SEX DIFFERENCES IN DIAPHRAGMATIC FATIGUE

by

Joseph Frank Welch

B.Sc. (Hons), University of Derby (UK), 2013
M.Res., University of Derby (UK), 2014

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

**Sex Differences in Diaphragmatic Fatigue**

Submitted by **Joseph Frank Welch** in partial fulfillment of the requirements for

the degree of **Doctor of Philosophy**

in **Kinesiology**

**Examining Committee:**

Dr. Bill Sheel  
Supervisor

Dr. Jordan Guenette  
Supervisory Committee Member

Dr. Christopher West  
Supervisory Committee Member

Dr. Tania Lam  
University Examiner

Dr. Colin Brauner  
University Examiner

**Additional Supervisory Committee Members:**

Supervisory Committee Member

Supervisory Committee Member
Abstract

Purpose: The purpose of the thesis was to: 1) establish the reliability of cervical magnetic stimulation and chest wall surface EMG in the assessment of the diaphragmatic compound muscle action potential (CMAP) in healthy men and women (Study #1, Chapter 3), and 2) explore sex-based differences in the mechanisms and consequences of diaphragmatic fatigue (DF), specifically, 2a) the cardiovascular response to inspiratory resistance (Study #2, Chapter 4), and 2b) the effect of DF on subsequent exercise performance (Study #3, Chapter 5).

Methods: Diaphragmatic fatigue was assessed in healthy men and women by measuring transdiaphragmatic twitch pressure using cervical magnetic stimulation. Surface electrodes were placed on the left and right hemi-diaphragm. Inspiratory pressure-threshold loading (PTL) was used to induce DF at rest, whilst a host of cardiovascular variables were measured (including: heart rate [HR], mean arterial [MAP] and low-frequency systolic blood pressure variability [LF\(_{SBP}\)]). A time-to-exhaustion cycle test was performed with and without the induction of DF.

Results: All CMAP characteristics demonstrated high reproducibility within and between experimental sessions. At PTL task failure, the degree of DF was not different between sexes (~23%); however, time to task failure was longer in women than men (27 vs. 16 min). Furthermore, women exhibited less of an increase in HR (13 vs. 19 bpm) and MAP (10 vs. 14 mmHg), and significantly lower LF\(_{SBP}\) (23 vs. 34 mmHg\(^2\)) during PTL compared to men. Prior-induced DF negatively and equally affected subsequent exercise performance in men and women (~15%).

Conclusions: Cervical magnetic stimulation is a reliable means to evaluate phrenic nerve conduction in healthy men and women. The female diaphragm is highly fatigue resistant, leading to an attenuation of the inspiratory muscle metaboreflex (i.e. cardiovascular consequences of DF). Yet, DF impairs exercise independent of sex.
Lay Summary

Diaphragmatic fatigue (DF) leads to a host of cardiovascular consequences that may contribute to exercise intolerance. The present thesis asked the following: 1) are women more resistant to DF during an isolated resistive breathing task compared to men, 2) do women exhibit an attenuated cardiovascular response to DF, and 3) what is the effect of DF on subsequent exercise performance. The rate of fatigue development was slower in women than men. Cardiovascular responses, including heart rate and mean arterial pressure were lower in women compared to men. Prior-induced DF led to the premature termination of exercise that did not differ between sexes. In conclusion, this thesis demonstrates the fatigue resistant properties of the female diaphragm and sheds light on sex-based differences in the inspiratory muscle metaboeflex.
Preface

This thesis contains the work of the candidate Joseph F. Welch, under the supervision of Dr. A. William Sheel. Joseph F. Welch and A. William Sheel conceptualised, designed and interpreted the work. Joseph F. Welch collected and analysed the data. All data were collected at the Health and Integrative Physiology Laboratory at the University of British Columbia.

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A version of Chapter 4 has been previously published as:


A version of Chapter 5 is currently under revision as:


Experiments contained herein received ethical approval from the University of British Columbia Clinical Research Ethics Board (approval number: H15-00801).
# Table of Contents

Abstract........................................................................................................................................ iii

Lay Summary ...................................................................................................................................... iv

Preface............................................................................................................................................... v

Table of Contents .............................................................................................................................. vi

List of Tables .................................................................................................................................... xi

List of Figures ..................................................................................................................................... xii

List of Abbreviations ...................................................................................................................... xiv

Acknowledgements ........................................................................................................................... xvii

Dedication .......................................................................................................................................... xviii

**Chapter 1: Introduction** .................................................................................................................. 1

**Chapter 2: Literature Review** .......................................................................................................... 4

2.1 Structure and Function of the Diaphragm....................................................................................... 4

2.1.1 Functional Anatomy .................................................................................................................. 4

2.1.2 Neural Innervation and Blood Supply ....................................................................................... 5

2.1.3 Histochemical Composition ..................................................................................................... 7

2.1.4 Respiratory Muscle Mechanics ............................................................................................... 8

2.1.5 Contractile Properties ............................................................................................................. 11

2.2 Respiratory Muscle Fatigue .......................................................................................................... 14

2.2.1 Respiratory System Limitations to Exercise ............................................................................ 14

2.2.2 Definition, Sites and Mechanisms of Fatigue ......................................................................... 15

2.2.3 Assessment of Respiratory Muscle Fatigue ............................................................................ 17

2.2.4 Respiratory Muscle Demands during Exercise ........................................................................ 22
Chapter 2: Respiratory Exercise Physiology

2.2.5 Exercise-Induced Diaphragmatic Fatigue.................................................. 23

2.3 Consequences of Respiratory Muscle Fatigue.............................................. 26
   2.3.1 Ventilation and Dyspnoea........................................................................ 26
   2.3.2 Cardiorespiratory Interactions .............................................................. 27
   2.3.3 Locomotor Muscle Fatigue and Exercise Performance......................... 29

2.4 Sex-Based Differences in Respiratory Exercise Physiology.......................... 33
   2.4.1 Sexual Dimorphism and Dysanapsis .................................................... 33
   2.4.2 Mechanical Ventilatory Constraints ..................................................... 33
   2.4.3 Work and Oxygen Cost of Breathing.................................................. 35
   2.4.4 Fatigue............................................................................................... 37

2.5 Purpose.......................................................................................................... 43

2.6 Research Questions....................................................................................... 43

2.7 Hypotheses.................................................................................................... 43


3.1 Introduction.................................................................................................... 45

3.2 Methods........................................................................................................ 48
   3.2.1 Subjects............................................................................................... 48
   3.2.2 Procedures........................................................................................... 48
   3.2.3 Cervical Magnetic Stimulation .............................................................. 49
   3.2.4 Surface EMG Recordings .................................................................... 49
   3.2.5 CMAP Analysis .................................................................................. 50
   3.2.6 Statistical Analysis .............................................................................. 52
Chapter 4: Sex Differences in Diaphragmatic Fatigue: The Cardiovascular Response to Inspiratory Resistance

4.1 Introduction

4.2 Methods

4.2.1 Subjects and Ethical Approval

4.2.2 Experimental Design

4.2.3 Inspiratory Pressure-Threshold Loading

4.2.4 Cervical Magnetic Stimulation

4.2.5 Electromyography

4.2.6 Cardiovascular Variables

4.2.7 Data Analysis

4.2.8 Statistics

4.3 Results
5.3.2 Diaphragmatic Fatigue ............................................................................. 102
5.3.3 Cardiovascular Responses to PTL ............................................................. 104
5.3.4 Breathing Pattern and Sensory Perceptions of Leg and Breathing Discomfort .. 104
5.3.5 Exercise Tolerance ..................................................................................... 105

5.4 Discussion ........................................................................................................ 112

5.4.1 Main Findings ............................................................................................... 112
5.4.2 Exercise Limitation ....................................................................................... 113
5.4.3 Ventilatory Response to Exercise ................................................................. 115
5.4.4 Dyspnoea ..................................................................................................... 117
5.4.5 Technical Considerations .............................................................................. 119
5.4.6 Conclusions .................................................................................................. 120

Chapter 6: Conclusions ........................................................................................ 121

6.1 Overall Summary ............................................................................................. 121
6.2 Significance ...................................................................................................... 122
6.3 Strengths and Limitations .................................................................................. 125
6.4 Future Directions ............................................................................................. 126
6.5 Conclusion ....................................................................................................... 127

Bibliography ............................................................................................................ 129
**List of Tables**

Table 3.1: Group Mean (± SD) Data for all CMAP Characteristics........................................54

Table 3.2: Full CMAP Reliability Analyses.............................................................................55

Table 4.1: Subject Characteristics..........................................................................................74

Table 4.2: Physiological Responses to Pressure-Threshold Loading....................................82

Table 5.1: Peak Responses during Maximal Incremental Exercise.......................................102

Table 5.2: Twitch Control Measures ....................................................................................107

Table 5.3: Reproducibility of Twitch Characteristics............................................................108

Table 6.1: List of Future Directions......................................................................................127
List of Figures

Figure 3.1: Illustration of CMAP Analysis Methodology .................................................. 51
Figure 3.2: Recruitment Curve .......................................................................................... 58
Figure 3.3: Within- (Thick Line) and Between-Session (Dashed Line) Correlations for all CMAP Characteristics .................................................................................................................. 60
Figure 3.4: Composite Average Raw CMAPs Demonstrating Within- and Between Reproducibility (One Representative Subject) ........................................................................ 61
Figure 4.1: Twitch Potentiation Protocol ............................................................................. 70
Figure 4.2: Recruitment Curves .......................................................................................... 75
Figure 4.3: Time to Task Failure and Severity of Diaphragmatic Fatigue ......................... 76
Figure 4.4: Relative Contributions of the Diaphragm and Ribcage Muscles to Changes in Transdiaphragmatic Twitch Pressure ....................................................................................... 78
Figure 4.5: Pressure-Time Product and Cumulative Diaphragmatic Work ......................... 79
Figure 4.6: Raw Cardiorespiratory Variables from One Representative Subject during Pressure-Threshold Loading .................................................................................................................. 80
Figure 4.7: Cardiovascular Responses to Pressure-Threshold Loading ............................. 81
Figure 5.1: Diaphragm Responses to Phrenic Nerve Stimulation ...................................... 100
Figure 5.2: Diaphragmatic Fatigue during Pre-Fatigue and Control Visits ......................... 106
Figure 5.3: Effect of Diaphragm Fatigue on the Ventilatory Response to Constant Load Exercise .................................................................................................................................................. 109
Figure 5.4: Effect of Diaphragm Fatigue on Ratings of Perceived Exertion and Dyspnoea during High-Intensity Constant Load Cycling to Exhaustion ......................................................... 110
Figure 5.5: Respiratory Muscle Pressure-Time Products during High-Intensity Constant Load Cycling to Exhaustion With and Without Prior-Induced Diaphragmatic Fatigue................. 111

Figure 5.6: Effect of Diaphragm Fatigue on Subsequent Exercise Performance in Healthy Men and Women.................................................................................................................. 112

Figure 6.1: Working Hypothesis for the Mechanisms and Consequences of Female Resistance to Diaphragmatic Fatigue................................................................. 124
# List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1RM</td>
<td>1 Repetition Maximum</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>BPNS</td>
<td>Bilateral Phrenic Nerve Stimulation</td>
</tr>
<tr>
<td>CMAP</td>
<td>Compound Muscle Action Potential</td>
</tr>
<tr>
<td>CMS</td>
<td>Cervical Magnetic Stimulation</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of Variation</td>
</tr>
<tr>
<td>DF</td>
<td>Diaphragmatic Fatigue</td>
</tr>
<tr>
<td>EELV</td>
<td>End-Expiratory Lung Volume</td>
</tr>
<tr>
<td>EFL</td>
<td>Expiratory Flow Limitation</td>
</tr>
<tr>
<td>EIAH</td>
<td>Exercise-Induced Arterial Hypoxaemia</td>
</tr>
<tr>
<td>EILV</td>
<td>End-Inspiratory Lung Volume</td>
</tr>
<tr>
<td>EMG</td>
<td>Electromyography</td>
</tr>
<tr>
<td>$f_b$</td>
<td>Breathing Frequency</td>
</tr>
<tr>
<td>FEV$_1$</td>
<td>Forced Expired Volume in One Second</td>
</tr>
<tr>
<td>FIO$_2$</td>
<td>Fraction of Inspired Oxygen</td>
</tr>
<tr>
<td>FRC</td>
<td>Functional Residual Capacity</td>
</tr>
<tr>
<td>FVC</td>
<td>Forced Vital Capacity</td>
</tr>
<tr>
<td>HR</td>
<td>Heart Rate</td>
</tr>
<tr>
<td>ICC</td>
<td>Intraclass Correlation Coefficient</td>
</tr>
<tr>
<td>IOC</td>
<td>International Olympic Committee</td>
</tr>
<tr>
<td>LVR</td>
<td>Limb vascular resistance</td>
</tr>
<tr>
<td>MAP</td>
<td>Mean Arterial Pressure</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>MIP</td>
<td>Maximal Inspiratory Mouth Pressure</td>
</tr>
<tr>
<td>MSNA</td>
<td>Muscle Sympathetic Nerve Activity</td>
</tr>
<tr>
<td>MVC</td>
<td>Maximal Voluntary Contraction</td>
</tr>
<tr>
<td>MVV</td>
<td>Maximum Voluntary Ventilation</td>
</tr>
<tr>
<td>PAV</td>
<td>Proportional Assist Ventilation</td>
</tr>
<tr>
<td>$P_{di}$</td>
<td>Transdiaphragmatic Pressure</td>
</tr>
<tr>
<td>$P_{di,tw}$</td>
<td>Transdiaphragmatic Twitch Pressure</td>
</tr>
<tr>
<td>$P_{ET}CO_2$</td>
<td>End-Tidal Partial Pressure of Carbon Dioxide</td>
</tr>
<tr>
<td>$P_{ga}$</td>
<td>Gastric Pressure</td>
</tr>
<tr>
<td>$P_i$</td>
<td>Inorganic Phosphate</td>
</tr>
<tr>
<td>$P_{oes}$</td>
<td>Oesophageal Pressure</td>
</tr>
<tr>
<td>PTL</td>
<td>Pressure-Threshold Loading</td>
</tr>
<tr>
<td>PTP</td>
<td>Pressure-Time Product</td>
</tr>
<tr>
<td>PTP$_{di}$</td>
<td>Diaphragm Pressure-Time Product</td>
</tr>
<tr>
<td>PTP$_{oes}$</td>
<td>Oesophageal Pressure-Time Product</td>
</tr>
<tr>
<td>$\dot{Q}$</td>
<td>Cardiac Output</td>
</tr>
<tr>
<td>$Q_{tw}$</td>
<td>Quadriceps Twitch Force</td>
</tr>
<tr>
<td>RER</td>
<td>Respiratory Exchange Ratio</td>
</tr>
<tr>
<td>$S_aO_2$</td>
<td>Arterial Oxygen Saturation</td>
</tr>
<tr>
<td>$S_pO_2$</td>
<td>Peripheral Oxygen Saturation</td>
</tr>
<tr>
<td>$T_i/T_{TOT}$</td>
<td>Inspiratory Duty Cycle</td>
</tr>
<tr>
<td>TTE</td>
<td>Time-to-Exhaustion</td>
</tr>
<tr>
<td>TTI</td>
<td>Tension-Time Index</td>
</tr>
<tr>
<td>Symbol</td>
<td>Definition</td>
</tr>
<tr>
<td>--------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td>$\dot{V}_A$</td>
<td>Alveolar Ventilation</td>
</tr>
<tr>
<td>$\dot{V}_{CO_2}$</td>
<td>Carbon Dioxide Production</td>
</tr>
<tr>
<td>$\dot{V}_E$</td>
<td>Minute Ventilation</td>
</tr>
<tr>
<td>$\dot{V}_{O_2}$</td>
<td>Oxygen Consumption</td>
</tr>
<tr>
<td>$\dot{V}_{O_2\text{max}}$</td>
<td>Maximal Oxygen Uptake</td>
</tr>
<tr>
<td>$\dot{V}_{O_2\text{RM}}$</td>
<td>Respiratory Muscle Oxygen Uptake</td>
</tr>
<tr>
<td>$V_T$</td>
<td>Tidal Volume</td>
</tr>
<tr>
<td>WOB</td>
<td>Work of Breathing</td>
</tr>
</tbody>
</table>
Acknowledgements

In September 2014, I arrived in Vancouver to commence a new chapter in my life, one that would take me on a journey I will always look back on proudly. I met many wonderful people and was able to not only pursue my passion for science, but experience a new country and culture. Naïve to the extent of the undertaking I was about to embark upon, I am indebted to those that helped me along the way.

First of all, I wish to thank Dr. Bill Sheel. I cannot express how fortunate I feel to have been afforded the opportunity to come to UBC and study in the HIP laboratory. I believe the standards set by those I worked with were exemplary and strongly reflects the mentorship we received. My gratitude goes to my colleagues and collaborators across many fields of physiology, for this is the part of being a graduate student I shall miss the most – the chance to speak with and learn from brilliant minds and people.

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In memory of Nanny M and Nanny B
Chapter 1: Introduction

Historically, research investigating the integrated physiologic response to exercise has been heavily biased by studies conducted exclusively in male subjects (Shephard, 2000). Reasons for this partiality are complex; one must consider the social attitudes, political viewpoints and cultural norms of the times (Sheel, 2016). In 1919, the founder of the International Olympic Committee (IOC), Baron Pierre de Coubertin, made the following statement regarding female involvement in the Olympic Games; in his view, female participation would be “impractical, uninteresting, unaesthetic and incorrect”. In this vein, just 22 women participated in the 1900 Olympic Games in Paris (2.2% of the complete athlete representation). More than one hundred years later, a total of 4676 female athletes competed at London 2012, accounting for a record 44.2% of all athletes competing at the Games (IOC, 2014).

It is argued that science reflects society (Kinloch & Mohan, 2000). Research philosophy is unique to every scholar; a culmination of viewpoints, ideologies, and values (axiology), directed by concepts of deontology and epistemology (Saunders et al., 2009). According to Iaccarino (2003), science is part of culture and culture influences how science is practiced. As female involvement in sport continues to expand, science has evolved accordingly, with research placing an emphasis on the role of inherent biological (i.e. sex-based) similarities and differences. The number of publications addressing sex differences in exercise physiology has increased from 12, before 1970, to as many as 1344 through October 2007 (Day, 2008). This tremendous expansion of scientific literature likely mirrors a cultural shift in our society today.

Sex-based differences with regard to the pulmonary system have important consequences for the development of flow, volume and pressure during dynamic exercise (Sheel & Guenette,
2008). Over the past few decades, significant structural and functional disparities have been noted between males and females, which profoundly alters our understanding of respiratory exercise physiology (Sheel et al., 2004) and the integrated whole-body response to exercise (Harms & Rosenkranz, 2008). For example, it is well acknowledged that when matched for lung size, women have narrower airways relative to men – a concept termed ‘dysanapsis’ (Mead, 1980; Thurlbeck, 1982; Sheel et al., 2009). This fundamental anatomical sex-based difference predisposes women to greater mechanical ventilatory constraints during heavy exercise (McClaran et al., 1998; Dominelli et al., 2011), leading to expiratory flow limitation and consequently, higher operating lung volumes (Guenette et al., 2007). In addition, women are more likely to experience exercise-induced arterial hypoxaemia (Dominelli et al., 2013) and for a given ventilation, have a greater resistive work and oxygen cost of breathing (Guenette et al., 2007; Guenette et al., 2009; Dominelli et al., 2015). Based upon the aforementioned evidence, it is logical to predict that women may be more susceptible to inspiratory muscle fatigue. However, it appears that women are in fact more resistant to fatigue of the diaphragm during intense whole-body exercise (Guenette et al., 2010) and inspiratory resistive breathing at rest (Gonzales & Scheuermann, 2006), leading to an attenuation of the inspiratory muscle metaboreflex (Smith et al., 2016), which may affect tolerance to exercise.

No longer regarded the “forgotten sex” (Haverkamp & Dempsey, 2004), this thesis attempts to further explore the mechanisms behind these sex-based differences by isolating fatigue of the diaphragm at rest. So called ‘pre-fatigue’ studies are designed to experimentally induce global inspiratory muscle fatigue in order to ascertain the influence of this variable upon subsequent exercise performance independent of confounding factors associated with whole-body
exercise, such as blood flow competition between the respiratory and locomotor muscles. Should a sex-based difference persist under this condition, it may shed light on the potential consequences of respiratory muscle fatigue, specifically as it pertains to the inspiratory muscle metaboreflex. Furthermore, this thesis will seek to provide a single benchmark process for the assessment of diaphragmatic function using cervical magnetic stimulation, establishing its efficacy via demonstrable and repeatable measurement of the compound muscle action potential, uniform between sexes.
Chapter 2: Literature Review

2.1 Structure and Function of the Diaphragm

2.1.1 Functional Anatomy

The diaphragm is a striated skeletal muscle, often considered the principle muscle of respiration (Mognoni et al., 1969; Macklem, 2014). Morphologically, the diaphragm may be described as a thin, flat, musculotendinous structure that assumes the shape of an elliptical cylinder capped by a dome, separating the thoracic cavity from the abdominal cavity (De Troyer & Loring, 1986; De Troyer & Estenne, 1988). The diaphragm should not be treated as a single muscle (De Troyer et al., 1981); rather, the diaphragm is composed of three main segments – a central non-contractile element (the central tendon); and two discrete muscular portions, the costal (also described as ventral) and crural (also described as dorsal) diaphragm (Poole et al., 1997). The cylindrical portion is apposed to the inner aspect of the lower ribcage and constitutes the zone of apposition.

Each hemi-diaphragm (left and right side separated by the central tendon) consists of a costal component, arising from the xiphoid process of the sternum and costal cartilage of the lower six ribs (ribs 7-12), and a crural component, projecting from lumbar vertebrae L1-L3 (Hallett & Chokroverty, 2005). The two costal margins of the hemi-diaphragms have an in-series arrangement of muscle fibres; both share a common origin (the central tendon) and together they form a continuous part of the abdominal wall (Pengelly et al., 1971; Bellemare et al., 1986). The mass of the human diaphragm is 283 ± 53 g, corresponding to approximately 0.5% of total body mass (Arora & Rochester, 1982). The diaphragm does not act in isolation; it is one of many synergistic respiratory muscles that work to rhythmically displace the chest wall in order to pump air in and out of the lungs for the paramount purpose of maintaining arterial blood-gas homeostasis.
2.1.2 Neural Innervation and Blood Supply

The diaphragm is innervated exclusively by the phrenic nerves (Sant'Ambrogio et al., 1963; De Troyer & Kelly, 1982). Phrenic motor neurones exit the spinal cord via the ventral horn between cervical roots three to five (C3-C5). Each phrenic nerve supplies its own hemi-diaphragm, including all the crural fibers that side of the oesophageal hiatus (Botha, 1957). The phrenic nerves are ~250-400 mm in length (McKenzie & Gandevia, 1985; Jiang et al., 2011). The left phrenic nerve is slightly longer than the right due to the course it must take around the heart (McKenzie & Gandevia, 1985). Conduction velocity of the phrenic motor neurones is ~48-78 m·s⁻¹ (Heinbecker et al., 1936; Newsom Davis, 1967). Hence, phrenic nerve conduction time is estimated between 3.2 and 8.3 ms.

The phrenic nerve contains relatively few proprioceptive afferent nerve fibres, including: fast-adapting group Ia (respond to changes in muscle length/stretch), Ib (respond to changes in muscle tension), and slow-adapting group II (length/stretch) (Road, 1990). Reduced numbers of fusorial sensory fibres (group II) are likely to correspond to the sparse number of muscle spindles found within the diaphragm (Duron et al., 1978). Spindles are predominantly situated in the crural region (Balkowiec et al., 1995). It is unsurprising therefore, that the majority of proprioceptive afferents arise from Golgi tendon organs (Corda et al., 1965).

In contrast, the diaphragm possesses an abundance of afferent C-fibres (Road, 1990). Thinely myelinated (group III) and unmyelinated (group IV) fibres are sensitive to mechanical deformation, vascular distension, and metabolite accumulation (Sinoway et al., 1993; Haouzi et al., 1999). When stimulated, these mechanically and metabolically sensitive thin-fibre afferents elicit increased efferent sympathetic nerve activity, producing widespread sympathetic vasomotor
outflow (Dempsey et al., 2002). This is a core concept of the respiratory muscle metaboreflex, which is discussed in greater detail in section 2.3.2: ‘Cardiorespiratory Interactions’.

Preservation of respiratory gas exchange during periods of increased respiratory muscle work requires an oxygen supply that meets demand. The oxygen requirement is met by a proportionate increase in blood flow. The ability of the respiratory muscles to generate force and thus ensure matching of alveolar ventilation with metabolic demand is dependent on the adequacy of its perfusion. Internal thoracic, caudal intercostal, and phrenic arteries provide the majority of arterial blood supply to the diaphragm (Comtois et al., 1987). The flow of blood to any tissue is governed by the perfusion pressure gradient and peripheral vascular resistance (Smith-Blair, 2002). Blood flow remains relatively constant over a wide range of perfusion pressures (60-120 mmHg) without significantly impairing diaphragm function (Bark et al., 1987). Therefore, blood flow is largely regulated by changes in vasomotor tone, accomplished via central cardiovascular and local vascular control mechanisms (Laughlin et al., 1996). Failure to meet the requirement of respiratory muscle oxygenation may result in fatigue with subsequent ventilatory failure.

Animal-based studies show that blood flow to the diaphragm is increased when breathing against high inspiratory resistances, a consequence of a high work of breathing, with no sign of limitation (Rochester & Bettini, 1976). However, Bellemare and Grassino (1982b) observed that the critical pressure necessary to produce diaphragmatic fatigue (DF) in humans increases with a decrease in the ratio of inspiratory contraction time to total respiratory cycle duration (i.e. duty cycle) – referred to by Bellemare and Grassino (1982a) as the tension-time index (TTI). It is suggested that the increase in diaphragmatic blood flow may be limited once the critical TTI threshold is reached (Bellemare et al., 1983). When combining a high force output with a
prolonged duty cycle, blood flow to the diaphragm was compromised in anaesthetised dogs (Bellemare et al., 1983; Buchler et al., 1985a; Buchler et al., 1985b), likely because rising abdominal pressure during inspiration compressed feed arteries to the diaphragm. As duty cycle increases, the relaxation time decreases; therefore, perfusion time decreases, causing substantial ischaemia of the diaphragm. It is postulated that a complex network of neural influences from central respiratory motor output (feed-forward) and feedback from the lung, respiratory pump muscles and chemoreceptors are responsible for the control of sympathetic vasomotor outflow in humans (Dempsey et al., 2002).

2.1.3 Histochemical Composition

The respiratory muscles are a unique subset of skeletal muscle; unlike any other skeletal muscle in the human body, these muscles of respiration must continuously contract and relax throughout life – it is a feat of endurance similar to that of the beating heart. As such, the physiology of the respiratory muscles must withhold this premiere function.

The respiratory system consists essentially of three parts: a gas exchange organ – the lungs; a means for air to flow into and out of the lungs – the airways; and a pump to ventilate the lungs – the respiratory muscles (Roussos, 1985). Failure of this ‘vital’ pump to generate pressure, secondary to fatigue, would result in ventilatory failure and alveolar hypoventilation would ensue, leading to hypercapnia and acidosis (Macklem, 1980). However, the muscle fibre composition of the diaphragm is well suited to the task it must perform (Faulkner et al., 1979).

