed using esophageal and gastric balloons that measure esophageal and gastric pressures (P_e and P_g), respectively (Moxham *et al.*, 1981). As the

diaphragm contracts, P_e decreases with a simultaneous rise in P_g. The difference between the two pressures represents P_{di}.

The phrenic nerves exclusively innervate the diaphragm, as such, a supramaximal stimulation of the phrenic nerves results in a maximal contraction that eliminates motivation as a contributing factor (American Thoracic Society/European Respiratory, 2002). The phrenic nerves can be stimulated electrically and magnetically; however, magnetic stimulation is better tolerated by participants. Magnetic stimulation is caused by producing magnetic pulses that, when passed through neural tissue, elicit an electrical current and a compound muscle action potential (CMAP or M-wave) if depolarization threshold is reached (Hovey & Jalinous, 2006). Phrenic nerves can be magnetically stimulated by unilateral and bilateral stimulation (Mills *et al.*, 1996), anterior pre-sternal stimulation (Polkey *et al.*, 2000), and cervical stimulation (Similowski *et al.*, 1989). Recently, it has been shown that cervical magnetic stimulation (CMS) serves as a reliable method to evaluate phrenic nerve stimulation by assessing the CMAP (Welch *et al.*, 2017).

1.1.9 Causes and Time Course of Exercise Induced Diaphragm Fatigue

In a study conducted by Johnson et al (1993), it was found that the diaphragm fatigues at constant work rate exercise of ≥85% of maximum. In addition, it has been shown that DF is mitigated when individuals exercise with a mechanical ventilator (Babcock *et al.*, 2002). This provides evidence that the diaphragm fatigues, in part, due to the substantial ventilatory work and force output needed to sustain heavy exercise. However, the high diaphragmatic power output during exercise may not be the only contributor to DF. Babcock et al. showed that when individuals mimicked the breathing pattern (matched P_{di}, frequency and duration) achieved

during exercise while at rest, there was no evidence of DF. The findings suggest that the ventilatory demand of whole-body exercise, by itself, is not sufficient enough to cause DF. Indeed, other factors contributing to exercise induced DF include metabolite accumulation (Fregosi & Dempsey, 1986) and competition for blood flow with the locomotor muscles (Harms *et al.*, 1997; Harms *et al.*, 1998).

Questions as to when DF occurs during exercise has been raised, with contradictory outcomes arising as a result. For instance, Kabitz *et al.* (2007) has suggested that DF is only present at the cessation of exercise, while others have proposed that DF occurs early in exercise and that are no additional decreases in fatigue as exercise continues towards peak (Walker *et al.*, 2011). Most recently, Archiza *et al.* (2018) and colleagues explored the temporal characteristics of DF by evaluating P_{di} at 100, 75, and 50% of TTE in healthy males. It was shown that DF occurs later in exercise than what was proposed by Walker *et al.* (2011) and that the level of fatigue is proportional to the cumulative work of breathing (WOB).

1.1.10 Consequences of Diaphragm Fatigue

1.1.10.1 Exercise Performance

"Pre-fatiguing" the respiratory muscles prior to whole body exercise has been used to determine the impact of respiratory muscle fatigue on exercise performance. Mador and Acevedo (1991b) explored the effect of respiratory muscle fatigue on performance by having individuals breathe against an inspiratory load at 80% of their maximal mouth pressure (P_m) until task failure, prior to performing whole body cycle exercise at 90% of their maximum wattage. Performance fell from 311±96 seconds to 238±69 seconds when compared to exercise without prior fatigue. There are a number of limitations to consider for all "pre-fatigue" studies that will

be discussed in the *Limitations* section of this thesis; however, other investigators have shown both a decrease in exercise time (Martin *et al.*, 1982; Harms *et al.*, 2000; Wuthrich *et al.*, 2013; Welch *et al.*, 2018a) and no difference (Sliwinski *et al.*, 1996) following pre-fatigue of the diaphragm. It has been proposed that exercising with a fatigued diaphragm will decrease performance because of an increased metaboreflex and increased perception of dyspnea (Romer & Polkey, 2008).

1.1.10.2 Respiratory Muscle Metaboreflex

The most likely mechanism of respiratory muscles decreasing exercise performance is the respiratory muscle metaboreflex (Romer & Polkey, 2008; Sheel & Romer, 2012). During high-intensity contractions of the respiratory muscles against resistive loads to the point of fatigue during rest, there is a time-dependent increase in muscle sympathetic nerve activity (MSNA) in the leg (St Croix *et al.*, 2000). The increase in MSNA is accompanied by a decrease in limb vascular conductance and blood flow (Sheel *et al.*, 2001; Sheel *et al.*, 2002). The increase in MSNA is caused by small diameter afferents of the respiratory muscles when stimulated mechanically or chemically.

There is evidence that increasing inspiratory effort via resistors decreases limb vascular conductance and blood flow, while the opposite effect occurs with mechanical ventilation (Harms *et al.*, 1997). During exercise, a decrease in blood flow and thus O_2 to the locomotor muscles would result in increased locomotor muscle fatigue and decreased performance. Indeed, Romer *et al.* (2006) showed an increase in quadriceps fatigue and perception of leg discomfort when individuals exercised while inspiring against resistors. In contrast, participants exercising with a proportional assist ventilator showed lower levels of quadriceps fatigue and decreased

perception of leg discomfort (Romer *et al.*, 2006). There is recent evidence that the decreased blood flow to the legs is redirected to the accessory respiratory muscles (Dominelli *et al.*, 2017), and it is possible that blood flow may also be redistributed to the diaphragm as well. As such, an increase in respiratory muscle work from high-intensity exercise, and competition for blood flow during exercise is partially responsible for locomotor muscle fatigue and reductions in exercise performance.

1.2 Dyspnea: The Sensory and Affective Dimensions

As defined previously, "dyspnea is a subjective experience of breathing discomfort that consists of qualitatively distinct sensations that vary in intensity" (Parshall *et al.*, 2012). Similar to the experience of pain, dyspnea was also previously regarded as unidimensional (Banzett *et al.*, 2015), commonly regarded as a single sensation of increased work and effort (Altose *et al.*, 1985). As such, researchers would simply record breathlessness on a 0-10 visual analogue scale (Gift, 1989) and not take into account the sensory dimensions, intensity and emotional response to the symptom (Banzett *et al.*, 2015). Research has since progressed to show that dyspnea is in fact multidimensional. Specifically, the literature suggests that 1) dyspnea consists of distinct sensory qualities caused by multiple afferent pathways; 2) these sensations may or may not occur in isolation; and 3) these sensations vary in their intensity, unpleasantness and emotional significance (Parshall *et al.*, 2012).

1.2.1 Measuring Dyspnea

According to the most recent American Thoracic Society (ATS) statement on dyspnea (2012), many researchers still use a single measurement approach when measuring dyspnea and

do not indicate whether the scale used is measuring the affective or sensory dimension. In order to fully understand dyspnea as a symptom, it is important that real time dyspnea measurements include all of its multidimensional components. Specifically, dyspnea measurement should explicitly include 1) sensory-perceptual; 2) affective distress; and 3) the impact and burden of dyspnea in clinical populations (Parshall *et al.*, 2012).

The sensory-perceptual dimension of dyspnea refers to what breathing "feels like" to the individual (Parshall *et al.*, 2012). Specifically, the sensory-perceptual dimension is comprised of the intensity of breathing discomfort, and the qualitative sensations experienced by the individuals. This intensity component is commonly evaluated using validated scales (e.g. the modified Borg (1982) scale), while the sensory qualities are evaluated by having the individual select the most appropriate qualitative dyspnea descriptors from a list (e.g. "My breathing feels heavy" and "I cannot get enough air in") (Simon *et al.*, 1989; Simon *et al.*, 1990; Elliott *et al.*, 1991; Mahler *et al.*, 1996). Simon and colleagues (1989) were among the first to systematically explore the qualitative descriptors of dyspnea by having individuals select appropriate descriptors from a list of 19 descriptors during breath holding, carbon dioxide (CO_2) inhalation, breathing against a resistive load, exercise, and other stimuli. The original list of 19 descriptors was compiled by asking individuals with various pulmonary and cardiac diseases who complained of shortness of breath to "describe the sensation(s) associated with their uncomfortable awareness of breathing" (Simon *et al.*, 1989). Lists of descriptors used in research today may vary among research laboratories; however, each descriptor typically falls under the category of 1) increased work or effort; 2) chest tightness; 3) unsatisfied inspiration or air hunger; and 4) unsatisfied expiration (O'Donnell *et al.*, 1997; Lansing *et al.*, 2009)

The affective dimension of dyspnea refers to how distressing or unpleasant breathing feels, as well as the emotional response to the symptom (Parshall *et al.*, 2012). The unpleasantness of dyspnea is commonly measured by using validated single item scales, such as the Borg Scale (Borg, 1982). While both the intensity and unpleasantness are measured using the same scale, the two dimensions are differentiated using standardized scripts. The emotional responses to dyspnea, for example, an increase in fear or anxiety, are measured using multi-item scales (Carrieri-Kohlman *et al.*, 2001; Carrieri-Kohlman *et al.*, 2010). One instrument to measure the emotional response to dyspnea is the Multi-Dimensional Dyspnea Profile (MDP) (Banzett *et al.*, 2015). The MDP was designed as an instrument to record all of the dimensions of dyspnea in a clinical or research setting. The MDP uses a series of "simple emotion scales" to record an individual's depression, anxiety, frustration, anger and fear resulting from their breathlessness from 0-10 (Banzett *et al.*, 2015).

Multiple studies have explored whether or not individuals can discern between the affective and sensory dimensions of dyspnea at rest (von Leupoldt, 2005; Banzett *et al.*, 2008; Wan *et al.*, 2009). For example, von Leupoldt (2005) explored whether healthy individuals could discern between the intensity (sensory dimension) and unpleasantness (affective dimension) during resistive breathing of increasing magnitude. The results showed that as dyspnea increased with increasing resistive loads, the perceived unpleasantness increased stronger than the perceived intensity, indicating that individuals can discern between the two dimensions (von Leupoldt, 2005). Similar research has shown that individuals can discern between the affective and sensory dimensions of dyspnea during CO_2 rebreathing (Wan *et al.*, 2009), and between sensory qualities (Banzett *et al.*, 2008), specifically that air hunger is more unpleasant than increased work and effort.

A few studies have also explored if individuals can discern between the affective and sensory components of dyspnea during exercise (Wilson & Jones, 1991; Carrieri-Kohlman *et al.*, 1996; Carrieri-Kohlman *et al.*, 2001; Carrieri-Kohlman *et al.*, 2010). For example, Wilson and Jones (1991) investigated whether healthy individuals were able to differentiate between "intensity" of their breathlessness and the amount of "distress" it evoked during cycling. Both intensity and distress were measured using a 0-10 modified Borg scale (Borg, 1982), and both dimensions were properly described before exercise. The slope of intensity/minute ventilation ($\dot{V}_E$) increased significantly more than distress/$\dot{V}_E$, indicating that at a given ventilation, the subjects perceived a lower level of distress than intensity. Other research groups have shown that individuals in clinical populations can also discern between the affective and sensory dimension of dyspnea during exercise (von Leupoldt *et al.*, 2007; Carrieri-Kohlman *et al.*, 2010). Carrieri-Kohlman and colleagues (2010) reported that during incremental treadmill and 6-minute walk tests, individuals with chronic obstructive pulmonary disease (COPD) reported higher levels of intensity compared to both dyspnea-related distress and dyspnea-related anxiety for any given ventilation. Another study conducted by the same group also showed that individuals with COPD reduced their dyspnea related anxiety scores after 12 weeks of supervised exercise training, while their dyspnea intensity scores remained the same (Carrieri-Kohlman *et al.*, 2001). The above studies provide direct evidence that healthy participants and patients with COPD can discern between the affective and sensory dimensions of dyspnea.

1.2.2 Exercise and Dyspnea

In healthy individuals, the respiratory system is regarded as overbuilt for exercise, with maximal exercise being limited by cardiovascular and locomotor muscle constraints (Dempsey,

1986). In fact, most healthy individuals select leg discomfort opposed to breathing discomfort as the primary symptom limiting exercise (Killian *et al.*, 1992; Hamilton *et al.*, 1996; Jones & Killian, 2000). During exercise the respiratory system must fulfil the role of matching alveolar ventilation with muscle metabolic demand (Sheel & Guenette, 2008). Specifically, ventilation must increase in proportion to metabolic requirements in order to compensate for the increased O_2 consumption and CO_2 production, as well compensate for the exercise-induced blood lactate acidosis. The respiratory system must match ventilation to metabolic demand while also 1) minimizing the work of breathing; 2) preserving the relationship between central respiratory motor drive and the mechanical response of the respiratory system (neuromechanical coupling); 3) minimizing dyspnea (Jensen *et al.*, 2009); and 4) generating large intrathoracic pressures without promoting respiratory muscle fatigue. The matching of the respiratory system to the demand of exercise is achieved by tightly controlled output from the respiratory centres of the brainstem, the NRD.

1.2.3 Neurophysiology of Exertional Dyspnea

The current theory is that exertional dyspnea results from a conscious awareness of increased NRD (Jensen *et al.*, 2016). When drive is matched by the mechanical response of the respiratory system, dyspnea intensity increases proportionally to increasing drive (Jensen *et al.*, 2009). Therefore, during exercise in healthy individuals, dyspnea is likely to increase with indices of central motor command including 1) ventilation, 2) absolute power output, 3) contractile respiratory muscle effort (tidal esophageal pressure swings expressed as % of maximum esophageal pressure obtained during a maximal maneuver ($\Delta P_e/P_{Imax}$)) (Killian *et al.*, 1984; Leblanc *et al.*, 1988; Killian *et al.*, 1992; O'Donnell *et al.*, 2000), and 4) EMG_{di} (a

surrogate of NRD) (Schaeffer *et al.*, 2014). The term "effort" is described as "the intensity of willed motor command" and is believed to reflect the conscious awareness of the central respiratory motor output command required to drive the active skeletal muscles (Killian 1984). Increases in breathing "effort", "work", or "heaviness" are the most common qualitative descriptors selected during exercise in healthy individuals (Simon *et al.*, 1989; O'Donnell *et al.*, 2000; Ofir *et al.*, 2007). It has been shown that the intensity of respiratory effort increases when ventilation and $\Delta P_e/P_{Imax}$ are increased with exercise or when the respiratory muscles are weakened by fatigue (Gandevia *et al.*, 1981).

The neurophysiology behind the sensation of increased work and effort is believed to be caused by increased central corollary discharge to the somatosensory cortex, secondary to increased cortical (voluntary) respiratory motor drive (Jensen *et al.*, 2009). Peripheral sensory information is also believed to play a role in the neurophysiology of increased work and effort. Specifically, afferent information from the muscle spindles, Golgi tendon organs, and *type III* and *type IV* mechanoreceptors and metaboreceptors of the respiratory muscles, including the diaphragm. Afferents from the above receptors project directly into the somatosensory cortex and are believed to increase the sensation of increased work and effort during intense exercise when the demand for ventilation is high (Jensen *et al.*, 2009).

Contrary to the above is when the mechanical response from the respiratory system is constrained below the level of outgoing drive. Under this condition, dyspnea increases in direct proportion to the growing disparity between drive and the mechanical response, known as neuromechanical uncoupling (O'Donnell & Webb, 2008), which is often perceived as "unsatisfied inspiration" or "air hunger" (O'Donnell *et al.*, 2009). Previously called "length-tension inappropriateness", Campbell and Howell (1963) proposed that when the change in the

respiratory muscle length, as sensed by the muscle spindles of the ribcage, is inappropriate for a given motor output, there is an increase in breathlessness. Recently, the theory has extended to include sensory afferents beyond just the ribcage, including the diaphragm, lungs and airways.

