Determinants and Physiological Consequences of Respiratory Muscle Fatigue: A Comprehensive Comparison of the Inspiratory and Expiratory Muscles

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The candidate confirms that the work submitted is his own and that appropriate credit has been given where reference has been made to the work of others.

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ABSTRACT

Exhaustive whole-body exercise elicits inspiratory and expiratory muscle fatigue, which may limit exercise tolerance through, at least in part, a reflexively-mediated reduction in leg blood flow ($\dot{Q}_L$). Despite phenotypical and functional differences between the inspiratory and expiratory muscles, an investigation of: 1) the cardiovascular consequences of; and 2) the factors that determine the development of inspiratory vs. expiratory muscle fatigue has not been conducted.

Presently, the cardiovascular responses to fatiguing inspiratory (IRL) and expiratory resistive loading (ERL), including leg vascular resistance (LVR) and $\dot{Q}_L$, were assessed in otherwise resting adults (*Chapters 5 and 6*). Despite a cardiovascular response consistent with a metabolically-induced sympathoexcitation, neither IRL nor ERL elicited the expected increase in LVR and reduction in $\dot{Q}_L$. The potential mechanisms underpinning this observation, including 'mechanical effects' of large swings in intra-thoracic and/or intra-abdominal pressure, are considered.

Next, the effect of exercise intensity and duration on the magnitude of exercise-induced respiratory muscle fatigue was assessed (*Chapter 7*). The magnitude of inspiratory muscle fatigue was a function of cumulative respiratory force output and exercise-intensity. Conversely, the magnitude of expiratory muscle fatigue was unaffected by exercise intensity or duration. This is likely due to a less fatigue-resistant phenotype and/or additional non-ventilatory roles of the expiratory versus inspiratory muscles.

Finally, the 'time-course' over which inspiratory and expiratory muscle fatigue develops during severe-intensity exercise was assessed (*Chapter 8*). The magnitude of inspiratory and expiratory muscle fatigue increased progressively with exercise

time (50% vs. 75% vs. 100% tolerable duration [T_{LIM}]). However, expiratory muscle fatigue became evident earlier than inspiratory muscle fatigue (50% vs. 75% T_{LIM}).

The findings from this thesis suggest that the fatigue threshold of the expiratory muscles may be lower than that of the inspiratory muscles; whether a more abrupt fatigue-induced sympathoexcitatory response occurs during increased expiratory vs. inspiratory work remains to be determined.

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ABBREVIATIONS

AP, arterial pressure

ADP, adenosine diphosphate

CO_2, carbon dioxide

COPD, chronic obstructive pulmonary disease

CP, critical power

CSA, cross-sectional area

CT, contraction time

CV, coefficient of variation

EELV, end-expiratory lung volume

EFL, expiratory flow limitation

EMF, expiratory muscle fatigue

EMG, electromyograms

ERL_{Pga}, expiratory resistive loading targeting gastric pressure

ERL, expiratory resistive loaded breathing

EFL, expiratory flow limitation

ERL_{Pm}, expiratory resistive loading targeting mouth pressure

FEV_1, forced expiratory volume in one second

f_R, respiratory frequency

FRC, functional residual capacity

FVC, forced vital capacity

H^+, hydrogen ions

HR, heart rate

HF, heart failure

IC, inspiratory capacity

IRL, inspiratory resistive loaded breathing

IRL_{Pdi}, inspiratory resistive loading targeting transdiaphragmatic pressure

IRL_{Pm}, inspiratory resistive loading targeting mouth pressure

K^+, potassium ions

LVR, leg vascular resistance

LT, lactate threshold

MAP, mean arterial pressure

MEP, maximal expiratory pressure

MIP, maximal inspiratory pressure

MRPD, maximal rate of pressure development

MRR, maximal rate of relaxation

MSNA, muscle sympathetic nerve activity

O_2, oxygen

PAV, proportional assist ventilation

PCr, phosphocreatine

Pdi, transdiaphragmatic pressure

Pdi_{MAX}, maximal transdiaphragmatic pressure

Pdi_{tw}, transdiaphragmatic twitch pressure

$P_{ET}CO_2$, end-tidal partial pressure of carbon dioxide

Pga, gastric pressure

Pga_{MAX}, maximal gastric pressure

Pga_{tw}, gastric twitch pressure

Pi, inorganic phosphate

Pm, mouth pressure

PoB, power of breathing

Poes, oesophageal pressure

P_{peak}, peak power output

PTPdi, diaphragm pressure time product

PTPga, gastric pressure time product

$\dot{Q}$, cardiac output

$\dot{Q}_L$, leg blood flow

Q_{tw}, quadriceps twitch pressure

RER, respiratory exchange ratio

RIST, ramp incremental sprint test

RMT, respiratory muscle training

ROS, reactive oxygen species

RPE, rating of perceived exertion

$RT_{0.5}$, one-half relaxation time

RV, residual volume

SFA, superficial femoral artery

SP, sprint power

T_{LIM}, time to the limit of tolerance

TTI, tension time index

$\dot{V}_E$, minute ventilation

V_{MEAN}, time-averaged mean blood velocity

$\dot{V}O_2$, rate of oxygen uptake

$\dot{V}O_{2peak}$, peak oxygen uptake

$\dot{V}O_{2max}$, maximal oxygen uptake

V_T, tidal volume

WoB, work of breathing

Δ25, 25% of the difference between CP and P_{peak}

Δ50, 50% of the difference between CP and P_{peak}

CHAPTER 1
Introduction

The primary function of the respiratory muscles during exercise is to increase alveolar ventilation in proportion to the metabolic requirements. With increasing exercise intensity, the respiratory muscles function to further drive alveolar ventilation such that arterial acidosis is minimised and arterial hypoxemia is prevented (Romer and Polkey, 2008). The increase in ventilation associated with exercise hyperpnoea places a substantial demand on the respiratory muscles. Despite a highly efficient neurally-regulated breathing pattern and recruitment strategy, a substantial work and O_2 cost of breathing is still incurred. Indeed, the O_2 cost of exercise hyperpnoea at peak exercise is ~8-10% of total body O_2 consumption ($\dot{V}O_2$) in healthy adults, and up to ~15-16% of $\dot{V}O_2$ in highly trained endurance athletes (Aaron et al., 1992b, Dominelli et al., 2015). Using objective, non-volitional measures of neuromuscular function, a now reasonable volume of evidence suggests that this high work and O_2 cost of breathing during high-intensity constant-power exercise (>85% of maximal $\dot{V}O_2$ [$\dot{V}O_{2max}$]) evokes both inspiratory and expiratory muscle fatigue in healthy males and females (e.g. Johnson et al., 1993, Guenette et al., 2010, Taylor et al., 2006, Verges et al., 2006). The genesis, or 'cause', of such exercise-induced *inspiratory* muscle fatigue appears to be a function of two factors: 1) a high power of breathing (i.e. the amount of energy required to ventilate the lung); and 2) a competition for limited available cardiac output. Indeed, diaphragmatic fatigue does not develop when the power of breathing during severe-intensity exercise is experimentally reduced by ~50% via proportional assist ventilation (Babcock et al., 2002), or when resting humans mimic the ventilatory (*and as such the diaphragm 'work'*) response to exhaustive high-intensity exercise (Babcock et al., 1995a). In addition, it has been suggested that exercise-induced diaphragmatic fatigue *may not* occur in response to lower intensity exercise (<85% of $\dot{V}O_{2max}$) possibly due to a lower power of breathing

combined with the absence of a competition for cardiac output (Johnson et al., 1993, Wetter et al., 1999); however, to date there is limited evidence to support such suggestions. Similarly, the absence of exercise-induced diaphragmatic fatigue in response to ramp incremental exercise (Romer et al., 2007) suggests that the time spent at a high power of breathing (i.e. cumulative force history) may also be an important factor in the development of respiratory muscle fatigue. The *expiratory* muscles exhibit a less fatigue-resistant phenotype and perform additional non-ventilatory roles during exercise in comparison to the diaphragm (Uribe et al., 1992, Haggmark and Thorstensson, 1979, Cresswell et al., 1992, Abraham et al., 2002). As such, it is possible that relative to the diaphragm the expiratory abdominal muscles may become fatigued in response to lower intensity and/or shorter-duration exercise. However, to date a critical comparison of exercise-induced inspiratory and expiratory muscle fatigue in response to exercise trials varying in intensity and tolerable duration has not yet been performed.

There is accumulating evidence to suggest that the development of exercise-induced respiratory muscle fatigue limits exercise performance. For example, experimentally inducing respiratory muscle fatigue prior to exercise impairs exercise tolerance by ~15-33% in comparison to control conditions (Taylor and Romer, 2008, Welch et al., 2018a, Mador and Acevedo, 1991b). Similarly, unloading the inspiratory muscles (i.e. reducing their power production) during severe-intensity constant-power exercise increases exercise performance time by ~14% (Harms et al., 2000). A primary mechanism by which respiratory muscle fatigue may limit exercise performance is via the activation of a respiratory muscle metaboreflex, whereby the accumulation of metabolites stimulates group III and IV respiratory muscle afferents eliciting a sympathetically-mediated response, characterised by a peripheral vasoconstriction and a reduction in blood flow to the locomotor muscles which may augment the rate of locomotor muscle fatigue (Sheel et al., 2018b). Indeed, fatiguing inspiratory muscle

work in otherwise resting individuals elicits a time-dependent sympathoexcitation characterised by an increase in muscle sympathetic nerve activity (MSNA), mean arterial pressure (MAP), leg vascular resistance (LVR) and a reduction in limb blood flow ($\dot{Q}_L$) (Sheel et al., 2001, Smith et al., 2016). In response to fatiguing expiratory muscle work there is a similar time-dependent increase in MSNA, and an increase in MAP (Derchak et al., 2002), however the cardiovascular effects in the resting limb are unknown.

Therefore a primary aim of this thesis was to compare and contrast the presence and magnitude of exercise-induced expiratory vs. inspiratory muscle fatigue in response to heavy-intensity exercise and severe-intensity exercise of short and long durations. A second primary aim was to compare and contrast the cardiovascular consequences of fatiguing inspiratory and expiratory muscle work.

The next chapter comprises a summary of the current literature regarding the inspiratory and expiratory muscle contribution to ventilation, and the neuromuscular and cardiovascular consequences of their work. In section 2.1 the anatomy, phenotype and morphology, and functional characteristics of the inspiratory and expiratory muscles are described. In section 2.2, the ventilatory responses to different exercise intensity domains is delineated, including differences in the breathing pattern and respiratory muscle energetics observed during exercise. In section 2.3 and 2.4, the aetiology of skeletal muscle fatigue and the factors contributing to the development of exercise-induced respiratory muscle fatigue are discussed. Finally, in section 2.5 and 2.6 the potential debilitating effect of exercise-induced respiratory muscle fatigue on exercise tolerance, and the potential mechanisms responsible, are evaluated.

CHAPTER 2
Literature review

2.1. STRUCTURE AND FUNCTION OF THE RESPIRATORY MUSCLES

The respiratory muscles are embryologically, morphologically, and functionally classified as striated skeletal muscles. The primary function of the respiratory muscles is to pump air in and out of the lungs in order to maintain homeostatic balance of blood-gas tensions. The respiratory muscles can be broadly defined as inspiratory or expiratory dependent on their mechanical actions. The inspiratory muscles, namely the diaphragm and the accessory inspiratory ribcage and 'neck' muscles, act to depress the abdominal contents and expand the chest wall, increasing volume of the lung. In contrast, the primary expiratory muscles are those of the ventrolateral abdominal wall, which act to decrease lung volume by compressing the abdominal wall and inwardly displacing the ribcage. This section of the literature review will briefly discuss the functional anatomy, the phenotype, and the morphology of the respiratory muscles. It is these characteristics that determine their mechanical action as well as their ability to sustain such actions when under load. A schematic representation of the anatomy of the primary and accessory respiratory muscles is displayed in Figure 2.1.

2.1.1. Functional Anatomy

2.1.1.1. Diaphragm

The diaphragm is a thin, flat, dome-shaped musculoskeletal structure that separates the thoracic and abdominal cavities (De Troyer and Estenne, 1988). Somewhat uniquely, the muscle fibres of the diaphragm radiate from a central tendinous structure (i.e. central tendon) to form sternal, vertebral and costal attachments. The diaphragm muscle is primarily divided into two distinct components: 1) the crural (vertebral) section with points of insertion on the ventrolateral aspect of the first three

lumbar vertebrae and the aponeurotic ligaments; and, 2) the costal component consisting of muscle fibres which insert on the xiphoid process of the sternum and upper margins of the lower six ribs (De Troyer and Loring, 2011). Historically, it was suggested that sections of the diaphragm were innervated by the intercostal nerves. However, it is now well established that the sole motor supply occurs from the phrenic nerve, originating at the level of the 3rd to 5th cervical nerve roots (C3-C5) (Sant'Ambrogio et al., 1963, Verin et al., 2011). Upon contraction, the muscle fibres of the diaphragm develop tension and shorten causing the dome of the diaphragm to descend relative to its costal insertions. This expands the thoracic cavity along its craniocaudal axis decreasing pleural pressure and, assuming that the airway is open, increasing lung volume (De Troyer and Loring, 2011). A second consequence of diaphragm contraction is a caudal displacement of the abdominal viscera, which increases abdominal pressure and causes an outward motion of the ventral abdominal wall. Through this piston-like movement, the diaphragm acts as the primary muscle of inspiration.

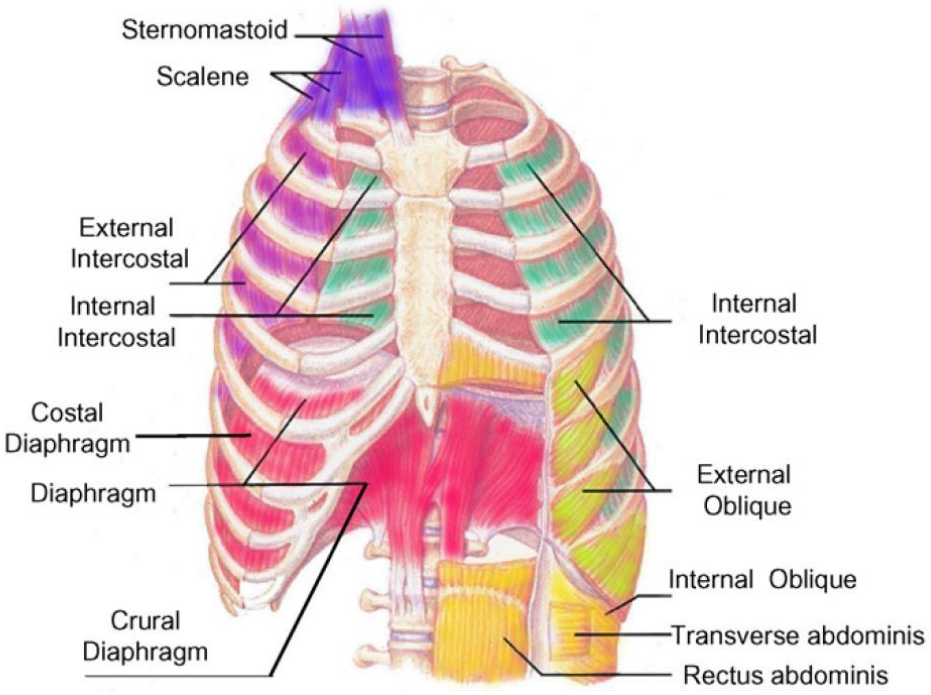


Figure 2.1. Anatomical arrangement of the inspiratory (left) and expiratory (right) muscles. Figure taken from Ratnovsky et al. (2008).

2.1.1.2. Accessory Inspiratory Muscles

In addition to the diaphragm, several additional muscles of the neck and ribcage contribute to inspiration. The two primary inspiratory 'neck' muscles are the sternocleidomastoids, a pair of muscles that descend from the mastoid process to the manubrium sterni and medial third of the clavicle, and the scalenes, a set of three muscles that connects the transverse process of the lower five cervical vertebrae to the first two ribs. The sternocleidomastoids are usually silent during rested breathing, but contribute to cranial displacement of the sternum and expansion of the upper ribcage as pulmonary ventilation increases (De Troyer et al., 1986). Conversely, the scalenes muscles demonstrate an inspiratory function at rest, predominantly acting to expand the upper ribcage by raising the sternum and upper two ribs (Legrand et al., 2003). The intercostal muscles are two thin sheets of muscle fibres that occupy the intercostal spaces and are innervated by the intercostal nerves (De Troyer et al., 2005). The external intercostal muscle is superficial to the internal intercostal muscle, and extends obliquely downward and ventrally from the rib above to the rib below. Conversely, the internal intercostals extend from the ribs dorsally to the sternocostal junctions, angled obliquely and backward from the rib above to the rib below. The parasternal intercostal muscles (i.e. interchrondal section of the internal intercostal) and, to a lesser extent, the external intercostal muscles display inspiratory activity at rest and appear to contribute to ribcage expansion by elevating the ribs (De Troyer et al., 2005).

2.1.1.3. Expiratory Abdominal Muscles

The primary expiratory muscles are those of the ventrolateral abdominal wall, namely transversus abdominis, internal oblique, external oblique and rectus abdominis. Although expiration is passive during eupnoea, active contraction of the expiratory muscles occurs as pulmonary ventilation increases, with recruitment typically occurring in order from the deepest to most superficial muscles. The muscle fibres of

transversus abdominis, the deepest expiratory muscle, run circumferentially around the abdominal visceral mass arising from the lower six ribs, lumbar fascia, and iliac crest, and insert at a ventral aponeurosis contributing to the rectus sheath (De Troyer and Estenne, 1988). Previous reports using fine-wire EMG electrodes suggest that transversus abdominis activity is evident in response to hyperoxic hypercapnia at a minute ventilation of ~10-18 $L \cdot min^{-1}$; considerably lower than the minute ventilation required for activation of the more superficial expiratory muscles (De Troyer et al., 1990). The muscle fibres of the internal oblique arise from the iliac crest and run superior-medially towards the costal margin, contributing to the rectus sheath. The most superficial of the lateral expiratory abdominal muscles, the external oblique originates above the costal margin of the lower eight ribs and inserts caudally to the iliac crest and inguinal ligament. The rectus abominis is the most ventral of the expiratory muscles and originates from the sternum and 5^{th}-7^{th} costal cartilages and inserting into the pubis, enclosed in a sheath formed by the other three muscles. These four expiratory muscles are innervated by the lower six thoracic nerve branches (T7-T12) and the first lumbar nerve (L1) (De Troyer and Estenne, 1988), and upon contraction have two primary expiratory functions. First, muscle contraction causes inward compression of the abdominal wall evoking an increase an abdominal pressure, which consequently pushes the diaphragm cranially into the thoracic cavity, resulting in an increase in pleural pressure and a reduction in lung volume (De Troyer and Loring, 2011). Second, due to their insertions at the lower ribcage, expiratory muscle contraction pulls the low ribs inwards, further aiding deflation (De Troyer and Estenne, 1988).

2.1.1.4. Accessory Expiratory Muscles

Both the interosseous component of the internal intercostal and the triangularis sterni display expiratory activity (De Troyer et al., 2005). The triangularis sterni is a flat muscle, innervated by the intercostal nerves, that lies deep to the sternum and

parasternal intercostals. Its fibres originate from the sternum and insert at the chondrocostal junction of the 3rd – 7th ribs, in an opposite orientation to the parasternal intercostals. Although both the triangularis sterni and internal intercostal are not considered as primary expiratory muscles and they do not exhibit marked activity in resting humans, their contraction pulls the ribcage downwards, and may aid lung deflation during expulsive manoeuvres by increasing pleural pressure (De Troyer et al., 2005, De Troyer et al., 1987).

2.1.2. Phenotype and Morphology

The contractile function of a muscle is primarily determined by its structural characteristics; 1) muscle fibre type; 2) motor unit organisation; and 3) morphology.

2.1.2.1. Inspiratory and Locomotor Muscles

Compared to most peripheral locomotor skeletal muscles, fibres of the diaphragm exhibit a higher proportion of slow-oxidative phenotypes, a higher oxidative capacity, and a greater capillary density, most likely because of an adaptation to the high frequency of their use. Histochemical analysis of the adult human diaphragm in individuals undergoing thoracic surgery suggests that the diaphragm consists of ~50-55% type I, ~20-30% type IIA, and ~25% type IIX muscle fibres (Lieberman et al., 1973, Sauleda et al., 1998, Mizuno, 1991). In contrast, the vastus lateralis in healthy humans exhibits ~45% type I, ~35% type IIA, and ~20% type IIX muscle fibres (Edgerton et al., 1975). Consistent with a high proportion of high-oxidative fibres, the diaphragm exhibits a ~twofold greater volume density of mitochondria (10-12% vs. 2-6%) and a substantially greater number of capillaries per cross-sectional area of muscle fibre when compared to the extensor, flexor or adductor locomotor limb muscle groups (Hoppeler et al., 1981). During exhaustive normoxic exercise, glycogen stores decline substantially in the limb locomotor muscles (>50% of control values) but remain at near-baseline levels in the diaphragm and intercostal muscles,

suggesting a predominant reliance on oxidative mechanisms of metabolism for the inspiratory muscles (Fregosi and Dempsey, 1986).

2.1.2.2. Expiratory Muscles

In comparison to the diaphragm, the expiratory abdominal muscles are characterised by a less-oxidative, but more variable proportion of muscle fibres. The proportion of type I and type II muscle fibres in the expiratory abdominal muscles ranges from 30-60% and 40-70%, respectively (Haggmark and Thorstensson, 1979, Johnson et al., 1973). Similarly, type IIA fibres are typically more abundant, larger in size, and more highly capilliarised in the expiratory compared to the inspiratory intercostal muscles (Mizuno and Secher, 1989). The expiratory abdominal muscle fibres also exhibit a lower oxidative capacity than those of the diaphragm. Citrate synthase activity, the enzyme responsible for the initial step in the Krebs cycle, is ~66% lower across the four primary expiratory abdominal muscles compared to that in the costal and crural diaphragm (Uribe et al., 1992). The less-oxidative morphology of the expiratory muscles likely reflects an adaptation to the different functional requirement of the muscle. Indeed, the primary expiratory abdominal muscles are silent during eupnoea in healthy individuals (De Troyer et al., 1990), and the greater proportion of type II muscle fibres may be beneficial for the performance of non-ventilatory manoeuvres such as speech, coughing, and flexion and rotation of the torso.

2.1.3. Inspiratory vs. Expiratory Functional Characteristics

The differences in phenotype and morphology between the inspiratory and expiratory muscles likely contribute to the endurance capability of each muscle group. Comparing the endurance properties of the respiratory and the limb muscles, Gandevia et al. (1983) reported that repeated maximal static contractions (5 s contractions with 5 s rest intervals for 6 min) of the elbow flexors, the expiratory muscles, and the inspiratory muscles reduced each muscle group’s maximal force

generating capacity by ~35%, ~30%, and ~8%, respectively. However, it remains unclear whether the differing endurance properties of the inspiratory and expiratory muscles translate into a different time-course of exercise-induced inspiratory and expiratory muscle fatigue (*see section* 2.4.3).

2.1.4. Summary

The diaphragm and the expiratory muscles of the ventrolateral abdominal wall are the primary muscles responsible for alveolar ventilation. The phenotype and morphology of the respiratory muscles appears to be tailored to suit their respective functions. Relative to the inspiratory muscles the expiratory muscles have a lower endurance capacity and/or are less fatigue-resistant. The following section provides and overview of the ventilatory responses and the metabolic demands of the respiratory muscles during whole-body exercise.

2.2. VENTILATORY AND CARDIOVASCULAR RESPONSES TO EXERCISE

The primary role of the respiratory muscles during exercise is to increase alveolar ventilation in proportion to O_2 consumption and CO_2 production, and as such regulate acid-base balance appropriately. Because the metabolic and ventilatory requirements differ depending on the intensity at which exercise is performed, this section will first briefly define the four exercise-intensity domains. Then, the ventilatory responses of healthy individuals exercising in the heavy- and severe-intensity domains, which are most relevant to this thesis, will be discussed. Specifically, breathing pattern, changes in operational lung volume, and respiratory muscle energetics will be addressed.

2.2.1. Definitions of Exercise Intensity Domains

Muscle metabolic, blood acid-base, and respiratory perturbation differs dramatically based on the prescribed exercise intensity, responses which have been categorised

into four domains demarcated by distinct physiological thresholds, namely: 1) moderate, 2) heavy, 3) severe, and 4) extreme (Jones and Poole, 2005; for alternative definition see: Whipp, 1996). Moderate intensity exercise refers to all power outputs performed below lactate threshold (LT), characterised by the achievement of a steady-state in pulmonary O_2 uptake ($\dot{V}O_2$) and the absence of any sustained metabolic acidosis (Whipp, 1996, Black et al., 2017). Heavy intensity exercise denotes power outputs between LT and critical power (CP), which evoke a progressive rise in $\dot{V}O_2$, arterial blood lactate concentration, H^+, and muscle acidosis that stabilises after ~15-20 min (Black et al., 2017, Casaburi et al., 1987). The severe intensity domain includes all power outputs above CP that elicit a substantial and progressive increase in $\dot{V}O_2$, arterial blood lactate concentration, and H^+, and a continued loss of muscle homeostasis (i.e. a decrease in muscle pH and phosphocreatine breakdown, and an accumulation of inorganic phosphate), which will result in the attainment of $\dot{V}O_{2max}$ if performed until exhaustion (e.g. Black et al., 2017, Jones et al., 2008). Therefore, CP represents the upper physiological threshold that separates power outputs that can be sustained with elevated but stable metabolic and ventilatory perturbation from power outputs that will elicit unstable responses rising progressively to reach maximum values. All power outputs performed above CP that terminate before the achievement of $\dot{V}O_{2max}$, are classified as the extreme intensity domain (Jones and Poole, 2005). An example of the pulmonary and metabolic responses to moderate, heavy, and severe-intensity exercise are displayed in Figure 2.2.

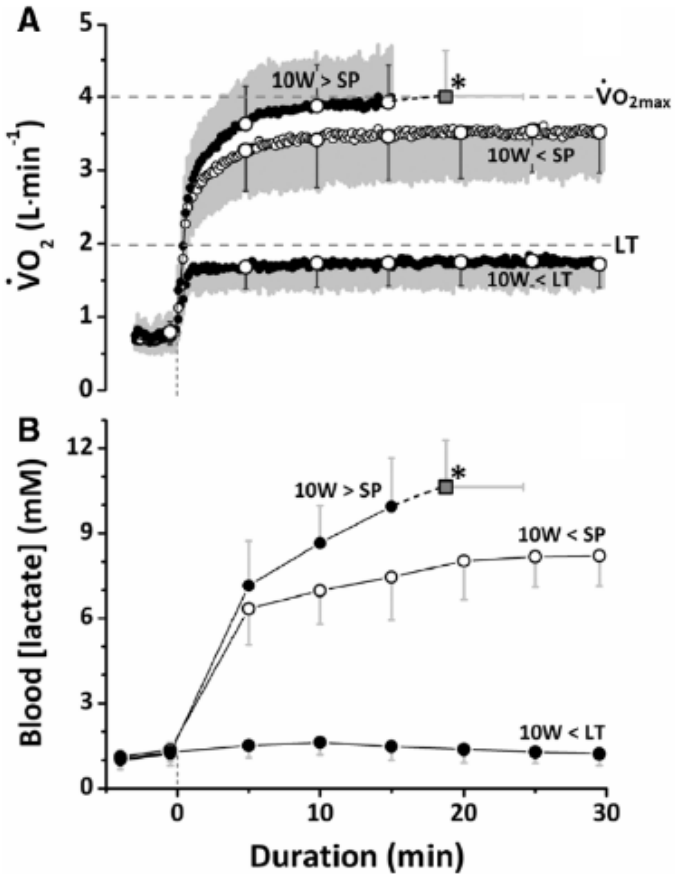


Figure 2.2. The V̇O2 and blood lactate responses to constant-power exercise in the severe domain (10 W >SP), heavy domain (10 W <SP), and moderate domain (10 W <LT). In this study SP refers to estimated critical power. Figure taken from Murgatroyd et al. (2014).

2.2.2. Breathing Pattern during Exercise

The specific breathing pattern adopted during exercise is carefully controlled to optimise pulmonary gas exchange, respiratory muscle energetics, respiratory sensations, and cardiac performance. During progressive ramp incremental exercise, minute ventilation ($\dot{V}_E$) rises due to an increase in both tidal volume (V_T) and respiratory frequency (f_R). The initial increase in V_T is caused by a decrease in end-expiratory lung volume (EELV) below functional residual capacity (FRC) and a slight rise in end-inspiratory lung volume (EILV) (*see* Figure 2.3, *panel A*). This reduction in EELV occurs with even minor increases in $\dot{V}_E$, and is caused by an increase in intra-abdominal pressure secondary to active expiratory muscle recruitment (Henke et al., 1988). The decrease in EELV enhances the efficiency of breathing, by: 1) increasing V_T over the linear (and most compliant) part of the respiratory pressure-

volume relationship, so that the minimal intrathoracic pressure is generated for a given change in volume (Rahn et al., 1946); 2) increasing the operating length of the diaphragm to nearer its optimal length-tension relationship (De Troyer and Wilson, 2009); and 3) storing elastic energy that contributes significantly to the work performed during subsequent inspirations (Henke et al., 1988). Similarly, by increasing V_T (as opposed to a sole change in f_R) alveolar ventilation is maximised by minimising dead space ventilation, and both the flow-rate and flow-resistive work of breathing are diminished (Sheel and Romer, 2012).

In response to higher exercise intensities (i.e. heavy- and severe-intensity), V_T progressively increases until reaching a plateau at ~50-60% of vital capacity (VC), whereby further increases in $\dot{V}_E$ are met by a rise in f_R (i.e. tachypnoea). In most healthy adults, a further decrease in EELV to ~0.4-0.7 L below FRC appears to contribute to the early phase of this increase in V_T during heavy-intensity exercise (*see* Figure 2.3, *panel B*). However, during the latter stages of heavy-intensity exercise as V_T approaches ~50-60% of VC, a rise in EELV above FRC (i.e. dynamic lung hyperinflation) is frequently observed (*see* Figure 2.3, *panel C*), particularly in fit healthy athletes (e.g. Johnson et al., 1992). This increase in EELV is beneficial for the generation of higher expiratory flow rates, but occurs at the expense of a greater inspiratory work and/or perception of dyspnoea secondary to the encroachment of end-inspiratory lung volume on TLC, which may contribute to exercise intolerance (Petrovic et al., 2012, Johnson et al., 1992, McClaran et al., 1999). Although the precise mechanism for the rise in EELV remains unclear, the development of expiratory flow limitation (EFL) and/or impending EFL may contribute (*see* Figure 2.3, *panel B*) (Babb, 2013). For example, Taylor et al. (2013) reported that during severe-intensity constant power cycling, EELV increased significantly above resting values in subjects that did not exhibit 'frank' EFL, but remained below FRC in individuals in whom EFL was observed. It is speculated that this rise in EELV may be caused by a

reflex inhibition of respiratory motor output leading to premature termination of expiration, secondary to the onset of dynamic hyperinflation and the subsequent increase in airway resistance (Pellegrino et al., 1993, Babb, 2013). However, it seems that some individuals may 'consciously override' this signal, choosing to tolerate lower expiratory flow rates and the occurrence of EFL.

After the plateau in V_T, the progressive rise in f_R is accomplished by a shortening of both inspiratory (T_I) and expiratory time (T_E). However, T_E decreases to a greater extent than T_I, resulting in an increase in the inspiratory duty cycle (T_I/T_{TOT}) from ~0.4 at rest to up to ~0.50-0.55 at maximal exercise (Sheel and Romer, 2012). It has been speculated that the increased T_I/T_{TOT} of ~0.50 adopted during exercise may be optimal for maintaining diaphragmatic blood flow (Hu et al., 1992, Dempsey et al., 1996), which may have implications for the development of fatigue.

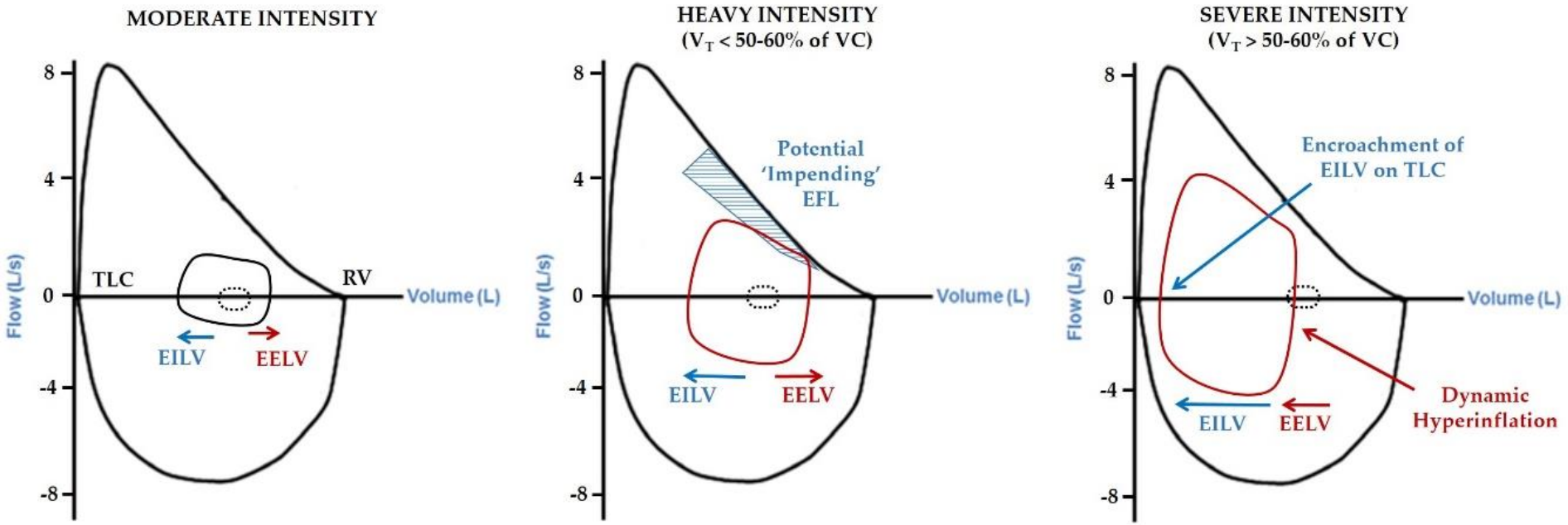


Figure 2.3. The typical response of operational lung volume to peak moderate-, heavy-, and severe-intensity exercise. TLC, total lung capacity; RV, residual volume; V_T, tidal volume; EILV, end-expiratory lung volume; EELV, end-expiratory lung volume; EFL, expiratory flow limitation. Note, impending EFL signals the onset of dynamic airway compression, and the potential initiation of dynamic hyperinflation.

2.2.3. Respiratory Muscle Energetics during Exercise

The ventilatory response associated with heavy- and severe-intensity exercise incurs a substantial mechanical work and O_2 cost of breathing. The work of breathing (WoB) represents the quantifiable degree of energy required for ventilation, and can be primarily split into two components: 1) elastic work, necessary to overcome the recoil of the lung and chest wall; and, 2) resistive work, required to overcome the opposition to airflow in the airways. The metabolic cost of exercise hyperpnoea can be estimated by comparing the $\dot{V}O_2$ of whole-body exercise to the $\dot{V}O_2$ exhibited in response to mimicked exercise hyperpnoea in otherwise resting individuals (i.e. replicating the $\dot{V}_E$, V_T, f_R, EELV that occurred during exercise). In response to incremental exercise, the increased ventilation elicits a disproportionate rise in both the work and O_2 cost of breathing (*see* Figure 2.4) (Aaron et al., 1992a, Aaron et al., 1992b, Johnson et al., 1992, Dominelli et al., 2015). Therefore, it is clear that the greater $\dot{V}_E$ associated with higher intensity exercise results in a greater respiratory muscle work and consequent $\dot{V}O_2$. For example, Aaron et al. (1992b) reported that in moderately fit males (i.e. $\dot{V}O_{2max}$ = 40-50 ml·kg·min^{-1}) cycling at 70% of $\dot{V}O_{2max}$ evoked a respiratory muscle $\dot{V}O_2$ ($\dot{V}O_{2RM}$) of 3-5% relative to total $\dot{V}O_2$, compared to 8-10% of total $\dot{V}O_2$ in response to maximal exercise. In select highly fit endurance trained males and females, the $\dot{V}O_{2RM}$ may be up to ~15% and ~25% of $\dot{V}O_{2max}$, respectively, at maximal exercise (Aaron et al., 1992b, Dominelli et al., 2015). The increased $\dot{V}O_{2RM}$ in females is likely explained by an increased prevalence of EFL and a greater WoB for a given $\dot{V}_E$, predominantly due to anatomical differences in lung and airway size compared to males (Dominelli et al., 2018).

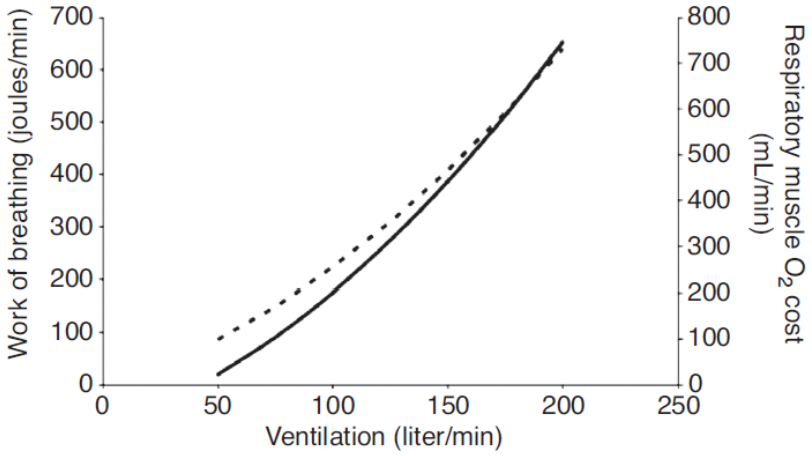


Figure 2.4. The relationship between ventilation and work of breathing (solid line) and the oxygen cost of breathing (dotted line) during exercise based on data from Aaron et al. (1992a), (Aaron et al., 1992b). Figure taken from Sheel and Romer (2012).

Similarly to the locomotor limb muscles, the contracting respiratory musculature requires sufficient perfusion to meet the $\dot{V}O_2$ demand. Due to the anatomical arrangement and complex vascular network, direct determination of respiratory muscle blood flow has not been possible in humans performing exercise. In maximally-exercising ponies, injection of radionuclide-labelled microspheres into the left ventricle has demonstrated that blood flow increases by more than 22-fold to the primary inspiratory and expiratory muscles relative to resting values, with ~15% of total cardiac output delivered to the respiratory muscles (~8% inspiratory vs. ~6% expiratory muscle) (Manohar, 1990). In humans, extrapolation of the fall in cardiac output exhibited in response to unloading the inspiratory WoB [via proportional assist ventilation (PAV)] during maximal exercise suggested that the respiratory muscles of fit adult males require a similar fraction of total cardiac output (~15%) (Harms et al., 1998). However, the less negative intrathoracic pressure exhibited during PAV and the consequent mechanical influence on stroke volume and cardiac output, may slightly confound this estimate. More recent methodological advances have resulted in the indirect assessment of respiratory intercostal muscle blood flow, by combining near infrared spectroscopy and the light-absorbing tracer indocyanine green dye

(Guenette et al., 2008). Such techniques have demonstrated that intercostal muscle blood flow increases progressively with ventilation during isocapnic hyperpnoea (Guenette et al., 2008, Vogiatzis et al., 2009) and is highly correlated to cardiac output, transdiaphragmatic pressure (Pdi), and the WoB (Guenette et al., 2008). However, during incremental exercise, Vogiatzis et al. (2009) reported that intercostal muscle blood flow increased towards a $\dot{V}_E$ of ~60-100 $L \cdot min^{-1}$, before subsequently falling to near-resting levels (~25 $ml \cdot min^{-1} \cdot 100g^{-1}$) as ventilation reached maximal values. In comparison, intercostal muscle blood flow was substantially higher at the equivalent ventilation of isocapnic hyperpnoea (~75 $ml \cdot min^{-1} \cdot 100g^{-1}$). The authors concluded that during maximal exercise blood flow does not increase to the respiratory intercostal muscles, potentially due to an increase in global sympathetic outflow and an inability of the circulatory system to supply the demands of both the locomotor and respiratory muscles. In contrast, it appears that vasodilatory capacity of the diaphragm in maximally-exercising equines is maximised as infusion of adenosine (a potent vasodilator) into the pulmonary artery elicits no further increase in diaphragm blood flow (Manohar, 1986). It has been suggested that the diaphragm may be less sensitive to the effects of increased sympathetic stimuli. Indeed, Aaker and Laughlin (2002) demonstrated that the degree of vasoconstriction in arterioles isolated from the diaphragm was significantly less in response to adrenergic stimulation in comparison to that exhibited in peripheral skeletal muscle. Therefore, while there is certainly a substantial demand for cardiac output from the respiratory muscles during maximal exercise, there may be heterogeneity in blood supply between respiratory muscles caused by differential responsiveness to sympathetic outflow. The potential hierarchy of blood flow distribution between the respiratory and locomotor muscles during maximal exercise is discussed later (*see section* 2.6.3).

2.2.4. Non-Ventilatory Responses to Exercise

In addition to their important ventilatory roles during exercise, there is evidence to suggest that the respiratory muscles perform additional non-ventilatory tasks that may contribute to their metabolic demands. Indeed the diaphragm may contribute to postural support, particularly in response to brief, intermittent disturbances to trunk stability (Hodges and Gandevia, 2000). However, during high-intensity whole-body exercise, evidence suggests that the diaphragm's contribution to ventilation may be protected by prioritising pontomedullary respiratory input and offloading its postural contribution to other muscle groups (Hodges et al., 2001). Active contraction of the expiratory abdominal muscles during whole-body exercise serves to maintain posture (Abraham et al., 2002), and is responsible for flexion and rotation of the vertebral column (Cresswell et al., 1992). In addition, tonic activity of the expiratory abdominal muscles during whole-body exercise reduces abdominal compliance, providing a stiff 'abdominal fulcrum' which enhances diaphragm contractility (Abdallah et al., 2017). Unlike the diaphragm however, the expiratory muscles do not appear to be subject to the same respiratory modulation of such non-ventilatory roles. It is possible that the metabolic requirements of performing such non-ventilatory tasks for the expiratory muscles may contribute to the development of fatigue at a lower exercise intensity or during tasks engendering lower exercise ventilations in comparison to the diaphragm. For example, expiratory muscle fatigue but not diaphragmatic fatigue (*see section* 2.3) develops in response to severe-intensity arm-crank exercise eliciting a peak $\dot{V}_E$ of only 99 ± 19 $L \cdot min^{-1}$ (Tiller et al., 2017).

2.2.5. Cardiovascular Responses to Exercise

An example of the cardiovascular response to incremental whole-body cycling exercise is presented in Figure 2.5. With the progressive increase in exercise intensity, there is a substantial increase in cardiac output to deliver increased blood flow to the locomotor muscles in proportion to metabolic demand. This increase in

cardiac output is met, in part, by an increase in heart rate, which is facilitated by a combination of withdrawal of vagal tone (up to ~100 beats·min^{-1}) and a catecholamine-driven increase in sympathetic nervous activity (Robinson et al., 1966). There is also an increase in cardiac stroke volume at the onset of exercise, which is achieved via an enhanced myocardial contractility and augmented venous return by contraction-induced central translocation of blood from the lower extremities (i.e. skeletal muscle pump). During submaximal exercise, the increase in cardiac output is closely coupled to the change in $\dot{V}O_2$ so that systemic O_2 delivery and utilisation are well matched. Indeed, based on a normal O_2 haemoglobin concentration and O_2 affinity, ~200 ml of O_2 are transported per litre of arterial blood, requiring a cardiac output of ~5 L to transport 1 L of blood (Joyner and Casey, 2015). Therefore, for each 1 L increase in $\dot{V}O_2$ during exercise, cardiac output increases by ~fivefold. In addition to the relatively linear increase in cardiac output from rest to moderate exercise intensities, there is a progressive rise in locomotor muscle blood flow and vascular conductance, and a rise in mean arterial pressure. However, due to marked vasodilation in the skeletal muscles, the change to mean arterial pressure remains relatively modest (usually <20-30 mmHg) despite a rise in cardiac output of between four- and eight-fold compared to resting values.

As exercise intensity reaches to ~70-90% of peak power, there is a plateau in locomotor blood flow and conductance. This cardiovascular response to higher intensity exercise appears to be driven by a blunting of cardiac output and the overriding influence of increased vasoconstrictor tone for the maintenance of systemic blood pressure (Saltin et al., 1998). Indeed, it is well established that the requirement for increased blood flow by contracting skeletal muscle, predominantly evoked by the release of vasodilatory metabolites (e.g. NO, CO_2, ATP, K^+), must compete with reflex vasoconstrictor mechanisms (Joyner and Casey, 2015). It appears that the exponential rise in muscle sympathetic nerve activity from mild to

severe exercise intensities may overcome such vasodilatory signals, contributing to the plateau in stroke volume, cardiac output, and locomotor limb blood flow and O_2 delivery (Ichinose et al., 2008).