Brooke and Kaiser (1970) define three basic skeletal muscle fibre types based upon their histochemical and morphological properties, they are: a) slow-twitch oxidative (type I), b) fast-twitch oxidative glycolytic (type IIa), and c) fast-twitch glycolytic (type IIb). These fibre types are
known to fatigue at different rates (Burke et al., 1971). Type IIb fibres are the least resistant to
fatigue, owing to their fast shortening velocities, low oxidative capacity, and high glycolytic
content (Sharp & Hyatt, 1986). Slow-twitch oxidative fibres are richly supplied with capillaries,
myoglobin and mitochondria; they are economical and ideally designed for endurance work.
Similarly, type IIa fibres have a high propensity for oxidative metabolism, however, they are also
equipped with the means of sustaining high-intensity work anaerobically, they are relatively
resistant to fatigue

The majority (55 ± 5%) of the fibres in the human diaphragm are type I oxidative, whilst
21 ± 6% of fibres can be classified as type IIa. The remaining 24 ± 3% of fibres are fast-twitch
glycolytic, which are susceptible to fatigue (Lieberman et al., 1973). In comparison, Gollnick et
al. (1972) found that the diaphragm contained twofold greater quantities of oxidative fibres (i.e.
type I and IIa) than limb skeletal muscles. Thus, the diaphragm has tremendous endurance
properties.

2.1.4 Respiratory Muscle Mechanics

The mechanics of breathing is concerned with one basic question: how does air get into and out of
the lungs? (Otis, 1986). It is a problem requiring on one hand, the “detailed knowledge of an
anatomist and on the other hand, the analytic understanding of an engineer” (Fenn, 1958). From
Claudius Galen to Leonardo da Vinci, to Hooke, Boyle and Newton, the study of respiratory
mechanics has a long history of scientific enquiry (see Otis, 1986 for full review). In the previous
section, an appreciation of respiratory muscle anatomy was established. The action of the
diaphragm with regard to the physical principles of pressure, flow and volume will now be
discussed.
Contraction of the diaphragm generates tension within its fibres, displacing the central tendon caudally. This has a number of consequences: a) pleural pressure falls, allowing air to flow into the airways and alveoli, thereby increasing lung volume; b) abdominal pressure increases, pushing the abdominal cavity downward, allowing the thorax to expand; c) the ribcage is lifted upwards (pump-handle motion) and outwards (bucket-handle motion) by action of the scalenes and sternocleidomastoid on the sternum, and external intercostal muscles on the ribs (Fry & Hyatt, 1960; West, 2012).

During incremental exercise, there is a progressive increase in tidal volume, accommodated by a decrease in end-expiratory lung volume and an increase in end-inspiratory lung volume. This can be achieved by different configurations of the ribcage and abdomen (Konno & Mead, 1967). The aforementioned respiratory muscles mediate this interaction. Early studies of chest wall kinematics during rest and exercise reveal that the volume displacement of the ribcage and abdomen are independent of one another and can be opposite to that of lung volume, which allows for the measurement of the elastic work required to overcome chest wall distortion (Grimby et al., 1968; Goldman et al., 1976).

In an investigation of the relative contribution of the ribcage and abdomen to lung volume during exercise, Grimby et al. (1968) demonstrated that between rest and moderate intensity exercise, the ribcage contributed little to the tidal volume. However, the transition from moderate to high-intensity exercise resulted in a much greater ribcage contribution to the volume displacement. It was posited that the diaphragm is assisted by recruitment of accessory inspiratory muscles in order to further elevate lung volume. Application of these findings are difficult given they are based upon a model that is limited by the assumption of a rigid ribcage, moving with only
two degrees of freedom. What’s more, the model considers the diaphragm a single muscle, acting upon the ribcage via its effect on abdominal pressure alone.

Improving upon this classical work, Ward et al. (1992) were able to separate the ribcage contribution to the volume displacement into its constituent compartments – the pulmonary/upper ribcage (apposed to the lung) and the abdominal/lower ribcage (apposed to the diaphragm), thus creating a three-compartmental model of chest wall kinematics. The mechanical arrangement of the diaphragm and ribcage muscles is presented in parallel. Therefore, tidal volume can be partitioned into a component due to contraction of the ribcage muscles and a second component due to diaphragmatic contraction. The diaphragm and abdominal muscles act upon the abdominal ribcage (Mier et al., 1985) whereas, inspiratory and expiratory ribcage muscles act upon the pulmonary ribcage (Kenyon et al., 1997). Importantly, this model takes into consideration the fact that the pulmonary and abdominal ribcages are exposed to substantially different pressures during inspiration (Agostoni & D'Angelo, 1988). Nevertheless, quantifying changes in tidal volume prove beyond the scope of the model proposed by Ward et al. (1992).

Modern investigations of respiratory mechanics have employed optoelectronic plethysmography to provide information on breath-by-breath alterations of the entire chest wall (including its ribcage and abdominal compartments) during exercise (Cala et al., 1996). In short, a number of reflective markers are positioned on anatomical reference sites of the ribcage and abdomen. Cameras are positioned to locate the markers and relay three-dimensional co-ordinates that can be used for motion analysis (Aliverti & Pedotti, 2014). Using this method, combined with ancillary measures of respiratory breathing pressures (oesophageal/pleural and gastric/abdominal pressure), Aliverti et al. (1997) were able to distinguish the pressures generated by each respiratory
muscle group (i.e. the diaphragm, abdominal muscles and ribcage muscles). Through this insightful work, it is now believed that the diaphragm serves primarily as a flow generator by modulating the transpulmonary pressure gradient. Conversely, the ribcage muscles and abdominal muscles function as pressure generators. Where the ribcage muscles aim to displace the pulmonary ribcage to increase end-inspiratory lung volume (EILV), the abdominal muscles decrease end-expiratory lung volume (EELV) by displacing the abdomen.

2.1.5 Contractile Properties

Similar to any other skeletal muscle, the ability of the diaphragm to generate force is largely dependent upon the mechanical contractile properties of the muscle, this includes: a) the initial length of the muscle (length-tension relationship), b) the velocity of shortening (force-velocity relationship), and c) the rate at which the muscle is stimulated (force-frequency relationship) (Rochester, 1985). Respiratory muscle shortening is measured as the change in volume of a structure that the muscle displaces (e.g. ribcage, abdomen, or lungs), whilst velocity or rate of shortening can be measured as flow and force as pressure. From these variables, the work (or power) of breathing can be estimated. The efficiency of the respiratory muscles to generate force and/or velocity of contraction has implications concerning the metabolic cost of breathing and respiratory muscle fatigue.

An important determinant of the pressure generating capacity of the diaphragm is the initial resting length from which it operates (McCully & Faulkner, 1983). Here, lung volume has a direct effect on diaphragm contractility, as the length-tension relationship predicts (Braun et al., 1982). The optimal resting length of the diaphragm is believed to lie between residual volume and functional residual capacity (FRC) (Farkas et al., 1996). During exercise, the initial fall in end-
expiratory lung volume lengthens diaphragm sarcomeres, thereby improving the length-tension behaviour of the muscle. However, at lung volumes greater than FRC (i.e. dynamic hyperinflation), the diaphragm must operate at a shorter disadvantageous sarcomere length, placing the muscle on a weaker portion of its length-tension curve. Consequently, the efficiency of the inspiratory muscles to generate pressure is compromised (Whipp & Pardy, 1986), leading to reduced respiratory system compliance. This has significant implications concerning the perception of dyspnoea (or breathlessness). More information on the neurophysiological mechanisms underpinning dyspnoea is provided in section 2.3.1: ‘Ventilation and Dyspnoea’. The force-velocity relationship may also have important implications regarding the pressure generating ability of the diaphragm.

The upper limits to maximum exercise ventilation are determined structurally by the resistive and elastic properties of the lung, in addition to the intrinsic force-velocity characteristics of the inspiratory muscles (Dempsey et al., 1996). Studies by Agostoni and Fenn (1960) and Hyatt and Flath (1966) indicate that the alveolar pressure developed by the respiratory muscles during maximum inspiratory efforts is inversely related to the velocity of inspired airflow. Thus, the faster the velocity of diaphragmatic contraction, the less pressure will be developed for a given lung volume (Pengelly et al., 1971; Goldman et al., 1978).

Finally, contractile force of a muscle increases as a function of the frequency at which the muscle is stimulated (Rochester, 1985). As the frequency increases from a single stimulus to a sequence of high-frequency stimuli, the muscle initially responds with a single twitch, followed by an oscillatory contraction, and ultimately, a fused tetanus (Roussos & Macklem, 1986). Transdiaphragmatic pressure (a surrogate for diaphragm force output) shows a similar force-
frequency response as other skeletal muscles, producing a sigmoidal shape curve (Edwards, 1979; Moxham et al., 1980). In humans, bilateral stimulation of the phrenic nerves begins to produce fusion of the transdiaphragmatic pressure contour, reaching a plateau at 8 Hz (Farkas et al., 1996). Fusion of twitches occurs at lower stimulation rates in slower muscles, as a result, the shape of the force-frequency curve is dependent upon contraction and relaxation time (Roussos, 1985). At less than optimal muscle length, the ascending limb of the force-frequency response curve shifts to the right (Farkas & Roussos, 1984). Therefore, at high lung volumes, a greater neural input/excitation of the muscle is required to produce an equivalent change in pressure.

The force-frequency response of the diaphragm has also been utilised as a means of assessing respiratory muscle fatigue (Moxham et al., 1981). Aubier et al. (1981) found a reduction in transdiaphragmatic pressure at low- and high-frequency stimulation intensities following a bout of inspiratory resistive breathing to the point of exhaustion. The mechanisms of peripheral fatigue are discussed further in the following section, titled: ‘Definitions, Sites and Mechanisms of Fatigue’.

In summary, the structural and functional properties of the human diaphragm excellently subserve the unique requirements of respiration. Despite the diaphragm possessing tremendous endurance characteristics, evidence exists that under certain conditions, the respiratory system is not without limitations, including the potential development of inspiratory muscle fatigue.
2.2 Respiratory Muscle Fatigue

2.2.1 Respiratory System Limitations to Exercise

The healthy pulmonary system has generally been regarded ‘overbuilt’ for the demands placed on gaseous exchange and ventilation during exercise (Dempsey, 1986). The respiratory system is able to maintain acid-base balance and blood-gas homeostasis near resting levels with remarkable ease (Sheel & Romer, 2012). Alveolar ventilation (\(V_A\)) and metabolic rate are precisely matched via a proportional increase in minute ventilation (\(V_E\)). This is achieved by a simultaneous rise in tidal volume (\(V_T\)) and breathing frequency (\(f_b\)) (i.e. exercise hyperpnoea). However, with training, the cardiovascular and musculoskeletal systems demonstrate progressive structural and functional adaptation compared to the lungs, airways and respiratory muscles, whereby (with few exceptions) no significant adaptations occur (McKenzie, 2012). To this end, the once ‘overbuilt’ lung becomes a so-called “limiting factor” affecting \(O_2\) transport and utilisation in highly trained endurance athletes (Dempsey et al., 1984). The mismatching of physiological systems is central to the demand vs. capacity theory (Dempsey, 1986).

This demand vs. capacity theory holds that there are exceptional circumstances whereby the substantial physiological requirements of exercise meet or exceed the capacity of the respiratory system to respond (Dempsey & Johnson, 1992). Respiratory system limitations (e.g. exercise-induced arterial hypoxaemia [EIAH, \(S_aO_2 < 95\%\)]) and mechanical ventilatory constraints of hyperpnoea (i.e. expiratory flow limitation [EFL]) each contribute to elevated respiratory muscle work and the development of inspiratory muscle fatigue (Dempsey et al., 2003).
2.2.2 Definition, Sites and Mechanisms of Fatigue

Fatigue is defined as “a condition in which there is a loss in the capacity for developing force and/or velocity of a muscle, resulting from muscle activity under load and which is reversible by rest” (NHLBI, 1990). Fatigue may result from failure of the muscle and/or neuromuscular junction (i.e. peripheral fatigue) or from a decreased output of the central nervous system (i.e. central fatigue) (Bigland-Ritchie et al., 1978).

Central fatigue is defined as a “progressive reduction in voluntary activation of muscle during exercise” (Gandevia, 2001). Evidence of central fatigue comes from the observation of a further increase in force generation when a maximal electrical or magnetic stimulation is superimposed upon a truly maximal voluntary contraction (Zakynthinos & Roussos, 2005).

Peripheral fatigue, on the other hand, is defined as “fatigue produced by changes at or distal to the neuromuscular junction (Gandevia, 2001). The mechanism of fatigue can be explained by high and/or low frequency stimulation. High frequency fatigue is characterised by: a fast recovery time (10-20 minutes), loss of force at high stimulation frequencies (typically 50-100 Hz), and a concurrent decrease in amplitude or slowing of the muscle compound action potential (i.e. M-wave) (Jones, 1996). Force loss at high frequencies is attributed to disruption of the action potential propagation along the muscle membrane/sarcolemma or down the transverse tubule (Edwards et al., 1977). Allen et al. (1992) states that this may be caused by changes in extracellular sodium and/or potassium ions. Whereas, low frequency fatigue is characterised by: a slow recovery time (hours) and loss of force at low stimulation frequencies (typically 10-30 Hz) (Edwards et al., 1977). The putative mechanism believed to be responsible for this type of fatigue is a reduction in calcium ion released from the sarcoplasmic reticulum; however, if low frequency fatigue persists,
Sarcomere muscle damage may be present (Jones, 1996). Reduced calcium ion release may be related to declining ATP levels (Westerblad & Allen, 1991) or increased production of reactive oxygen species (Shindoh et al., 1990; Anzueto et al., 1992). Calcium is responsible for the propagation of muscle excitation and relaxation stages, also known as excitation-contraction coupling (Berchtold et al., 2000).

There are two conjectures pertaining to peripheral fatigue, they are the exhaustion and accumulation hypotheses (Allen et al., 2008). Briefly, the exhaustion hypothesis refers to the depletion of phosphagens, such as: ATP, creatine phosphate and glycogen (Bergstrom et al., 1967; Astrand et al., 2003). The accumulation hypothesis refers to a build up of intracellular inorganic acids, including: lactate, hydrogen ions (H\(^+\)), inorganic phosphate (P\(_i\)), potassium, and adenosine diphosphate (ADP) (Allen et al., 2008). Inhibition of excitation-contraction coupling is thought to be caused by: a) release of H\(^+\) and P\(_i\), which prevent the transition from low- to-high force states, and b) the release of ADP, which limits crossbridge cycle speed and thus fibre velocity (Kent-Braun et al., 2012).

Fatigue may be caused anywhere along the brain-muscle pathway. It is likely that the three types of fatigue do not occur in isolation, but rather coexist when the respiratory muscles face excessive work, with the relative importance of each depending on the duration of respiratory loading other physiologic variables (e.g. arterial pressure and blood-gas concentrations) (Zakynthinos & Roussos, 2005). There are many methods that can be adopted to assess DF, each with their own unique set of advantages and disadvantages. The complexity of muscle fatigue means no single test is able to universally detect all three types of fatigue. The limitations of a given test must be carefully considered (ATS/ERS, 2002).
2.2.3 **Assessment of Respiratory Muscle Fatigue**

Central and peripheral fatigue of the respiratory muscles in response to an imposed load was first identified almost a century ago in Oxford, England (Davies *et al.*, 1919). Davies, Haldane and Priestley (1919) sought to answer how breathing adapts itself to increased resistance, and when the adaptation begins to fail. The authors packed canisters with cotton wool in order to increase inspiratory and expiratory resistance to airflow. Initially, the authors found respiration to slow and become deeper. When the resistance was increased beyond that could be sustained, breathing frequency increased and tidal breaths became progressively shallower (i.e. tachypnoea). Eventually, this led to the observation of cyanosis in some individuals, a rudimentary diagnosis of arterial hypoxaemia. Today, assessment of respiratory rhythm and rate can still be used to indicate a reflex response to an increase in respiratory workload (Tobin *et al.*, 1986), but is not indicative of peripheral respiratory muscle fatigue (Mador & Tobin, 1992).

A second approach that may provide insight into the potential development of respiratory muscle failure is through inspection of thoracoabdominal motion. The previously mentioned two-compartmental model of inspiratory muscle action (see section 2.1.4: ‘Respiratory Muscle Mechanics’) developed by Konno and Mead (1967), allows the volume displacement during respiration to be segmented into ribcage and abdominal contributions. Respiratory inductance plethysmography gives insight into recruitment and function of the respiratory muscles, in particular, the diaphragm, inspiratory ribcage muscles and abdominal muscles. Paradoxical motion of the abdomen (inward displacement) may represent weak, absent, or ineffective contraction of the diaphragm, perhaps secondary to DF (ATS/ERS, 2002).
The tension-time index (TTI) of the diaphragm, briefly mentioned in section 2.1.2, is an approximation of muscle energy demands, calculated as the average tension generated by a muscle over time, expressed as a fraction of the maximum tension available to the muscle at rest. Bellemare and Grassino (1982b) proposed a fatigue threshold exists for the respiratory muscles; based on their findings, a critical TTI of 0.15-0.18 implies insufficient energy supplies are available, ultimately leading to contractile failure of the muscle. Grassino and Macklem (1984) state that the TTI should be considered a conceptual framework rather than a precise numerical instrument, as there are many conditions that may shift the fatigue threshold, such as: oxygen delivery (e.g. hypoxia), perfusion pressure (e.g. shock), and changes in the oxygen dissociation curve (e.g. temperature and pH).

It is difficult to obtain direct measurements of DF due to invasive procedures; nonetheless, force development across the muscle (i.e. transdiaphragmatic pressure \(P_{di}\)) can be estimated by measuring the difference in gastric \(P_{ga}\) and oesophageal \(P_{oes}\) pressures with the use of balloon tipped catheters (Baydur et al., 1982; Henke et al., 1988). When used in conjunction with electromyography (EMG), the measurement of \(P_{di}\) in response to bilateral phrenic nerve stimulation (BPNS) provides the most objective method of evaluating diaphragm function. Unlike maximal volitional pressures (e.g. inspiratory mouth pressure [MIP]), “the technique of BPNS allows objective assessment of diaphragm function without influence of motivation, whole-body fatigue, or other central factors” (Dempsey et al., 1996).

The phrenic nerves can be stimulated by electrical or pulsed magnetic fields eliciting synchronous motor unit activation. Electrical stimulation may be performed using needle (Mier & Brophy, 1991), implanted wire (Hubmayr et al., 1989), or transcutaneous (Bellemare & Bigland-
Ritchie, 1984) procedures. Early studies employed unilateral single-twitch electrical stimulations (Aubier et al., 1981; Bellemare & Bigland-Ritchie, 1984) to detect and quantify peripheral DF; unfortunately, due to the unilaterality of the stimulations, chest wall geometry was difficult to control, resulting in diaphragm distortion. Aubier et al. (1985) introduced BPNS in an attempt to optimise right and left hemi-diaphragm contribution to contraction and thus eliminate diaphragm distortion. Needle and implanted wire stimulations are invasive with risk of haematoma and phrenic nerve damage; consequently, neither is currently recommended (ATS/ERS, 2002). Transcutaneous stimulation has minimal side effects, but has several technical challenges; furthermore, stimulations performed at supramaximal intensities can be uncomfortable for subjects to tolerate.

Magnetic stimulation is based upon the principle that a time-varying magnetic field will induce an electrical current in any tissue through which it passes; therefore, magnetic fields can be used to cause current to flow in nervous tissue, resulting in depolarisation of the cell membrane and initiation of a compound muscle action potential (CMAP, or M-wave) (Miranda, 2005). This represents the summated electrical activity produced by all motor units synchronously activated (Man et al., 2004). Similar to electrical, magnetic stimulation may be performed in a variety of different ways, this includes: cervical (Similowski et al., 1989), unilateral and bilateral anterolateral (Mills et al., 1996) and anterior pre-sternal (Polkey et al., 2000). Each of the aforementioned techniques has their own set of advantages and disadvantages.

Cervical magnetic stimulation (CMS) may be better tolerated than electrical stimulation, and reduces the risk of potentiation, whilst retaining reproducibility (Wragg et al., 1994). A drawback of the magnetic stimulation technique is specificity; there is greater risk of co-activation
of accessory inspiratory muscles and muscles innervated by the brachial plexus compared to electrical stimulation, all of which could contribute to twitch $P_{di}$ and contaminate the diaphragm EMG/CMAP, particularly when obtained using surface electrodes (Laghi et al., 1996). Importantly, supramaximal phrenic nerve stimulation can never be guaranteed.

Despite its limitations, there are several lines of evidence against signal contamination. First, patients with phrenic nerve palsy do not demonstrate any electrical activity using chest wall electrodes in response to phrenic nerve stimulation, suggesting that the signal cannot possibly be contaminated due to the unavoidable stimulation of extra-diaphragmatic muscles (Chokroverty et al., 1995). If the signal were contaminated, electrical activity from other muscles would have been detected. Secondly, the latencies measured by surface electrodes closely match that recorded by needle electrodes (Verin et al., 2002). Over time and pragmatic experimentation, many possible electrode-recording sites have been reported (Glerant et al., 2006); however, there remains no consensus as to optimal electrode positioning and the technique of CMS continues to vary among different laboratories due to the lack of standardisation (ATS/ERS, 2002). Nevertheless, taken collectively, it appears that electrodes should be positioned closer together and at a more anterior and medial site.

In order to negate the difficulties of chest wall EMG, specially designed oesophageal electrodes have been developed with the aim of reducing the likelihood of signal contamination and influence of subcutaneous adipose tissue (Agostoni et al., 1960; Petit et al., 1960). A major limitation of this method is the fixed distance between oesophageal and gastric balloons. This becomes a problem when measuring pressure because of the inability to withdraw one balloon without affecting the other (a particular concern when using subjects of varying stature). Thus,
there seems to be a tradeoff with chest wall versus oesophageal electrodes, such that the primary outcome variable (either EMG or $P_{di}$) may dictate which method is most suitable.

As stated earlier, the most objective measure of DF remains transdiaphragmatic twitch pressure ($P_{di,tw}$) in response to BPNS. In order to guarantee supramaximal stimulation of the phrenic nerves, the diaphragm CMAP must be monitored from both the left and right hemidiaphragm, thus either chest wall or oesophageal electrodes should accompany measures of pressure. A plateau in $P_{di,tw}$ or M-wave amplitude with rising stimulation intensity indicates that the diaphragm is supramaximally stimulated.

There are some important technical (e.g. post-activation potentiation) and functional (e.g. lung volume and flow rate) considerations that must be acknowledged when measuring respiratory muscle fatigue. Firstly, there is risk of transient increase in the mechanical response to low frequency stimulation resulting from previous contractile activity – this is known as post-activation potentiation and is easily demonstrated in the diaphragm (Botelho & Cander, 1953). The potentiated twitch is more sensitive to small discrepancies in DF (Kufel et al., 2002) and can be used to rule out a potentiation effect (Laghi et al., 1995). Secondly, the maximal force generation of the respiratory muscles is determined in part by sarcomere length (i.e. the length-tension relationship) and velocity of contraction (i.e. force-velocity relationship). Therefore, measurements should be taken at the end of a normal tidal expiration (i.e. EELV) with the airway closed. Diaphragmatic fatigue is typically assumed present if there is a reduction in $P_{di,tw}$ of $\geq 15\%$ compared to baseline.
2.2.4 Respiratory Muscle Demands during Exercise

The ventilatory response to exercise is governed by the principle of ‘minimum effort’, such that breathing pattern and operating lung volumes optimise respiratory system compliance and thus, the work performed by the respiratory muscles (Rahn et al., 1946). As the primary muscle of inspiration, uncompromised diaphragm function is essential to support the ventilatory demands associated with physical exertion (Poole et al., 1997). By design, the diaphragm has a high oxidative capacity, a short capillary-mitochondria diffusion area for O₂, and a natural recruitment order of fast (type IIa [oxidative glycolytic]) and slow-twitch (type I [oxidative]) muscle fibres that enhances the diaphragm’s ability to resist fatigue relative to most locomotor muscles (Lieberman et al., 1973; Bellemare & Bigland-Ritchie, 1987; McKenzie & Gandevia, 1991; Mizuno, 1991). However, as mentioned previously, the respiratory system is not without limits; due to a combination of a high mechanical and energetic work of breathing (WOB), metabolite accumulation and competition for blood flow between the respiratory and locomotor muscles, the inspiratory muscles fatigue (Dempsey et al., 2008).

During heavy exercise, \( V_A \) must parallel metabolic demand; consequently, \( V_E \) may increase 20-fold above resting levels. Exercise hyperpnoea is the respiratory systems first line of defence to meet these substantial demands and results in an increased WOB (Otis, 1954). This high WOB can command between 10-15% of maximal oxygen consumption depending on training status and sex (Aaron et al., 1992; Dominelli et al., 2015). The increased oxygen transport is accommodated by increases in respiratory muscle blood flow. Harms et al. (1997) quantified changes in respiratory muscle perfusion using the thermodilution technique (Andersen & Saltin, 1985). Harms and colleagues mechanically reduced the WOB by unloading the respiratory muscles during high-
intensity exercise using a proportional assist ventilator (PAV). The PAV provides feedback-controlled, positive-pressure ventilation, proportional to inspiratory flow and/or volume; thereby, reducing inspiratory muscle work by 40-50% (Younes, 1992). This method of mechanical unloading decreased vascular conductance and cardiac output to working limb locomotor muscles by 14-16% (Harms et al., 1998b). Critically, the authors imply that blood flow is redirected away from the legs and redistributed towards the respiratory muscles via a sympathetically mediated metaboreflex (details provided in section 2.3.2: ‘Cardiorespiratory Interactions’). Direct measurements of blood flow are not feasible in humans; nevertheless, this study does provide insightful data that are commensurate with microsphere studies conducted in maximally exercising ponies (Manohar, 1986, 1990).

2.2.5 Exercise-Induced Diaphragmatic Fatigue
Respiratory muscle fatigue has been proposed to be a key factor involved in the determination of exercise tolerance during prolonged submaximal and/or short-term maximal exercise (Loke et al., 1982; Martin et al., 1982). The first studies to objectively assess exercise-induced DF were conducted by researchers in the John Rankin Laboratory of Pulmonary Medicine at the University of Wisconsin (Johnson et al., 1993), and the Division of Pulmonary Medicine at State University of New York at Buffalo (Mador et al., 1993). Johnson et al. (1993) assessed DF in healthy male cyclists (n = 12) of various fitness levels following constant load exercise at 85% and 95% of maximal oxygen uptake (\(\dot{V}O_2\text{max}\)) to exhaustion. Fatigue was determined by the change in \(P_{di,\text{tw}}\) in response to BPNS – a non-volitional measure of fatigue. Transdiaphragmatic twitch pressure decreased significantly from baseline at both exercise intensities (95% \(\dot{V}O_2\text{max}\): -20 ± 3%; 85% \(\dot{V}O_2\text{max}\): -15 ± 5%). The authors concluded that whole-body endurance exercise \(\geq\)85% \(\dot{V}O_2\text{max}\)
induces significant fatigue of the diaphragm. Exercise intensity and thus, diaphragmatic work appear to have a salient role in the development of fatigue, as moderate-intensity submaximal exercise (50% and 75% V\text{O}_2\text{max}) does not significantly alter diaphragmatic force output, limb vascular resistance (LVR), or cardiac output to the legs (Wetter et al., 1999). Further, DF appears to occur in healthy young subjects of all fitness levels (Babcock et al., 1996).