1.2.3.1 Assessing Neural Drive

It is not possible to directly measure the brain's NRD. Previous surrogates to assess neural drive include minute ventilation (Mador & Tobin, 1992; Yan *et al.*, 1993), P_e or P_{di} output, and mouth occlusion pressure in 0.1 second (Whitelaw *et al.*, 1975). However, these measurements have limited usefulness in a clinical population (Sinderby *et al.*, 2001). EMG_{di}, recorded from a multi-pair esophageal catheter, has been used as an alternative method to estimate respiratory drive (Lourenco *et al.*, 1966; Lopata *et al.*, 1977; Sinderby *et al.*, 1999; Luo *et al.*, 2001; Sinderby *et al.*, 2001; Luo & Moxham, 2005; Luo *et al.*, 2014). Using EMG_{di} to assess NRD is based on the fact that the phrenic nerves exclusively innervate the diaphragm and that there is strong positive correlation between phrenic nerve activity and EMG_{di} in dogs (Lourenco *et al.*, 1966; Aubier *et al.*, 1981). In addition, multiple studies have shown increases in EMG_{di} during CO_2 rebreathing (Lourenco *et al.*, 1966; Lopata *et al.*, 1977; Onal *et al.*, 1981; Luo *et al.*, 2001; Luo & Moxham, 2005) and that there is a linear relationship between EMG_{di} and ventilation in healthy subjects (Luo *et al.*, 2001) and patients with COPD (Luo & Moxham, 2005).

However, the technique of using EMG_{di} from an esophageal electrode catheter to assess NRD has been called into question. Gandevia and McKenzie (1986) showed a change in the amplitude of the diaphragm compound muscle action potential (CMAP) with changing lung volume. The changes represented artificial changes due to the changing distance between the

muscle fibres and the recording electrodes (Gandevia & McKenzie, 1986). However, with the addition of more electrode pairs in catheters as described elsewhere (Luo *et al.*, 2008), a number of studies have shown that the CMAP measured by an esophageal catheter at the electrically active region of the diaphragm is independent of lung volume (Beck *et al.*, 1998; Luo *et al.*, 1998). Despite this, it is important to acknowledge that EMG_{di} is an indirect surrogate of NRD.

1.2.4 Dyspnea and Diaphragm Fatigue

As previously mentioned, afferent feedback from the respiratory system, including the chest wall, airways, lungs, and respiratory muscles plays an important role in the manifestation of dyspnea. The diaphragm is known to fatigue during high intensity constant work rate exercise in healthy humans (Johnson *et al.*, 1993; Babcock *et al.*, 1996; Guenette *et al.*, 2010). Thus, contribution of the afferent pathway from the diaphragm to cause dyspnea, specifically afferent feedback from the diaphragm as it fatigues, should be further explored.

Gandevia *et al.* (1981) were among the first to explore respiratory sensations after inducing DF. The investigators found that individuals increased their perception of work and effort during resistive breathing after performing fatiguing inspiratory muscle work. McConnell and Romer (2004) suggest there are two interpretations of this finding. First, DF results in increased ventilatory drive in order to maintain a given ventilation (central hypothesis) (Gandevia *et al.*, 1981; Supinski *et al.*, 1987). Second, there is a decrease in proprioceptive discharge in parallel to increased *type III* and *type IV* metaboreceptor activity when DF occurs (peripheral hypothesis), as shown in previous studies (Balzamo *et al.*, 1992; Jammes & Balzamo, 1992). As such, DF could potentially cause increases in both the sensory and affective dimensions of dyspnea. The afferent feedback from DF could potentially contribute to increased

neural drive, thus creating an increase in the conscious awareness of the work and effort needed to breathe. Simultaneously, DF itself may potentially act as a constraint on the respiratory system, limiting its ability to match outgoing motor command. The constraint of DF would result in neuromechanical uncoupling, potentially leading to sensations such as "unsatisfied inspiration" or "air hunger", as well as potentially induce distress, unpleasantness and discomfort.

While exploring sex differences in exercise tolerance following DF, Welch *et al.* (2018a) showed a mean increase in breathing discomfort for both men and women, and only a mean increase in leg discomfort for the women. Both sexes additionally showed a decrease in exercise performance, indicating that an increased in breathing discomfort, at least in part, impacted exercise performance. Similar studies have shown the same effect (Mador & Acevedo, 1991a; Sliwinski *et al.*, 1996; Verges *et al.*, 2006).

1.2.5 Diaphragm Fatigue and Neural Drive

It has previously been suggested that when the diaphragm fatigues, NRD to the diaphragm increases in order to sustain ventilation (Moxham *et al.*, 1980; Moxham *et al.*, 1981). To our knowledge, there have been two studies exploring the role of DF on neural drive, assessed by the ventilatory response to CO_2 (Mador & Tobin, 1992; Yan *et al.*, 1993). However, the results were contradictory to each other. Mador and Tobin (1992) reported that after inspiratory muscle fatigue, the slope of the ventilatory response to CO_2 was significantly decreased. Alternatively, Yan *et al.* (1993) reported no alteration in ventilatory response to CO_2 after respiratory muscle fatigue.

The abovementioned contradictory results prompted Luo *et al.* (2001) to further explore the relationship between DF and neural drive. Neural drive was assessed using a multi-pair esophageal electrode catheter that recorded EMG_{di} during CO_2 rebreathing (Luo *et al.*, 2001; Luo *et al.*, 2008). The authors suggest that EMG_{di} serves as more reliable surrogate for neural drive than changes in ventilation. DF was induced in two ways. First, DF was induced via 2-minutes of maximum isocapnic voluntary ventilation (MIVV). Second, during a follow-up experiment, DF was induced via 3, 5-minute trials of inspiratory respiratory loading (IRL). During both experiments DF was assessed via bilateral anterior magnetic stimulation of the phrenic nerves (BAMPS) 10 minutes before and after inducing fatigue. CO_2 rebreathing was conducted 15-minutes prior and 15-minutes following the fatiguing protocol. The study showed no significant difference in EMG_{di} before and after DF for both protocols. However, there are a number of considerations to take into account. First, only 6 individuals participated in the original study. Of those 6, only 4 participants returned to perform the follow up IRL experiment. The small sample size may not have yielded enough statistical power for the study. Second, CO_2 re-breathing was conducted 5-minutes following the assessment of fatigue (and 15-minutes following the fatiguing protocol) rather than immediately after. Therefore, the diaphragm may have partially recovered during that time. Third, fatiguing the diaphragm via 2-minutes of MIVV may not have been severe enough to significantly alter neural drive, something the authors suggest in their discussion and the primary reason for conducting the IRL follow-up experiment. However, during the three 5-minute trials of IRL, the participants rested for 10-minutes between each trial. It is possible that the diaphragm recovered enough during these rest period to not significantly alter NRD. As such, the relationship between DF and neural drive,

assessed with a multi-pair esophageal catheter, should be further explored, particularly during clinically relevant conditions such as whole-body exercise.

1.2.6 Diaphragm, Dyspnea and Neural Drive

Previous studies have found that cycling with pre-induced inspiratory muscle fatigue results in higher ventilation compared to control, believed to be contributed by excess drive to the respiratory muscles (Martin *et al.*, 1982; Mador, 1991; Mador & Acevedo, 1991b, a; Sliwinski *et al.*, 1996). A few of these studies (Martin *et al.*, 1982; Mador & Acevedo, 1991b; Sliwinski *et al.*, 1996) have also shown that exercising with a pre-fatigued diaphragm results in the increased perception of breathing work and effort. The results from the above studies support the hypothesis that diaphragm fatigue contributes to increased ventilatory drive and dyspnea (McConnell & Romer, 2004). It is important to note, however, that these abovementioned studies only evaluated dyspnea as a single sensation of increased effort, and not as a multidimensional symptom including the sensory, affective and emotional components. Additionally, respiratory drive was retrospectively assumed to increase based on an increase in ventilation and not otherwise quantified.

Chapter 2: Thesis Study

2.1 Purpose

The purpose of this study was to determine the effect of DF on EMG_{di} and the sensory perceptions of exertional dyspnea, including its intensity and unpleasantness during exercise in healthy humans.

2.2 Objectives

The ***primary*** objective of this study was to examine the effects of DF on the intensity and unpleasantness of dyspnea during exercise, and whether the dyspnea sensations can be attributed to EMG_{di}. The ***secondary*** objective was to determine what sensory qualities of dyspnea are experienced during exercise with a fatigued diaphragm.

2.3 Hypothesis

1) The DF trial will result in an increase in EMG_{di} relative to the control condition throughout any given submaximal exercise time and ventilation.

2) Individuals will select higher ratings for both the intensity and unpleasantness of dyspnea during exercise in the pre-fatigue trial compared to the control condition.

3) The increase in EMG_{di} during exercise following the fatigue trial will be significantly correlated with the increase in dyspnea intensity ratings.

4) Individuals that select the descriptor of "unsatisfied inspiration" will see an earlier onset of this descriptor in the pre-fatigue trial compared to the control condition.

2.4 Methods

Healthy recreationally active males (N=16) underwent three sessions at the Cardiopulmonary Exercise Physiology Laboratory at St. Paul's Hospital. Each session took approximately 3 hours, with all visits taking place at least 48 hours apart to allow appropriate recovery time. Only males were recruited since women are known to have greater resistance to diaphragm fatigue relative to men (Guenette *et al.*, 2010). During Visit 1, participants provided written informed consent, underwent anthropometric measurements and completed a Physical Activity Readiness Questionnaire (PAR-Q+) (Warburton *et al.*, 2011), International Physical Activity Questionnaire (Craig *et al.*, 2003) and medical history questionnaire. Visit 1 concluded with pulmonary function testing for screening purposes, as well as an incremental cycling test to determine peak work rate and to familiarize subjects with testing procedures. Visits 2 and 3 involved either a weighted breathing task to induce diaphragm fatigue or no breathing task (control), prior to performing a high intensity constant load cycling test. The order of the study conditions was randomized for each participant. The constant load cycling test for Visits 2 and 3 were performed at a wattage equal to the individual's calculated gas exchange threshold (GET) plus 60% of the difference between their peak work rate and GET (60%Δ) (Lansley *et al.*, 2011), until exhaustion. Familiarization of the constant load exercise test took place at the end of Visit 1. Subjects were instrumented with a multi-pair esophageal electrode catheter to measure EMG_{di}, P_g, P_e and P_{di}. The P_{di} achieved in response to CMS of the phrenic nerves was used to measure DF (Similowski *et al.*, 1989; Guenette *et al.*, 2010). Dyspnea intensity and unpleasantness of breathing was measured every minute throughout exercise using the 0-10 category-ratio Borg scale (Borg, 1982). Subjects also selected the most dominant qualitative dyspnea descriptors throughout exercise as described previously (Cory *et al.*, 2015), as well as

applicable dyspnea descriptors at peak exercise from a list of 15 descriptive phrases (Schaeffer *et al.*, 2014; Cory *et al.*, 2015). Standard cardiopulmonary measurements were obtained using a commercially available metabolic cart (TrueOne 2400; Parvo Medics, Sandy, UT, USA). A detailed description of the methodology can be found below.

2.4.1 Participants

Table 1. Participant inclusion and exclusion criteria

Participant inclusion criteria:	***Participant exclusion criteria:***
• Male	• History of or currently smoking
• Ability to read and understand English	• History of or current symptoms of cardiopulmonary disease including asthma and exercise-induced asthma
• Recreationally active (High Category on the International Physical Activity Questionnaire).	• Contraindications to exercise testing defined as anything that would limit your ability to properly and safely perform exercise (i.e. A problem with the heart or lungs, muscle or bone injury, a serious infection).
• Forced expiratory volume in 1 second (FEV_1) to forced vital capacity (FVC) ratio >0.70	• Ulcer or tumor in the esophagus, nasal septum deviation, or recent nasopharyngeal surgery
• FEV_1 ≥80% predicted	• Allergies to latex or local anesthetic
• Body mass index (BMI) greater than 18 or less than 30 kg/m^2	• Cardiac pacemaker, implanted defibrillators or implanted neurostimulators
• Able to ride an upright stationary bicycle	• Any metal or electronics inside of the body
• Questionnaire). 19-40 years of age (inclusive)	

Abbreviations: FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity.

2.4.2 Pulmonary Evaluation

Routine spirometry measurements were obtained on Visit 1 using a commercially available cardiopulmonary testing system (Vmax 229d with Autobox 6,200 DL; SensorMedics, Yorba Linda, CA). All testing was performed according to recommended guidelines (American Thoracic Society/European Respiratory, 2002; Miller *et al.*, 2005).

2.4.3 Exercise Protocol

2.4.3.1 Incremental Cycle Test

The incremental cycle test was performed on an electronically braked ergometer. Prior to exercise, the participants were provided time to adjust the ergometer to fit their personal measurements, including seat height and position, as well as handle bar height and angle. All measurements were recorded by the study team at the end of Visit 1 and were used for subsequent exercise testing on Visits 2 and 3. Individuals were afforded the opportunity to use their personal cycling pedals and shoes if preferred, and were instructed to bring them on every subsequent visit.

The incremental test began with a 6-minute steady-state resting period to collect metabolic, ventilatory and sensory baseline measurements, followed by a 1-minute warm-up of unloaded pedaling. Following warm-up the participant pedaled at an initial work rate of 50W with a 25W stepwise increase every two minutes. During each 2-minute stages, blood pressure was collected at the onset of the stage; dyspnea intensity, unpleasantness and leg discomfort were all collected at both the 30 second and 90 second mark; the most prominent breathing sensation was selected at the 90 second mark; and an inspiratory capacity maneuver was collected at the end of the stage. Cessation of the test occurred following a drop in pedaling

cadence to below 60 revolutions per minute (rpm) or until the individual indicated they were no longer able to continue. Maximal work rate was determined as the highest work rate sustained for at least 30-seconds.

2.4.3.2 Constant Load Cycle Test

Both experimental constant load exercise tests were performed on the same electronically braked ergometer as the incremental test set up with the same measurements. Each test began with a steady-state 1-minute resting period followed by a 1-minute warm-up of unloading pedaling. Only 1-minute of baseline measures were obtained to avoid any fatigue recovery. Following warm-up, work rate increased to 60%Δ determined on Visit 1. 60%Δ is equal to the participant's GET, plus 60% of the difference between their GET and peak work rate. The participant's GET was determined by using the dual criteria method (Caiozzo *et al.*, 1982). Two relationships were plotted using this method. First, the individual's oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) were plotted against one another. Second, the participant's ventilatory equivalent for oxygen ($\dot{V}_E/\dot{V}O_2$) and ventilatory equivalent for carbon dioxide ($\dot{V}_E/\dot{V}CO_2$). The inflection point between $\dot{V}O_2$ and $\dot{V}CO_2$, as well as the rise in $\dot{V}_E/\dot{V}O_2$ without an associated rise in $\dot{V}_E/\dot{V}CO_2$ were used to determine GET. GET was confirmed by calculating the second derivative of the $\dot{V}O_2$-$\dot{V}CO_2$ plot to determine the point of inflection. The 60%Δ work rate was maintained for the remainder of the test until pedal cadence fell below 60rpm or symptoms limited the participant's ability to continue. Dyspnea evaluation and ICs were collected at the same time points as the incremental test. The two conditions for the constant load exercise test were 1) pre-induced DF and 2) no pre-induced DF trial. Both conditions took place on different days at least 48-hours apart in a randomized order.

Familiarization with the constant load exercise test took place at the end of Visit 1 following 30-minutes of rest after the incremental cycle test. The familiarization was identical to the experimental constant load exercise test described above; however, no data was collected.