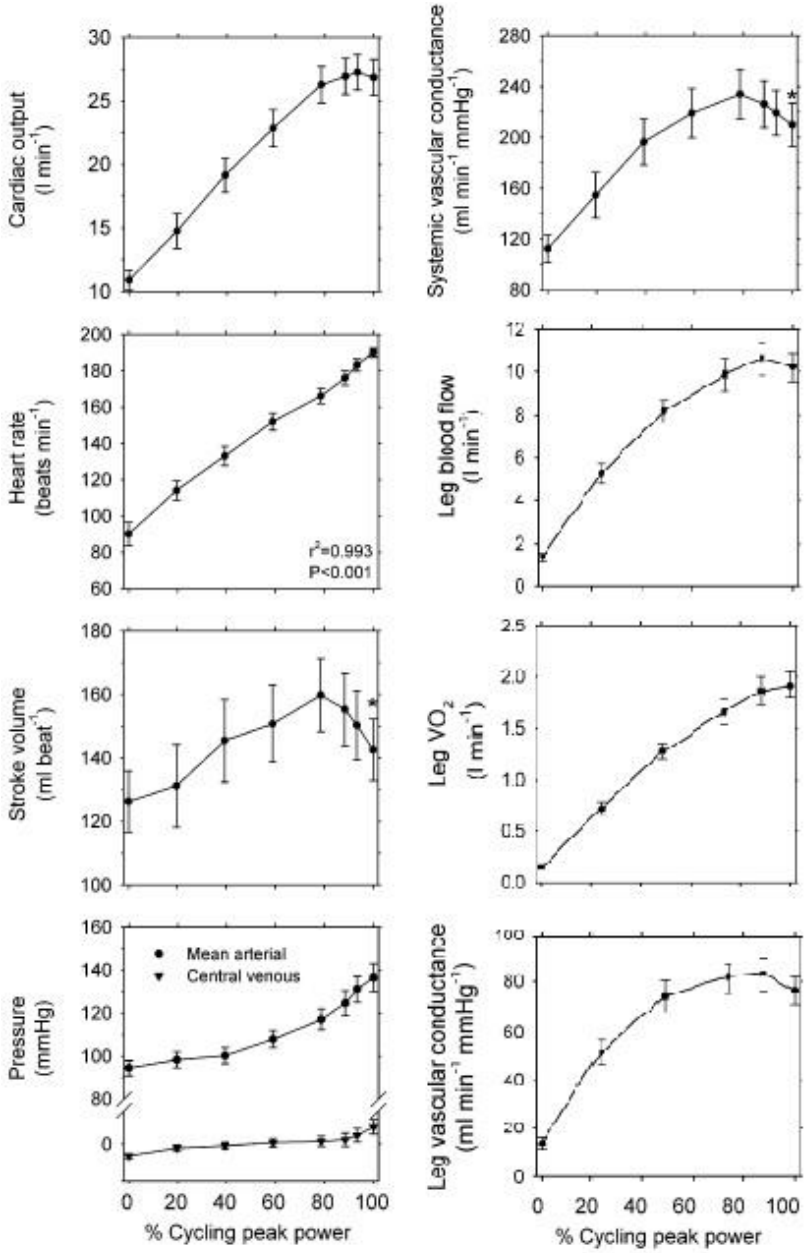


Figure 2.5. The cardiovascular responses to incremental cycling exercise. Figure modified from Mortensen et al. (2005).

2.2.6. Summary

It is clear that the ventilatory response to heavy- and severe-intensity exercise engenders a substantial work and cost of breathing, which demands up to ~15% of cardiac output and ~15-25% of total $\dot{V}O_2$ at maximal exercise in highly trained males

and females. Despite a phenotype and morphology of the respiratory muscles that is adapted to enhance muscular endurance, fatigue of these muscles may occur under conditions of an inadequate energy supply to meet demand. Such sources of inadequate energy supply include: insufficient respiratory muscle blood flow, low arterial O_2 content, impaired O_2 extraction, and alterations in substrate concentration.

2.3. RESPIRATORY MUSCLE FATIGUE

2.3.1. Definitions of Skeletal Muscle fatigue

In this thesis, skeletal muscle fatigue is defined as a reduction in the force- and/or velocity-generating capacity of a muscle that has been under load and is relieved with rest (NHLBI, 1990). As such, respiratory muscle fatigue is considered evident from a reduction in pressure production relative to baseline values, whereby pressure is measured during maximal volitional inspiratory or expulsive efforts, or objectively via measurement of the transdiaphragmatic or gastric twitch pressure response to artificial nerve stimulation of the phrenic and thoracic nerve roots, respectively.

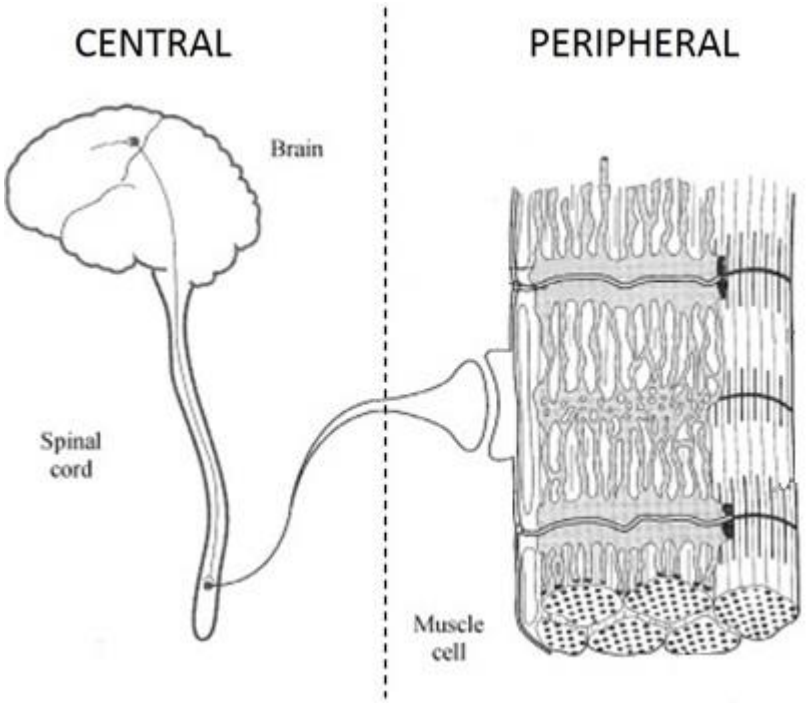


Figure 2.6. A schematic illustration of the sites of central and peripheral neuromuscular fatigue. Figure adapted from Boyas and Guevel (2011).

2.3.2. Central Fatigue

Central fatigue is defined as a reduction in muscle contractility caused by processes within the central nervous system, originating proximally to the neuromuscular junction (*see* Figure 2.6) (Taylor et al., 2016). The development of central fatigue results in a decrease in neural drive to the muscle, manifesting as a reduction in voluntary activation. To assess central fatigue of the respiratory muscles the interpolated twitch technique has been used, whereby impairments in voluntary activation are estimated by measuring changes to the twitch amplitude performed during a maximal voluntary contraction, normalised to resting post-contraction twitch pressure (Merton, 1954) (*see* Chapter 3.8.2). Because central fatigue recovers rapidly following short-duration high-intensity exercise (typically <2 min) (Carroll et al., 2017), measurements are usually performed immediately after the task. A reduction in diaphragm voluntary activation has been previously reported in response to inspiratory resistive loading (Bellemare and Bigland-Ritchie, 1987), but few studies have assessed changes in diaphragm voluntary activation via the interpolated twitch technique in response to whole-body exercise. For the expiratory abdominal muscles, central fatigue does not appear to develop as voluntary activation is unchanged in response to severe-intensity constant-power exercise (Taylor et al., 2006). However, pre- to post-exercise reductions in voluntary activation for the locomotor muscles increase in relation to tolerable exercise duration (Thomas et al., 2016); therefore, it remains to be determined whether central fatigue of the respiratory muscles develops after prolonged heavy-intensity exercise.

Impairments in neural activation of the diaphragm originating from the supraspinal region have also been measured by inspecting changes in motor evoked potentials elicited by transcranial magnetic stimulation of the motor cortex. Previous studies report that in response to incremental or moderate-intensity constant-power exercise diaphragm motor-evoked potentials are depressed, reflecting a decreased excitability

of the motor cortex (Verin et al., 2004, Dayer et al., 2007, Jonville et al., 2005). However, the functional consequences of a fall in motor-evoked potential remain to be determined. Indeed, such reductions in diaphragm motor-evoked potential appear to be unrelated to diaphragmatic work (Dayer et al., 2007), and occur despite the absence of peripheral neuromuscular fatigue or any ergogenic effect of unloading inspiratory muscle work in response to incremental exercise (Romer et al., 2007).

2.3.3. Peripheral Fatigue

Peripheral muscle fatigue is defined as a reduction in muscle contractility caused by processes at or distal to the neuromuscular junction (*see* Figure 2.6) (Gandevia, 2001). An impairment in excitation-contraction (E-C) coupling (i.e. the conversion of an action potential to sarcomere cross-bridge formation) appears to be a major cause of peripheral muscle fatigue. This may be attributable to several different factors, including: 1) diminished action potential propagation to the T-tubules; 2) a failure of coupling between the action potential and consequent calcium release; and 3) dysregulation of calcium within the contractile elements (Jones, 1996). Failure of E-C coupling may result in the primary loss of force or pressure at high frequencies of stimulation (50-100 Hz) or in response to low frequencies of stimulation (1-30 Hz).

2.3.3.1. High-frequency fatigue

The development of high-frequency fatigue is characterised by a loss of force or pressure in response to high-frequency stimulation (50-100 Hz). Evidence suggests that the accumulation of extracellular potassium (K^+) in the interfibre spaces is a primary cause of high frequency fatigue, by preventing action potential transmission across the sarcolemma and impairing conduction within the T-tubules (Jones, 1996). Indeed, in skinned muscle fibres high-frequency stimulation elicits a non-uniform calcium (Ca^{2+}) distribution whereby Ca^{2+} is predominantly reduced in the centre compared to the outer part of the muscle fibre, congruent with an impaired T-tubule

transmission (Westerblad et al., 1990). High frequency fatigue has been reported in the diaphragm following inspiratory resistive loading in response to 50 and 100 Hz electrical phrenic nerve stimulation (Aubier et al., 1981). Because of the technical difficulty and subject discomfort associated with tetanic stimulation at such frequencies, subsequent studies have investigated high-frequency diaphragmatic fatigue using either electrically or magnetically evoked paired pulses, by varying the interstimulus duration to achieve the desired frequency. Using this technique, high-frequency (50-100 Hz) fatigue of the diaphragm has been reported in response to incremental and severe-intensity constant-power exercise (Babcock et al., 1998, Romer et al., 2007). However, it is questionable whether the development of high-frequency fatigue, elicited by stimulation frequencies in excess of most human motor unit firing frequencies (~30 Hz), represents a 'normal' mechanism of fatigue and as such it remains debated whether high-frequency fatigue has any 'real' physiological impact (Jones, 1996).

2.3.3.2. Low Frequency Fatigue

Low frequency fatigue refers to a decrease in force or pressure generation in response to low-frequency stimuli (1-30 Hz). Such fatigue is characterised by a slow recovery, which may take several hours for complete restoration of force or pressure despite the absence of a gross mechanical or electrical disturbance to the muscle (Carroll et al., 2017). The underlying mechanisms of impaired E-C coupling occurring distal to the sarcolemma (i.e. in the absence of a change in t-tubule propagation) are thought to be caused by impairments in: Ca^{2+} release from the sarcoplasmic reticulum, myofibrillar Ca^{2+} sensitivity, or the force production of active actin-myosin cross-bridges (Place et al., 2010). Such derangements in cellular contractile function occurring in response to exhaustive whole-body exercise can be caused by a variety of factors which are dependent on the modality, intensity and duration of the task.

In response to severe-intensity exercise, the utilisation of non-oxidative energy systems including anaerobic glycolysis and hydrolysis of phosphocreatine (PCr) results in the accumulation of metabolites, namely lactate, H^+, and inorganic phosphate (Pi). Although the accumulation of lactate and the consequent muscle acidosis shares a similar time-course to reductions in muscular force (Allen et al., 2008), there is a poor correlation between changes in muscle pH and contractile function in skinned muscle fibres (Westerblad et al., 1997), and the recovery of force production occurs more quickly than muscle pH following exhaustive exercise (Sahlin and Ren, 1989). Therefore, muscle acidosis is unlikely to be the primary mediator of low-frequency peripheral fatigue.

The breakdown of PCr and subsequent accumulation of Pi has multiple effects within muscle cells that could influence contractility, predominantly by reducing the availability and sensitivity of Ca^{2+} and decreasing actin-myosin cross-bridge formation. Indeed, increasing Pi in permeabilised fibers elicits a decrease in tetanic force production (Coupland et al., 2001) predominantly attributed to a decrease in cross-bridge formation (Allen et al., 2008). It has also been suggested that entry of Pi into the sarcoplasmic reticulum may impair Ca^{2+} release by direct inhibition of ryanodine receptors or via precipitation with free Ca^{2+} (Allen and Westerblad, 2001). Moreover, the accumulation of Pi is directly implicated in a reduction in myofibrillar Ca^{2+} sensitivity by limiting its binding to troponin C (Martyn and Gordon, 1992). Therefore, there is increasing evidence implicating Pi as a leading factor in the impaired E-C coupling associated with low-frequency fatigue.

The release of reactive oxygen and nitrogen species (ROS) during exercise may contribute to low-frequency peripheral fatigue, particularly by decreasing myofibrillar Ca^{2+} sensitivity (Andrade et al., 2001, Moopanar and Allen, 2005), although effects are both time and concentration dependent. Indeed, it is speculated that brief

exposure to ROS may enhance force production during the initial stages of exercise, but prolonged exposure can negatively affect contractile function (Reid et al., 1992, Place et al., 2010). In response to high-load fatiguing inspiratory resistive breathing that induces diaphragmatic fatigue, systemic oxidative stress is elevated as evidenced by an increase in plasma F_2-isopropanes (Briskey et al., 2020). Further evidence that ROS may be implicated in the development of respiratory muscle fatigue is that N-acetylcysteine supplementation (a potent antioxidant) attenuates the magnitude of low-frequency diaphragmatic fatigue in response to repetitive diaphragm contraction in anaesthetised rabbits (Shindoh et al., 1990), and blunts global inspiratory muscle fatigue (~14%) versus placebo conditions during constant-power cycling in healthy adults (Kelly et al., 2009).

2.3.4. Summary

There is some, but limited, evidence to suggest that central and high-frequency peripheral muscle fatigue may occur in the diaphragm in response to exercise or resistive loaded breathing. However, the primary cause the reduction in respiratory muscle contractility is more likely low-frequency fatigue. Such impairments in muscular force/pressure production appear to be primarily caused by the accumulation of metabolites (i.e. Pi) or ROS associated with fatigue and their consequent inhibitory effects on Ca^{2+} release, sensitivity and actin-myosin cross-bridge formation. However, to date the vast majority of studies have investigated the cellular mechanisms of fatigue in limb locomotor muscle fibre preparations, and it is possible that there may be differences in the cellular mechanism(s) that underpin fatigue of the diaphragm and expiratory abdominal muscles.

2.4. EXERCISE-INDUCED RESPIRATORY MUSCLE FATIGUE

2.4.1. Volitional Assessment of Exercise-Induced Respiratory Muscle Fatigue

Exercise-induced respiratory muscle fatigue has previously been assessed by measuring the pre- to post-exercise reduction in the volitional production of maximal inspiratory (MIP) or expiratory mouth pressure (MEP), and maximal transdiaphragmatic (Pdi_{MAX}) or gastric pressure (Pga_{MAX}) (*see section* 3.8). Benefits of measuring MIP and MEP are that it does not require invasive instrumentation, and it allows for the assessment of respiratory muscle function immediately after exercise. In recreationally active, healthy humans, exhaustive exercise at >85% of $\dot{V}O_{2max,}$ as well as prolonged whole-body endurance exercise (e.g., marathon running), elicits a 5-30% reduction in MIP and MEP (Loke et al., 1982, Lomax and McConnell, 2003, Griffiths and McConnell, 2007, Lomax et al., 2013, Smith et al., 2014, Wuthrich et al., 2015). In isolation, such reductions in MIP and MEP reflect global inspiratory and expiratory muscle fatigue, and the relative contribution of central and peripheral mechanisms cannot be distinguished. An important consideration of volitional changes in respiratory muscle pressure production is the requirement of a consistent technique and maximal effort from subjects; therefore such measures may be susceptible to changing subject motivation. Moreover, muscle length (i.e. lung volume) is self-estimated by the subject, which without independent verification (i.e. by measuring Poes), may introduce significant error into the measurement. Indeed, it is well established that particularly at FRC, small changes in lung volume have a large effect on pressure generation (Agostoni, 1964).

2.4.2. Non-Volitional Assessment of Exercise-Induced Respiratory Muscle Fatigue

2.4.2.1. Exercise-Induced Inspiratory Muscle Fatigue

Pressure development across the diaphragm (Pdi) can be estimated as the difference between gastric and oesophageal pressure (Pga and Poes, respectively).

In response to cervical or antero-bilateral magnetic or electrical stimulation of the phrenic nerve roots, diaphragm muscle function can be objectively assessed by analysis of the amplitude and secondary characteristics of the diaphragm twitch response (Pdi_{tw}) (*see section* 3.8.2). In a seminal study by Johnson et al. (1993), the Pdi_{tw} response to electrical bilateral stimulation of the phrenic nerves was measured in twelve young healthy males before and after either treadmill running or cycling at a work rate eliciting 80-85% and 90-95% of $\dot{V}O_{2max}$. A pre-to post-exercise reduction in mean Pdi_{tw} amplitude across stimulation frequencies of 1, 10 and 20 Hz was reported following exercise at 80-85% of $\dot{V}O_{2max}$ (−15 ± 5%) and 90-95% of $\dot{V}O_{2max}$ (−20 ± 3%), although the magnitude and consistency of diaphragmatic fatigue was lower at the lower work rate. In combination with several subsequent studies, it is now well established that a reduction in Pdi_{tw} (1 Hz) of 15-35% occurs in healthy males in response to exhaustive high-intensity cycling or running (*see* Figure 2.7 for example) (Johnson et al., 1993, Babcock et al., 1996, Mador et al., 1993, Guenette et al., 2010, Archiza et al., 2018, Wuthrich et al., 2014a). Although the vast majority of previous studies have recruited solely male subjects, it is now clear that females also exhibit diaphragmatic fatigue in response to exercise, although the magnitude of fatigue is blunted versus males (−21 ± 2% vs. −31 ± 2%; Pdi_{tw} at 1Hz) (Guenette et al., 2010). Such sex-based differences in diaphragm fatigability likely relate to differences in the ventilatory response, respiratory muscle recruitment patterns, muscle morphology, and substrate utilisation. For example, females exhibit a smaller increase in minute ventilation and accessory respiratory muscle recruitment in comparison to males (Guenette et al., 2010), and typically rely on an increased contribution of beta oxidation of fatty acids to meet the energy requirement of exercise (Hicks et al., 2001).

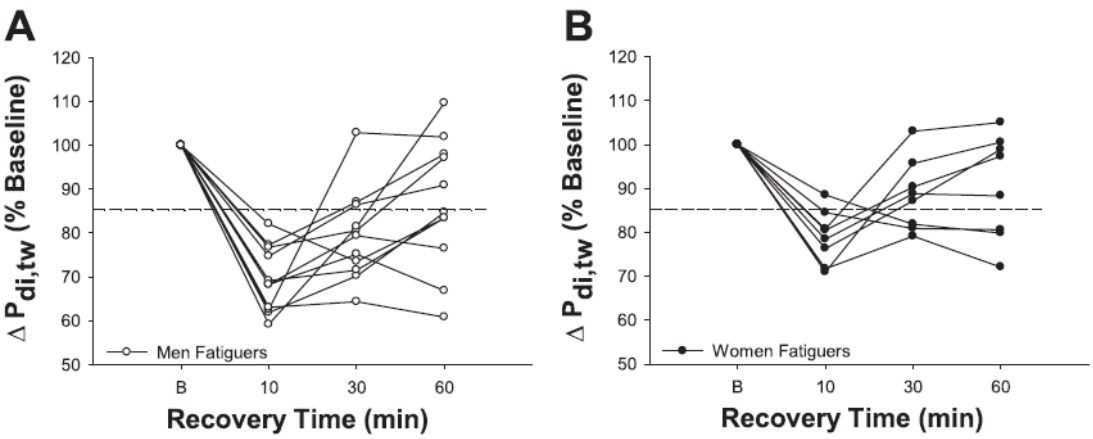


Figure 2.7. An example of the development of inspiratory muscle fatigue in males and females in response to constant-power cycling at 90% of peak incremental power. Transdiaphragmatic twitch pressure (Pdi_{tw}) was measured in response to 1 Hz stimulation of the phrenic nerves at baseline ('B' on x-axis), and 10, 30, and 60 min after exercise termination. Figure taken from Guenette et al. (2010).

2.4.2.2. Exercise-Induced Expiratory Abdominal Muscle Fatigue

Magnetic stimulation of the thoracic nerve roots and interrogation of the gastric twitch pressure (Pga_{tw}) response provides an objective, non-volitional measure of expiratory abdominal muscle function. Using this nerve stimulation technique, Taylor et al. (2006) assessed the presence of expiratory muscle fatigue in healthy adult males following exhaustive cycling at 90% of peak power. In response to exercise, the authors identified a mean reduction in the gastric pressure response to magnetic thoracic nerve stimulation (Pga_{tw}) of 25 ± 4% across a series of stimulation frequencies (1-25 Hz) (*see* Figure 2.8). The cause of such low-frequency fatigue was likely due to peripheral mechanisms, as voluntary activation of the expiratory muscles was unaffected. Moreover, recovery of the Pga_{tw} following 30 minutes of rest, and the lack of change in M wave characteristics, suggested that neither muscle damage nor impaired action potential transmission were responsible for the impairment in expiratory muscle function, but instead likely an inhibition in excitation-contraction coupling. Similarly, Verges et al. (2006) reported a significant but slightly smaller

magnitude of expiratory muscle fatigue (−13 ± 7% Pga_{tw}) immediately following exhaustive exercise at 85% of peak ramp incremental power.

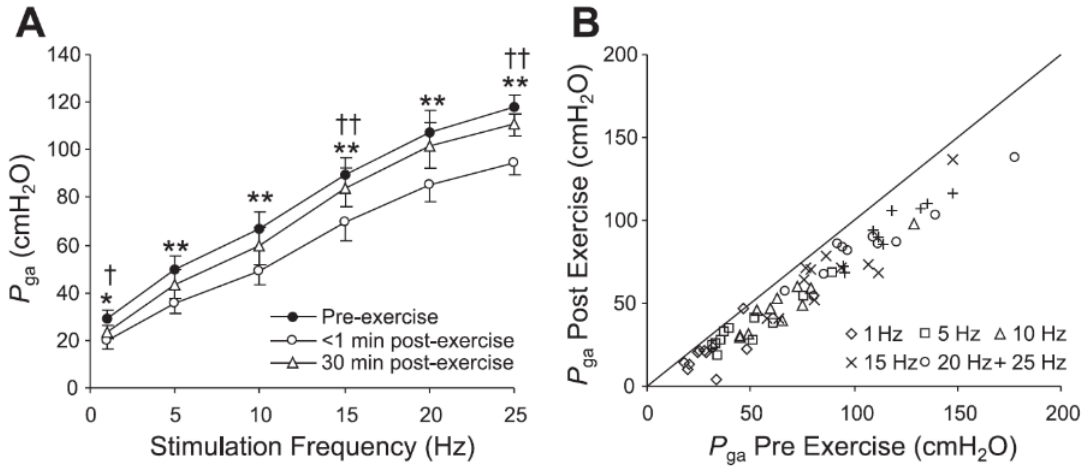


Figure 2.8. An example of the development of expiratory abdominal muscle fatigue (i.e. reduction in gastric twitch pressure) across 1-25 Hz stimulation of the thoracic nerves in response to exhaustive constant-power cycling at >90% of $\dot{V}O_{2peak}$. Panel A shows group mean responses (*n* = 11) before, and up to 30 min post-exercise and Panel B shows an identity plot of individual response (pre vs. immediately post-exercise). Figure taken from Taylor et al. (2006).

2.4.3. Factors Affecting the Development of Exercise-Induced Respiratory Muscle Fatigue

There appears to be two critical determinants of exercise-induced respiratory muscle fatigue: 1) an elevated and sustained work (or power) of breathing; and, 2) a limited availability of cardiac output to supply the metabolic demands of the locomotor and respiratory muscles. Indeed, exercise-induced diaphragmatic fatigue is abolished when the inspiratory power of breathing is reduced by 40-50% during severe-intensity exercise (Babcock et al., 2002). Similarly, previous studies have reported a significant correlation between diaphragm pressure production and the magnitude of post-exercise diaphragmatic fatigue (Johnson et al., 1993), suggesting that individuals that incurred the greatest diaphragm force output during exercise exhibited the greatest magnitude of fatigue. The importance of the degree of the respiratory force output for the development of fatigue is also evident from inspiratory resistive loading studies. Indeed, it is well established that time to task failure is

shortened (and presumably the rate of fatigue augmented) when inspiratory resistive loading is performed at higher pressure outputs at the same inspiratory duty cycle. This is most likely due to the progressive reduction in diaphragm blood flow that occurs at higher diaphragm force outputs secondary to a greater compression of the phrenic arteries and increased ischemia (Bellemare et al., 1983, Bellemare and Grassino, 1982). However, high respiratory muscle pressure production alone during exercise appears insufficient to elicit respiratory muscle fatigue. Indeed, in a study by Babcock et al. (1995b), diaphragmatic fatigue did not occur when resting humans voluntarily mimicked the magnitude, frequency, and duration of diaphragmatic work experienced during exercise. Furthermore, fatigue of the diaphragm was not present until the resting subjects produced transdiaphragmatic pressures that were almost twofold greater than achieved during the fatiguing exercise trial. The most likely explanation for these findings is that during rested hyperpnoea there is an adequate supply of blood flow to meet the metabolic demands of the diaphragm, whereas during exercise there appears to be a competition for the limited available cardiac output between the respiratory and locomotor muscles (Sheel et al., 2018a). The importance of the degree of respiratory muscle work and the competition for blood flow therefore raises the possibility that the magnitude of respiratory muscle fatigue may be blunted in response to lower intensity exercise.

2.4.3.1. Exercise Intensity

During heavy-intensity exercise (i.e. at power outputs between LT and CP), it appears that the normally-occurring demand for oxygen uptake ($\dot{V}O_2$) and cardiac output does not elicit a competition for blood flow between the respiratory and locomotor muscles. For example, in response to cycling at a power output evoking ~75-80% of $\dot{V}O_{2peak}$, unloading the inspiratory power of breathing via proportional assist ventilation had no effect on noradrenaline spillover, leg blood flow ($\dot{Q}_L$) or leg vascular resistance (LVR) (Wetter et al., 1999). It has been suggested that the lower absolute pressure

demands of the respiratory muscles combined with the absence of a competition for blood flow between the locomotor and the respiratory muscles may therefore result in the absence, or smaller magnitude of respiratory muscle fatigue in lower intensity exercise. For example, Johnson et al. (1993) reported that $\dot{V}O_2$ was negatively associated with the diaphragmatic fatigue index (r = −0.67) (Figure 2.9), and that the likelihood of developing diaphragmatic fatigue increased when 'exercise intensity' exceeded 80-85% of $\dot{V}O_{2max}$.

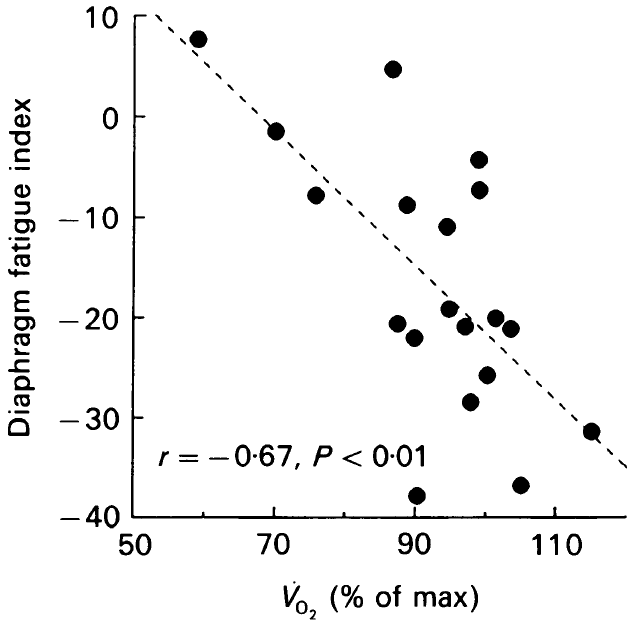


Figure 2.9. The relationship between the relative oxygen consumption (% of $\dot{V}O_{2max}$) during exercise vs. the magnitude of exercise-induced diaphragmatic fatigue. Figure taken from Johnson et al. (1993).

Similarly, the magnitude of diaphragmatic fatigue was slightly greater in response to a 15- vs. 30-minute cycling or running time trial (−24 ± 8% vs. −20 ± 9%) (Wuthrich et al., 2014a); it was suggested that the augmented respiratory muscle power (+27%) and greater competition for blood flow were leading factors responsible for the greater severity of fatigue. However, perhaps due to early suggestions of the importance of exercise intensity, there remains a paucity of data objectively investigating the development of inspiratory or expiratory muscle fatigue in response to power outputs eliciting <80% of $\dot{V}O_{2max}$ (Janssens et al., 2013), or systematically in response to

heavy vs. severe-intensity constant-power exercise (i.e. at power outputs between LT and CP vs. power outputs >CP). Indeed, in the study by Johnson et al. (1993), there was a vast range in the average $\dot{V}O_2$ across the exercise trials (60-112% of $\dot{V}O_{2max}$), modality was not standardised, and relatively few subjects exercised at power outputs eliciting <85% of $\dot{V}O_{2max}$ (*n* = 3) (*see* Figure 2.9). Moreover, prescribing exercise-intensity as a percentage of $\dot{V}O_{2max}$ may fail to standardise physiological responses between subjects, as there may be drastic inter-individual differences in the relative point at which important physiological thresholds (i.e. LT and CP) occur as a percentage of peak values (Poole et al., 1988, Katch et al., 1978). For example, LT and CP may vary between-subjects from 40-85% and 50-95% of $\dot{V}O_{2max}$, respectively (Murgatroyd et al., 2014, Iannetta et al., 2020). This method of prescribing exercise-intensity is also particularly problematic for exercise performed at 75-85% of $\dot{V}O_{2max}$ where there is a significant chance of 'domain overlap' (Iannetta et al., 2020, Jamnick et al., 2020). As a result, there is a high probability that subjects exhibited varying physiological responses when exercise was set at 80-85% of $\dot{V}O_{2max}$ in the study by (Johnson et al., 1993), which may have affected the magnitude of fatigue. Therefore, to date the effect of exercise intensity (and specifically intensity domain), on the development of inspiratory and expiratory muscle fatigue remains to be systematically investigated.

2.4.3.2. Tolerable Exercise Duration

In addition to an elevated force output of the respiratory muscles, there appears to be a minimum cumulative work history (i.e. a minimum exercise duration at an elevated WoB) required to evoke respiratory muscle fatigue. Exhaustive incremental exercise does not cause fatigue of the diaphragm, most likely because the duration of exercise at a very-high ventilation and work of breathing is short (Romer et al., 2007, Verin et al., 2004, Levine and Henson, 1988). For example, in response to a slow incremental cycling test (98 W + 11 W·min^{-1}; T_{LIM} ~24 min), Romer et al. (2007)

reported no significant change in pre- to post-exercise Pdi_{tw} at 1 Hz (57 ± 6 cmH_2O vs. 50 ± 4 cmH_2O; P = 0.051), potentially because subjects cycled at >90% of $\dot{V}O_{2max}$ for only ~4 min. However, diaphragm function was only assessed in 5 subjects, the reduction in Pdi_{tw} closely approximately significance, and impairments in diaphragm function were noted at higher frequencies of stimulation (50 and 100 Hz). Moreover, several studies have reported volitional indications of respiratory muscle fatigue in response to ramp incremental cycling or running exercise, evidenced by a pre- to post-exercise decrease in MIP (9-17%) and MEP (13-19%) (Oueslati et al., 2018, Oueslati et al., 2017, Oueslati et al., 2016). In response to constant-power exercise, a reduction in Pdi_{tw} (−24 ± 6%) has been reported after only 6 minutes of non-exhaustive severe-intensity exercise (Archiza et al., 2018). Therefore, although respiratory muscle fatigue may develop in response to exercise protocols inducing only short periods of very high respiratory muscle work, the magnitude and consistency of fatigue likely increase as the tolerable duration of high-intensity is prolonged.

2.4.4. Summary

The data presented in this section demonstrates that exercise-induced inspiratory and expiratory muscle fatigue develops in healthy individuals under specific exercise conditions. Indeed, a vast number of studies have reported reductions in Pdi_{tw} and Pga_{tw} of 15-35% in response to high-intensity exercise (>85% of $\dot{V}O_{2max}$). The primary factors responsible for the development of respiratory muscle fatigue appear to be a sustained increase in the work incurred by the respiratory muscles in combination with a competition for cardiac output between the limb locomotor and respiratory muscles. Therefore, it has been suggested that the lack of competition for blood flow and the lower power of breathing during less-intense exercise, or the lower cumulative respiratory work history during short-duration exercise, may lead to a lower prevalence or blunted magnitude of respiratory muscle fatigue. However, to

date systematic investigation of the effect of exercise-intensity domain, or exercise duration in the same domain, has not been performed.

2.5. FUNCTIONAL CONSEQUENCES OF RESPIRATORY MUSCLE FATIGUE

One of the primary consequences of exercise-induced respiratory muscle fatigue is an impairment in exercise tolerance. In an attempt to determine the contribution of respiratory muscle fatigue to exercise intolerance, previous studies have measured the tolerable duration of exercise either: 1) with and without prior induction of respiratory muscle fatigue; and 2) with and without experimental manipulation (*increasing and decreasing*) of the power of breathing during high-intensity exercise.

2.5.1. Effects of Inspiratory Muscle Unloading and Pre-fatigue on Exercise Tolerance

Pre-fatigue of the inspiratory muscles has been previously induced via inspiratory threshold/resistive loading performed until task failure or by isocapnic hyperpnoea sustained for a set time period. Several studies have demonstrated that inspiratory muscle pre-fatigue impairs subsequent exercise time to the limit of tolerance (T_{LIM}) by 14-23% in both male and female subjects (Welch et al., 2018a, Wuthrich et al., 2013, Mador and Acevedo, 1991b, Martin et al., 1982). For example, following the induction of diaphragmatic fatigue (−23-25% Pdi_{tw}) after unsustainable inspiratory pressure-threshold loading at 60% of Pdi_{MAX}, Welch et al. (2018a) observed a ~15% reduction in severe-intensity constant-power T_{LIM} in both males and females in comparison to control conditions (males: 10.9 ± 3.5 min vs. 13.0 ± 3.0 min; females: 10.1 ± 2.4 min vs. 12.2 ± 3.3 min). Such previous studies have also reported a concomitant increase in submaximal sensations of dyspnoea and leg discomfort, suggesting that a cardiorespiratory interaction may be contributing to the change in exercise time (*see section* 2.6). In contrast, several studies have also reported no change in exercise tolerance after inspiratory muscle pre-fatigue (Dodd et al., 1989,

Spengler et al., 2000, Sliwinski et al., 1996); however, inspiratory muscle fatigue was not always objectively verified prior to exercise, and may not have been of a sufficient magnitude prior to the initiation of exercise. Indeed, it is well established that task failure from inspiratory resistive loading may occur in the absence of inspiratory muscle fatigue (Gorman et al., 1999, McKenzie et al., 1997).

Proportional assist ventilation (PAV) has been used to unload the inspiratory power of breathing during exercise, which gives insight into the impact of the 'normally-occurring' respiratory muscle work on exercise performance. Using this technique, Harms et al. (2000) reported that a 40-50% decrease in inspiratory muscle power resulted in a 14 ± 5% increase in severe-intensity exercise time (~90% $\dot{V}O_{2max}$) versus control conditions; in contrast, increasing inspiratory muscle power by adding a resistance to inspiration decreased T_{LIM} by 15 ± 3%. However, such ergogenic effects of inspiratory muscle unloading are not universal findings. Indeed, both Marciniuk et al. (1994) and Krishnan et al. (1996) demonstrated no difference in exercise time between control conditions and inspiratory unloading by PAV. Although it had been suggested that the aforementioned studies may not have found any effect due to a lower exercise-intensity (Sheel and Romer, 2012), both trials were performed in the severe-intensity domain with a T_{LIM} of 10 ± 1 min and 11 ± 1 min respectively (i.e. tolerable durations associated with the consistent development of diaphragm and expiratory muscle fatigue). However, PAV in the latter studies may have been less effective due to a lower aerobic fitness in the subjects recruited, diminishing the O_2 cost of breathing and therefore the effectiveness of reducing the power of breathing. For example, the O_2 cost of breathing is ~15-16% of $\dot{V}O_{2max}$ in highly fit athletes versus ~8-10% of $\dot{V}O_{2max}$ in moderately fit individuals (Aaron et al., 1992b).

2.5.2. Effect of Expiratory Muscle Pre-Fatigue on Exercise Tolerance

To determine the effect of expiratory muscle fatigue on exercise tolerance, Taylor and Romer (2008) examined exercise T_{LIM} with and without prior induction of expiratory muscle fatigue. Expiratory muscle fatigue was induced by performance of expiratory resistive loading (ERL) at 60% of MEP until task failure, resulting in pre- to post-ERL decrease of 27 ± 5% in Pga_{tw}. Immediately after the development of expiratory muscle fatigue, participants cycled to exhaustion at 90% of peak power output (P_{peak}). In comparison to the control condition, T_{LIM} was reduced by 33 ± 10% (6.9 ± 2.9 min vs. 9.9 ± 2.9 min). Similarly, Verges et al. (2007) induced expiratory muscle fatigue by 'exhaustive' expiratory loading (i.e. performed until MEP fell below 80% of baseline values), prior to completion of a maximal 12 minute run. In comparison to the control test, the mean distance covered immediately following expiratory muscle pre-fatigue was reduced by ~3 ± 2% (2872 ± 256 m vs. 2957 ± 325 m), and the degree of expiratory muscle fatigue was positively correlated with the reduction in distance achieved (r = 0.73). While there is strong evidence to suggest that expiratory muscle pre-fatigue limits exercise tolerance, no study has successfully reduced (or 'unloaded') expiratory muscle power during exercise, most likely due to greater technical difficulties than for the inspiratory muscles.

2.5.3. Effects of Heliox on Exercise Tolerance

Administration of heliox (HeO_2) during exercise may unload respiratory muscle power by reducing gas viscosity, and consequently reducing turbulent airflow and airway resistance, which may prevent or blunt the degree of expiratory flow limitation (Sheel and Romer, 2012). In response to high-intensity constant-power exercise (85-95% $\dot{V}O_{2max}$), breathing a heliox gas mixture (e.g. 79% HeO_2: 21% O_2) increases peak minute ventilation, $\dot{V}O_{2max}$, and exercise tolerance (Aaron et al., 1985, Powers et al., 1986, Wilson and Welch, 1980). However, heliox appears to have no ergogenic effect for lower intensity exercise. Indeed, in the study by Aaron et al. (1985) rowing

performance time was unchanged from control conditions with heliox at 80% of $\dot{V}O_{2max}$ (24 ± 5 min vs. 24 ± 5 min), whereas exercise time increased by ~40% at 90-95% of $\dot{V}O_{2max}$. The mechanism for such divergent findings may relate to a diminished magnitude of respiratory muscle fatigue and the consequent cardiovascular consequences in response to less-intense exercise (*see section* 2.4.3.1). However, MIP and MEP were reduced from pre-exercise values to a similar extent (~6-7%), regardless of exercise intensity or breathing trial (normoxia vs. heliox) (Aaron et al., 1985). Alternatively, heliox administration may improve high-intensity performance by reducing the sensation of dyspnoea secondary to a reduction in expiratory flow limitation, which is less common during lower intensity exercise.

2.5.4. Summary

Previous evidence from studies that have pre-fatigued the respiratory muscles or manipulated the power of breathing during exercise suggest that respiratory muscle work and/or fatigue may negatively impact severe-intensity exercise tolerance. The next section will discuss the potential mechanisms responsible for a limiting role of respiratory muscle fatigue on exercise performance.

2.6. MECHANISMS BY WHICH RESPIRATORY MUSCLE FATIGUE MAY LIMIT EXERCISE PERFORMANCE

Four primary mechanisms by which respiratory muscle fatigue may limit exercise performance have been explored: 1) a failure to maintain an adequate ventilatory response (alveolar hypoventilation); 2) increased sensations of dyspnoea; 3) metabolic cardiorespiratory interactions; and 4) mechanical cardiorespiratory interactions. Although exercise-induced respiratory muscle fatigue could theoretically induce relative alveolar hypoventilation by causing a failure to sustain the required respiratory muscle pressures, or by substantially increasing dead space ventilation secondary to a highly tachypnoeic breathing pattern, previous data suggests that

ventilation is generally appropriate for metabolic demand in studies where exercise-induced respiratory muscle fatigue is present (i.e. end-tidal pressure of CO_2 and O_2 saturation are maintained at <40 mmHg and >94%, respectively (Romer and Polkey, 2008). Several studies have reported that experimentally pre-fatiguing the inspiratory or expiratory muscles via resistive loaded breathing evokes an increased sensation of dyspnoea during subsequent exercise, which is associated to the reduction in exercise tolerance compared to control conditions (Boyle et al., 2020, Suzuki et al., 1992, Taylor and Romer, 2008). Thus, an increase in the sensation of dyspnoea secondary to the development of respiratory muscle fatigue likely contributes to the impairment in exercise tolerance, the causes of which have been reviewed in detail elsewhere (Sheel et al., 2011, Sheel and Romer, 2012).

2.6.1. Metabolic Cardiorespiratory Reflexes

2.6.1.1. Overview

One of the primary mechanisms by which respiratory muscle work may limit exercise performance is via a respiratory muscle metaboreflex, whereby the development of respiratory muscle fatigue evokes a sympathetically-mediated response characterised by a reduction in locomotor muscle perfusion and O_2 delivery, which may accelerate and/or augment the development of locomotor muscle fatigue (Figure 2.10) (Dempsey et al., 2006). It is hypothesised that the accumulation of metabolites (e.g. lactate, adenosine diphosphate, potassium) secondary to the development of respiratory muscle fatigue stimulates an increased discharge of group III and IV phrenic and expiratory muscle afferents, promoting this efferent sympathetic response (Figure 2.10). Indeed, the respiratory muscles, including the diaphragm, expiratory abdominal muscles, and the accessory inspiratory muscles, are richly innervated with metaboreceptors (Duron, 1981). In resting canines, the infusion of lactic acid into the phrenic (inspiratory) or deep circumflex iliac (expiratory) arteries elicits an increase in mean arterial pressure (MAP) and a decrease in leg blood flow

($\dot{Q}_L$) and leg vascular conductance at rest and during exercise (Rodman et al., 2003). Evidence suggests that this metabolically-induced sympathoexcitation may be triggered by activation of respiratory muscle afferents as these cardiovascular responses were prevented by adrenergic receptor blockade (Rodman et al., 2003) and electrically-evoked diaphragm contractions in anaesthetised cats evokes an increase in group III and IV phrenic afferent activity after the onset of fatigue (Hill, 2000).

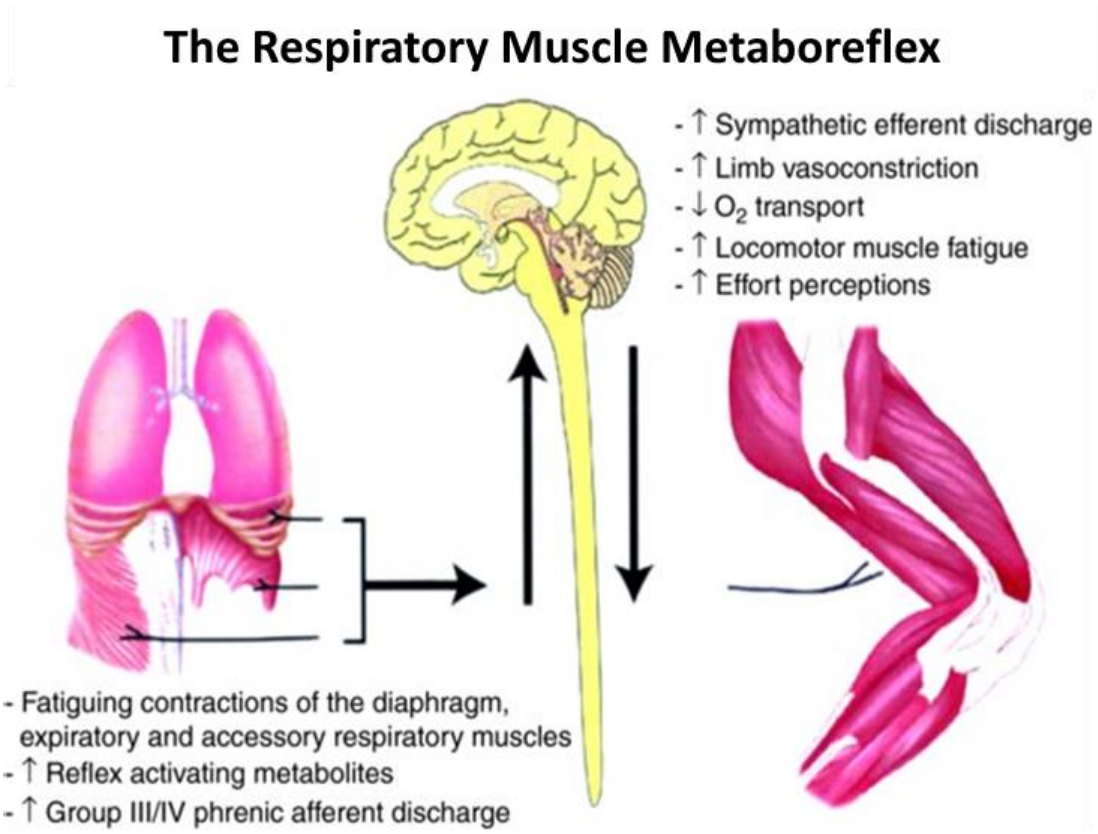


Figure 2.10. A schematic of the proposed respiratory muscle metaboreflex. Figure taken from Sheel and Romer (2012).