In light of Johnson et al.’s (1993) findings, the same laboratory began to ask, what are the underlying causes of this exercise-induced DF? Accordingly, Babcock et al. (1995) set out to determine whether the mechanical work generated and sustained by the diaphragm during heavy endurance exercise was sufficient in lieu of confounding factors associated with whole-body exercise, to explain their observations. Babcock et al. (1995) conducted an experiment similar in design to Johnson and co-workers (1993); however, an experimental condition was applied, whereby participants would be required to mimic the exercise hyperpnoea achieved during high-intensity endurance exercise at rest. By accurately mimicking the V\text{T}, f_b, pressure-time product (PTP) of the diaphragm and duty cycle (ratio of inspiratory time to total respiratory cycle duration), the authors were able to replicate the exercise ventilation and pressure-generating work of the diaphragm sustained during dynamic exercise, while at rest, enabling them to answer their question. Constant load endurance exercise to exhaustion (85-90% V\text{O}_2\text{max}) elicited significant fatigue of the diaphragm, as measured by the change in P_{di, tw} compared to baseline (-26.0 ± 2.9%), whereas voluntary hyperpnoea did not (-9.5 ± 4.8%). In addition, blood lactate concentration was much greater following whole-body exercise than mimicking exercise ventilation at rest (exercise: 6.5 ± 1.1 mmol\cdot l^{-1}; mimic: 1.1 ± 0.3 mmol\cdot l^{-1}). These findings suggest that the mechanical work done by the diaphragm during intense whole-body exercise is not sufficient by itself to explain all
of the exercise-induced DF. The authors postulate two ways in which whole-body exercise contributes to DF: 1) increasing levels of circulating metabolites originating from contracting locomotor muscles, and 2) compromised blood flow to the diaphragm.

In support of this notion, Fregosi and Dempsey (1986) found that endurance exercise to exhaustion in rodents increased circulating metabolites many-fold above resting values, whilst diaphragm glycogen content remained relatively unchanged. Although limited, this study provides some evidence to suggest that metabolite accumulation, not glycogen depletion is the primary cause of peripheral fatigue in the exercising diaphragm. Moreover, volitional hyperpnoea at rest brings about a 3–5 l·min$^{-1}$ increase in cardiac output (Anholm et al., 1987). In theory, the multitude of central and local haemodynamic vascular control mechanisms, such as functional sympatholysis (Remensnyder et al., 1962) would ensure that there is adequate blood flow to the respiratory muscles in order to meet metabolic demand; however, during whole-body exercise, the diaphragm must compete with exercising locomotor muscles for finite cardiac output and may therefore be compromised.

Perhaps the most distinct mediator of exercise-induced DF is WOB. Babcock et al. (2002) used the experimental model of manipulating the WOB using PAV to partially unload the respiratory muscles during heavy endurance exercise to determine the role of diaphragm force output per se on exercise-induced DF. A control group cycling at 85% $\dot{V}$O$_2$max to the limit of volitional tolerance experienced a 20.3 ± 3.1% drop in $P_{di,\text{tw}}$ compared to pre-exercise values. In comparison, when the WOB was mechanically alleviated during inspiration and expiration, fatigue did not occur. Furthermore, ventilation decreased by 9.6 ± 3.4%, and there were significant reductions in diaphragm (PTP$_{di}$) and oesophageal (PTP$_{oes}$) pressure-time products.
Collectively, these findings suggest that the development of exercise-induced DF is a function of the relationship between the magnitude of diaphragmatic work and the adequacy of its blood supply – the less blood flow available, the less diaphragmatic work is required to produce fatigue (Romer & Polkey, 2008). Inspiratory muscle fatigue is associated with many physiological consequences, including the redistribution of blood flow from active locomotor muscles, changes in ventilation and the perception of dyspnoea, in addition to alterations in exercise tolerance.

2.3 Consequences of Respiratory Muscle Fatigue

2.3.1 Ventilation and Dyspnoea

A reduction in the capacity of the respiratory muscles to generate force of contraction may lead to inadequate force generating pressures, particularly when the demand for ventilation is high. In turn, this could lead to relative alveolar hypoventilation and high dead space ventilation, secondary to DF induced by an excessive mechanical WOB (Romer & Polkey, 2008). However, many studies have documented DF where $V_A$ was appropriate for metabolic demand as demonstrated by indirect measures of arterial blood gases (e.g. Johnson et al., 1993; Babcock et al., 1995, 1996).

Exercise hyperpnoea is associated with a progressive recruitment of accessory inspiratory muscles, which may distort the chest wall (Grimby et al., 1968; Goldman et al., 1976) reduce the mechanical efficiency of breathing (Dodd et al., 1988) and contribute to an elevated sensation of breathing discomfort (or dyspnoea). Dyspnoea is defined as the “subjective experience of breathing discomfort that consists of qualitatively distinct sensations that vary in intensity” (ATS, 1999). The ‘length-tension inappropriateness’ paradigm (Campbell & Howell, 1963) (also referred to as: ‘efferent-reafferent disassociation’, Schwartzstein et al., 1990; and ‘neuromechanical uncoupling’, O’Donnell & Webb, 2008) posits that dyspnoea is the result of a
“disparity between the central reflexive drive to breathe and the appropriate mechanical response of the respiratory system” (Ambrosino & Vagheggini, 2006). The theory is based on the principle that when changes in respiratory muscle length (i.e. volume) or tension (i.e. pressure) are inappropriate for the outgoing motor command, the intensity of breathing discomfort increases (McConnell, 2005). It is argued that conscious appreciation of effort associated with breathing against externally applied mechanical loads play an important role in the genesis of exertional dyspnoea (Sheel & Romer, 2012). The combined effects of mechanical constraints and respiratory system limitations elevate dyspnoeic sensations and ultimately impair exercise tolerance through central fatigue.

2.3.2 Cardiorespiratory Interactions

Similar to limb skeletal muscles, the diaphragm is richly innervated by group III and IV afferent nerve fibres (Duron, 1981). Balzamo et al. (1992), and Jammes and Balzamo (1992) demonstrated that fatiguing contractions of the diaphragm caused increased activation of group III phrenic nerve afferents; similarly, Hill (2000) found that group IV afferent fibres are stimulated with the development of DF. Thinly myelinated (group III) and unmyelinated (group IV) fibres are sensitive to mechanical deformation, vascular distension, and metabolite accumulation (Road, 1990; Sinoway et al., 1993; Haouzi et al., 1999). When stimulated, these mechanically and metabolically sensitive thin-fibre afferents elicit increased efferent sympathetic nerve activity, producing widespread vasoconstrictor influences via supraspinal pathways (Dempsey et al., 2002).

A series of studies completed at the start of this century provide insights into the role of respiratory muscle fatigue in mediating muscle sympathetic nerve activity (MSNA) and sympathetic vasoconstrictor outflow, resulting in a metaboreflex. St. Croix et al. (2000) measured
MSNA whilst subjects inspired against flow resistive loads equal to 60% of MIP. Duty cycle 
($T_i/T_{TOT}$), $f_b$ and $V_T$ were carefully monitored ($T_i/T_{TOT} = 0.7; f_b = 15$ breaths-min$^{-1}$, $V_T = 1$ l). St. Croix et al. (2000) found a time-dependent increase in MSNA (+77%), heart rate ([HR] +27 beats-min$^{-1}$), and mean arterial blood pressure ([MAP] +12 mmHg) at the time of task failure. What’s more, MSNA was relatively unchanged (+5.5%) in a mimic non-fatiguing control group (MIP = 2%). In light of this finding, Sheel et al. (2001) asked whether the rise in MSNA during fatiguing contractions of the diaphragm was accompanied by limb muscle vasoconstriction and reduced limb blood flow. Sheel et al. (2001) measured limb blood flow at the femoral artery using Doppler ultrasound techniques and calculated limb vascular conductance in a variety of loading protocols. The authors found that cardiac output to the legs decreased by 23% and LVR increased by 45% in a fatiguing trial (60% MIP) at 0.7 duty cycle and 15 breaths-min$^{-1}$. In a second fatiguing trial (60% MIP), performed at a shorter duty cycle ($T_i/T_{TOT} = 0.4$) and increased $f_b$ (20 breaths-min$^{-1}$), limb blood flow fell by 30% and LVR increased by 52%. In non-fatiguing (2% MIP) mimic control trials, blood flow and vascular conductance remained unchanged. The similarity in results between the two fatiguing trials suggests that the important trigger of the systemic vasoconstrictor effect was metabolite accumulation coupled with fatigue of the diaphragm, rather than muscle ischaemia per se.

Aaker and Laughlin (2002b) found that this sympathetically mediated limb vasoconstriction was caused by the stimulation of $\alpha$-1 adrenergic receptors. The authors noted that nor-adrenaline (or norepinephrine) binds to adrenergic receptors, which causes locomotor blood vessels to vasoconstrict. Importantly, Aaker and Laughlin (2002a) found that in the rodent, diaphragm arterioles are less responsive to $\alpha$-1 adrenergic vasoconstriction than the gastrocnemius
muscle. Based on this evidence, a global increase in sympathetic activity would result in greater vasoconstriction in the locomotor than respiratory vasculature (Romer & Polkey, 2008). The notion made by Sheel et al. (2002) was supported by the work of Rodman et al. (2003) in which a bolus infusion of lactic acid was injected into the phrenic artery of resting and exercising canines, eliciting a transient reduction in limb blood flow and vascular conductance – an effect that was prevented via pharmacological blockade of the adrenergic receptors. The fundamental purpose of this metaboreflex is thought to be protection of O₂ delivery to the diaphragm and ensuring the ability to maintain pulmonary ventilation (Seals, 2001).

2.3.3 Locomotor Muscle Fatigue and Exercise Performance

The reductions in local O₂ transport to working limb muscle due to sympathetically mediated vasoconstriction would likely augment fatigue of the limb muscles. Romer et al. (2006b) determined the effect of respiratory muscle work on exercise-induced quadriceps fatigue using femoral nerve stimulation. The authors conducted two constant load (90% \( \dot{V}O_2\text{max} \)) time-to-exhaustion (TTE) exercise tests, one with added inspiratory resistance (loading) and one without (unloading). The loading trial was compared to a control trial, with the resistance removed, conducted for the same duration and intensity (i.e. iso-time). The unloading trial was followed by an iso-TTE test with mechanical unloading of the respiratory muscles. Force output of the respiratory muscles was reduced by 56% using PAV and was increased by 80% with the application of inspiratory resistive loads. The magnitude of quadriceps fatigue was reduced almost one-third when the inspiratory muscles were unloaded and exacerbated almost two-fold when the inspiratory muscles were loaded. These findings indicate a significant effect of WOB upon peripheral locomotor fatigue during sustained high-intensity exercise. Romer et al. (2006b)
contend this reflects corresponding changes in limb blood flow and O$_2$ transport, secondary to increased sympathetic vasoconstrictor outflow.

In a follow-up study, Romer et al. (2006a) sought to explore the peripheral effects of exercise-induced arterial hypoxaemia (EIAH) by assessing locomotor muscle fatigue. It was hypothesised that preventing EIAH (defined as a drop in arterial oxygen saturation [S$_a$O$_2$] below pre-exercise resting levels) would decrease the magnitude of fatigue, whilst arterial hypoxaemia would enhance fatigue. The authors measured quadriceps fatigue in trained endurance athletes using magnetic stimulation of the femoral nerve. Two constant load exercise tests were performed; the first conducted in normoxia (fraction of inspired oxygen [FIO$_2$] = 0.21) served as a control and was performed to the limit of volitional tolerance. The second constant load test was conducted for the same duration as the control; however, supplemental O$_2$ was added to the inspirate (FIO$_2$ = 0.25) in order to attenuate any arterial oxygen desaturation. In normoxia, S$_a$O$_2$ fell to between 84-95% at the end of exercise, resulting in a 33% decrease in quadriceps twitch torque (Q$_{tw}$). Hyperoxia prevented EIAH by maintaining S$_a$O$_2$ above 98% and the magnitude of quadriceps fatigue was more than halved (15% decrease in Q$_{tw}$). What’s more, blood lactate concentrations and ratings of perceived exertion were all lower at the end of exercise in hyperoxia.

Romer and co-workers speculate that EIAH may have caused fatigue directly (via decreased O$_2$ transport) and indirectly through systemic effects of hypoxia. For example, attenuation of hypoxaemia curtailed the ventilatory drive to breathe, thereby reducing respiratory muscle work and based upon the work of Harms et al. (1998b), should improve limb blood flow and vascular conductance. Finally, it is believed that EIAH contributes to exercise limitation by
intensifying effort perceptions, such that subjects ‘choose’ to reduce their work output and terminate exercise.

In order to address whether respiratory muscle fatigue directly affects exercise performance, some authors have employed so-called ‘pre-fatigue’ study designs. By inducing fatigue of the respiratory muscles prior to exercise, researchers are able to identify the influence of this variable upon subsequent whole-body exercise performance. A number of modalities have been implemented to experimentally induce DF, including: 150 min volitional hyperpnoea (separated by a 4 min break every 15 min) at the maximal sustainable ventilation (58-82% maximal voluntary ventilation [MVV]) (Martin et al., 1982), 10 min volitional hyperpnoea at 80% of maximal exercise $\dot{V}_E$ (Dodd et al., 1989), 60% MVV until exhaustion (Mador et al., 1996) and resistive breathing at 80% of MIP (Mador & Acevedo, 1991a, b; Sliwinski et al., 1996). Authors have either found a decrease (Mador & Acevedo, 1991b) or no change (Sliwinski et al., 1996) in subsequent exercise tolerance. The protocol adopted by Mador and Acevedo (1991b) failed to control breathing pattern and therefore, inspiratory duty cycle ranged from 0.50-0.71.

A key limitation with the aforementioned studies is that subjects were allowed to choose their own breathing pattern. As a result, all studies observed an increase in $f_b$ with no change in tidal volume (i.e. rapid but not shallow breathing), in addition to a heightened sensation of dyspnoea. Furthermore, there were differences in chosen loading protocol (e.g. flow-resistive vs. pressure-threshold) between studies and typically, DF was assessed using volitional methods.

In an attempt to overcome these limitations, Harms et al. (2000) mechanically reduced the WOB by unloading the respiratory muscles during high-intensity exercise using PAV. This method of mechanical unloading increased vascular conductance and blood flow to working limb
locomotor muscles by 14-16% (Harms et al., 1997; Harms et al., 1998b) and significantly improved endurance-exercise performance by 14% compared to a control (Harms et al., 2000).

More recently, Wuthrich et al. (2013) induced fatigue at rest using flow-resistive loading and determined subsequent cycling performance at 85% of peak power output. Wuthrich et al. (2013) assessed inspiratory muscle fatigue and quadriceps muscle fatigue using magnetic stimulation applied to the cervical spine and femoral nerve, respectively. Inspiratory resistive breathing lasted for 27.8 ± 8.8 min on average. Transdiaphragmatic twitch pressure and $Q_{tw}$ were significantly reduced (23.4 ± 13.3% and 32.0 ± 11.8%, respectively), leading to a 13.5 ± 15.3% reduction in exercise performance. Importantly, this study failed to measure neural activation of the respiratory muscles (diaphragm EMG); therefore, the reduction in $P_{di, tw}$ could have been attributed to a loss in inhibition of the diaphragm compound muscle action potential propagation and not necessarily impaired excitation-contraction coupling.

To summarise, the onset of DF is associated with a sympathetically mediated metaboreflex, caused by three primary factors: 1) excessive respiratory muscle work, 2) metabolite accumulation, and 3) competition for finite cardiac output during near-maximal exercise. This unique phenomenon occurs when the respiratory muscles ‘steal’ blood from exercising limbs, thereby catalysing peripheral locomotor fatigue. A greater perception of effort induces central fatigue and in doing so, confines peripheral fatigue to a sensory tolerance limit (or critical threshold). Exercise is terminated before failure of organism homeostasis may occur, ultimately leading to impaired performance. However, there is still debate as to whether DF limits exercise performance. The following section will explore sex-based differences with regard to the key principles outlined thus far.
2.4  Sex-Based Differences in Respiratory Exercise Physiology

2.4.1  Sexual Dimorphism and Dysanapsis

Originally conceptualised in the early 1970’s (Green et al., 1974), Mead (1980) and Thurlbeck (1982) made the distinction of non-isotropic growth of the airways and lung parenchyma (also referred to as dysanapsis). It was noted that women had smaller airways relative to lung size in comparison to men. However, the disparity between airway size and lung size could be attributed to variation in lung volume. To correct this shortcoming, Sheel et al. (2009) found that men had 14-25% greater luminal areas of the trachea and upper generations of the bronchial tree (conducting zone) when matched for women with equivalent lung volume. Based upon the Poiseuille equation, which states that airway radius is a major determinant of airway resistance when flow is laminar, it stands to reason that women may experience a greater mechanical and energetic WOB during exercise, due in part to flow limitation. More recent work has shown that the shape of the lungs also differs between men and women. Using 3D geometric morphometrics, Torres-Tomayo et al. (2017) found that the lungs of men have a pyramidal shape, whilst women have a prismatic shape. It is suggested that women have a predominant ‘pump-handle’ ribcage movement, whereas men show a ‘bucket-handle’ movement due to greater diaphragmatic action, which expands the lower lungs mediolaterally. It is thought that greater ribcage inclination would accommodate large abdominal volume displacement during pregnancy (Bellemare et al., 2003).

2.4.2  Mechanical Ventilatory Constraints

Dysanapsis explains why there is an increased prevalence of expiratory flow limitation (EFL) in women of all fitness levels during heavy exercise compared with men (McClaran et al., 1998). In addition, EFL correlates with degree of fitness, as more flow limitation was shown to be present.
in highly fit ($\dot{V}O_2\text{max} > 57 \text{ l} \cdot \text{min}^{-1}$) vs. less fit women (McClaran et al., 1998). Expiratory flow limitation occurs with the demonstration of an increase in transpulmonary pressure (via oesophageal balloon), without further increase in expiratory flow (Hyatt & Flath, 1966; Calverley & Koulouris, 2005). According to Dominelli et al. (2011) dysanapsis precedes aerobic fitness as the principle cause of EFL during exercise in women; this is in contrast to our understanding of the determinants of EFL men.

Furthermore, research to date indicates that EIAH may be present in women with unremarkable aerobic capacity, unique to this gender (Hopkins & Harms, 2004). Harms et al. (1998a) found that women of varying fitness levels ($\dot{V}O_2\text{max} \text{ range} = 30-70 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) developed EIAH ($S_aO_2$) more often (75.8%) at submaximal intensities and at a lower relative $\dot{V}O_2\text{max}$ than men. It should be appreciated however, that men were not directly assessed in this study, but sex-based comparisons were made based upon previous work in endurance-trained healthy young males (Dempsey et al., 1984). Conversely, Hopkins et al. (2000) found that only 23.5% of moderately fit (mean $\dot{V}O_2\text{max} = 51 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) women developed EIAH ($S_aO_2$). This seems strange considering both studies were conducted using women of similar aerobic fitness and followed similar exercise protocols. Laursen et al. (2002) found that prepubescent females do not develop EIAH ($S_pO_2$) during maximal exercise; therefore, a potential explanation for the discrepancy between studies relates to hormonal differences (specifically, progesterone and oestrogen). Finally, Richards et al. (2004) found that 67% of women developed EIAH ($S_pO_2$) during maximal incremental cycle test, slightly higher than reported values for men. Harms et al. (1998a) standardised testing to the mid-follicular phase of the menstrual cycle, whereas subjects from Hopkins et al. (2000) were studied randomly. Sansores et al. (1995) states that lower
diffusing capacity of carbon monoxide can vary by up to 13% during the menstrual cycle (with highest values occurring during the mid-late luteal phase) and may predispose women to O₂ diffusion limitation. Clearly warranting further investigation, Olfert et al. (2004) found that despite lower diffusion capacities, active women (tested during the follicular phase of their menstrual cycle) did not experience greater exercise-induced gas exchange abnormalities compared to age, height, aerobic capacity and lung size matched men. Therefore, it was concluded that gas exchange abnormalities were more likely a consequence of variance in fitness level or lung sizes as opposed to sex per se.

In a large cohort of women with ranging fitness levels (n = 30; \( \dot{V}O_{2\text{max}} \) range = 28-62 ml·kg\(^{-1}\)·min\(^{-1}\)), Dominelli et al. (2013) found that EIAH (\( S_aO_2 \)) occurred in 93% of trained subjects and 65% of women total, during constant load submaximal exercise (80-90% \( \dot{V}O_{2\text{max}} \)). Furthermore, breathing heliox gas partially reversed the EIAH in subjects who developed EFL. Heliox inspiration (21% O₂: 79% He) induces greater flows by making airflow more laminar (Babb, 1997). Dominelli et al. (2013) discuss tolerating EIAH as a ‘strategy’ to minimise the potential negative consequences of increasing exercise hyperpnoea and an excessive WOB. The author’s continue by stating that lung and chest wall mechanics may be preserved at the expense of a worsening arterial hypoxaemia, DF, and \( \dot{V}O_2 \) of breathing.

### 2.4.3 Work and Oxygen Cost of Breathing

Guenette et al. (2007) examined sex-based differences in respiratory mechanics during incremental cycle exercise to exhaustion in endurance-trained athletes. Several key findings were made: 1) at maximal exercise intensity, EFL occurred more frequently in women (90%) than in men (43%); 2) women exhibited higher relative end-expiratory and end-inspiratory lung volumes
(EELV and EILV, respectively); and finally, 3) at high levels of ventilation ($\dot{V}_E > 90 \text{ l}\cdot\text{min}^{-1}$), the total WOB for women increases almost two-fold relative to men. It appears these findings are interrelated. The presence of EFL during exercise may lead to an increase in operating lung volumes (such as EELV and EILV) and promotes gas trapping or dynamic hyperinflation. As a consequence, the inspiratory muscles are functionally weakened, less efficient at generating pressure and operate on the flatter portion of the pressure-volume curve (lower compliance), resulting in an increased mechanical work and $\dot{V}O_2$ of breathing (Dominelli et al., 2015).

The WOB can be divided into its constituted components using modified Campbell diagrams. Guenette et al. (2009) took their previous findings a step further by using this technique to determine the elastic and resistive contributions to inspiratory and expiratory work. Guenette et al. (2009) hypothesised that women would have a greater resistive WOB due to the laws of fluid dynamics through a tube (i.e. Poiseuille’s law). Standardising the total WOB into a ratio of work rate to body mass avoided complications associated with men achieving higher absolute work rates and ventilations during incremental cycling exercise. Guenette and colleagues (2009) found that the total WOB was significantly greater in women at 3.5 W·kg$^{-1}$ and 4.0 W·kg$^{-1}$, and this was owed to the inspiratory and expiratory resistive components exclusively. What’s more, the inspiratory resistive WOB was 67, 89, and 109% higher in women at 50, 75, and 100 l·min$^{-1}$, respectively, whilst the expiratory resistive WOB was only significantly higher in women at 75 l·min$^{-1}$. This study reveals that women must generate substantially higher pressures in order to generate an equivalent volume of flow compared to men and this difference is likely caused by inherently narrower diameter airways.
A higher WOB for a given $\dot{V}_E$ would theoretically result in a greater respiratory muscle $\dot{V}O_2 (\dot{V}O_{2\text{RM}})$. Recently, Dominelli et al. (2015) made the observation that the oxygen cost of exercise hyperpnoea is greater in women, compared to men. Dominelli and co-workers (2015) report that $\dot{V}O_{2\text{RM}}$ represents a significantly greater fraction of total $\dot{V}O_2$ in females ($13.8 \pm 1.5\%$) compared to males ($9.4 \pm 1.1\%$). It is postulated therefore, that women will have a greater fraction of their cardiac output directed to the respiratory muscles in order to meet metabolic demand.

### 2.4.4 Fatigue

Based upon the evidence stated above, logic points to a female diaphragm that would perhaps be more susceptible to fatigue, given the critical variable related to fatigue development is the WOB. However, contrary to their original hypothesis, Guenette et al. (2010) found that the magnitude and prevalence of exercise-induced DF was significantly greater in men than women during whole-body constant load exercise to exhaustion ($90\% \dot{V}O_{2\text{max}}$). Fifty-eight percent of men and 42% of women were defined as being fatigued. Of those, the magnitude decrease in $P_{\text{di, tw}}$ 10 minutes following the cessation of exercise was $30.6\%$ in males and $21.0\%$ in females.

The reasons for this apparent female fatigue resistance are unknown; nevertheless, Guenette and colleagues (2010) theorise that their findings may reflect the higher absolute work rate accomplished by men ($327 \text{ W vs. } 242 \text{ W}$) and therefore, a higher demand for ventilatory work. This is based upon the finding that men had a significantly larger total respiratory pressure-time product (sum of $\text{PTP}_{\text{oes}}$ and $\text{PTP}_{\text{di}}$) throughout the exercise bout. Interestingly, men were able to increase $\dot{V}_E$ linearly over time, whereas women reached a plateau towards the end of exercise. Coincidentally, the female $\text{PTP}_{\text{oes}}$ remained relatively flat for the duration of the test, but increased dramatically in men, approximately two-thirds of total time elapsed. These findings suggest that
men were able to recruit additional inspiratory muscles in order to keep increasing ventilation, likely as a contingency for a fatiguing diaphragm. Women on the other hand, were able to share the load placed upon the respiratory muscles relatively well over time. Further explanation of the female fatigue resistance comes from experiments conducted on other isolated skeletal muscles, in which a female resistance to fatigue is well established. An exception to this rule was thought to be the respiratory muscles due to the structural and functional disparities acknowledged previously. Further confusion arises when comparisons are made during large muscle mass (including quadriceps) dynamic exercise, in which sex differences are eliminated (Hunter, 2016; Dominelli et al., 2017b; Sundberg et al., 2017), as other factors likely override any sex-specific influence.

In their review of sex differences in skeletal muscle fatigue, Hicks et al. (2001) posited that females are more resistant to fatigue than men. A discussion of the studies that informed their conclusions follows; in addition, recent advances in the field will be examined and the putative mechanisms associated with female fatigue resistance explored.

Petrofsky et al. (1975) is credited as being the first to acknowledge that men are more prone to fatigue in relation to their opposite sex. Several studies have corroborated Petrofsky’s findings and demonstrated that when men and women perform a submaximal fatiguing contraction of the same relative intensity, women are able to sustain the contraction for a longer duration than men. This has been shown in various muscles groups, such as: knee extensors (Maughan et al., 1986), elbow flexors (Miller et al., 1993), handgrip muscles (West et al., 1995) and the adductor pollicis muscle (Fulco et al., 1999). Similarly, women exhibit less of a reduction in maximal force output during sustained or intermittent maximal contractions (Russ & Kent-Braun, 2003; Hunter et al.,
Several mechanisms have been put forth to explain the observed sex differences in fatigue resistance. Hicks et al. (2001) broadly classifies these mechanisms into three themes: 1) muscle mass and morphology, 2) substrate utilisation, and 3) neuromuscular activation.