2.4.4 Experimental Conditions

2.4.4.1 Pre-Induced Diaphragm Fatigue

DF was be induced through pressure threshold loading (PTL). The participant breathed on a weighted plunger loading device, and in order to initiate inspiration, had to produce enough negative pressure to overcome the weighted load. The loader for this study was custom built and can be seen in Appendix C. The participant was instructed to inspire at a target P_{di} of 60% of their maximum P_{di} achieved during a maximal inspiratory effort against an occluded mouthpiece at functional residual capacity (FRC) on the same day. The target pressure was displayed in front of them on a digital monitor. The participant inspired to a target P_{di} rather than a target P_m to specifically load the diaphragm. This is because it is possible to generate inspiratory pressures at the airway opening via the ribcage and other accessory inspiratory muscles without diaphragm contribution (American Thoracic Society/European Respiratory, 2002). Participants were verbally instructed to emphasize the use of their diaphragm and ensure their abdomen protrudes during each inspiration. Participants were also instructed to place one hand on their abdomen and another along the anterior axillary line of the ribcage and breathe so that the hand on their ribcage remains relatively stationary, while attempting to only move their abdomen. Familiarization with diaphragmatic breathing occurred on Visit 1. Subjects were also instrumented with wireless surface EMG (MyoSystem 1400A, Noraxon, USA) on their sternocleidomastoid, scalene and parasternal muscles to monitor extradiaphragmatic muscle

recruitment during the fatiguing task. P_{di} was calculated as the difference between P_g and P_e, which were both recorded using differential pressure transducers (model DP15-34, Validyne Engineering, Northridge, CA, USA) and displayed on a digital monitor with a marker indicating the target pressure (Roussos *et al.*, 1979; Bellemare & Grassino, 1982a, b). During this breathing task, the participant breathed with a prolonged 0.7 duty cycle and a breathing frequency of 15 breaths per minute to further promote fatigue (Bellemare & Grassino, 1982a; Sheel *et al.*, 2001; Witt *et al.*, 2007). That is, for one full breath cycle of inspiration and expiration, the individuals inspired for 70% of that time to keep the diaphragm under tension. Duty cycle and breathing frequency were maintained by distinct inspiratory and expiratory auditory tones. End-tidal partial pressure of CO_2 ($P_{ET}CO_2$) was monitored throughout the loading task and manual adjustments to the inspired fraction of CO_2 were made in the event of hypocapnia. The breathing task occurred until task failure, defined as an inability to reach the target P_{di} for three consecutive breaths. A reduction in P_{di} by ≥15% or double the coefficient of variation between a block of potentiated twitches following PTL, whichever the bigger number, was used to define DF (Kufel *et al.*, 2002; Guenette *et al.*, 2010). If DF did not occur, then the subject repeated the procedure in 5-minute intervals until fatigue was present. A detailed methodology of CMS can be found below.

2.4.4.2 Control Condition

On a separate visit subjects performed the same constant load exercise test without performing PTL. This trial served as a control similar to previous pre-fatigue studies (Martin *et al.*, 1982; Dodd *et al.*, 1989; Mador & Acevedo, 1991b, a; Sliwinski *et al.*, 1996; Taylor &

Romer, 2008; Wuthrich *et al.*, 2013). No true placebo to the fatigue condition has been established and serves as a limitation to all pre-fatigue studies, including the present study.

2.4.5 Measurements

2.4.5.1 Anthropometric Measurements

Body mass, height and body mass index (BMI) were all measured to create an anthropometric profile during Visit 1 using a commercially available measuring station (Seca 769; Seca, Chino, CA).

2.4.5.2 Diaphragmatic EMG

EMG_{di} of the crural diaphragm was measured with a multipair electrode esophageal catheter. The catheter contains 10 1cm silver coils forming 5 EMG recording pairs. A detailed configuration of the catheter has been described previously by Luo *et al.* (2008) and was inserted by a trained member of the study team. Briefly, lidocaine (Lidodan Endotracheal Spray, Odan Laboratories LTD. Montreal, QC, Canada) was sprayed to numb the participant's nostril and back of the throat. Following, the catheter was inserted through the nostril, down the esophagus and into the stomach. The placement of the EMG was determined based on the strength of the EMG signals as described previously (Luo *et al.*, 2008). Raw EMG_{di} signals were converted to root mean square (RMS) and expressed as a percentage of $EMG_{di,max}$ obtained during inspiratory capacity (IC) maneuvers at FRC (Sinderby *et al.*, 1998; Jensen *et al.*, 2011; Luo *et al.*, 2011; Schaeffer *et al.*, 2014). The ratio of EMG_{di} to $EMG_{di,max}$ (EMG_{di}% max) was used as an index of NRD. All EMG signals were amplified (Biomedical Amplifier, Guangzhou Yinghui Medical), converted from analogue to digital (Power Lab 16s, ADInstruments Pty, Castle Hill, Australia)

and collected using LabChart software (LabChart v7.3.7, ADInstruments; Colorado Springs, CO, USA). The characteristics of the diaphragm M-wave were calculated as previously described (Welch *et al.*, 2017)

2.4.5.3 Respiratory Pressures

The catheter used to measure EMG_{di} was also equipped with esophageal and gastric balloons to simultaneously measure P_e and P_g, respectively. The balloons were connected to calibrated pressure transducers for recording. P_{di} was calculated as the difference between P_g and P_e.

2.4.5.4 Cervical Magnetic Stimulation

A transient magnetic field passing through neural tissue has the ability to create an electrical current inducing an action potential if a threshold is reached (Hovey & Jalinous, 2006). When an action potential is induced in the phrenic nerves a diaphragmatic contraction occurs. In this study, the participant's phrenic nerves were stimulated by a single magnetic pulse via a magnetic stimulator using a 90 mm circular coil (MagStim 200^2, The MagStim Company Ltd.; Whitland, Wales) as we have used previously in our laboratory (Ramsook *et al.*, 2016). The Magstim 200^2 is a magnetic nerve stimulator intended for the stimulation of cortical and peripheral nerves for diagnostic and research purposes. The Magstim enables deep and otherwise inaccessible nerves to be stimulated by inducing small currents in the nerve using a brief pulse of electromagnetic energy. Stimulation was performed by a trained member of the study team. Briefly, the optimal site of stimulation was found by measuring P_{di} in response to low frequency CMS between cervical vertebrae C_3 and C_7. Because lung volume can influence

twitch amplitude, all stimulations occurred at the end of expiration with the glottis closed. To find the optimal site of stimulation, C_7 was located and marked with ink on the participant's neck, along with 1cm points from C_7 up and down the neck. Each 1cm point, including C_7, was stimulated via CMS with each stimulation taking place at least 30-seconds apart to avoid twitch potentiation. The position that produced the largest identifiable P_{di} was marked with a separate colour of ink and used as the site of stimulation for the remainder of the study. Following, maximal stimulation was assessed by increasing the stimulator output in the following order: 60%, 70%, 80%, 90%, 95%, 100%. At least three stimulations were performed at each intensity separated by 30-seconds to avoid twitch potentiation. A plateau in mean twitch P_{di} ($P_{di,tw}$) with rising stimulus output indicated maximal stimulation of the phrenic nerves. Each subsequent stimulation was performed at 100% output.

2.4.5.5 Evaluation of Diaphragmatic Fatigue

To evaluate fatigue, potentiated twitches $P_{di,tw}$ were measured before and after PTL. Fatigue was also evaluated immediately after exercise, as well as 15-minutes post-exercise. $P_{di,tw}$ was recorded after a series of maximal inspiratory maneuvers. Briefly, each participant inspired maximally against an occluded mouthpiece at FRC. After the second tidal expiration, magnetic stimulation of the phrenic nerves occurred at 100% output. This sequence was repeated five times (Figure 1).

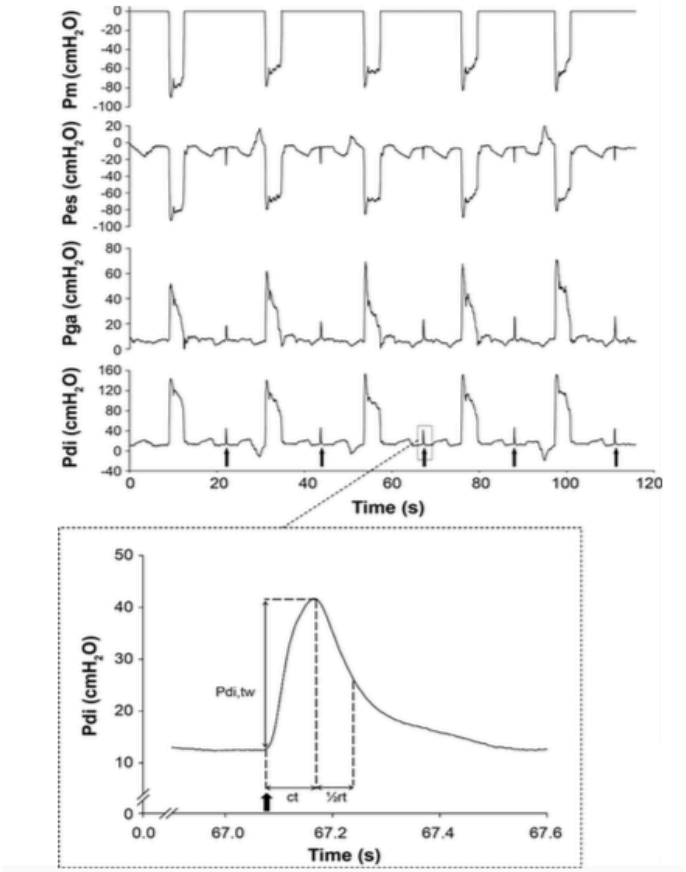

Figure 1. Potentiated twitches protocol. *Top: P_m (mouth pressure), P_{es} (esophageal pressure), P_{ga} (gastric pressure), P_{di} (transdiaphragmatic pressure) traces. Bottom: example of an individual twitch following CMS (cervical magnetic stimulation) (Guenette et al., 2010).*

A reduction in $P_{di,tw}$ by ≥15%, or double the coefficient of variation between the set of $P_{di,tw}$ (whichever value was higher), from baseline measures indicated that DF was successfully induced.

2.4.5.6 Dyspnea Evaluation

Both dyspnea intensity and unpleasantness were evaluated at rest, during every minute of exercise, and at peak exercise. Participants provided answers to the following questions used in previous work (Schaeffer *et al.*, 2014): "how intense is your sensation of breathing overall?" and "How unpleasant or badly does your breathing make you feel?" A script was read from Banzett

et al. (2015) on all visits to help participants distinguish between dyspnea intensity and unpleasantness (Appendix A). During this time, individuals were also asked their overall sensation of leg discomfort. Breathing intensity, breathing unpleasantness and leg discomfort were all evaluated using a 0-10 modified Borg scale (Borg, 1982) by having the individual point to their desired number. Each selection was verbally confirmed by a member of the study team, and the participants were instructed to repoint if the rating was incorrect. Through a standardized script, participants were informed that 0 represented no sensation of breathing intensity, breathing unpleasantness or leg discomfort at all and 10 represented the most intense breathing, maximal breathing unpleasantness or maximal leg discomfort one has ever experienced or could ever imagine experiencing.

Every 2-minutes during exercise participants were also asked to describe their breathing compared to rest by selecting from the following phrases: 1) "My breathing requires more work and effort"; 2) "I cannot get enough air in"; 3) "I cannot get enough air out"; and 4) "None apply" (Cory *et al.*, 2015). Following exercise, individuals were asked to give their reason for stopping as 'breathing discomfort', 'leg discomfort' or 'other' (Cory *et al.*, 2015). Participants were asked further to contribute a percentage for stopping to breathing and leg discomfort, totaling 100%. Following cessation of exercise, participants then selected qualitative dyspnea descriptors experienced at peak exercise from a list of 15 as used previously (Schaeffer *et al.*, 2014; Cory *et al.*, 2015). To conclude, the multidimensional dyspnea profile was administered at the end of the visit (Banzett *et al.*, 2015).

2.4.5.7 Cardiopulmonary Measurements

Standard cardio-respiratory measures were recorded on a breath-by-breath basis using a commercially available metabolic measuring system (TrueOne 2400, Parvo Medics, Utah, USA). The metabolic cart used was customized by the manufacturer to provide an output of the raw expired flow signal. Inspired flow was collected through a separate pneumotachometer (Series 3813, Hans Rudolph, Shawnee, KS, USA) connected to an amplifier (PA-1 Series 1110, Hans Rudolph, Shawnee, KS, USA). Both inspired and expired pneumotachometers were connected to a two-way-non-rebreathing valve (Series 2700, Hans Rudolph, Shawnee, KS, USA) via 150cm of large bore tubing. Other variables recorded included: $\dot{V}_E$, $\dot{V}O_2$, $\dot{V}CO_2$, tidal volume (V_t), and breathing frequency (f_b). In addition, operating lung volumes were determined by having individuals perform dynamic IC maneuvers as previously described (Guenette *et al.*, 2013). End-expiratory lung volume (EELV) was calculated as the difference between FVC and IC. FVC was used instead of total lung capacity (TLC) since TLC was not measured in this study. End-inspiratory lung volume (EILV) was calculated as the sum of EELV and V_t. Heart rate (HR) was recorded continuously using a commercially available heart rate monitor (Polar T34, Polar Electro Canada, Quebec, Canada). Lastly, blood oxygen saturation was estimated using pulse oximetry (Radical-7 Rainbow CO-Oximetry, Masimo Corp., Irvine, California, USA).

2.4.6 Data Analysis

All physiological measurements were averaged in 30 second epochs when no dyspnea scores were being reported. Dyspnea intensity, unpleasantness and leg discomfort scores were collected between these epochs to ensure that the subjective ratings did not influence

physiological variables. Since four sensory scores were reported at once, both testers recorded the scores for comparison. The Borg scale was held in the exact same position throughout the test during sensory score collection.

Respiratory pressures, ventilatory parameters and EMG were collected using LabChart (7.3.7 Pro; ADInstruments Inc., USA). Data was converted from analogue to digital with a 16-channel acquisition system (PowerLab 16/35; ADInstruments Inc., USA). EMG_{di} was sampled at 10,000 Hz, while all other parameters were sampled at 2,000 Hz. Raw EMG_{di} signals were amplified, but collected without filtering to ensure that the M-wave was not filtered from the signal. Post-acquisition filtering occurred between 20 Hz and 500 Hz using the LabChart software when M-waves were not being analyzed. RMS for all EMG signals were calculated using a 0.1 second moving average window. EMG_{di} RMS values were selected on a breath-by-breath basis during the 30 second analysis window to remove cardiac artifact. EMG_{di} was expressed as a percentage of the highest value obtained during an inspiratory capacity maneuver for a given visit. EMG_{di} selections occurred via a file de-identified to the participant and the exercise condition to avoid selection bias. M-waves were analyzed objectively using a customized script (MATLAB, MathWorks, USA) that analyzed amplitude, latency, duration and area of the wave. $P_{di,tw}$ were analyzed using the same LabChart software mentioned previously. $P_{di,tw}$ was identified as the amplitude from the stimulation onset to the twitch peak.

2.4.7 Statistical Analysis

Differences in EMG_{di}, Borg dyspnea ratings, and selected cardiopulmonary measurements between conditions at standardized exercise times were performed using a repeated measures ANOVA with Bonferroni adjusted post hoc comparisons. DF was assessed

using an independent sample *t*-test following PTL. Spearman's correlation coefficient was used to examine the association between EMG_{di} and dyspnea scores, and tested for significance using a linear regression. Dyspnea descriptors were compared across conditions using McNemar's test. Statistical significance was set at $p<0.05$. All values are expressed in mean ± SD unless otherwise stated.

2.4.8 Sample Size

The primary endpoint for this study was dyspnea intensity and unpleasantness ratings during both constant work rate exercise tests. With $\alpha=0.05$ and $\beta=0.80$, we estimated that at least 16 participants were needed to detect a minimally clinically important difference of ±1 Borg 0-10 scale units (Ries, 2005) at the longest equivalent exercise time between tests, assuming a standard deviation of ±1 Borg 0-10 scale units.

2.5 Results

2.5.1 Participant Characteristics

Anthropometrics, spirometry and peak exercise responses of the 16 individuals who completed all three study visits are displayed in Table 2. One individual was excluded because he did not meet the spirometry inclusion criteria. One individual showed no measure of fatigue following over an hour of loading. Two individuals did not return following their second visit due to abnormal responses to the esophageal catheter or the magnetic stimulation. Of the two, one participant experienced nose bleeds as a response to insertion of the esophageal catheter, while another experienced shoulder pain due to the magnetic stimulation. One individual withdrew for personal reasons.