In healthy humans, it has been shown that high-intensity voluntary contractions of the inspiratory muscles to the point of task failure or fatigue engenders a time-dependent increase in mean arterial pressure (MAP) that is accompanied by an increase in muscle sympathetic nerve activity (MSNA) and a decrease in $\dot{Q}_L$ (Sheel et al., 2001, Smith et al., 2016, Smith et al., 2017). Similar time-dependent increases

in MAP and MSNA in the resting leg have been shown in response to fatiguing contractions of the expiratory abdominal muscles (Derchak et al., 2002). However, the effect of fatiguing expiratory muscle contractions on $\dot{Q}_L$ has yet to be examined.

2.6.1.2. Evidence for an Inspiratory Muscle Metaboreflex during Exercise

During whole-body exercise, any increase in locomotor muscle blood flow is dependent upon the opposing effects of sympathetic vasoconstrictor activity and local vasodilatory mediators. Therefore, the question becomes, is the respiratory muscle metaboreflex sufficiently powerful to overcome local vasodilatory effects and redistribute blood flow from the locomotor muscles to the respiratory muscles during exercise? In highly fit humans performing very high-intensity exercise (>95% $\dot{V}O_{2max}$), reducing the power of breathing of the inspiratory muscles via proportional assist ventilation elicits an increase in $\dot{Q}_L$ and a decrease in LVR (Harms et al., 1997). Conversely, increasing inspiratory muscle power attenuates $\dot{Q}_L$ and LVR compared to control conditions (Harms et al., 1997). Moreover, alterations in $\dot{Q}_L$ with changes in inspiratory muscle power were inversely correlated with changes in norepinephrine spillover, implying that changes in $\dot{Q}_L$, and thus LVR, are likely sympathetically-mediated. A considerable reduction in blood flow and O_2 transport to the locomotor muscles during exercise would be expected to exacerbate exercise-induced locomotor muscle fatigue and thus limit exercise tolerance. Indeed, Romer et al. (2006) found that reducing inspiratory muscle power during very high-intensity exercise (≥90% $\dot{V}O_{2max}$) attenuated both the perception of leg discomfort and the severity of exercise-induced quadriceps muscle fatigue compared to control conditions (−20% vs −28% reduction in quadriceps twitch force). Therefore, these data suggest that exercise tolerance and locomotor muscle fatigue may be negatively influenced by the normal 'work' of inspiration during severe-intensity exercise.

However, whether there is a hierarchy of blood flow distribution between the respiratory and locomotor muscles remains less clear. Indeed, it has been previously highlighted that the arterioles of the diaphragm may be less resistant to vasoconstrictor influences and in maximally-exercising equines the diaphragm, but not the limb vasculature, appears to be maximally dilated (*see section* 2.2.3). Moreover, it has been speculated that the increasing T_I/T_{TOT} may serve to better maintain diaphragm blood flow as exercise progresses. However, direct assessment of diaphragm or the primary expiratory abdominal muscle blood flow during exercise in humans remains methodologically complex. In a recent study, Dominelli et al. (2017) measured the effect of increasing or decreasing the inspiratory power of breathing during severe-intensity exercise on quadriceps and sternocleidomastoid blood flow, assessed using near-infrared spectroscopy and indocyanine green dye. The authors reported that when the power of breathing was decreased by PAV, respiratory muscle blood flow decreased and locomotor muscle blood flow increased; conversely, when the power of breathing was increased respiratory muscle blood flow increased and locomotor muscle blood flow decreased (*see* Figure 2.11). Therefore, these data suggest that respiratory muscle power influences the distribution of blood flow to the respiratory and locomotor muscles during high-intensity exercise. Although yet to be empirically verified, it is possible that the accessory respiratory muscles are able to access a greater share of cardiac output under conditions of increased respiratory muscle power via a similar attenuated responsiveness to adrenergic stimulation to the diaphragm.

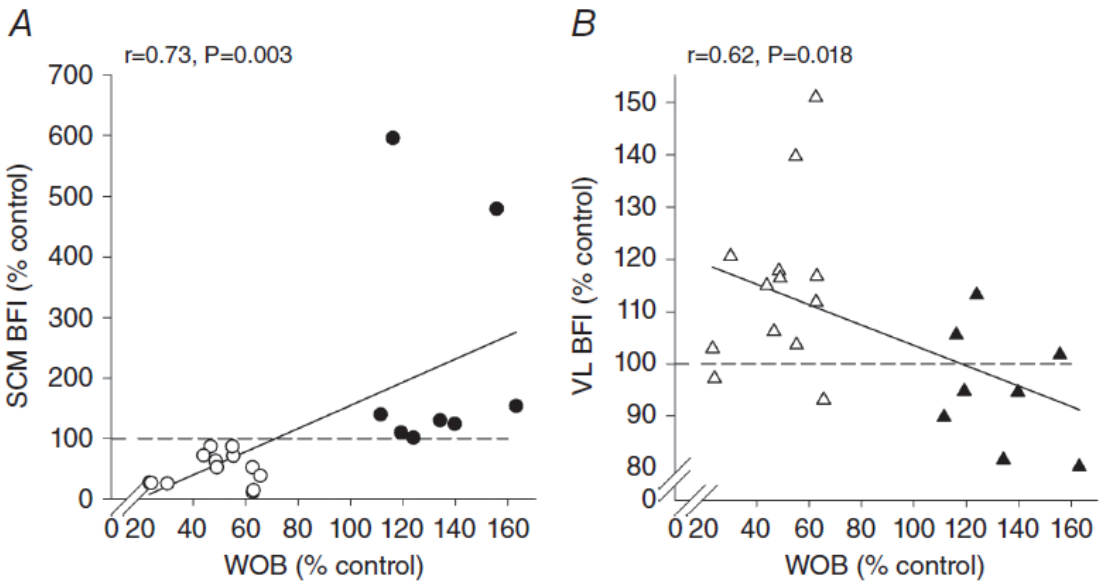


Figure 2.11. The relationship between the inspiratory work (power) of breathing and (A) sternocleidomastoid blood flow, and (B) vastus lateralis blood flow during exercise trials at 90% of peak ramp power. Open symbols represent inspiratory unloading by proportional assist ventilation and filled symbols represent inspiratory loading by increasing airway resistance. Figure taken from Dominelli et al. (2017).

2.6.1.3. Evidence for an Expiratory Muscle Metaboreflex during Exercise

There is substantially less evidence implicating an expiratory muscle contribution to the respiratory muscle metaboreflex during whole-body exercise. However, it has been suggested that expiratory muscle loading during submaximal exercise in healthy humans may lower $\dot{Q}_L$ while increasing accessory respiratory muscle blood flow (Athanasopoulos et al., 2010). In addition, increasing expiratory muscle power by adding an expiratory resistance during submaximal exercise (40% $\dot{V}O_{2peak}$) was associated with an augmentation in MAP and MSNA compared to exercise without added expiratory resistance (MAP: +5 vs. +29%; MSNA: +22 vs. +44%) (Katayama et al., 2015a). Moreover, in the study by Katayama et al. (2015a) there was a significant reduction in MEP from pre- to post-exercise (−19%) with but not without added expiratory resistance, suggesting the expiratory muscle fatigue may contribute to the sympathetic response. Finally, it has been shown that prior induction of expiratory muscle fatigue increases the severity of exercise-induced quadriceps

muscle fatigue in response to severe-intensity constant-power exercise compared to time-matched control conditions (–28 ± 7% vs. –14 ± 6% change in pre- to post-exercise quadriceps twitch force) (Taylor and Romer, 2008). In combination, the aforementioned findings suggest that, similar to the inspiratory muscles, elevated expiratory muscle activity and exercise-induced expiratory muscle fatigue may also lead to an increase in sympathetic vasomotor outflow, a reduction in $\dot{Q}_L$, and an exacerbation of exercise-induced limb locomotor muscle fatigue during whole-body exercise.

2.6.1.4. Evidence from Respiratory Muscle Training Studies

There is also accumulating evidence to suggest that respiratory muscle training (RMR) may be able to attenuate the cardiovascular responses associated with the activation of a respiratory muscle metaboreflex. For example, Witt et al. (2007) reported that 5 weeks of inspiratory muscle training at 50% of MIP blunted the increase in HR and MAP (~8 beats·min^{-1} and ~10 mmHg) observed during an IRL task. Similarly, during exercise in heart failure patients and healthy individuals respectively, RMT increases limb blood flow and vascular conductance (Chiappa et al., 2008) and reduces the magnitude of locomotor limb muscle fatigue (McConnell and Lomax, 2006). Theoretically, RMT may attenuate and/or delay the onset of respiratory muscle fatigue, postponing the accumulation of fatigue associated metabolites and the consequent sympathetically-mediated responses.

2.6.2. Mechanical Cardiorespiratory Effects

During severe-intensity exercise, large swings in intrathoracic and intra-abdominal pressure across the respiratory cycle exert a complex mechanical effect that influences cardiac output via alterations in venous return, ventricular afterload, left ventricular (LV) filling, and LV emptying (Cheyne et al., 2020). For example, a more negative inspiratory intrathoracic pressure augments venous return but increases LV

afterload; effects which are reversed as intrathoracic pressure increases during expiration (Permutt and Wise, 2011). In contrast, although increased abdominal pressure secondary to expiratory muscle recruitment may increase venous return from the abdominal compartment (Aliverti et al., 2010), it limits femoral venous return and may result in a reduction in inferior vena cava flow (Miller et al., 2005a). There is evidence to suggest that during exercise such an increase in intra-abdominal pressure may impair cardiac performance. For example, Stark-Leyva et al. (2004) reported that an increase in gastric pressure of 7-18 cmH_2O during submaximal exercise with expiratory loading was negatively associated to a ~1 L reduction in cardiac output (r = −0.60), predominantly mediated by a reduction in stroke volume.

Interestingly, the development of expiratory muscle fatigue may augment the rise in expiratory abdominal pressure during exercise. Pre-fatigue of the expiratory muscles increases peak expiratory Pga during subsequent severe-intensity exercise by ~9 cmH_2O versus time-matched control conditions, likely secondary to an increase in expiratory flow rate (Taylor and Romer, 2008). Such an increase in peak expiratory gastric pressure may decrease femoral venous return and could theoretically reduce locomotor blood flow and O_2 delivery secondary to a decrease in cardiac output, augmenting the rate of locomotor muscle fatigue. Indeed, the pre- to post-exercise reduction in locomotor twitch force is ~twofold greater (−28 ± 9% vs. –14 ± 6%) in response to expiratory muscle pre-fatigue vs. time-matched conditions (Taylor and Romer, 2008).

2.6.3. Summary

Exercise-induced respiratory muscle fatigue may limit exercise performance by contributing to an increased sensation of dyspnoea or influencing the normally-occurring mechanical cardiorespiratory interaction observed during exercise. However, the primary performance-limiting consequence of respiratory muscle

fatigue appears to be an increased sympathetic outflow which may blunt locomotor blood flow and O_2 delivery by activation of a respiratory muscle metaboreflex, with a resultant increase in the rate and/or severity of locomotor muscle fatigue. A greater severity of locomotor muscle fatigue would be expected to limit exercise performance via a direct effect on locomotor force generation, or via an increase sensation of exertion of the working locomotor muscles and a consequent reduction in centre motor drive.

2.7. SUMMARY

There is extensive evidence that inspiratory and expiratory abdominal muscle fatigue occur in response to exhaustive constant-power exercise sustained at ≥85% of $\dot{V}O_{2max}$ (*see section* 2.4.2). However, although it has been suggested that the development of diaphragmatic fatigue may be less prevalent and of a lower magnitude at power outputs eliciting <85% of $\dot{V}O_{2max}$ (*see section* 2.4.2), the effect of exercise intensity on the development of inspiratory and expiratory abdominal muscle fatigue has yet to be systematically investigated. Moreover, it has been suggested that respiratory muscle fatigue may not develop in response to exercise trials in which a high power of breathing is sustained for only a short period of time (e.g. ramp incremental exercise; *see section* 2.4.2). However, the effect of the tolerable duration of exercise on the presence and magnitude of exercise-induced inspiratory and expiratory muscle fatigue remains to be empirically assessed.

Additionally, despite a plethora of evidence reporting that diaphragm and expiratory abdominal muscle fatigue occur *in response* to severe-intensity exercise (*see section* 2.4.2), the temporal characteristics or 'time-course' of exercise-induced respiratory muscle fatigue remains somewhat unclear. There is emerging evidence suggesting that diaphragmatic fatigue occurs prior to exercise intolerance; however, it is unclear whether the magnitude of fatigue remains stable or progressively increases towards

exercise intolerance. To date, no study has investigated the time-course of exercise-induced expiratory muscle fatigue.

Based on previous literature, it is hypothesised that the development of respiratory muscle fatigue may limit exercise tolerance in part via a fatigue-induced sympathoexcitation that compromises locomotor blood flow and O_2 delivery (i.e. the respiratory muscle metaboreflex), ultimately resulting in an earlier development of locomotor limb muscle fatigue and exercise termination (*see section* 2.6.3). The inspiratory contribution to this reflex is supported by observations that fatiguing inspiratory resistive loaded breathing (IRL) in healthy otherwise-resting humans elicits a time-dependent increase in MSNA, MAP and LVR and a time-dependent decrease in $\dot{Q}_L$ (*see section* 2.6.3). In response to fatiguing expiratory resistive loaded breathing (ERL) there is a similar response of MSNA and MAP; however, the $\dot{Q}_L$ and LVR response remains unknown.

2.8. THESIS DESIGN

The overall aim of this thesis was to comprehensively compare and contrast the determinants and physiological consequences of exercise-induced inspiratory versus expiratory muscle fatigue. Two primary research strategies were used to address this aim. In Chapters 5 & 6, the cardiovascular responses to fatiguing IRL and ERL tasks were measured in healthy otherwise resting individuals. This approach allowed for the experimental isolation of the effect of inspiratory or expiratory muscle fatigue on sympathetically-induced changes in peripheral limb vasomotor tone, blood flow and vascular resistance. That is, IRL and ERL facilitates the assessment of the physiological consequences of inspiratory or expiratory muscle fatigue alone without the competing influence of local vasodilatory metabolites in the peripheral skeletal muscle. Importantly, the degree of inspiratory and expiratory muscle fatigue elicited by IRL and ERL, respectively, appears to be similar to, and representative of, the

magnitude of exercise-induced inspiratory and expiratory muscle fatigue (Johnson et al., 1993, Guenette et al., 2010, Taylor et al., 2006, Taylor and Romer, 2008). In Chapters 7 & 8, the effect of varying exercise intensity and duration on the magnitude of inspiratory and expiratory muscle fatigue was assessed and compared. This approach allowed elucidation of the importance of respiratory muscle work, cumulative respiratory force output, and the availability of cardiac output during dynamic whole-body exercise on the magnitude of exercise-induced respiratory muscle fatigue. Collectively, this thesis design allowed comprehensive investigation of the exercise conditions in which inspiratory and expiratory muscle fatigue develop, and the cardiovascular consequences of inspiratory and expiratory muscle fatigue, which may have important implications for exercise tolerance.

2.9. AIMS AND OBJECTIVES

The primary aims of this thesis were to:

1. Investigate the cardiovascular consequences of fatiguing expiratory and inspiratory muscle work in healthy humans who were otherwise at rest. It was hypothesised that: a) in comparison to baseline conditions, heart rate (HR), MAP, and LVR would significantly increase and $\dot{Q}_L$ would significantly decrease in a time-dependent manner during IRL and ERL; and b) the time taken for significant changes in HR, MAP, LVR and $\dot{Q}_L$ to become evident would be shorter for ERL vs. IRL.
2. Compare and contrast the presence and magnitude of exercise-induced inspiratory and expiratory abdominal muscle fatigue in response to heavy-intensity exercise, short-duration (~5 min) severe-intensity exercise and long-duration (~10 min) severe-intensity exercise. It was hypothesised that: a) in comparison to long-duration severe-intensity exercise, heavy-intensity exercise and short-duration severe-intensity exercise would evoke a significantly lower magnitude of inspiratory muscle fatigue; and b) the magnitude of expiratory

muscle fatigue would not be significantly different in response to heavy-intensity, or short- and long-duration severe-intensity exercise.

3. To compare and contrast the time-course or ‘temporality’ of exercise-induced inspiratory and expiratory muscle fatigue during long-duration (~10 min) severe-intensity exercise. It was hypothesised that: a) exercise-induced expiratory muscle fatigue would occur at a significantly earlier time-point than inspiratory muscle fatigue; and b) inspiratory and expiratory muscle contractility would decrease significantly with increasing exercise time.

CHAPTER 3
General Methods

3.1. ETHICAL APPROVAL

All testing protocols received ethical approval from the Faculty of Biological Sciences Research Ethics Committee (Certificate Number: BIOSCI 16-020; BIOSCI 17-016) and conformed to the Declaration of Helsinki.

3.2. SUBJECTS

Full subject demographics are presented in Appendix II. Prior to participation in experimental procedures, all subjects completed a medical health questionnaire and provided written informed consent after receiving an information sheet of the study protocols. For all studies in this thesis, healthy subjects aged 18-45 years were recruited. Exclusion criteria included the presence of cardiovascular, pulmonary or metabolic disease. Additionally, for any protocol including assessment of oesophageal or gastric pressure in response to magnetic nerve stimulation, individuals with metallic implants, allergies to local anaesthetic, dysphagia, pregnancy, recent nasal trauma (i.e. a broken nose) or a viral illness within the previous 2 weeks were excluded.

3.3. PULMONARY FUNCTION

3.3.1. Forced Vital Capacity and Forced Expiratory Volume

Pulmonary function was assessed in accordance with the American Thoracic Society (ATS) and European Respiratory Society (ERS) guidelines (Laveneziana et al., 2019b, Miller et al., 2005b). Subjects were seated and breathed through a flanged mouthpiece with the nose occluded. The maximal volume of air that could be expired from total lung capacity (TLC) (forced vital capacity, FVC) and the maximal volume of air that could be expired in one second (forced expiratory volume over 1 s, FEV_1)

were assessed via performance of maximal flow volume loops (MFVL). Subjects were coached to: 1) inhale rapidly and fully from functional residual capacity (FRC); 2) perform a maximal “blast” expiration from TLC maintained until residual volume (RV) was reached; and 3) repeat the initial rapid and maximal inhalation to TLC. During each manoeuvre, the lead experimenter ensured appropriate technique and effort was maintained. Subjects performed a minimum of three and a maximal of seven manoeuvres. The test was considered acceptably repeatable once three values were within 150 ml of the highest reported FVC and FEV_1. The greatest FVC and FEV_1 were reported, regardless of whether they came from the same curve, and all other indices (e.g. peak expiratory flow) were reported from the manoeuvre with the highest sum of FVC and FEV_1.

3.3.2. Maximal Voluntary Ventilation

The maximal voluntary ventilation (MVV) reflects the maximal volume of air that a subject can inhale and exhale over a set period of time. In this thesis, MVV manoeuvres were maintained for 12 s and extrapolated to 60 s. After three tidal breaths, subjects were instructed to breathe rapidly and deeply, so that a breathing frequency of ~90 breaths·min^{-1} and a tidal volume of ~50% of vital capacity were achieved. Subjects were encouraged and coached during the manoeuvre to maintain a maximal effort and to adopt an appropriate breathing pattern. MVV manoeuvres with a breathing frequency of between 80-100 breaths·min^{-1} and tidal volume (V_T) exceeding twice the resting value were considered acceptable. The maximum value of two valid manoeuvres was reported.

3.4. DOPPLER ULTRASOUND

3.4.1. Principles of Doppler Ultrasound

Ultrasound is the generation of high-frequency sound waves caused by the conversion of electrical energy to mechanical (acoustic) energy (and vice versa) from

vibration of piezoelectric crystals inside the ultrasound transducer. Such sound waves propagate through human body tissue and are partially reflected by boundaries in which there is an abrupt change in acoustic impedance. In brightness mode (B mode) such ultrasonic wave reflections can be used to distinguish between bone, muscle and soft tissue. In pulse-wave Doppler mode, ultrasonic beams emitted from the transducer are reflected and back-scattered by moving blood cells. The principle of Doppler ultrasound is determined by the Doppler effect – a phenomenon in which observed changes in the frequency of energy wave transmission occur with relative motion between the source of wave transmission and the observer (Maulik, 1989). Frequency shifts in the backscattered sound waves are processed using Equation 3.1, in order to calculate the magnitude and direction of blood velocity (f_d, Doppler shift frequency; f_t, transmitted frequency; V, velocity of blood cells; c, velocity of sound in blood).

$$f_d = \frac{2 f_t V cos\theta}{c} \qquad \textbf{Eqn 3.1}$$

In the current study, Duplex mode was used to simultaneously record arterial diameter (B-Mode) and blood velocity (pulse-wave Doppler mode) in order to calculate blood flow.

3.4.2. Practical Application

In the present thesis, Doppler ultrasound was used to measure leg blood flow ($\dot{Q}_L$) before, during, and after fatiguing expiratory resistive loaded breathing (ERL) and fatiguing inspiratory resistive loaded breathing (IRL) in Chapters 5 and 6, respectively. This allowed for the investigation of the cardiovascular effects in the resting limb of ERL and IRL, and as such was crucial in addressing the first aim of this thesis: investigating the $\dot{Q}_L$ response to 'high-intensity' contractions of the expiratory and the inspiratory muscles sustained to the point of task failure in otherwise resting individuals.

3.5. FINGER PHOTOPLETHYSMOGRAPHY

In this thesis, finger photoplethysmography (Finapres Nova, Finapres Medical Systems, Amsterdam, The Netherlands) was used to non-invasively and continuously (beat-by-beat) measure finger arterial blood pressure and heart rate.

3.5.1. Principles of Finger Photoplethysmography

The principle of finger photoplethysmography is based on the 'volume-clamp' method (Penaz, 1973). Finger arterial pressure is measured via an inflatable finger cuff in combination with an infrared plethysmograph, comprised of a light emitting diode and a light-sensitive receiver. The diameter of the artery under the cuff is maintained at a constant (clamped) diameter by measuring the proportion of blood absorbed by the light detector and adjusting the pressure applied to the cuff using a servo-controller. To ensure a high level of accuracy, the 'unloaded' diameter of the finger artery is first determined, at which point finger cuff pressure and intra-arterial pressure are equal and transmural pressure across the finger arterial wall is zero (Imholz et al., 1998). Any subsequent change in inter-arterial pressure is then proportional to the change in finger-cuff pressure. For example, an increase in arterial blood pressure (and arterial diameter) would cause a consequent decrease in infrared light detected by the photo-sensitive receiver, resulting in an increase in pressure applied by the servo-controller to maintain a constant arterial diameter. The unloaded diameter of the finger diameter is affected by haematocrit, smooth muscle tone and stress, and may therefore vary during measurement periods (Bogert and van Lieshout, 2005). As a result, the unloaded diameter of the finger artery is verified and adjusted at regular intervals by automatic analysis of the amplitude and shape of the plethysmograph signal in response to different levels of constant pressure (PhysioCal) (Wesseling, 1995).

3.5.2. Reconstruction of Brachial Artery Pressure

Arterial waveforms become transformed as they travel along the arterial network, due to differences in vessel compliance and wave reflection, causing pressure differences between brachial artery pressure and the directly measured finger arterial pressure. The Finapres (Finapres Medical Systems, Amsterdam, The Netherlands) corrects for such changes in pressure gradient and pulse wave distortion using a generalised waveform filter and level correction method (Truijen et al., 2012, Bos et al., 1996), resulting in accurate reconstruction of brachial pressure (see Figure 3.1). All blood pressure measurements in this thesis (i.e. MAP) refer to resynthesized brachial artery pressure.

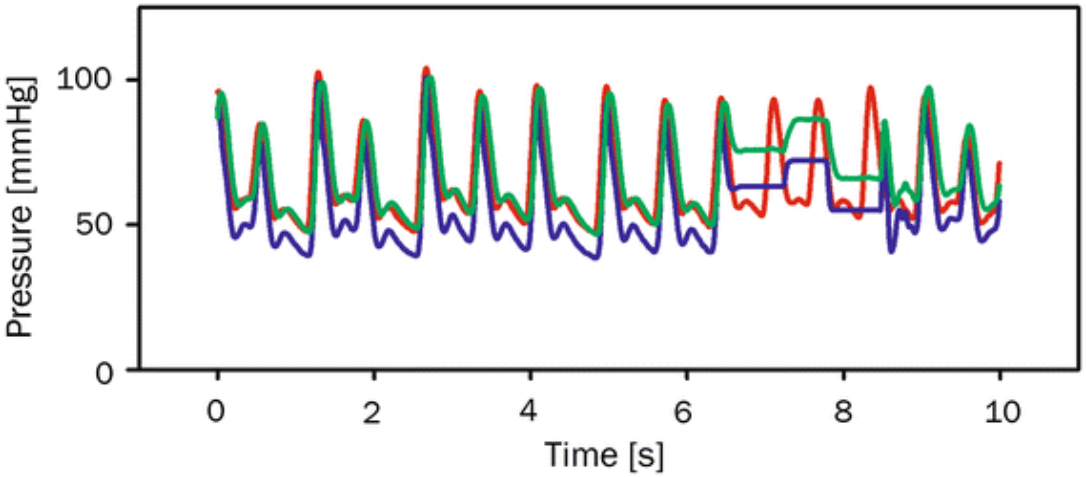


Figure 3.1. An example of the reconstruction of radial (red) and brachial artery pressure (green) reconstruction from finger arterial pressure (blue). An example period of PhysioCal shown between 6 and 9 s. Figure taken from Truijen et al. (2012).

3.5.3. Practical Application

In the present thesis, finger photoplethysmography was used to measure heart rate (HR) and mean arterial pressure (MAP) before, during, and after fatiguing expiratory resistive loaded breathing (ERL) and fatiguing inspiratory resistive loaded breathing (IRL) in Chapters 5 and 6, respectively. This allowed for the investigation of the systemic cardiovascular effects, and in combination with the $\dot{Q}_L$ data the limb vascular resistance (LVR) effects, of ERL and IRL and as such was crucial in addressing the first aim of this thesis: investigating the HR, MAP and LVR response

to ‘high-intensity’ contractions of the expiratory and the inspiratory muscles sustained to the point of task failure in otherwise resting individuals.

3.6. PULMONARY GAS EXCHANGE, AIRFLOW, AND PRESSURE

3.6.1. Pulmonary Gas Exchange

Respired gases were analysed via a calibrated gas exchange system (Ultima Cardio 2, MGC Diagnostics, St Paul, MN, USA). Gases were sampled via a bidirectional flow sensor connected to a Pitot tube (‘preVent’ flow sensor; dead space = 39 ml) via an umbilical for volume measurement and respired gases were passed to galvanic (O_2) and non-dispersive infrared (CO_2) sensors for gas analysis. Because the flow sensor responds faster than the time taken for gas analysis to occur, gas concentrations were aligned on-line to the start and end of each breath as detected by the inspiratory and expiratory signals of the flow sensor. Prior to each test, the flow sensor and gas analysers were calibrated across the physiological range. Atmospheric air pressure, temperature, and humidity were logged to convert gas volumes into body temperature and pressure saturated values. The flow sensor was then calibrated using a 3 L syringe (Hans Rudolph, Kansas City, MO, USA). Five inspired and five expired strokes were performed at various flow rates across the physiological range from rest to maximal exercise (0.2-6 L/s) using the entire 3 L volume of the syringe. This calibration procedure was repeated until mean inspired and expiratory volumes were between 2.99 and 3.01 L. Gas sensors were calibrated by sampling known concentrations of O_2 and CO_2 from calibration gases spanning the physiological range of exercise (gas mixture 1 = O_2, 21%; CO_2, 0%, N_2, 79%; gas mixture 2 = O_2, 12%; CO_2, 5%, N_2, 83%).

3.6.2. Airflow

Inspiratory and expiratory airflow at the mouth were measured using a bidirectional pressure differential pneumotachometer (PNT) (model 4813, Hans Rudolph, Kansas

City, MO, USA) integrated into the mouthpiece assembly, connected to a PNT amplifier (model 1110, Hans Rudolph, Kansas City, MO, USA). The analogue output from the PNT amplifier was collected onto a data acquisition system (*see* Data Capture). The PNT amplifier was turned on and left running for 60 min before each use, to account for "warm-up" drift in the zero offset of the PNT. Prior to each test, the PNT was calibrated using a standard 3 L syringe (Hans Rudolph, Kansas City, MO, USA). A minimum of 20 inspiratory and expiratory strokes were performed at low (<3 $L \cdot s^{-1}$), medium (3-6 $L \cdot s^{-1}$) and high (>6 $L \cdot s^{-1}$) flow rates. The raw voltage output was calibrated and converted into airflow (in $L \cdot s^{-1}$) using the polynomial least squares method (Tang et al., 2003) from a commercially available script (*PTcal.s2s*, Cambridge Electronic Design). The PNT was considered adequately calibrated when average flow errors were <1%.

3.6.3. Pressure

Mouth pressure (Pm) was measured by connecting the pressure port of the PNT amplifier (model 1110, Hans Rudolph, Kansas City, USA) to a side port in the mouthpiece via a 1 m length of tygon tubing. Gastric (Pga) and oesophageal pressure (Poes) were measured via two balloon-tipped catheters (47-9005; Akrad Laboratories, Cooper Surgical, CT, USA) passed per nasally into the stomach and lower one-third of the oesophagus, respectively, after application of 2% topical anaesthetic to the naris. Each catheter was connected to a differential pressure transducer (model DP15, Validyne, Northridge, CA, USA) by a single length of 90 cm polyethylene tubing for optimal frequency response characteristics. The gastric and oesophageal catheters were filled with 2 ml and 1 ml of air, respectively. The gastric catheter was positioned so that a positive pressure was recorded throughout each breath; typically requiring 55-65 cm of the catheter to be inserted. The oesophageal catheter was initially inserted so that a negative deflection in pressure occurred during each inspiration (usually ~10 cm less than subject height multiplied by 0.288).

Final catheter positioning was then validated using the occlusion technique (Baydur et al., 1982). A series of static inspiratory and expiratory manoeuvres were performed against an occluded mouthpiece. The oesophageal catheter was then repositioned until the inspiratory-to-expiratory swing in Poes closely aligned to the swing in Pm (i.e. Poes/Pm of >0.97 and <1.03). Each pressure transducer was calibrated across the physiological range using a digital pressure manometer (model 621 Test Products International Inc., Beaverton, CA, USA).

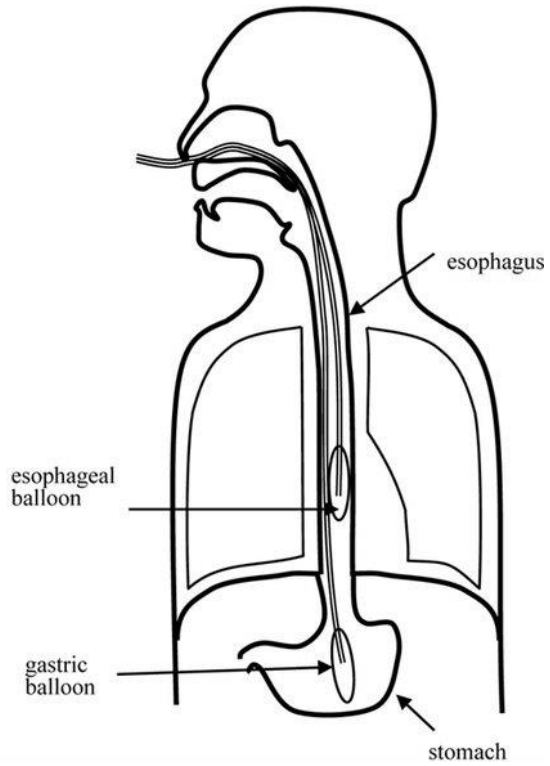


Figure 3.2. A schematic demonstrating the positioning of balloon catheters. Figure taken from (Mase et al., 2018).

3.7. MAGNETIC NERVE STIMULATION

All magnetic stimuli in this thesis were delivered from a Magstim BiStim 2 via a 90-mm circular coil (Magstim Company, Whitland, Wales). Specific stimulation protocols used to assess respiratory muscle function are presented in each relevant chapter.

3.7.1. Principles of Magnetic Nerve Stimulation

Magnetic stimulators comprise of a high-pulse generator unit and a stimulating coil consisting of one or more tightly-wound copper coils. Inside the generator unit, a capacitor is charged from a high-voltage power-supply with a transformer under microprocessor control, so that the power level can be adjusted from 0-100%. When the magnetic stimulator is triggered, stored energy is rapidly transmitted into the stimulating coil, creating an intense rapidly changing magnetic field that, if in close proximity to the human body, can penetrate soft tissue and bone to reach deep nervous tissue. The resultant effect of such magnetic stimulation of the human nervous system is determined by Faraday's law of electromagnetic induction; when a time-varying magnetic field is applied in the vicinity of a conductive structure an electrical field is induced, of which the amplitude is determined by the rate of change of the magnetic field and the geometry of the conductive structure (Faraday, 1839). If the subsequent electrical current is of a sufficient amplitude and duration, neuromuscular tissue is stimulated in a similar manner to electrical stimulation leading to depolarisation of cell membranes and the initiation of an action potential (Man et al., 2004). The practical application of magnetic stimulation in this thesis is detailed in section 3.8.2.

3.8. ASSESSMENT OF INSPIRATORY AND EXPIRATORY MUSCLE FUNCTION

3.8.1. Volitional Assessment of Respiratory Muscle Function

Maximal static inspiratory and expiratory pressure at the mouth (MIP, MEP) were measured by performance of maximal Müeller and Valsalva manoeuvres performed against an occluded airway using a handheld mouth pressure meter with a flanged mouthpiece and a 1 mm air leak to prevent closure of the glottis (MicroRPM, Carefusion, CA, USA). Each manoeuvre was maintained for ~5 s, and the maximal 1 s average was reported. During the Valsalva manoeuvre, subjects' cheeks were supported to minimise discomfort and the recruitment of the buccal muscles. MIP and

MEP were measured at residual volume (RV) and total lung capacity (TLC), respectively. At such lung volumes, small changes in volume have a minimal effect on pressure and the length-tension relationship of the inspiratory and expiratory muscles are optimised. Prior to collection of experimental data, subjects were coached to ensure appropriate technique and effort, to minimise any learning effect. Subjects were considered familiarised once three manoeuvres could be performed within 10% of the maximum value. When maximal expulsive or inspiratory manoeuvres were performed with the insertion of balloon catheters maximal Pga (Pga_{MAX}) and Pdi (Pdi_{MAX}) were defined as the highest 1 s average during each expiratory Valsalva or inspiratory Müeller manoeuvre, respectively.

3.8.2. Non-Volitional Assessment of Expiratory Abdominal and Diaphragm Muscle Function by Magnetic Nerve Stimulation

3.8.2.1. Subject and Coil Positioning

Diaphragm muscle function was assessed with subjects sat upright with the neck flexed and shoulders relaxed (Figure 3.3). The coil was positioned over the midline between the 3rd and 7th (C3-C7) cervical vertebrae (Similowski et al., 1989) (Figure 3.3). Expiratory abdominal muscle function was assessed with subjects sat facing forwards on an incline bench set to an angle of ~30 degrees past the vertical, with their chest and abdomen supported (Figure 3.4). A 90 mm circular coil was positioned firmly against the skin over the vertebral column between the 8th and 11th thoracic vertebrae (T8-T11) (Kyroussis et al., 1996a) (Figure 3.4). The optimal respective coil positions were defined as the location that elicited the greatest gastric twitch (Pga_{tw}) amplitude and transdiaphragmatic twitch (Pdi_{tw}) amplitude from a series of non-potentiated 1 Hz stimulations delivered at 100% of the stimulators maximum power along the vertebral axis of each specified region. Non-potentiated stimulations were separated by ~30 s to avoid minimise the influence of twitch potentiation. Each location was marked with indelible ink and used for all further stimulations. In this

thesis, all non-potentiated stimulations were delivered at functional residual capacity (FRC) against a semi-occluded airway, as judged via a stable and relaxed end-expiratory Poes, and a positive hand signal from the subject.

Figure 3.3. An example of the subject and coil positioning for magnetic stimulation of the cervical nerve roots.

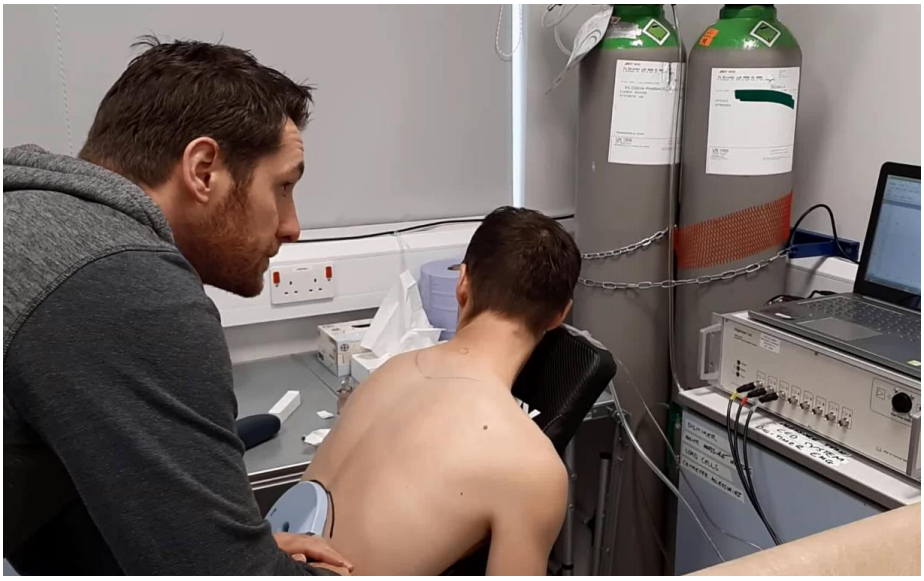


Figure 3.4. An example of the subject and coil positioning for magnetic stimulation of the thoracic nerve roots.

3.8.2.2. Potentiated Twitch Protocol

In comparison to the non-potentiated twitch, the potentiated twitch is a more sensitive measure of fatigue, likely due to a greater susceptibility to low frequency fatigue for the mechanisms that determine potentiated vs. non-potentiated twitch amplitude (i.e. phosphorylation of myosin light chain subunits) (Laghi et al., 1998, Kufel et al., 2002b). Therefore, potentiated twitches were used to assess expiratory abdominal and diaphragmatic muscle fatigue in this thesis. Accordingly, potentiated Pga_{tw} was measured once subjects has returned to FRC, ~5 s after a 5 s maximal expiratory manoeuvre (i.e. Valsalva) initiated from TLC. Similarly, potentiated Pdi_{tw} was measured at FRC ~5 s after a 5 s maximal inspiratory manoeuvre (i.e. Müeller) initiated from RV. For each assessment of expiratory and inspiratory muscle function, six potentiated twitches were performed. Pdi_{tw} and Pga_{tw} were defined as the average of the highest 3 or 4 twitches that met the acceptability criteria (*see section* 3.8.2.4).

3.8.2.3. Voluntary Activation

Voluntary activation of the diaphragm and expiratory abdominal muscles was assessed during the Müller and Valsalva manoeuvres, respectively, using the interpolated twitch technique (Strojnik and Komi, 1998). During the maximal expulsive (Valsalva) and inspiratory (Müeller) manoeuvres, a twitch was delivered approximately 2 seconds into the manoeuvre (i.e. the superimposed twitch). Voluntary activation of the was determined as the ratio of the superimposed twitch to the following control twitch (Strojnik and Komi, 1998), using equation 3.2:

$$\text{Voluntary Activation} = 100 - P_{SUP} * (\frac{P_{PRE}}{P_{MAX}})/P_{TW} * 100 \qquad \textbf{Eqn 3.2}$$

In this equation: P_{SUP} denotes amplitude of the superimposed twitch; P_{PRE} is the pressure immediately before the twitch; P_{MAX} is the maximum pressure during the maximal effort; and P_{TW} is the amplitude of the pressure response to the following control twitch.

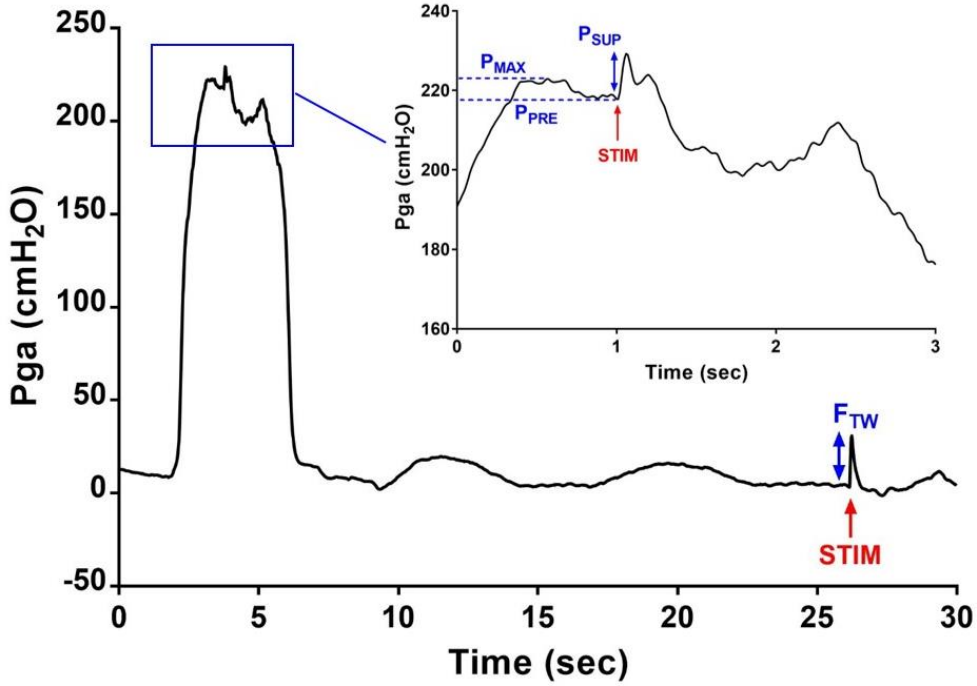


Figure 3.5. An annotated example of the interpolated twitch technique to assess voluntary activation of the expiratory abdominal muscles.

3.8.2.4. Within-Twitch Analysis

All gastric and diaphragmatic twitches were analysed for: amplitude (baseline-to-peak), contraction time (CT), maximal rate of pressure development (MRPD), one-half relaxation time ($RT_{0.5}$) and maximal rate of relaxation (MRR) (*see* Figure 3.2). Because MRPD and MRR are pressure dependent, values were normalised to Pga_{tw} and Pdi_{tw} twitch amplitude. Twitches were excluded if they met any on the following criteria: 1) the twitch was not initiated at a relaxed end-expiratory Poes (i.e. FRC); 2) cardiac artefact occurred at the time of stimulation; 3) oesophageal peristalsis was evident at the initiation of a twitch; or 4) there was a substantial rise in Pga or Pdi prior to stimulation (indicative of 'abdominal bracing').

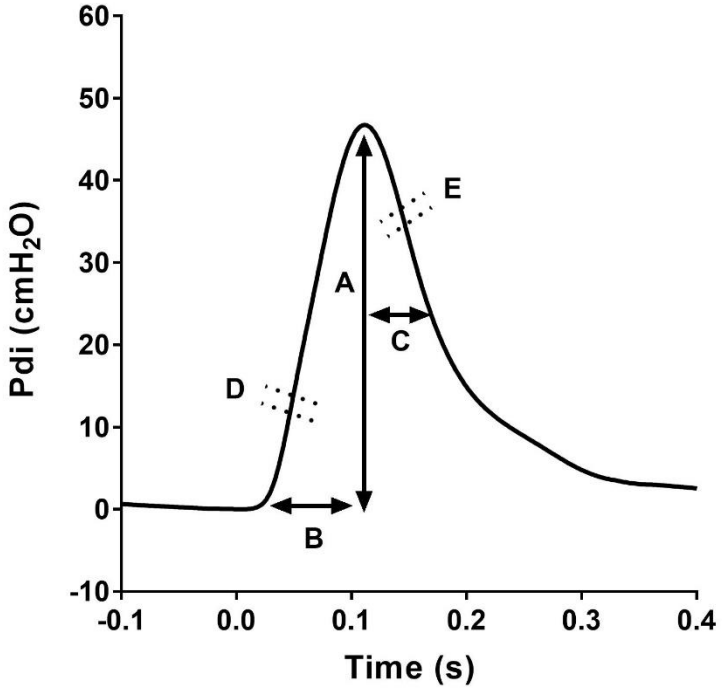


Figure 3.6. Schematic illustrating the determination of twitch characteristics; A, twitch amplitude (baseline-to-peak); B, contraction time (time of stimulus initiation to peak amplitude); C, one-half relaxation time (time from peak amplitude to one-half peak amplitude); D, maximal rate of pressure development (slope of the tangent drawn at the steepest slope between stimulus initiation and peak pressure); E, maximal rate of relaxation (slope of the tangent at the steepest slope between peak amplitude and return to baseline pressure).

3.9. ELECTROMYOGRAPHY

3.9.1. Principles of Electromyography

Electromyography (EMG) is the recording of myoelectric signals generated at the sarcolemma during transmission of action potential depolarisation along the surface of a muscle fibre. Each EMG electrode records all superimposed motor unit action potentials under the specific site of placement. In bipolar surface EMG systems, the voltage (potential) difference between each electrode is measured; because the action potential depolarisation wave arrives at each electrode (of the bipolar pair) at a different time the EMG response appears biphasic (i.e. M-wave).

3.9.2. Surface EMG Application

The skin under each muscle site was shaved, lightly abraded and cleansed with alcohol prior to application of electrodes to minimise baseline signal noise. EMG electrodes (Trigno Avanti, Delsys Inc., Natick, MA, USA) were placed over each respective muscle belly, in agreement with recommended guidelines. For the rectus abdominis (EMG_{RA}), a single bipolar electrode was placed over the muscle belly of the rectus abdominis, 2-4 cm laterally and ~2 cm superior to the umbilicus, in alignment with the orientation of the muscle fibre (*see* Figure 3.7) (Ng et al., 1998). Diaphragm EMG activity (EMG_{DI}) was recorded between the sixth and eighth intercostal spaces along the anterior-axillary line (*see* Figure 3.7). The vastus lateralis (EMG_{VL}) and vastus medialis (EMG_{VM}) electrodes were placed ~2/3 and ~4/5 of the distance from the anterior spina iliaca superior the patella and the anterior border of the medial ligament, respectively (*see* Figure 3.8).