In general, women have less muscle mass than men and this in itself is proposed to be one of the key contributors to explain the greater fatigue resistance found in women. When performing at the same relative intensity, lower muscle mass translates directly into lower absolute force generation. This means there will be a decreased demand for O₂, a decrease in mechanical compression of the local vasculature and less intramuscular occlusion of blood flow. Maughan et al. (1986) compared fatigability in untrained males and females during an isometric and a dynamic exercise protocol. Sustained leg extension at varying intensities (percent of maximal voluntary contraction [MVC]) was performed for the isometric trial and elbow flexion to task failure at varying intensities was performed for the dynamic trial. As expected, men had greater absolute strength for both trials. Sex differences were present in endurance capacity during leg extension at 20% MVC, with females demonstrating a 29% increase in time to task failure. Interestingly, as contraction intensity increased (50 and 80% MVC), the sex difference declined. The time to task failure during elbow flexion was also greater in females at lower intensities (50, 60 and 70% MVC), but not at higher intensities (80 and 90% MVC). These results support the notion that lower absolute force production (such as that exhibited by females) results in less vascular occlusion of blood flow, leading to increased perfusion to the working muscles which in turn, prevents the onset of fatigue. Reduced blood supply to an active muscle will result in accelerated muscle fatigue and earlier task failure because of the reduced oxygen delivery to the muscle and rapid accumulation of metabolites that interfere with the contractile function (Russ & Kent-Braun, 2003).
fundamental problem when interpreting the findings from this study is that men and women were not matched for absolute force of submaximal contraction; therefore, sex differences may be due to contraction conditions eliciting a greater degree of imbalance between muscle O$_2$ demand and supply. Fulco et al. (1999) addressed this issue by comparing muscle performance when men and women were matched for one-repetition maximum (1RM) of the adductor pollicis muscle. Despite controlling for absolute strength, the authors still found that females were more resistant to fatigue than males.

With regard to muscle morphological differences, there is evidence to suggest that females possess a greater percentage of slow-twitch type I muscle fibres than men (Nygard, 1981; Simoneau & Bouchard, 1989). Type I fibres have a slower rate of contraction and thus, a slower rate of metabolism (Hamada et al., 2003). As a result, type I fibres fatigue at slow rates compared to glycolytic fast-twitch type II fibres. Froberg and Pedersen (1984) examined sex-based differences in glycogen utilisation during high intensity aerobic cycle exercise when males and females were matched for physical activity participation. Females had a significantly greater endurance capacity at 80% $\dot{V}$O$_2$max (females: 53.8 ± 12.7 minutes; males 36.8 ± 12.2 minutes). Mid-exercise and final stage respiratory exchange ratio (RER) values were significantly lower for females. This suggests that females rely more on $\beta$-oxidation of free fatty acids relative to men, thus preserving glycogen stores. Further, blood lactate concentration was also lower in females at 80% $\dot{V}$O$_2$max compared to males. However, at 90% $\dot{V}$O$_2$max, these sex-based differences were eliminated. The authors concluded that females had greater endurance due to spared glycogen and believed that this sparing was countered at high exercise intensities, when metabolic milieu evoked contractile failure. However, muscle biopsy studies have shown that lower activities of common
glycolytic enzymes (such as: pyruvate kinase, phosphofructokinase, and lactate dehydrogenase) have been reported in women, which would decrease their potential for anaerobic glycolysis (Tarnopolsky, 1999). Moreover, type I muscle fibres possess a greater fraction of vasodilatory $\beta_2$-adrenergic receptors, thereby improving $O_2$ transport kinetics (Roatta & Farina, 2010).

Finally, sex differences in skeletal muscle fatigue may relate to failure of neuromuscular activation. West et al. (1995) measured electromyogram (EMG) activity in males and females during various intensities of forearm, wrist and hand flexion. Subjects performed sustained isometric contraction on a handgrip dynamometer at a predetermined percentage of MVC until task failure. EMG was monitored throughout. The authors found that females displayed longer time to task failure at all three intensity levels (30, 50 and 75% MVC) compared to males. Neuromuscular activity measured via EMG also increased with intensity, suggesting a relationship between the variables.

Hakkinen (1993) examined acute neuromuscular fatigue and recovery in men and women following heavy resistance exercise. Subjects performed twenty, 1RM squat-lifts. Despite similar declines in force production (females: $20.5 \pm 11.8\%$; males: $24.1 \pm 14.4\%$), maximal voluntary integrated EMG activity of the leg extensor muscles was significantly reduced in men, but not in women. What’s more, maximal force recovered more quickly during the first hour of rest in women. Recovery between sexes took place gradually and to the same degree thereafter. The authors summarised their findings by stating that males may have greater impairment of neuromuscular activation during strenuous loading and take longer to recover compared with females.
Hunter (2014) acknowledges that female hormone fluctuations throughout the menstrual cycle may alter fatigability. The menstrual cycle can influence pulmonary function, substrate metabolism, thermoregulation, and ventilation during exercise (Harms, 2006). The sex-specific hormones, progesterone and oestrogen, are at their highest concentration during the luteal phase of menstruation (Dombovy et al., 1987). Effects of progesterone and oestrogen on the pulmonary system include: hyperventilation, an increase in the hypercapnic and hypoxic ventilatory responses (Schoene et al., 1981; Moore et al., 1987), partial compensation of respiratory alkalosis (England & Farhi, 1976), augmented central drive to breathe (Dombovy et al., 1987), and changes in resting diffusing capacity (reduced during the early follicular phase of menstrual cycle when oestrogen concentration are lowest) (Sansores et al., 1995). Recently, however, MacNutt et al. (2012) found that the ventilatory response to submaximal exercise was unchanged regardless of hormone concentration in both normoxia and hypoxia, while resting ventilation and end-tidal partial pressure of CO₂ were influenced by reproductive hormone concentrations. Thus, there is still debate among researchers as to control for hormonal status during exercise testing as the many feed-forward and feedback mechanisms may override any possible modulating effects of naturally occurring changes in sex hormones.

Sex-based differences in the respiratory system have important implications concerning the physiology of exercise (Guenette & Sheel, 2007). By virtue of their narrower airways relative to lung size compared to men, women experience EFL more often, develop EIAH more frequently, breathe at higher lung volumes, have a higher mechanical WOB and \( \dot{V}O_{2\text{RM}} \) for a given \( \dot{V}_E \), and yet, are more resistant to fatigue of the diaphragm.
2.5 Purpose

The primary purposes of the present thesis were two-fold. First, to determine the reliability of cervical magnetic stimulation in the assessment of the diaphragmatic compound muscle action potential (i.e. M-wave) recorded by chest wall surface EMG and to ensure its efficacy in sex-based comparisons of diaphragmatic neuromuscular function. Second, to explore sex-based differences in the mechanisms and consequences of DF, specifically: a) the cardiovascular response to inspiratory resistance, and b) the effect of prior-induced DF on subsequent exercise performance.

2.6 Research Questions

1) Is cervical magnetic stimulation an effective means of producing reliable and reproducible results in the analysis of diaphragm motor action potentials when recorded via chest wall surface EMG and is this uniform between the sexes?

2) Are there sex differences in the prevalence and magnitude of DF when experimentally induced at rest using inspiratory pressure-threshold loading performed to task failure?

3) Are there sex differences in the sympathetic response of the cardiovascular system to imposed inspiratory resistance (i.e. the inspiratory muscle metaboreflex)?

4) What is the effect of prior-induced DF on subsequent exercise performance in healthy young men and women?

2.7 Hypotheses

1) The technique of cervical magnetic stimulation will yield reproducible results within and between experimental sessions, whilst no statistically significant sex-based differences in any of the M-wave characteristics shall be found.
2) Women will be more resistant to DF when induced at rest compared to their male counterparts.

3) By virtue of their fatigue resistance, women will experience an attenuated inspiratory muscle metaboreflex.

4) A blunted sympathetic response of the cardiovascular system to inspiratory resistance in women will result in mild exercise performance decrements relative to men.
Chapter 3: Reliability of the Diaphragmatic Compound Muscle Action Potential Evoked by Cervical Magnetic Stimulation and Recorded via Chest Wall Surface EMG

3.1 Introduction

The diaphragm is the principle muscle of inspiration, contributing to around 70% of lung volume displacement during regular tidal breathing (Mead & Loring, 1982). Failure of this ‘vital pump’ to generate pressure, due to fatigue or pathology, has implications concerning gaseous exchange that may contribute to alveolar hypoventilation and respiratory acidosis. Accurate measurement of the electrical and mechanical properties of the diaphragm is therefore warranted in order to monitor diaphragmatic neuromuscular function.

Electrical or magnetic stimulation of the phrenic nerve elicits a compound muscle action potential (CMAP) that allows for objective assessment of diaphragm neural activation, without influence of central factors. The CMAP represents the summation of electrical activity travelling across the muscle fibre sarcolemmas preceding mechanical force production. Several techniques of phrenic nerve stimulation have been described. Electrical stimulation can be delivered through indwelling needle (Mier & Brophy, 1991) or wire electrodes (Hubmayr et al., 1989), or transcutaneously (Bellemare & Bigland-Ritchie, 1984). The insertion of indwelling electrodes presents a risk of haematoma and phrenic nerve damage; consequently, neither are recommended for patient evaluation (ATS/ERS, 2002). Transcutaneous stimulation has minimal risks, but poses some technical challenges such as maintaining the spatial relationship between stimulating electrode and nerve; furthermore, the stimulation intensity needed to maximally activate the phrenic nerves can be considered painful for some subjects due to the high-density current at the
stimulation site (ATS/ERS, 2002). Magnetic stimulation, on the other hand, uses a time-varying magnetic field to induce an electrical field that activates underlying peripheral nerve axons. Importantly, magnetic fields penetrate non-conductive tissue unimpeded and preferentially target large efferent motor fibers rather than high threshold afferent fibres that mediate pain sensation (Mills, 1999). Regardless of the specific technique utilised (e.g. cervical [CMS], unilateral or bilateral anterolateral, or anterior pre-sternal), magnetic stimulation is hindered by the potential inability to achieve maximal phrenic nerve activation, which could render the technique sensitive to small changes in coil position and introduce variability in evoked potentials. Generally, CMS is preferred in most clinical and research settings because it is well described with normal values being published in both young and elderly populations (Similowski et al., 1989; Polkey et al., 1997). However, few studies have reported stimulation thresholds using CMS and the criteria for maximal activation has not been clearly defined, as researchers typically rely on twitch pressure to infer phrenic nerve activation (Bellemare & Poirier, 2005). Another potential drawback of the magnetic stimulation technique is specificity; there is greater risk of co-activation of accessory inspiratory muscles and muscles innervated by the brachial plexus compared to electrical, all of which could contribute to contamination of diaphragm EMG (Laghi et al., 1996). Nonetheless, crosstalk from non-diaphragmatic muscles can be minimised depending on the recording method employed.

Multiple methods of recording evoked respiratory muscle activity are available (e.g. intramuscular, oesophageal, and surface electrodes placed on the chest wall). Yet, there remains no consensus regarding optimal electrode positioning as the technique of CMS continues to vary among laboratories due to lack of standardisation (ATS/ERS, 2002). Indwelling electrodes have
the advantage of recording directly from the diaphragm; however, the method is impractical for clinical use because of the risks associated with invasive procedures. Oesophageal electrodes record crural region diaphragm EMG, thereby reducing the likelihood of detecting non-diaphragmatic activity. Should one be interested in both electrical (i.e. CMAP) and mechanical (i.e. transdiaphragmatic twitch pressure) responses of the diaphragm to phrenic nerve stimulation, the fixed distance separating oesophageal and gastric balloons may compromise optimal balloon and/or electrode placement, leading to inaccurate pressure measurements and/or EMG recordings. An alternative recording technique involves the application of surface electrodes placed on the chest wall. A concern with using surface EMG is the susceptibility to contamination, which can be mitigated through diligent electrode placement; for example, keeping inter-electrode distance to a minimum and placing electrodes at a more anterior, medial, and lower site (Verin et al., 2002; Demoule et al., 2003; Glerant et al., 2006).

Of the stimulation and recording techniques available for assessing the diaphragm CMAP, CMS combined with chest wall surface EMG represents the most practical approach for both clinical and research purposes. Given the importance of accurately monitoring diaphragmatic neuromuscular function and some of the potential limitations accompanying CMS and surface EMG, it is surprising that research investigating the reliability of the method is scarce. To date, only one study has explored the reliability of CMS evoked CMAPs using surface recordings of the diaphragm. Whilst providing preliminary evidence supporting the efficacy of the technique, the study examined within-session reliability of two CMAP characteristics (onset latency and peak-to-peak amplitude) exclusively in men (Chien et al., 2008). A more comprehensive analysis of the temporal and spatial characteristics of the diaphragmatic CMAP is required. In addition,
information on between-session reliability of the technique is relevant to experimenters and clinicians monitoring long-term changes in diaphragm function and may prove valuable in the diagnosis, treatment, and management of patients with phrenic neuropathies, such as Guillain-Barré syndrome (Zifko et al., 1996a). To this end, the primary aim of the present experiment was to establish the within- and between-session reliability of CMS to evoke the diaphragm CMAP recorded by chest wall surface EMG.

3.2 Methods

3.2.1 Subjects

Healthy young males (n = 10; age = 24.7 ± 2.5 years; height = 178.4 ± 8.1 cm; weight = 73.6 ± 8.2 kg) and females (n = 10; age = 25.9 ± 1.5 years; height = 168.8 ± 9.6 cm; weight = 62.9 ± 6.4 kg) were recruited for the study. Subjects were non-smokers and free from any known cardiorespiratory disorder, with the exception of two subjects who reported mild asthma. All subjects provided written informed consent prior to testing. The study protocol adhered to ethical guidelines set forth by the University of British Columbia Research Ethics Board.

3.2.2 Procedures

Subjects reported to the laboratory where anthropometrics were measured and standard pulmonary function testing took place. To assess within-session reliability of the CMS protocol, two blocks of five stimuli were delivered 30 minutes apart at 100% stimulator output (equivalent to 2.0 Tesla). A subgroup of subjects (n = 9 [5 male]) returned to the laboratory on a second occasion, approximately two months later, for examination of between-session reliability. One block of stimuli was delivered on the second day. In order to determine if the phrenic nerves were maximally stimulated, approximate twitch thresholds were measured and recruitment curves
plotted by progressively increasing the intensity of the stimulus from 20-100% (in 5-10% increments) of the maximal stimulator output. Five twitches were delivered at each intensity with a 30 s interval between successive stimuli to minimise the effect of twitch potentiation.

3.2.3 Cervical Magnetic Stimulation

CMS was performed according to the original technique described by Similowski et al. (1989). A handheld 90 mm circular coil (P/N 3192-00; peak electric field strength = 530 V/m, peak magnetic field strength = 2.0 Tesla, inductance = 23.5 µH) powered by a magnetic stimulator (Magstim 200-2, Magstim; Wales, UK) was used to stimulate the phrenic nerves at the level of the third-seventh cervical vertebrae. The coil was held horizontal and centered over the midline of the cervical spine with the current flowing in a clockwise direction, as diaphragm CMAPs are not affected by the direction of current (Similowski et al., 1997). Subjects were seated comfortably with the neck flexed. Stimuli were delivered at end-expiration with the glottis closed to preclude lung volume influence on diaphragm EMG. The optimal site of stimulation was identified by gradually moving the coil along cervical vertebrae C3-C7 until the largest CMAP amplitude was observed at submaximal stimulation intensities (70% stimulator output). This location was marked and used for all subsequent stimuli.

3.2.4 Surface EMG Recordings

To record electrical activity of the left and right costal diaphragm, self-adhesive surface Ag/AgCl electrodes (H59P, Kendall; Mansfield, MA, USA) were placed on the chest wall in bipolar arrangement (2 cm apart) between the sixth and eighth intercostal spaces along the anterior-axillary line. The ground electrode was placed on the acromion process of the scapula. The skin was lightly abraded and cleansed with alcohol to minimise electrical impedance. Electrodes were further
secured using surgical tape and repositioned if necessary. Signals were amplified (x 200) and band-pass filtered (0.1 Hz to 3 kHz; Model P511, Grass Instruments; Warwick, RI, USA), sampled at 10 kHz (PowerLab 16SP, AD Instruments; Colorado Springs, CO, USA), and monitored online using LabChart software (AD Instruments; Colorado Springs, CO, USA).

### 3.2.5 CMAP Analysis

CMAP characteristics (onset latency, duration, peak-to-peak amplitude, and total rectified area) were calculated in MATLAB (MathWorks, USA) using a custom algorithm (see Figure 3.1 for analyses details). To objectively determine onset latency, EMG data were differentiated to generate an instantaneous slope profile and CMAP onset was identified as the point when the EMG slope exceeded a mean ± 10 SD threshold (calculated over a 50 ms pre-stimulus window) and remained above this threshold for a minimum of 20 of the subsequent 30 data points. CMAP offset was determined as the first data point of full wave rectified (non-differentiated) EMG that fell below a mean ± 10 SD threshold (calculated over a 50 ms pre-stimulus window). CMAP duration was calculated as the time difference between CMAP onset and offset, and peak-to-peak amplitude as the voltage difference between positive and negative EMG peaks. Integrated area (minus background noise) was calculated from rectified EMG as $\int_o^e (f(x)-b)\,dt$, where $o =$ CMAP onset, $e =$ CMAP offset and $b =$ background noise. Twitches were excluded for any of the following reasons: 1) the subject was not resting at functional residual capacity immediately prior to stimulation (determined by oesophageal pressure and/or the subject providing a hand-signal at end-expiration), 2) if a cardiac artefact was superimposed upon a stimulus, and 3) if there was lack of diaphragmatic relaxation evidenced by noticeable diaphragm EMG. The first three acceptable twitches were used for CMAP analysis.
Figure 3.1: Illustration of CMAP Analysis Methodology

(A) Raw M-wave data were differentiated to create an instantaneous slope profile. (B) M-wave onset was identified as the time when slope of the M-wave exceeded a threshold based upon background EMG (+/- 5 SDs) and remained above this threshold for a select number of subsequent data points (≥ 20 of 30). (C) M-wave offset was determined from the rectified M-wave data as the point when rectified EMG returned below the threshold criteria.
3.2.6 Statistical Analysis

Normality was assessed prior to statistical comparisons using the Shapiro-Wilk test. Independent samples t-tests were used to determine if any bilateral (right-left) and/or sex-based (male-female) differences existed for any of the CMAP characteristics. A one-way repeated measures analysis of variance (ANOVA) was used to determine maximal phrenic nerve activation by comparing CMAP amplitude and area at all intensities of stimulation with the maximal stimulator output. To assess within-session reliability, coefficients of variation (CV) were calculated between subsequent stimuli within a single block, and intraclass correlation coefficients (ICC[3,k]) were calculated between the two blocks of stimuli performed on the same day for all dependent measures (latency, duration, amplitude, and area). Between-session reliability was assessed between the first block of stimuli delivered on days 1 and 2. Only stimulations delivered at maximal stimulator output were used for the assessment of CMAP reliability. The level of significance was set at $p < 0.05$ for all statistical comparisons.

3.3 Results

3.3.1 CMAP Characteristics

At maximal stimulation (2.0 Tesla), mean (± SD) CMAP onset latency was slightly longer for the left (5.6 ± 0.9 ms) versus right (5.1 ± 0.8 ms) hemi-diaphragm and longer in males (5.6 ± 0.8 ms) compared to females (5.1 ± 0.9 ms); however, these differences were not statistically significant ($p = 0.34$ and 0.43, respectively). Mean CMAP duration was 47.3 ± 11.3 ms and did not differ between the right and left hemi-diaphragms or between sexes ($p = 0.45$ and 0.32, respectively). Peak-to-peak amplitude and area tended to be larger in males (amplitude = 594.9 ± 328.8 mV; area = 5.3 ± 2.0 mV·ms) compared to females (amplitude = 387.6 ± 220.0 mV; area = 4.4 ± 2.0 mV·ms),
but were also not statistically significant \( (p = 0.14 \text{ and } 0.25, \text{ respectively}) \). CMAP spatial characteristics were similar between the right and left hemi-diaphragms, where peak-to-peak amplitude and area differed by 7.2 and 3.2\%, respectively \( (p = 0.78 \text{ and } 0.72, \text{ respectively}) \). Descriptive statistics (mean ± SD, range and CV) of all CMAP parameters, including sex-based (male-female) and bilateral (right-left) comparisons are provided in Table 3.1. All CMAP temporal and spatial characteristics showed low within-subject variability between subsequent stimuli (CVs < 5\%), as well as between the two blocks of stimuli delivered within (CVs < 7\%) and between (CVs < 11\%) experimental sessions.

Approximate CMAP threshold was 40.6 ± 8.7\% of the maximal stimulator output. There was a sigmoidal increase in peak-to-peak amplitude as CMS intensity increased with a plateau observed at 1.8 and 1.9 Tesla (no statistically significant differences compared to 2.0 Tesla; \( p = 0.21 \text{ and } 0.35, \text{ respectively} \)). Inspection of individual subject data revealed clear evidence of a plateau in eight of nine subjects (see Figure 3.2 for full recruitment curve).

### 3.3.2 Reproducibility

Within-session reliability of the CMS protocol was high for all temporal (latency ICC = 0.98; duration ICC = 0.96; \( p < 0.001 \)) and spatial (amplitude ICC = 0.99; area ICC = 0.99; \( p < 0.001 \)) CMAP characteristics. CMAPs were similarly reproducible between days for both temporal (latency ICC = 0.96; duration ICC = 0.97; \( p < 0.001 \)) and spatial (amplitude ICC = 0.89; area = 0.89; \( p < 0.05 \)) characteristics, as illustrated in Figure 3.3 and Figure 3.4. Full reliability analyses and statistics are shown in Table 3.2.
### Table 3.1: Group Mean (± SD) Data for all CMAP Characteristics

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Bilateral Differences</th>
<th></th>
<th>Sex Differences</th>
<th></th>
<th>Group Average</th>
<th>Range</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RHD</td>
<td>LHD</td>
<td>Male</td>
<td>Female</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latency (ms)</td>
<td>5.1 ± 0.8</td>
<td>5.6 ± 0.9</td>
<td>5.6 ± 0.8</td>
<td>5.1 ± 0.9</td>
<td>5.3 ± 0.9</td>
<td>3.5-7.3</td>
<td>3.1</td>
</tr>
<tr>
<td>Duration (ms)</td>
<td>49.7 ± 11.3</td>
<td>44.8 ± 10.9</td>
<td>44.3 ± 14.2</td>
<td>50.2 ± 6.7</td>
<td>47.3 ± 11.3</td>
<td>22.6-64.9</td>
<td>4.5</td>
</tr>
<tr>
<td>Amplitude (mV)</td>
<td>497.8 ± 275.4</td>
<td>482.3 ± 320.4</td>
<td>594.9 ± 328.8</td>
<td>387.6 ± 220.0</td>
<td>490.3 ± 297.7</td>
<td>118.1-1221.3</td>
<td>4.2</td>
</tr>
<tr>
<td>Area (mV·ms)</td>
<td>5.0 ± 1.7</td>
<td>4.6 ± 2.4</td>
<td>5.3 ± 2.0</td>
<td>4.4 ± 2.0</td>
<td>4.8 ± 2.1</td>
<td>1.4-8.8</td>
<td>4.6</td>
</tr>
</tbody>
</table>

*Abbreviations: RHD = right hemi-diaphragm; LHD = left hemi-diaphragm; CV = coefficient of variation (within-subject and between subsequent stimuli). Values are mean ± S.D. No statistically significant bilateral or sex-based differences found for any CMAP characteristic (p > 0.05).*
Table 3.2: Full CMAP Reliability Analyses

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Within-Day</th>
<th></th>
<th>Between-Day</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>ICC</td>
<td>Mean ± SD</td>
<td>ICC</td>
</tr>
<tr>
<td>Latency (ms)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute Difference</td>
<td>0.2 ± 0.1</td>
<td>0.98*</td>
<td>0.3 ± 0.2</td>
<td>0.96*</td>
</tr>
<tr>
<td>% Difference</td>
<td>3.6 ± 3.0</td>
<td></td>
<td>5.6 ± 3.9</td>
<td></td>
</tr>
<tr>
<td>Duration (ms)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute Difference</td>
<td>3.2 ± 2.0</td>
<td>0.96*</td>
<td>3.4 ± 2.7</td>
<td>0.97*</td>
</tr>
<tr>
<td>% Difference</td>
<td>6.2 ± 3.3</td>
<td></td>
<td>7.5 ± 5.5</td>
<td></td>
</tr>
<tr>
<td>Amplitude (mV)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute Difference</td>
<td>18.0 ± 0.2</td>
<td>0.99*</td>
<td>60.7 ± 70.3</td>
<td>0.89*</td>
</tr>
<tr>
<td>% Difference</td>
<td>5.1 ± 3.6</td>
<td></td>
<td>10.1 ± 6.7</td>
<td></td>
</tr>
<tr>
<td>Area (mV·ms)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute Difference</td>
<td>0.1 ± 0.1</td>
<td>0.99*</td>
<td>0.6 ± 0.6</td>
<td>0.89*</td>
</tr>
<tr>
<td>% Difference</td>
<td>3.3 ± 3.1</td>
<td></td>
<td>10.8 ± 9.9</td>
<td></td>
</tr>
</tbody>
</table>

*Abbreviations: ICC = intraclass correlation coefficient. Values are mean ± S.D. *p < 0.001

3.4 Discussion

The primary aim of the current investigation was to examine the reliability of CMS to evoke the diaphragmatic CMAP recorded by chest wall surface EMG. The temporal and spatial characteristics of evoked potentials were highly reproducible within a single experimental session. These results extend previous reports by demonstrating that the diaphragmatic CMAP can also be reliably assessed across testing sessions conducted on different days in both men and women, without systematic bias toward unilateral phrenic nerve activation.

3.4.1 CMAP Characteristics and Stimulation Thresholds

The measurements of CMAP onset latency (5.3 ± 0.9 ms) correspond well with previous studies that used magnetic stimulation (Similowski et al., 1989; Luo et al., 1998), and are slightly shorter compared to those of electrical (Chen et al., 1995; Zifko et al., 1996b). It is posited that CMS may
depolarise the phrenic nerves at a more distal site (Similowski et al., 1997), hence the discrepancies between electrical and magnetic stimulation. The values of phrenic nerve conduction time are within normal limits based upon estimates of phrenic nerve length (250-400 mm) (McKenzie & Gandevia, 1985; Jiang et al., 2011) and conduction velocity (48-78 m⋅s⁻¹) (Heinbecker et al., 1936; Newsom Davis, 1967). High between-subject variability in peak-to-peak amplitude and area is not uncommon, as normal values typically vary over a 10-fold range (Verin et al., 2002). Thus, the magnitude of the CMAP serves as a reliable measure of diaphragm motor unit recruitment only under circumstances whereby each individual subject acts as his or her own control.