Table 2. Participant characteristics

Anthropometrics	
Age, years	27 ± 5
Height, cm	177 ± 8
Mass, kg	82 ± 10
BMI, kg/m^2	26 ± 3
Spirometry	
FVC, l	5.73 ± 0.81
FVC, % predicted	106 ± 10
FEV_1, l	4.57 ± 0.64
FEV_1, % predicted	105 ± 11
FEV_1/FVC, %	80 ± 6
Peak Incremental Exercise	
Work Rate, W	291 ± 73
$\dot{V}O_{2Max,}$ $l \cdot min^{-1}$	3.66 ± 0.77
$\dot{V}O_{2Max}$, $ml \cdot kg^{-1} \cdot min^{-1}$	45.8 ± 9.8
$\dot{V}CO_2$, $l \cdot min^{-1}$	4.10 ± 0.80
RER	1.13 ± 0.04
HR, $beats \cdot min^{-1}$	187 ± 9
$\dot{V}_E$, $l \cdot min^{-1}$	143.1 ± 32.7
V_t, l	2.68 ± 0.45
f_b, $breaths \cdot min^{-1}$	53.2 ± 8.1
$P_{ET}CO_2$, mmHG	26.7 ± 2.84
$\dot{V}_E/\dot{V}O_2$	39.2 ± 4.2
$\dot{V}_E/\dot{V}CO_2$	34.8 ± 3.7

Abbreviations: BMI, body mass index; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 second; W, watts; $\dot{V}O_{2max}$, maximum oxygen consumption; $\dot{V}CO_2$, carbon dioxide production; RER, respiratory exchange ratio; HR, heart rate; $\dot{V}_E$, minute ventilation; $\dot{V}_t$, tidal volume; f_b, breathing frequency; $P_{ET}CO_2$, end-tidal partial pressure of carbon dioxide; $\dot{V}_E/\dot{V}O_2$, ventilatory equivalent for oxygen; $\dot{V}_E/\dot{V}CO_2$, ventilatory equivalent for carbon dioxide. Values are mean ± SD.

2.5.2 Response to Magnetic Stimulation

A plateau in $P_{di,tw}$ was observed at 90% and 95% of stimulator output (Figure 2). Inspecting individual data, three of sixteen participants did not reach a clear plateau. The average coefficient of variation for all study participants between consecutive stimuli was 5.1 ± 2.9%. There was no change in twitch control parameters, including M-wave characteristics and end-expiratory esophageal pressure, before and after PTL.

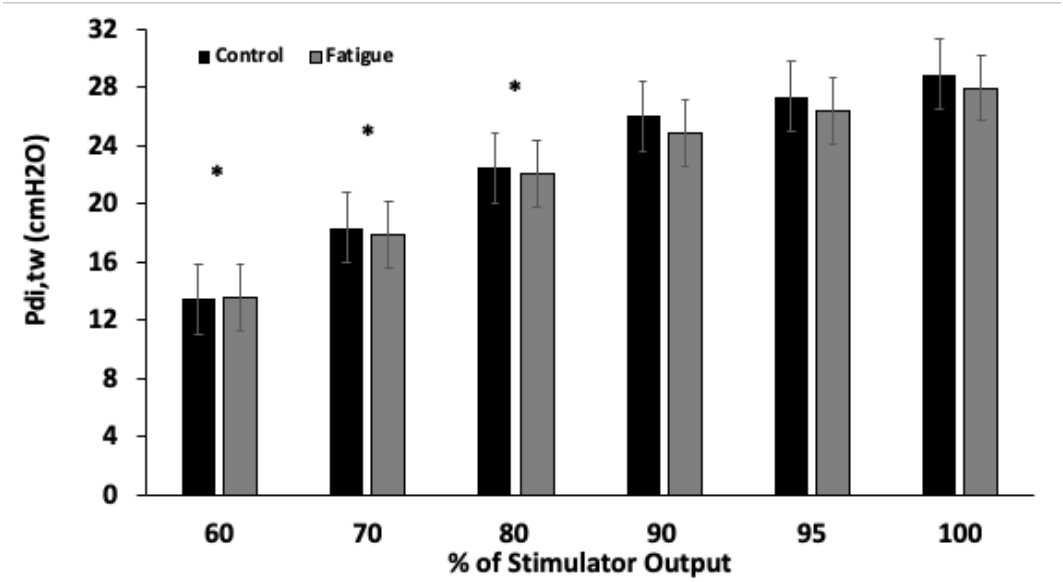

Figure 2. $P_{di,tw}$ in response to increasing stimulator output. *All values are mean ± SD. Abbreviations: $P_{di,tw}$, transdiaphragmatic twitch pressure.*, $p<0.05$ statistically different from 100%.*

2.5.3 Diaphragm Fatigue

Baseline twitch pressures on the control visit was 41.8 ± 11.6 cmH_2O. Following exercise, twitch pressures decreased 17.6 ± 15.2% (Figure 3). Of the 16 participants, half did not fatigue following the CWR exercise test on the control day using the DF criteria for the study. Following 15-minutes of recovery, twitch pressures were -6.0 ± 9.9% of baseline values.

During the pre-fatigue visit, baseline twitch pressures were 43.4 ± 13.5 cmH_2O. Following PTL, $P_{di,tw}$ decreased significantly by 31.7 ± 12.9%. All sixteen participants met the

DF criteria (≥15% decrease in $P_{di,tw}$ or twice the coefficient of variation during potentiated twitches, whichever was the higher percentage). Based on this criteria, 13 participants needed to decrease $P_{di,tw}$ by ≥15%, while three participants needed to decrease by 26.4, 15.5 and 15.2% respectively. The average PTL time until DF was confirmed was 15.3 ± 5.1 min. Seven participants did not demonstrate DF following their first assessment after PTL task failure. Each participant continued PTL for 5-minute intervals or volitional cessation of PTL, until fatigue was successfully assessed. Following pre-fatigue CWR exercise, $P_{di,tw}$ remained decreased by 29.8 ± 14.2% of baseline values. $P_{di,tw}$ values following 15-minutes of recovery were -20.7 ± 13.9% of baseline values.

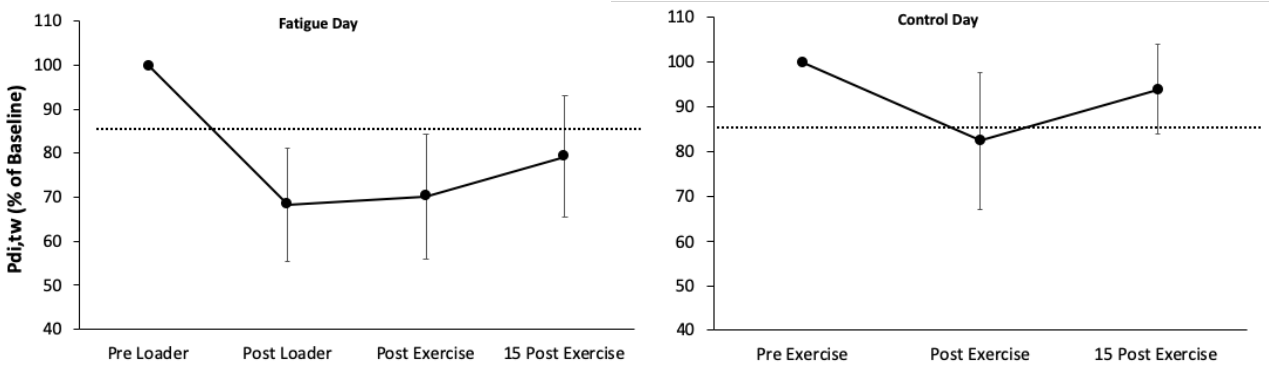

Figure 3. Diaphragm twitch pressures following PTL, exercise and recovery. *Dashed line represents fatigue threshold of -15%. Values are mean ± SD. Abbreviations: $P_{di,tw}$, transdiaphragmatic twitch pressure.*

2.5.4 Exercise Response

TTE for both CWR exercise tests can be found in Figure 4. Using the 60%Δ method to set exercise intensity for the current study, the average intensity for the study was 258 ± 62 W ranging from 76-91% maximum. The average TTE for all 16 participants was 10.7 ± 7.5 min for the control condition and 9.0 ± 5.5 min for the pre-fatigue condition. This equated to a decrease in TTE of 1.7 min (-19%) in the pre-fatigue condition compared to the control (p=0.04).

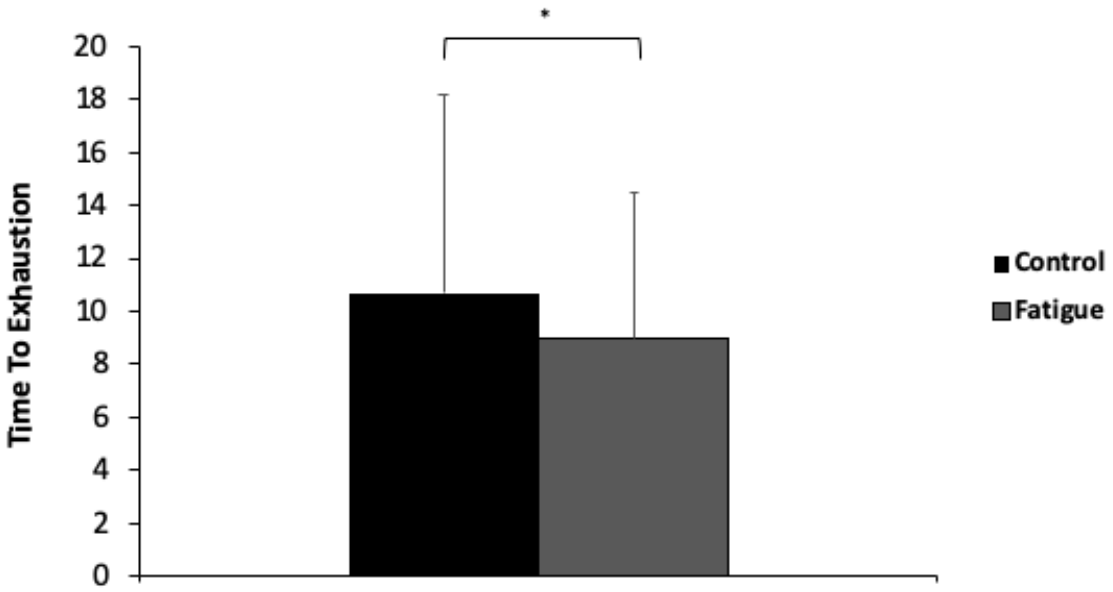

Figure 4. Exercise response during constant work rate exercise with and without a pre-fatigued diaphragm. *Values are mean ± SD.* *, $p<0.05$ *statistically different between conditions.*

2.5.5 Cardiorespiratory and Metabolic Responses

Cardiorespiratory parameters, iso-time and peak exercise can be found in Table 3. Iso-time is defined as the highest time point achieved on both tests for all participants. Absolute and relative $\dot{V}O_2$ was significantly higher at baseline only in the pre-fatigue condition compared to control (p=0.03, p=0.02, respectfully). $\dot{V}CO_2$ was modestly, but significantly higher in the control condition compared to the pre-fatigue condition at peak exercise (p=0.04). RER was significantly lower in the pre-fatigue condition compared to the control at baseline, iso-time and peak (p=0.0002, p=0.04, p=0.01, respectfully). There were no differences in HR across all time points between the two conditions.

2.5.6 Breathing Pattern

The ventilatory response during exercise is shown in Table 3. V_t was lower and f_b was higher at baseline and iso-time during the pre-fatigue trial compared to the control (both $p<0.05$) with no differences in either variable at peak ($p>0.05$). There were no differences in $\dot{V}_E$ across all time points. EELV as a percentage of FVC tended to be higher at baseline ($p=0.06$) and iso-time ($p<0.05$). No differences were present in EILV across all time points ($p>0.05$).

Table 3. Cardiorespiratory and ventilatory responses to constant load exercise

Parameter	Iso-time		Peak	
	Control	**Fatigue**	**Control**	**Fatigue**
$\dot{V}O_2$, l·min^{-1}	2.70 ± 0.65	2.70 ± 0.65	3.34 ± 0.71	3.45 ± 0.77
$\dot{V}O_2$, ml·kg^{-1}·min^{-1}	33.4 ± 8.4	33.4 ± 8.4	41.3 ± 8.7	41.3 ± 9.3
$\dot{V}CO_2$, l·min^{-1}	2.49 ± 0.60	2.40 ± 0.60	3.54 ± 0.65	3.43± 0.69 *
RER	0.93 ± 0.10	0.89 ± 0.08 *	1.07 ± 0.11	1.04 ± 0.11 *
HR, beats·min^{-1}	142 ± 7	136 ± 13	178 ± 9	174 ± 10 *
$\dot{V}_E$, l·min^{-1}	63.5 ± 17.4	63.9 ± 17.8	120.0 ± 24.5	116.4 ± 22.4
V_t, l	2.51 ± 0.53	2.36 ± 0.51 *	2.70 ± 0.47	2.66 ± 0.58
f_b, breaths·min^{-1}	25.4 ± 4.6	27.2 ± 5.3 *	44.6 ± 7.0	44.4 ± 0.9
$P_{ET}CO2$, mmHG	36.7 ± 2.6	35.1 ± 3.5 *	27.8 ± 3.4	27.5 ± 2.6

$\dot{V}_E/\dot{V}O_2$	23.7 ± 3.6	23.7 ± 3.2	36.3 ± 4.5	35.4 ± 4.2
$\dot{V}_E/\dot{V}CO_2$	25.4 ± 1.7	26.6 ± 2.6 *	33.9 ± 4.0	34.2 ± 3.6
EELV (%FVC)	34 ± 6	37 ± 7 *	37 ± 7	37 ± 7
EILV (%FVC)	79 ± 8	79 ± 9	84 ± 5	84 ± 5

*All values are expressed as mean ± SD. *, $p<0.05$ statistically significant from control condition at same time point. Abbreviations: $\dot{V}O_2$, oxygen consumption; $\dot{V}CO_2$, carbon dioxide production; RER, respiratory exchange ratio; HR, heart rate; $\dot{V}_E$, minute ventilation; V_t, tidal volume; f_b, breathing frequency; PCO_2, partial pressure of carbon dioxide; $\dot{V}_E/\dot{V}O_2$, ventilatory equivalent for oxygen; $\dot{V}_E/\dot{V}CO_2$, ventilatory equivalent for carbon dioxide; EELV, end expiratory lung volume; EILV, end inspiratory lung volume; FVC, forced vital capacity.*

2.5.7 Sensory Responses

2.5.7.1 Breathing Intensity

Breathing intensity scores versus exercise time can be found in Figure 5. Breathing intensity scores did not differ between the pre-fatigue and the control condition at baseline (0.1 ± 0.3 vs. 0.0 ± 0.0, $p>0.05$) or peak exercise (6.4 ± 2.5 vs. 6.7 ± 2.5, $p>0.05$). Submaximal breathing intensity scores at all three measurement points achieved by all participants, as well as one additional time point achieved by 15 participants, tended to be higher in the pre-fatigue trial compared to control; however, no statistical significance was observed ($p=0.19$, $p=0.13$, $p=0.16$, $p=0.05$, respectively).

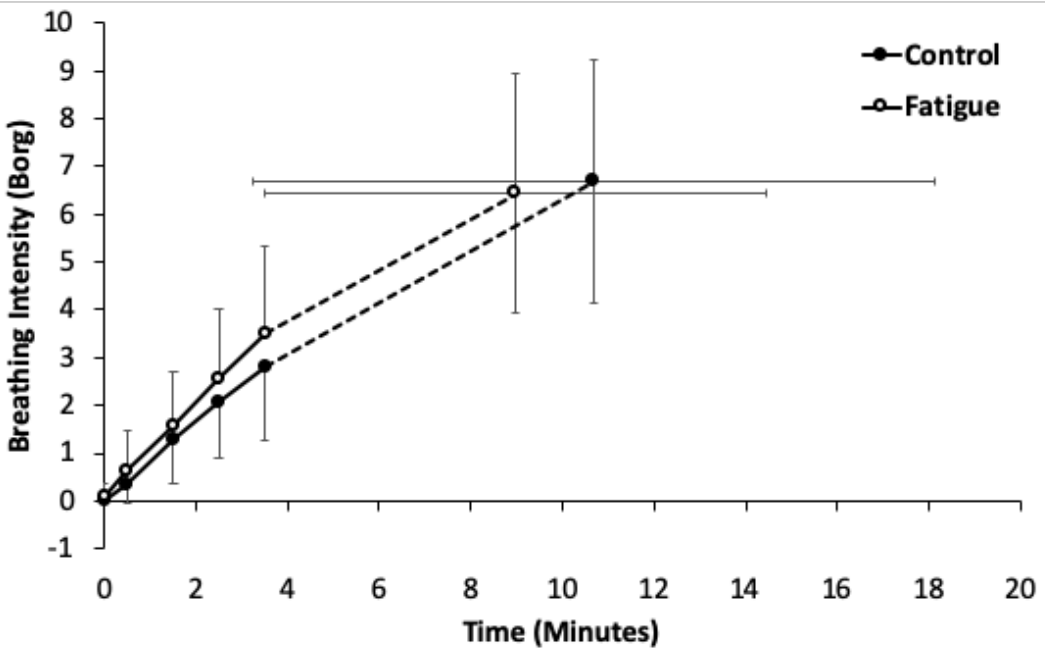

Figure 5. Breathing intensity with and without pre-diaphragm fatigue. *Values are plotted at measurement time points [baseline (0 minutes), 0.5 minutes, 1.5 minutes, 2.5 minutes, 3.5 minutes and peak]. Value at 3.5 minutes has n=15. Values are mean ± SD.*

2.5.7.2 Breathing Unpleasantness

Breathing unpleasantness scores versus exercise time can be found in Figure 6. Breathing unpleasantness scores did not significantly differ between the pre-fatigue and control conditions at baseline (0.1 ± 0.3 vs. 0.0 ± 0.0) or peak (4.6 ± 3.8 vs. 5.1 ± 3.3) (both $p>0.05$). Breathing unpleasantness scores in the pre-fatigue condition increased by 0.2 units (p=0.09), 0.6 units ($p=0.04$) and 0.6 units ($p=0.04$) at all three measurement points achieved by all participants. At one additional measurement point achieved by fifteen participants, breathing unpleasantness scores increased by 0.9 units ($p=0.03$) in the pre-fatigue compared to the control condition.