3.9.3. Data Processing

EMG signals were sampled at 2000 Hz. M-waves were unanalysed as raw unprocessed signals. EMG signals for the quadriceps muscles (EMG_{VL} and EMG_{VM}) were band-passed filtered (Butterworth second order) from 20-500 Hz, and a RMS smoothed with a time-constant of 25 ms (Ross et al., 2010). RMS amplitude was reported for each pedal stroke of averaged across minute bins.

3.9.4. Data Analysis

M-waves were analysed for peak-to-peak amplitude, duration, and area according to the procedures of (Sandiford et al., 2005). Peak-to-peak amplitude was determined as the difference between the maximum and minimum points of the biphasic M-wave, duration was defined as the time from the initiation of the M-wave (i.e. a rise in EMG amplitude of 2 SD's above the baseline value) to the end of the biphasic M-wave (a

return to baseline EMG values), and area was calculated as the integral across the entire M-wave.

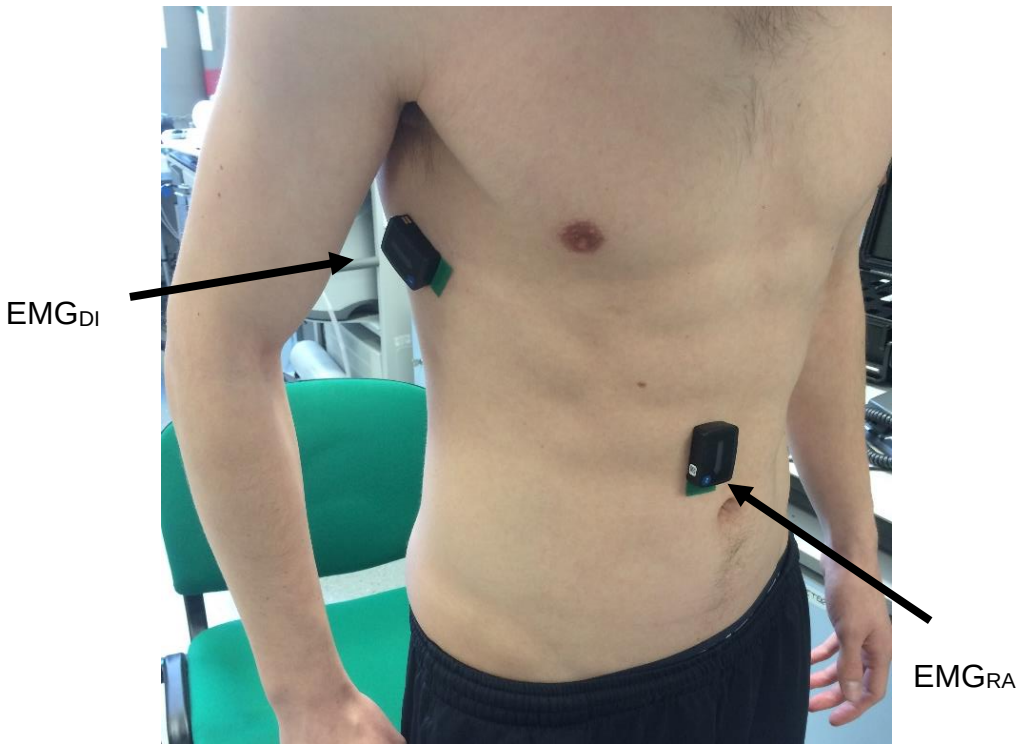


Figure 3.7. EMG electrode positions for the respiratory muscles. EMG_{DI}, diaphragm; EMG_{RA}, rectus abdominis.

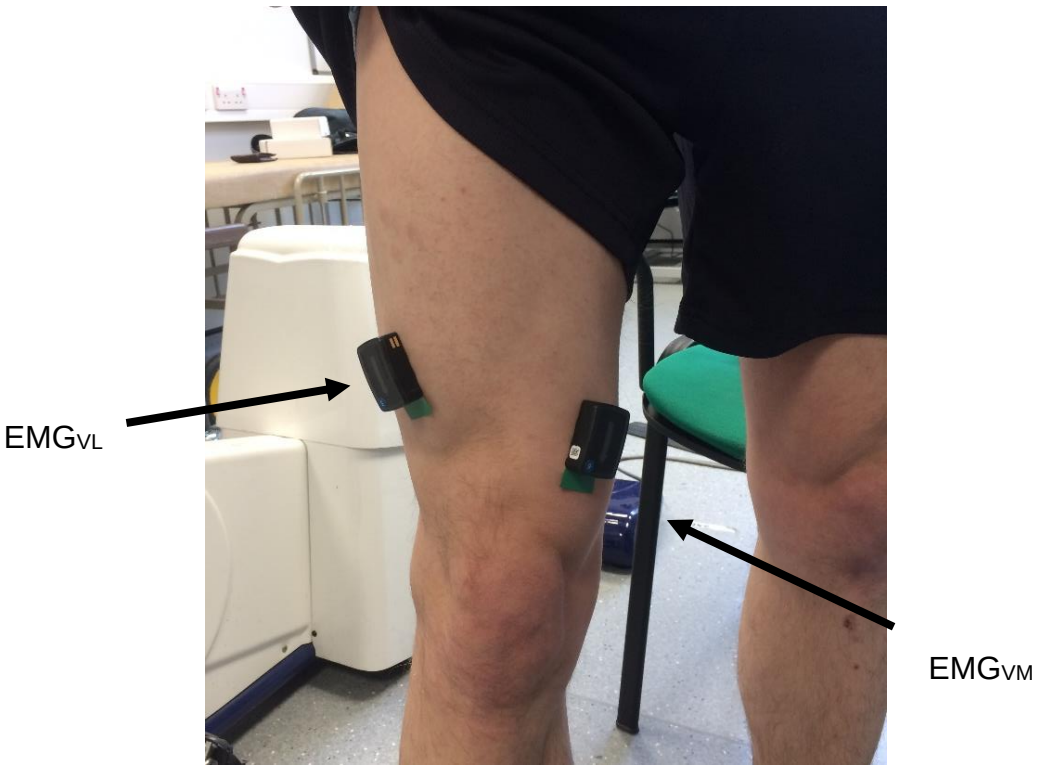


Figure 3.8. EMG electrode positions for the locomotor muscles. EMG_{VL}, vastus lateralis; EMG_{VM}, vastus medialis.

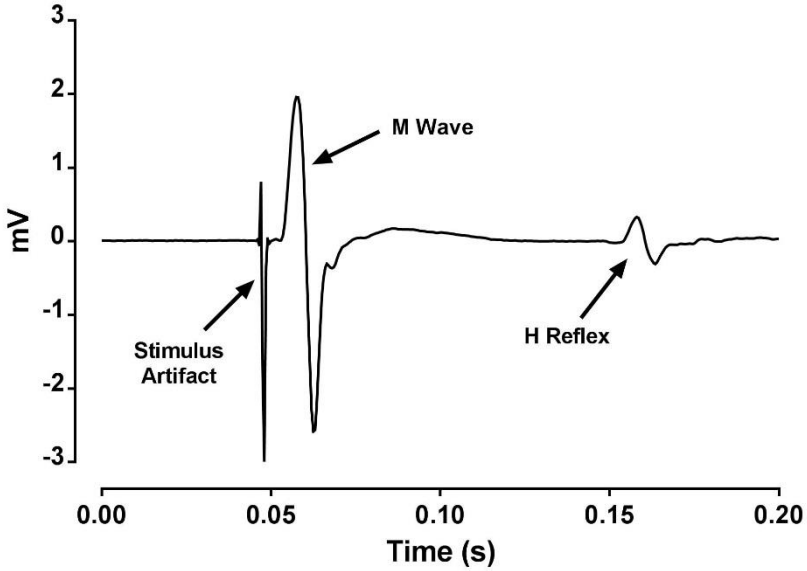


Figure 3.9. Example of an M wave and H reflex of the rectus abdominis in response to magnetic stimulation (1 Hz) of the thoracic nerve roots taken from a representative subject. The H reflex was not analysed or interpreted in this thesis.

3.9.5. Practical Application

The M-wave response to magnetic stimulation of the cervical and thoracic nerve roots was used to assess changes in membrane excitability for the diaphragm and expiratory abdominal muscles (rectus abdominis) respectively during *all* non-volitional assessments of respiratory muscle function (i.e. before, and up to 30 minutes after exercise or resistive loaded breathing tasks). As such, inspection of the M-wave response was important to elucidate the mechanisms of respiratory muscle fatigue, and specifically the potential contribution of impairments in action potential transmission, in Chapters 5-8 of this thesis.

3.10. CYCLE ERGOMETRY

An electromagnetically-braked cycle ergometer (Excalibur Sport PFM, Lode BV, Groningen, The Netherlands) was used for all exercise protocols. The ergometer was pre-programmed with the required power output (hyperbolic mode) or the pre-determined linear factor (linear mode) from a computer running Lode Ergometer

software (Lode Ergometer Manager, Lode BV, Groningen, The Netherlands). The ergometer position was optimised for each participant by vertically and horizontally adjusting the saddle and handlebar height, which was digitally recorded for all subsequent visits.

3.10.1. Cadence-independent Cycling (Hyperbolic Mode)

In hyperbolic (cadence-independent) mode, the prescribed power output is determined by the electromagnetic braking force applied to the flywheel, so that power can be maintained independently to cycling cadence. Upon increases in cycling cadence, the braking force applied to the ergometer is reduced. Conversely a reduction in cadence generates a greater braking force to maintain target power. Such alterations in braking force are determined by electromagnetic currents generated from a conductive structure (the flywheel) moving past a stationary magnet in the cycle ergometer, such that the drag force translated to the flywheel is proportional to its velocity.

3.10.2. Cadence-dependent Cycling (Linear Mode)

In linear (cadence-dependent) mode the ergometer operates similarly to a mechanically-braked ergometer, whereby the power output is determined as the product of cycling cadence and a pre-set and constant linear factor (α) which acts as the flywheel breaking resistance (*see* Equation 3.3).

$$Power = \alpha * rpm^2 \qquad \textbf{Eqn 3.3}$$

Therefore, in linear mode increasing cadence results in a higher power output. The linear factor is pre-determined as the intended power output divided by the square of target cadence (*see* Equation 3.4 and *section* 4.2.3).

$$\alpha = \frac{power\ output}{cadence^2} \qquad \textbf{Eqn 3.4}$$

3.10.3. Practical Application

In Chapters 7 and 8, a ramp-incremental sprint test (RIST) was used to determine critical power (CP) and peak power output (P_{peak}) (*see section* 4.2 and 7.2.3 for a detailed protocol description). During the sprint phase of the RIST, the cycle ergometer was operated in linear (cadence-dependent) mode. The estimate of CP derived from the RIST was then used to prescribe power outputs for constant-power exercise tests that were in the heavy domain (i.e. <CP) and in the severe domain (>CP) in Chapters 7 and 8. Therefore, an accurate RIST-derived estimate of CP was of vital importance to the second aim of this thesis: 'To compare and contrast the presence and magnitude of exercise-induced diaphragmatic and expiratory abdominal muscle fatigue in response to *heavy intensity* exercise, short-duration (~5 min) *severe-intensity exercise* and long-duration (~10 min) *severe-intensity* exercise'. The accuracy and validity of the RIST-derived estimate of CP was compared to CP modelled from the performance of 3-5 exhaustive constant-power exercise tests (i.e. the 'gold standard' method) in a subset of individuals in Chapter 4. All constant power tests in Chapters 7 and 8, and the ramp incremental phase of the RIST were performed in cadence-independent (hyperbolic) mode.

3.11. CAPILLARY BLOOD SAMPLING

Capillary blood lactate concentrations were sampled from the earlobe at regular intervals during all constant-power exercise tests. Prior to sampling, the earlobe was disinfected using a swab containing 70% isopropyl alcohol and allowed to dry to prevent haemolysis. The earlobe was subsequently punctured using a lancing device (Safe T Pro Plus, Accu-Chek, Msida, Malta). After removing the first drop of blood (to avoid debris contaminating the sample) a small drop of blood (~5 µL) was sampled using a portable analyser (Lactate Pro 2, Arkray Global Business Inc., Shiga, Japan) with a test range of 0.5-25.0 mmol/L. During each blood sample, a single-use electrode strip ('test strip') was used to aspirate the blood. Each electrode strip

contains an enzyme sensor, potassium ferricyanide, which reacts with lactate oxidase and lactate in the blood sample to form potassium ferrocyanide and pyruvate. Upon application of a set voltage (+0.5 V), ferrocyanide becomes oxidised and releases a current of electrons proportional to the lactate concentration in the blood sample, which is detected by an ammeter within the blood lactate test meter.

3.11.1. Practical Application

The blood lactate concentration was assessed at rest, at regular intervals during exercise, and within 10 s of exercise termination during all constant power exercise tests in Chapter 7 and 8 of this thesis. Because it is well established that metabolic perturbation differs drastically according to exercise intensity (Black et al., 2016), blood lactate concentration was used (among other cardiopulmonary measures) to verify that exercise trials in Chapter 7 were performed in the heavy-intensity and severe-intensity domains. The blood lactate concentration was also used to show that metabolic perturbation was similar at common time points in response to severe-intensity exercise performed at the same power output but for different durations (i.e. 50% vs. 75% vs. 100% T_{LIM}) in Chapter 8.

CHAPTER 4
Methodological Observations and Considerations

This brief experimental pre-chapter addresses key technical considerations related to some of the methodological techniques that are central to the work presented in this thesis. In Chapters 5 and 6, blood flow in the superficial femoral artery is measured using Doppler ultrasound as the product of vessel cross-sectional area (CSA) (derived from diameter as πr^2) and the time-averaged mean blood velocity (V_{MEAN}). In this chapter, I will consider the impact of manual experimenter measurement vs. automated software measurement of vessel diameter for the computation of blood flow. In Chapters 7 and 8, exercise trials are prescribed in the heavy- and severe-intensity domains following estimation of critical power (CP) via performance of a 'ramp incremental sprint test' (RIST). To accurately prescribe such exercise intensities it is important that the RIST-derived estimate of CP closely approximates CP. Therefore, in this chapter I will establish the validity of the estimate of CP derived from the RIST by comparison to CP modelled directly from a power-duration relationship. In Chapters 5-8, diaphragm and expiratory abdominal muscle contractility are assessed by measuring the pressure and EMG responses to 1-Hz magnetic stimulation of the phrenic and thoracic nerve roots, respectively. Two critical considerations when using magnetic-evoked pressure and EMG responses to assesses changes in muscle contractility over time are: 1) whether the motor nerve input to the muscle is supramaximal; and 2) the within-day between-occasion reproducibility of the measures, both of which will be addressed in this chapter.

4.1. VALIDATION OF MEASUREMENTS OF FEMORAL ARTERY DIAMETER

In Chapters 5 and Chapter 6 of this thesis, leg blood flow ($\dot{Q}_L$) was calculated as the product of superficial femoral artery cross sectional area (CSA) and time-averaged mean blood velocity (V_{MEAN}). Similarly to several previous studies, CSA in these

chapters was determined from the 2D brightness-mode image of the femoral artery by positioning on-screen callipers across the diameter of the artery lumen (i.e. from near to far wall tunica intima) during diastole. V_{MEAN} was determined by integrating the area under the curve of the entire velocity envelope using EchoPAC software (EchoPAC, GE Healthcare, Milwaukee, WI, USA) (Smith et al., 2016, Smith et al., 2017). However, manual positioning of callipers by an experimenter may introduce observer error and/or bias and could theoretically result in in poor reproducibility and validity of the measurement. Additionally, recording diameter at end-diastole may lead to an under-estimation of $\dot{Q}_L$ due to arterial expansion during systole that is not reflected in the measurement. Therefore, manual experimenter-derived determination of vessel diameter was compared to that calculated by automated wall-tracking edge-detection software (Brachial Analyzer, Medical Imaging Applications LLC, Coralville, IA, USA).

Data from the expiratory resistive loading trials targeting mouth and gastric pressure (ERL_{Pm} and ERL_{Pga}) in Chapter 5 were used for this validation (*see* Chapter 5 for detailed description of the experimental protocol). Briefly, Doppler ultrasound recordings were recorded for 16 s for the final 3 min of rest, during every odd min of ERL (i.e. min 1, min 3, and min 5), at task failure, and for 3 min of recovery. There were 11 subjects in the ERL_{Pm} trial and 5 subjects in the ERL_{Pga} trial, giving a total of 160 samples of $\dot{Q}_L$ for the validation. Femoral artery diameter was first determined manually by an experimenter positioning on-screen callipers across the diameter of the artery lumen at end-diastole of each recording (as described above) (DIA_{MAN}). In addition, artery diameter was determined over the entire cardiac cycle using commercially-available automated edge-detection software (DIA_{AUTO}) (Brachial Analyzer). For each measurement, CSA (determined as πr^2) was multiplied by V_{MEAN}, to give $\dot{Q}_L$ derived by manual diameter measurement ($\dot{Q}_{LMAN}$) and $\dot{Q}_L$ derived by automated diameter measurement ($\dot{Q}_{LAUTO}$). The agreement between manual and

automated measurements of diameter and $\dot{Q}_L$ was assessed via Bland-Altman plots, and significant differences were assessed via a one-sample T-test.

The agreement between manual and automated determination of femoral artery diameter is presented in Figure 4.1. On average, manual assessment of femoral artery diameter resulted in an underestimation of 0.009 ± 0.13 cm (95% LoA: −0.034 cm to +0.016 cm) compared to automated methods (Figure 4.1, *panel A*); this mean difference was significantly different to 0 ($P < 0.001$). Similarly, $\dot{Q}_L$ computed from manual diameter was 5 ± 8 ml (95% LoA: −21 ml·min^{-1} to +10 ml·min^{-1}) less than $\dot{Q}_L$ determined from automated diameters (Figure 4.1, *panel B*), a mean difference that was also significantly different to 0 ($P < 0.001$). These findings support the hypothesis that superficial femoral artery diameter using manual calliper-based assessment at end-diastole would be slightly underestimated, as arterial expansion during systole is not factored into the measurement. However, considering a group mean superficial femoral diameter of 0.60 ± 0.06 cm and mean $\dot{Q}_L$ of 165 ± 81 ml·min^{-1} and the small and consistent difference in diameter and $\dot{Q}_L$, I am confident that manual calliper measures provided an accurate measure of superficial femoral artery diameter.

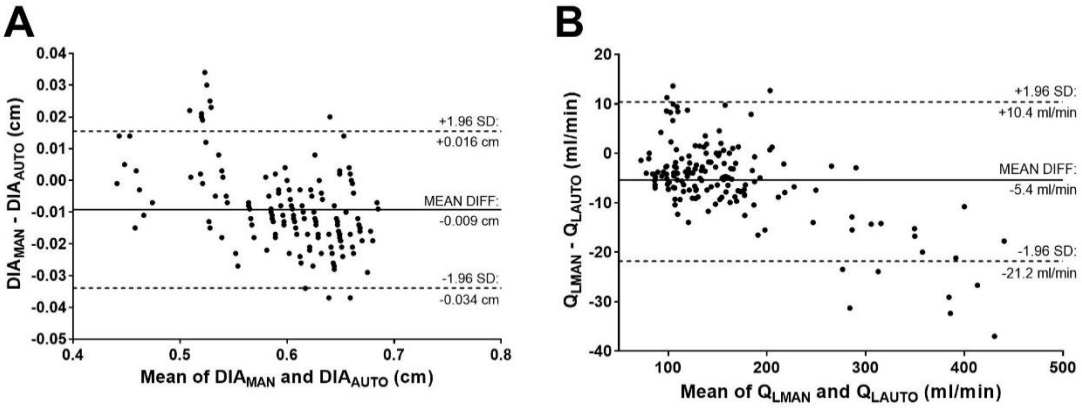


Figure 4.1. Panel A: A bland-altman plot demonstrating agreement between femoral artery diameter derived manually by an experimenter (DIA_{MAN}) vs. via automated edge-detection software (DIA_{AUTO}). Panel B: A bland-altman plot showing agreement between leg blood flow ($\dot{Q}_L$) calculated using manual ($\dot{Q}_{LMAN}$) vs. automated ($\dot{Q}_{LAUTO}$) femoral artery diameters.

Why was automated wall-tracking software not used to determine femoral artery diameter? Brachial Analyser was not used to determine $\dot{Q}_L$ in Chapters 5 and 6 of this thesis for the following reasons. First, the ability to detect both antegrade and retrograde blood flow was of vital importance to the study outcomes. We anticipated that any 'downstream' peripheral vasoconstriction (i.e. distal to the superficial femoral artery) caused by fatiguing expiratory or inspiratory resistive loading breathing tasks would likely result in/be evidenced by an increase in retrograde blood velocity and flow. Indeed, previous studies have suggested that limb blood flow is regulated primarily by changes in vasomotor tone of smaller resistance arteries rather than the large conduit arteries (Joyner and Casey, 2015). As one example, previous work investigating the cardiovascular consequences of fatiguing inspiratory muscle work found a reduction in leg blood flow (Q_L) *without a change in femoral artery diameter* (Sheel et al., 2001). Unfortunately, Brachial Analyser is unable to determine retrograde Doppler velocity. This is because the software package only detects flow above the line of zero flow. Second, Brachial Analyser determines Doppler velocity by using the *peak* Doppler shift, measuring the fastest moving blood cells usually located in the centre of the artery. It is then assumed that half the peak velocity is representative of the mean velocity, derivable from the assumption of a parabolic distribution of laminar flow (Thijssen et al., 2011). However, in the present study, the (expected) large retrograde flows were likely somewhat turbulent, and therefore it was more appropriate to use a software package that can determine Doppler velocity from the intensity-weighted mean Doppler shift (V_{MEAN}), whereby blood velocity from all of the Doppler shifts across the cross-section of the artery is analysed. Therefore, a software package in which diameter and the full blood velocity profile using an intensity-weighted mean Doppler shift could be measured (i.e. EchoPAC) was chosen in the present thesis. Based on the results of the diameter comparison, I am confident the manually-derived femoral artery diameter at end-diastole in EchoPAC

closely approximates that calculated by automated software across the entire cardiac cycle.

4.2. VALIDATION OF THE RAMP INCREMENTAL SPRINT TEST

In Chapters 7 and 8 of this thesis, a 'ramp incremental sprint test' (RIST) was used to determine $\dot{V}O_{2peak}$, P_{peak}, and CP (see Figure 4.2), in order to accurately prescribe heavy- and severe-intensity exercise which are characterised by differing and distinct physiological responses (*see section* 2.2.1 for description of exercise intensity domains). Previous data have shown that the RIST yields a reproducible and valid estimate of CP in healthy habitually active males and trained athletes. Indeed, RIST-derived CP (CP_{RS}) estimates directly measured CP (from performance of 3-5 exhaustive constant-power tests) to within 1-5 W (Wells et al., 2014, Goulding et al., 2018, Murgatroyd et al., 2014), with 95% confidence limits as low as −4.7 to 6.5 W (Murgatroyd et al., 2014). Moreover, the performance of constant-power exercise 10 W above and 10 W below CP_{RS} elicits cardiopulmonary and haemodynamic responses characteristic of exercise in the severe- and heavy-intensity domains, respectively (Murgatroyd et al., 2014).

4.2.1. Experimental Overview

In the present thesis, the accuracy and validity of the CP_{RS} was compared against CP modelled directly from determination of individual power-duration relationships (i.e. the 'gold standard' method) in a sub-sample of participants (*n* = 4, 1 female; 26 ± 1 years; 1.77 ± 0.03 m; 66 ± 5 kg). Each participant visited the laboratory on a minimum of five different occasions, each separated by at least 48 h. The participants abstained from food for 3 h, caffeine for 12 h, and alcohol and exercise for 24 h before each laboratory visit.

4.2.2. CP Modelled from a Power-duration Relationship

The participants performed 4-5 constant power tests to the limit of tolerance (T_{LIM}) at a series of different pre-determined power outputs selected to elicit a range of exercise durations from ~3-15 min (Jones et al., 2010). Following a 4 min warm-up (2 min at 20 W and 2 min at 30% P_{peak}) the power output was increased to the designated level for each test, and each participant maintained a self-selected pedal cadence (80-100 rpm) to within ± 5 rpm until exercise intolerance, defined as a fall in pedal cadence below 60 rpm despite strong verbal encouragement. CP was calculated using the intercept of the linear power-time relationship ($P = W' * [1/t] + CP$) from least squares regression (Jones et al., 2010).

4.2.3. CP Estimated from the RIST

4.2.3.1. RIST protocol

During the ramp incremental phase of the test, the ergometer was operated in hyperbolic mode. Participants cycled at 20 W for 4-6 min before power was increased as a linear function of time at a rate of 25-30 $W \cdot min^{-1}$. The participants increased their pedal cadence progressively until they reached a sustainable self-determined rate (80-100 rpm). Each participant maintained this pedal cadence to within ± 5 rpm until the limit of tolerance, defined as the point at which pedal cadence fell below 60 rpm despite strong verbal encouragement. At the point of exercise intolerance, the cycle ergometer was switched instantaneously to cadence-dependent (linear) mode. The participants accelerated their pedal cadence quickly and performed 3 min of maximal effort cycling (Burnley et al., 2006, Vanhatalo et al., 2007). CP_{RS} was calculated as the mean power output after a plateau in power had occurred between consecutive 30 s bins.

4.2.3.2. Determination of the RIST linear factor

In cadence-dependent mode, the linear factor prescribed for the RIST must be computed prior to the test (Equation 3.4). It is important to select an appropriate alpha factor for each individual, as if cadence is allowed to vary excessively internal power production may not accurately reflect external power (Vanhatalo et al., 2008). Because preferred cadence was unknown prior to the test, and 80 rpm represents the optimum of the parabolic relationship between power and cadence in the fatigued state (Beelen and Sargeant, 1991, Elmer et al., 2013) a target cadence of 80 rpm was selected (e.g. Murgatroyd et al., 2014). From pilot work conducted for this thesis and previous investigations of the relationship between body mass and CP (van der Vaart et al., 2014), it was determined that a power output of 2.5 and 3 times body mass in untrained and trained females, and 3 and 3.5 times body mass in untrained and trained males respectively, would result in a cadence of ~80 rpm during the sprint phase.

4.2.4. Results

There was no significant difference between CP (212 ± 59 W, SEE = 3.1 W) and CP_{RS} (216 ± 54 W) ($P > 0.05$) and there was an intraclass correlation coefficient (ICC) of 0.996 ($P < 0.001$). The 95% limits of agreement for the difference between CP_{RS} and CP were +17.3 to −9.8 W, and CP_{RS} overestimated CP by an average of 3.8 W (*see* Figure 4.3). Accordingly, to prescribe heavy-intensity exercise that would elicit exercise intolerance within 25-45 minutes (Chapter 7), the prescribed power output for the <CP trial in Chapter 7 was set at 5% <CP_{RS}. In contrast to previous studies (Murgatroyd et al., 2014), we utilised a percentage of CP_{RS} rather than an absolute reduction from CP_{RS} (e.g. CP_{RS} −10 W), to better account for differences in absolute CP among participants of varying fitness levels. In this thesis, CP_{RS} was also verified by performance of an exercise test to T_{LIM} at a power output 5% below CP_{RS} in 12 subjects (3 females; 26 ± 4 years; 1.75 ± 0.08 m; 71 ± 9 kg; $\dot{V}O_{2peak}$ = 55 ± 9

ml·kg·min^{-1}). All participants exceeded a tolerable duration of 25 min (mean ± SD = 34.8 ± 6.0 min) and exhibited a delayed steady-state in blood lactate concentration and $\dot{V}O_2$ resulting in a submaximal end-exercise $\dot{V}O_2$ (3.59 ± 0.80 L·min^{-1}, 87 ± 5% of $\dot{V}O_{2peak}$; *see* Chapter 7).

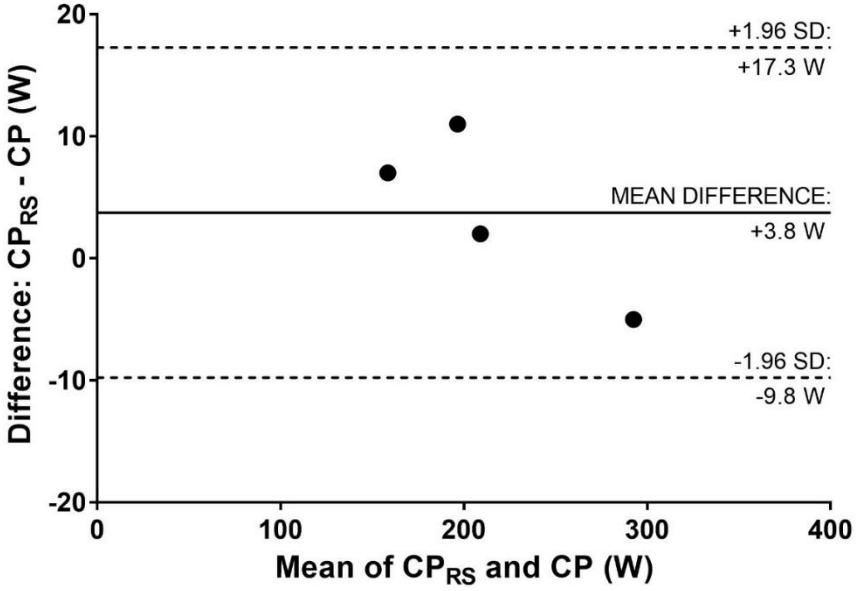


Figure 4.3. A Bland-Altman plot demonstrating the agreement between CP_{RS} and CP in a sub-population of participants (*n* = 4). In these participants, CP_{RS} overestimated CP by 4 ± 7 W with 95% limits of agreement of −10 to 17 W. The mean change (CP_{RS} − CP) was not significantly different from 0 (*P* = 0.36).

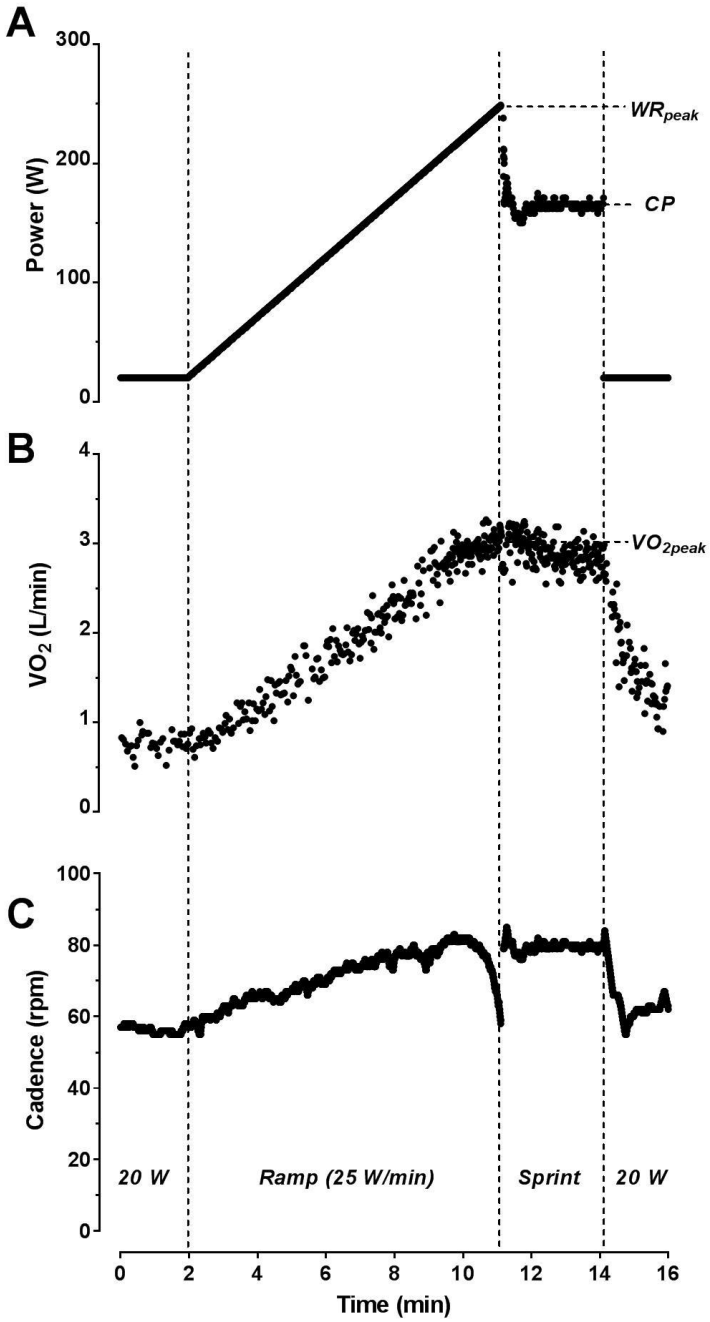


Figure 4.2. The power output (A), pulmonary oxygen uptake ($\dot{V}O_2$) (B) and cycling cadence (C) response to the ramp incremental sprint test (RIST). After unloaded cycling at 20 W, power, cadence and $\dot{V}O_2$ increased linearly as a smooth function of time (25 W·min^{-1}). Immediately following exercise intolerance (i.e. a drop in cadence below 60 rpm; second dashed vertical line) the ergometer was switched to linear mode. During the subsequent 3 min maximal effort 'sprint', power, cadence and $\dot{V}O_2$ were stabilised within 30 s. The average power of the final 150 s of the 'sprint' (165 W) was reported as estimated critical power (CP_{RS}). WR_{peak}, peak ramp incremental work rate; $\dot{V}O_{2peak}$, maximal oxygen uptake.

4.3. ASSESSMENT OF DIAPHRAGM AND EXPIRATORY ABDOMINAL NEUROMUSCULAR FUNCTION

4.3.1. Supramaximality

An important consideration of magnetic stimulation of the phrenic and thoracic nerve roots is whether stimulations are supramaximal, referring to a lack of change in electrical (i.e. action potential amplitude) and mechanical (twitch pressure amplitude) output in response to increasing stimulator intensities. If the assumption of supramaximality is not met, then stimulus reproducibility may not be guaranteed (Davies and White, 1982). Additionally, for the determination of changes in neuromuscular function over time it is important that the recruitment of motor units in response to nerve stimulation is kept constant (*see section* 4.2.2). Supramaximality of stimulation may provide a 'buffer' to protect against any decrease in motor axon excitability induced by repetitive loading of the muscle, ensuring that any change in muscle contractility over time can be attributed to muscle fatigue.

4.3.1.1. Protocol

Supramaximality of electrical (M-wave amplitude) and mechanical (twitch pressure amplitude) responses to magnetic stimulation of the thoracic and phrenic nerve roots was measured separately in Chapters 5 and 6 (Figure 4.3, *panels A-B* and Figure 4.4, *panels A-B*), and in Chapters 7 and 8 (Figure 4.3, *panels C-D* and Figure 4.4, *panels C-D*) of this thesis. At separate visits and after 10 minutes of seated rest, three 1 Hz twitches were delivered to the cervical or thoracic nerve roots at a series of increasing stimulator intensities (50, 60, 70, 80, 85, 90, 95 and 100%). Each stimulation was separated by ~30 s to minimise any effect of twitch potentiation. The amplitude of the transdiaphragmatic and gastric twitch pressure responses, respectively, were analysed as described previously (*see section* 3.8.2). Repeated measures ANOVA with Bonferroni pairwise comparisons were performed to identify significant differences between twitch pressure responses and EMG M-wave

responses at 100% stimulator power vs. all other stimulation intensities. Supramaximality of stimulation was defined as a plateau (i.e. lack of significant differences) in twitch amplitude between 90% and 100% of the stimulators maximum power output.

When the data from all subjects in this thesis were combined, Pga_{tw} amplitude increased progressively in response to increasing stimulator intensities (Figure 4.4, *panel E*), although there was a clear plateau in rectus abdominis M-wave amplitude from 70% to 100% of stimulator power (Figure 4.4, *panel F*). These data suggest that although activation of the rectus abdominis was supramaximal, recruitment of the additional expiratory abdominal muscles (i.e. transversus abdominis, internal oblique, external oblique) was likely submaximal, resulting in the absence of a plateau in Pga_{tw}. In contrast, there was no significant difference in the Pdi_{tw} response to phrenic nerve stimulation at stimulator intensities of ≥90% and a clear plateau was evident (Figure 4.5, *panel E*), suggesting that phrenic nerve depolarisation was supramaximal.

4.3.1.2. Considerations for Submaximal Depolarisation of the Phrenic and Thoracic Nerve Roots

Similarly to the majority of previous studies (e.g. Kyroussis et al., 1996b, Taylor et al., 2006, Geary et al., 2019), magnetically-evoked depolarisation of the thoracic nerve roots was likely submaximal in this thesis. This finding is likely explained by the diffuse spread of thoracic nerve endings (T7-L1), which despite the relatively wide stimulation field of magnetic nerve stimulation (Man et al., 2004), may not be fully activated by nerve stimulation over the vertebral column. A potential consequence of submaximal stimulation of the thoracic nerve roots may be poor reliability of the gastric twitch response (Davies and White, 1982). However, this concern was mitigated by keeping thoracic nerve stimulation constant throughout all experimental

procedures. Specifically, for determination of fatigue, stimulation was always performed at 100% of the stimulators power output and the coil position was repositioned in exactly the same location for all testing periods (identified by application of indelible ink to the skin). Moreover, it is well established that small variations in coil position at the lower thoracic vertebral level do not significantly influence Pga_{tw} responses (Kyroussis et al., 1996a). In this thesis, a very high-level of between-occasion within-day reproducibility was observed (*see section* 4.3.2), and previous data show a similar reliability of Pga_{tw} responses from before to after exercise (Verges et al., 2006).

An additional consideration of submaximal stimulation is that in response to repeated muscle contraction (i.e. during exercise), there may be a change in motor axon excitability which influences the number of motor axons recruited for a given stimulus. For example, in response to repetitive muscle loading axonal hyperpolarisation may occur, characterised by an increase in the activation threshold of motor axons and a consequent reduction in motor axon recruitment, most likely due to the ionic imbalance caused by activation of the Na^+-K^+ pump (Kiernan et al., 2004). A reduction in motor axon excitability and the consequent decrease in force or pressure observed could lead to the overestimation of the magnitude of fatigue. For example, Kiernan et al. (2004) demonstrated that the activation threshold of the motor axons that innervate the wrist increased by ~10-16% following 10 minutes of repetitive supramaximal stimulation of the median nerve. However, this effect may be slightly blunted for the respiratory muscles in response to exercise, as peak exercising inspiratory and expiratory muscle pressures are typically far below maximum values. Additionally, the increase in muscle temperature may have a adverse effect by increasing conductance and theoretically strengthening the magnetic current and consequent twitch response for a given stimulator power. However, to protect against the potential change in motor axon excitability, respiratory muscle fatigue was

conservatively considered as a change in Pga_{tw} and Pdi_{tw} of more than twice the between-occasion within-day CV of each measure (3.9% and 3.7%, respectively).

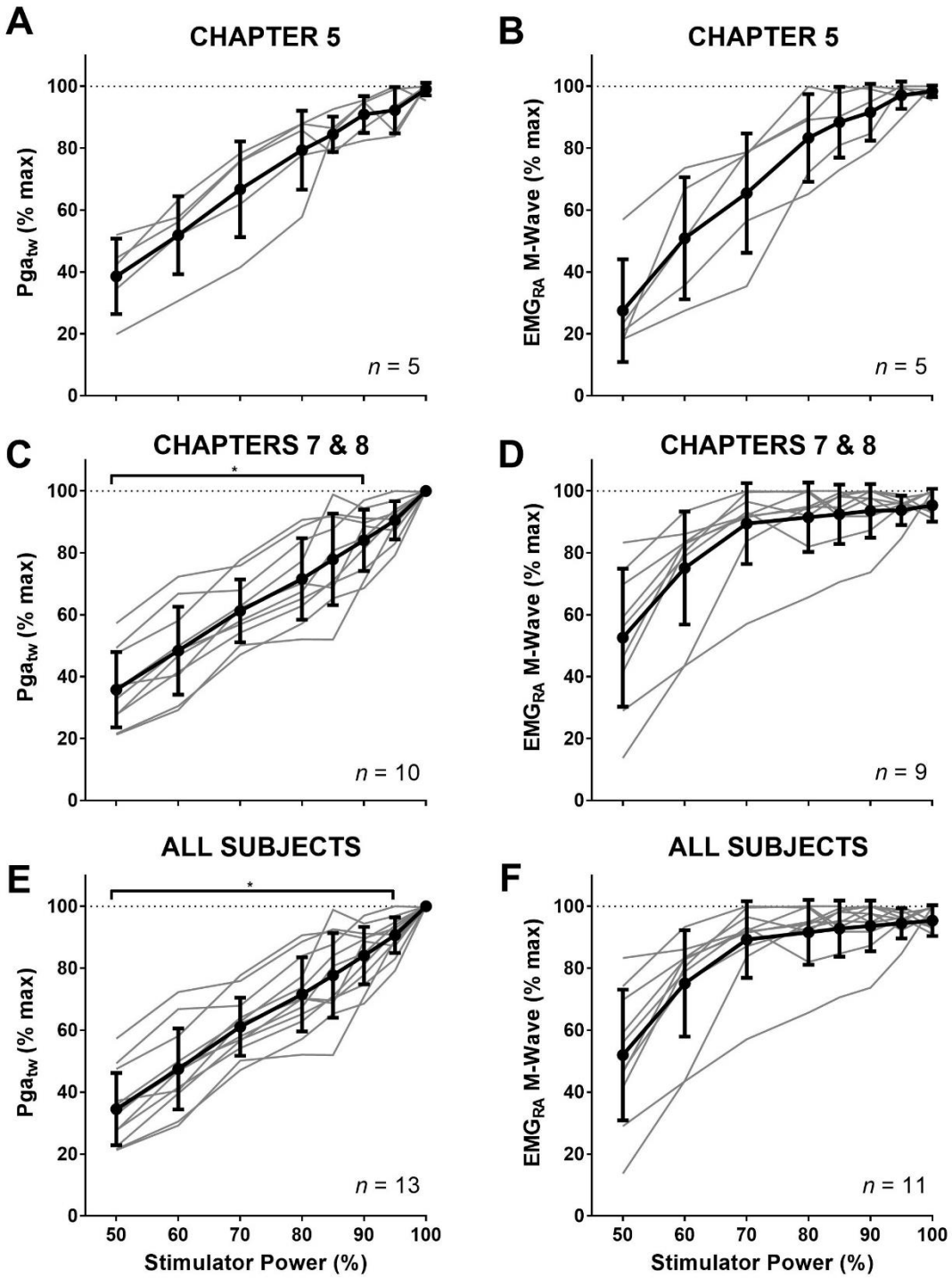


Figure 4.4. Expiratory supramaximality curves for each experimental Chapter of this thesis. Group mean and individual mechanical (Pga_{tw}) and electrical (EMG_{RA} M-Wave) twitch responses to 1 Hz stimulation of the thoracic nerve roots at progressive stimulator intensities. Pga_{tw}, gastric twitch pressure; EMG_{RA}, rectus abdominis electromyography. * $P < 0.05$; significantly different to 100% stimulator power (one-way repeated measures ANOVA with Bonferroni correction).

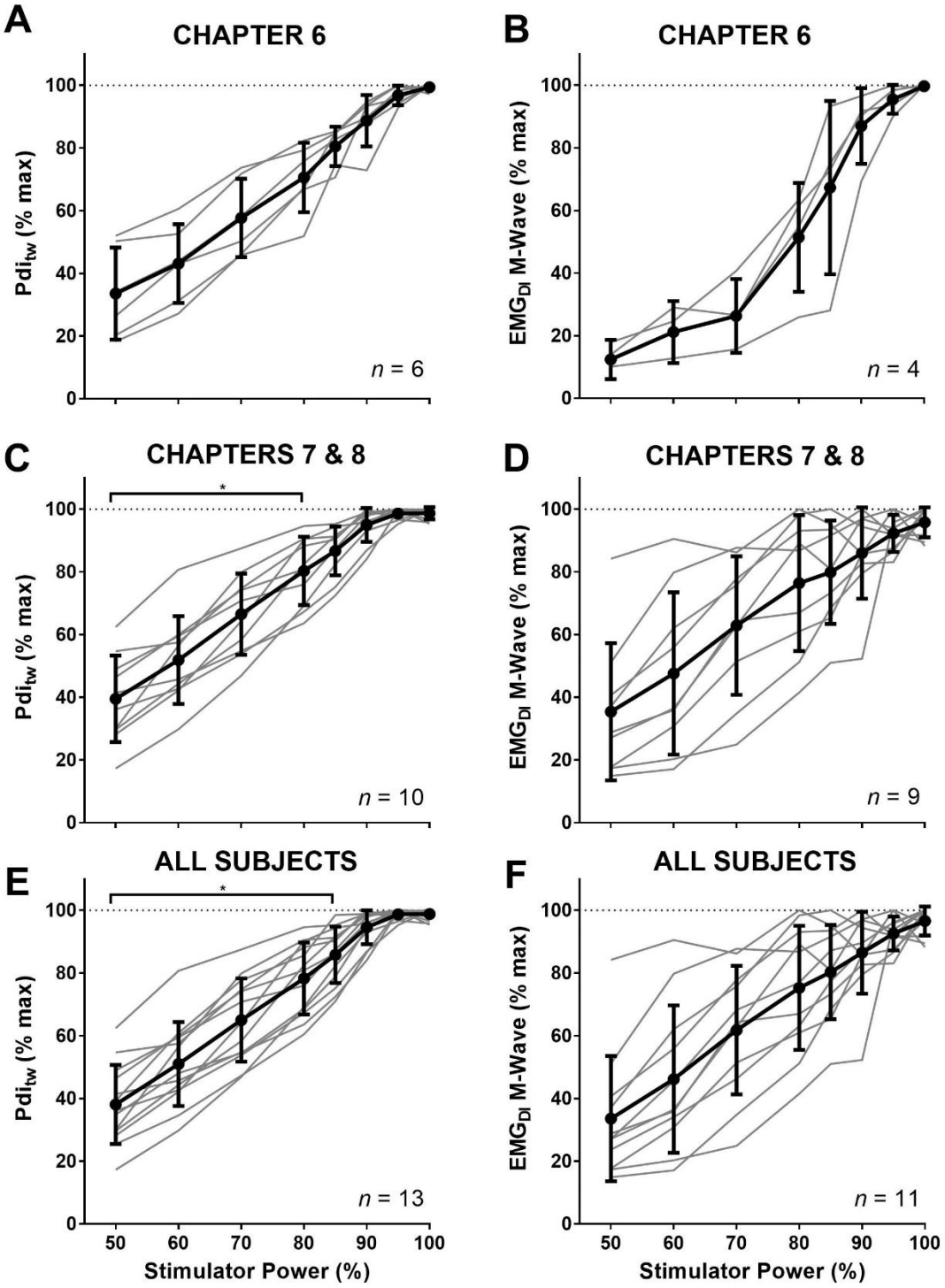


Figure 4.5. Inspiratory supramaximality curves for each experimental Chapter of this thesis. Group mean and individual mechanical (Pdi_{tw}) and electrical (EMG_{DI} M-Wave) twitch responses to 1 Hz stimulation of the thoracic nerve roots at progressive stimulator intensities. Pdi_{tw}, transdiaphragmatic twitch pressure; EMG_{DI}, diaphragm electromyography. * $P < 0.05$; significantly different to 100% stimulator power (one-way repeated measures ANOVA with Bonferroni correction).