A potential drawback of the magnetic stimulation technique is the inability to increase the stimulus intensity beyond the stimulator capacity. A plateau in CMAP amplitude was observed at 90 and 95% of peak stimulator output, providing reasonable certainty of maximal phrenic nerve activation. In the current experiment, eight of nine subjects (88.8%) demonstrated a plateau. Failure to evoke maximal twitch responses could introduce variability between stimuli and across testing sessions because subtle changes in stimulus location could alter the number of recruited nerve fibers. Nevertheless, the one subject that failed to demonstrate a plateau in CMAP amplitude did show reproducible results within (CV = 5.6%) and between (CV = 9.7%) sessions. In line with previous research, the approximate excitability threshold of the phrenic nerves was 40.6 ± 8.7% of the maximal stimulator output, equal to 0.8 Tesla (Zifko et al., 1996b). Interestingly, there was no evidence of the H-reflex during construction of recruitment curves, possibly due to the relatively few proprioceptive afferent phrenic nerve fibres and muscle spindles found within the diaphragm (Duron et al., 1978; Road, 1990). Moreover, the majority of proprioceptive afferents
arise from Golgi tendon organs and spindles are predominately located in the crural, not costal region (Corda et al., 1965; Balkowiec et al., 1995).

3.4.2 Within- and Between-Session Reliability

Diaphragmatic CMAPs recorded using chest wall surface EMG were highly reproducible within and between experimental sessions. High within-session reliability of CMS evoked CMAPs has been previously demonstrated for measures of peak-to-peak amplitude and onset latency (Chien et al., 2008), ICCs reported therein are similar to those of the current investigation (amplitude ICC = 0.99 vs. 0.99; latency ICC = 0.97 vs. 0.98). The results extend those of Chien and colleagues (2008) by providing evidence of between-session reproducibility, pertinent to researchers and clinicians alike wishing to monitor phrenic nerve conduction over a prolonged period of time (e.g. efficacy of treatment).

3.4.3 Bilateral and Sex-Based Differences

The right and left hemi-diaphragms were co-activated equally, as shown by comparable CMAP spatial characteristics – confirming effective bilateral phrenic nerve stimulation. It is well acknowledged that the length of the left phrenic nerve is longer than the right due to the path it must take around the heart (McKenzie & Gandevia, 1985). In support of this notion, CMAP onset latency was slightly shorter for the right vs. left hemi-diaphragm. Compared to visual inspection, the ability of the more rigorous and objective analysis methodology used to detect small differences in onset latency in the present study, could be beneficial in certain situations whereby such sensitivity is required (e.g. detection of unilateral phrenic nerve lesions).
Figure 3.2: Recruitment Curve

(A) Group average (± S.E.M.) CMAP peak-to-peak amplitudes at each stimulus level. A plateau was observed at 90 and 95% of peak power output. (B) Raw CMAP traces from one subject. Thick black line represents maximal stimulator output. Dashed lines are submaximal stimulation intensities of progressively increasing intensity.
Despite the finding of a large difference in peak-to-peak amplitude and area between sexes, statistical significance was not observed due to the large variability among subjects. Chen and co-workers (1995) found a correlation between chest circumference and CMAP amplitude; the authors attribute their findings to larger muscle mass and lung volumes in males. Whilst chest circumference was not measured in the current study, lung volumes were indeed larger in men compared to women (data not presented), which may partially explain these observations. Subjects were resting at functional residual capacity immediately prior to each stimulation – an important consideration given that lung volumes are known to increase CMAP amplitude (Bellemare & Bigland-Ritchie, 1984). Males also had marginally longer onset latencies, likely as a consequence of phrenic nerve length.

3.4.4 **Clinical Utility and Application**

Electrophysiological examination of diaphragm activation can provide important diagnostic information that may be used to manage patients with neuromuscular disease. Furthermore, the diaphragmatic CMAP is a key component of isolating the specific mechanisms contributing to inspiratory muscle fatigue. When combined with measurements of mechanical force production (i.e. transdiaphragmatic pressure), the CMAP can be used to identify where along the neuromuscular pathway failure may have occurred. Loss of force and simultaneous reduction or slowing of the CMAP implies a disruption of the action potential propagation along the muscle membrane/sarcolemma or down the transverse tubule. Conversely, a decline in transdiaphragmatic twitch pressure with no concurrent alteration in CMAP properties infers a loss of force caused by impaired excitation-contraction coupling (Edwards *et al.*, 1977; Jones, 1996).
Figure 3.3: Within- (Thick Line) and Between-Session (Dashed Line) Correlations for all CMAP Characteristics
Each scatter plot compares two blocks of stimuli delivered at different time points. Results from block 1 (B1) are plotted against results from block 2 (B2). Each data point represents the mean value of three stimulations delivered at 100% stimulator output for one subject. Statistically significant relationships were found for all variables (latency, duration, amplitude, and area), both within and between experimental sessions ($p < 0.05$). *Abbreviations:* ICC = intraclass correlation coefficient.
3.4.5 Technical Considerations

As stated previously, CMS is non-focal; several muscle groups may be activated at once, resulting in the possibility of extra-diaphragmatic contribution to the EMG. It cannot be excluded the possibility that EMG signals were contaminated; however, pragmatic design and experimentation has led to an approach that may reduce the chance of crosstalk from non-diaphragmatic muscles. Electrodes were positioned close together and situated near the costochondral junction in compliance with recommendations by Verin et al. (2002). Additionally, patients with phrenic
nerve palsy do not demonstrate any electrical activity using chest wall electrodes in response to phrenic nerve stimulation, suggesting that the signal was not contaminated due to the unavoidable stimulation of non-diaphragmatic muscles (Chokroverty et al., 1995). Where possible, it may be of benefit to place multiple electrode pairs along the anterior chest wall in order to determine the most suitable electrode configuration on a subject-by-subject basis. Glerant et al. (2006) provide evidence that the same electrode configuration should not be used for all subjects as CMAP amplitudes vary depending where along the chest wall electrodes are placed. Specially designed oesophageal electrodes circumvent the limitations associated with surface EMG and may be preferred depending on the application and feasibility. Under circumstances whereby pressure measurements are priority (such as in the assessment of diaphragmatic fatigue), surface electrodes placed on the chest wall may offer a practical advantage. The decision to use chest wall or oesophageal electrodes may best be dictated depending on the primary outcome variable of interest, with chest wall EMG favoring mechanical properties and oesophageal favoring electrical.

3.4.6 Conclusions

In conclusion, CMS is an effective means of evoking the diaphragmatic CMAP. Surface electrodes placed on the chest wall provide a minimally invasive and reliable method to record diaphragm EMG in healthy young men and women. Crucially, this study demonstrates that the technique of CMS may be used to achieve reliable results between testing sessions, thereby validating its practice in the longitudinal assessment of diaphragmatic neuromuscular function.
Chapter 4: Sex Differences in Diaphragmatic Fatigue: The Cardiovascular Response to Inspiratory Resistance

4.1 Introduction

The conjecture that the diaphragm, the principle muscle of respiration, could begin to fail as a ventilatory pump due to fatigue of central or peripheral structures was first established in the early 20th Century by British physiologists, Davies, Haldane and Priestley (Davies et al., 1919). Upon excessive respiratory resistance, the authors made the observation that breathing becomes shallow and more frequent, leading to inadequate alveolar ventilation and the clinical appearance of anoxaemia and hypercapnia. It was not until 60 years thereafter, that a series of experiments demonstrated the relationship between respiratory muscle work/demand and capacity. It was theorised that when the work required to breathe exceeds the capacity of the respiratory muscles to perform that work, fatigue will ensue (Roussos & Macklem, 1977; Bellemare & Grassino, 1982a, b).

Diaphragmatic fatigue (DF) is associated with a sympathetically mediated metaboreflex. As the diaphragm contracts more forcefully, mechanically (thinly myelinated group III) and metabolically (unmyelinated group IV) sensitive thin-fibre phrenic afferents are stimulated (Hussain et al., 1990; Jammes & Balzamo, 1992; Haouzi et al., 1999; Hill, 2000; Rodman et al., 2003), causing a time-dependent increase in muscle sympathetic nerve activity, heart rate, mean arterial blood pressure (St. Croix et al., 2000), limb vascular resistance and a concurrent reduction in limb blood flow (Sheel et al., 2001). In the rodent, diaphragm arterioles are less sensitive to α-1 adrenergic vasoconstriction than the gastrocnemius muscle (Aaker & Laughlin, 2002a). Theoretically, a global increase in sympathetic outflow would result in a preferential redistribution
of blood flow away from the periphery and directed towards the respiratory muscles (Harms et al., 1997; Dominelli et al., 2017a). Thus, a major consequence of DF may be considered cardiovascular in nature.

Much of the aforementioned research was conducted in animal models or men exclusively. Whilst studies investigating sex-based differences in DF are few, there is a growing body of evidence to suggest there are important distinctions. For example, Guenette et al. (2010) found the magnitude of exercise-induced DF to be significantly less in women than men. Recently, Smith et al. (2016) asked whether or not female resistance to DF was accompanied by an attenuation of the inspiratory muscle metaboreflex. Femoral artery blood flow ($Q_L$) and limb vascular resistance (LVR) were assessed during inspiratory resistive breathing at rest until mean arterial pressure (MAP) reached a plateau. The authors found less of an increase in MAP (7 vs. 11 mmHg) and LVR (17\% vs. 47\%), as well as less of a decrease in $Q_L$ (7\% vs. 23\%) in women compared to men. Crucially, Smith and colleagues (2016) did not assess DF, nor did they report any indices of respiratory muscle force output during the loading task. Hence, it is unclear if the differences observed were due to women experiencing less fatigue, performing less work, a combination of both, or other undetermined mechanisms.

To this end, the aims of the study were: 1) to determine if there are sex differences in DF when the work of breathing is increased at rest, and 2) to monitor the cardiovascular responses of men and women to imposed inspiratory resistance. We hypothesised that women would be more resistant to DF following inspiratory pressure-threshold loading (PTL) to task failure than men. By virtue of this fatigue resistance, we further hypothesised that women would experience a
blunted sympathetic response of the cardiovascular system, leading to an attenuation of the inspiratory muscle metaboreflex.

4.2 Methods

4.2.1 Subjects and Ethical Approval

Eighteen subjects (nine men and nine women) were recruited for the study. Subjects were young, healthy (normal height and body mass, no history of smoking or presence of any known cardiovascular, neurological or pulmonary disease) and recreationally active. Written informed consent was obtained from all subjects prior to testing. Women were tested during the early follicular phase of the menstrual cycle (determined by self-report) to minimise any potential effect of circulating sex hormones on autonomic function. Experimental procedures were approved by the Clinical Research Ethics Board at the University of British Columbia (approval number: H15-00801) and conformed to the Declaration of Helsinki.

4.2.2 Experimental Design

Familiarisation sessions were conducted prior to the PTL trial. Subjects were afforded sufficient time to become accustomed to the breathing apparatus. Once subjects could achieve 2-3 min of uninterrupted PTL they were deemed adequately prepared. On this general familiarisation visit (which included a maximal incremental exercise test), anthropometrics were taken and basic spirometry (Spirolab II, Medical International Research; Rome, Italy) performed according to established guidelines (ATS/ERS, 2002). At least 24 h separated the familiarisation visit and PTL trial.

During the experimental PTL trial, subjects completed a single bout of constant load isocapnic PTL to task failure. Diaphragm contractility was assessed before and immediately after
the loading protocol by measuring transdiaphragmatic twitch pressure ($P_{di,tw}$) in response to cervical magnetic stimulation. Force development across the muscle was estimated by calculating the difference in gastric ($P_{ga}$) and oesophageal ($P_{oes}$) pressure with the use of balloon-tipped catheters (no. 47-9005, Ackrad Laboratory; Cranford, NJ, USA). Topical anaesthetic (2% lidocaine hydrochloride, AstraZeneca; Mississauga, ON, Canada) was applied to the nasal and pharyngeal passages to minimise discomfort during catheter insertion. Catheters were directed intranasally and positioned in the stomach and lower one-third of the oesophagus to measure $P_{ga}$ and $P_{oes}$, respectively. Each catheter was connected to a piezoelectric pressure transducer (Raytech Instruments; Vancouver, BC, Canada), which was independently calibrated using a digital pressure manometer (2021P, Digitron; Torquay, UK). Subjects were asked to perform a Valsalva manoeuvre in order to evacuate air from the balloons. Oesophageal and gastric balloons were then filled with 1 and 2 ml of air, respectively. Correct placement was confirmed using the occlusion technique. Phrenic nerve activation was determined by inspection of the compound muscle action potential (CMAP, or M-wave) using surface recordings of the diaphragm electromyogram (EMG). Continuous beat-by-beat arterial blood pressure was taken throughout PTL via finger pulse photoplethysmography for measurement of cardiovascular responses.

4.2.3 Inspiratory Pressure-Threshold Loading

Pressure-threshold loading is an indirect means of evoking DF without the confounding effects of whole-body exercise (e.g. acidosis). A bespoke PTL device was used for the experiment. The design was based on the weighted plunger model described by Nickerson and Keens (1982). In brief, subjects were required to generate an inspiratory pressure sufficient to overcome a threshold load in order to initiate inspiration – expiration was unimpeded. Unlike flow-resistive loading,
PTL allows greater control of pressure/force production, as flow is pressure dependent until the threshold pressure is generated. Once pressure degrades below the threshold required to lift the weighted plunger, flow stops entirely.

Subjects were seated comfortably in the upright position. A customised two-way non-rebreathing valve was connected to the PTL device on the inspired side and a pneumotachograph (no. 3813, Hans Rudolph; Kansas City, MO, USA) for measurement of flow and volume on the expired side. Resistance was added to the weighted plunger such that inspiratory pressure was equal to 60% of predetermined maximal inspiratory mouth pressure (MIP). A minimum of five Mueller manoeuvres were performed from residual volume with the average of the three highest values defined as the MIP. A metronome was used to control breathing pattern. Breathing frequency \( (f_b) \) was set to 15 breaths \( \cdot \text{min}^{-1} \) and inspiratory duty cycle (i.e. ratio of inspiratory contraction time to total respiratory cycle duration \( [T_i/T_{TOT}] \)) at 0.7. Target inspiratory pressure was displayed on a computer screen to provide continuous visual feedback. Subjects were instructed to breathe diaphragmatically as natural breathing strategies during resistive inspirations can preferentially target accessory inspiratory muscles (Ramsook et al., 2016). The task was terminated when subjects failed to generate the target inspiratory pressure for four consecutive breaths or the second occasion of three missed consecutive breaths despite verbal encouragement.

Mouth pressure \( (P_m) \) and end-tidal partial pressure of CO\(_2\) \( (P_{ETCO_2}) \) were measured via a side-port in the mouthpiece, which was connected to a piezoelectric pressure transducer (Raytech Instruments; Vancouver, BC, Canada) and CO\(_2\) gas analyser (no. 17630, VacuMed; Ventura, CA, USA), respectively. Manual adjustments to the inspired fraction of CO\(_2\) were made in the event of hypocapnia \( (P_{ETCO_2} < 30 \text{ mmHg}) \) using a 7% CO\(_2\) gas mixture. Prior to commencement of the
PTL trial, 5-10 min of resting cardiorespiratory data were collected. Cardiovascular responses were measured throughout PTL and diaphragm contractile function was assessed before the trial began and immediately after task failure.

4.2.4 **Cervical Magnetic Stimulation**

A handheld 90 mm circular coil (P/N 9784-00; peak magnetic field strength = 2.0 T, average inductance = 23.3 µH) powered by a magnetic stimulator (200-2, Magstim; Wales, UK) was used to stimulate the phrenic nerve roots at the level of the third-seventh cervical vertebrae, according to the original technique described by Similowski *et al.* (1989). The coil was held horizontal and centred over the midline of the cervical spine with the current flowing in a clockwise direction. Subjects were seated comfortably with the neck flexed. Stimuli were delivered at end-expiration (determined by end-expiratory *P* _oes_) with the glottis closed to preclude lung volume influence on diaphragm EMG and twitch pressure responses. The optimal site of stimulation was identified by gradually moving the coil along cervical vertebrae C₃-C₇ until the largest twitch pressure was observed. This location was marked and used for all subsequent stimuli.

Phrenic nerve excitability was determined by gradually increasing the intensity of the stimulator output from 60 to 100%. Three twitches were delivered at each intensity with a 30 s interval between successive stimuli to minimise the effect of twitch potentiation. A series of 1 Hz potentiated (preceded by ~5 s maximal inspiratory effort [i.e. Mueller manoeuvre]) twitches were performed at 100% of peak stimulator output at baseline and immediately after PTL to determine the presence of DF. Skeletal muscle fatigue may be defined as “a condition in which there is a loss in the capacity for developing force and/or velocity of a muscle, resulting from muscle activity under load and which is reversible by rest” (NHLBI, 1990). Fatigue of the inspiratory muscles was
assumed present if there was a $\geq 15\%$ reduction in $P_{di,tw}$ relative to resting baseline levels. Twitches were excluded for the following reasons: 1) the twitch was not initiated at functional residual capacity, 2) if oesophageal peristalsis was evident at the time of the twitch, 3) if a cardiac artefact was superimposed upon a stimulus, and 4) if there was lack of diaphragmatic relaxation evidenced by noticeable diaphragm EMG. An example of the twitch potentiation protocol is provided in Figure 4.1.

4.2.5 Electromyography

To record electrical activity of the left and right costal diaphragm, self-adhesive surface Ag/AgCl electrodes (H59P, Kendall; Mansfield, MA, USA) were placed on the chest wall in bipolar arrangement (~2 cm apart) between the sixth and eighth intercostal spaces along the anterior-axillary line. The ground electrode was placed on the acromion process of the scapula. The skin was lightly abraded and cleansed with alcohol to minimise electrical impedance. Electrodes were further secured using surgical tape and repositioned if necessary. Signals were amplified (x 200) and band-pass filtered (0.1 Hz to 3 kHz; P511 Series, Grass Instruments; Warwick, RI, USA). M-waves were analysed for: onset latency, duration, peak-to-peak amplitude and total rectified area (see below for analysis details).
Figure 4.1: Twitch Potentiation Protocol
Pressure (mouth, oesophageal, gastric and transdiaphragmatic) and EMG (left and right hemi-diaphragm) responses during maximal inspiratory manoeuvres separated by magnetic stimulation (1 Hz) of the phrenic nerve roots. Abbreviations: $P_m =$ mouth pressure; $P_{oes} =$ oesophageal pressure; $P_{ga} =$ gastric pressure; $P_{di} =$ transdiaphragmatic pressure; LHD = left hemi-diaphragm, RHD = right hemi-diaphragm.
4.2.6 Cardiovascular Variables

Heart rate (HR) and arterial blood pressure were measured beat-by-beat using finger pulse photoplethysmography (Finometer, Finapres Medical Systems BV; Arnhem, Netherlands). An automated sphygmomanometer (BPM-100, VSM MedTech Ltd; Vancouver, BC, Canada) was used to calibrate the Finometer during resting conditions. Physiological calibrations were made frequently during inspiratory loading (approximately once per minute). Power-frequency spectrum analysis of the raw blood pressure trace was used to calculate low-frequency systolic blood pressure variability (LF_{SBP}) over the PTL bout. The first two minutes of loading were not included. Low-frequency oscillations (around 0.1 Hz) in arterial blood pressure (i.e. Meyer waves) are believed to reflect efferent sympathetic nerve activity indicative of changes in vasomotor tone (Malliani et al., 1991).

4.2.7 Data Analysis

Transdiaphragmatic twitch pressure was determined as the change in pressure from stimulus onset to peak pressure. The ratio of twitch \( P_{oes} \) to twitch \( P_{ga} (P_{oes, tw}/P_{ga, tw}) \) was used to discriminate diaphragm and ribcage muscle fatigue. Cumulative force output of the diaphragm (i.e. pressure-time product) was calculated by integrating \( P_{di} \) down to end-inspiratory pressure over the periods of inspiratory flow for the entire duration of the PTL trial. Pressure was maintained in approximate square-wave fashion during each inspiratory effort, such that \( P_{di} \) was equal to mean \( P_{di} (\bar{P}_{di}) \). The highest \( P_{di} \) generated throughout loading or during Mueller manoeuvres was defined as \( P_{di, max} \). Diaphragm tension-time index (TTI_{di}) was calculated for each minute of PTL as the product of \( \bar{P}_{di}/P_{di, max} \) and \( T/T_{TOT} \). M-wave characteristics (latency, duration, amplitude, and area) were calculated in MATLAB (R2015a, MathWorks; Natick, MA, USA) using a custom algorithm
Cardiorespiratory data were averaged over 30-60 s throughout threshold loading. Systolic peaks underwent fast Fourier transformation to produce a spectrum; the power of the systolic peaks was calculated by measuring the area under the spectra curve. Low-frequency components of the resulting power spectrum ranged from 0.04-0.15 Hz. Flow, pressure and EMG signals were amplified, A/D converted (PowerLab 16SP, AD Instruments; Colorado Springs, CO, USA), sampled at 10 kHz, and monitored online using LabChart data acquisition software (v8.1, AD Instruments; Colorado Springs, CO, USA).

4.2.8 Statistics

Descriptive characteristics were compared using independent-samples t-tests. Repeated measures ANOVA was used to determine if the phrenic nerves were maximally activated by comparing $P_{di,tw}$ at all intensities of stimulation (60, 70, 80, 90, 95%) with the maximal stimulator output (100%). If there were significant main effects, pairwise comparisons were made using Tukey’s post hoc test. On an individual basis, a plateau was considered present if the average $P_{di,tw}$ at submaximal and maximal stimulation intensities was separated by equal to or less than the within block coefficient of variation for all twitches. Diaphragmatic fatigue ($P_{di,tw}$) was tested by independent samples t-test post-PTL. Sex-based differences in cardiovascular responses and DF were assessed using a two-way mixed factorial ANOVA. Time to task failure was compared using independent-samples t-tests. M-wave characteristics were compared at baseline and post-PTL by repeated measures ANOVA. For all statistical tests, normality was assessed qualitatively by visually inspecting descriptive statistics, histograms, and Q-Q plots and quantitatively using the Shapiro-Wilk test for small samples. An ANOVA on ranks was used in the event of failed
normality. Significance was set at $p < 0.05$ for all statistical comparisons (SigmaPlot v12, Systat Software Inc.; San Jose, CA, USA). Results are expressed as mean ± SD, unless otherwise stated.

4.3 Results

4.3.1 Subjects

Subject characteristics, including anthropometrics and spirometry are presented in Table 4.1. Groups were of similar age and spirometry was within normal limits based upon predictive equations (Tan et al., 2011). Men had significantly greater maximal static inspiratory pressures than women ($p = 0.011$), but no differences were observed ($p = 0.230$) when expressed as percentages of predicted values (Black & Hyatt, 1969).

4.3.2 Diaphragmatic Neuromuscular Function

A plateau in $P_{di,\text{tw}}$ was observed at 80, 90 and 95% of peak stimulator output in both men and women (Figure 4.2). Upon inspection of individual data, clear evidence of a plateau existed in 83% of all subjects (eight of nine men and seven of nine women). Coefficients of variation between subsequent stimuli were 6.8% for men and 6.6% for women. There was no change in M-wave characteristics (spatial or temporal) pre-post threshold loading in men or women.

Baseline twitch pressures were 38.0 ± 8.2 and 35.9 ± 7.2 cmH$_2$O for men and women, respectively. At task failure, twitch pressures were significantly reduced in both sexes and to a similar extent (M = 28.9 ± 8.0 cmH$_2$O [-24.3%], W = 27.8 ± 6.3 cmH$_2$O [-22.8%]; $p = 0.328$) (see Figure 4.3). There were no sex differences in delta $P_{oes,\text{tw}}/P_{ga,\text{tw}}$ post-PTL compared to baseline (M = -0.63 ± 0.23, W = 0.02 ± 0.36; $p = 0.355$). Predominant diaphragm fatigue (increase delta $P_{oes,\text{tw}}/P_{ga,\text{tw}}$) was found in four men and six women (Figure 4.4).
Time to task failure was significantly longer (27.3 ± 11.2 vs. 15.6 ± 10.5 min; \( p = 0.018 \)) and consequently, the rate of fatigue development slower in women than men (M = -0.69 ± 0.30 cmH\(_2\)O·min\(^{-1}\), W = -0.34 ± 0.16 cmH\(_2\)O·min\(^{-1}\); \( p = 0.009 \)). A main effect of time (\( p < 0.001 \)) and sex (\( p = 0.001 \)) was found for cumulative diaphragm force output (M = 32,288 ± 20,752 cmH\(_2\)O·s, W = 46,805 ± 16,723 cmH\(_2\)O·s) (Figure 4.5). Of note, at the point of task failure, two subjects (one male and one female) did not reach our definition of fatigue (i.e. \( \geq 15\% \) reduction in \( P_{di,\text{tw}} \) post-PTL).

Table 4.1: Subject Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Men (( n = 9 ))</th>
<th>Women (( n = 9 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>24 ± 3</td>
<td>24 ± 3</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>181 ± 8</td>
<td>170 ± 8*</td>
</tr>
<tr>
<td>Mass (kg)</td>
<td>76 ± 11</td>
<td>62 ± 9*</td>
</tr>
<tr>
<td>FVC (l)</td>
<td>6.1 ± 1.1</td>
<td>4.4 ± 1.1*</td>
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<tr>
<td>(% predicted)</td>
<td>113 ± 12</td>
<td>111 ± 17</td>
</tr>
<tr>
<td>FEV(_1) (l·s(^{-1}))</td>
<td>4.8 ± 0.6</td>
<td>3.8 ± 0.9*</td>
</tr>
<tr>
<td>(% predicted)</td>
<td>106 ± 7</td>
<td>108 ± 16</td>
</tr>
<tr>
<td>FEV(_1)/FVC (% predicted)</td>
<td>80 ± 6</td>
<td>85 ± 6</td>
</tr>
<tr>
<td>PEF (l·s(^{-1}))</td>
<td>10.8 ± 1.1</td>
<td>8.1 ± 1.4*</td>
</tr>
<tr>
<td>(% predicted)</td>
<td>106 ± 14</td>
<td>107 ± 18</td>
</tr>
<tr>
<td>FEF(_{25,75}) (l·s(^{-1}))</td>
<td>4.3 ± 0.8</td>
<td>4.2 ± 1.4</td>
</tr>
<tr>
<td>(% predicted)</td>
<td>84 ± 15</td>
<td>97 ± 29</td>
</tr>
<tr>
<td>MIP (cmH(_2)O)</td>
<td>-138 ± 32</td>
<td>-100 ± 23*</td>
</tr>
<tr>
<td>(% predicted)</td>
<td>108 ± 22</td>
<td>123 ± 27</td>
</tr>
</tbody>
</table>

Abbreviations: FVC = forced vital capacity; FEV\(_1\) = forced expired volume in one second; PEF = peak expiratory flow; FEF\(_{25,75}\) = forced expiratory flow at 25-75\% of FVC; MIP = maximal inspiratory mouth pressure. * Significantly different from men (\( p < 0.05 \)).
Figure 4.2: Recruitment Curves
Bar graph depicting mean $P_{di, tw}$ ± SEM as a percentage of peak $P_{di, tw}$ in response to gradually increasing intensities of phrenic nerve stimulation. A plateau was observed at 80, 90 and 95% of peak power output in men and women. Group mean data is shown in panel A, individual curves for men and women are displayed in panels B and C, respectively. Abbreviations: $P_{di, tw}$ = transdiaphragatic twitch pressure. * Significantly different from 100% ($p < 0.05$).
4.3.3 Respiratory Responses to PTL

The PTL protocol was identical for men and women, equating to a target TTI of 0.42. Parameters used to control breathing pattern were not different between sexes (\( p > 0.05 \)) and were well maintained throughout the trial, this includes: \( f_b \), \( T_i/T_{TOT} \), target \( P_m \), and TTI\(_{di} \) (Table 4.2). In the final minute, pressure generation began to decay, resulting in a small decrease in TTI\(_{di} \). Oesophageal (PTP\(_{oes} \)) and transdiaphragmatic (PTP\(_{di} \)) pressure-time products are shown in Figure 4.5. Diaphragm contribution to total respiratory muscle pressure production (i.e. PTP\(_{di} \)/PTP\(_{oes} \)) did not change during the loading protocol in men nor women (\( p > 0.05 \));
nevertheless, PTP_\text{di}/PTP_\text{oex} was greater in women throughout (p = 0.006). There were no sex differences in PTP_\text{di} relative to body mass (p = 0.675).