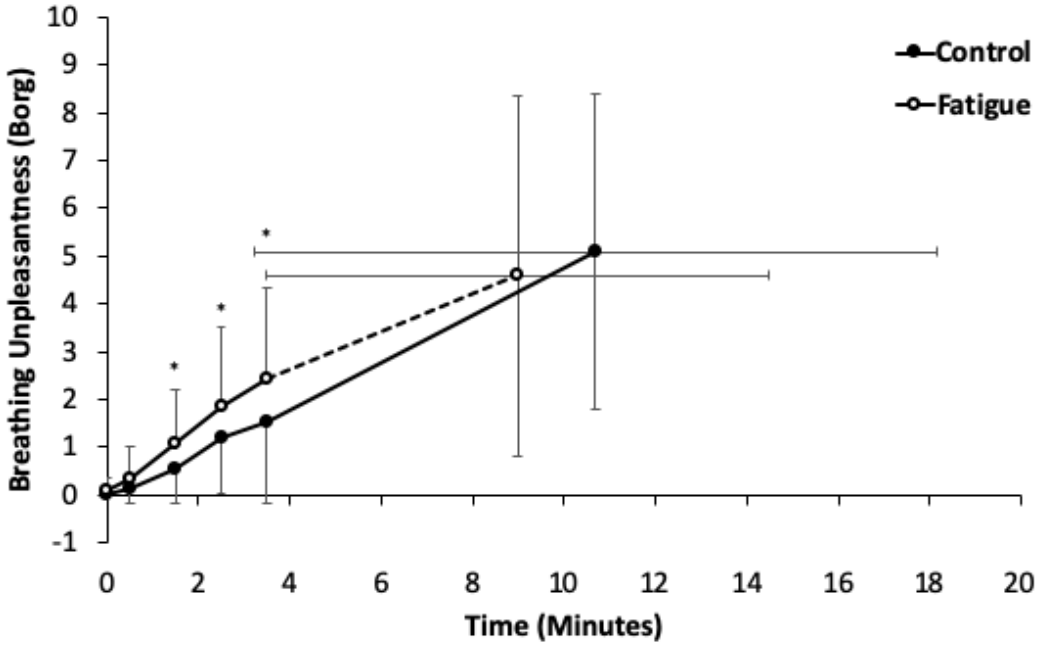

Figure 6. Breathing unpleasantness with and without pre-diaphragm fatigue. *Values are plotted at measurement time points [baseline (0 minutes), 0.5 minutes, 1.5 minutes, 2.5 minutes, 3.5 minutes and peak]. Value at 3.5 minutes has n=15. Values are mean ± SD. *, p<0.05 statistically different between conditions.*

2.5.7.3 Leg Discomfort

Leg discomfort scores versus exercise time can be found in Figure 7. There were no differences in leg discomfort scores at baseline, any exercise time point or peak (all $p>0.05$).

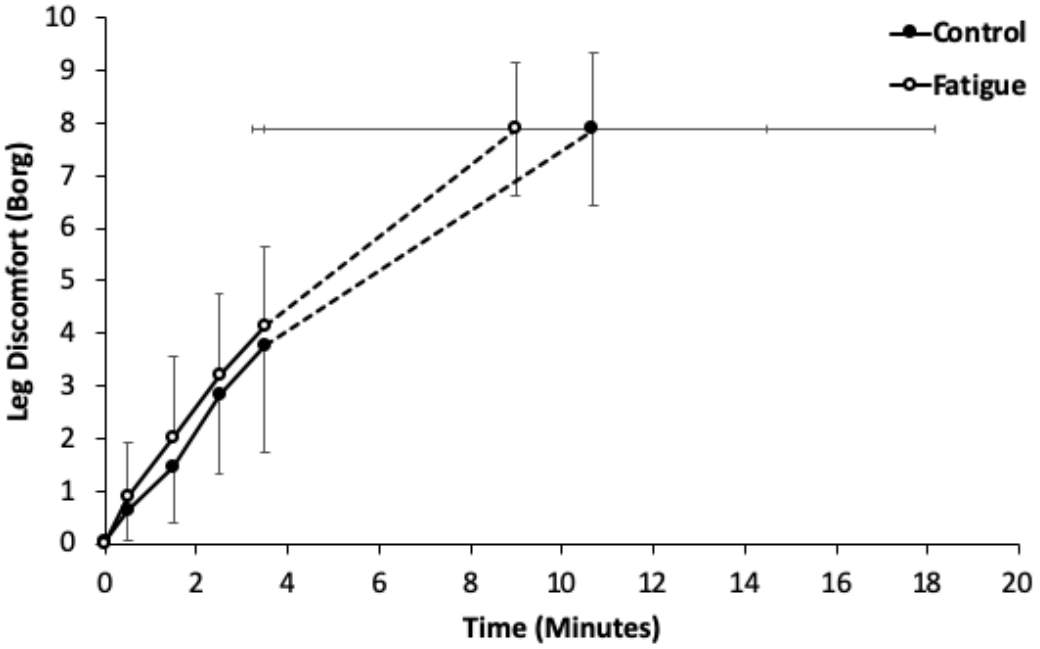

Figure 7. Leg discomfort with and without pre-diaphragm fatigue. *Values are plotted at measurement time points [baseline (0 minutes), 0.5 minutes, 1.5 minutes, 2.5 minutes, 3.5 minutes and peak]. Value at 3.5 minutes has n=15. Values are mean ± SD.*

2.5.7.4 Dyspnea Descriptors During Exercise

69% of participants selected "My breathing requires more work and effort" as the most prominent descriptor in the pre-fatigue trial compared to 44% of participants in the control at the highest equivalent exercise time achieved by all participants in which dyspnea descriptors were measured (1.5-minutes). Of the 15 participants who were able to cycle for 3.5 minutes during both conditions, 93% selected "My breathing requires more work and effort" as the most prominent descriptor during the pre-fatigued condition compared to 73% during the control condition. 100% of the participants who were able to cycle for 5.5-minutes (N=10) selected "My breathing requires more work and effort" compared to 82% in the control. At peak (N=16), 88% of participants during the fatigue trial, compared to 75% during the control, selected "My breathing requires more work and effort" as the descriptor best describing their breathing.

Across both conditions, "my breathing requires more work and effort" was the most selected descriptor. No measurement time showed statistically differences between the control and pre-fatigue trials (p>0.05).

No significant differences were seen in the selection frequency of "I cannot get enough air in" throughout both CWR exercise tests. However, 31% of individuals selected this descriptor as most prominent in the control condition compared to 19% in the pre-fatigue condition at peak. Only three individuals selected "I cannot get enough air in" in both conditions, with the selection occurring, on average, 3.3 minutes sooner in the pre-fatigue trial. There were no differences in the selection of "I cannot get enough air out" during both exercise tests, including peak.

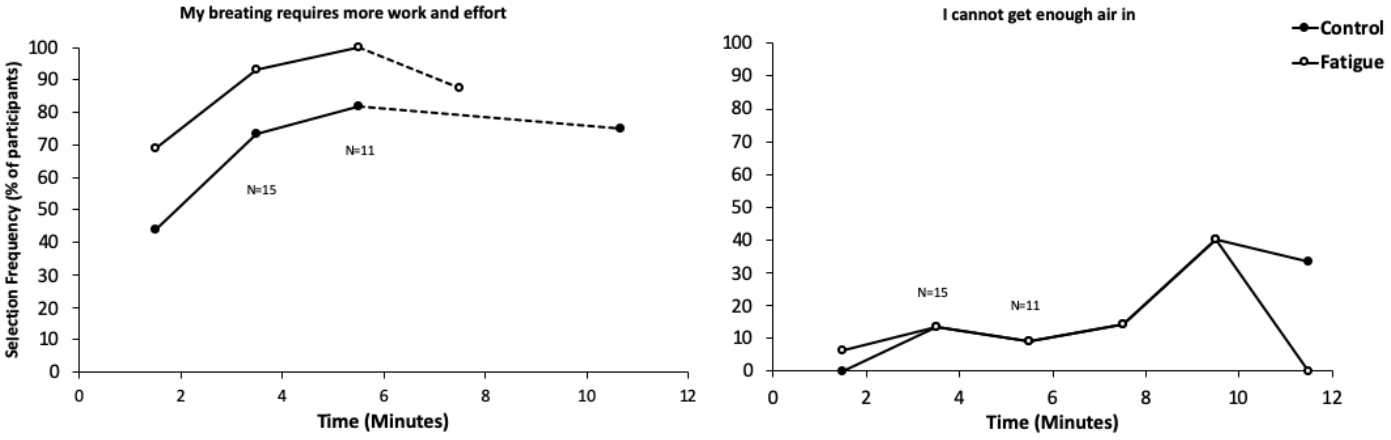

Figure 8. Selection frequency of dyspnea descriptors during exercise. *Left panel: selection frequency of "my breathing requires more work and effort". Right panel: selection frequency of "I cannot get enough air in." Values are plotted across measurement time points (1.5 minutes, 3.5 minutes, 5.5 minutes and peak). n=15 at 3.5 minutes and n= 11 at 5.5 minutes.*

2.5.7.5 Reasons for Stopping

The primary reason for cessation of exercise during both the pre-fatigue and control CWR exercise tests can be found in Figure 9. In the pre-fatigue condition, 19%, of participants cited breathing discomfort, 50% cited leg discomfort, and 31% cited a combination of breathing

and leg discomfort as their primary reason for stopping exercise. No individuals selected "other" as their primary reason for stopping in the fatigue condition. Comparatively, 13% of participants cited breathing discomfort, 56% cited leg discomfort, 25% cited a combination of breathing and leg discomfort, and 6% cited "other" as their primary reason for stopping exercise during the control condition. The one individual who stated "other" for his primary reason for stopping stated he stopped exercise because of an "abdominal cramp." When asked to give a percentage contribution for stopping exercise to breathing and leg discomfort, individuals in the pre-fatigue condition attributed 37% to breathing discomfort and 63% to legs, while in the control conditions individuals attributed 28% and 72%, respectively, with no significant differences between conditions.

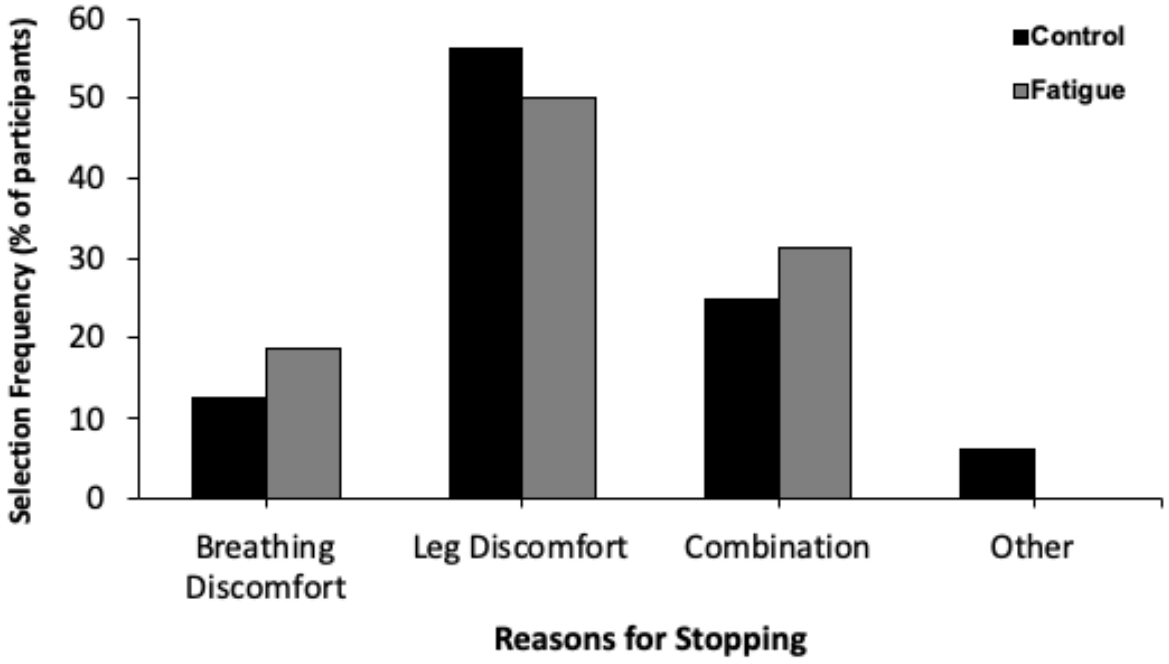

Figure 9. Reasons for stopping exercise. *Selection frequency for stopping exercise for breathing discomfort, leg discomfort, a combination of both breathing and leg discomfort, or other. Values are % of participants.*

2.5.7.6 Dyspnea Descriptors Post Exercise

Dyspnea descriptors selected following exercise can be found in Figure 10. Following exercise, descriptors related to "increased work and effort", "heavy breathing" and "rapid breathing" were the most prominent descriptors selected. 89% of participants during the pre-fatigue trial compared to 69% during control trial selected descriptors related to "unsatisfied inspiration" (p>0.05). 63% of participants selected descriptors relating to "air hunger" in the pre-fatigue condition compared to 44% in the control (p>0.05). Lastly, in the pre-fatigue trial, 44% of participants selected descriptors relating to "shallow breathing" compared to 13% in the control condition (p=0.06).