4.3.2. Reproducibility

For nerve stimulation to provide a valid measure of neuromuscular fatigue, it is important to demonstrate a high level of consistency and reproducibility over time. It is well established that several methodological factors can affect the twitch response to magnetic stimulation of the phrenic or thoracic nerve roots, including: the operating length of the diaphragm and expiratory abdominal muscles (estimated by Poes), the degree of muscle potentiation, and abdominal wall compliance. These methodological factors were controlled for in this thesis before and after exercise or resistive loading by: 1) performing all stimulations at an end-expiratory Poes with the airway closed; 2) pre-potentiating the twitch response via performance of an inspiratory Müeller or expiratory Valsalva manoeuvre; and 3) performing stimulations in the seated position with the abdomen supported (*see section* 3.8.2).

Within-visit between-occasion reproducibility was determined for inspiratory and expiratory muscle contractility, before and after a period of quiet seated rest prior to constant-power exercise on the fourth or fifth laboratory visit in Chapters 7 and 8. There were no systematic differences in inspiratory and expiratory neuromuscular function from before to after 30 minutes of quiet rest (Table 4.1). The test-retest coefficient of variation (CV) was ≤6.0% and the ICC was ≥0.90 for all variables. The within-day between-occasion test-retest CV for Pdi_{tw} amplitude (3.7%) and Pga_{tw} amplitude (3.9%) demonstrated excellent reproducibility and values were within the lower range of previously reported values (Pdi_{tw}: 5.6-9.4%; Pga_{tw}: 2.8-9.4%) (Archiza et al., 2018, Taylor and Romer, 2008, Welch et al., 2018a, Kyroussis et al., 1996a, Taylor and Romer, 2009). Therefore the Pga_{tw} and Pdi_{tw} were deemed highly reproducible in this thesis.

Table 4.1. Within-day between-occasion reproducibility of neuromuscular function assessments.

Twitch Characteristic	Trial 1	Trial 2	CV (%)	ICC
Pdi_{tw}, cmH_2O	41.7 ± 10.5	40.0 ± 10.3	3.7	0.97
CT, ms	115 ± 7	113 ± 7	1.5	0.92
MRPD/Pdi_{tw}, s/cm	18.6 ± 1.6	18.3 ± 1.6	2.4	0.92
$RT_{0.5}$, ms	69 ± 11	67 ± 13	5.0	0.92
MRR/Pdi_{tw}, s/cm	9.9 ± 1.9	9.8 ± 1.6	5.4	0.91
DIA M-Wave Amplitude, mV	1.7 ± 1.3	1.7 ± 1.3	2.1	0.99
DIA M-Wave Duration, ms	33.1 ± 5.4	32.6 ± 5.2	1.6	0.99
DIA M-Wave Area, $mV \cdot ms^{-1}$	11.1 ± 7.3	10.7 ± 7.2	2.6	0.99
MIP, cmH_2O	140 ± 33	143 ± 31	3.1	0.98
Pga_{tw}, cmH_2O	48.8 ± 26.2	47.6 ± 25.8	3.9	0.99
CT, ms	112 ± 18	113 ± 18	1.6	0.99
MRPD/Pga_{tw}, s/cm	16.9 ± 4.1	16.5 ± 3.8	6.0	0.93
$RT_{0.5}$, ms	123 ± 17	122 ± 20	4.8	0.90
MRR/Pga_{tw}, s/cm	5.4 ± 1.3	5.4 ± 1.2	5.6	0.94
RA M-Wave Amplitude, mV	2.6 ± 1.4	2.6 ± 1.4	5.3	0.99
RA M-Wave Duration, ms	23.6 ± 5.2	23.7 ± 5.5	6.6	0.92
RA M-Wave Area, $mV \cdot ms^{-1}$	13.2 ± 8.0	13.3 ± 7.7	5.1	0.99
MEP, cmH_2O	173 ± 49	170 ± 43	4.9	0.97

Values are group means ± SD for 12 subjects (n = 9 and 11 for diaphragm and rectus abdominis EMG). Trial 1 and trial 2 represent measurements take before and directly after 30 min of seated rest, respectively. CV, coefficient of variation; ICC, intraclass correlation coefficient; Pdi_{tw}, transdiaphragmatic twitch pressure; CT, contraction time, MRPD, maximal rate of pressure development; $RT_{0.5}$, one-half relaxation time; DIA, diaphragm; MIP, maximal inspiratory pressure; Pga_{tw}, gastric twitch pressure; RA, rectus abdominis; MEP, maximal expiratory pressure.

CHAPTER 5
The Cardiovascular Consequences of Fatiguing Expiratory Muscle Work in Healthy Otherwise Resting Humans

5.1. INTRODUCTION

The respiratory and cardiovascular systems are functionally linked. The interactions between these two organ systems play a critical role in determining blood flow and O_2 delivery to the body tissues in relation to oxygen consumption ($\dot{V}O_2$), particularly during exercise. One such cardiorespiratory interaction is a fatigue-induced respiratory muscle metaboreflex (Dempsey et al., 2006, Sheel et al., 2018a). Previously, it has been shown that fatiguing 'high-intensity' voluntary contractions of the inspiratory muscles in otherwise resting humans trigger a reflexively-mediated sympathoexcitation that is associated with a time-dependent increase in muscle sympathetic nerve activity (MSNA) and mean arterial pressure (MAP), and with vasoconstriction and a reduction in blood flow and O_2 delivery in the resting limb (St Croix et al., 2000, Sheel et al., 2001, Smith et al., 2016, Smith et al., 2017, Welch et al., 2018b). This inspiratory muscle metaboreflex also appears to be active during severe-intensity whole-body exercise. Indeed, relative to control conditions, attenuation of the inspiratory work of breathing via proportional assist ventilation during such exercise is associated with a decrease in noradrenaline spillover that is significantly related to a reduction in vascular resistance (LVR) and an increase in blood flow ($\dot{Q}_L$) in the exercising leg (Harms et al., 1997, Dominelli et al., 2017). These findings suggest that sympathetically-mediated alterations in LVR and $\dot{Q}_L$ can be triggered by changes in inspiratory muscle work.

The present interest of this study is in the effect of fatiguing expiratory muscle work on cardiovascular function and systemic O_2 transport. Rhythmic contractions of the expiratory muscles sustained to the point of task failure in otherwise resting humans elicit an increase in MSNA burst frequency and MAP (i.e. a sympathoexcitation) that

is similar in magnitude and time-dependency to that caused by fatiguing contractions of the inspiratory muscles (Sheel et al., 2001, Derchak et al., 2002). Moreover, in the resting canine, infusion of the metabolite lactic acid into the expiratory muscle circulation versus the diaphragm circulation causes a marked and comparable increase in MAP and decrease in leg vascular conductance and $\dot{Q}_L$ (Rodman et al., 2003). During whole-body exercise the expiratory muscles of the rib cage and abdominal wall contribute substantially to ventilation (Henke et al., 1988, Aaron et al., 1992b, Aliverti et al., 1997, Taylor et al., 2006, Dominelli et al., 2015) and, like the diaphragm, the expiratory muscles fatigue in response to severe-intensity exercise performed to the limit of tolerance (Taylor et al., 2006, Verges et al., 2006). Interestingly, during submaximal exercise, augmentation of expiratory muscle work via voluntary hyperpnoea combined with an expiratory resistance is associated with greater increases in MSNA and MAP relative to the addition of voluntary hyperpnoea alone (Katayama et al., 2015b). In addition, it has been shown that the severity of exercise-induced quadriceps fatigue, quantified as the reduction relative to prior baseline values in magnetically evoked quadriceps twitch force (Q_{tw}), is greater after exercise of the same intensity and duration with compared to without prior induction of expiratory muscle fatigue (EMF) (Taylor and Romer, 2008). It was suggested that this exacerbation of exercise-induced quadriceps fatigue with prior EMF was likely the consequence of attenuated $\dot{Q}_L$ and O_2 delivery to the working muscles secondary to a sympathetically-mediated vasoconstriction in the exercising limb muscles.

In combination, the aforementioned findings suggest that fatiguing expiratory muscle work elicits a sympathoexcitation (i.e. expiratory muscle metaboreflex) that is remarkably similar to that induced by very high inspiratory muscle work and/or fatigue. However, while it is well accepted that the sympathoexcitatory response to fatiguing inspiratory muscle work results in vasoconstriction and impaired blood flow and O_2 delivery to the resting and exercising limb (Harms et al., 1997, Sheel et al.,

2001, Chiappa et al., 2008, Katayama et al., 2012, Smith et al., 2016, Smith et al., 2017), the cardiovascular response to activation of an expiratory muscle metaboreflex is yet to be fully addressed. Accordingly, the aim of this study was to investigate the cardiovascular consequences of fatiguing expiratory work in healthy humans. Specifically, the LVR and $\dot{Q}_L$, as well as the MAP and heart rate (HR), response to 'high-intensity' contractions of the expiratory muscles sustained to the point of task failure in otherwise resting individuals was determined. It was hypothesised that HR, MAP and LVR would increase and that $\dot{Q}_L$ would decrease in a time-dependent manner during fatiguing expiratory muscle work.

5.2. METHODS

5.2.1. Subjects

Eleven recreationally active adults participated in the study (2 females, mean ± SD: age 25 ± 4 y; stature 1.76 ± 0.06 m; body mass 73.8 ± 9.5 kg). All subjects were healthy, had no history of respiratory, cardiovascular or metabolic disease, and had pulmonary function within normal limits (forced vital capacity: 5.4 ± 0.9 L, 103 ± 9% of predicted; forced expiratory volume in 1 s: 4.5 ± 0.7 L, 104 ± 9% of predicted). The subjects abstained from food for 3 h, caffeine for 12 h, and alcohol and exercise for 48 h before each laboratory visit. Both female subjects had been using a monophasic oral contraceptive pill for >6 months prior to starting the study, and continued their oral contraceptive pill throughout the experimental period. All of the experimental procedures were approved by the University of Leeds Faculty of Biological Sciences Research Ethics Committee and conformed to the Declaration of Helsinki (approval REF: BIOSCI 16-020). Each subject provided written informed consent prior to the commencement of any testing procedures.

5.2.2. Experimental Procedures

Each subject initially visited the laboratory on two separate occasions. At the first visit, the subjects were thoroughly familiarized with all of the experimental procedures and measurements, including the expiratory resistive loaded breathing (ERL) tasks and the determination of maximal expiratory mouth pressure (MEP). To ensure familiarisation with the ERL tasks, each subject performed short bouts (1-2 min) of ERL until they could generate a consistent 'square-wave' in expiratory mouth pressure (Pm) for >2 min at the target pressure and in accordance with the prescribed duty cycle and respiratory frequency without coaching. On the second visit, the subjects performed ERL targeting a Pm of 2% of MEP for 5 min (*control*). Following 30 min of quiet rest, the subjects then performed ERL targeting 65% of MEP until task failure (ERL_{Pm}). The control trial was performed first to avoid any residual effect of peripheral muscle fatigue and potential sensitisation of the metaboreflex becoming apparent during the control trial. During ERL_{Pm}, the subjects were instructed to maintain a constant Pm at the target level throughout each expiration but *were not* given any specific instructions on how to recruit the expiratory muscles. $\dot{Q}_L$ and LVR were measured for the final 16 s of every minute at rest, during ERL, and in recovery; HR and MAP were recorded continuously.

Despite the presence of ERL-induced expiratory muscle fatigue and the expected increase in MAP (*see below*: "ERL_{Pm}-induced expiratory muscle fatigue" and "Cardiovascular measurements during ERL_{Pm}"), no change in group mean LVR or $\dot{Q}_L$ in response to ERL_{Pm} was observed. At this time, it was considered possible that specifically targeting the primary expiratory muscles (*i.e. the muscles of the abdominal wall*) during ERL may increase the severity of ischemia in these muscles, hasten the onset of their fatigue, augmenting the initiation of an expiratory muscle metaboreflex and the associated cardiovascular consequences. Accordingly, a sub-sample of subjects (n = 5) attended the laboratory on a third occasion and performed

ERL but this time targeted 2% (5 min, *control*) and 65% (to task failure) of maximal expiratory gastric pressure (Pga_{max}) (ERL_{Pga}). At the start of the experimental visit subjects practiced and were coached through the performance of ERL_{Pga}; the subjects were instructed to maintain a constant Pga at the target level throughout each expiration, and were explicitly instructed to 'target the abdomen' during each expiration. Once ERL_{Pga} could be performed accurately at the intended duty cycle and respiratory frequency, subjects rested quietly for 30 min prior to performance of the ERL_{Pga} control trial. During ERL_{Pga} trials, $\dot{Q}_L$ and LVR were measured for the final 16 s of every minute at rest, during ERL, and in recovery; HR and MAP were recorded continuously.

5.2.3. Expiratory Resistive Loaded Breathing

All ERL trials were performed with the subjects in the semi-recumbent position and breathing through a custom-built two-way valve with a variable diameter resistor incorporated into the expiratory port; inspiration was completely unimpeded. A calibrated pressure transducer (DP45, Validyne Engineering, Northridge, CA, USA) was connected into the mouthpiece to allow continuous measurement of Pm. During each ERL trial, the target expiratory pressure (Pm or Pga) was displayed on a computer screen, and the subjects maintained a respiratory frequency (f_R) of 15 breaths·min^{-1} and an expiratory duty cycle (T_E/T_{TOT}) of 0.5 by following a computer generated audio signal with distinct inspiratory and expiratory tones. The subjects were instructed to maintain a constant Pm or Pga at the target level throughout each expiration; inspiration was unresisted. The subjects were monitored closely by the researchers during each ERL trial to ensure proper timing, breathing technique, and effort. An experimenter supported the subject's cheeks throughout ERL to minimize use of the buccal muscles. Airflow (no. 4813, Hans Rudolph Inc.; Shawnee, KS, USA) and end-tidal partial pressure of carbon dioxide ($P_{ET}CO_2$) (Ultima Cardio 2, MGC Diagnostics, St Paul, MN, USA) were measured throughout each ERL trial, and

$P_{ET}CO_2$ was maintained within ± 3 mmHg of eucapnic control values by manually adjusting the inspired fraction of CO_2. It is unlikely that such a small change in $P_{ET}CO_2$ would have significant vasomotor effects (Heistad and Wheeler, 1970). Doppler ultrasound (Vivid iq, GE Healthcare, Milwaukee WI, USA) was used to measure $\dot{Q}_L$ during the last three minutes of each period of eupnoea (*rest*), every minute during all ERL trials, and during the first three minutes after each ERL trial (*recovery*). MAP and HR were measured beat-by-beat using finger photoplethysmography (Finapres Nova, Finapres Medical Systems, Amsterdam, The Netherlands), and LVR was subsequently calculated as MAP/$\dot{Q}_L$. As per the manufacturer guidelines, physiological calibration (PhysioCal) was used during ERL to maintain the accuracy of Finapres recordings; measurements were averaged over 60 s and calibration periods were excluded from the analysis. In addition, surface electromyography (EMG) (Trigno Avanti, Delsys Inc., Natick, MA, USA) was recorded from the vastus lateralis and vastus medialis of the right leg to confirm that no limb muscular contraction occurred during the ERL trials.

5.2.3.1. Expiratory resistive loading breathing targeting expiratory mouth pressure (ERL_{Pm})

The subjects rested quietly with breathing completely un-resisted for 15 min to allow accurate baseline cardiovascular measurements to be made. Next, each subject expired against the resistive load for 5 min whilst targeting 2% of MEP (*control*) before resting quietly with breathing completely unresisted for a further 30 min. Then, each subject performed ERL whilst targeting 65% of MEP until task failure (ERL_{Pm}), defined as an inability to generate the target pressure for three consecutive breaths despite strong verbal encouragement. Once task failure was achieved, the subjects were given strong verbal encouragement to continue attempting to generate their target Pm for one additional minute (defined as the ‘task failure’ minute). By having subjects perform this additional minute of ERL_{Pm}, it was ensured that: 1) each subject was

truly failing the task rather than having a 'few bad breaths' and 2) a full sample of ultrasound data was collected without interruption during the final 'task failure' minute. The presence and severity of ERL_{Pm}-induced expiratory muscle fatigue was quantified as the reduction relative to prior baseline values in the MEP response to maximal Valsalva manoeuvres initiated from total lung capacity (Laveneziana et al., 2019a). The maximum value of 3-5 attempts that varied by <10% was reported.

5.2.3.2. Expiratory resistive loaded breathing targeting expiratory gastric pressure (ERL_{Pga})

As in ERL_{Pm}, the subjects rested quietly for 15 min before expiring against the resistive load for 5 min but this time targeting 2% of Pga_{max} (*control*). Following a further 30 min of quiet rest each subject performed ERL targeting 65% of Pga_{max} (ERL_{Pga}) until task failure; again, subjects continued to perform ERL_{Pga} for one minute after task failure was reached. The presence and severity of ERL_{Pga}-induced expiratory muscle fatigue was determined as the pre- to post-ERL reduction in MEP and the gastric twitch pressure (Pga_{tw}) response to magnetic stimulation of the thoracic nerve roots.

5.2.4. Leg Blood Flow via Doppler Ultrasound

$\dot{Q}_L$ was measured using Doppler ultrasound (Vivid iq, GE Healthcare, Milwaukee, WI, USA). A 10 Hz linear probe (9L-RS, GE Healthcare, Milwaukee, WI, USA) was positioned over the superficial femoral artery of the right leg ~3-5 cm below the bifurcation of the deep and superficial femoral artery. The position of the probe was marked with indelible ink and measured from the knee to ensure accurate and consistent re-positioning across ERL trials. The Doppler sample volume was set to the full width of the artery and the angle of insonation was fixed to 60°. Video recordings were obtained for the last 16 s of: a) each of the last three minutes of each period of eupnoea (*rest*); b) every minute during each ERL trial; and c) the first three

minutes after each ERL trial (*recovery*). Because of the 0.5 expiratory duty cycle during ERL, there was an equal period of inspiration and expiration for each recording. All data analysis was performed offline by the same investigator using EchoPac software (EchoPAC, GE Healthcare, Milwaukee, WI, USA). Femoral artery diameter was determined during diastole by positioning on-screen callipers, and CSA was computed as πr^2 (Smith et al., 2016). Time-averaged mean blood velocity (V_{MEAN}) was determined for each cardiac cycle by integrating the area under curve of the entire velocity profile. Antegrade blood velocity (V_{ANT}) was calculated by integration of positive blood velocity for each cardiac cycle, and retrograde blood velocity was determined as $V_{ANT}-V_{MEAN}$. $\dot{Q}_L$ was calculated for each cardiac cycle as the product of vessel CSA and V_{MEAN}.

5.2.5. Electromyography

To confirm that the subjects avoided contraction of the non-respiratory muscles during ERL, the EMG activity of the vastus medialis (VM) and vastus lateralis (VL) of the right leg was recorded continuously (Trigno Avanti, Delsys Inc., Natick, MA, USA) according to standard guidelines (Hermens et al., 1999). Subjects performed three maximal isometric contractions prior to the rested breathing phase, and all subsequent signals were normalized to the maximum EMG response. EMG signals were band-pass filtered and full-wave rectified, and the peak root mean square was calculated using a time constant of 0.1 s (VM_{RMS} and VL_{RMS}).

5.2.6. Expiratory Abdominal Function via Magnetic Nerve Stimulation

For ERL_{Pga}, gastric (Pga) and oesophageal (Poes) pressure were measured using two balloon-tipped catheters (47-9005, Ackrad Laboratory, Berlin, Germany) that were passed via the nares and into the stomach and lower one-third of the oesophagus, respectively. The oesophageal balloon was filled with 1 ml of air and positioned using the occlusion technique (Baydur et al., 1982). The gastric balloon

was filled with 2 ml of air and positioned so that Pga was positive during eupnoeic breathing in the seated position. Each catheter was connected to a differential pressure transducer (DP45, Validyne Engineering, Northridge, CA, USA) that was calibrated across the physiological range using a digital pressure manometer (no. 621, Test Products International Inc., Beaverton, OR, USA).

Magnetic stimuli were delivered to the thoracic nerve roots between the 8th (T8) and 11th (T11) thoracic vertebrae via a 90-mm circular coil powered by a magnetic stimulator (Magstim BiStim 2, Magstim, Whitland, Wales), as described before (Kyroussis et al., 1996a, Taylor et al., 2006). The area of stimulation that evoked the greatest Pga_{tw} was located and marked for use for all subsequent stimulations, and all stimulations were delivered at a consistent relaxed end-expiratory lung volume (i.e. FRC), as judged by end-expiratory Poes. Similarly to previous findings, magnetic stimulation of the thoracic nerves likely elicited a submaximal response from the expiratory abdominal muscles; these data and the inherent considerations for submaximal stimulations are presented in the 'Methodological Observations and Considerations' Chapter (*see section* 4.3) (Kyroussis et al., 1996a, Taylor et al., 2006, Verges et al., 2006).

Expiratory abdominal muscle contractility was assessed at baseline, ~5 min after *control* ERL, and 5 min after ERL_{Pga}. The potentiated twitch is a more sensitive measure of muscle fatigue relative to the non-potentiated twitch (Kufel et al., 2002b). Accordingly, the Pga_{tw} response to a 1-Hz magnetic stimulation that was delivered at 100% of the stimulator's power output ~5 s after a 5 s maximal expulsive manoeuvre that was initiated from total lung capacity, was measured. This procedure was repeated six times such that six potentiated Pga_{tw} values were obtained, with the first two measurements discarded because the degree of potentiation was slightly smaller after the first and second expulsive manoeuvres. Each potentiated twitch was

assessed for amplitude (baseline to peak), maximal rate of pressure development (MRPD), maximal relaxation rate (MRR), contraction time (CT) and one-half relaxation rate ($RT_{0.5}$). Pga_{MAX} was calculated as the peak Pga (across 1 s) during each expulsive manoeuvre; Pga_{MAX} was reported as the maximum of three values that varied by ≤10%. The within-day between occasion reproducibility coefficients (coefficient of variation, CV) were 3.9, 7.7, 5.7, 1.6, 4.8 and 2.1% for Pga_{tw}, MRPD, MRR. CT, $RT_{0.5}$ and MEP, respectively (*see section* 4.4).

5.2.7. Data capture

All pressure, airflow, hemodynamic and EMG signals were digitized at sampling rates of 150 Hz and 2 kHz (EMG only) using an analogue-to-digital converter (Micro3 1401, Cambridge Electronic Design, Cambridge, UK) and captured using commercially available software (Spike 2 version 8.0, Cambridge Electronic Design). For ERL_{Pga}, airflow and the Doppler ultrasound signal were time-aligned according to corresponding clock-times in the data acquisition software (*Spike 2*) and the ultrasound machine for each video recording. If a cardiac cycle crossed the inspiratory-expiratory cycle, it was allocated to the respiratory phase that it was initiated in.

5.2.8. Statistical analysis

Based on a previously reported reduction in $\dot{Q}_L$ in response to IRL of 23 ± 10% (rest vs. task failure) (Smith et al., 2016), it was determined that 5 subjects would be needed to detect a significant change in $\dot{Q}_L$ at an alpha error probability of 0.05 and a statistical power of 0.90. Normality of distribution was assessed qualitatively via visual inspection of descriptive statistics, Q-Q plots and histograms, and quantitatively using the Shapiro-Wilk test and the determination of Z-scores for skewness and kurtosis. All data that violated the assumption of normality were assessed using a Friedman's ANOVA with Bonferroni post hoc comparisons

performed for significant main effects. For all normally distributed data, repeated measures ANOVA were used to compare absolute hemodynamic and cardiovascular measurements ($\dot{Q}_L$, LVR, MAP, HR), as well as EMG measurements (VM_{RMS} and VL_{RMS}) across time for ERL_{Pm} (rest vs. min 1 vs. min 3 vs. min 5 vs. task failure vs. recovery). When significant main effects were shown, post-hoc pairwise comparisons with a Bonferroni correction were made. To limit the occurrence of type 2 error for hemodynamic variables in the ERL_{Pga} trial (i.e. HR and MAP), the number of comparisons across time were limited to rest vs. min 1 vs. task failure. Repeated measures ANOVA with Bonferroni correction were also used to compare absolute MEP (ERL_{Pm} and ERL_{Pga}) and Pga_{tw} (ERL_{Pga} only) across time (baseline vs. ~5 min after control vs. 5 min after ERL). For ERL_{Pm} only, Pearson's product moment correlation coefficients (*r*) were computed to determine the relationship between the absolute change in $\dot{Q}_L$ from baseline (eupnoea) to task failure and: 1) baseline expiratory muscle strength (i.e. MEP); 2) the magnitude of ERL-induced expiratory muscle fatigue (i.e. the pre- to post-ERL_{Pm} change in MEP); and 3) the time to task failure. Results are expressed as group mean ± SD and all statistical analysis was performed in SPSS Statistics 24 (SPSS Inc, Chicago, IL). The acceptable type 1 error was set at $P < 0.05$.

5.3. RESULTS

5.3.1. Cardiovascular measurements during eupnoea

The CV and intraclass correlation coefficients for all cardiovascular parameters ($\dot{Q}_L$, LVR, MAP and HR) during resting eupnoeic breathing were ≤5.9% and ≥0.92, respectively. Due to the random variation in $\dot{Q}_L$ and LVR across time and the lack of external validation of absolute flow values against phantom artery preparations (Thijssen et al., 2011), the $\dot{Q}_L$ and LVR responses were compared to values measured during prior eupnoeic control periods. However, the absolute resting values of superficial femoral artery $\dot{Q}_L$ (119 ± 52 $ml \cdot min^{-1}$; ERL_{Pm}) were well within

the normal range reported for young healthy adults (70-196 ml·min^{-1}) (Hussain et al., 1996, Holland et al., 1998, Smith et al., 2016, Smith et al., 2017).

5.3.2. Expiratory resistive loaded breathing targeting expiratory mouth pressure: ERL_{Pm}

5.3.2.1. Cardiovascular measurements during control ERL (2% of MEP)

There was no change in group mean MAP, $\dot{Q}_L$, LVR (Figure 5.1), HR, superficial femoral artery diameter and V_{MEAN} (Table 5.1) across time in response to ERL at 2% of MEP (all $P \geq 0.05$). Neither VM_{RMS} nor VL_{RMS} increased from rest to during ERL at 2% of MEP, confirming no contraction of the non-respiratory muscles ($P \geq 0.05$; Table 5.1).

5.3.2.2. Cardiovascular measurements during ERL_{Pm} (65% of MEP)

The ERL_{Pm} trial was performed for 9.3 ± 2.7 min. Before task failure, expiratory Pm was maintained at 97 ± 4% of the target value (Table 5.1). There was an immediate and sustained increase in group mean HR from rest to during ERL_{Pm} ($P < 0.05$; Table 5.1). Similarly, relative to resting baseline values, group mean MAP increased by 19 ± 6 mmHg ($P < 0.001$) and 31 ± 10 mmHg ($P < 0.001$) at the first minute and at task failure, respectively, during ERL_{Pm} (Figure 5.1). Conversely, there was no change in group mean superficial femoral artery diameter, V_{MEAN}, $\dot{Q}_L$ or LVR across time from baseline to during ERL_{Pm} ($P \geq 0.05$) (Figure 5.1, Table 5.1). The $\dot{Q}_L$ and LVR response to ERL_{Pm} was, however, highly variable between the subjects. Indeed, the change in $\dot{Q}_L$ and LVR from baseline to task failure during ERL_{Pm} ranged from –38% to +70% ($\dot{Q}_L$) and from –19% to +93% (LVR) (Figure 5.1). As in the control trial targeting 2% of MEP, there was no evidence of leg muscle contraction throughout ERL_{Pm} (Table 5.1).

Table 5.1. Group mean ventilatory, cardiovascular and muscular responses to expiratory resistive loading targeting 2% and 65% of maximal expiratory mouth pressure.

	Rest	Min 3	Min 5	Task Failure	Recovery
ERL at 2% of MEP (control)					
Expiratory Pm, cmH_2O	1 ± 0	2 ± 1	5 ± 2	-	2 ± 1
f_R, $br \cdot min^{-1}$	12 ± 3	15 ± 1	15 ± 0	-	13 ± 4
$P_{ET}CO_2$, mmHg	42 ± 4	38* ± 4	39* ± 3		42 ± 4
Heart Rate, $bt \cdot min^{-1}$	61 ± 9	62 ± 10	65 ± 9	-	62 ± 10
SFA V_{MEAN}, $cm \cdot s^{-1}$	7.7 ± 3.6	7.6 ± 4.0	8.1 ± 3.8	-	7.6 ± 4.0
SFA Diameter, cm	0.58 ± 0.05	0.58 ± 0.06	0.58 ± 0.06	-	0.58 ± 0.06
VM_{RMS}, % of max	0.8 ± 0.8	0.9 ± 0.7	1.0 ± 0.8	-	1.0 ± 0.7
VL_{RMS}, % of max	0.8 ± 1.0	0.9 ± 0.9	1.0 ± 1.1	-	1.0 ± 1.1
ERL at 65% of MEP (ERL_{Pm})					
Expiratory Pm, cmH_2O	2 ± 0	128* ± 19	121* ± 17	112* ± 21	3 ± 1
f_R, $br \cdot min^{-1}$	12 ± 3	15 ± 1	15 ± 1	15 ± 1	16 ± 5
$P_{ET}CO_2$, mmHg	43 ± 5	45 ± 4	46 ± 4	46 ± 4	42 ± 4
Heart Rate, $bt \cdot min^{-1}$	63 ± 12	83* ± 9	86* ± 9	91* ± 9	69 ± 10
SFA V_{MEAN}, $cm \cdot s^{-1}$	8.3 ± 3.2	9.9 ± 2.8	10.0 ± 3.2	9.2 ± 3.3	10.2 ± 4.3
SFA Diameter, cm	0.59 ± 0.07	0.57 ± 0.06	0.57 ± 0.06	0.57 ± 0.06	0.58 ± 0.06
VM_{RMS}, % of max	0.5 ± 0.2	0.9 ± 0.3	0.9 ± 0.3	0.9 ± 0.4	0.7 ± 0.4
VL_{RMS}, % of max	0.6 ± 0.6	1.0 ± 0.7	1.1 ± 0.8	1.0 ± 0.8	0.9 ± 0.8

Values are mean ± SD in 11 subjects (HR and $\dot{Q}$, $n = 9$). Pm, mouth pressure; T_E/T_{TOT}, expiratory duty cycle; V_T, tidal volume; SFA, superficial femoral artery; V_{MEAN}, time averaged mean blood velocity; VM, vastus medialis; VL, vastus lateralis; RMS, root mean square. *significantly different to rest ($P < 0.05$).

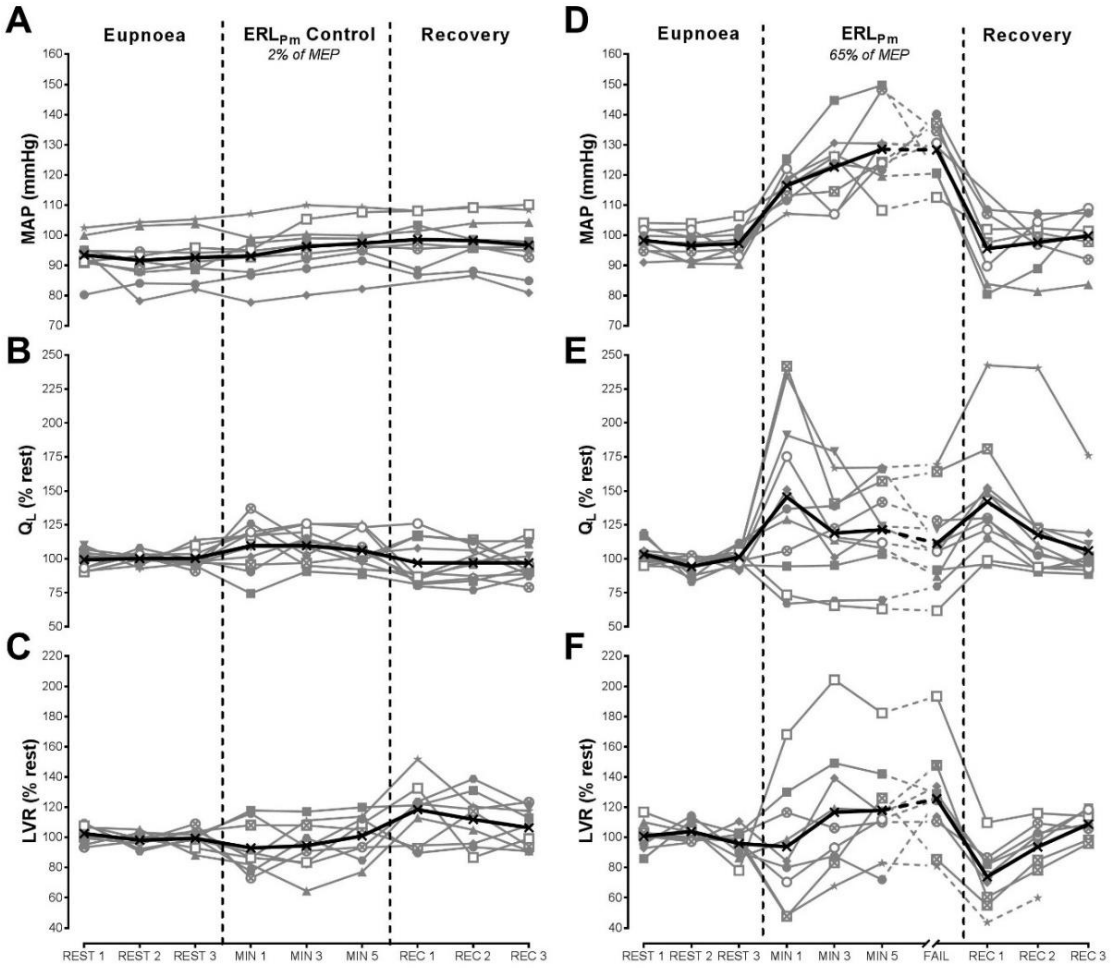


Figure 5.1. Mean arterial pressure (MAP, *n* = 9) (top panels), leg blood flow ($\dot{Q}_L$, *n* = 11) (middle panels), and leg vascular resistance (LVR, *n* = 9) (bottom panels) responses to expiratory resistive loading targeting 2% (ERL_{Pm} control) (A-C) and 65% (ERL_{Pm}) of maximal expiratory mouth pressure (D-F). Data are group means (black) and individual values (grey). Females are presented as clear open symbols (*n* = 2).

5.3.2.3. ERL_{Pm}-induced expiratory muscle fatigue

There was no change in MEP from pre- to post-5 min of control ERL at 2% of MEP (200 ± 28 vs. 196 ± 28 cmH_2O, *P* = 1.000). There was, however, a significant reduction relative to prior baseline values in MEP following ERL_{Pm} at 65% of MEP (196 ± 28 vs. 163 ± 28 cmH_2O, *P* = 0.001) (Figure 5.2, *panel A*).

5.3.3. Expiratory resistive loaded breathing targeting expiratory gastric pressure: ERL_{Pga}

Figure 5.3 shows breath-by-breath Pm and Pga, beat-by-beat finger arterial pressure (AP) and HR, and mean $\dot{Q}_L$ and LVR data for one individual subject during ERL targeting 2% (Figure 5.3, *panel A*) and 65% of Pga_{MAX} (Figure 5.3, *panel B*).

5.3.3.1. Cardiovascular measurements during control ERL (2% of Pga_{max})

Group mean MAP, $\dot{Q}_L$, LVR (Figure 5.4), HR, superficial femoral artery diameter and V_{MEAN} (Table 5.2) did not change across time during ERL at 2% of Pga_{MAX} ($P \geq 0.05$). Similarly, neither VM_{RMS} nor VL_{RMS} increased from rest to during ERL at 2% of Pga_{MAX}, confirming contraction of the non-respiratory muscles did not occur ($P \geq 0.05$) (Table 5.2).

5.3.3.2. Cardiovascular measurements during ERL_{Pga} (65% of Pga_{max})

ERL_{Pga} was performed for 9.5 ± 2.3 min. Prior to task failure, expiratory Pm and Pga were maintained at 99 ± 5% and 87 ± 11% of the target values, respectively (Table 5.2). There was a progressive increase in HR that was significantly elevated versus resting values by task failure (+31 ± 10 beats·min^{-1}, $P = 0.007$) (Table 5.2). Similarly, group mean MAP was not different to resting values at the first minute of ERL_{Pga} (+20 ± 15 mmHg; $P = 0.132$), but was significantly elevated by task failure (18 ± 9 mmHg, $P = 0.028$) (Figure 5.4). There was no change in group mean superficial femoral artery diameter, V_{MEAN}, $\dot{Q}_L$ or LVR across time from baseline to during ERL_{Pga} (all $P \geq 0.05$) (Figure 5.4; Table 5.2). However, as in ERL_{Pm}, the $\dot{Q}_L$ and LVR response to ERL_{Pga} was highly variable between the subjects (Figure 5.4). There was no evidence of leg muscle contraction throughout ERL_{Pga} ($P \geq 0.05$; Table 5.2).

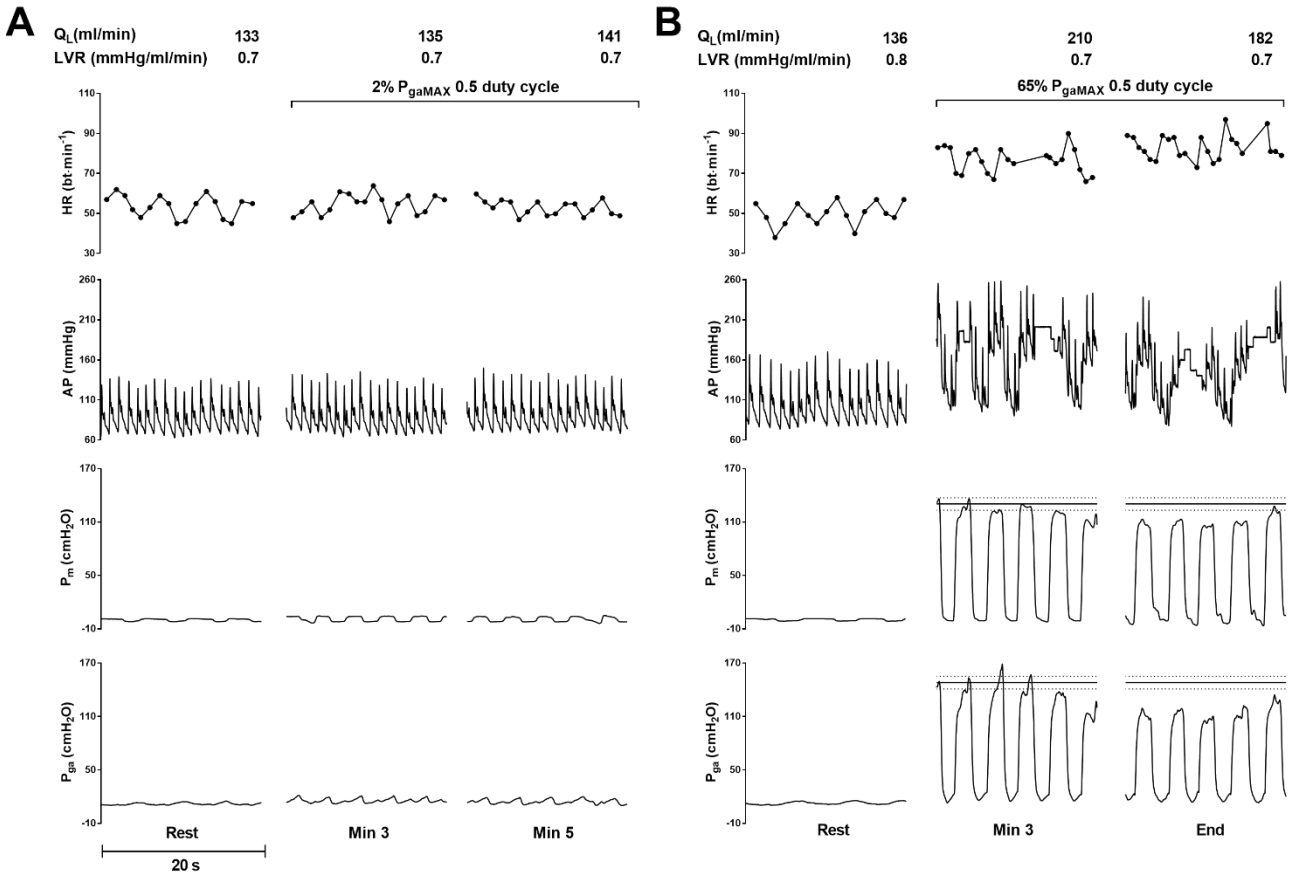


Figure 5.3. Cardiovascular and ventilatory responses to (A) ERL_{Pga} control and (B) ERL_{Pga} for one subject, represented by black squares in all other figures. Cardiovascular and ventilatory parameters were unchanged in response to ERL_{Pga} control. There was a time-dependent increase in HR and MAP in response to ERL_{Pga}. Pm and Pga were maintained at the target level until the final minute of ERL_{Pga}. $\dot{Q}_L$, leg blood flow; LVR, leg vascular resistance; HR, heart rate; AP, arterial pressure; Pm, mouth pressure; Pga, gastric pressure.

Table 5.2. Group mean ventilatory, cardiovascular and muscular responses to expiratory resistive loading targeting 2% and 65% of maximal gastric pressure.

	Rest	Min 3	Min 5	Task Failure	Recovery
ERL at 2% of Pga_{MAX} (control)					
Expiratory Pm, cmH_2O	2 ± 1	4* ± 0	4* ± 1	-	2 ± 0
f_R, breath·min^{-1}	12 ± 2	15 ± 0	15 ± 0	-	12 ± 2
$P_{ET}CO_2$, mmHg	48 ± 4	46 ± 4	47 ± 4		48 ± 4
Heart Rate, beat·min^{-1}	61 ± 9	63 ± 10	62 ± 11	-	61 ± 12
SFA V_{MEAN}, cm·s^{-1}	7.0 ± 1.6	7.2 ± 1.8	6.9 ± 2.1	-	7.1 ± 2.4
SFA Diameter, cm	0.63 ± 0.04	0.63 ± 0.03	0.62 ± 0.03	-	0.62 ± 0.04
VM_{RMS}, % of max	0.9 ± 0.2	0.9 ± 0.3	0.9 ± 0.2	-	1.0 ± 0.3
VL_{RMS}, % of max	0.9 ± 0.3	0.9 ± 0.2	0.9 ± 0.3	-	0.9 ± 0.3
ERL at 65% of Pga_{MAX} (ERL_{Pga})					
Expiratory Pm, cmH_2O	2 ± 0	122* ± 19	121* ± 18	111* ± 15	2 ± 0
f_R, breath·min^{-1}	12 ± 3	14 ± 1	15 ± 1	15 ± 0	17 ± 5
$P_{ET}CO_2$, mmHg	48 ± 4	49 ± 2	51 ± 4	52 ± 6	47 ± 6
Heart Rate, beat·min^{-1}	64 ± 12	86 ± 15	87 ± 15	95* ± 20	72 ± 18
SFA V_{MEAN}, cm·s^{-1}	8.6 ± 3.4	10.0 ± 4.9	9.2 ± 5.6	9.6 ± 6.2	11.8 ± 6.3
SFA Diameter, cm	0.62 ± 0.04	0.61 ± 0.04	0.61 ± 0.04	0.62 ± 0.04	0.62 ± 0.03
VM_{RMS}, % of max	1.0 ± 0.2	0.9 ± 0.2	0.9 ± 0.3	0.8 ± 0.2	0.9 ± 0.2
VL_{RMS}, % of max	0.8 ± 0.3	0.8 ± 0.3	0.8 ± 0.3	0.9 ± 0.4	0.8 ± 0.3

Values are presented as mean ± SD in 5 subjects. Pm, mouth pressure; T_E/T_{TOT}, expiratory duty cycle; V_T, tidal volume; SFA, superficial femoral artery; V_{MEAN}, time averaged mean blood velocity; VM, vastus medialis; VL, vastus lateralis; RMS, root mean square. *significantly different to rest ($P < 0.05$).