4.3.4 Cardiovascular Responses to PTL

An illustration of raw cardiorespiratory traces collected during PTL is provided in Figure 4.6. There was a time-dependent increase in HR and MAP until the final minute of the trial, whereby small decreases in both variables were observed (Figure 4.7). A main effect of time was found for all variables, including: HR (p < 0.001), SBP (p < 0.001), DBP (p < 0.001) and MAP (p < 0.001). At the time of task failure, HR (M = +19 ± 12 bpm, W = +13 ± 8 bpm), SBP (M = +22 ± 12 mmHg, W = +16 ± 17 mmHg), DBP (M = +10 ± 8 mmHg, W = +7 ± 4 mmHg) and MAP (M = +14 ± 9 mmHg, W = +10 ± 8 mmHg) were well above resting levels. A main effect of sex was found for HR (p = 0.009), SBP (p = 0.019) and MAP (p = 0.024), but not DBP (p = 0.095). No significant interactions existed for any dependent variable (p > 0.05). Post hoc tests did not reveal any statistically significant differences in HR, SBP or MAP at 20 (p = 0.65, 0.49 and 0.57, respectively), 40 (p = 0.19, 0.18 and 0.18, respectively), 60 (p = 0.10, 0.14 and 0.14, respectively), 80 (p = 0.08, 0.14 and 0.14, respectively) or 100% (p = 0.18, 0.42 and 0.46, respectively) of time to task failure. Finally, LF_{SBP} was significantly lower in women compared to men (23.2 ± 11.1 vs. 33.9 ± 7.7 mmHg^2; p = 0.038).
Figure 4.4: Relative Contributions of the Diaphragm and Ribcage Muscles to Changes in Transdiaphragmatic Twitch Pressure

Relative Contributions of the Diaphragm and Ribcage Muscles to Changes in Transdiaphragmatic Twitch Pressure. Box and whisker plot showing the ratio of $P_{oes,tw}$ to $P_{ga,tw}$ in men and women at baseline and pressure-threshold loading task failure. An increase in $P_{oes,tw}/P_{ga,tw}$ following PTL indicates predominant diaphragmatic fatigue. No sex differences were observed in delta $P_{oes,tw}/P_{ga,tw}$ ($p > 0.05$).
Figure 4.5: Pressure-Time Product and Cumulative Diaphragmatic Work
Panels A and B represent PTP\textsubscript{oes} and PTP\textsubscript{di} in absolute units of pressure and time. Panel C represents the ratio of PTP\textsubscript{di} to PTP\textsubscript{oes} during threshold breathing. Men generated significantly greater absolute pressure; however, PTP\textsubscript{di}/PTP\textsubscript{oes} was greater in women than men throughout. 

Abbreviations: PTP = pressure-time product. * Main effect of time; † main effect of sex; ‡ main effect of sex on PTP\textsubscript{oes} and PTP\textsubscript{di}. 
Figure 4.6: Raw Cardiorespiratory Variables from One Representative Subject during Pressure-Threshold Loading

Raw Traces of Cardiorespiratory Variables from One Representative Subject during Pressure-Threshold Loading. A time-dependent increase in HR and MAP was observed. Isocapnia was maintained throughout as shown by end-tidal PCO₂. Approximate square-wave pressure generation was achieved until the final minute of loading. Abbreviations: HR = heart rate; AP = arterial pressure; PCO₂ = partial pressure of CO₂; Pₘ = mouth pressure; Pₐᵩ = transdiaphragmatic pressure.
Figure 4.7: Cardiovascular Responses to Pressure-Threshold Loading

Cardiovascular Responses to Pressure-Threshold Loading. Heart rate and blood pressure responses are reported as group mean ± SEM. Panels A-D represent relative time (% of time to task failure) during a bout of inspiratory pressure-threshold loading to task failure and panels E-H are the corresponding results in absolute time (minutes). A main effect of time was found for all variables and a main effect of sex for HR, SBP and MAP. Post hoc tests revealed no significant differences between men and women for any dependent variable at any time point. Abbreviations: HR = heart rate; MAP = mean arterial pressure; SBP = systolic blood pressure; DBP = diastolic blood pressure. * Main effect of time; † main effect of sex.
Table 4.2: Physiological Responses to Pressure-Threshold Loading

Men

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>Final</th>
</tr>
</thead>
<tbody>
<tr>
<td>$f_b$ (breaths·min$^{-1}$)</td>
<td>10 ± 3</td>
<td>15 ± 0.1*</td>
<td>15 ± 0.4*</td>
<td>15 ± 0.2*</td>
<td>15 ± 0.8*</td>
<td>14 ± 2*</td>
<td></td>
</tr>
<tr>
<td>$V_T$ (l)</td>
<td>1.3 ± 0.3</td>
<td>1.6 ± 0.7</td>
<td>1.3 ± 0.4</td>
<td>1.5 ± 0.6</td>
<td>1.4 ± 0.6</td>
<td>1.5 ± 0.6</td>
<td>1.7 ± 0.6</td>
</tr>
<tr>
<td>$V_T/T_I$ (l·s$^{-1}$)</td>
<td>0.69 ± 0.23</td>
<td>0.48 ± 0.20</td>
<td>0.42 ± 0.12</td>
<td>0.46 ± 0.20</td>
<td>0.45 ± 0.20</td>
<td>0.45 ± 0.19</td>
<td>0.52 ± 0.21</td>
</tr>
<tr>
<td>$V_E$ (l·min$^{-1}$)</td>
<td>14 ± 4</td>
<td>24 ± 9*</td>
<td>20 ± 6*</td>
<td>23 ± 9*</td>
<td>22 ± 8*</td>
<td>22 ± 9*</td>
<td>24 ± 9*</td>
</tr>
<tr>
<td>$P_{ETCO_2}$ (mmHg)</td>
<td>38 ± 5</td>
<td>32 ± 4*</td>
<td>35 ± 3</td>
<td>37 ± 5†</td>
<td>37 ± 4†</td>
<td>38 ± 4†</td>
<td>38 ± 3†</td>
</tr>
<tr>
<td>HR (beats·min$^{-1}$)</td>
<td>59 ± 8</td>
<td>72 ± 8*</td>
<td>71 ± 7*</td>
<td>72 ± 9*</td>
<td>73 ± 15*</td>
<td>74 ± 11*</td>
<td>79 ± 9*†</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>90 ± 5</td>
<td>93 ± 13</td>
<td>97 ± 14</td>
<td>99 ± 14</td>
<td>100 ± 18</td>
<td>101 ± 16*</td>
<td>104 ± 12†</td>
</tr>
<tr>
<td>$P_m$ (cmH$_2$O)</td>
<td>-1.1 ± 0.4</td>
<td>-82 ± 16*</td>
<td>-80 ± 16*</td>
<td>-81 ± 18*</td>
<td>-81 ± 17*</td>
<td>-82 ± 17*</td>
<td>-81 ± 17*</td>
</tr>
<tr>
<td>$P_{di}/P_{di,max}$ (%)</td>
<td>8 ± 3</td>
<td>58 ± 6*</td>
<td>56 ± 9*</td>
<td>59 ± 10*</td>
<td>60 ± 7*</td>
<td>59 ± 6*</td>
<td>53 ± 9*</td>
</tr>
<tr>
<td>$T_i/T_{TOT}$</td>
<td>0.34 ± 0.03</td>
<td>0.72 ± 0.02*</td>
<td>0.72 ± 0.01*</td>
<td>0.69 ± 0.02*</td>
<td>0.70 ± 0.01*</td>
<td>0.70 ± 0.02*</td>
<td>0.70 ± 0.03*</td>
</tr>
<tr>
<td>TTI$_{di}$</td>
<td>0.03 ± 0.01</td>
<td>0.42 ± 0.05*</td>
<td>0.40 ± 0.06*</td>
<td>0.41 ± 0.07*</td>
<td>0.42 ± 0.05*</td>
<td>0.41 ± 0.05*</td>
<td>0.37 ± 0.07†</td>
</tr>
</tbody>
</table>

Breathing pattern, heart rate, mean arterial pressure and respiratory muscle pressure generation during PTL. Values are presented as mean ± SD. Abbreviations: $f_b$ = breathing frequency; $V_T$ = tidal volume; $V_T/T_I$ = mean inspiratory flow rate; $V_E$ = minute ventilation; $P_{ETCO_2}$ = end-tidal partial pressure of CO$_2$; HR = heart rate; MAP = mean arterial pressure; $P_m$ = mouth pressure; $P_{di}$ = transdiaphragmatic pressure; $T_i/T_{TOT}$ = inspiratory duty cycle; TTI$_{di}$ = tension-time index of the diaphragm. * Significantly different from rest; † significantly different from first minute; ‡ significantly different from men ($p < 0.05$).
Table 4.2: Physiological Responses to Pressure-Threshold Loading
Women

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>Time to Task Failure (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>(f_b) (breaths·min(^{-1}))</td>
<td>14 ± 2‡</td>
<td>15 ± 0.1</td>
</tr>
<tr>
<td>(V_T) (l)</td>
<td>0.8 ± 0.3‡</td>
<td>1.2 ± 0.4*</td>
</tr>
<tr>
<td>(V_T/T_I) (l·s(^{-1}))</td>
<td>0.50 ± 0.15</td>
<td>0.41 ± 0.18</td>
</tr>
<tr>
<td>(V_e) (l·min(^{-1}))</td>
<td>12 ± 5</td>
<td>18 ± 6</td>
</tr>
<tr>
<td>(P_{ETCO_2}) (mmHg)</td>
<td>38 ± 3</td>
<td>32 ± 5*</td>
</tr>
<tr>
<td>HR (beats·min(^{-1}))</td>
<td>65 ± 13</td>
<td>73 ± 15</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>86 ± 5‡</td>
<td>87 ± 9</td>
</tr>
<tr>
<td>(P_m) (cmH(_2)O)</td>
<td>-0.9 ± 0.2</td>
<td>-63 ± 10‡†</td>
</tr>
<tr>
<td>(P_{di}/P_{di,max}) (%)</td>
<td>6 ± 3</td>
<td>54 ± 7*</td>
</tr>
<tr>
<td>(T_I/T_{TOT})</td>
<td>0.37 ± 0.05</td>
<td>0.71 ± 0.02*</td>
</tr>
<tr>
<td>TTI(_{di})</td>
<td>0.02 ± 0.01</td>
<td>0.38 ± 0.05*</td>
</tr>
</tbody>
</table>

Breathing pattern, heart rate, mean arterial pressure and respiratory muscle pressure generation during PTL. Values are presented as mean ± SD. **Abbreviations:** \(f_b\) = breathing frequency; \(V_T\) = tidal volume; \(V_T/T_I\) = mean inspiratory flow rate; \(V_e\) = minute ventilation; \(P_{ETCO_2}\) = end-tidal partial pressure of CO\(_2\); HR = heart rate; MAP = mean arterial pressure; \(P_m\) = mouth pressure; \(P_{di}\) = transdiaphragmatic pressure; \(T_I/T_{TOT}\) = inspiratory duty cycle; TTI\(_{di}\) = tension-time index of the diaphragm. * Significantly different from rest; † significantly different from first minute; ‡ significantly different from men (\(p < 0.05\)).
4.4 Discussion

4.4.1 Main Findings

The main findings of the present study are three-fold. Firstly, inspiratory muscle endurance time was significantly longer in women relative to men. Secondly, the severity of PTL-induced DF did not differ between sexes at the point of task failure. Finally, notwithstanding a similar degree of DF, women experienced a blunted cardiovascular response to inspiratory resistance, emphasised by less of an increase in HR and MAP, and significantly lower LF$_{SBP}$. This is the first study to demonstrate an attenuation of the inspiratory muscle metaboreflex in women subjected to the same reflex-evoking stimulus as men (i.e. degree of DF). We interpret our collective findings to mean that sex-based differences in diaphragmatic function have important implications concerning sympathetic vasomotor outflow that may affect limb and respiratory muscle haemodynamics.

4.4.2 Task Failure

On average, women were able to perform inspiratory PTL for 75% longer than men before reaching task failure. Only two previous studies have compared inspiratory muscle endurance time between men and women. Using flow-resistive loads (70% MIP, 18 breaths·min$^{-1}$, 0.5 duty cycle; calculated TTI = 0.35), Gonzales and Scheuermann (2006) found no significant sex-based difference in time to task failure despite women on average lasting 13% longer (M = 12.3 min, W = 14.1 min). Similarly, Shimizu et al. (2017) did not observe a sex-based difference in respiratory muscle endurance time (M = 12.1 min, W = 11.6 min) with an incremental hyperpnoea challenge (30% of maximal voluntary ventilation for 3 min plus 10% every 3 min until task failure). These observations are in contrast to findings presented in the current study. While the incremental protocol employed by Shimizu and colleagues likely nullified any differences between groups in
endurance time, it is unclear why our results vary from that of Gonzalez and Scheuermann. Interestingly, the authors point out that the absence of a sex-based difference in time to task failure was surprising given what is known about sex differences in muscle fatigue and task specificity.

During intermittent isometric contractions (similar to the present study) of various muscle groups (including: elbow and finger flexors, thumb adductors, and ankle dorsiflexors), women on average, are able to sustain the task for 33% longer than men (Hunter, 2009), regardless of the absolute strength exerted by the muscle (Hunter et al., 2004). Our results are the first to demonstrate a sex-based difference in endurance time of the human inspiratory muscles. However, task failure has been shown to occur during inspiratory resistive breathing without the presence of DF (McKenzie et al., 1997).

Two of our subjects did not display peripheral DF at the time of task failure. Hence, alternative mechanisms that have been proposed include: hypo/hypercapnia, central fatigue, and/or breathing discomfort. None of our subjects became hypercapnic during loading and \( P_{ET}CO_2 \) was maintained within 3-4 mmHg of resting values. Dyspnoea, on the other hand, was not measured in the current study. Women breathed with a higher diaphragmatic contribution to total inspiratory muscle force output (i.e. the ratio of \( PTP_{di} \) to \( PTP_{oes} \), see Figure 5) in relation to men. Thus, it is possible that by generating pressure primarily through diaphragm activation and not synergist inspiratory muscles, the disassociation between mechanical input and central respiratory motor output was minimised. As a result, women were able to sustain the task for longer without experiencing extreme dyspnoea. This notion remains speculative and warrants further enquiry. Although beyond the scope of the present investigation, it is posited that the relative influence of central and peripheral factors to task failure are contingent upon exercise intensity, as denoted by
the power-duration relationship (Burnley & Jones, 2018). Anticipatory feedforward regulation of skeletal muscle recruitment to ensure organism homeostasis (St Clair Gibson & Noakes, 2004), attainment of a ‘sensory tolerance limit’ modulated by peripheral feedback (Amann et al., 2011), and psychobiological constructs such as perception of effort and potential motivation (Marcora & Staiano, 2010), are all believed to play a role in the aetiology of task failure.

4.4.3 Diaphragmatic Fatigue

At the cessation of PTL, the magnitude of DF in men and women was within 2% of each other. Thus, despite similar reductions in diaphragm force output, women were able to sustain the task for a longer duration. As a result, women produced greater cumulative diaphragmatic pressure during the loading task. Depending on the time course of fatigue development (linear or exponential decay), it is possible that the rate of fatigue development was slower in women than men. In this regard, Laghi et al. (1996) found the diaphragm to progressively fatigue during mechanical loading in healthy young men. To our knowledge, only one study has previously compared sex-based differences in DF during a resistive breathing protocol at rest. In accordance with our results, Gonzales and Scheuermann (2006) did not observe any differences between men and women at the time of task failure in the severity of DF (M = 16.9%, W = 14.8%), albeit using volitional techniques (i.e. MIP). Moreover, the authors found that women fatigued at slower rates compared to men (-1.5 cmH₂O·min⁻¹ vs. -2.9 cmH₂O·min⁻¹). We speculate that the reason for parallel reductions in diaphragm contractility between sexes in the current study was because the task was terminated before fatigue reached a so-called ‘critical threshold’ (Amann et al., 2011).

Fatigue may occur anywhere along the brain-muscle pathway. Stimulation of the phrenic nerve roots using single 1 Hz stimuli allows for the assessment of low-frequency peripheral DF.
A reduction in muscle force output without any concurrent alteration in the M-wave configuration implies disruption to the excitation-contraction coupling mechanism. The accumulation of metabolites within skeletal muscle inhibits the binding of calcium with troponin-tropomyosin, preventing the formation of actin-myosin crossbridges (Edwards et al., 1977). End-organ fatigue (i.e. at the level of the diaphragm) was present in all but two subjects. Although we must reject our first hypothesis, that the magnitude of fatigue was not less in women compared to men, the rate of fatigue development was slower in women. Intracellular changes within the muscle are not independent of the central nervous system in vivo (Kent-Braun et al., 2012). The absence of central measures in the present study prohibits our ability to comment on sex-differences in neural activation. Several mechanisms have been put forth to explain female resistance to skeletal muscle fatigue, in particular, differences in muscle morphology, substrate utilisation and sex hormones (Hicks et al., 2001; Hunter, 2014).

Women have less muscle mass than men and this alone may provide some insight into the greater fatigue resistance observed in women. When performing work at the same relative intensity, smaller muscle mass translates directly into lower absolute force generation. Consequently, there is less O$_2$ demand, a decrease in mechanical compression of the local vasculature and less intramuscular occlusion of blood flow, which averts the accumulation of metabolites that interfere with contractile machinery (Russ & Kent-Braun, 2003). The diaphragm itself has a remarkable ability to maintain perfusion in the face of high inspiratory loads. In an animal model, blood flow to the diaphragm increased 26-fold with a 15-fold increase in the work of breathing (Robertson et al., 1977). However, blood flow to the diaphragm is impeded once the critical TTI of 0.20 is reached (Bellemare et al., 1983). It has been shown in anesthetised dogs that
combining a high diaphragm force output with a prolonged inspiratory duty cycle compromises diaphragmatic perfusion, rendering the muscle ischaemic (Buchler et al., 1985b; Bark et al., 1987). Conversely, increasing phrenic artery blood flow partially reverses DF in situ (Supinski et al., 1988). The relationship between muscle metabolic demand and delivery of blood-borne substrates is clearly a major determinant of DF. In our study, TTI\textsubscript{di} was not different between men and women at any point during PTL and far exceeded the threshold for DF to occur, ranging between 0.34 and 0.42. Nonetheless, the TTI should be considered a conceptual framework rather than a precise numerical instrument. The protocol used in the present study (targeted TTI of 0.42) may have provided a disproportionate stimulus for fatigue development in men and women and therefore, endurance time was not equal.

With regard to muscle morphological differences, there is evidence (muscle biopsy of vastus lateralis and deltoid) to suggest that women possess a greater percentage of slow-twitch type I muscle fibres than men (Nygard, 1981). Type I fibres have a slower rate of contraction and thus, a slower rate of metabolism. As a result, type I fibres fatigue at slower rates compared to glycolytic fast-twitch type II fibres. Additionally, type I muscle fibres possess a greater fraction of vasodilatory $\beta_2$-adrenergic receptors, thereby improving $O_2$ transport kinetics. Approximately 75% of diaphragm muscle fibres are oxidative (Lieberman et al., 1973). It is not known if the histochemical composition of costal or crural diaphragm regions differs between men and women. Anecdotally, in the present study, contraction time (time difference between stimulus onset and peak pressure) was slower for women than men, which may indicate a higher proportion of slow-twitch muscle fibres.
4.4.4 Inspiratory Muscle Metaboreflex

Fatiguing diaphragmatic contractions elicits increased efferent sympathetic activity, producing widespread vasoconstrictor influences via supraspinal pathways (Dempsey et al., 2006). Inspiratory PTL was used to evoke reflex effects secondary to DF. Women exhibited an attenuated cardiovascular response to PTL. The changes in HR (+13 vs. +19 bpm), SBP (+16 vs. +22 mmHg) and MAP (+10 vs. +14 mmHg) were significantly lower in women versus men. Furthermore, women had lower LF_{SBP} (23 vs. 34 mmHg^2), suggesting repressed sympathetic vascular transduction and improved conductance. Recent work indicates that the interaction between pressor and carotid baroreflex control of HR and MAP may be additive and independent of central command (Hureau et al., 2018). It remains to be elucidated if attenuation of group III/IV afferent feedback compromises cardiovascular adjustments during exercise differently in men and women.

Two previous investigations have examined sex-based differences in the cardiovascular consequences to elevated inspiratory muscle work. Smith et al. (2016) found less of an increase in HR (4 vs. 14 bpm), MAP (7.3 vs. 11.1 mmHg) and LVR (17.7% vs. 47.9%), as well as less of a decrease in \(\dot{Q}_L\) (7.5% vs. 23.3%) in women compared to men during inspiratory resistive loading (65% MIP, 20 breaths·min^−1, 0.5 duty cycle; calculated TTI = 0.33). Similarly, Shimizu et al. (2017) found the change in MAP (14.9 vs. 32.1 mmHg) to be lower in women during incremental isocapnic voluntary hyperpnoea. While both studies corroborate findings presented here, there are fundamental differences in experimental design that should be acknowledged. Firstly, DF was not assessed in either study. Furthermore, no information regarding inspiratory muscle pressure generation (including: \(P_{m}, P_{di}, \) or TTI) was provided. Inferences made are based on the premise that men and women were subjected to the same reflex-evoking stimulus. However, without any
indication of the amount of work done by the diaphragm during inspiratory loading, conclusions drawn are limited. Thus, the findings of the present study add much needed mechanistic insight into sex-differences in DF and the associated metaboreflex. In combination with the findings of Smith and Shimizu, it appears that women develop DF at slower rates than men and experience an attenuated cardiovascular response to inspiratory resistance. This is highlighted by less of an increase in MAP, LVR, and less of a reduction in $Q_L$. Sex differences in autonomic control of the circulation may assist in explaining these observations.

According to Darcy’s law, MAP is equal to the product of cardiac output ($\dot{Q}$) and total peripheral resistance (TPR). The relationship between $\dot{Q}$, TPR and MAP is dependent upon age and sex (Charkoudian et al., 2005; Hart et al., 2009; Hart et al., 2011; Hart et al., 2012). For example, the positive relationship between muscle sympathetic nerve activity (MSNA) and TPR that exists in young men does not exist in pre-menopausal women due to the sympatho-inhibitory effects of oestrogen upon the central nervous system and peripheral vasculature (Joyner et al., 2015; Barnes, 2017). Increased quantity and sensitivity of $\beta_2$-adrenergic receptors, heightened expression of endothelial nitric oxide synthase, and enhanced functional sympatholysis promote vasodilation, which offsets $\alpha$-adrenergic vasoconstriction (Hart et al., 2011; Just & DeLorey, 2017). The attenuated blood pressure response to DF shown by women in the present study may be attributed to the physiological mechanisms outlined above, which not only work to counter vasoconstrictor influences, but preserve muscle oxygenation and thus, delay the development of fatigue.
4.4.5 Technical Considerations

A primary limitation of the present study is the use of HR and MAP to imply changes in sympathetic nerve activity. Importantly, MAP is not correlated with MSNA in healthy young men or women (Narkiewicz et al., 2005; Hart et al., 2009). Therefore, a blunted blood pressure response in women may not accurately reflect changes in MSNA. A clear demonstration of the relationship between DF and MSNA is needed. Furthermore, record resting limb and respiratory muscle haemodynamics were not measured during threshold loading. Sonography and near-infrared spectroscopy coupled with fluorescent tracer dye are highly informative; however, with the addition of cervical magnetic stimulation and invasive balloon catheters, the former techniques were not utilised. Instead, power spectrum analysis of arterial waveforms (i.e. LF\textsubscript{SBP}) provided a non-invasive means of gaining insight into the peripheral vasculature.

Women generated inspiratory pressure with a greater relative contribution of the diaphragm than did men. Respiratory inductance plethysmography would have allowed a more comprehensive assessment of diaphragmatic and ribcage muscle action on lung volume displacement. In addition, prescribing a workload based upon TTI permits comparisons with previous literature, but does withhold certain limitations (e.g. \( f_b \) is not factored into calculations). An incremental protocol to establish peak inspiratory muscle strength may also reduce variability between groups in target workload.

In an attempt to mitigate the effect of sex hormones of autonomic function, women were tested during the early follicular phase of the menstrual cycle, when sex hormone concentrations reach a nadir. However, self-reported menstrual history poorly predicts circulating hormone concentrations and therefore, it is difficult to predict the influence of this variable upon results.
Axonal hyperpolarisation may have led to a depression in phrenic nerve excitability due to repeated near-maximal contractions. A plateau in $P_{di,tw}$ was observed in men and women at 80% of the maximal stimulator capacity, demonstrating effective and supramaximal bilateral phrenic nerve stimulation. Thus, the potential effect of axonal hypoexcitability was minimised if not entirely removed. Lastly, there is evidence that when matched for absolute diaphragm force output and/or duration of inspiratory loading performed at equal relative intensities, the magnitude of DF is less in women than men. These postulations require further scrutiny. Differences in absolute force may also affect post-ganglionic sympathetic discharge patterns, including differential recruitment of low- (rate coding) and high-threshold (population coding) axons, which modulate neurotransmitter release and the post-neurovascular junction response (Badrov et al., 2016).

4.4.6 Conclusions

In conclusion, men and women experience time-dependent sympathoexcitation in response to fatiguing contractions of the diaphragm. These cardiorespiratory interactions are attenuated in women, despite the absence of a sex-based difference in DF at the time of PTL task failure. Women are able to breathe against externally applied inspiratory resistance for significantly longer and generate greater cumulative diaphragmatic pressure than do men. Thus, the results indicate that the female diaphragm is highly fatigue resistant, leading to blunted increases in HR, MAP and LF$_{SBP}$ attendant to high levels of inspiratory muscle work. An attenuation of the inspiratory metaboreflex may influence limb and respiratory muscle haemodynamics with implications for exercise performance.
Chapter 5: Effect of Diaphragm Fatigue on Subsequent Exercise Performance

5.1 Introduction

The concept of symmorphosis denotes that structural design should be matched to functional demand (Weibel et al., 1991). In this regard, the healthy pulmonary system is generally considered ‘overbuilt’ for the demands placed on gaseous exchange and ventilation during muscular exercise (Olafsson & Hyatt, 1969). The respiratory system is able to maintain acid-base balance and blood-gas homeostasis near resting levels with remarkable ease due to the tight coupling of alveolar ventilation and metabolic rate. However, with exercise training, the cardiovascular and musculoskeletal systems demonstrate progressive structural and functional adaptation compared to the lungs, airways and respiratory muscles, whereby (with few exceptions) no significant adaptations occur (McKenzie, 2012). To this end, the once ‘overbuilt’ lung becomes a so-called “limiting factor” affecting O₂ transport and utilisation in highly trained endurance athletes (Dempsey et al., 1984). The mismatching of physiological systems is central to the demand vs. capacity theory (Dempsey, 1986).