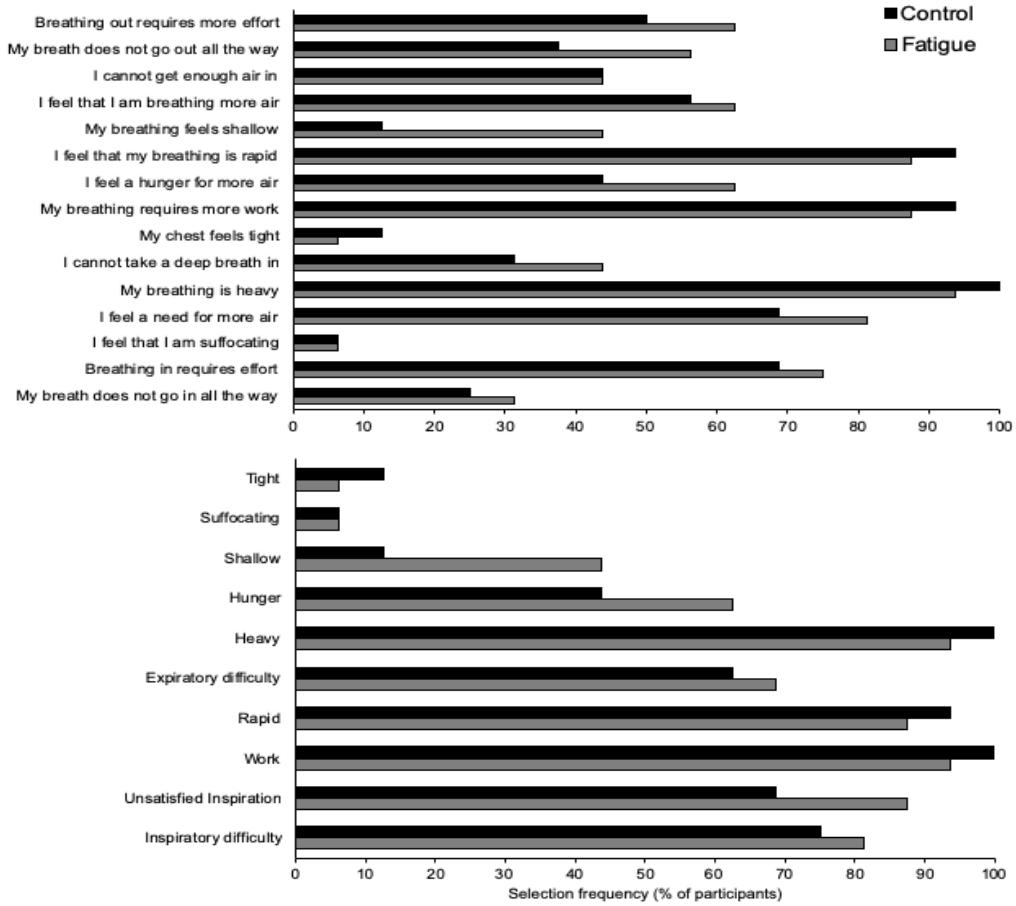

Figure 10. Dyspnea descriptors post exercise. *Top panel: selection frequency of 15 applicable dyspnea descriptors post exercise. Bottom panel: cluster analysis of descriptors into appropriate categories.*

2.5.7.7 Multidimensional Dyspnea Profile

All four components of the Multidimensional Dyspnea Profile can be found in Figure 11. There were no differences in peak unpleasantness between the pre-fatigue and control condition (p>0.05). 81% of participants selected "breathing requires muscle work or effort" as an applicable descriptor of breathlessness compared to 56% in the control condition (p>0.05). The intensity of this sensation was rated 4.8 in the pre-fatigue condition and 3.7 in the control (p>0.05). 69% of participants selected "I am not getting enough air or I am smothering or I feeling a hunger for more air" with an intensity rating of 4.5 in the pre-fatigue exercise test compared to a 56% selection frequency (p>0.05) and an intensity rating of 3.2 (p=0.09) in the control condition. There was no difference in the selection frequency of "my breathing requires mental effort or concentration"; however, the intensity rating for this descriptor was 4.4 in the pre-fatigue test and 3.6 in the control (p>0.05). There were no differences in selection frequency or intensity ratings of "my chest and lungs feel tight or constricted" or "I am breathing a lot." Averaged intensity ratings for all five descriptors were significantly higher in the pre-fatigue condition compared to the control condition (4.1 ± 3.4 vs. 3.4 ± 3.3, p=0.04). The immediate perception domain, consisting of average intensity rating and peak unpleasantness, was significantly higher in the pre-fatigue condition compared to control (4.3 ± 1.9 vs. 3.6 ± 1.8, p=0.04). Participants rated their feeling of anxiety and frustration 0.8 (p=0.08) and 1.0 (p=0.06) units higher, respectively, in the pre-fatigue condition compared to control. No differences were reported in their feelings of depression, anger and fear. Lastly, there were no differences in the emotional perception domain (average score for all emotions)

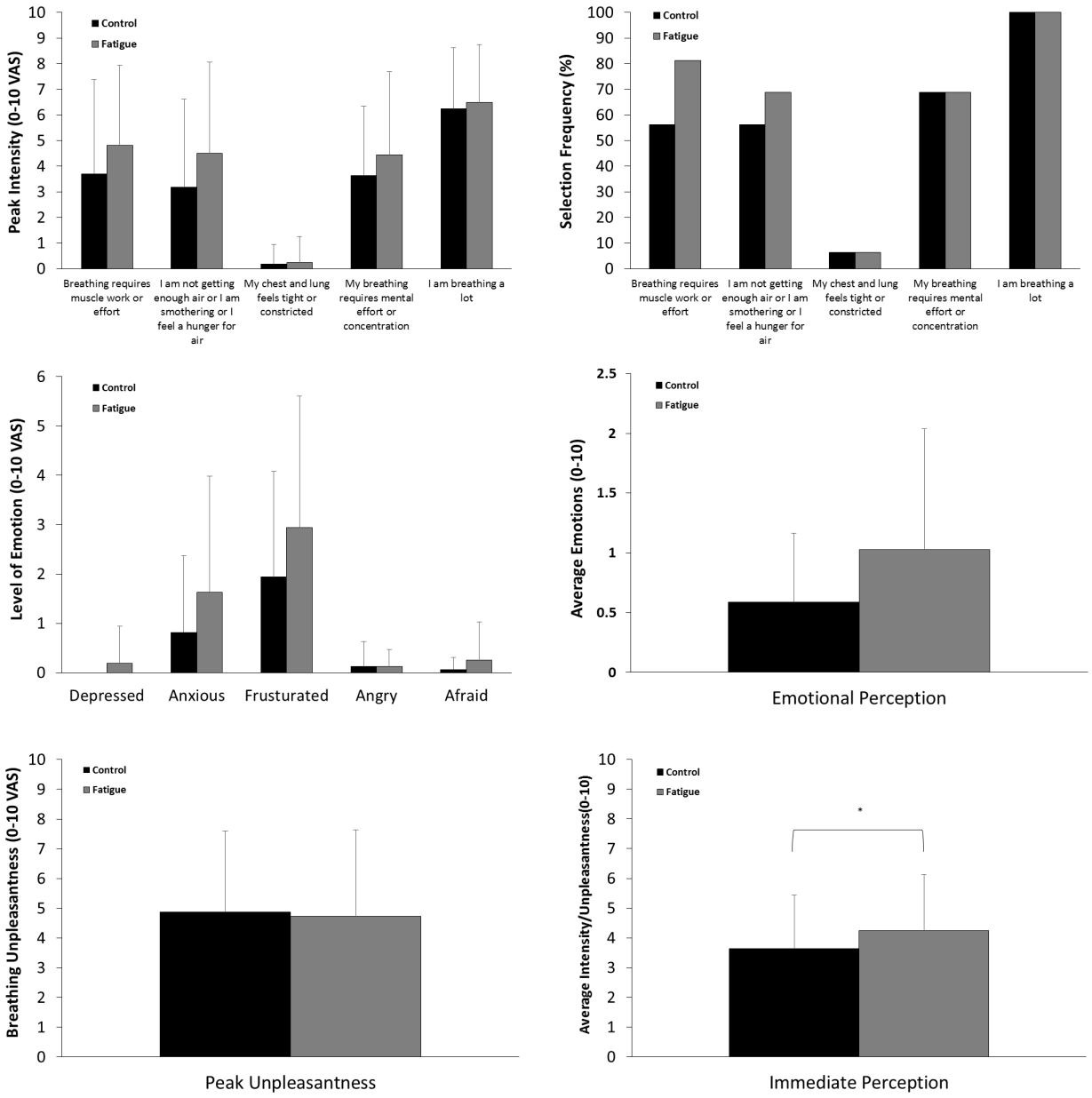

Figure 11. The Multidimensional Dyspnea Profile. *Top left: peak intensity for dyspnea descriptors. Values are mean ± SD. Top right: selection frequency for peak dyspnea descriptors. Values are % of participants. Middle left: level of dyspnea associated emotion from 0-10. Values are mean ± SD. Middle right: emotional perception domain consisting of scores averaged from all five emotions measured. Values are mean ± SD. Bottom left: peak unpleasantness score rated from 0-10 at the end of exercise. Values are mean ± SD. Bottom right: immediate perception domain consisting of average peak unpleasantness and intensity scores for each dyspnea descriptor. Values are mean ± SD. *, p<0.05 statistically significant across conditions.*

2.5.8 Diaphragmatic EMG

Diaphragmatic EMG can be found in Figure 12. One individual was excluded from EMG_{di} analysis due to the catheter sliding out of nose during exercise. Examining the data, the exact point the catheter moved was not clearly identifiable, as such, EMG_{di} data for this participant was excluded for both of his CWR exercise tests. There were no differences at baseline or any submaximal exercise time. Diaphragm EMG tended to be higher at peak exercise in the pre-fatigue condition compared to the control (56.8 ± 14.6 vs. 50.3 ± 9.8 % of maximum) although this did not reach statistical significance (p=0.08).

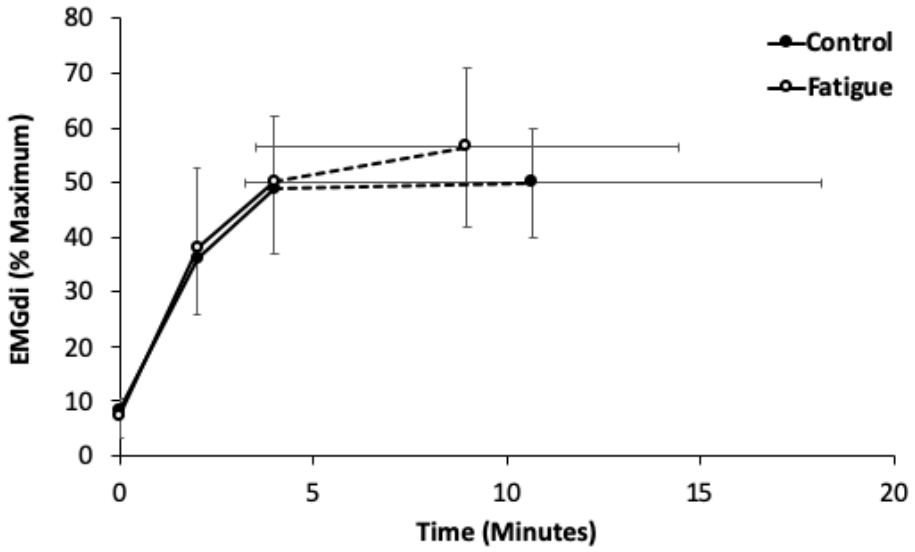

Figure 12. Diaphragm EMG during exercise. *Values are plotted at analysis time points [baseline (0 minutes),1.5 minutes, 3.5 minutes and peak]. Value at baseline and 1.5 minutes have n=15, value at 3.5 minutes has n=13. Abbreviations: EMG_{di}, diaphragm electromyography.*

EMG_{di} correlations with breathing intensity, breathing unpleasantness and $\dot{V}_E$ are presented in Figure 13. EMG_{di} was significantly associated with breathing intensity and unpleasantness scores, as well as ventilation across both conditions (all p<0.001). No statistically significant differences were present across conditions (p>0.05).

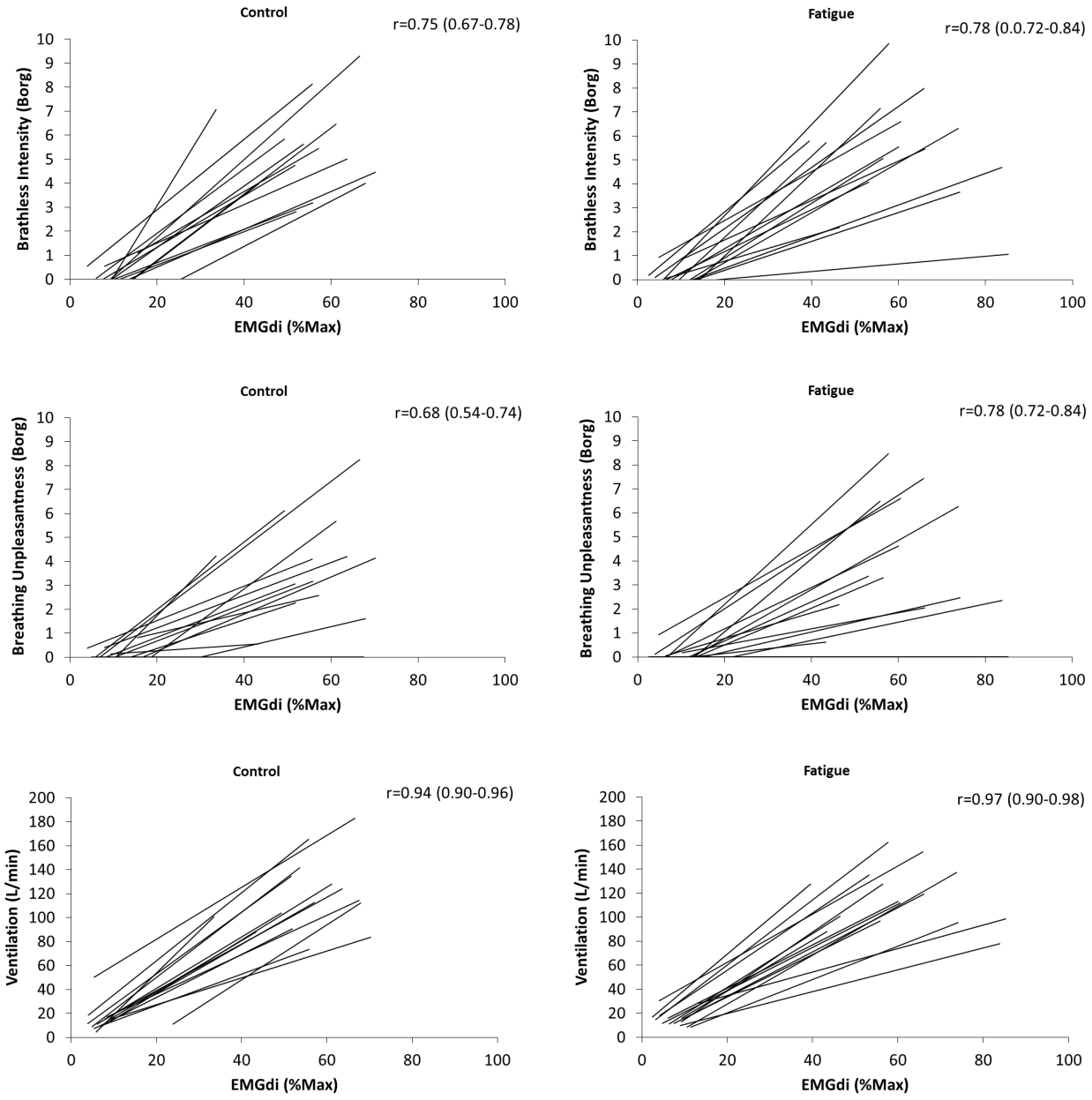

Figure 13. Diaphragmatic EMG correlations. *Top panels: correlation between EMG_{di} and breathing intensity with and without a pre-fatigued diaphragm. Middle: correlation between EMG_{di} and breathing unpleasantness with and without a pre-fatigued diaphragm. Bottom: correlation between EMG_{di} and minute ventilation with and without a pre-fatigued diaphragm. r values are expressed as median (interquartile range) in the top right corner of each plot. Abbreviations: EMG_{di}, diaphragmatic electromyography.*

2.5.9 Surface EMG

Surface EMG versus exercise time for the sternocleidomastoid and scalene muscles can be found in Figure 14. There were no differences in EMGscm or EMGsca at baseline or peak (both $p>0.05$). EMG activation in both the sternocleidomastoid and scalene were significantly higher in the second minute of exercise in the pre-fatigue condition compared to control (6.3 ± 3.7 vs. 4.3 ± 2.4 % of maximum, $p=0.02$ and 14.9 ± 8.5 vs. 11.8 ± 7.4 % of maximum, $p<0.01$), but no other submaximal exercise time.