5.3.3.3. ERL_{Pga}-induced expiratory muscle fatigue

From before to after control ERL at 2% of Pga_{MAX}, there was no change in either MEP or Pga_{tw} (MEP: 190 ± 27 vs. 188 ± 27 cmH_2O, P = 1.000; Pga_{tw}: 46.1 ± 20.2 vs. 51.0 ± 26.3 cmH_2O, P = 0.554). There were no differences in twitch characteristics or M-wave responses from before to after control ERL_{Pga} (all P > 0.05). ERL_{Pga} at 65% of Pga_{MAX} did, however, elicit expiratory muscle fatigue as evidenced by a 16.0 ± 11.6% reduction in Pga_{tw} (P = 0.092) (Figure 5.2, *panel B*) and a 16.2 ± 5.8% reduction in MEP (P = 0.025) (Figure 5.2, *panel C*). Although the group mean change in Pga_{tw} was not statistically significant, 4 of the 5 subjects exhibited a percent reduction that was > 2× greater than the CV of the measure, which is conservatively indicative of fatigue. There were no changes in twitch characteristics in response to ERL_{Pga}: CT, 154 ± 23 vs 153 ± 26 ms; MRPD/Pga_{tw}, 13.5 ± 2.7 vs. 14.2 ± 2.2 s/cmH_2O; MRR/Pga_{tw}, −4.7 ± 0.8 vs. −5.2 ± 0.5 s/cmH_2O; $RT_{0.5}$, 160 ± 32 vs. 153 ± 11 ms (all P > 0.05). Similarly, M-wave characteristics were unchanged in response to ERL_{Pga}: amplitude, 2.6 ± 2.3 vs. 3.0 ± 2.9 mV; duration, 22.5 ± 4.5 vs. 24.1 ± 7.4 ms; area, 10.7 ± 8.6 vs. 13.3 ± 10.6 $mV \cdot s^{-1}$ (n = 3, all P > 0.05). Pre-twitch end-expiratory Poes was unchanged from baseline versus post-ERL_{Pga} control or post-ERL_{Pga} (−4.0 ± 1.5 cmH_2O vs. −4.4 ± 2.0 cmH_2O vs. −3.6 ± 1.8 cmH_2O; P = 0.476), suggesting that all stimulations were performed at a similar lung volume across time.

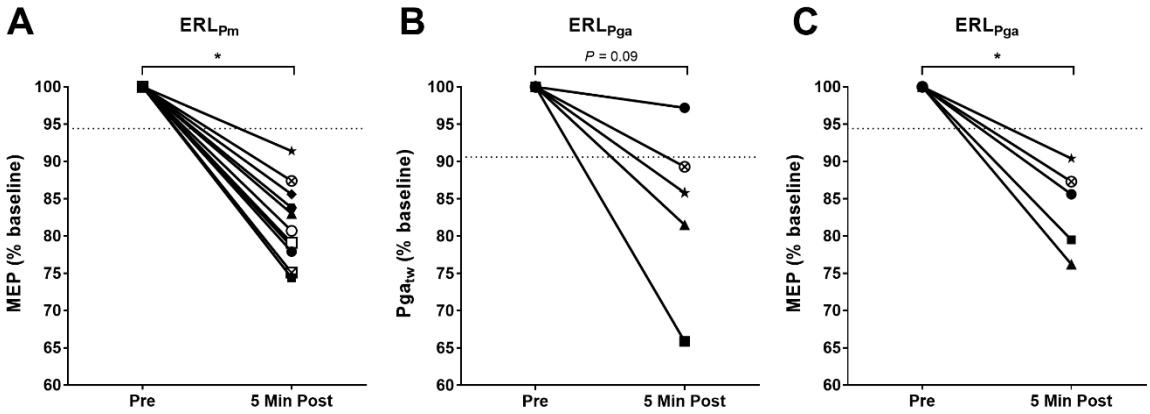


Figure 5.2. Individual expiratory muscle function responses to expiratory resistive loading (ERL) targeting mouth pressure (ERL_{Pm}) (A) and gastric pressure (ERL_{Pga}) (B-C). MEP, maximal expiratory pressure; Pga_{tw}, gastric twitch pressure. Dotted lines represent 2 x CV. * P < 0.05 vs. pre-ERL values.

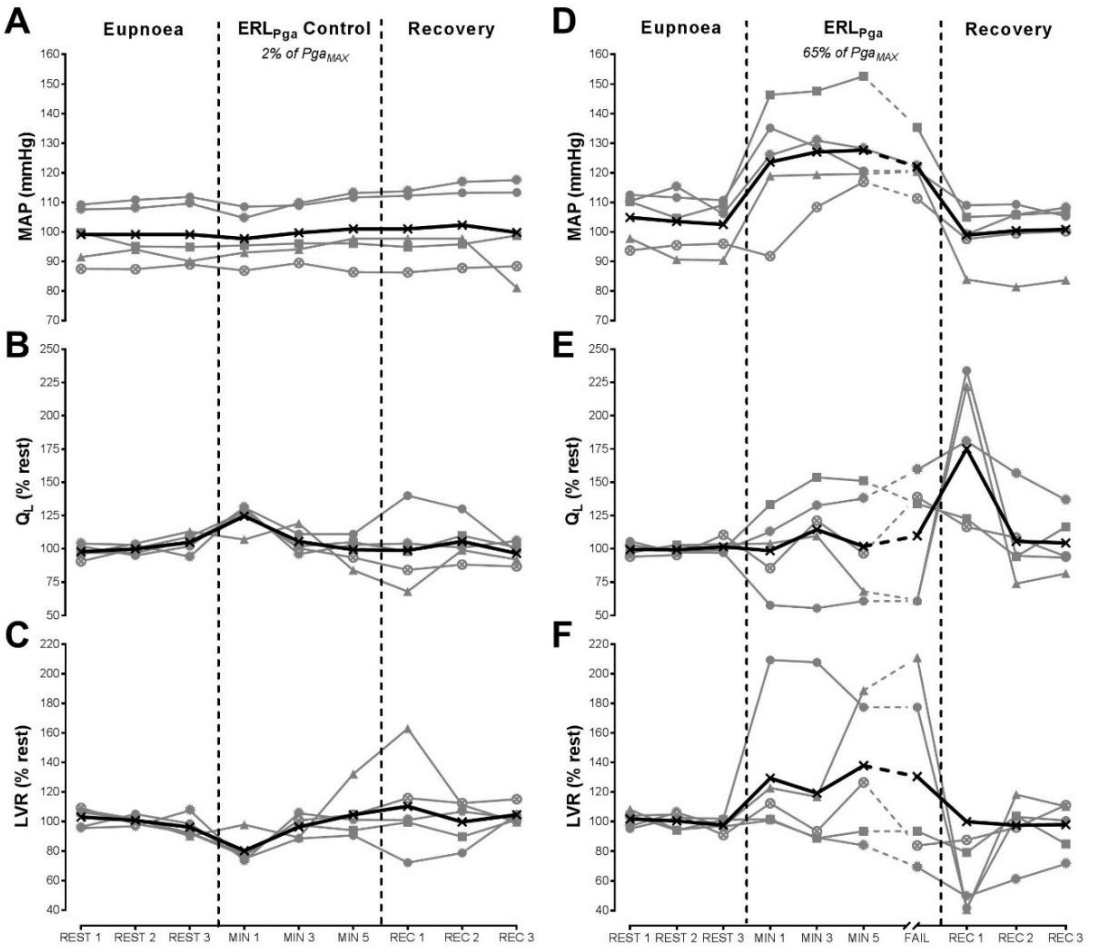


Figure 5.4. Mean arterial pressure (MAP, *n* = 5) (top panels), leg blood flow ($\dot{Q}_L$, *n* = 5) (middle panels), and leg vascular resistance (LVR, *n* = 5) (bottom panels) responses to expiratory resistive loading targeting 2% (ERL_{Pga} control) (A-C) and 65% (ERL_{Pga}) of maximal gastric pressure (D-F). Data are group means (black) and individual values (grey).

5.3.4. Within-breath cardiovascular responses to ERL

In response to ERL_{Pm} and ERL_{Pga}, group mean $\dot{Q}_L$ was not significantly different from resting eupnoeic values (Figure 5.1 and Figure 5.4). However, further analyses of ERL_{Pga} showed that $\dot{Q}_L$ varied cyclically in time with each respiratory phase of each breath (see Figure 5.5 for an individual example during ERL_{Pga} and Table 5.3 for group mean values). For example, when measured across an entire breath (*i.e. inspiration and expiration*), group mean $\dot{Q}_L$ over the duration of ERL_{Pga} was effectively unchanged relative to resting baseline values (7 ± 38%; *P* = 0.688). However, when apportioned to each respiratory phase within a breath, $\dot{Q}_L$ during each resisted expiration increased by 66 ± 49% (*P* = 0.054) (range +9% to +123%) relative to $\dot{Q}_L$

measured across the entire respiratory cycle at rest. This increase was mediated predominantly by an increase in antegrade flow (mean change: +65 ± 28%; P = 0.015). By contrast, $\dot{Q}_L$ during each unresisted inspiration decreased by 49 ± 27% (P = 0.018) (range –85% to –22%) relative to $\dot{Q}_L$ measured across the entire respiratory cycle at rest. This decrease was, predominantly, the result of an increase in retrograde flow (mean change: +244 ± 157%; P = 0.002). The potential mechanistic cause of such phasic swings in $\dot{Q}_L$ is discussed in section '*Why did ERL have no effect on LVR and $\dot{Q}_L$: metabolic vs. mechanical factors'* of the discussion.

5.3.5. Correlations between variables

For ERL_{Pm} only, there was no significant correlation between the change in $\dot{Q}_L$ from rest to task failure and 1) baseline MEP (r = 0.28); 2) the change in MEP from pre to post-ERL (r = 0.11); or 3) time to task failure for ERL (r = −0.27) (all P > 0.05). Similarly, the change in LVR from rest to task failure during ERL_{Pm} was not associated with 1) baseline MEP (r = 0.33); 2) the change in MEP from pre to post-ERL (r = 0.04); or 3) time to task failure of ERL (r = −0.16) (all P > 0.05). These data indicate that the individual variability in cardiovascular responses to ERL_{Pm} was not associated with baseline expiratory muscle strength and/or the severity of ERL-induced expiratory muscle fatigue.

Table 5.3. Group mean leg blood flow ($\dot{Q}_L$) during each respiratory phase in response expiratory resistive loading targeting 65% of maximal expiratory gastric pressure (ERL_{Pga}).

	Rest	Min 1	Min 3	Min 5	Task Fail	Recovery
Mean $\dot{Q}_L$ ($ml \cdot min^{-1}$)	160 ± 48	160 ± 72	178 ± 80	170 ± 97	179 ± 110	216 ± 110
Inspiratory	164 ± 51	75 ± 34	91 ± 62	79 ± 67	85 ± 65	217 ± 99
Expiratory	156 ± 44	252 ± 110	270 ± 113	261 ± 123*	267 ± 159	214 ± 95
Insp/Exp Ratio	1.05 ± 0.07	0.30 ± 0.05*	0.32 ± 0.14*	0.27 ± 0.10*	0.31 ± 0.15*	1.02 ± 0.02
Antegrade $\dot{Q}_L$ ($ml \cdot min^{-1}$)	193 ± 49	235 ± 65	250 ± 67	240 ± 84	250 ± 92	253 ± 101
Inspiratory	198 ± 54	169 ± 39	189 ± 47	178 ± 56	188 ± 45	253 ± 104
Expiratory	189 ± 46	308 ± 96	313 ± 93	302 ± 108	308 ± 137	251 ± 100
Retrograde $\dot{Q}_L$ ($ml \cdot min^{-1}$)	33 ± 14	74 ± 35	72 ± 35	70 ± 30	71 ± 27*	38 ± 11
Inspiratory	33 ± 14	94 ± 41	98 ± 38	99 ± 30*	103 ± 25*	36 ± 12
Expiratory	33 ± 13	56 ± 40	43 ± 37	41 ± 35	41 ± 37	38 ± 12

Values are presented as mean ± SD in 5 subjects. $\dot{Q}_L$, leg blood flow. *significantly different to rest ($P < 0.05$).

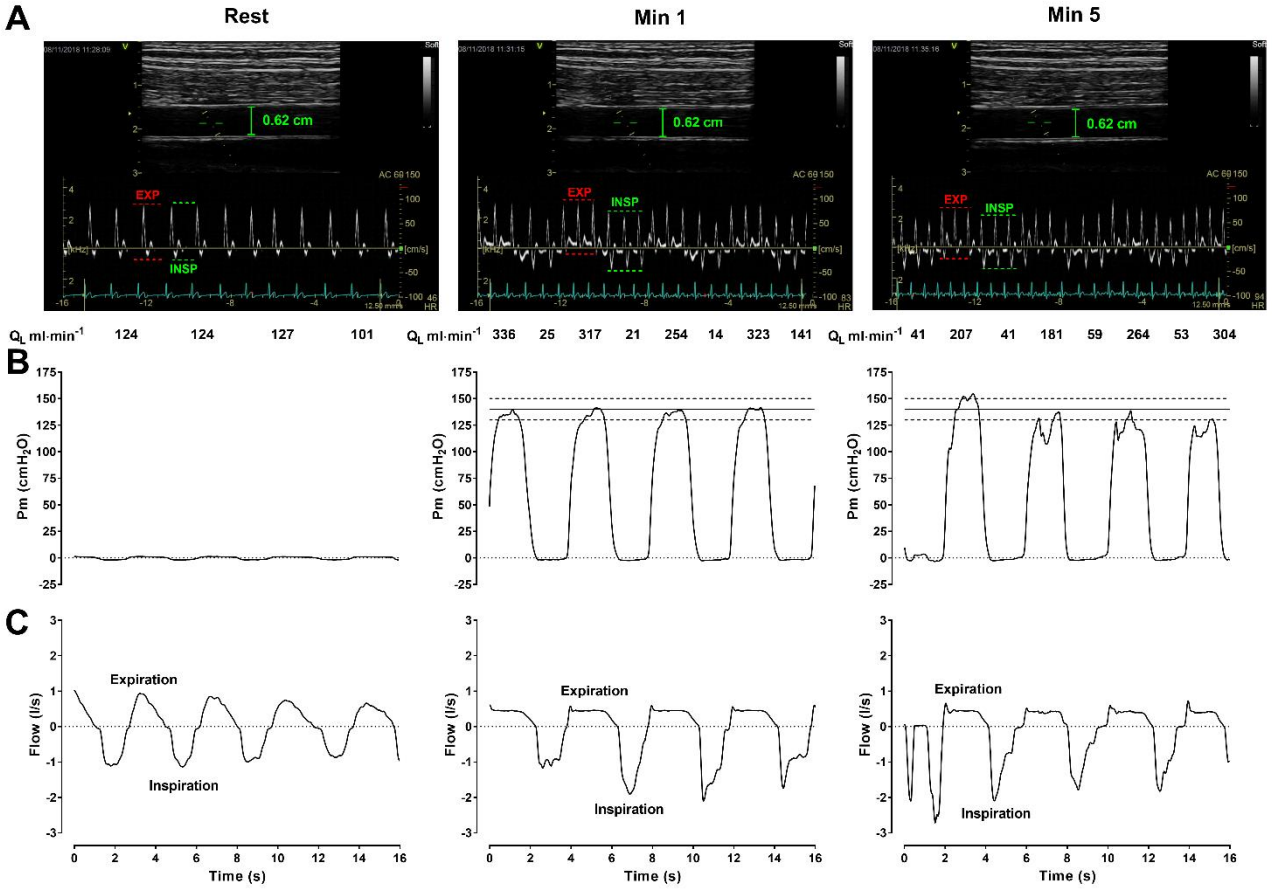


Figure 5.5. An individual example of the effect of large swings in mouth pressure (Pm) on resting $\dot{Q}_L$ during fatiguing expiratory resisted loading targeting 65% of maximal gastric pressure (ERL_{Pga}). A, raw Doppler ultrasound images of the superficial femoral artery; B, Pm; C, airflow. $\dot{Q}_L$ (ml·min^{-1}) calculated as the product of vessel cross sectional area (cm^2) x time averaged mean blood velocity (cm·s^{-1}) x 60. Example periods of high antegrade and retrograde flow, during each loaded expiration and non-resisted inspiration, respectively, are highlighted.

5.4. DISCUSSION

5.4.1. Main Findings

This study investigated the cardiovascular consequences of fatiguing expiratory muscle work in healthy humans. To the author's knowledge, this is the first study to measure leg vascular resistance (LVR) and leg blood flow ($\dot{Q}_L$) in response to 'high-intensity' contractions of the expiratory muscles sustained to the point of task failure in otherwise resting individuals. The major findings were that expiratory resistive loaded breathing (ERL) targeting 65% of either expiratory mouth pressure or expiratory gastric pressure caused: 1) a substantial and sustained increase in mean arterial pressure (MAP) and heart rate (HR); and 2) fatigue of the expiratory muscles, as evidenced by a significant pre- to post-ERL reduction in maximal expiratory mouth pressure (MEP) and/or gastric twitch pressure (Pga_{tw}); but 3) no change in group mean LVR or $\dot{Q}_L$ relative to prior resting baseline values. There was, however, a large degree of between-subject variability in LVR (−31 to +111%) and $\dot{Q}_L$ (−39 to +70%) in response to ERL. Interestingly, it was observed that $\dot{Q}_L$ oscillated cyclically across the respiratory phases within each breath. Indeed, relative to $\dot{Q}_L$ measured across the entire respiratory cycle at rest (*i.e. inspiration and expiration*), $\dot{Q}_L$ increased by ~66% during each resisted expiration but decreased by ~49% during each unresisted inspiration. In combination, these data suggest that while fatiguing expiratory muscle work had no effect on group mean LVR or $\dot{Q}_L$ in otherwise resting humans, it is possible that any sympathetically-mediated peripheral vasoconstriction and reduction in $\dot{Q}_L$ was counteracted by transient increases in $\dot{Q}_L$ due to the mechanical effects of high intra-abdominal pressure during ERL (*see 'mechanical effects of expiratory resisted loading'*).

5.4.2. Expiratory Muscle Metaboreflex

It is well accepted that fatiguing inspiratory muscle work in otherwise resting humans is associated with neural and cardiovascular consequences. Characterised by time-dependent increases in MSNA, MAP and LVR, with a consequent decrease in $\dot{Q}_L$, this inspiratory muscle metaboreflex is thought to be triggered by fatigue-induced metabolite accumulation and stimulation of metabosentive group IV and, to a lesser extent, group III muscle afferents secondary to 'high-intensity' contractions (≥60% of maximal inspiratory mouth pressure) of the inspiratory muscles (Hussain et al., 1991, Hill, 2000, Sheel et al., 2001, Rodman et al., 2003, Smith et al., 2016). Contrary to this, there was no evidence for a change in either group mean LVR or group mean $\dot{Q}_L$ in response to ERL performed to task failure. This raises two important questions: 1) were the experimental conditions required to initiate an expiratory muscle metaboreflex met in the present study; and 2) mechanistically, why did fatiguing ERL have no effect on group mean LVR and $\dot{Q}_L$ in otherwise resting humans?

5.4.3. Was an expiratory muscle metaboreflex initiated?

The critical tension-time index (TTI) for the diaphragm is the product of muscle tension and duty cycle (*or time*) above which blood flow to the diaphragm is limited, metabolite accumulation occurs and fatigue ensues (Bellemare and Grassino, 1982, Bellemare et al., 1983). The critical TTI for the expiratory abdominal muscles is unknown. However, if the expiratory TTI during loaded breathing in the present study was too low to induce expiratory muscle fatigue and metabolite accumulation, and as such an expiratory muscle metaboreflex, then it would perhaps be unsurprising that ERL did not cause a reduction in group mean LVR and $\dot{Q}_L$. However, the 'lack' of a significant LVR and $\dot{Q}_L$ response to ERL in this study is not likely to be due to an insufficient TTI for several reasons. The expiratory TTI during ERL in the present study was 0.325, which is more than two-times greater than the critical TTI previously reported for the diaphragm (~0.15) (Bellemare and Grassino, 1982, Bellemare et al.,

1983). Moreover, the cardiovascular consequences of inspiratory loading, including an increase in LVR and reduction in $\dot{Q}_L$, have been reported in response to inspiratory resistive loaded breathing (IRL) with a TTI as low as 0.24 (Sheel et al., 2001). Given that the expiratory muscles are less fatigue-resistant compared to the inspiratory muscles (Gandevia et al., 1983), it is likely that the present ERL protocols were above the critical TTI for the expiratory muscles and thus induced neuromuscular fatigue and metabolite accumulation. Indeed, a 16-17% reduction in MEP and a 16% decrease in Pga_{tw} (ERL_{Pga} only) was observed in response to ERL trials (Figure 5.2). In addition, the magnitude and temporality of the MAP response to ERL (Figure 5.1 and Figure 5.4) was remarkably comparable to that observed during similar ERL protocols. For example, during ERL_{Pm} there was an immediate and substantial increase in MAP (~19 mmHg; rest vs. min 1) that, although not significant, progressively increased over time (~12 mmHg; min 1 vs. task failure; P = 0.12). Similarly, in response to ERL (60% MEP, T_E/T_{TOT} = 0.7, f_R = 15 breaths/min), Derchak et al. (2002) reported a sharp initial rise in MAP (~17 mmHg; rest vs. min 1) that increased progressively but non-significantly over time (~11 mmHg; rest vs. task failure); a cardiovascular response that was concomitant with a time-dependent increase in MSNA. As such, although sympathetic outflow was not directly measured in the present study, based on the striking similarity between the magnitude and time-dependency of the MAP response to ERL compared to the study by Derchak et al. (2002) who also reported a time-dependent sympathoexcitation, I am confident that an expiratory muscle metaboreflex was indeed elicited.

Another consideration is whether it is necessary to specifically target the expiratory abdominal muscles during ERL to elicit an expiratory muscle metaboreflex. In previous IRL studies, subjects were instructed to isolate the diaphragm during resisted inspiratory efforts (Sheel et al., 2001) to avoid the recruitment of accessory inspiratory muscles which may be heavily utilised when using ‘natural’ breathing

techniques (Ramsook et al., 2016). Whether specifically targeting the diaphragm versus the inspiratory accessory muscles during IRL elicits a different cardiovascular response is currently unknown. It is possible that the diaphragm has a higher density of metaboreceptors in comparison to the accessory inspiratory muscles, eliciting a greater sympathetic response for a similar metabolic 'insult'. However, type III and IV afferent fibers have been identified in the intercostal nerve (Duron, 1981), and such metaboreceptors are ubiquitous among other skeletal muscles. In addition, in exercising healthy humans, the inverse relationship of sternocleidomastoid blood flow to manipulations in the inspiratory work of breathing (Dominelli et al., 2017) supports the idea that the accessory respiratory muscles contribute to, or are at least responsive to, a respiratory muscle metaboreflex. In the present study, performing fatiguing expiratory muscle work without specific breathing instructions (ERL_{Pm}) led to no change in LVR and $\dot{Q}_L$. Subsequently, and in line with IRL studies that specifically targeted the diaphragm, the cardiovascular responses to ERL whilst subjects specifically targeted Pga (i.e. recruited the primary expiratory muscles of the abdominal wall) were examined. While it has been shown previously that the severity of expiratory muscle fatigue is increased when expiratory duty cycle (and presumably the duration of ischemia) is lengthened (Taylor and Romer, 2009), there was still no consistent group mean change in LVR or $\dot{Q}_L$ during ERL_{Pga}. Therefore, it is clear that the conditions required to trigger an expiratory muscle metaboreflex were met in the present study. The speculative mechanisms for why this did not translate into a reduction in $\dot{Q}_L$ are considered in more detail, below.

5.4.4. Why did ERL have no effect on LVR and $\dot{Q}_L$: metabolic vs. mechanical factors

5.4.4.1. Metabolic effects of expiratory resistive loading

It was hypothesised that, like for the inspiratory muscles, fatigue-induced accumulation of metabolic by-products (e.g. lactate, potassium, adenosine

diphosphate) in the expiratory musculature would lead to a sympathetically-mediated time-dependent peripheral vasoconstriction with a resultant increase in LVR and decrease in $\dot{Q}_L$ (St Croix et al., 2000, Sheel et al., 2001, Rodman et al., 2003). Contrary to this hypothesis it was presently found that group mean LVR and $\dot{Q}_L$ were not different compared to eupnoeic values in response to ERL. Despite this, upon further analyses, a 49 ± 27% reduction in $\dot{Q}_L$ during the *unresisted inspiratory* phase of each breathing cycle was observed, compared to resting values across the entire breath, that was largely due to an increase in retrograde flow (mean change: +244 ± 157%) (Figure 5.5; Table 5.3). Although speculative, it is possible that this reduction in $\dot{Q}_L$ during the inspiratory phase of each breath, in the absence of a mechanical consequence of high intra-abdominal pressure, is indicative of an underlying peripheral vasoconstriction in the resting limb. The increase in inspiratory retrograde $\dot{Q}_L$, despite the lack of a change in superficial femoral artery diameter, is congruent with constriction of resistance arteries and arterioles downstream of the superficial femoral artery, which, rather than the larger conduit arteries, are primarily responsible for the regulation of limb blood flow (Joyner and Casey, 2015, Hanson et al., 2020). Therefore, it is likely that peripheral vasoconstriction in the arterioles downstream of the superficial femoral artery was responsible for, or at least contributed to, the change in MAP, and the reduction in $\dot{Q}_L$ during unresisted inspirations in the present study. In further support of this assertion, a hyperaemic response was displayed by the majority of subjects immediately following cessation of ERL trials (Figure 5.1), characteristic of arterial vasodilation following a period of vasoconstriction. So, the question becomes: if ERL did elicit a metabolite-induced and sympathetically-mediated vasoconstriction in the resting limbs, why did group mean LVR and $\dot{Q}_L$ not change across time in response to expiratory resisted breathing?

5.4.4.2. Mechanical effects of expiratory resistive loading

It is well documented that stroke volume fluctuates during the respiratory cycle due to changes in intrathoracic and intra-abdominal pressure, with consequent effects on venous return, right ventricular preload and left ventricular emptying (Miller et al., 2005a). It could be speculated that the very-high intra-abdominal pressures during ERL in the present study may have resulted in a mechanical cardio-pulmonary interaction that transiently influenced the recordings of $\dot{Q}_L$. Indeed, when measured across an entire breath (*i.e. inspiration and expiration*), group mean $\dot{Q}_L$ during ERL_{Pga} was effectively unchanged relative to resting baseline values (7 ± 38%). However, when apportioned to each respiratory phase within a breath, there was a 66 ± 49% increase $\dot{Q}_L$ during each resisted expiration that was primarily mediated by an increase in antegrade flow (+65 ± 28%). So, why did $\dot{Q}_L$ during each loaded expiration increase during ERL? Typically, increases in abdominal pressure achieved via abdominal compression, diaphragmatic breathing, or Valsalva manoeuvres have been shown to attenuate venous return, cardiac filling and stroke volume (Richardson and Trinkle, 1976, Willeput et al., 1984, Miller et al., 2005a). Conversely, previous reports have also suggested that expulsive manoeuvres generating a very-high intra-abdominal pressure with concomitant diaphragm contraction (i.e. ERL) cause a substantial blood-volume shift from the trunk to the extremities, likely originating from the splanchnic region (Aliverti et al., 2010). For example, a 'square-wave' increase in abdominal pressure of ~100 cmH_2O maintained for 1 s (followed by 2 s relaxation) produced a 'stroke volume' from the splanchnic bed of 350 ml and an output of 6.8 $L \cdot min^{-1}$ compared to a resting cardiac output of 5.6 $L \cdot min^{-1}$ (Aliverti et al., 2010). It could be speculated that the highly comparable Pga exhibited during ERL in the present study (~120 cmH_2O; Table 2) may have similarly transiently increased splanchnic outflow and stroke volume during the expiratory phase of each breath. Indeed, an increase in cardiac ejection and driving pressure secondary to a blood volume shift could theoretically increase antegrade $\dot{Q}_L$, even if underlying

vasoconstriction is present. Because this effect was present immediately and was sustained for the duration of ERL (Figure 5.5 and Table 5.3), it is predicted that the splanchnic reservoir was completely refilled during each un-resisted inspiration of ERL, as found previously (Aliverti et al., 2010).

5.4.5. Individual Variability in LVR and $\dot{Q}_L$

There was substantial between-subject variability in LVR and $\dot{Q}_L$ in response to ERL (Figure 5.1 and Figure 5.4). Although the range of $\dot{Q}_L$ and LVR during ERL was large relative to eupnoeic values ($\dot{Q}_L$ range: −39 to +70%), such between-subject variability is not novel. For example, previous data show individual changes in $\dot{Q}_L$ ranging from approximately −40 to +5% by task failure of IRL (Sheel et al., 2001). So, what are the potential mechanisms for the between-subject variability in cardiovascular responses to ERL? First, it is possible that subjects exhibiting a greater relative increase in $\dot{Q}_L$ during ERL may have failed to incite a sufficient severity of expiratory muscle fatigue to elicit the respiratory muscle metaboreflex. However, presently there was no correlation with the change in $\dot{Q}_L$ or LVR and the magnitude of expiratory muscle fatigue, baseline MEP or time to task failure. In addition, MEP decreased in every subject during ERL (by > 2× CV) indicating that expiratory muscle fatigue was present in response to all trials. It remains to be determined whether, for a given magnitude of respiratory muscle fatigue, the activation of group III and IV muscle afferents varies among subjects. As sex or training status was not controlled for in the present study, it is likely that muscle morphology and substrate utilization varied between subjects, which may have led to differences in fatigue-associated metabolite accumulation. For example, females exhibit an attenuated inspiratory muscle metaboreflex compared to males (Smith et al., 2016), and also demonstrate blunted increases in H^+ and $H_2PO_4^-$ in response to handgrip exercise with post-exercise forearm occlusion (Ettinger et al., 1996). Second, differences in the magnitude of sympathetic outflow or vascular transduction between subjects may affect the extent of sympathetically-

mediated vasoconstriction for a given afferent stimulus. Indeed, the change in MSNA in response to ERL in young healthy males is highly variable, ranging from approximately -20 to +700% from baseline to task failure (Derchak et al., 2002). Third, there may be individual differences in the magnitude of splanchnic outflow and the consequent blood-volume shift for a given increase in abdominal pressure. For example, depending on the conditions of the circulatory system, the abdominal venous compartment can act as a capacitor augmenting venous return (zone 3 conditions) or less commonly, as a starling resistor dynamically compressing the inferior vena cava limiting venous return (zone 2 conditions) (Takata et al., 1990).

5.4.6. Temporality of the Expiratory Muscle Metaboreflex

It is well accepted that the autonomic and cardiovascular consequences of IRL do not become apparent until IRL has been performed for ≥2 min (Sheel et al., 2001, St Croix et al., 2000). This 'time-dependency' likely reflects the time taken for fatigue-associated metabolites to accumulate and for the subsequent stimulation of type III and IV phrenic afferents to occur. However, in response to ERL an abrupt initial rise in MAP that increased somewhat progressively over time was observed (*see* Figure 5.1, *panel D*). This poses the question; is the temporality of respiratory muscle metaboreflex initiation different for fatiguing inspiratory vs. fatiguing expiratory muscle work? It is highly likely that central expiratory motor command increased during ERL and in theory could have contributed to the observed MAP response. However, it has been shown previously that performance of ERL with a very-high expiratory Pm in the absence of expiratory muscle fatigue (95% MEP; T_E/T_{TOT} = 0.35; f_R = 12 breaths·min^{-1}) does not evoke a change in MSNA or MAP, and that central command only has a "minor, variable contribution" to the cardiovascular response to ERL (Derchak et al., 2002). Perhaps a more likely mechanism underpinning the different temporality of the cardiovascular responses to ERL vs. IRL relates to differences in the fatigability of the expiratory vs. inspiratory muscles. Indeed, it is

well established that the expiratory muscles are phenotypically and functionally less fatigue-resistant than the inspiratory muscles (especially the diaphragm) (Gandevia et al., 1983, Uribe et al., 1992, Haggmark and Thorstensson, 1979). As such, it could be speculated that the development of expiratory muscle fatigue, the subsequent accumulation of fatigue-associated metabolites, and the consequent activation of a respiratory muscle metaboreflex may occur more abruptly during ERL vs. during IRL.

5.4.7. Conclusions

In response to fatiguing expiratory muscle work in healthy otherwise resting humans, there was no change in group mean leg blood flow ($\dot{Q}_L$) or leg vascular resistance, despite substantial expiratory muscle fatigue and an increase in heart rate and mean arterial pressure suggestive of an increase in sympathetic outflow. The lack of cardiovascular changes in the resting limb may be the result of substantial cyclical increases in expiratory $\dot{Q}_L$, coincident with a mechanical effect of very-high intra-abdominal pressures, which may have masked underlying sympathetically-mediated vasoconstriction caused by an expiratory muscle metaboreflex.

CHAPTER 6
The Cardiovascular Consequences of Fatiguing Inspiratory Muscle Work in Healthy Otherwise Resting Humans

6.1. INTERIM SUMMARY

In the previous chapter, it was demonstrated that fatiguing expiratory muscle work in healthy otherwise resting humans had no effect on group mean leg blood flow ($\dot{Q}_L$) or leg vascular resistance (LVR), despite substantial expiratory muscle fatigue and an increase in heart rate and mean arterial pressure (MAP) suggestive of an increase in sympathetic outflow. It is possible that the lack of cardiovascular changes in the resting limb was the result of substantial cyclical increases in expiratory $\dot{Q}_L$, coincident with a mechanical effect of very-high intra-abdominal pressures, which may have masked underlying sympathetically-mediated vasoconstriction caused by an expiratory muscle metaboreflex. To my knowledge, this was the first study to directly assess the $\dot{Q}_L$ and LVR response to such fatiguing expiratory muscle work.

This original aim of this line of research was to compare the magnitude and temporality of changes in cardiovascular parameters (i.e. MAP, LVR, and $\dot{Q}_L$) during expiratory resistive loaded breathing (ERL) to the previously reported time-dependent increases in MAP and LVR and the decrease in $\dot{Q}_L$ in response to fatiguing inspiratory resistive loaded breathing (IRL). Given that the data presented in this thesis suggest that ERL *does not* cause the hypothesised changes to limb vascular resistance or blood flow, in this chapter I will simply present a summary of the cardiovascular responses observed during IRL, examining the methodological and physiological factors affecting the development of the inspiratory muscle metaboreflex, providing careful considerations for future studies.

6.2. INTRODUCTION

In healthy otherwise resting humans, inspiratory resistive loaded breathing (IRL) sustained until task failure has been shown to elicit a time-dependent sympathoexcitation, characterised by an increase in muscle sympathetic nerve activity (MSNA) and heart rate (St Croix et al., 2000) leading to a consequent peripheral vasoconstriction in the resting limb (characterised by a rise in MAP, LVR, and a decrease in $\dot{Q}_L$ (Sheel et al., 2001, Smith et al., 2016, Welch et al., 2018b). Importantly, the aforementioned cardiovascular responses are time-dependent, with changes in LVR and $\dot{Q}_L$ not evident until at least the second minute of IRL (Sheel et al., 2001, Smith et al., 2016), congruent with the time required to accumulate fatigue-associated metabolites that likely trigger such neurovascular responses. The consistency of such findings in the resting limb has led to the assertion that the presence of a respiratory muscle metaboreflex can be measured by increases in heart rate and MAP alone during IRL (Witt et al., 2007, Geary et al., 2019); responses that are coincident with changes in MSNA, $\dot{Q}_L$ and LVR.

Compared to the diaphragm, the expiratory abdominal muscles are phenotypically less fatigue-resistant. For example, relative to the diaphragm, the expiratory muscles exhibit a lower (but more variable) proportion of type I muscle fibres (40-70 vs. 55-60%) (Keens et al., 1978, Haggmark and Thorstensson, 1979) and a lower oxidative capacity. Indeed, citrate synthase activity, which is a marker of mitochondrial content, is ~65% lower in the expiratory abdominal muscles versus the diaphragm (Uribe et al., 1992). In addition, in response to sustained repeated maximal contractions there is a greater functional impairment in the ability to volitionally generate expiratory versus inspiratory pressure at the mouth (Gandevia et al., 1983). Because of the greater fatigability of the expiratory muscles, it could be speculated that the cardiovascular responses characteristic of a respiratory muscle metaboreflex (i.e. a time-dependent increase in LVR and decrease in $\dot{Q}_L$) would occur more abruptly in

response to fatiguing expiratory versus inspiratory muscle work. To date, however, this assertion has not been experimentally examined.

The purpose of the present chapter was to assess the cardiovascular responses to fatiguing inspiratory muscle work in heathy humans. Specifically, the systemic (HR and MAP) and peripheral (LVR, $\dot{Q}_L$) cardiovascular responses to 'high-intensity' contractions of the inspiratory muscles sustained until task failure were measured. It was hypothesised that in response to fatiguing inspiratory work, there would be: 1) a significant time-dependent increase in HR, MAP and LVR; and 2) a decrease in $\dot{Q}_L$. Although the intention was to also compare the temporality and magnitude of cardiovascular responses during IRL and ERL, the lack of a group mean change in LVR and $\dot{Q}_L$ during ERL (Chapter 5) precluded this comparison.

6.3. METHODS

6.3.1. Methods Common to Chapters 5 & 6

Leg blood flow (via Doppler ultrasound) and electromyographic recordings of the quadriceps muscles were measured as detailed in Chapter 5 (*see section* 5.4.4 *and* 5.4.5). Similarly, the data capture and statistical analysis procedures in this chapter were the same as described in Chapter 5 (*see section* 5.4.7 *and* 5.4.8). Therefore, these methods will not be detailed in this Chapter.

6.3.2. Subjects

The subject cohort who participated in Chapter 5 (*see section* 5.2.1) also participated in this study.

6.3.3. Experimental Procedures

As in Chapter 5 (*see section* 5.4.2), each subject initially visited the laboratory on two separate occasions. At the first visit, the subjects were thoroughly familiarised with

all of the experimental procedures and measurements, including the inspiratory resistive loaded breathing (IRL) tasks and the determination of maximal inspiratory mouth pressure (MIP). To ensure familiarisation with the IRL tasks, each subject performed short bouts (1-2 min) of IRL until they could consistently generate inspiratory mouth pressure (Pm) for >2 min as a 'square wave' at the target pressure and in accordance with the prescribed duty cycle and respiratory frequency without coaching. On the second visit, the subjects performed IRL targeting a Pm of 2% of MIP for 5 min (*control*). Following 30 min of quiet rest, the subjects then performed IRL targeting 65% of MIP until task failure (IRL_{Pm}). The control trial was performed first to avoid any residual effect of peripheral muscle fatigue and potential sensitisation of the metaboreflex becoming apparent during the control trial. During IRL_{Pm}, the subjects were instructed to maintain a constant Pm at the target level throughout each inspiration but *were not* given any specific instructions on how to recruit the inspiratory muscles. $\dot{Q}_L$ and LVR were measured for the final 16 s of every minute at rest, during IRL, and in recovery; HR and MAP were recorded continuously.

Despite the presence of IRL-induced inspiratory muscle fatigue and the expected time-dependent increase in MAP (*see section* 6.4.1), there was no change in group mean LVR or $\dot{Q}_L$ in response to IRL_{Pm}. At this time, it was considered possible that specifically targeting the diaphragm (i.e. the primary inspiratory muscle) during IRL may increase the severity of ischemia, hasten the onset of fatigue, and augment the initiation of an inspiratory muscle metaboreflex and the associated cardiovascular consequences. Accordingly, a sub-sample of subjects (n = 6) attended the laboratory on a third occasion and performed IRL but this time targeted 2% (5 min, *control*) and 65% (to task failure) of maximal inspiratory transdiaphragmatic pressure (Pdi_{max}) (IRL_{Pdi}). At the start of the experimental visit subjects practiced and were coached through the performance of IRL_{Pdi}; the subjects were instructed to maintain a constant Pdi at the target level throughout each inspiration, and were explicitly instructed to

'target the diaphragm' during each inspiration. Once IRL_{Pdi} could be performed accurately at the intended duty cycle and respiratory frequency, subjects rested quietly for 30 min prior to performance of the IRL_{Pdi} control trial. During IRL_{Pdi} trials, $\dot{Q}_L$ and LVR were measured for the final 16 s of every minute at rest, during IRL, and in recovery; HR and MAP were recorded continuously.

6.3.4. Inspiratory Resistive Loaded Breathing

The IRL trials were performed using the same experimental set-up as detailed for the ERL trials in Chapter 5 (*see section* 5.4.3). Briefly, the subjects lay in a semi-recumbent position and breathed through a custom-built two-way valve with a variable diameter resistor incorporated into the inspiratory port; expiration was completely unimpeded. A calibrated pressure transducer (DP45, Validyne Engineering, Northridge, CA, USA) was connected into the mouthpiece to allow continuous measurement of Pm. During IRL, the target inspiratory pressure was displayed on a computer screen, and the subjects maintained a respiratory frequency (f_R) of 15 breaths·min^{-1} and an inspiratory duty cycle (T_I/T_{TOT}) of 0.5 by following a computer generated audio signal with distinct inspiratory and expiratory tones. The subjects were instructed to maintain a constant Pm at the target level throughout each inspiration. The subjects were monitored closely by the researchers during IRL to ensure proper timing, breathing technique, and effort. Airflow (no. 4813, Hans Rudolph Inc.; Shawnee, KS, USA) and end-tidal partial pressure of carbon dioxide ($P_{ET}CO_2$) (Ultima Cardio 2, MGC Diagnostics, St Paul, MN, USA) were measured throughout each IRL trial. Doppler ultrasound (Vivid iq, GE Healthcare, Milwaukee WI, USA) was used to measure $\dot{Q}_L$ during the last three minutes of each period of eupnoea (*rest*), every minute during IRL, and during the first three minutes after IRL (*recovery*). MAP and HR were measured beat-by-beat using finger photoplethysmography (Finapres Nova, Finapres Medical Systems, Amsterdam, The Netherlands), and LVR was subsequently calculated as MAP/$\dot{Q}_L$. As per the

manufacturer guidelines, physiological calibration (PhysioCal) was used during IRL to maintain the accuracy of Finapres recordings; measurements were averaged over 60 s and calibration periods were excluded from the analysis. In addition, surface electromyography (EMG) (Trigno Avanti, Delsys Inc., Natick, MA, USA) was recorded from the vastus lateralis and vastus medialis of the right leg to confirm that no limb muscular contraction occurred during IRL.

Following 15 min of quiet rest (*breathing un-resisted*), each subject inspired against the resistive load for 5 min whilst targeting 2% of MIP (*control*) before resting quietly with breathing completely un-resisted for a further 30 min. Next, each subject performed IRL whilst targeting 65% of MIP until task failure (IRL_{Pm}), defined as an inability to generate the target pressure for three consecutive breaths despite strong verbal encouragement. For the same reasons as detailed in Chapter 5 (*see section* 5.4.3), once task failure was achieved, the subjects were given strong verbal encouragement to continue attempting to generate their target Pm for one additional minute (defined as the 'task failure' minute). The presence and severity of IRL_{Pm}-induced inspiratory muscle fatigue was quantified as the reduction relative to prior baseline values in the MIP response to maximal Müeller manoeuvres initiated from residual volume (Laveneziana et al., 2019a). The maximum value of 3-5 attempts that varied by <10% was reported.

6.3.5. Diaphragm Muscle Function by Magnetic Nerve Stimulation

For IRL_{Pdi}, gastric (Pga) and oesophageal (Poes) pressure were measured via insertion of air-filled catheters, as described previously in this thesis (*see section* 3.2.6). Pdi was calculated as Pga−Poes. Magnetic stimuli were delivered to the phrenic nerve roots between the level of the 3rd (C3) and 7th (C7) cervical vertebrae via a 90-mm circular coil powered by a magnetic stimulator (Similowski et al., 1989). The area of stimulation that evoked the greatest Pdi_{tw} amplitude was located and

marked for use for all subsequent stimulations, and all stimulations were delivered at a consistent relaxed end-expiratory lung volume (i.e. FRC), as judged by end-expiratory Poes. Similarly to previous findings, magnetic stimulation of the cervical nerves elicited a supramaximal response from the diaphragm; these data are presented in the 'Methodological Observations and Considerations' Chapter (*see section* 4.3).

Diaphragm contractility was assessed at baseline, ~5 min after *control* IRL, and 5 min after IRL_{Pdi}. The Pdi_{tw} response to a 1-Hz magnetic stimulation that was delivered at 100% of the stimulator's power output ~5 s after a 5 s maximal Müeller manoeuvre that was initiated from residual volume was measured (i.e. potentiated twitch). This procedure was repeated six times such that six potentiated Pdi_{tw} values were obtained. The average of the highest 3 or 4 twitches that met the acceptability criteria was reported. Each potentiated twitch was assessed for amplitude (baseline to peak), maximal rate of pressure development (MRPD), maximal relaxation rate (MRR), contraction time (CT) and one-half relaxation time ($RT_{0.5}$). Pdi_{MAX} was calculated as the peak Pdi (across 1 s) during each inspiratory Müller manoeuvre; Pdi_{MAX} was reported as the maximum of three values that varied by ≤10%. The within-day between occasion reproducibility coefficients (coefficient of variation, CV) were 3.7, 4.3, 7.0, 1.5, 5.0 and 3.1% for Pdi_{tw}, MRPD, MRR, CT, $RT_{0.5}$ and MIP, respectively (*see section* 4.4).

6.4. RESULTS

6.4.1. Inspiratory Resistive Loaded Breathing Targeting Inspiratory Mouth Pressure: IRL_{Pm}

6.4.1.1. Cardiovascular Measurements during Control IRL_{Pm} (2% of MIP)

There was no change in group mean MAP, $\dot{Q}_L$ or LVR (Figure 6.1) over time in response to IRL at 2% of MIP ($P \geq 0.05$). Similarly, HR, superficial femoral artery

diameter and V_{MEAN} remained unchanged in response to IRL at 2% of MIP ($P \geq 0.05$) (Table 6.1). VM_{RMS} and VL_{RMS} were not different to resting values during IRL at 2% of MIP, suggesting that there was no contraction of the non-respiratory muscles (Table 6.1).