The demand vs. capacity theory holds that there are exceptional circumstances whereby the physiological requirements of exercise meet or exceed the capacity of the respiratory system to respond (Dempsey & Johnson, 1992). For example, during heavy exercise, ventilation may increase 20-fold above resting levels and as such, the mechanical and energetic work of breathing rises over time, placing substantial demands upon the respiratory muscles. The hyperpnoea of exercise can command between 10-15% of total oxygen consumption depending on training status and sex (Aaron et al., 1992; Calbet et al., 2007; Dominelli et al., 2015) and 14-16% of maximum cardiac output (Manohar, 1986, 1990; Harms et al., 1997; Harms et al., 1998b). Due to a
combination of a high work of breathing (Babcock et al., 2002), accumulation of metabolites (Fregosi & Dempsey, 1986) and competition for finite cardiac output (Babcock et al., 1995), the inspiratory muscles fatigue (Johnson et al., 1993), resulting in a sympathetically mediated metaboreflex (Dempsey et al., 2002, 2006). The inspiratory muscle metaboreflex posits that blood flow is preferentially redistributed away from active limb locomotor muscles in favour of the fatiguing inspiratory muscles (Dominelli et al., 2017a), thereby catalysing the onset of locomotor muscle fatigue and enhancing the perception of dyspnoea (Romer et al., 2006b). The net effect of these cardiovascular and sensory consequences on exercise tolerance is debated.

Studies have reported that diaphragmatic fatigue (DF) results in either no change (Dodd et al., 1989; Sliwinski et al., 1996) or a decrease in exercise performance (Martin et al., 1982; Mador & Acevedo, 1991b; Harms et al., 2000; Wuthrich et al., 2013). Discrepancies in findings are due in part to the variability in study designs, which include: voluntary isocapnic hyperpnoea (Martin et al., 1982; Dodd et al., 1989), inspiratory resistive (Sliwinski et al., 1996; Wuthrich et al., 2013) or pressure-threshold loading (Mador & Acevedo, 1991b), and mechanical unloading (Harms et al., 2000). So-called ‘pre-fatigue’ studies attempt to isolate the independent effects of DF on exercise performance by inducing fatigue at rest, without the confounding influences associated with whole-body exercise (such as blood flow competition, temperature and acidosis). This modality is preferred because any exercise impairment can be attributed directly to limitations of the ventilatory pump.

To date, much of the research investigating respiratory system limitations to exercise have focused on men exclusively. More recently, it has become clear that sex-based differences in respiratory physiology have implications concerning the integrated whole-body response to
exercise (Sheel, 2016). For example, there is evidence to suggest that women have narrower airways relative to men matched for lung size (Mead, 1980; Sheel et al., 2009) – a concept termed ‘dysanapsis’ (unequal growth). Poiseuille’s law states that resistance to airflow is inversely proportional to the radius of a tube; thus, women experience a significantly greater resistive component of the work of breathing for a given ventilation during exercise (Guenette et al., 2007; Guenette et al., 2009). The work of breathing has been shown to be a major determinant of DF (Babcock et al., 2002; Archiza et al., 2018); therefore, it is logical to predict that women would be more likely to develop fatigue than men. However, contrary to this hypothesis, Guenette et al. (2010) found that women were in fact more resistant to exercise-induced DF.

In Chapter 4, the superior endurance capacity of the female diaphragm was confirmed. In addition, women experienced an attenuated cardiovascular response to DF (i.e. metaboreflex). Collectively, these results suggest that women are less susceptible to the detrimental consequences of DF, specifically as it pertains to cardiorespiratory interactions, which may preserve their ability to perform exercise. The aim of the present investigation was to examine the effect of DF on exercise performance in healthy men and women. It was hypothesised that DF induced prior to subsequent exercise would cause less of a reduction in exercise time in women relative to men.

5.2 Methods

5.2.1 Subjects

The subjects and experimental design used in the current study were the same as presented in Chapter 4. Eighteen healthy men ($n = 9$) and women ($n = 9$) were recruited. Written informed consent was obtained from all subjects prior to testing. Women were tested during the early follicular phase of the menstrual cycle (determined by self-report). Experimental procedures were
approved by the Clinical Research Ethics Board at the University of British Columbia (approval number: H15-00801) and conformed to the Declaration of Helsinki.

5.2.2 Experimental Design

Testing took place over three days. On day 1, subjects completed a maximal incremental exercise test to determine peak work rate. Anthropometrics and spirometry (Spirolab II, Medical International Research; Rome, Italy) were conducted prior to testing. Fatigue was defined as “a condition in which there is a loss in the capacity for developing force and/or velocity of a muscle, resulting from muscle activity under load and which is reversible by rest” (NHLBI, 1990). On days 2 and 3, subjects performed a constant load exercise test at 85% of the predetermined peak work rate to the limit of volitional tolerance with or without prior-induced DF. Subjects were randomly and equally assigned to either day 2 or day 3. Day 2 will be hereon regarded as the ‘pre-fatigue’ trial, in which subjects performed inspiratory pressure-threshold loading (PTL) to task failure immediately prior to exercise; day 3 will be regarded as the ‘control’ trial, in which no fatigue inducing task was performed prior to exercise.

The loading protocol conducted on day 2 has been described in Chapter 4 and will not be further described. During the PTL trial, cardiovascular responses were measured, including: heart rate (HR), systolic (SBP), diastolic and mean arterial blood pressure (MAP). Fatigue of the inspiratory muscles was assessed on days 2 and 3 using cervical magnetic stimulation, and diaphragm EMG recorded as detailed in Chapter 4. During the pre-fatigue trial, a series of potentiated twitches were performed before and immediately after PTL. Fatigue was assessed again at 5, 15 and 30 min post exercise. During the control visit, potentiated twitches were performed before and after exercise at the same time points. All subjects were familiarised with
the loading protocol and time-to-exhaustion (TTE) exercise test. A minimum of 48 h separated the
two experimental testing sessions. Subjects refrained from caffeinated beverages for 8 h and
exercise for 24 h before each testing session.

5.2.3 Flow, Volume and Pressure

Inspired and expired airflow was measured using two independently calibrated lilly-type
pneumotachographs (no. 3813, Hans Rudolph; Kansas City, MO, USA). The expired pneumotach
was heated to prevent condensation accumulation from expired gases. Volume was determined by
numerical integration of inspiratory and expiratory flow. Respiratory pressures were assessed
using previously described procedures (Milic-Emili et al., 1964; Baydur et al., 1982). Force
development across the diaphragm (i.e. transdiaphragmatic pressure \( P_{dil} \)) was estimated by
calculating the difference in gastric \( P_{ga} \) and oesophageal \( P_{oes} \) pressure with the use of balloon-
tipped catheters (no. 47-9005, Ackrad Laboratory; Cranford, NJ, USA). Topical anaesthetic (2% lidocaine hydrochloride, AstraZeneca; Mississauga, ON, Canada) was applied to the nasal and
pharyngeal passages to minimise discomfort during catheter insertion. Catheters were directed
intrasalynally and positioned in the stomach and lower one-third of the oesophagus to measure \( P_{ga} \)
and \( P_{oes} \), respectively. Each catheter was connected to a piezoelectric pressure transducer (Raytech
Instruments; Vancouver, BC, Canada), which was independently calibrated using a digital pressure
manometer (2021P, Digitron; Torquay, UK).

5.2.4 Maximal Incremental Exercise Test (Day 1)

Five to ten minutes of resting ventilatory data was acquired prior to exercise. Subjects completed
a warm-up at a self-selected work rate on an electronically braked cycle ergometer (Velotron,
RacerMate; Seattle, WA, USA). Men and women began cycling at 125 W and 75 W, respectively
at their preferred cadence. Work rate increased in a stepwise fashion by 25 W every two minutes. The test was terminated when cadence dropped below 60 rpm despite verbal encouragement. Peak work rate was calculated as the sum of the final completed exercise stage, plus an extrapolated work rate depending on the time spent in the final uncompleted stage.

Subjects breathed through a customised two-way non-rebreathing valve (2700B, Hans-Rudolph; Kansan City, MO, USA) attached to a mixing chamber. Mixed expired gases were sampled and analysed using calibrated O₂ and CO₂ gas analysers (S-3-A/I and CD-3A, respectively, Applied Electrochemistry; Pittsburgh, PA, USA). End-tidal partial pressure of CO₂ ($P_{ET}CO₂$) was measured via a side-port in the mouthpiece, which was connected to a CO₂ gas analyser (CD-3A, Applied Electrochemistry; Pittsburgh, PA, USA). Graded FVC manoeuvres were performed before and after exercise for construction of the maximum expiratory flow-volume envelope (MEFV). An inspiratory capacity manoeuvre was performed at rest and at least once per stage during exercise for offline analysis of ventilatory mechanics.

5.2.5 **Constant Load Exercise Tests (Days 2 and 3)**

Constant load exercise was performed using a similar design to Verges *et al.* (2006). Subjects began cycling at 40% of peak work rate for two minutes followed by a further two minutes of cycling at 60% to standardise the warm-up prior to constant load cycling. After four minutes of low-moderate intensity cycling, subjects began the TTE at 85% of peak work rate. Exercise was terminated when cadence dropped below 60 rpm despite verbal encouragement. Subjects wore a nose clip and breathed through the same circuit as day 1. Leg and breathing discomfort was assessed at two minute intervals using the modified 0-10 Borg scale (Borg, 1982).
5.2.6 Data Analysis

Gas exchange variables were averaged every 30 s throughout exercise. The mechanical work of breathing (WOB) was estimated using oesophageal pressure-volume loop integration according to methods described by Dominelli and Sheel (2012). Pressure-time products were calculated during periods of inspiratory flow for oesophageal (PTP<sub>oes</sub>) and transdiaphragmatic (PTP<sub>di</sub>) pressure by integrating the area under the P<sub>oes</sub> and P<sub>di</sub> curves, respectively. Non-representative breaths (e.g. coughs, swallows etc.) were excluded. Operating lung volumes and the presence of expiratory flow limitation (EFL) were determined by superimposing the tidal FVL within the MEFV curve. Diaphragmatic fatigue was assumed present if there was an ≥15% drop in twitch P<sub>di</sub> (P<sub>di,tw</sub>) compared to baseline measures. Twitches were excluded from analysis for any of the following reasons: 1) the subject was not resting at functional residual capacity (determined by end-expiratory P<sub>oes</sub>) immediately prior to stimulation, 2) if oesophageal peristalsis was evident at the time of the twitch, 3) if a cardiac artefact was superimposed upon a stimulus, and 4) if there was lack of diaphragmatic relaxation evidenced by noticeable diaphragm EMG. Twitch (contraction time [CT] and half-relaxation time [½RT]) and M-wave characteristics (amplitude, area, latency and duration) were analysed for all baseline and post-PTL/post-exercise stimulations. An example of the mechanical and electrical responses of the diaphragm to CMS is shown in Figure 5.1. Within and between-occasion coefficients of variation (CV) were calculated for men and women from baseline stimuli.
Figure 5.1: Diaphragm Responses to Phrenic Nerve Stimulation
Illustration of the electrical (EMG) and mechanical ($P_{di, tw}$) response of the diaphragm to cervical magnetic stimulation (one representative subject). Initiation of the diaphragmatic CMAP occurs between 3-8 ms after the delivered stimuli (shown as a noise artefact in the EMG trace). *Abbreviations*: EMG = electromyography; $P_{di}$ = transdiaphragmatic pressure.

5.2.7 Statistics

Descriptive characteristics (including metabolic data) were compared using independent-samples $t$-tests. Diaphragmatic fatigue was tested by independent samples $t$-test post-PTL. A two-way repeated measures ANOVA was used to assess sex-based differences in DF post-exercise. Ventilatory and sensory data measured during exercise, including perception of breathing/leg discomfort, were compared between sexes over time using a two-way ANOVA. Sex differences
in TTE during constant load cycling was assessed with an independent samples \( t \)-test. Variables related to PTL (cardiovascular and respiratory) were analysed and reported in full in Chapter 4. Twitch and M-wave characteristics were compared at baseline, post-PTL and post-exercise by repeated measures ANOVA. For all statistical tests, normality was assessed quantitatively using the Shapiro-Wilk test for small samples. An ANOVA on ranks was used in the event a physiological variable failed the test of normality. Significance was set at \( p < 0.05 \) for all statistical comparisons (SigmaPlot v12, Systat Software Inc.; San Jose, CA, USA). Results are expressed as mean ± SD, unless otherwise stated.

5.3 Results

5.3.1 Subjects

Participant demographics, anthropometrics and spirometry are presented in Chapter 4. Groups were of similar age and spirometry was within normal limits based upon predictive equations (Tan et al., 2011). Maximal exercise data, including: metabolic, ventilation and respiratory mechanics are shown in Table 5.1. Men achieved a higher peak work rate (310 ± 57 vs. 243 ± 35 W, \( p = 0.015 \)) and greater absolute peak O\(_2\) uptake (\( \dot{V}O_2 \), \( p = 0.008 \)) and CO\(_2\) production (\( \dot{V}CO_2 \), \( p = 0.003 \)) compared to women. Relative peak \( \dot{V}O_2 \) was not different between sexes and there were no differences in aerobic power when expressed as a percentage of predicted (\( p > 0.05 \)) (Jones et al., 1985). Minute ventilation (\( \dot{V}_E \)) and the absolute WOB were greater in men than women (\( p = 0.001 \) and 0.030, respectively); however, operating lung volumes and the presence of EFL was not different between sexes (\( p > 0.05 \)).
Table 5.1: Peak Responses during Maximal Incremental Exercise

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Men (n = 9)</th>
<th>Women (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Metabolic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\dot{V}O_2$ (l·min$^{-1}$)</td>
<td>4.2 ± 0.8</td>
<td>3.2 ± 0.5*</td>
</tr>
<tr>
<td>$\dot{V}O_2$ (ml·kg$^{-1}$·min$^{-1}$)</td>
<td>56.5 ± 4.8</td>
<td>52.3 ± 7.0</td>
</tr>
<tr>
<td>$\dot{V}O_2$ (% predicted)</td>
<td>123 ± 24</td>
<td>139 ± 22</td>
</tr>
<tr>
<td>$\dot{V}CO_2$ (l·min$^{-1}$)</td>
<td>4.6 ± 0.8</td>
<td>3.5 ± 0.5*</td>
</tr>
<tr>
<td>RER</td>
<td>1.10 ± 0.05</td>
<td>1.10 ± 0.04</td>
</tr>
<tr>
<td>HR (beats·min$^{-1}$)</td>
<td>187 ± 10</td>
<td>186 ± 13</td>
</tr>
<tr>
<td><strong>Ventilation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V_T$ (l)</td>
<td>2.8 ± 0.3</td>
<td>2.1 ± 0.5*</td>
</tr>
<tr>
<td>$f_b$ (breaths·min$^{-1}$)</td>
<td>58 ± 6</td>
<td>56 ± 10</td>
</tr>
<tr>
<td>$\dot{V}E$ (l·min$^{-1}$)</td>
<td>182 ± 31</td>
<td>130 ± 19*</td>
</tr>
<tr>
<td>$P_{ETCO_2}$ (mmHg)</td>
<td>24 ± 4</td>
<td>29 ± 4*</td>
</tr>
<tr>
<td>$\dot{V}E/\dot{V}CO_2$</td>
<td>39 ± 5</td>
<td>38 ± 3</td>
</tr>
<tr>
<td>$\dot{V}E/\dot{V}O_2$</td>
<td>44 ± 5</td>
<td>41 ± 4</td>
</tr>
<tr>
<td><strong>Respiratory Mechanics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EELV (% FVC)</td>
<td>31 ± 7</td>
<td>33 ± 7</td>
</tr>
<tr>
<td>EILV (% FVC)</td>
<td>85 ± 4</td>
<td>86 ± 7</td>
</tr>
<tr>
<td>$\dot{V}Ecap$ (l·min$^{-1}$)</td>
<td>227 ± 35</td>
<td>182 ± 34*</td>
</tr>
<tr>
<td>$\dot{V}E/\dot{V}Ecap$ (%)</td>
<td>80 ± 8</td>
<td>73 ± 16</td>
</tr>
<tr>
<td>WOB (J·min$^{-1}$)</td>
<td>607 ± 199</td>
<td>397 ± 146*</td>
</tr>
<tr>
<td>EFL (n)</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>

Ventilation, metabolic and pulmonary mechanics data from a maximal incremental exercise test to exhaustion. **Abbreviations:** $\dot{V}O_2$ = oxygen consumption; $\dot{V}CO_2$ = carbon dioxide production; RER = respiratory exchange ratio; HR = heart rate; $V_T$ = tidal volume; $f_b$ = breathing frequency; $\dot{V}E$ = minute ventilation; EELV = end-expiratory lung volume; EILV = end-inspiratory lung volume; $\dot{V}Ecap$ = ventilatory capacity; WOB = work of breathing; EFL = expiratory flow limitation. * Significantly different from men ($p < 0.05$).

5.3.2 Diaphragmatic Fatigue

On the pre-fatigue visit, baseline twitch pressures were 38.0 ± 8.2 and 35.9 ± 7.2 cmH$_2$O for men and women, respectively ($p = 0.579$). At the time of PTL task failure, twitch pressures were significantly reduced and to a similar extent in men and women (M = 28.9 ± 8.0 cmH$_2$O [-24.3%], W = 27.8 ± 6.3 cmH$_2$O [-22.8%]; $p = 0.328$). Thus, no differences existed in the severity of PTL-
induced DF. However, two subjects did not meet the definition of DF ($\geq 15\%$ decrease in $P_{\text{di, tw}}$). In this instance, subjects continued PTL for a further 5 min at which point DF was assessed again. Once subjects met or exceeded the threshold for DF, subsequent exercise performance could be determined. After exercise, twitch pressures were $28.3 \pm 9.4 (-25.7\%)$ cmH$_2$O for men and $30.3 \pm 6.9 (-16.9\%)$ cmH$_2$O for women. Diaphragm function gradually returned towards baseline values after 15 min ($M = -20.3 \pm 12.5\%$, $W = -12.5 \pm 11.9\%$) and 30 min ($M = -13.2 \pm 9.1\%$, $W = -8.7 \pm 10.1\%$) recovery. There was a main effect of sex ($p = 0.030$) and time ($p < 0.001$) for all stimuli delivered on day 2 (Figure 5.2), but no interaction ($p = 0.589$).

On the control visit, baseline twitch pressures were $39.1 \pm 8.2$ and $38.0 \pm 5.3$ cmH$_2$O for men and women, respectively ($p = 0.752$). After exercise, twitch pressures were reduced by $24.8 \pm 15.0\%$ in men and $13.5 \pm 13.2\%$ in women (see Figure 5.2). Seven out of nine men (78%) and three out of nine women (33%) met the criteria for DF. Twitch pressures returned towards baseline after 15 min ($M = -17.2 \pm 12.0\%; W = -8.4 \pm 10.1\%$) and 30 min ($M = -11.1 \pm 13.7\%, W = -3.5 \pm 9.4\%$) recovery. A main effect of sex ($p = 0.010$) and time ($p < 0.001$) was found for all stimuli delivered on day 3, but no interaction ($p = 0.436$).

Table 5.2 demonstrates twitch control measures, including: end-expiratory $P_{\text{oes}}$ at the time of stimulation, twitch and M-wave characteristics. Post-PTL and/or post-exercise end-expiratory $P_{\text{oes}}$ was unchanged from baseline on days 2 and 3. M-wave amplitude and area were unchanged from baseline for all stimulations delivered on days 2 and 3 in men and women. Contraction time ($139 \pm 12\ ms vs. 133 \pm 4\ ms, p = 0.308$) was slightly longer in women compared to men, whilst $\frac{1}{2}$ RT ($82 \pm 13\ ms vs. 83 \pm 11\ ms, p = 0.900$) was similar between sexes for baseline stimulations. Threshold loading and exercise prolonged CT and $\frac{1}{2}$ RT in men and women. Within- and between-
session CV between subsequent stimuli were 6.8% and 8.5% for men, and 6.6% and 9.1% for women (reproducibility of twitch characteristics shown in Table 5.3).

5.3.3 Cardiovascular Responses to PTL

The cardiovascular response to PTL is described in detail in Chapter 4. To summarise, a time-dependent increase in HR and MAP was observed for both sexes. At the time of task failure, HR (M = +19 ± 12 beats·min⁻¹, W = +13 ± 8 beats·min⁻¹) and MAP (M = +14 ± 9 mmHg, W = +10 ± 8 mmHg) were well above resting levels. A main effect of sex was found for HR (p = 0.009) and MAP (p = 0.024), which was driven solely by differences in SBP (p = 0.019). In addition, low-frequency systolic blood pressure was significantly lower in women compared to men (23.2 ± 11.1 vs. 33.9 ± 7.7 mmHg²; p = 0.038).

5.3.4 Breathing Pattern and Sensory Perceptions of Leg and Breathing Discomfort

The ventilatory response to exercise is shown in Figure 5.3. There was a main effect of sex on all indices of ventilation, including: fₒ, Vᵱ and Vₑ (p < 0.05). Breathing frequency was greater in women compared to men across both trials (p = 0.009). Tidal volume and Vₑ were greater in men than women (p < 0.001). Men exhibited a hyperventilatory response to DF. Mean exercise Vₑ was raised by 5 l·min⁻¹ during the pre-fatigue trial compared to control (p = 0.428), owing to increased fₒ. Ventilation was unaffected in women (-1 l·min⁻¹, p = 0.845). Metabolic data (including VO₂, VCO₂ and the respiratory exchange ratio [RER]) were not different between trials in either sex (p > 0.05). Absolute values for VO₂ (p < 0.05) and VCO₂ (p < 0.05) were greater in men than women. The RER and HR response to exercise was not different between sexes at any time point during either trial (p > 0.05). End-tidal PCO₂ declined throughout exercise and did not differ between sexes or trials (p > 0.05). The WOB was elevated with pre-fatigue by 54 J·min⁻¹ in men (p = 0.140)
and 11 J·min⁻¹ in women \((p = 0.363)\) compared to control conditions. A main effect of sex was found for WOB across both conditions \((p < 0.001)\).

A main effect of time was found for leg and breathing discomfort across both conditions in men \((p < 0.001)\) and women \((p < 0.001)\). There was a main effect of condition (pre-fatigue vs. control) on leg and breathing discomfort in women \((\text{leg}: p < 0.001, \text{breathing}: p = 0.006)\), and only breathing discomfort in men \((p = 0.031)\). No sex differences were found in either condition \((p > 0.05)\). The mean increases in leg and breathing discomfort with pre-fatigue were 0.5 and 0.7 units in men, respectively, and 1.5 and 1.0 units in women, respectively \((\text{Figure 5.4})\).

Respiratory muscle pressure-time products during exercise under control and experimental conditions are shown in Figure 5.5. There was a main effect of time on PTP\(_{\text{oes}}\) and PTP\(_{\text{di}}\) during control TTE \((p < 0.05)\), and PTP\(_{\text{oes}}\) during pre-fatigue TTE only \((p = 0.013)\). There was no effect of sex on PTP\(_{\text{oes}}\) or PTP\(_{\text{di}}\) in either control \((p = 0.426 \text{ and } 0.068, \text{respectively})\) or pre-fatigue \((p = 0.779 \text{ and } 0.126)\) trials. However, there was a main effect of sex on PTP\(_{\text{di}}/\text{PTP}_{\text{oes}}\) during the pre-fatigue trial \((p = 0.024)\).

5.3.5 Exercise Tolerance

Control TTE was 13.0 ± 3.2 min and 12.2 ± 3.3 min in men and women, respectively \((p = 0.610)\). Pre-fatigue resulted in similar reductions in exercise duration for both sexes. Men cycled for 10.9 ± 3.5 min, whilst women cycled for 10.1 ± 2.4 min. The percent change was -15.8 ± 19.5\% for men and -14.5 ± 19.2\% for women \((\text{Figure 5.6, } p = 0.888)\). All but four subjects (two male and two female) demonstrated a reduction in exercise performance with prior-induced DF.
Figure 5.2: Diaphragmatic Fatigue during Pre-Fatigue and Control Visits
Panels A (Pre-Fatigue) and B (Control) demonstrate the change in $P_{\text{di,tw}}$ relative to baseline measures following PTL to task failure and constant load exercise to exhaustion. Abbreviations: $P_{\text{di,tw}}$ = transdiaphragmatic twitch pressure; B = resting baseline; PTL = pressure-threshold loading; Ex = exercise. * Main effect of time, † main effect of sex ($p < 0.05$).
Table 5.2: Twitch Control Measures

<table>
<thead>
<tr>
<th>Twitch Characteristics</th>
<th>Men (n = 9)</th>
<th>Women (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Post-PTL</td>
</tr>
<tr>
<td>$P_{dt, tw}$ (cmH$_2$O)</td>
<td>38.0 ± 8.2</td>
<td>28.9 ± 8.0*</td>
</tr>
<tr>
<td>CT (ms)</td>
<td>133 ± 4</td>
<td>127 ± 5*</td>
</tr>
<tr>
<td>½ RT (ms)</td>
<td>83 ± 11</td>
<td>73 ± 12*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>M-wave Characteristics</th>
<th>Men (n = 9)</th>
<th>Women (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Post-PTL</td>
</tr>
<tr>
<td><strong>Right Hemi-Diaphragm</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amplitude (V)</td>
<td>3.4 ± 0.8</td>
<td>3.5 ± 0.1</td>
</tr>
<tr>
<td>Area (V·ms)</td>
<td>34.4 ± 6.0</td>
<td>34.3 ± 6.8</td>
</tr>
<tr>
<td>Latency (ms)</td>
<td>4.5 ± 0.5</td>
<td>4.6 ± 0.5</td>
</tr>
<tr>
<td>Duration (ms)</td>
<td>50.8 ± 1.0</td>
<td>49.1 ± 6.1</td>
</tr>
<tr>
<td><strong>Left Hemi-Diaphragm</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amplitude (V)</td>
<td>4.2 ± 0.8</td>
<td>4.4 ± 1.4</td>
</tr>
<tr>
<td>Area (V·ms)</td>
<td>38.9 ± 9.4</td>
<td>39.9 ± 8.7</td>
</tr>
<tr>
<td>Latency (ms)</td>
<td>5.1 ± 0.3</td>
<td>5.1 ± 0.4</td>
</tr>
<tr>
<td>Duration (ms)</td>
<td>46.6 ± 8.2</td>
<td>47.1 ± 3.8</td>
</tr>
</tbody>
</table>

*(A) Contraction and half-relaxation times decreased after PTL and exercise in both sexes. (B) M-wave characteristics were unchanged from baseline in men and women. Abbreviations: PTL = pressure-threshold loading; $P_{dt, tw}$ = transdiaphragmatic twitch pressure; CT = contraction time; ½ RT = half-relaxation time. *Significantly different from baseline ($p < 0.05$).
Table 5.3: Reproducibility of Twitch Characteristics

<table>
<thead>
<tr>
<th>Twitch Parameter</th>
<th>Men (n = 9)</th>
<th>Women (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 2</td>
</tr>
<tr>
<td><strong>Mechanical</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$P_{\text{di,tw}}$ (cmH$_2$O)</td>
<td>38.0 ± 8.2</td>
<td>39.1 ± 8.2</td>
</tr>
<tr>
<td>CT (ms)</td>
<td>133 ± 4</td>
<td>129 ± 10</td>
</tr>
<tr>
<td>$\frac{1}{2}$ RT (ms)</td>
<td>83 ± 11</td>
<td>80.4 ± 13</td>
</tr>
<tr>
<td><strong>Electrical</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amplitude (V)</td>
<td>3.8 ± 0.8</td>
<td>4.3 ± 0.6</td>
</tr>
<tr>
<td>Area (V·ms)</td>
<td>36.6 ± 7.7</td>
<td>40.6 ± 6.0</td>
</tr>
<tr>
<td>Latency (ms)</td>
<td>4.8 ± 0.4</td>
<td>5.0 ± 0.6</td>
</tr>
<tr>
<td>Duration (ms)</td>
<td>48.7 ± 4.6</td>
<td>52.0 ± 2.8</td>
</tr>
</tbody>
</table>

Mechanical (CT and $\frac{1}{2}$ RT) and electrical (M-wave) characteristics of the diaphragm to CMS on days 2 (pre-fatigue) and 3 (control). *Abbreviations*: WD = within-day; BD = between-day; CV = coefficient of variation; $P_{\text{di,tw}}$ = transdiaphragmatic twitch pressure; CT = contraction time; $\frac{1}{2}$ RT = half-relaxation time.
Figure 5.3: Effect of Diaphragm Fatigue on the Ventilatory Response to Constant Load Exercise

(A-C) Breathing pattern, (D-F) gaseous exchange, (G) HR, (H) $P_{ET}CO_2$, and (I) WOB during exercise in men and women on days 2 (pre-fatigue) and 3 (control). † Main effect of sex ($p < 0.05$).
Figure 5.4: Effect of Diaphragm Fatigue on Ratings of Perceived Exertion and Dyspnoea during High-Intensity Constant Load Cycling to Exhaustion

Sensory perceptual experience of leg (A and C) and breathing (B and D) discomfort during exercise on days 2 (A and B) and 3 (C and D) in men and women. * Main effect of time, § main effect of condition ($p < 0.05$).
Figure 5.5: Respiratory Muscle Pressure-Time Products during High-Intensity Constant Load Cycling to Exhaustion With and Without Prior-Induced Diaphragmatic Fatigue

Oesophageal (PTP_{oes}) and transdiaphragmatic (PTP_{di}) pressure-time products during (A-C) pre-fatigue and (D-F) control exercise. * Main effect of time, † main effect of sex (p < 0.05).
Figure 5.6: Effect of Diaphragm Fatigue on Subsequent Exercise Performance in Healthy Men and Women

(A) Individual TTE data points from days 2 and 3. Diagonal line represents the line of identity. (B) Bar graph showing pre-fatigue and control TTE in men and women. Abbreviations: TTE = time-to-exhaustion; NS = not significant (p > 0.05). * Significantly different from control (p < 0.05).