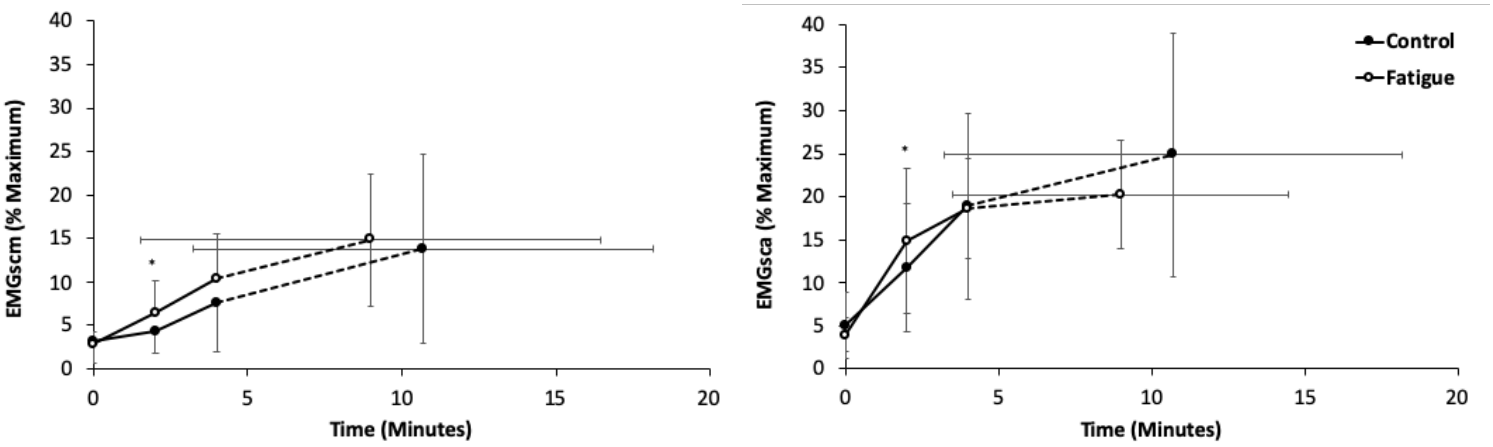

Figure 14. Surface EMG. *Left: EMG_{sca} plotted across measurement time points [baseline (0 minutes), 1.5 minutes, 3.5 minutes and peak]. Value at baseline and 1.5 minutes have n=16, value at 3.5 minutes have n=15. Right: EMG_{sca} plotted across measurement time points [baseline (0 minutes), 1.5 minutes, 3.5 minutes and peak]. Values at baseline and 1.5 minutes have n=16, value at 3.5 minutes have n=15. *, $p<0.05$ statistically different between conditions.*

2.6 Discussion

2.6.1 Primary Findings

To our knowledge, this was the first study to examine the effect of DF on the multidimensional components of dyspnea and diaphragm EMG during exercise. The main findings are as follows: DF in healthy males does not act as a sufficient enough stimulus in isolation to increase diaphragm EMG (an index of NRD) and global breathing intensity during

exercise. However, DF does appear to constrain the respiratory system enough to decrease exercise performance, potentially caused by an increase in dyspnea unpleasantness. To our knowledge, this is the first study to show the emotional dyspnea response to exercising with DF.

2.6.2 Diaphragm Fatigue and PTL

The pressure-time product during loading for this study was 0.42, but may have slightly declined towards task failure due to the onset of fatigue and an inability to reach the target pressure. As such, the PT_{di} for this study was far beyond the 1-hour critical PT_{di} of 0.15-0.18 suggested by Bellemare and Grassino (1982a). As such, task failure with the presence of DF occurred rapidly. The duration of PTL was roughly 15-minutes before DF was successfully assessed. Seven participants needed additional PTL following their first presence of task failure. The most likely mechanisms for task failure without the occurrence of DF has been suggested to be hypocapnia and dyspnea. Baseline $P_{ET}CO_2$ was measured for all participants on the loader before PTL began, and $P_{ET}CO_2$ was maintained within ±10% of that baseline throughout the entirety of loading. As such, dyspnea was the more likely mechanism for PTL task failure without the presence of DF. It should be noted however that dyspnea was not recorded during PTL.

The severity of DF in the present study was ~8% higher than what has been shown previously in men using a similar loading setup (Welch *et al.*, 2018a). The increase in severity is likely the result of our participants targeting P_{di} during loading opposed to targeting P_m as done by Welch *et al.* (2018a). The investigators showed that both men and women fatigued to a similar amount following PTL (~23%). However, following exercise with DF, men declined further by 1.1% and women improved by 6.2%. The interpretation for this finding was that

exercise served as a recovery period for the women following the demanding task of PTL. In the current study of 16 men, DF recovered on average by 1.9% following exercise. Individually, half of the men showed an improvement in DF following exercise, while the other half showed a further reduction in DF. This finding could potentially indicate that sex is not the sole factor determining who recovers during exercise with pre-induced DF.

2.6.3 Breathing Pattern and Ventilatory Response

The high demand to exchange O_2 and CO_2 during exercise requires a large increase in ventilation from resting values. Fatigue of the diaphragm may result in decreased pressure generating capacity of the muscle to meet the high ventilatory demands of exercise, potentially leading to high dead space ventilation, alveolar hypoventilation and subsequent respiratory acidosis. Previous studies have found that ventilation was preserved following DF at rest during CO_2 rebreathing (Yan *et al.*, 1993; Luo *et al.*, 2001). During maximal and submaximal exercise, other studies have shown increases in $\dot{V}_E$ during exercise following DF (Mador & Acevedo, 1991a; Sliwinski *et al.*, 1996; Verges *et al.*, 2006; Welch *et al.*, 2018a). While exploring the sex differences DF has on subsequent exercise performance, Welch *et al.* (2018a) showed a mean increase of 5 $l \cdot min^{-1}$ across the duration of exercise in the pre-fatigue trial compared to control in men, although these results were not significant. Similarly, Verges *et al.* (2006) showed an increase in mean exercise $\dot{V}_E$ of 6.1 during pre-fatigue exercise at 85% of maximum capacity.

In the present study, ventilation was preserved during the pre-fatigue trial compared to control at baseline and throughout exercise. Ventilation was preserved at baseline and early in exercise by adopting a tachypneic and shallower breathing pattern, as reflected by an increase in f_b and decrease in V_t ($p<0.05$). These differences disappeared as exercise progressed towards

peak, despite an increase in selection frequency of "my breathing feels shallow" in 31% of participants in the pre-fatigue condition at peak (p=0.06). Interpretations of this phenomenon are as follows. 1) Following PTL, DF reduced the pressure generating ability of the diaphragm resulting in decreased V_t, forcing a tachypenic breathing pattern in order to maintain ventilation. The differences could have disappeared once the diaphragm began to fatigue in the control condition. Alternatively, as exercise progressed in the fatigue condition, a feedforward mechanism forced a return to similar patterns when compared to the control. 2) The decreased V_t and increased f_b immediately following PTL (baseline measures) and early in exercise was caused by the forced change in breathing pattern and increased respiratory muscle workload during PTL, and not fatigue itself (Tobin *et al.*, 1986). During PTL, participants are required to adopt a fixed breathing pattern while targeting a high inspiratory pressure, which may temporarily alter breathing mechanics immediately following PTL cessation. A study exploring breathing mechanics following PTL to the point of fatigue and PTL without fatigue (accomplished by reducing target pressure, but not breathing pattern), is thus proposed. The preservation of $\dot{V}_E$ following diaphragm fatigue highlights the high reserve capacity of the respiratory system. In the present study, this reserve capacity is reflected in the increase in extradiaphragmatic EMG early in exercise.

2.6.4 Diaphragm EMG and Neural Respiratory Drive

EMG_{di} has been previously used to indirectly assess NRD during both exercise and rest (Lopata *et al.*, 1977; Luo *et al.*, 2001; Schaeffer *et al.*, 2014). To our knowledge, only one current study has assessed the effect diaphragm fatigue has on EMG_{di}, as discussed earlier in the Literature Review section of this thesis (Luo *et al.*, 2001). Similar to the findings of Luo *et al.*

(2001), the present study showed no differences in EMG_{di} following DF. No change in EMG_{di} may indicate that DF in isolation is not sufficient enough to increase NRD, which speaks to the large reserve capacity of the respiratory system to compensate in healthy individuals. Mechanistically, this compensation could have occurred via an increase in motor neuron firing frequency. Additionally, EMG_{di} may not have increased, at least in part, because of an increased activation of the extradiaphragmatic inspiratory muscles. In the present study, both the scalene and sternocleidomastoid showed increased relative activation in the pre-fatigue trial compared to control early in exercise. However, this difference disappeared as exercise progressed.

2.6.5 Exercise Performance

Multiple studies have previously shown a decrease (Martin *et al.*, 1982; Mador & Acevedo, 1991b; Harms *et al.*, 2000; Wuthrich *et al.*, 2013; Welch *et al.*, 2018a) or no change (Sliwinski *et al.*, 1996) in exercise time with pre-induced DF. The current study presents an average decrease in exercise performance of 19%. This decrease in TTE following fatigue is comparable to what has been observed in the literature for men (14-23%). Of the sixteen participants in the present study, five participants did not demonstrate a reduction in exercise performance following pre-induced DF. Three of those individuals replicated their TTE within four seconds, while two improved.

Various mechanisms for DF to impact exercise performance have been proposed, including the respiratory muscle metaboreflex and increased dyspnea (Romer & Polkey, 2008). Dyspnea will be discussed in detail later, while the respiratory muscle metaboreflex (although outside of the scope of this thesis) will be discussed briefly here. High intensity contraction of the diaphragm to the point of fatigue results in an increase in group III and IV nerve afferents of

the diaphragm (Hill, 2000), resulting in a time-dependent increase of MSNA. This increase in MSNA is accompanied by reduced blood flow and vascular conductance of the working muscles (St Croix *et al.*, 2000; Sheel *et al.*, 2001). Ultimately, it is proposed that blood flow is redirected, at least in part, towards the respiratory muscles (Dominelli *et al.*, 2017). Consequently, a reduction of blood flow to the legs during cycle exercise would result in faster locomotor muscle fatigue and a subsequent decrease in exercise performance due to inadequate oxygen supply. In the present study, it is possible that PTL induced the respiratory muscle metaboreflex as shown previously using the same technique (Welch *et al.*, 2018b), ultimately contributing to a decrease in subsequent exercise performance. It is important to note, however, that cardiovascular variables were not evaluated during PTL. In addition, there were no differences in HR during baseline prior to exercise, nor differences in leg discomfort throughout the duration of the cycle exercise test.

Two individuals in the current study had abnormal TTE results. One individual had a dramatic increase in exercise performance during the pre-fatigue exercise test compared to the control, believed to be caused by a change in self-selected cadence during the test. In the control condition, which took place before the fatigue condition, the participant's cadence ranged from 71-77 rpm. In the following pre-fatigue exercise test, the participant's cadence ranged from 71-107, with the shift to a higher cadence occurring at the same time point he ceased exercise in the fatigue condition. Given that the test occurred on an electronically braked ergometer, and that wattage is the product of cadence and resistance, the resistance on the bike lowered following the shift to a higher cadence, despite a constant power output. It is possible that the reduced resistance on the cycle ergometer allowed him to exercise for a longer duration. The second individual experienced a large increase in TTE in both conditions compared to the rest of the

participants, believed to be caused by an underestimation of his GET that was used to set his exercise intensity using the 60%Δ method. As such, the individual exercised at 80% of his maximum, and although 80% fell within the range of the study (76-91%), it is evident that 80% was too low for this individual. With both outliers removed, the decrease in absolute time to exhaustion and statistical significance does not change (-1.7 min, p=0.04), but the standard deviations are more comparable to what is in the current literature (9.2 ± 4.0 control vs. 7.5 ± 3.0 minutes pre-fatigue).

2.6.6 Sensory Perception

The sensory component of dyspnea is made up of the descriptive sensory qualities of breathing, for example "air hunger" and "physical breathing effort", as well as the intensity of those sensations (Banzett *et al.*, 2015). In the present study, breathing intensity was defined as "how strong, or how much breathing sensation you feel." Intensity ratings trended slightly higher in the pre-fatigue CWR exercise test compared to the control condition; however, the increase was not significant. The lack of significant change in breathlessness intensity scores between the conditions may reflect a lack of change in EMG_{di}, which serves as an index of NRD (Schaeffer *et al.*, 2014). Similarly, Luo *et al.* (2001) found no difference in EMG_{di} following diaphragm fatigue after inspiratory resistive loading. As such, it is probable that DF may not be a strong enough stimulus to alter NRD, at least when measured using an esophageal electrode catheter.

It's important to note that during exercise, breathlessness intensity was rated globally (i.e. no particular sensation was rated), given the inadequate time to measure each qualitative descriptor. However, following peak exercise, the intensity of "breathing requires muscle work

or effort", "I am not getting enough air or I am smothering or I feeling a hunger for air", "my chest and lungs feel tight or constricted", "my breathing requires mental effort or concentration" and "I am breathing a lot" were all rated individually. Collectively, the average score across all sensations were significantly higher in the pre-fatigue condition compared to the control, while, each individual descriptor trended higher in the pre-fatigue CWR exercise test compared to the control. Interestingly, the increase in average intensity scores across all descriptors at peak in the pre-fatigue trial corresponds with the largest disparity in EMG_{di}. The descriptors most impacted at peak exercise include "breathing requires muscle work and effort", "I am not getting enough air, or I am smothering, or I feeling a hunger for more air", and "my breathing requires mental effort or concentration".

The current theory of dyspnea suggests that an increase in corollary discharge sensed at the somatosensory cortex of the brain contributes to an increase in work and effort (Jensen *et al.*, 2009). Simon *et al.* (1989) was one of the first to show that healthy humans select increased work and effort as the primary breathlessness descriptor during exercise, with others showing similar results (O'Donnell *et al.*, 2000; Ofir *et al.*, 2008). Recently, Cory *et al.* (2015) showed that this phenomenon holds true regardless of sex. It is known that afferents from the mechanoreceptors and metaboreceptors of the diaphragm directly project into the somatosensory cortex. As such, by reducing the capacity of the diaphragm to generate pressure via fatigue, an increased in the perception of work and effort should be expected. Gandevia *et al.* (1981) was one of the first to show an increase in the perception of work and effort to breathe following respiratory muscle fatigue during IRL. The increase in the intensity score of "my breathing requires more work and effort" at peak, and an increase in selection frequency of the same

descriptor throughout exercise (although not significant) in the present study therefore follows suit with what has been shown in the literature.

Given that the diaphragm serves as the primary muscle of inspiration, it was interesting that there were no differences in the selection of "I cannot get enough air in" during exercise. Of the sixteen participants, three of those selected this descriptor during both the control and pre-fatigue CWR exercise tests, occurring on average 3.3 minutes sooner in the pre-fatigue condition. It has been proposed that the sense of "unsatisfied inspiration" or "air hunger" is most likely caused by the stimulation of chemoreceptors than mechanical constraint, such as with breathing hypoxia or during CO_2 rebreathing (Banzett *et al.*, 1989; Banzett *et al.*, 1990). In the present study, there were some statistically significant differences in cardiorespiratory variables in the pre-fatigue condition compared to control. However, the absolute changes across variables were small. In addition, ventilation was similar across both conditions throughout the entirety of exercise. As such, given that individuals were asked to only select the most prominent descriptor and not every sensation they were feeling, it is likely that DF did not induce enough chemoreceptor stimulation for "unsatisfied inspiration" to be more prominent than "my breathing requires more work and effort" during exercise. It is possible that individuals may have experienced more unsatisfied inspiration in the pre-fatigue trial compared to the control; however, at no time point was it more prominent than the sensation of work and effort. For instance, at peak, there was a higher selection frequency of "I am not getting enough air or I am smothering or I am feeling a hunger for more air" when assessed using the MDP. The intensity rating of this sensation was 1.3 units higher in the pre-fatigue condition compared to the control (p=0.09). However, these intensity ratings were still lower than "my breathing requires muscle work or effort" at peak exercise in both conditions. This provides evidence that if individuals are

only asked to select the most prominent descriptor, it may hide any disparities between other sensations across conditions. This fact remains a limitation of this study and others using this similar methodology. Further research should explore evaluating specific sensations from 0 to 10 during exercise as done previously (O'Donnell *et al.*, 2000).

No differences were present in the selection of "breathing out requires effort" when participants were asked to select the most prominent descriptor during exercise. When asked to select applicable descriptors from a list of 15 as used previously (Schaeffer *et al.*, 2014; Cory *et al.*, 2015), slightly more individuals selected "breathing out requires more effort" and "my breath does not go out all the way" in the pre-fatigue condition compared to the control, although not significant. It is known that there is a co-activation between inspiratory and expiratory muscles. Taylor and Romer (2009) showed a decrease in both P_{di} and P_{ga} following a bout of expiratory resistive loading. Given that the recruitment of abdominal muscles is increased when the load of the respiratory muscles are high (Martin & De Troyer, 1982; Abbrecht *et al.*, 1991), the question arises as to whether or not the expiratory muscles are recruited to the point of fatigue following inspiratory loading. Peters *et al.* (2017) explored this question in healthy humans by having individuals perform IRL. P_{di} significantly reduced following IRL without a reduction in P_{ga}, indicating that DF was present without expiratory muscle fatigue. It should be noted that in the present study, unlike Peters *et al.* (2017), individuals were targeting P_{di} rather than P_m during loading. In addition, to reduce the co-activation of accessory inspiratory muscles, participants were asked to place one hand on the abdomen and one hand on the ribs to ensure that only the abdomen protruded during inspiration. Thus, it could be possible that this focus on diaphragmatic breathing during inspiration to high target pressures may have caused individuals to overly protrude their abdomen, potentially to the point of fatigue. Further research should

explore the relationship between inspiratory and expiratory muscle activation and fatigue during different loading techniques.