6.4.1.2. Cardiovascular Measurements during IRL_{Pm} (65% of MIP)

Task failure during IRL_{Pm} occurred at 16.2 ± 6.5 min. Prior to task failure, inspiratory Pm was maintained at 105 ± 6% of the target value (Table 6.1). There was an immediate and sustained increase in group mean HR from rest to during IRL_{Pm} (Table 6.1). Relative to eupnoeic baseline values, group mean MAP was unchanged at the first minute of IRL_{Pm}, but increased by 15 ± 9 mmHg ($P = 0.032$) at task failure ($n = 8$; Figure 6.1). Conversely, there was no change in group mean superficial femoral artery diameter, V_{MEAN}, $\dot{Q}_L$ or LVR across time from baseline to during IRL_{Pm} (Figure 6.1, Table 6.1). The $\dot{Q}_L$ and LVR response to IRL_{Pm} was, however, highly variable between the subjects. Indeed, the change in $\dot{Q}_L$ and LVR from baseline to task failure during IRL_{Pm} ranged from –52% to +71% ($\dot{Q}_L$) and from –40% to +61% (LVR) (Figure 6.1). As in the control trial targeting 2% of MIP, there was no evidence of leg muscle contraction throughout IRL_{Pm} (Table 6.1).

Table 6.1. Group mean ventilatory, cardiovascular and muscular responses to inspiratory resistive loading targeting 2% and 65% of maximal inspiratory *mouth* pressure.

	Rest	Min 3	Min 5	Task Failure	Recovery
IRL at 2% of MIP (control)					
Inspiratory Pm, cmH_2O	−2 ± 1	−5 ± 3	−4 ± 2	-	−2 ± 0
f_R breaths·min^{-1}	13 ± 3	15 ± 0	15 ± 0	-	13 ± 3
HR, beat·min^{-1}	61 ± 11	62 ± 11	62 ± 9	-	62 ± 11
SFA V_{MEAN}, cm·s^{-1}	7.6 ± 3.4	8.3 ± 4.0	8.2 ± 3.8	-	7.8 ± 4.3
SFA Diameter, cm	0.59 ± 0.06	0.58 ± 0.06	0.58 ± 0.06	-	0.58 ± 0.06
VM_{RMS}, % of max	1.6 ± 1.5	1.7 ± 1.4	1.8 ± 1.5	-	1.7 ± 1.5
VL_{RMS}, % of max	1.5 ± 1.3	1.6 ± 1.5	1.6 ± 1.4	-	1.3 ± 1.4
IRL at 65% of MIP (IRL_{Pm})					
Inspiratory Pm, cmH_2O	−2 ± 1	−102* ± 16	−103* ± 16	−98* ± 18	−2 ± 2
f_R breaths·min^{-1}	13 ± 2	15 ± 1	15 ± 1	15 ± 1	16 ± 5
HR, beat·min^{-1}	63 ± 11	84* ± 9	84* ± 9	89* ± 10	71 ± 15
SFA V_{MEAN}, cm·s^{-1}	8.5 ± 2.6	9.4 ± 1.5	9.4 ± 2.3	10.9 ± 3.4	10.1 ± 3.8
SFA Diameter, cm	0.59 ± 0.06	0.58 ± 0.06	0.58 ± 0.06	0.59 ± 0.06	0.59 ± 0.06
VM_{RMS}, % of max	1.2 ± 1.7	1.4 ± 2.0	1.4 ± 1.7	1.5 ± 1.6	1.1 ± 0.7
VL_{RMS}, % of max	0.8 ± 0.7	1.0 ± 0.6	1.4 ± 1.1	1.8 ± 2.2	1.3 ± 1.4

Values are mean ± SD in 11 subjects (HR and $\dot{Q}$, n = 8). Pm, mouth pressure; HR, heart rate; SFA, superficial femoral artery; V_{MEAN}, time averaged mean blood velocity; VM, vastus medialis; VL, vastus lateralis; RMS, root mean square. *significantly different to rest ($P < 0.05$).

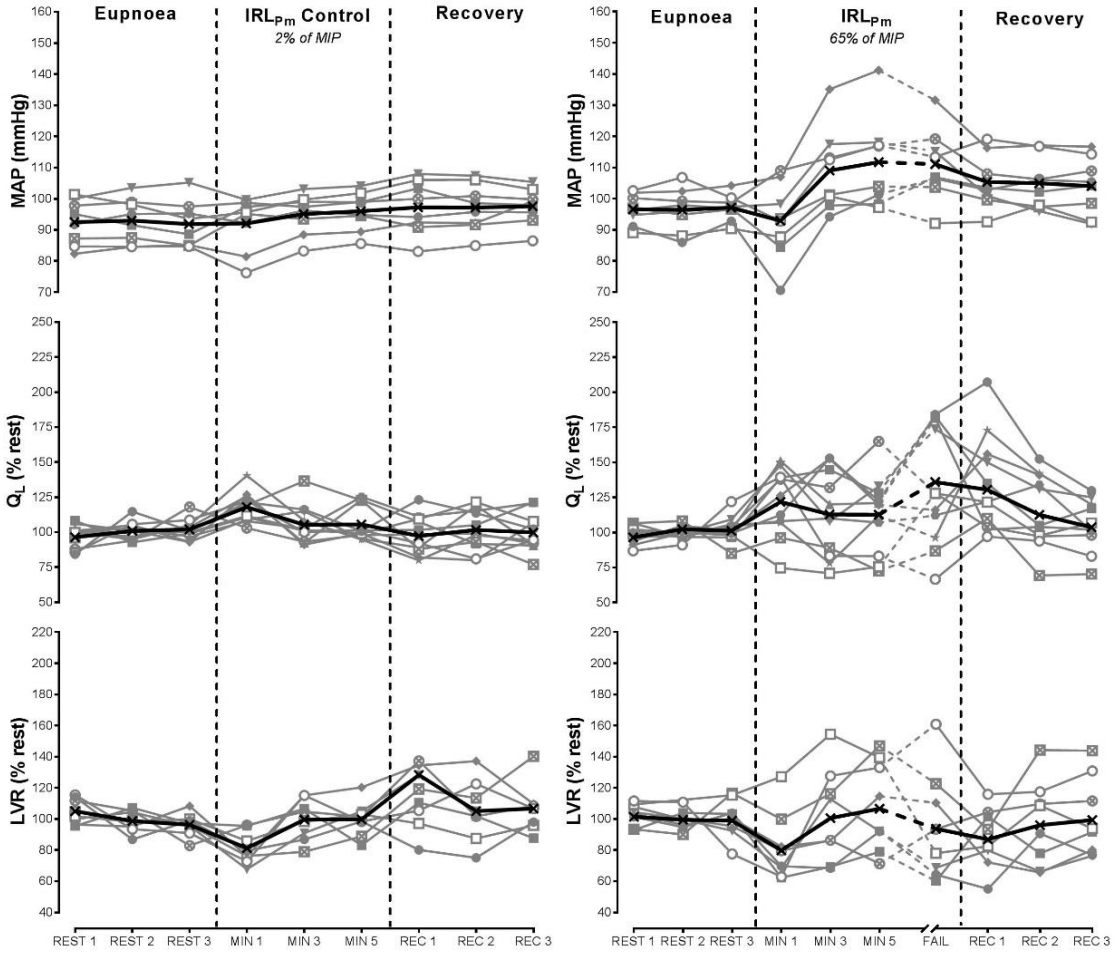


Figure 6.1. Mean arterial pressure (MAP, *n* = 8) (top panels), leg blood flow ($\dot{Q}_L$, *n* = 11) (middle panels), and leg vascular resistance (LVR, *n* = 8) (bottom panels) responses to inspiratory resistive loading targeting 2% (IRL_{Pm} control) (A-C) and 65% (IRL_{Pm}) of maximal inspiratory mouth pressure (D-F). Data are group means (black) and individual values (grey).

6.4.1.3. IRL_{Pm}-induced Inspiratory Muscle Fatigue

There was no change in MIP from pre- to post-5 min of control IRL at 2% of MIP (150 ± 24 vs. 150 ± 20 cmH_2O, *P* = 1.000). There was, however, a significant reduction relative to prior baseline values in MIP following IRL_{Pm} at 65% of MIP (150 ± 20 vs. 134 ± 28 cmH_2O, *P* = 0.026) (Figure 6.2, *panel A*).

6.4.2. Inspiratory Resistive Loaded Breathing Targeting Inspiratory Diaphragmatic Pressure: IRL_{Pdi}

An representative subject's cardiovascular responses to IRL targeting 2% and 65% of peak diaphragm pressure (Pdi_{MAX}) are presented in Figure 6.3, including breath-by-breath Pm, beat-by-beat arterial pressure (AP) and HR, and mean $\dot{Q}_L$ and LVR data.

6.4.2.1. Cardiovascular measurements during control IRL (2% of Pdi_{max})

There was no change in group mean MAP, $\dot{Q}_L$ or LVR over time in response to IRL at 2% of Pdi_{MAX} ($P \geq 0.05$) (Figure 6.4). Similarly, HR, superficial femoral artery CSA and V_{MEAN} remained unchanged during IRL at 2% of Pdi_{MAX} ($P \geq 0.05$) (Table 6.2). VM_{RMS} and VL_{RMS} were not different to resting values during IRL at 2% of Pdi_{MAX}, suggesting that there was no contraction of the non-respiratory muscles ($P \geq 0.05$) (Table 6.2).

6.4.2.2. Cardiovascular Measurements during IRL_{Pdi} (65% of Pdi_{MAX})

Task failure during IRL_{Pdi} occurred at 16.1 ± 3.6 min. Prior to task failure, peak inspiratory Pm and Pdi were maintained at 103 ± 8% and 136 ± 25% of the target value (Table 6.2). From eupnoea to task failure of IRL_{Pdi}, there was a significant group mean increase in HR (Table 6.2) and MAP (Figure 6.4) (*both* $P < 0.05$). Conversely, there was no change in group mean superficial femoral artery diameter, V_{MEAN}, $\dot{Q}_L$ or LVR across time from baseline to during IRL_{Pdi} (Figure 6.4, Table 6.2). The $\dot{Q}_L$ and LVR response to IRL_{Pdi} was, however, highly variable between the subjects. Indeed, the change in $\dot{Q}_L$ and LVR from baseline to task failure during IRL_{Pm} ranged from –41% to +51% ($\dot{Q}_L$) and from –30% to +44% (LVR) (Figure 6.4). As in the control trial targeting 2% of MIP, there was no evidence of leg muscle contraction throughout IRL_{Pdi} (Table 6.2).

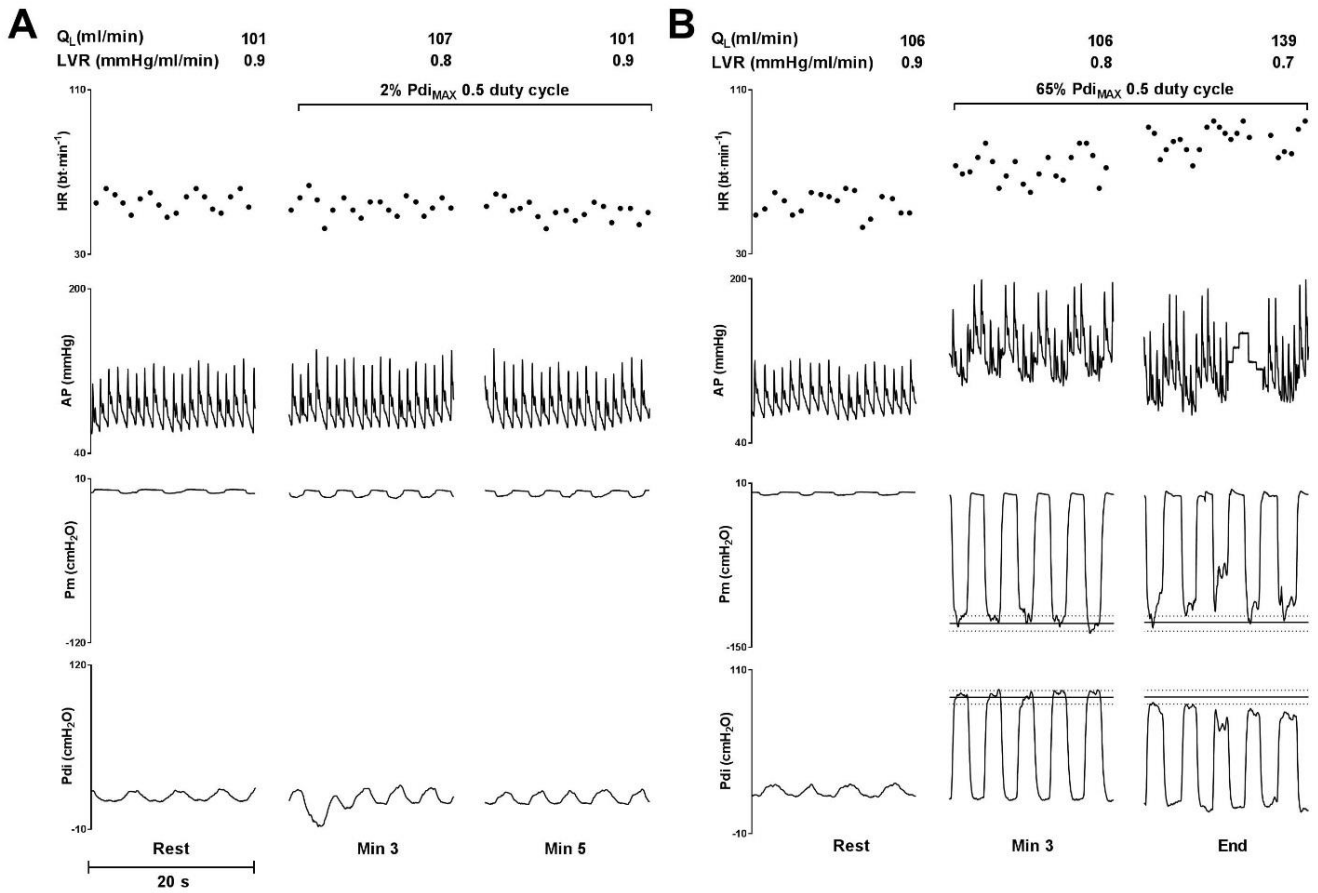


Figure 6.3. Cardiovascular and ventilatory responses to (A) IRL_{Pdi} control and (B) IRL_{Pdi} for one subject, represented by black squares in all other figures. Cardiovascular and ventilatory parameters were unchanged in response to IRL_{Pdi} control. There was a time-dependent increase in HR and MAP in response to IRL_{Pdi}. Pm and Pdi were maintained at the target level until the final minute of IRL_{Pdi}. $\dot{Q}_L$, leg blood flow; LVR, leg vascular resistance; HR, heart rate (derived from AP signal); AP, arterial pressure; Pm, mouth pressure; Pdi, diaphragm pressure.

Table 6.2. Group mean ventilatory, cardiovascular and muscular responses to inspiratory resistive loading targeting 2% and 65% of maximal inspiratory *diaphragm* pressure.

	Rest	Min 3	Min 5	Task Failure	Recovery
IRL at 2% of MIP (control)					
Inspiratory Pm, cmH_2O	−2 ± 0	−3 ± 1	−3 ± 1	-	−2 ± 0
f_R, breaths·min^{-1}	12 ± 2	15 ± 0	15 ± 0	-	12 ± 2
Heart Rate, beat·min^{-1}	63 ± 8	66 ± 8	67 ± 8	-	63 ± 7
SFA V_{MEAN}, cm·s^{-1}	6.8 ± 1.7	7.9 ± 2.1	7.8 ± 2.7	-	7.2 ± 2.7
SFA Diameter, cm	0.62 ± 0.04	0.61 ± 0.05	0.61 ± 0.04	-	0.61 ± 0.05
VM_{RMS}, % of max	1.1 ± 0.5	1.0 ± 0.4	1.1 ± 0.4	-	1.1 ± 0.5
VL_{RMS}, % of max	1.0 ± 0.4	1.1 ± 0.4	1.1 ± 0.4	-	1.1 ± 0.4
IRL at 65% of Pdi_{MAX} (IRL_{Pdi})					
Inspiratory Pm, cmH_2O	−2 ± 0	−95 ± 16	−98 ± 16	−96* ± 15	−2 ± 0
f_R, breaths·min^{-1}	11 ± 2	15 ± 1	15 ± 1	14 ± 1	17 ± 6
Heart Rate, beat·min^{-1}	65 ± 8	86 ± 16	87 ± 17	93 ± 15	75 ± 16
SFA V_{MEAN}, cm·s^{-1}	8.1 ± 3.8	6.9 ± 1.1	6.7 ± 1.1	7.3 ± 1.2	9.4 ± 5.1
SFA Diameter, cm	0.61 ± 0.05	0.61 ± 0.05	0.61 ± 0.05	0.61 ± 0.05	0.62 ± 0.05
VM_{RMS}, % of max	1.1 ± 0.3	1.1 ± 0.3	1.1 ± 0.3	1.1 ± 0.3	1.1 ± 0.3
VL_{RMS}, % of max	1.1 ± 0.4	1.2 ± 0.4	1.1 ± 0.4	1.1 ± 0.4	1.1 ± 0.4

Values are mean ± SD in 6 subjects (HR and $\dot{Q}$, n = 4). Pm, mouth pressure; SFA, superficial femoral artery; V_{MEAN}, time averaged mean blood velocity; VM, vastus medialis; VL, vastus lateralis; RMS, root mean square. * Significantly different to rest ($P < 0.05$).

6.4.2.3. Additional leg blood flow analyses for IRL_{Pdi}

In comparison to resting values during IRL_{Pdi} (161 ± 31 ml·min^{-1}), antegrade $\dot{Q}_L$ across the entire respiratory cycle was ~50% higher in the first minute of IRL (227 ± 57 ml·min^{-1}), and remain elevated by task failure (221 ± 32 ml·min^{-1}); however these absolute increases did not attain statistical significance (P > 0.05). Retrograde $\dot{Q}_L$ across the entire respiratory cycle increased progressively during IRL, and was significantly greater than resting values by task failure (31 ± 14 vs. 95 ± 28 ml·min^{-1}; +285%) (P = 0.042). Unlike the response to ERL_{Pga} in Chapter 5, there was no substantial within-breath fluctuation in $\dot{Q}_L$ during IRL_{Pdi} (Figure 6.5), and therefore these data were not analysed further.

6.4.2.4. IRL_{Pdi}-induced Inspiratory Muscle Fatigue

From before to after control IRL at 2% of Pdi_{MAX}, there was no change in either MIP or Pdi_{tw} (MIP: 146 ± 22 vs. 147 ± 17 cmH_2O, P = 0.537; Pdi_{tw}: 29.4 ± 7.6 vs. 27.8 ± 9.6 cmH_2O, P = 0.970). IRL_{Pdi} did, however, elicit inspiratory muscle fatigue as evidenced by a 23 ± 12% reduction in Pdi_{tw} (P = 0.014) (Figure 6.2, *panel B*), although MIP was not significantly reduced (P = 0.089) (Figure 6.2, *panel C*). There were no changes in twitch characteristics in response to IRL_{Pdi}: CT, 143 ± 17 ms vs 147 ± 21 ms; MRPD/Pdi_{tw}, 13.5 ± 3.0 s/cmH_2O vs. 14.6 ± 1.4 s/cmH_2O; MRR/Pdi_{tw}, −6.1 ± 1.6 s/cmH_2O vs. −6.5 ± 1.8 s/cmH_2O; $RT_{0.5}$, 113 ± 28 vs. 116 ± 29 (all P > 0.05). Similarly, M-wave characteristics were unchanged in response to IRL_{Pdi}: amplitude, 0.9 ± 0.6 mV vs. 0.9 ± 0.6 mV; duration, 28.6 ± 18.5 ms vs. 27.6 ± 19.1 ms; area, 5.1 ± 5.0 mV·s^{-1} vs. 5.5 ± 5.6 mV·s^{-1} (n = 5, all P > 0.05). Additionally, there were no differences in twitch or M-wave responses to the control trial (all P > 0.05).

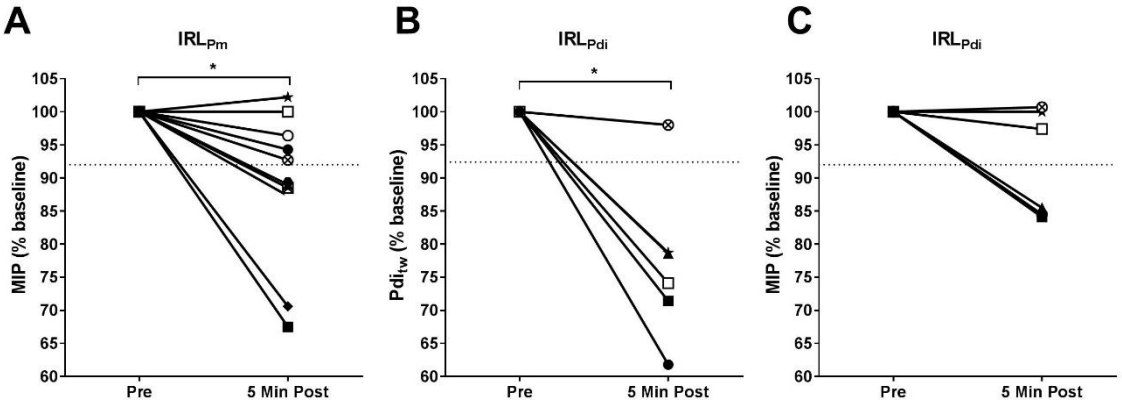


Figure 6.2. Individual inspiratory muscle function responses to inspiratory resistive loading (IRL) targeting mouth pressure (IRL_{Pm}) (A) and diaphragm pressure (IRL_{Pdi}) (B-C). MIP, maximal inspiratory pressure; Pdi_{tw}, diaphragm twitch pressure. Dotted lines represent 2 x CV. * *P* < 0.05 vs. pre-IRL values.

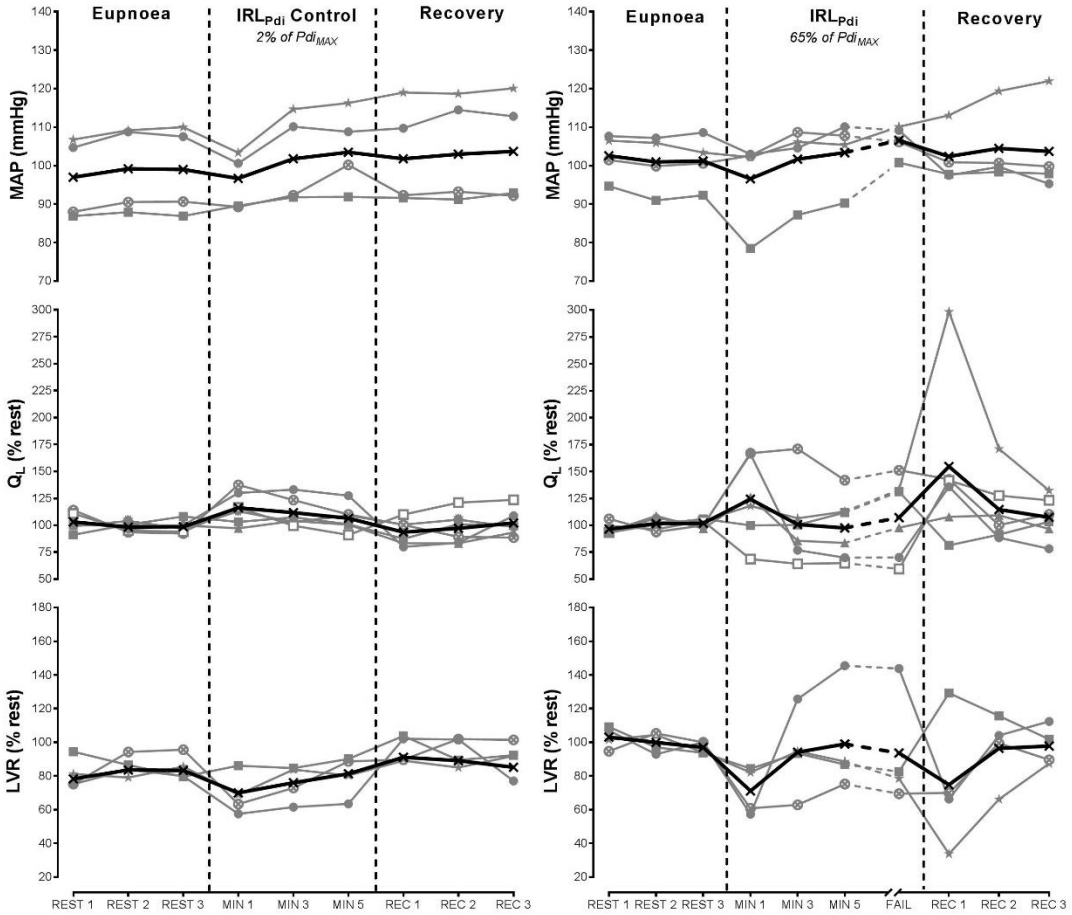


Figure 6.4. Mean arterial pressure (MAP, *n* = 4) (top panels), leg blood flow ($\dot{Q}_L$, *n* = 5) (middle panels), and leg vascular resistance (LVR, *n* = 4) (bottom panels) responses to inspiratory resistive loading targeting 2% (IRL_{Pdi} control) (A-C) and 65% (IRL_{Pdi}) of maximal inspiratory diaphragm pressure (D-F). Data are group means (black) and individual values (grey).

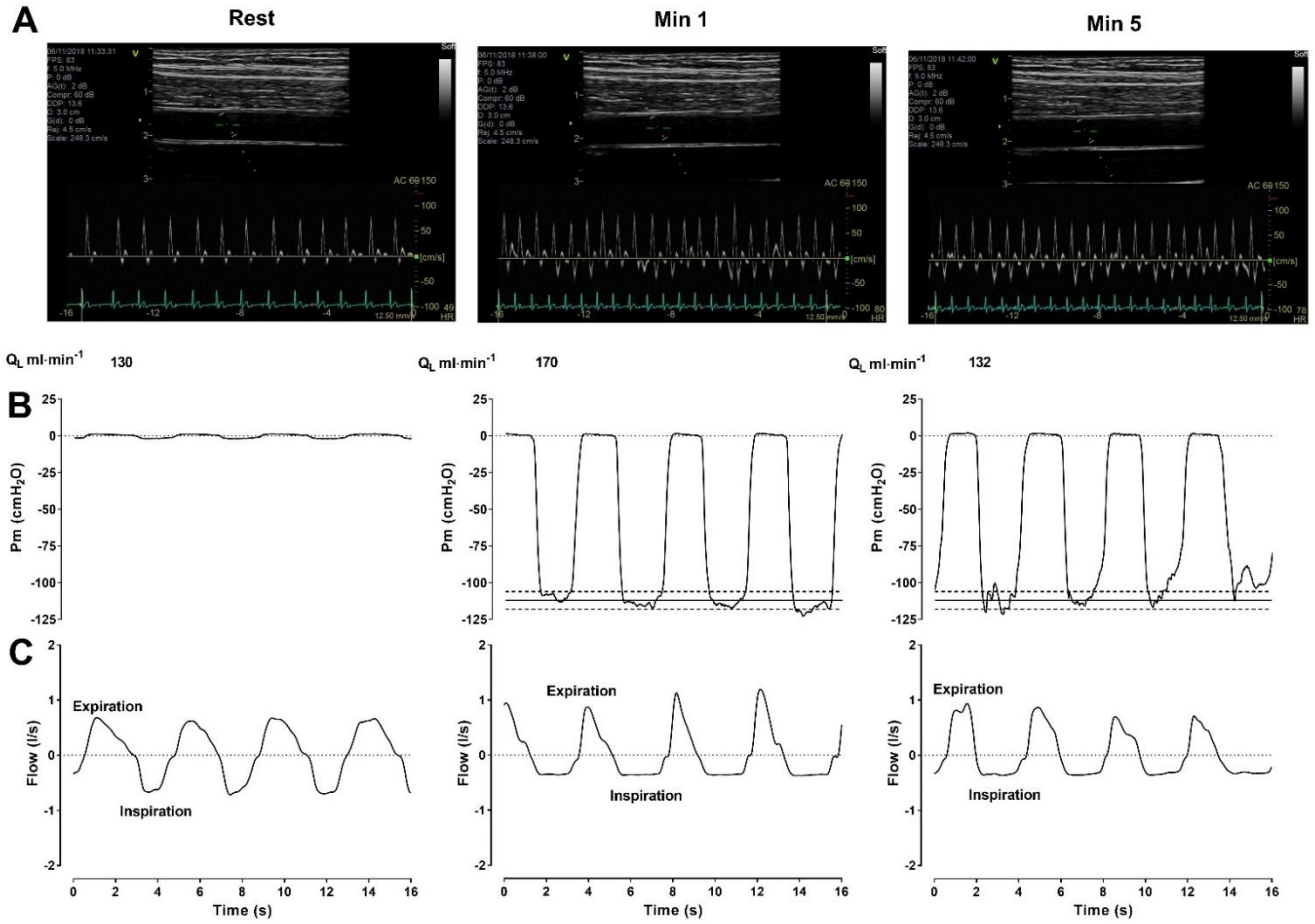


Figure 6.5. An individual example of beat-by-beat leg blood flow ($\dot{Q}_L$) (A), mouth pressure (Pm) (B), and airflow at the mouth (C) during fatiguing inspiratory resisted loading targeting 65% of maximal transdiaphragmatic pressure (IRL_{Pdi}). $\dot{Q}_L$ (ml·min^{-1}) calculated as the product of vessel cross sectional area (cm^2) x time averaged mean blood velocity (cm/s) x 60. Note, within-breath responses were not determined for IRL_{Pdi}.

6.4.3. Correlations Between Variables

For IRL_{Pm}, there was no significant correlation between the change in $\dot{Q}_L$ from rest to task failure and 1) baseline MIP (r = 0.024); 2) the change in MIP from pre to post-IRL (r = −0.14); or 3) time to task failure for IRL (r = 0.36) (all P > 0.05). Similarly, the change in LVR from rest to task failure during IRL_{Pm} was not associated with 1) baseline MIP (r = 0.45); 2) the change in MIP from pre to post-IRL (r = −0.07); or 3) time to task failure of IRL (r = 0.44) (all P > 0.05). These data indicate that the individual variability in cardiovascular responses to IRL_{Pm} was not associated with baseline inspiratory muscle strength and/or the severity of IRL-induced inspiratory muscle fatigue. During IRL_{Pm}, the change in $\dot{Q}_L$ from rest to task failure was strongly and positively associated to the change in HR (r = 0.82, P = 0.013).

6.5. DISCUSSION

6.5.1. Main Findings

In this study, the cardiovascular consequences of fatiguing inspiratory muscle work in healthy otherwise resting humans were investigated. The major findings were that inspiratory resistive loaded breathing (IRL) targeting 65% of inspiratory mouth pressure (IRL_{Pm}) or transdiaphragmatic pressure (IRL_{Pdi}) elicited: 1) a significant increase in heart rate (HR) and a time-dependent increase in mean arterial pressure (MAP); and 2) fatigue of the inspiratory muscles, as evidenced by a significant pre- to post-IRL reduction in maximal inspiratory mouth pressure (MIP) and transdiaphragmatic twitch pressure (Pdi_{tw}); but 3) no change in group mean LVR or $\dot{Q}_L$ relative to prior resting values. There was, however, considerable between-subject variability in the LVR (−40 to +61%) and $\dot{Q}_L$ (−52 to 71%) response to IRL. In contrast to the cardiovascular response to ERL (Chapter 5), there was no substantial within-breath fluctuation in $\dot{Q}_L$ during IRL. However, the progressive and significant rise in retrograde $\dot{Q}_L$ (+285% task failure vs. rest), which may be suggestive of an

underlying time-dependent peripheral vasoconstriction, was likely counteracted by a consistent absolute rise in antegrade $\dot{Q}_L$.

In response to fatiguing inspiratory muscle work, previous studies have demonstrated a sympathoexcitatory response, characterised by a time-dependent increase in MSNA, MAP, LVR and a decrease in $\dot{Q}_L$ (St Croix et al., 2000, Sheel et al., 2001, Smith et al., 2016, Welch et al., 2018b). Presently, an initial aim of this thesis was to compare the magnitude and temporality of changes in cardiovascular parameters (i.e. MAP, LVR, and $\dot{Q}_L$) during ERL to the previously reported time-dependent increases in MAP and LVR and the decrease in $\dot{Q}_L$ in response to fatiguing IRL. However, there was no evidence for a change in either group mean LVR or $\dot{Q}_L$ in response to IRL performed to task failure. Therefore, the temporality of changes in cardiovascular responses of IRL to ERL cannot be directly compared. Thus, as in Chapter 5, two important questions must be considered: 1) were the experimental conditions required to initiate an inspiratory muscle metaboreflex met in the present study; and 2) mechanistically, why did fatiguing IRL have no effect on group mean LVR and $\dot{Q}_L$ in otherwise resting humans?

6.5.2. Was an inspiratory muscle metaboreflex initiated?

The provocation of an inspiratory muscle metaboreflex is hypothesised to be critically dependent upon the accumulation of metabolic by-products secondary to fatiguing muscular contractions. In the resting canine, infusion of the lactic acid into the phrenic artery elicits a substantial increase in MAP, and a reduction in $\dot{Q}_L$ and leg vascular conductance (Rodman et al., 2003). In addition, the discharge rate of metabosensitive group IV phrenic afferent fibres is increased in response to electrically-stimulated rhythmic contractions of the diaphragm in anaesthetised rats when the diaphragm is in a fatigued versus non-fatigued state (Hill, 2000). In otherwise resting humans performing IRL, the importance of inspiratory muscle

fatigue for the initiation of the inspiratory muscle metaboreflex is highlighted by the *time-dependence* of changes in MAP, MSNA, LVR, and $\dot{Q}_L$ (i.e. significant changes only become evident by the second minute of IRL) (St Croix et al., 2000, Sheel et al., 2001, Smith et al., 2016), which is congruent with the time required for metabolic by-products to accumulate (Mark et al., 1985). The importance of the accumulation of fatigue-associated metabolites is further highlighted by the absence of a change in LVR and $\dot{Q}_L$ in the resting limb in response to IRL protocols performed at lower relative pressures (i.e. 30-50% of MIP vs. 60% of MIP) that do not result in the attainment of inspiratory muscle fatigue (Sheel et al., 2002).

In the present study, a TTI (i.e. the product of duty cycle and pressure generation) of the inspiratory muscles (IRL_{Pm}) and the diaphragm specifically (IRL_{Pdi}) was prescribed that substantially exceeded the critical TTI of the diaphragm (0.325 vs 0.15); hence it was hypothesised that the IRL trials would be unsustainable, terminating under conditions of inspiratory muscle fatigue (Bellemare and Grassino, 1982, Bellemare et al., 1983). Indeed, in response to IRL sustained until task failure, there was a substantial decrease in MIP (−11%; IRL_{Pm}), and a reduction in Pdi_{tw} (−23%; IRL_{Pdi}) that is highly comparable to previous IRL trials in healthy young individuals (Pdi_{tw}: −23 to −27%) (Welch et al., 2018b, Geary et al., 2019). In addition, there was no significant change in M-wave characteristics during potentiated resting twitches. Therefore, the reduction in Pdi_{tw} (at 1 Hz stimulation frequency) in response to IRL_{Pdi} is most consistent with low-frequency peripheral muscle fatigue (Jones, 1996, Allen et al., 2008), rather than an inhibition of action potential transmission to the T-tubule system. The cause of low-frequency muscle fatigue is complex and multifaceted, but evidence suggests that alterations in the Ca^{2+} sensitivity of myofibrillar proteins and impaired Ca^{2+} release from the sarcoplasmic reticulum are predominant mechanisms (Leppik et al., 2004). Such impairments in Ca^{2+} function are strongly linked to metabolic perturbation, including increases in inorganic

phosphate, reactive oxygen species, adenosine diphosphate and a reduction in pH (Allen et al., 2008). Therefore, it is highly likely that the an accumulation of metabolites were present in the diaphragm causing, or at least contributing to, the development of low frequency diaphragmatic fatigue. Collectively, these findings suggest that the conditions required to initiate an inspiratory muscle metaboreflex were met in the present study.

6.5.3. Why did IRL-induced diaphragm fatigue have no effect on LVR and $\dot{Q}_L$?

As diaphragm fatigue is reportedly a prerequisite for the initiation of an inspiratory muscle metaboreflex, and considering that the IRL protocols used in this study clearly elicited such inspiratory muscle fatigue, the question then becomes: why did group mean LVR and $\dot{Q}_L$ not change across time in response to inspiratory resisted breathing?

6.5.3.1. Methodological Factors

There are two key methodological factors that have the potential to influence peripheral vasomotor tone in response to IRL. First, co-contraction of the 'otherwise resting' limb muscles would be expected to increase blood flow in relation to metabolic demand and the contractile work performed (Hamann et al., 2005). However, there was no difference in EMG activity of the vastus lateralis or vastus medialis during IRL in comparison to rest, and mean EMG activity was below the minimum threshold classified as muscle activity (2% of MVC) (Tikkanen et al., 2013). Second, an increase in CO_2 tension may cause vasodilation and a consequent reduction in vascular resistance in the limbs. However, across both IRL trials, $P_{ET}CO_2$ was remained within ± 3 mmHg.

6.5.3.2. Metabolic Factors

It was hypothesised that the development of diaphragmatic fatigue and the associated accumulation of fatigue-associated metabolites would stimulate group III and IV phrenic afferents, evoking a peripheral vasoconstriction secondary to an increase in sympathetic motor-outflow. In the present study, there was a substantial and *time-dependent* increase in MAP (Figure 6.1) which is consistent with the time taken for such metabolite accumulation to occur. Although sympathetic outflow was not directly measured in the present study, the magnitude and time-dependency of the change in MAP in very similar to that previously reported in response to IRL data, in which a concomitant increase in MSNA in the resting limb was reported (St Croix et al., 2000). Therefore it is highly likely that a time-dependent increase in sympathetic motor-outflow and a consequent peripheral vasoconstriction contributed to the MAP response observed in response to IRL in the present study. Moreover, upon further analysis of the present $\dot{Q}_L$ data in response to IRL_{Pdi}, a substantial progressive increase in retrograde $\dot{Q}_L$ across the entire respiratory cycle was observed compared to resting values (~285% task failure vs. rest; *see section* 6.4.2.3). It could be speculated that a sympathetically-mediated peripheral vasoconstriction, particularly in the resistance arteries and arterioles that primarily regulate blood flow, is the primary cause of such an increase in retrograde flow (Joyner and Casey, 2015, Hanson et al., 2020). Indeed, the superficial femoral artery flow profile during IRL compared closely to that found during external compression of the lower limb (Heffernan et al., 2013), which is a model that 'mimics' an increase in distal vascular tone (Liu et al., 2011). *So, now it must be asked: if retrograde $\dot{Q}_L$ increased during IRL, why was mean $\dot{Q}_L$ not lower than resting values?*

6.5.3.3. Mechanical Factors

The majority of previous evidence supports an underlying peripheral vasoconstriction as the primary cause of the reduction in $\dot{Q}_L$ in response to IRL. However, it is also

likely that a mechanical cardio-pulmonary interaction caused by very-high swings in intrathoracic pressure also *positively and negatively* affected cardiac output, and as such $\dot{Q}_L$ (*see below*), during such loaded breathing protocols (Convertino, 2019). In the present study, in combination with a substantial decrease in retrograde $\dot{Q}_L$, antegrade $\dot{Q}_L$ increased by 41% over the duration of IRL_{Pdi}. In peripheral conduit arteries such as the superficial femoral artery blood flow is triphasic; the predominance of antegrade flow occurs during systole, primarily determined by left ventricular (LV) contractile force (Scissons, 2008). In contrast, the retrograde waveform (phase 2) occurs during early diastole and is caused by a reflection from the high-resistance outflow bed. The second smaller antegrade waveform (phase 3) occurs due to a refection from the closed aortic valve during late diastole. Therefore, it is possible that a mechanical effect of highly negative intrathoracic pressure, and to a lesser extent the increased intra-abdominal pressure associated with diaphragmatic breathing, during resistive inspiratory efforts may have transiently influenced *antegrade* $\dot{Q}_L$ (phase 1), primarily via an effect on LV function. Indeed, in resting humans, small reductions in intrathoracic pressure (~10 cmH_2O below normal resting values) augment cardiovascular function via an enhancement of cardiac preload and LV stroke volume (Brecher and Hubay, 1955, Robotham and Peters, 1989, Innes et al., 1993). By contrast, larger reductions in intrathoracic pressure (i.e. >20 cmH_2O) may also elicit adverse cardiac effects, such as 1) limiting flow in the inferior vena cava (Condos et al., 1987, Kimura et al., 2011); 2) impairing right ventricular cardiac filling (Cheyne et al., 2018); 3) compromising LV geometry (Iliceto et al., 1988); 4) increasing afterload secondary to an increase in LV transmural pressure or direct ventricular interaction (Robotham et al., 1978, Karam et al., 1984); and ultimately, 5) reducing stroke volume (Weber et al., 1982, Cheyne et al., 2018). In the present study, Poes was highly negative during resistive inspiratory efforts (−55 to −80 cmH_2O). Due to the complex and somewhat contrasting mechanical effects of generating high intrathoracic pressures on cardiac function, it is difficult to ascertain

any consequent effect on $\dot{Q}_L$. However, the preponderance of literature would suggest that very-high intrathoracic pressures likely *impair* rather than augment LV stroke volume, and are therefore unlikely to explain the increase in antegrade $\dot{Q}_L$ observed in the present study.

6.5.3.4. Heart Rate

An increase in heart rate primarily shortens diastolic rather than systolic time during each cardiac cycle (Salvi, 2012), meaning that systole becomes a greater proportion of each cardiac cycle. Therefore, an increase in heart rate predominantly affects antegrade blood flow in peripheral arteries (i.e. because only antegrade flow is predominantly present during systole in triphasic waveforms – *see section* 6.5.3.3). In response to both IRL trials we found an increase in group mean heart rate of ~40% (26-28 beats·min^{-1}) that considerably exceeds the group mean change reported by previous studies (e.g. 4-17 beats·min^{-1}) (Sheel et al., 2001, Smith et al., 2016). Assuming the absence of a substantial change in LV stroke volume (*see section* 6.5.3.3), such an increase in heart rate likely contributed substantially to the group mean increase in antegrade $\dot{Q}_L$ (+41%), which may have counteracted the metabolically-induced increase in retrograde $\dot{Q}_L$ (*see section* 6.5.3.2), resulting in the absence of a group mean change in mean $\dot{Q}_L$. Below, the importance of heart rate in the determination of $\dot{Q}_L$ is highlighted via theoretical calculations of $\dot{Q}_L$ (ml·min^{-1}) as the product of vessel cross sectional area (CSA), V_{MEAN} (cm·beat^{-1}) and heart rate. We first demonstrate the calculation of $\dot{Q}_L$ using fixed values of CSA, V_{MEAN}, and heart rate obtained from IRL_{Pdi} at (1) rest and (2) task failure. Additionally (3), we predict the decrease in V_{MEAN} required to elicit a 20% reduction in $\dot{Q}_L$ at the heart rate and vessel CSA observed at task failure.

Table 6.3. Theoretical example calculations of the required change in V_{MEAN} to elicit a 20% reduction in $\dot{Q}_L$ from the IRL data obtained in the present study.

	Rest (1)	Task Failure (2)	Task Failure −20% (3)
CSA, cm^2	0.30	0.30	0.30
V_{MEAN}, $cm \cdot beat^{-1}$	7.4	7.4	**4.1**
HR, $beats \cdot min^{-1}$	65	93	93
Q_L, $ml \cdot min^{-1}$	**144**	**206**	115

These calculations highlight that, for a decrease in $\dot{Q}_L$ to occur under circumstances of an unchanging arterial CSA and a rise in heart rate of ~40%, a reduction in V_{MEAN} of ~45% is required to induce a decrease in $\dot{Q}_L$ of 20% below resting values. In several subjects performing IRL in the present study, the magnitude of peripheral vasoconstriction in the resistance arterioles downstream of the superficial femoral artery may have been insufficient to overcome the substantial sympathetically-mediated increase in heart rate and antegrade flow to cause such a resultant reduction in $\dot{Q}_L$. In support, presently there was a strong significant correlation between the change in heart rate and the change in $\dot{Q}_L$ from rest to task failure of IRL_{Pm} (r = 0.82); i.e. subjects that exhibited a smaller increase in heart rate demonstrated a decreased $\dot{Q}_L$ response to IRL. *Why was the magnitude of change in heart rate in the present study ~twofold greater than previous reports?* The greater increase in heart rate during IRL in the present study may indicate that subjects were working at a higher relative intensity, sustained for a longer duration than previous studies (16 min vs. 7-13 min) (Sheel et al., 2001, Smith et al., 2016). There were minor methodological differences between IRL protocols including: the method used to determine MIP (i.e. maximum vs. mean value; familiarisation included or not), the relative load prescribed (60 vs. 65% of MIP), whether subject Pm was directly verified during IRL, and the criteria for task failure (failure to maintain Pm or a plateau in MAP). As a result, in the highly familiarised subjects of the present study, absolute peak Pm was ~40 cmH_2O higher than reported by Sheel et al. (2001), maintained consistently at the target value until task failure, which may have elicited an augmented cardiovascular response to the task.

6.5.4. Natural vs. Diaphragm Breathing

This study also assessed whether specifically targeting the diaphragm during IRL augmented the inspiratory muscle metaboreflex response in a subsample of subjects. Although prior evidence suggests that the accessory inspiratory muscles respond to- and that accessory inspiratory muscle fatigue like stimulates- the respiratory muscle metaboreflex (*see section* 5.4.3), it is possible that selectively targeting diaphragm pressure may incite greater phrenic afferent feedback, increasing the sympathetic response to IRL. Moreover, it is well established that diaphragmatic breathing impedes venous return (and likely stroke volume) in comparison to ribcage/accessory muscle breathing (Miller et al., 2005a). However, net venous return remains unaffected by such alterations in breathing pattern due to a compensatory increase in expiratory venous return; therefore, although diaphragm breathing may augment any cyclical effects of breathing cycle, net stroke volume likely remains unaffected (Miller et al., 2005a). In the subset of subjects in the present study (n = 6), specifically targeting Pdi vs. Pm attenuated changes in $\dot{Q}_L$ from rest to task failure of IRL (+7 vs +53%). Although the Pdi_{tw} response to IRL_{Pm} was not assessed, it is likely that IRL_{Pdi} increased the severity of diaphragm vs. global inspiratory muscle fatigue via greater ischemia of the phrenic artery, which may have augmented afferent signalling and consequent sympathetically mediated vasoconstriction in the resting limb.