5.4 Discussion

5.4.1 Main Findings

The present study is the first to examine sex-based differences in the effect of DF on subsequent exercise performance. At the time of PTL task failure, men and women exhibited a similar degree of DF. Yet, women were able to sustain the task for a significantly longer duration and experienced a blunted cardiovascular response throughout PTL. Prior-induced DF negatively and equally affected exercise performance in men and women. Leg and breathing discomfort was also exaggerated with DF in both sexes, but was amplified in women. These findings suggest that DF contributes to exercise impairment independent of biological sex; however, the mechanisms by which DF impairs exercise tolerance may differ between men and women.
5.4.2 Exercise Limitation

At the time of task failure, PTL-induced DF was similar between sexes (~23% decrease in \( P_{di, tw} \)). Contrary to the hypothesis, the effect of DF on subsequent exercise performance did not differ on the basis of sex (~15% decrease in TTE). No previous study has examined the effect of DF on exercise tolerance in women. Nevertheless, the current results are in line with the 14-23% reduction in exercise performance reported in men (Martin et al., 1982; Mador & Acevedo, 1991b; Harms et al., 2000; Wuthrich et al., 2013).

The mechanisms by which DF can influence exercise tolerance include: cardiorespiratory interactions, locomotor muscle fatigue, central fatigue, ventilation and dyspnoea (Romer & Polkey, 2008). Diaphragmatic fatigue elicits a sympathetically mediated metaboreflex through activation of group III and IV phrenic nerve afferents (Hill, 2000). A time-dependent increase in muscle sympathetic nerve activity (MSNA) follows, concurrent with a decrease in limb blood flow and vascular conductance (St. Croix et al., 2000; Sheel et al., 2001; Sheel et al., 2002). It is believed that blood flow is at least partially redirected toward the fatiguing inspiratory muscles (Harms et al., 1997; Harms et al., 1998b; Dominelli et al., 2017a), although this hypothesis remains to be fully elucidated. Reduced limb blood flow exacerbates peripheral quadriceps fatigue by impairing \( O_2 \) delivery to active locomotor muscles (Romer et al., 2006b). Central motor drive is consequently reduced and exercise promptly ceases before a catastrophic failure of organism homeostasis occurs (Amann & Dempsey, 2008).

A blunted cardiovascular response to inspiratory resistance was observed in women that may have led to sex-based differences in respiratory and locomotor muscle haemodynamics. In support of this theory, Smith et al. (2016) found less of a reduction in limb blood flow during isolated inspiratory resistive breathing in women relative to men. However, it stands to reason that
women may be more likely to exhibit an increase in respiratory muscle perfusion at the expense of locomotor muscle blood flow given the ~4% greater \( \bar{VO}_2 \) of breathing (Dominelli et al., 2015). During intense whole-body exercise without prior-induced DF, the severity of locomotor muscle fatigue is not different between men and women (Dominelli et al., 2017b) as other factors related to whole-body exercise likely override any sex-specific influence on skeletal muscle fatigability. Recently, Katayama et al. (2018) found that women show less of an increase in MSNA (peroneal nerve) during low-intensity leg exercise with inspiratory resistance compared to men. The complex interaction of cardiorespiratory control mechanisms leading to changes in respiratory and locomotor muscle blood flow during whole-body exercise with or without prior-induced DF make it difficult to determine the effect of an attenuated inspiratory muscle metaboreflex on exercise tolerance.

The present study confirmed the findings of Guenette et al. (2010), in which the magnitude of exercise-induced DF (without prior-induced DF, i.e. control conditions) was less in women than men during high-intensity submaximal exercise to exhaustion. The current findings are in agreement with that of Guenette and co-workers; at the cessation of exercise, the percent change in \( P_{di,tw} \) relative to baseline measures was -20.4% and -13.0% for men and women, respectively, compared to -24.8% and -13.5% in the current study. Thus, it appears that whole-body dynamic exercise, known to elicit DF in men, is insufficient a stimulus to significantly impair diaphragm contractility in women.

Strikingly, exercise seemed to serve as a recovery period after the induction of DF in women. The severity of DF was closely matched in men and women (~23%) at the time of PTL task failure, yet following subsequent exercise, diaphragm function was improved by 5.9% in women, but slightly declined by 1.4% in men. The fact that diaphragm force output returned
towards baseline in women is counterintuitive and not anticipated. Several explanations have been put forward to explain sex-based differences in human skeletal muscle fatigability, including: differences in muscle morphology, substrate utilisation and sex hormones. Excellent reviews can be found elsewhere (Hicks et al., 2001; Hunter, 2009, 2014). It is speculated that enhanced inspiratory muscle endurance speeds lactate recovery kinetics, thereby facilitating subsequent bouts of exercise (Brown et al., 2010). Additional explanations are put forward by examining the ventilatory response to exercise and pattern of respiratory muscle recruitment.

5.4.3 Ventilatory Response to Exercise

A reduction in the capacity of the respiratory muscles to generate force of contraction may lead to inadequate force generating pressures, particularly when the demand for ventilation is high. In turn, this could lead to relative alveolar hypoventilation and high dead space ventilation. However, many studies have documented DF where alveolar ventilation was appropriate for metabolic demand as demonstrated by indirect measures of arterial blood gases (i.e. $P_{ET\text{CO}_2}$) (Johnson et al., 1993; Babcock et al., 1995; Babcock et al., 1996). Men and women were able to maintain adequate ventilation (or at least unchanged relative to control conditions) despite developing fatigue of the inspiratory muscles. In women, $\dot{V}_E$ was almost identical in pre-fatigue and control conditions. Whereas in men, there was a 5 l·min$^{-1}$ increase in mean exercise $\dot{V}_E$ during TTE with pre-fatigue compared to control TTE. Similar observations have been made during constant load short-term submaximal and maximal exercise (Mador & Acevedo, 1991a; Sliwinski et al., 1996; Verges et al., 2006).

At lower work rates (25 and 50% of peak work rate), Mador and Acevedo (1991a) found exercise ventilation to be unaffected by DF. However, at higher intensities ($\geq 75\%$ of peak work
rate), exercise $\dot{V}_E$ was elevated by $\sim 8$ l⋅min$^{-1}$. The increase in ventilation was caused by increased $f_b$ only. Sliwinski et al. (1996) reported remarkably similar values at low and high intensities of exercise. After the induction of DF, exercise at 90% of peak work rate resulted in an increased $f_b$ of 5 breaths⋅min$^{-1}$, which fully accounted for a 10 l⋅min$^{-1}$ increase in exercise $\dot{V}_E$. Likewise, Verges et al. (2006) reported an increase in $\dot{V}_E$ of 6 l⋅min$^{-1}$ in men during constant load cycling at 85% of peak work rate relative to control values. The present observations closely mirror those previously described – that a tachypnoeic breathing pattern follows PTL-induced DF. An explanation for the small increase in $\dot{V}_E$ accompanying DF has not been fully delineated, but may be related to activation of thin-fibre phrenic nerve afferents (Hussain et al., 1990).

Interestingly, $\dot{V}_E$ increased linearly over time in men, but tended to plateau in women. A similar observation was made by Guenette and co-workers (2010). Hence, women spend less time at maximal exercise ventilatory capacity. It is argued that men recruit accessory inspiratory muscles (e.g. sternocleidomastoid) in order to elevate ventilation even further during exercise, whereas women maintain a similar pattern of respiratory muscle activation. In line with the findings of Guenette et al. (2010), the ratio of PTP$_{di}$ to PTP$_{oes}$ (i.e. the fractional utilisation of $P_{di}$ to total inspiratory muscle force production) gradually declined during exercise in men, but remained constant in women, thereby demonstrating recruitment of extra-diaphragmatic muscles. Recently, Mitchell et al. (2017) found a greater reliance upon the scalene and sternocleidomastoid muscles during high-intensity cycling in women compared to men. It is posited that women preserve diaphragmatic function at the expense of developing mechanical ventilatory constraints during exercise. This apparent hypoventilation may be linked with the development of exercise-induced arterial hypoxaemia (EIAH). Support of this notion is made by Dominelli et al. (2013), who speculated that women tolerate EIAH at submaximal intensities in order to minimise
diaphragmatic work and thus prevent the negative consequences associated with DF. In addition to differences in ventilation and respiratory muscle recruitment, there are feedback effects from peripheral fatigue (of both respiratory and limb locomotor muscles) on the brain’s perception of effort (of both dyspnoea and limb discomfort), which may have contributed to exercise limitation.

5.4.4 Dyspnoea

Breathlessness, or dyspnoea, is a term generally applied to sensations of respiratory distress culminating in shortness of breath (ATS, 1999). Dyspnoea derives from “multiple physiological, psychological, social, and environmental factors, and may induce secondary physiological and behavioural responses” (Parshall et al., 2012). The pathogenesis of dyspnoea has been conceptualised by many theories. The ‘length-tension inappropriateness’ paradigm (Campbell & Howell, 1963), also referred to as ‘efferent-reafferent disassociation’ or ‘neuromechanical uncoupling’, theorises that dyspnoea is the result of a disparity between the central reflexive drive to breathe and the appropriate mechanical response of the respiratory system (Ambrosino & Vagheggini, 2006). The theory is based on the principle that when changes in respiratory muscle length (i.e. volume) or tension (i.e. pressure) are inappropriate for the outgoing motor command, the intensity of breathing discomfort increases. An increase in the perception of both leg and respiratory exertion was found in men and women during pre-fatigue TTE relative to control conditions.

An increase of 0.5 and 0.7 Borg units was observed in men for leg and breathing discomfort, respectively, in contrast to 1.6 and 1.0 units, respectively for women. Hence, sensory perceptions are accentuated particularly in women. Other pre-fatigue studies conducted in predominantly in men have made similar observations. Verges et al. (2006) found an increased
breathing discomfort of 1.4 units during whole-body high-intensity exercise to exhaustion. Likewise, Sliwinski et al. (1996) found an increased breathing discomfort of ~2 units, and Mador and Acevedo (1991a) reported an increased intensity of breathlessness equal to 13 mm using a visual analogue scale. Thus, the current results are in accordance with the aforementioned.

The increase in respiratory and limb exertion are likely due to the effects of DF on respiratory muscle recruitment and limb blood flow. Exercise hyperpnoea is associated with a progressive recruitment of accessory inspiratory muscles, which may distort the chest wall (Grimby et al., 1968) reduce the mechanical efficiency of breathing (Dodd et al., 1988) and contribute to an elevated sensation of breathing discomfort. Furthermore, fatiguing respiratory muscle work compromises active limb locomotor blood flow and catalyses locomotor muscle fatigue as described previously. In turn, exacerbation of effort perception would be expected to precipitate a reduction in central motor output (i.e. central fatigue), thereby facilitating the premature termination of exercise. A disturbance of the normal relationship between information on muscle length and tension in the control system can be envisaged in all conditions associated with breathlessness (Rankin & Dempsey, 1967). Stimulation of group III/IV muscle afferents increases central fatigue, but attenuates peripheral fatigue to a ‘sensory tolerance limit’ (Taylor et al., 2016). The effect of central motor drive on modulating peripheral muscle fatigue is key the central feedback loop hypothesis (Amann, 2011).

Women are believed to be predisposed to greater mechanical ventilatory constraints (i.e. EFL) during exercise due in part to dysanapsis of the lungs and airways. Consequently, at high-intensity exercise, women tend to operate at higher lung volumes relative to men and may have relatively greater increases in end-expiratory lung volume in an attempt to avoid impending flow limitation (Guenette et al., 2007). The presence of EFL and higher operating lung volumes would
likely manifest as an increased perception of dyspnoea. Sex differences in exertional dyspnoea are poorly understood and somewhat contradictory. For a given absolute metabolic requirement, women report higher intensity dyspnoea than men, but this disparity often disappears at equal relative intensities of submaximal exercise. Furthermore, at maximal exercise, women tend to report more inspiratory difficulty/unsatisfied inspiration and shallow breathing, which may be related to differences in respiratory mechanics (Ofir et al., 2008; Cory et al., 2015). The present study was not designed to address the complex multifactorial components of dyspnoea. However, the severe effect of DF on breathlessness observed in the current study provide rationale for future research.

5.4.5 Technical Considerations

A primary limitation of this work is prescribing an exercise intensity based on a fraction of peak power output. Previous investigations have adopted a similar protocol and allow comparisons to be made; however, the method does not take into consideration inter-individual differences in O₂ uptake kinetics. Critical power or 60% delta are two alternative methods that have been shown to reduce TTE variability by incorporating the anaerobic threshold into calculations of exercise intensity (Lansley et al., 2011). Secondly, operating lung volumes or EFL were not measured during constant load exercise. Thus, changes in breathing pattern or respiratory muscle activation due to mechanical ventilatory constraints are speculative and require further exploration. Finally, blood lactate and arterial blood gases were also not measured. It is highly likely that as exercise continued toward exhaustion, changes in the strong-ion difference created an acidic environment that catalysed the development of inspiratory and limb muscle fatigue.
5.4.6 Conclusions

In combination with the results of Chapter 4, it has been shown that the mechanisms and consequences of DF vary between men and women. Women have significantly greater inspiratory muscle endurance compared to men, and experience an attenuated inspiratory muscle metaboreflex despite experiencing a similar degree of PTL-induced DF at the time of task failure. Crucially, high-intensity submaximal exercise does not appear to be a sufficient stimulus to induce DF in healthy young women. The induction of DF prior to subsequent exercise negatively affects exercise tolerance equally in men and women. However, the ventilatory response to exercise differs on the basis of sex. With pre-fatigue, men had a higher breathing frequency, $\dot{V}_E$ and WOB for any given exercise time relative to men. On the other hand, perceptions of breathing and leg discomfort are intensified in both sexes, but particularly in women. Diaphragmatic fatigue has implications regarding whole-body exercise performance independent of sex; yet, the mechanisms contributing to exercise impairment appear to be different in men and women.
Chapter 6: Conclusions

6.1 Overall Summary

Much of our understanding of basic human physiology is based on work conducted exclusively in men. The reasons for this sex bias are multifactorial and relates in part to historical, sociological and cultural factors (Sheel, 2016). Over the past 20-30 years, important anatomical differences have been observed between men and women with regard to the respiratory system that have important implications concerning the integrated whole-body response to exercise. Dysanapsis of the lungs and airways precedes a host of functional disparities that exist between men and women when examining respiratory mechanics during exercise. As a direct consequence of smaller conducting airways relative to lung size, women are more likely to develop mechanical ventilatory constraints during exercise, leading to an increased resistive work of breathing that demands a higher fraction total \( \text{VO}_2 \) compared to men (Guenette et al., 2007; Guenette et al., 2009; Dominelli et al., 2015). It is logical to presume based upon the former evidence that women would be more susceptible to experience fatigue of the inspiratory muscles than men. However, some evidence exists supporting the view of female resistance to diaphragmatic fatigue (DF) (Gonzales & Scheuermann, 2006; Guenette et al., 2010). If women are more resistant to DF, the deleterious effects associated with the inspiratory muscle metaboreflex may be void. This theory formed the conceptual basis of the current thesis.

In Chapter 3, the reliability of cervical magnetic stimulation (CMS) to evoke the diaphragmatic compound muscle action potential was established. With a valid and reliable means of assessing diaphragm neuromuscular function, sex-based differences in human inspiratory muscle fatiguability were then determined in Chapter 4. Women were able to perform isocapnic
inspiratory pressure-threshold loading (PTL) for significantly longer before reaching task failure than men. Yet, at the time of task failure, the magnitude of DF was not different between sexes. Thus, women tended to experience less fatigue for a given cumulative diaphragmatic force output and developed fatigue at slower rates compared to men. Women experienced an attenuated inspiratory muscle metaboreflex, evidenced by a blunted increase in heart rate, mean arterial pressure and low-frequency systolic blood pressure. However, despite this observed sex-based difference, prior-induced DF negatively and equally affected subsequent exercise performance in Chapter 5.

6.2 Significance

The notion of female fatigue resistance is not novel. It is widely held that women are able to sustain intermittent isometric contractions for significantly longer than men (Hunter, 2014). This has been observed for many muscle groups, including: knee extensors (Maughan et al., 1986), elbow flexors (Miller et al., 1993) and handgrip muscles (West et al., 1995). It was believed that the human respiratory muscles may be an exception due to the high work of breathing women demonstrate during high-intensity exercise. However, the findings of this thesis further support the findings of Guenette et al. (2010) and Gonzales and Scheuermann (2006) that the female muscles of inspiration are highly fatigue resistant.

Diaphragmatic fatigue is associated with a time-dependent increase in muscle sympathetic nerve activity and vascular resistance along with a concurrent decrease in limb blood flow (Dempsey et al., 2002; Dempsey et al., 2006). Resistance to DF attenuates this cardiovascular response, which may influence haemodynamics, limb locomotor muscle fatigue, dyspnoea, central fatigue and ultimately, exercise tolerance. Despite women being more resistant to DF than men
and experiencing an attenuated inspiratory muscle metaboreflex, the effect of DF on subsequent exercise performance is not different between men and women. A schematic is shown in Figure 6.1 to demonstrate the consequences of resistance to DF in relation to the findings of the present thesis. It appears that the mechanisms by which DF contributes to exercise intolerance are different depending on sex. Exercise may be impaired due to alterations in limb blood flow and hastened locomotor muscle fatigue in men, whilst dyspnoea and central fatigue may augment exercise impairment in women. The findings of this thesis do not confirm the former postulations; however, mechanisms beyond those associated with the inspiratory muscle metaboreflex must account for some of the observations.

Several mechanisms have been put forward to explain the female resistance to skeletal muscle fatigue, which among many others, include sex differences in muscle morphology, substrate utilisation, and metabolism (Hicks et al., 2001). It is evident that female inspiratory muscles possess excellent endurance characteristics. Therefore, it is unclear how specific inspiratory muscle training programmes may benefit. Inspiratory muscle training improves the strength and endurance of the inspiratory muscles and has been shown to improve symptoms of dyspnoea, attenuate the inspiratory muscle metaboreflex (Witt et al., 2007), and increase exercise capacity in healthy subjects (Illi et al., 2012; HajGhanbari et al., 2013). However, the physiological mechanisms underpinning such ergogenic characteristics are poorly understood and remain controversial (Sheel, 2002; McConnell & Romer, 2004).
Figure 6.1: Working Hypothesis for the Mechanisms and Consequences of Female Resistance to Diaphragmatic Fatigue

Red indicates results from this thesis. Dotted lines indicate potential modifying effects. Abbreviations: HR = heart rate; MAP = mean arterial blood pressure; LVR = limb vascular resistance; LF<sub>SBP</sub> = low-frequency systolic blood pressure variability; MSNA = muscle sympathetic nerve activity. * Without prior-induced diaphragmatic fatigue.

If the respiratory muscles fail in their role as pressure-generators, the resulting effects upon gaseous exchange are potentially grave. It is possible that fatigue represents a final common pathway in the development of hypercapnic respiratory failure from a variety of conditions (Cohen <i>et al.</i>, 1982). Any condition that results in an increased work of breathing may cause respiratory muscle fatigue, when energy demand exceeds energy supply. Macklem (1980) broadly classifies
sources of inspiratory muscle energy demands into: 

- \textit{a}) work of breathing (minute ventilation, breathing frequency and tidal volume, compliance and resistance), 
- \textit{b}) strength (lung volume, atrophy, neuromuscular disease, nutritional status), and 
- \textit{c}) efficiency.

Factors that determine sources of energy supply include: 

- \textit{a}) arterial oxygen content (haemoglobin concentration and O$_2$ saturation), 
- \textit{b}) inspiratory muscle blood flow (cardiac output, distribution of perfusion and force of contraction), 
- \textit{c}) blood substrate concentration, 
- \textit{d}) energy stores, and 
- \textit{e}) ability to extract energy sources.

Thus, chronic obstructive pulmonary disease (work of breathing, efficiency), asthma (work of breathing), myasthenia gravis (strength), cachexia (strength), inducible laryngeal obstruction (work of breathing), and Guillain-Barré syndrome (strength) are examples of conditions that may lead to DF.

### 6.3 Strengths and Limitations

Many limitations are presented in each of the three respective study chapters. However, there are strengths and weaknesses of the research beyond those discussed previously. The measurements of diaphragmatic function were highly reproducible and well controlled. Notwithstanding the conventional difficulties with magnetic stimulation and surface EMG, nearly 90% of all subjects demonstrated maximal bilateral phrenic nerve activation. Moreover, twitch characteristics, including mechanical and electrical responses, were analysed and compared between men and women, giving further insight into sex differences in diaphragmatic neuromuscular function. In addition, low coefficients of variation solidify the aptitude of the stimulation and measurement technique.

The primary limitations of the thesis are the absence of blood flow and muscle sympathetic nerve activity (MSNA) measurements in Chapter 4 and the use of percent peak work rate to
prescribe exercise intensity in Chapter 5. The results from this thesis would have been improved with some indication of MSNA or respiratory and limb muscle blood flow. Inferences are made through other measures of sympathetic activity, such as heart rate and mean arterial blood pressure. Low-frequency systolic pressure was used in order to provide an estimate of sympathetic vasomotor tone. Whilst effective in demonstrating the activation of the inspiratory muscle metaboreflex, microneurography and/or sonography would have added to the results found. Previous work from our laboratory and others have used percent peak work rate for constant load time-to-exhaustion exercise trials. Clearly, this allows comparisons to be made; however, the method lacks a tailored approach that considers inter-individual differences in anaerobic threshold. Therefore, 85% peak work rate may be more difficult for some subjects than others. The 60% delta method has been shown to reduce variability between subjects and consequently offers a more valid means to compare groups. Finally, subjects were not matched for inspiratory muscle strength and the sample sizes were too small to compare sub-groups.

6.4 Future Directions

The results from the current thesis provide a small but significant step forward in the literature. A list of research questions that arise from the work are provided in Table 6.1. One of the main questions that remain from this thesis is how does DF resistance affect MSNA and the distribution of blood flow? An attenuated blood pressure response to inspiratory pressure-threshold loading observed in the present thesis suggests that women may experience less MSNA for a given severity of DF. Do women distribute blood flow to the respiratory muscles more effectively than men (functional sympatholysis)? The issue of sex vs. size remains a limitation of much work in the field.
Table 6.1: List of Future Directions

<table>
<thead>
<tr>
<th>Question</th>
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<tbody>
<tr>
<td>Are women more resistant to diaphragmatic fatigue when matched for absolute strength?</td>
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<td>How does low O₂ content (i.e. hypoxia) influence diaphragmatic fatigue?</td>
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<td>Do women experience less fatigue if the task is performed for the same duration?</td>
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<td>Does this fatigue resistance disappear during menopause?</td>
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<tr>
<td>Do women have less muscle sympathetic nerve activity during inspiratory loading?</td>
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<td>What is the effect of inspiratory loading on blood flow distribution?</td>
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<td>How does diaphragmatic fatigue affect lung and chest wall kinematics?</td>
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<td>Are there sex differences in dyspnoea due to diaphragmatic fatigue?</td>
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<tr>
<td>Are there sex differences in central fatigue due to diaphragmatic fatigue?</td>
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<tr>
<td>Does inspiratory muscle training prevent exercise-induced diaphragmatic fatigue in men?</td>
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<tr>
<td>Does inspiratory muscle training relieve dyspnoea in women?</td>
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6.5 Conclusion

The purpose of the present thesis was to determine the efficacy of CMS and chest wall surface EMG in the assessment of the diaphragm compound muscle action potential (CMAP) in healthy men and women, and to explore sex-based differences in the mechanisms and consequences of DF, specifically: a) the inspiratory muscle metaboreflex, and b) the effect of diaphragm fatigue on subsequent exercise performance. The method of CMS reliably evoked the diaphragm CMAP within and between experimental sessions without systematic bias toward unilateral phrenic nerve activation or sex. There were no sex differences in DF at the time of PTL task failure; however, women sustained the task for significantly longer than men and tended to develop less fatigue for a given cumulative diaphragm force output during loading, thereby highlighting the fatigue resistant characteristics of the female diaphragm. Women also experienced an attenuated inspiratory metaboreflex, possibly due to sex differences in autonomic blood pressure regulation. Lastly, prior-induced DF negatively and equally affected subsequent exercise performance in men.
and women. Thus, sex differences in the inspiratory muscle metaboreflex do not explain the current observations. Furthermore, the inspiratory metaboreflex is only one mechanism by which DF influences exercise tolerance. Future studies should attempt to isolate sex differences in the effect of DF on dyspnoea and central motor drive.
Bibliography