Unlike the sensory dimension of dyspnea, the affective dimension is made up of breathing discomfort (or unpleasantness) and the emotional response of such discomfort. In this study, the affective dimension of dyspnea was defined as "how bad or how distressed" their breathing made them feel. This idea of breathing discomfort is the traditional measure of dyspnea among laboratories. Similar to what has been presented in this thesis, previous research has shown an increase in breathing discomfort or unpleasantness following DF. The current thesis has shown a maximum increase in 0.9 Borg units at any time point during exercise. Most recently, Welch *et al.* (2018a) showed a mean increase in breathing discomfort of 0.7 Borg units across exercise during a TTE exercise test at 85% of maximum. Indeed, other laboratories have shown larger increases in Borg unit change. For example, Verges *et al.* (2006) reported a mean increase of 1.4 Borg units across exercise following diaphragm fatigue. However, it is important to note that this study targeted a P_m of at least 80% of maximum during the resistance protocol. A combination of a higher target pressure and targeting of P_m may have contributed to fatigue of respiratory muscles beyond the diaphragm. For instance, Sliwinski *et al.* (1996) reported an increase of 2 units during exercise following intentional global respiratory muscle fatigue.

To our knowledge, this will be the first study to record the emotional response to dyspnea in individuals exercising with pre-induced DF. Measuring the emotional response of a symptom is an important, often over looked, factor. To no surprise, feelings of depression, anger and fear were rarely ranked beyond a zero and showed no differences across conditions. However, individuals in the pre-fatigue condition tended to report higher ratings for anxiety and frustration, (p=0.08 and p=0.06, respectively). The increase in anxiety during exercise during the pre-fatigue

condition was a surprise to the research team given that anxiety during physical activity is more often rated in clinical populations (Carrieri-Kohlman *et al.*, 1996; Carrieri-Kohlman *et al.*, 2001). This thesis shows short term muscle fatigue plays a role in the emotional dyspnea response of even healthy individuals, which may have potential consequences for behavior change and activity avoidance.

2.6.7 Limitations

A limitation of this study is that it was conducted in only male participants. It has been shown that women are more resistant to DF during exercise (Guenette *et al.*, 2010), and that they demonstrate different DF responses during loaded breathing as well (Welch *et al.*, 2018b). Given that the goal of this thesis was to explore mechanisms of dyspnea, specifically the impact of DF, only one sex was chosen. As such, men were chosen because of their lower resistance to DF.

There are three major limitations to all "pre-fatigue" studies. First, the number and type of motor recruitment during PTL may be different than that recruited during exercise. Second, during loaded breathing there may be coactivation of expiratory muscles (the abdominals) to assist the respiratory muscle as discussed in detail above. Third, there is no true placebo condition to PTL.

Another limitation is the fixed distance between the gastric and esophageal balloons in the multi-pair esophageal catheter. The fixed distance does not allow the alteration of one balloon without the movement of the other. However, a primary outcome for this project was EMG_{di}; therefore, the catheter was positioned to optimize the EMG_{di} signal. The limitations of

EMG_{di} as a surrogate of NRD have been discussed earlier in the *Assessing Neural Drive* and *Electromyography* sections of this thesis.

There are a few considerations when stimulating the phrenic nerves via CMS. Despite being better tolerated than electrical stimulation, CMS does not guarantee maximal stimulation. As such, $P_{di,tw}$ recorded from the multi-pair esophageal electrode was evaluated for a plateau as evidence of maximal stimulation. Second, stimulation of the phrenic nerves may cause co-activation of the sternocleidomastoid and scalene that may have contributed to both the M-wave and P_{di}.

It's important to acknowledge that this study only presents evidence for peripheral fatigue, specifically low-frequency peripheral fatigue. That is, fatigue occurring as a result of mechanisms distal to the neuromuscular junction. It is known however that fatigue can occur at any point along the neural network proximal to this junction. Further research should explore how central fatigue impacts dyspnea as a multidimensional experience and its effect on EMG_{di}.

There are a few limitations with the exercise protocol. First, by setting an individual's exercise intensity using the 60%Δ method, it may be more beneficial to use a ramp incremental exercise test rather than a step-wise increase as used in the present study. By using a ramped protocol, pinpointing the GET of an individual would be more exact to a specific wattage, rather than a 25-increment range. By having a more specific GET wattage, it would limit the likelihood of over or underestimating exercise intensity.

Lastly, although the sample size was calculated to detect differences in dyspnea intensity and unpleasantness scores, the study may have benefited from a larger sample size. There were a number of variables trending towards significance that may have been detected in a larger sample.

Chapter 3: Conclusion

To our knowledge, this is the first study to explore the effect DF has on the multidimensional components of dyspnea and EMG_{di} during exercise. The study adds to the body of knowledge in both the dyspnea, DF, and exercise performance literature. By evaluating both the sensory and affective dimensions of dyspnea during exercise with and without a pre-fatigued diaphragm, we have increased our understanding of how DF impacts exercise in healthy men.

This study showed that DF may not have a strong influence in the sensory component of dyspnea. Specifically, DF does not increase the intensity of dyspnea, nor increase the prevalence of sensations such as "increased work and effort", "unsatisfied inspiration" or "air hunger" throughout exercise. This may be reflected in the fact that DF fatigue in isolation was not a strong enough stimulus to alter EMG_{di}, an indirect surrogate of NRD. However, DF does appear to act upon the affective dimension of dyspnea during exercise by increasing the unpleasantness of breathing sensations. Although it was not significant, a trend was shown that exercising with a pre-fatigued diaphragm increases the feeling of anxiety and frustration. Thus, it is possible that this increase in the affective dimension of dyspnea may contribute to decreased exercise performance. Further research in this field should explore the sex differences DF imposes on the various dimensions of dyspnea, as well as the impact of central fatigue.

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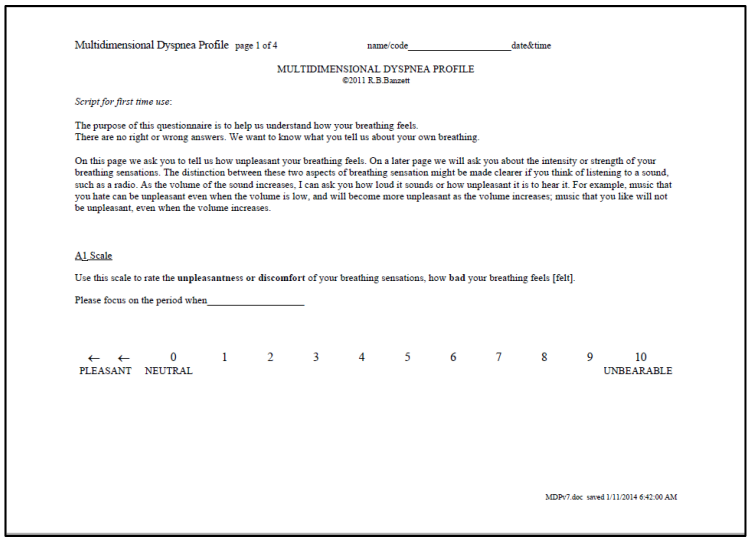
Appendices

Appendix A Intensity Vs. Unpleasantness Script

"During bicycle exercise, you will be asked to rate various aspects of your breathing sensation. Some ratings relate specifically to the **intensity** of your breathing sensation, while others relate specifically to the **unpleasantness** of your breathing sensation. You will be asked to **SEPARATELY** rate the **intensity** and **unpleasantness** of your breathing sensations. The **intensity** of the sensation is **how strong** or **how much** breathing sensation you feel, while the **unpleasantness** of the sensation is **how bad** or **how distressed** it makes you feel. The distinction between these two aspects of breathing sensation might be made clearer if you think of listening to a sound, such as a radio. As the volume and content of the sound changes, I can ask you how loud it sounds or how **unpleasant** it is to hear it. For example, music that you hate can be **unpleasant** even when the volume is low, and will become more **unpleasant** as the volume increases. Alternatively, music that you like will not be **unpleasant**, even when the volume increases. The **intensity** of breathing sensation is like sound volume, whereas the **unpleasantness** of breathing sensation depends not only on **intensity** but also on how good or bad the sensation is."

Appendix B Multidimensional Dyspnea Profile



Multidimensional Dyspnea Profile page 1 of 4 name/code_____date&time

MULTIDIMENSIONAL DYSPNEA PROFILE
©2011 R.B.Banzett

Script for first time use:

The purpose of this questionnaire is to help us understand how your breathing feels.
There are no right or wrong answers. We want to know what you tell us about your own breathing.

On this page we ask you to tell us how unpleasant your breathing feels. On a later page we will ask you about the intensity or strength of your breathing sensations. The distinction between these two aspects of breathing sensation might be made clearer if you think of listening to a sound, such as a radio. As the volume of the sound increases, I can ask you how loud it sounds or how unpleasant it is to hear it. For example, music that you hate can be unpleasant even when the volume is low, and will become more unpleasant as the volume increases; music that you like will not be unpleasant, even when the volume increases.

A1 Scale

Use this scale to rate the **unpleasantness** or **discomfort** of your breathing sensations, how **bad** your breathing feels [felt].

Please focus on the period when_____

← ← 0 1 2 3 4 5 6 7 8 9 10
PLEASANT NEUTRAL UNBEARABLE

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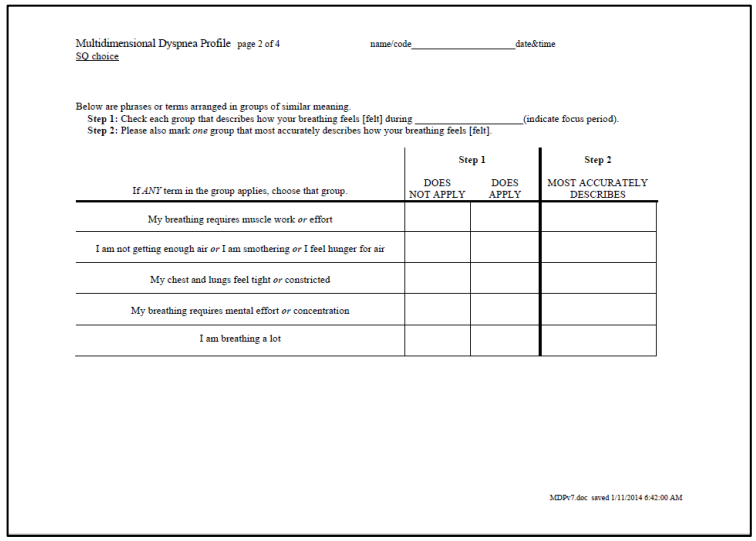

Multidimensional Dyspnea Profile page 2 of 4 name/code_____date&time
SQ choice

Below are phrases or terms arranged in groups of similar meaning.
Step 1: Check each group that describes how your breathing feels [felt] during _____(indicate focus period).
Step 2: Please also mark *one* group that most accurately describes how your breathing feels [felt].

If *ANY* term in the group applies, choose that group.	Step 1 DOES NOT APPLY	Step 1 DOES APPLY	Step 2 MOST ACCURATELY DESCRIBES
My breathing requires muscle work *or* effort			
I am not getting enough air *or* I am smothering *or* I feel hunger for air			
My chest and lungs feel tight *or* constricted			
My breathing requires mental effort *or* concentration			
I am breathing a lot			

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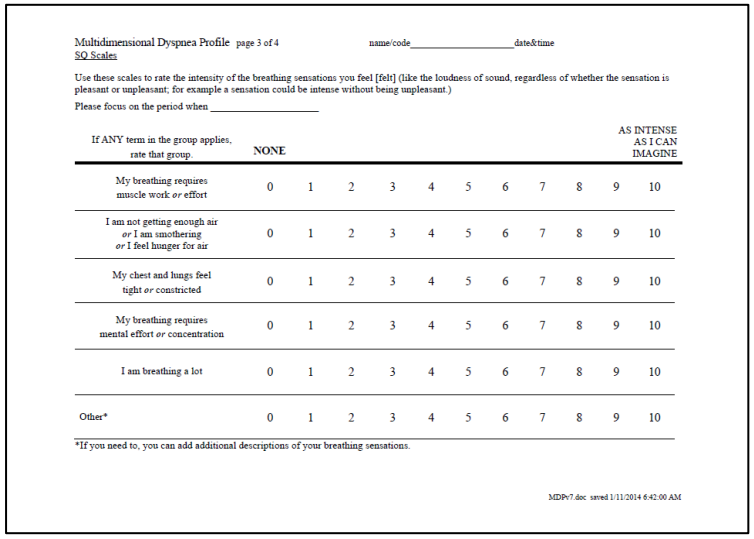

Multidimensional Dyspnea Profile page 3 of 4 name/code_____date&time
SQ Scales

Use these scales to rate the intensity of the breathing sensations you feel [felt] (like the loudness of sound, regardless of whether the sensation is pleasant or unpleasant; for example a sensation could be intense without being unpleasant.)

Please focus on the period when _____

If ANY term in the group applies, rate that group.	NONE										AS INTENSE AS I CAN IMAGINE
My breathing requires muscle work *or* effort	0	1	2	3	4	5	6	7	8	9	10
I am not getting enough air *or* I am smothering *or* I feel hunger for air	0	1	2	3	4	5	6	7	8	9	10
My chest and lungs feel tight *or* constricted	0	1	2	3	4	5	6	7	8	9	10
My breathing requires mental effort *or* concentration	0	1	2	3	4	5	6	7	8	9	10
I am breathing a lot	0	1	2	3	4	5	6	7	8	9	10
Other*	0	1	2	3	4	5	6	7	8	9	10

*If you need to, you can add additional descriptions of your breathing sensations.

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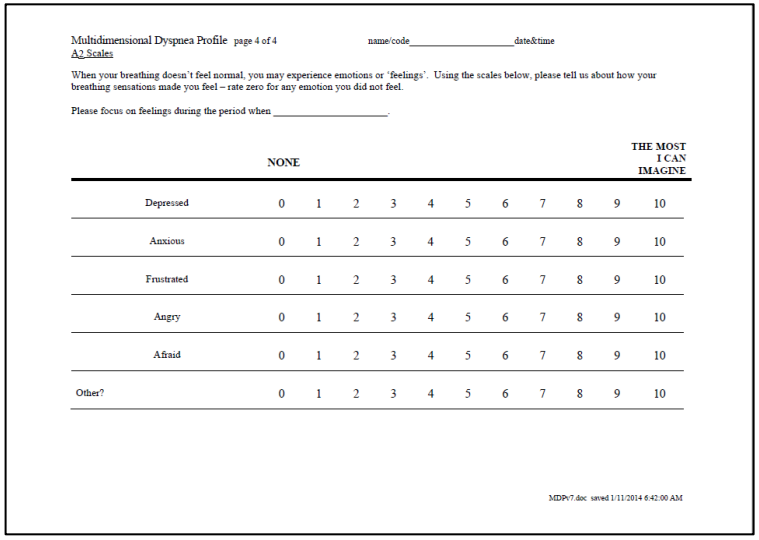

Multidimensional Dyspnea Profile page 4 of 4 name/code_____date&time
A2 Scales

When your breathing doesn't feel normal, you may experience emotions or 'feelings'. Using the scales below, please tell us about how your breathing sensations made you feel – rate zero for any emotion you did not feel.

Please focus on feelings during the period when _____.

	NONE										THE MOST I CAN IMAGINE
Depressed	0	1	2	3	4	5	6	7	8	9	10
Anxious	0	1	2	3	4	5	6	7	8	9	10
Frustrated	0	1	2	3	4	5	6	7	8	9	10
Angry	0	1	2	3	4	5	6	7	8	9	10
Afraid	0	1	2	3	4	5	6	7	8	9	10
Other?	0	1	2	3	4	5	6	7	8	9	10

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Appendix C Pressure Threshold Loader