6.5.5. Considerations

Although a measure of sympathetic outflow (i.e. MSNA) would have further supported the interpretation of the activation of an inspiratory muscle metaboreflex in the present study, the absence of such a measure is not considered as a crucial limitation. Indeed, to the authors knowledge only one previous study has measured MSNA during IRL in healthy otherwise resting humans (St Croix et al., 2000). As such, the vast majority of previous studies that have concluded on the presence (or

otherwise) of a respiratory muscle metaboreflex in response to fatiguing inspiratory muscle work have done so without a direct measure of MSNA (Sheel et al., 2001, Witt et al., 2007, Smith et al., 2016, Smith et al., 2017, Geary et al., 2019). However, in support of the present findings, the change in MAP observed from rest to task failure of IRL (15 ± 9 mmHg; IRL_{Pm}) was highly comparable to that observed by previous research in which a time-dependent increase in MSNA was observed (12 ± 7 mmHg) (St Croix et al., 2000). The measurement of diaphragmatic fatigue via assessment of the Pdi_{tw} response to phrenic nerve stimulation (vs. MIP alone) and the direct determination of Pdi during IRL were considered strengths of the present research design in comparison to previous studies (Sheel et al., 2001, Smith et al., 2016, Smith et al., 2017).

6.5.6. Conclusions

In response to fatiguing inspiratory muscle work in healthy otherwise resting humans, there was no change in group mean LVR or $\dot{Q}_L$, despite substantial inspiratory muscle fatigue and an increase in heart rate and mean arterial pressure characteristic of an increase in sympathetic outflow. The mechanisms for the lack of cardiovascular changes in the resting limb are currently unclear, but there are likely competing influences of metabolically-induced peripheral vasoconstriction, mechanical effects of a very-negative intrathoracic pressure, and the magnitude of sympathetically-driven increases in heart rate. It is suggested that a time-dependent rise in heart rate and MAP alone in response to fatiguing inspiratory muscle work does not necessarily indicate the initiation of an inspiratory muscle metaboreflex.

CHAPTER 7
The Effect of Exercise Intensity and Tolerable Duration on Exercise-induced Inspiratory and Expiratory Muscle Fatigue

7.1. INTRODUCTION

Whole-body exercise performed above 85% of maximal oxygen uptake ($\dot{V}O_{2max}$) to volitional exhaustion elicits diaphragmatic and expiratory abdominal muscle fatigue (Johnson et al., 1993, Taylor et al., 2006, Babcock et al., 1996, Mador et al., 1993, Guenette et al., 2010, Verges et al., 2006). The prerequisite factors that contribute to such exercise-induced *diaphragmatic* muscle fatigue are thought to be well understood. It has been shown that experimental attenuation of inspiratory muscle power (~40-50%) prevents the development of diaphragmatic fatigue in response to exhaustive 'high-intensity' exercise (Babcock et al., 2002), suggesting that exercise-induced diaphragmatic fatigue is, at least in part, due to the magnitude of inspiratory muscle power (i.e. the amount of energy required to ventilate the lung). However, volitional mimicking of the tidal integral of diaphragm pressure at the respiratory frequency ($\int Pdi \cdot f_R$) engendered by exhaustive 'high-intensity' exercise does not result in significant diaphragmatic fatigue in otherwise resting men (Babcock et al., 1995b). In fact, it has been suggested that diaphragmatic fatigue does not occur until volitional production of $\int Pdi \cdot f_R$ exceeds ~180% of exercising values in non-exercising individuals (Babcock et al., 1995b). The likely explanation for the aforementioned findings is that the development of exercise-induced diaphragmatic fatigue is a function of the balance between the magnitude of its work (power) and the adequacy of its blood supply, with a lower available blood flow due to competition between the respiratory and the limb-locomotor muscles for available cardiac output ($\dot{Q}$) during 'high-intensity' exercise being associated with a decrease in the diaphragmatic work required to produce fatigue (Sheel et al., 2018a). This fatigue-favouring imbalance between diaphragm muscle force output and the adequacy of its blood flow and/or O_2

delivery appears to occur most consistently when whole-body exercise is performed at ≥85% $\dot{V}O_{2max}$ to the point of volitional exhaustion (Johnson et al., 1993).

It is well established that the high respiratory muscle power and/or the development of exercise-induced respiratory muscle fatigue during high-intensity exercise evokes distinct cardiovascular consequences. For example, previous data demonstrate that unloading the inspiratory power of breathing during cycling exercise at >95% of $\dot{V}O_{2max}$ causes an increase in limb locomotor blood flow and a decrease in limb vascular resistance in comparison to control conditions; conversely increasing inspiratory muscle power elicits a decrease in limb locomotor blood flow and an increase in limb vascular resistance (Harms et al., 1997). It is hypothesised that such cardiovascular responses associated with the 'normally-occurring' work of breathing during high-intensity exercise are secondary to a *fatigue-induced* metabolic perturbation of the respiratory muscles that increases group III and IV afferent firing rates, leading to a consequent increase in global sympathetic motor-outflow that causes vasoconstriction in the locomotor muscles (Dempsey et al., 2006). However, it appears that the $\dot{V}O_2$ and $\dot{Q}$ demands associated with the power of breathing during submaximal exercise (<80% $\dot{V}O_{2max}$) are insufficient to cause substantial competition for blood flow between the respiratory and the limb-locomotor muscles, and may therefore be of an inadequate level to cause a fatigue-induced sympathoexcitation. Indeed, Wetter et al. (1999) reported that neither increasing nor decreasing inspiratory muscle power during non-exhaustive cycling at 75% of $\dot{V}O_{2peak}$ had any significant effect on leg vascular resistance (LVR), arterial pressure, or leg blood flow (Wetter et al., 1999). The authors postulated that the 'normally occurring' inspiratory power of breathing associated with submaximal exercise makes insufficient demands for $\dot{V}O_2$ and $\dot{Q}$ to require any cardiovascular adjustment (Wetter et al., 1999). Theoretically, the lack of competition for blood flow in combination with a lower power of breathing during submaximal exercise may preclude the development of

respiratory muscle fatigue in such conditions. In support of this, Johnson et al. (1993) suggested that the likelihood of developing diaphragmatic fatigue increases when 'exercise-intensity' exceeds 80-85% of $\dot{V}O_{2peak}$, although in the previous study exercise intensity (range: 60-112% $\dot{V}O_{2peak}$) and modality were not standardised and relatively few subjects exercised below 85% of $\dot{V}O_{2max}$ (n = 3) (Johnson et al., 1993). As such, the effect of exercise intensity, and specifically intensity domain (i.e. heavy- vs. severe-intensity) on the development of respiratory muscle fatigue has not been systematically assessed.

It has also been suggested that diaphragmatic fatigue does not consistently occur in response to exercise protocols that induce a relatively short period of very-high respiratory muscle power, such as ramp incremental exercise or short-duration constant power protocols (Romer et al., 2007, Verin et al., 2004, Babcock et al., 1995a). That is, there may be a minimum duration and intensity of respiratory work under conditions of a limited available cardiac output in order for respiratory muscle fatigue to develop. In contrast, it is theorised that the peripheral vasoconstriction and attenuation of limb locomotor blood flow that occurs within only 2.5 to 3 min of very-high intensity constant-load cycling (>95% $\dot{V}O_{2max}$), is likely contributed to by a respiratory muscle metaboreflex originating from fatiguing respiratory muscle work (Harms et al., 1997, Dempsey et al., 2006). However, as yet no study has directly measured the development of exercise-induced respiratory muscle fatigue in response to short-duration severe-intensity exercise.

Because the expiratory muscles are phenotypically and functionally less fatigue-resistant in comparison to the diaphragm (Haggmark and Thorstensson, 1979, Uribe et al., 1992) and perform additional non-ventilatory roles during exercise (Abraham et al., 2002, Cresswell et al., 1992), it is possible that this 'fatigue threshold' (i.e. cumulative work and power of breathing) may be lower for the expiratory abdominal

muscles vs. the diaphragm. As such, it could be speculated that the expiratory muscles could become fatigued at a lower exercise intensity than previously suggested for the diaphragm (i.e. <85% of $\dot{V}O_{2max}$). Indeed, previous studies have reported reductions in maximal volitional expiratory pressure generation at the mouth following prolonged endurance exercise (Loke et al., 1982, Wuthrich et al., 2015, Tiller et al., 2019). However, whether exercise-induced expiratory muscle fatigue is more prevalent and of a greater magnitude than diaphragmatic fatigue in response to heavy-intensity exercise (most likely <85% $\dot{V}O_{2max}$), and as such whether there is a differing magnitude of expiratory vs. inspiratory muscle fatigue in response to exercise of the same intensity and duration, has not yet been empirically investigated.

The purpose of the present study was therefore to compare and contrast the presence and magnitude of exercise-induced inspiratory and expiratory abdominal muscle fatigue in response to heavy-intensity exercise, short-duration (~5 min) severe-intensity exercise and long-duration (~10 min) severe-intensity exercise. It was hypothesised that: a) in comparison to long-duration severe-intensity exercise, heavy-intensity exercise and short-duration severe-intensity exercise would evoke a significantly lower magnitude of inspiratory muscle fatigue; and b) the magnitude of expiratory muscle fatigue would not be significantly different in response to heavy-intensity, or short- and long-duration severe-intensity exercise.

7.2. METHODS

7.2.1. Subjects

Ten physically active adults (2 females) participated in the study (age, 25 ± 5 years; stature, 1.77 ± 0.07 m; body mass, 72 ± 10 kg). All participants were healthy, had no history of respiratory, cardiovascular, or metabolic disease, and had pulmonary function within normal limits (forced vital capacity, 103 ± 12% of predicted; forced expiratory volume in 1 s, 98 ± 10% of predicted; maximum voluntary ventilation, 113

± 18% predicted). The experimental procedures were approved by the University of Leeds Faculty of Biological Sciences Research Ethics Committee and conformed to the Declaration of Helsinki (approval REF: BIOSCI 17-016). Each participant provided written informed consent prior to commencement of any testing procedures.

7.2.2. Experimental Procedures

An overview of the experimental procedures is presented in Figure 7.1. Each participant visited the laboratory on four different occasions, each separated by at least 48 h. The participants abstained from food for 3 h, caffeine for 12 h, and alcohol and exercise for 24 h before each laboratory visit. At the first visit, resting pulmonary function was assessed according to standard procedures (Laveneziana et al., 2019b). Next, the participants performed exhaustive ramp incremental exercise followed immediately by a maximal 3 min effort against a fixed resistance (ramp incremental sprint test, RIST) (Murgatroyd et al., 2014) on an electromagnetically braked cycle ergometer (Excalibur, Lode, Groningen, The Netherlands) for the determination of maximal oxygen uptake ($\dot{V}O_{2max}$) and associated cardiometabolic parameters, peak ramp power (P_{peak}), and critical power (CP). The participants were then familiarised with the protocols used to assess respiratory neuromuscular function, described below. During the next three visits, the participants performed constant-power cycle exercise to the limit of tolerance at: 1) 5% <CP (*heavy intensity exercise;* <CP); 2) ~25% of the difference (Δ) between CP and P_{peak} (‘*long-duration’ severe intensity exercise*; Δ25); or 3) ~50% Δ (‘*short-duration’ severe intensity exercise*; Δ50) (Figure 7.1). The order of visits 2, 3, and 4 was randomised between the participants. Diaphragm and expiratory abdominal muscle contractility were assessed before and up to 30 min after each constant-power exercise test by measuring the transdiaphragmatic (Pdi_{tw}) and gastric (Pga_{tw}) twitch pressure response to magnetic stimulation of the phrenic nerve roots and thoracic nerve roots, respectively.

7.2.3. Ramp Incremental Sprint Test

Participants cycled at 20 W for 4-6 min before power was increased as a linear function of time at a rate of 25-30 $W \cdot min^{-1}$. The participants increased their pedal cadence progressively until they reached a sustainable self-determined rate (80-100 rpm). Each participant maintained this pedal cadence to within ± 5 rpm until the limit of tolerance, defined as the point at which pedal cadence fell below 60 rpm despite strong verbal encouragement. At the point of exercise intolerance, the cycle ergometer was switched instantaneously to cadence-dependent (linear) mode, in which power is the product of cadence and the 'linear factor' (flywheel resistance). The participants accelerated their pedal cadence quickly and performed 3 min of maximal effort cycling (Burnley et al., 2006, Vanhatalo et al., 2007). For each subject, the linear factor, calculated as power output/cadence^2, was pre-determined to elicit a cadence of ~80 rpm (i.e. the optimum of the parabolic relationship between power and cadence in the fatigued state) while subjects cycled at CP (Beelen and Sargeant, 1991, Elmer et al., 2013). From pilot work conducted as part of this thesis and previous investigations of the relationship between body mass and CP (van der Vaart et al., 2014), it was determined that a power output of 2.5 and 3 times body mass in untrained and trained females, and 3 and 3.5 times body mass in untrained and trained males respectively, would result in a cadence of ~80 rpm during the sprint phase. Sprint power (SP), which provides an estimate of CP (Murgatroyd et al., 2014), was calculated as mean power after a plateau in cadence had occurred between consecutive 30 s bins.

7.2.4. Pressure, Flow and Volume

Gastric (Pga) and oesophageal pressure (Poes) were measured using two balloon-tipped catheters (47-9005; Akrad Laboratories, Cooper Surgical, CT, USA) that were passed intranasally into the stomach and lower one-third of the oesophagus, respectively. The oesophageal balloon was filled with 1 ml of air and positioned

according to the occlusion technique (Baydur et al., 1982). The gastric balloon was filled with 2 ml of air and positioned so that Pga was positive throughout eupnoeic breathing with the participant in the seated position. Each catheter was connected to a differential pressure transducer (DP15; Validyne, Northridge, CA, USA) that was calibrated across the physiological range using a digital pressure manometer (no. 621, Test Products International Inc., Beaverton, OR, USA). Transdiaphragmatic pressure (Pdi) was obtained by subtracting Poes from Pga.

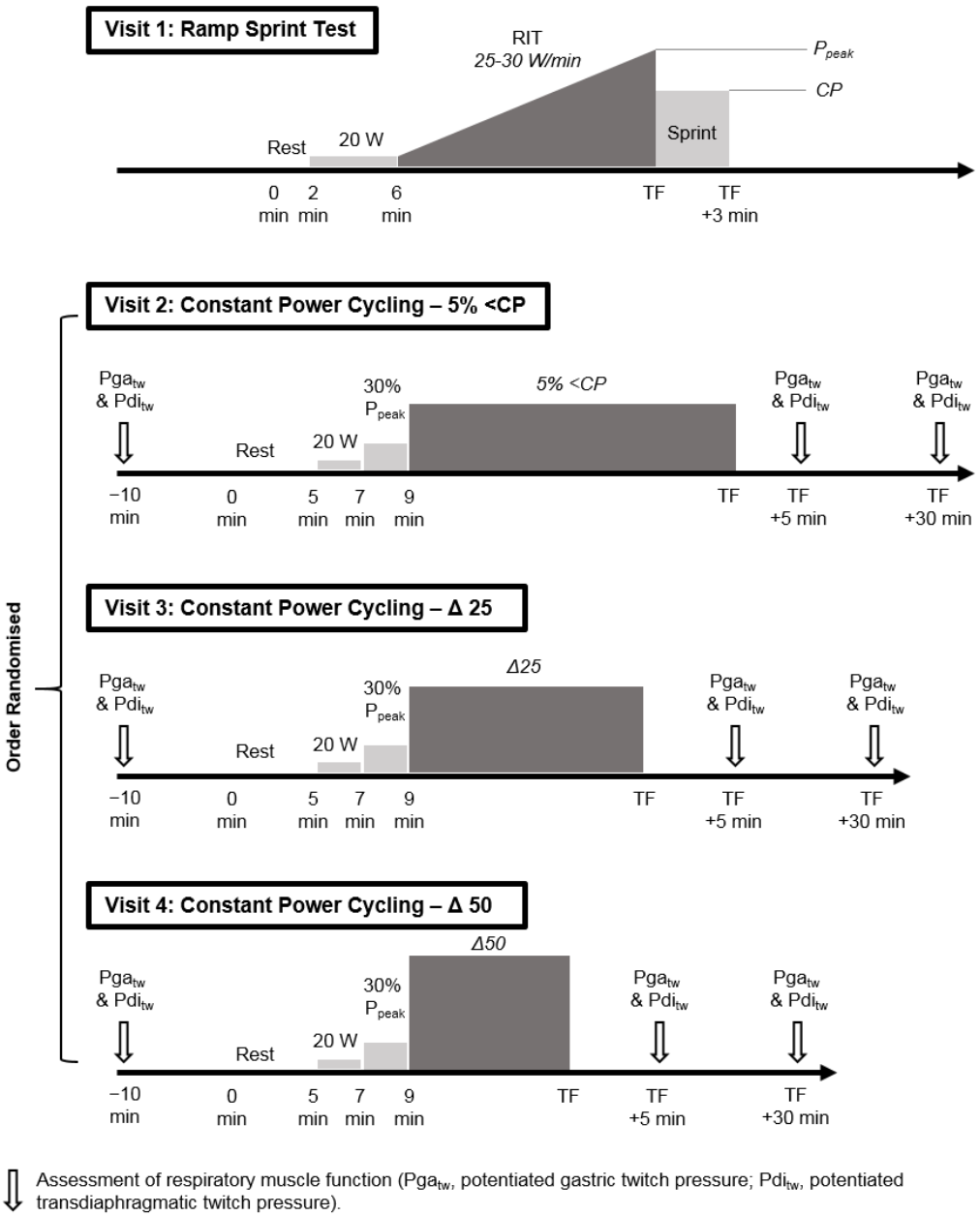


Figure 7.1. Experimental exercise protocols. RIT, ramp incremental test; TF, task failure; P_{peak}; peak power; CP, critical power; Pga_{tw}, gastric twitch pressure; Pdi_{tw}, transdiaphragmatic twitch pressure.

7.2.5. Electromyography

7.2.5.1. Respiratory muscles

Electromyograms (EMG) were recorded from the right hemi-diaphragm (EMG_{DI}) and rectus abdominis (RA; EMG_{RA}) using bipolar surface electrodes (Trigno Avanti, Delsys Inc.; Natick, MA, USA). For EMG_{DI}, the electrodes were positioned between the 6th and the 8th intercostal space along the anterior axillary line on the right-hand side of the thorax (Glerant et al., 2006). For EMG_{RA}, the electrodes were positioned over the muscle belly on the right-hand side of the abdomen, 2-4 cm lateral and ~2 cm superior to the umbilicus, and in the direction of the muscle fibres (Ng et al., 1998, Fuller et al., 1996). The final diaphragm and RA electrode positions were determined according to the optimal M-wave response to magnetic stimulation.

7.2.5.2. Quadriceps

EMG were also recorded from vastus lateralis (VL; EMG_{VL}) and vastus medialis (VM; EMG_{VM}) of the right leg. Bipolar surface electrodes (Trigno Avanti, Delsys Inc.; Natick, MA, USA) were placed over the muscle belly at ~2/3 of the linear distance between the anterior spina iliaca and lateral side of the patella for the VL, and ~4/5 of the linear distance between the anterior spina iliaca and the joint space prior to the anterior border of the medial ligament for the VM (Hermens et al., 1999). Correct placement of the VL and VM electrodes was verified via the EMG response to a maximal voluntary contraction of the quadriceps.

After verification of correct electrode positioning, all of the electrodes were secured in place using double-sided adhesive interfaces and hypoallergenic medical tape. The position of each electrode was marked with indelible ink to ensure that it was placed in the same location at subsequent visits.

7.2.6. Respiratory Muscle Function

7.2.6.1. Magnetic nerve stimulation

Magnetic stimuli (1-Hz) were delivered to the nerve roots supplying the respiratory muscles using a 90 mm circular coil powered by a magnetic stimulator (Magstim BiStim^2; The Magstim Company Ltd, Whitland, UK). For the diaphragm, the participants sat upright with the neck flexed, and the coil was positioned between the 3rd (C3) and 7th (C7) cervical vertebrae (Similowski et al., 1989). For the expiratory abdominal muscles, the participants sat facing an inclined bench (~30° past vertical) with their chest and abdomen supported, and the coil was positioned between the 8th (T8) and 11th (T11) thoracic vertebrae (T8-T11) (Kyroussis et al., 1996a). The areas of stimulation that evoked the greatest Pdi twitch pressure (Pdi_{tw}) and the greatest Pga twitch pressure (Pga_{tw}) were located and marked for use for all subsequent stimulations. All stimulations were delivered at 100% of the stimulator's power output and at a consistent relaxed end-expiratory lung volume (i.e. functional residual capacity), as judged by end-expiratory Poes.

7.2.6.2. Neuromuscular function

Inspiratory and expiratory abdominal muscle contractility were assessed before (~10 min) and after (5 min and 30 min) each of the constant-power exercise tests (Figure 7.1). The potentiated twitch is a more sensitive measure of muscle fatigue in comparison to the non-potentiated twitch, particularly when the degree of fatigue is small (Kufel et al., 2002b). Accordingly, Pdi_{tw} was measured ~5 s after a maximal Müeller manoeuvre that was initiated from residual volume and maintained for ~5 s. This procedure was repeated six times such that six measures of potentiated Pdi_{tw} were obtained. Similarly, Pga_{tw} was measured 5 s after a maximal expulsive manoeuvre. These expiratory manoeuvres were initiated from total lung capacity, maintained for ~5 s, and repeated six times such that six measures of potentiated Pga_{tw} were obtained. The mean of the highest 3 or 4 valid twitches was used for

analysis. Any twitch response that was initiated from an unstable end-expiratory Poes, Pdi, and/or Pga, or in the presence of subject 'bracing' (evidenced by EMG_{DI} and/or EMG_{RA} activity immediately prior to the stimulation) were excluded from subsequent analysis. Voluntary activation of the diaphragm and expiratory abdominal muscles was determined using the interpolated twitch technique (Strojnik and Komi, 1998). The order of diaphragm and expiratory abdominal muscle assessment was randomised and counterbalanced between participants but remained constant within each participant across all of the constant-power exercise trials.

The amplitude (baseline to peak) of the Pdi_{tw} and Pga_{tw} response were analysed for each stimulation. In addition, contraction time (CT), one-half relaxation time ($RT_{0.5}$) maximal rate of pressure development (MRPD), and maximal relaxation rate (MRR) was assessed for each stimulation of the diaphragm and the expiratory abdominal muscles. Membrane excitability was determined by measuring the magnetically evoked peak-to-peak amplitude (mV), duration (ms), and area (mV/ms) of the EMG_{DI} and EMG_{RA} M-waves.

7.2.7. Constant-power Exercise Tests

Following 5 min of quiet rest in the cycling position, the participants cycled for 2 min at 20 W and 2 min at 30% of P_{peak} before power output was increased to either: 1) 5% <CP; 2) Δ25; or 3) Δ50 (Figure 7.1). Each participant pedalled at a self-selected cadence (80-100 rpm) and maintained this cadence during all of the constant-power exercise tests. During each test, the participants exercised until volitional exhaustion, which was defined as the point at which pedal cadence fell below 60 rpm despite strong verbal encouragement. Inspiratory and expiratory airflow was measured breath-by-breath using a non-heated linear pneumotachometer (model 4813, Hans Rudolph, Kansas City, MO, USA). Additionally, ventilatory and pulmonary gas exchange indices were measured breath-by-breath using a calibrated bidirectional

Pitot tube sensor for volume measurement and galvanic (O_2) and non-dispersive infrared (CO_2) sensors for gas analysis (Ultima Cardio 2, MGC Diagnostics, St Paul, MN, USA). Heart rate (HR) was measured beat-by-beat via 12-lead electrocardiogram (X12, Montara Instrument; Milwaukee, WI, USA). Capillary blood was sampled from an earlobe at rest, every 2.5 min for the first 10 min of exercise, at 20 and 30 min (i.e. during <CP exercise), and within 15 s of exercise termination for the determination of blood lactate concentration (Lactate Pro 2, Arkray Factory Inc., Shiga, Japan). Ratings of perceived leg discomfort and dyspnoea were obtained at rest, at the end of the 'warm-up', every 2 min during exercise, and within 15 s of exercise termination using a modified 'Borg' CR10 scale (see *Appendix I*). Pga, Poes, and Pdi were measured throughout exercise and time-aligned to the airflow signal

7.2.8. Data Capture

The raw pressure signals (Poes and Pga) were passed through a carrier demodulator (Validyne model CD15, Northridge, CA, USA), and the EMG signals were band-pass filtered (10-850 Hz) (Trigno Avanti, Delsys Inc.; Natick, MA, USA). For EMG_{VL} and EMG_{VM} signals, a second order butterworth band-pass filter (20-500 Hz) was applied, and signals were RMS smoothed using a 25 ms time-window. The pressure, airflow, and EMG signals were digitised at sampling rates of 150 Hz and 2kHz (EMG only) (Micro 1401-3, Cambridge Electronic Design, Cambridge, UK), and captured and analysed using commercially available software (Spike 2 version 8.0, Cambridge Electronic Design, Cambridge, UK).

7.2.9. Data Analysis

Cardiorespiratory parameters including: oxygen uptake ($\dot{V}O_2$), minute ventilation ($\dot{V}_E$), respiratory frequency (f_R), tidal volume (V_T), respiratory exchange ratio (RER), and heart rate (HR) were averaged at rest, during each entire minute of exercise, and during the final 60 s of exercise (*end-exercise*) for all trials. In addition, $\dot{V}O_{2peak}$ was

determined during the ramp incremental phase of the RIST test and during all constant-power exercise tests as the highest 12 breath average from the final 25 breaths. Diaphragm and expiratory abdominal pressure-time products were calculated by integrating Pdi and Pga over the periods of inspiratory flow (PTP_{di}) and expiratory flow (PTP_{ga}), respectively. PTP_{di} and PTP_{ga} was determined per minute as the sum of every breath performed over each 60 s time bin, and cumulative PTP_{di} and PTP_{ga} were determined as the sum of all breaths performed during each exercise trial. To quantify the progression of limb motor unit recruitment throughout the constant-power exercise tests, the sum of the RMS amplitude of the EMG burst (EMG_{RMS}) recorded from VL and VM during each contraction was calculated.

7.2.10. Statistical Analysis

Changes in respiratory muscle contractility in response to the constant-power exercise trials was assessed using two-way repeated measures ANOVA, with the time and interaction effects (exercise trial × time) inspected. When a significant main effect of time was observed, one-way repeated measures ANOVA with Bonferroni correction was used to asses changes in respiratory muscle contractility across time (pre-exercise vs. 5 min post-exercise vs. 30 min post-exercise) within each of the constant-power exercise trials (<CP, ∆25, and ∆50). When a significant interaction effect was observed, one-way repeated measures ANOVA with a Holm-Sidak correction was used to compare the percentage change in respiratory muscle contractility from pre-exercise to 5 min post-exercise (i.e. magnitude of exercise-induced respiratory muscle fatigue) between the exercise trials (<CP vs. ∆25 vs. ∆50). To compare differences in exercise responses over time within each trial (cardiopulmonary, metabolic, ventilatory, and electromyography), one-way repeated-measures ANOVA with Bonferroni correction were performed across 1 minute bins and end-exercise values for Δ25 and Δ50, and 5 minute bins and end-exercise values for <CP. A one-way repeated-measures ANOVA with a Bonferroni correction was

used to assess differences in final minute exercise responses between trials (<CP vs. Δ25 vs. Δ50). A Friedman's ANOVA was used to assess differences in end-exercise perceptual responses between trials (<CP vs. Δ25 vs. Δ50). Pearson's product-moment correlation coefficients (*r*) were computed to assess the relationship between diaphragm and expiratory abdominal PTP (expressed as the percent increase from the first to final min) and the severity of exercise-induced respiratory muscle fatigue across all of the constant-power exercise trials. The acceptable type I error was set at $P < 0.05$. Data are expressed as group means ± SD. Statistical analyses were performed using SPSS version 26 for Windows (SPSS Inc., Chicago, IL).

7.3. RESULTS

7.3.1. Physiological Responses to the Ramp Incremental Sprint Test

During the ramp incremental section of the RIST, peak $\dot{V}O_2$, $\dot{V}_E$, HR, respiratory exchange ratio (RER), and P_{peak} were 4.15 ± 0.92 L·min^{-1} (57.1 ± 8.8 ml·kg^{-1}·min^{-1}), 150 ± 34 L·min^{-1}, 183 ± 11 beats·min^{-1}, 1.19 ± 0.10 and 321 ± 57 W, respectively. During sprint phase of the RIST, pedal cadence was maintained at 76 ± 8 rpm and CP was estimated as 202 ± 55 W.

7.3.2. Physiological Responses to the Constant-power Exercise Trials

7.3.2.1. Cardiopulmonary, metabolic, and perceptual responses

The participants cycled at 191 ± 52 W, 234 ± 53 W, and 263 ± 53 W during <CP, Δ25, and Δ50, respectively. The tolerable duration of constant-power exercise decreased in accordance with increasing exercise task power (<CP: 34.6 ± 6.2 min vs. Δ25: 10.2 ± 2.6 min vs. Δ50: 4.9 ± 0.7 min; all $P < 0.001$). Selected cardiopulmonary, metabolic, ventilatory, and perceptual responses to the constant-power exercise trials are shown in Figure 7.2. $\dot{V}O_2$ and blood lactate concentration increased progressively throughout exercise until the point of intolerance during each constant-power test. In

<CP, there was clear evidence of a $\dot{V}O_2$ slow component (i.e. a slowly developing increase in $\dot{V}O_2$), and peak $\dot{V}O_2$ at end-exercise was submaximal (3.59 ± 0.80 $L \cdot min^{-1}$, 87 ± 5% of $\dot{V}O_{2peak}$). By comparison, $\dot{V}O_2$ increased more rapidly during Δ25 and Δ50, and $\dot{V}O_{2peak}$ was attained in both of these trials (4.04 ± 0.88 $L \cdot min^{-1}$, 99 ± 5% of $\dot{V}O_{2peak}$; and 4.07 ± 0.82 $L \cdot min^{-1}$, 98 ± 4% of $\dot{V}O_{2peak}$). Similarly, blood lactate concentration was greater at end-exercise in Δ25 and Δ50 vs. <CP (Figure 7.2). Importantly, the aforementioned exercise responses are consistent with exercise in the severe-intensity domain (Δ25 and Δ50) and with exercise in the heavy-intensity domain (<CP) (Hill et al., 2002, Jones et al., 2010). End-exercise $\dot{V}_E$ was greater in Δ50 and Δ25 vs. <CP (both $P < 0.001$) and, but not different in Δ50 vs. Δ25 ($P = 0.096$). The greater end-exercise $\dot{V}_E$ in Δ50 was due primarily to a larger V_T, with the participants adopting a more tachypnoeic breathing pattern as tolerable exercise duration increased (Figure 7.2). Group mean HR, dyspnoea, and leg discomfort were not different at end-exercise between the three constant-power exercise trials (Figure 7.2).

7.3.2.2. Respiratory Muscle Pressure Production

There was a progressive increase in PTP_{di} and PTP_{ga} during all exercise trials (Figure 7.3). End-exercise PTP_{di} was greater during Δ50 and Δ25 vs. <CP (Figure 7.3, *panel A*) and end-exercise PTP_{ga} was greater during Δ50, and to a lesser extent Δ25, vs. <CP (Figure 7.3, *panel B*). Cumulative PTP_{di} and PTP_{ga} increased with tolerable exercise duration, and both were higher during <CP vs. Δ25 and Δ50, and during Δ25 vs. Δ50 (Figure 7.4). There was no association between the change from minute 1 to end-exercise in PTP_{di} or PTP_{ga} across all trials and the magnitude of diaphragmatic and expiratory muscle fatigue, respectively ($r = 0.25$ and $r = 0.10$, $P > 0.05$). Similarly, cumulative PTP_{di} and PTP_{ga} during severe-intensity exercise trials (Δ25 and Δ50) were not associated to the magnitude of diaphragmatic and expiratory muscle fatigue ($r = -0.03$ and $r = -0.31$, $P > 0.05$).

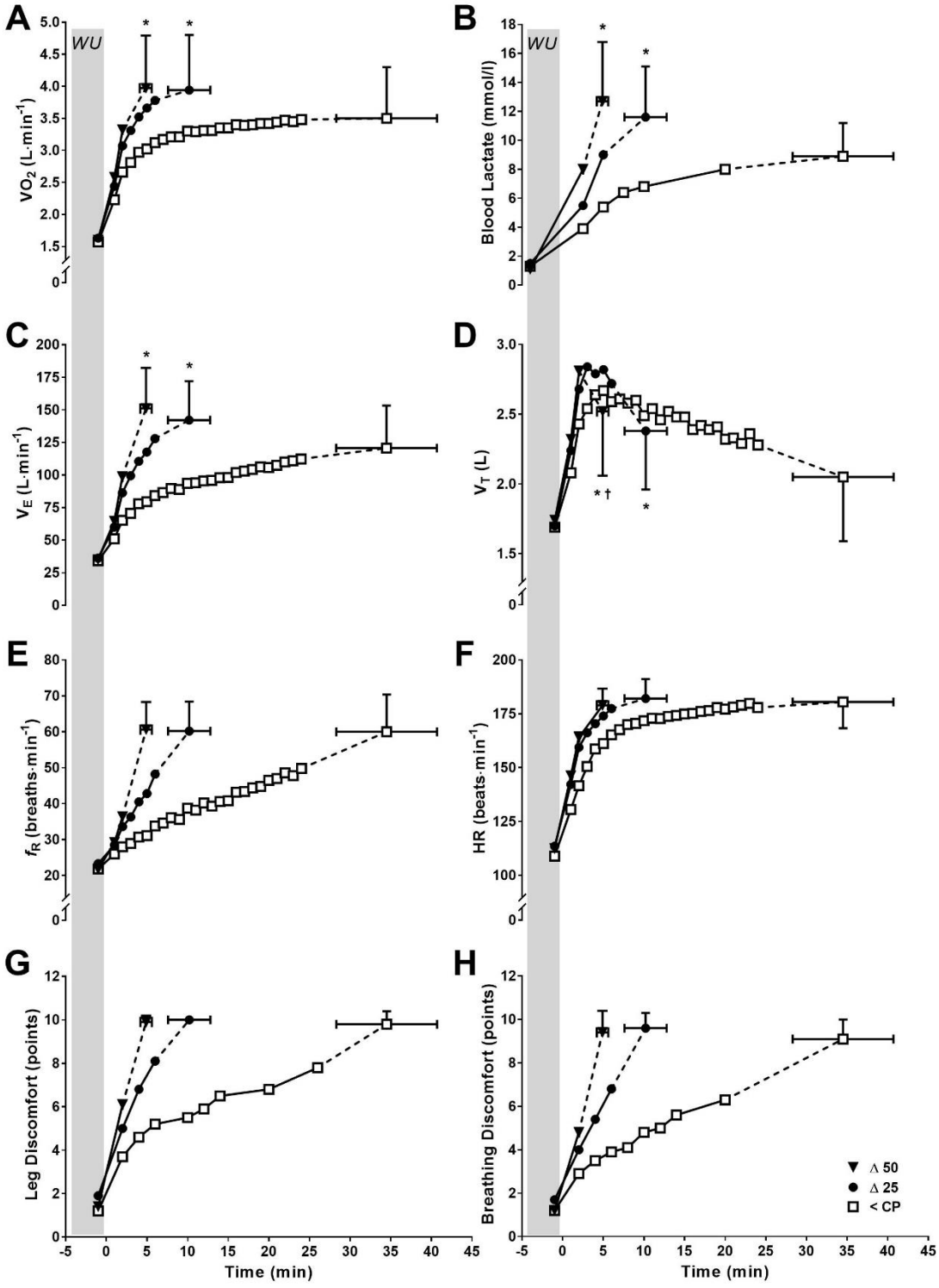


Figure 7.2. Cardiopulmonary responses to constant-power exercise trials. A, oxygen uptake ($\dot{V}O_2$); B, blood lactate (n = 8); C, minute ventilation ($\dot{V}_E$); D, tidal volume (V_T); E, respiratory frequency (f_R); F, heart rate (HR); G, leg discomfort; H, breathing discomfort. Symbols represent a statistically significantly difference (P < 0.05) in end-exercise values compared with the following: *different from <CP; †different from Δ25.

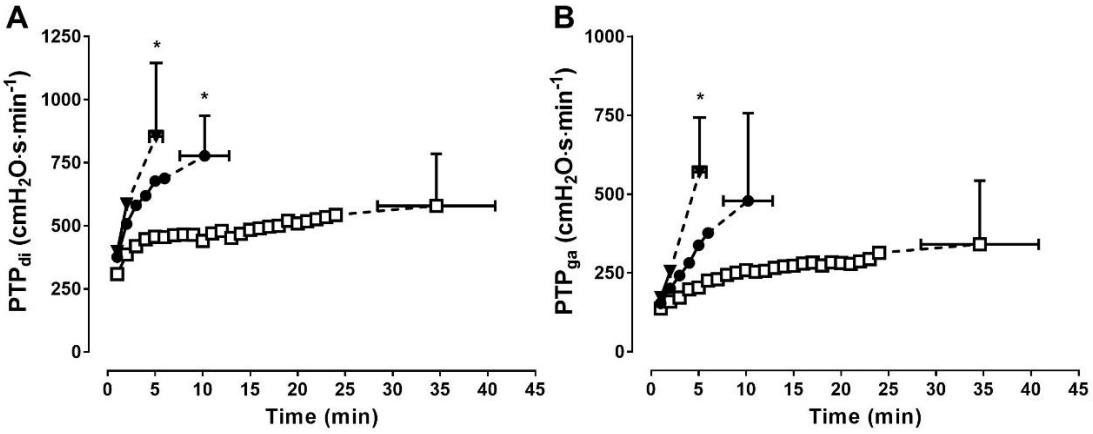


Figure 7.3. Group mean pressure-time product (PTP) per minute during constant-power exercise trials. A, transdiaphragmatic PTP (PTP_{di}); B, gastric PTP (PTP_{ga}); *Statistical difference in end-exercise value compared to <CP (*P* < 0.05).

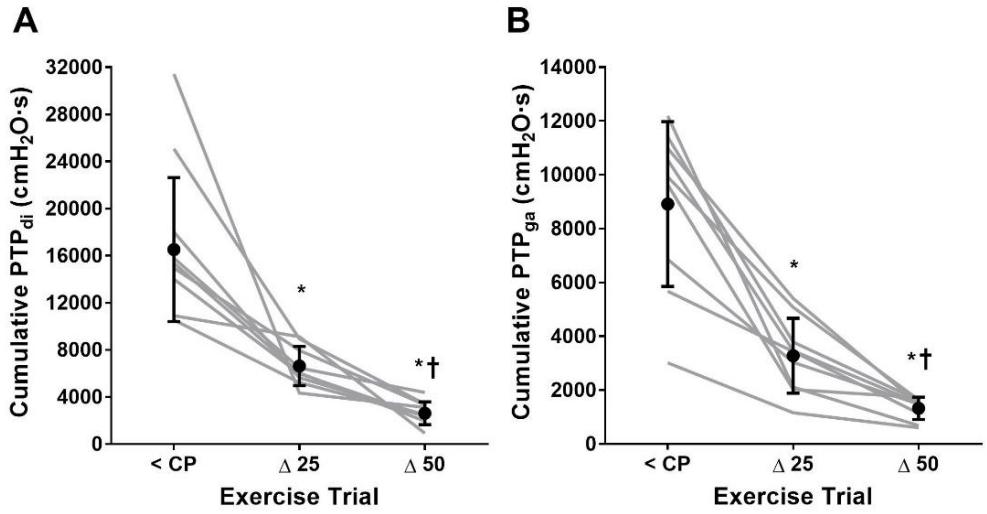


Figure 7.4. Total cumulative pressure time product (PTP) during constant-power exercise trials. A, transdiaphragmatic PTP (PTP_{di}); B, gastric PTP (PTP_{ga}). Symbols represent a significant difference: **P* < 0.05 vs. <CP; †*P* < 0.05 vs. Δ25.

7.3.2.3. Quadriceps EMG

EMG_{RMS} activity of the quadriceps (VL and VM summed) increased over time from minute 1 to end-exercise during Δ50 and Δ25 (27 ± 24% and 22 ± 22%, respectively, *P* < 0.05) and to a lesser extent during heavy-intensity exercise (9 ± 19%, *P* = 0.072). This percent increase in EMG_{RMS} was of a greater magnitude for Δ50 and Δ25 vs. <CP (both *P* < 0.05), but was not different between Δ50 and Δ25 (*P* > 0.05).

7.3.3. Exercise-induced Respiratory Muscle Fatigue

The mechanical and electrical responses to magnetic stimulation of the cervical and thoracic nerve roots before and after <CP, ∆25, and ∆50 in a representative participant are shown in Figure 7.5.

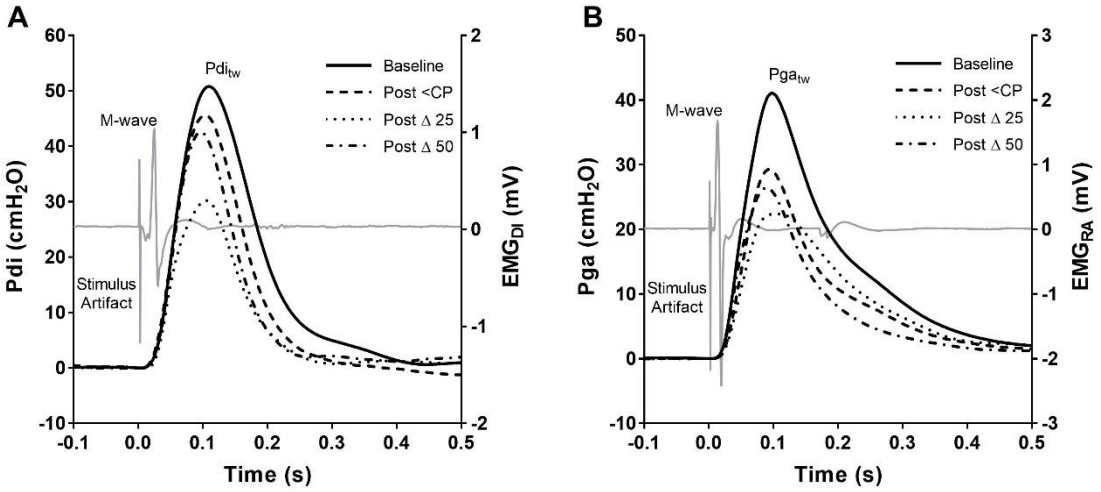


Figure 7.5. Individual representative ensemble average traces of diaphragm (A) (Pdi_{tw}) and gastric twitch pressure (B) (Pga_{tw}) at baseline (mean value across all time points), and in response to each exercise trial. Diaphragm (EMG_{DI}) and rectus abdominis (EMG_{RA}) electromyography signals are also shown.

7.3.3.1. Inspiratory Muscle Fatigue

Immediately after exercise in <CP, ∆25, and ∆50, group mean Pdi_{tw} was reduced below pre-exercise baseline values (Figure 7.6). At 30 min post-exercise, Pdi_{tw} had recovered fully in <CP, but remained reduced relative to pre-exercise values in ∆25 and ∆50, although this is statistically significant for ∆25 only (Figure 7.6). The magnitude of the exercise-induced percent decrease in Pdi_{tw} was greater in ∆25 (−22 ± 12%) compared to <CP (−13 ± 8%; P = 0.050) and Δ50 (−14 ± 12%; P = 0.045) (Figure 7.7). The pre- to post-exercise response of secondary diaphragm twitch characteristics (CT, MRPD/Pdi_{tw}, $RT_{0.5}$, MRR/Pdi_{tw}) and end-expiratory Poes and Pdi are reported in Table 7.1; M-wave responses are reported in Table 7.2. There was a pre- to post-exercise decrease in MIP in response to <CP and ∆25, but not ∆50 (Table

7.1). Voluntary activation of the diaphragm was unchanged from baseline values in response to all trials (Table 7.1).

7.3.3.2. Expiratory Muscle Fatigue

There was also a pre- to post-exercise reduction in group mean Pga_{tw} for <CP, ∆25, and ∆50 (Figure 7.6). Despite some recovery, Pga_{tw} remained below baseline values at 30 min after exercise in all three constant-power exercise trials (Figure 7.6). Unlike for the magnitude of exercise-induced diaphragm fatigue, the pre- to post-exercise percent reduction in Pga_{tw} was not different between <CP vs. ∆25 vs. ∆50 (−23 ± 15% vs. −29 ± 15% vs. −25 ± 16%, $P > 0.05$) (Figure 7.7). The pre- to post-exercise response of secondary expiratory twitch characteristics (CT, $MRPD/Pga_{tw}$, $RT_{0.5}$, MRR/Pga_{tw}) and end-expiratory Poes and Pga are reported in Table 7.1; M-wave responses are reported in Table 7.2. There was a pre- to post-exercise decrease in MEP in response to <CP, ∆25, and ∆50 (Table 7.2). Voluntary activation of the expiratory muscles decreased from pre- to post-exercise in response to ∆25, but not <CP or ∆50 (Table 7.2).

7.3.3.2. Exercise-induced Inspiratory vs. Expiratory Muscle Fatigue

The magnitude of exercise-induced expiratory muscle fatigue tended to be greater than the magnitude of inspiratory muscle fatigue in response to <CP (−23 ± 15% vs. −13 ± 8%, $P = 0.051$), and to a lesser extent in response to ∆50 (−25 ± 16% vs. −14 ± 12%, $P = 0.057$), but there was no difference in response to ∆25 (−29 ± 15% vs. −22 ± 12%, $P = 0.106$) (Figure 7.7).

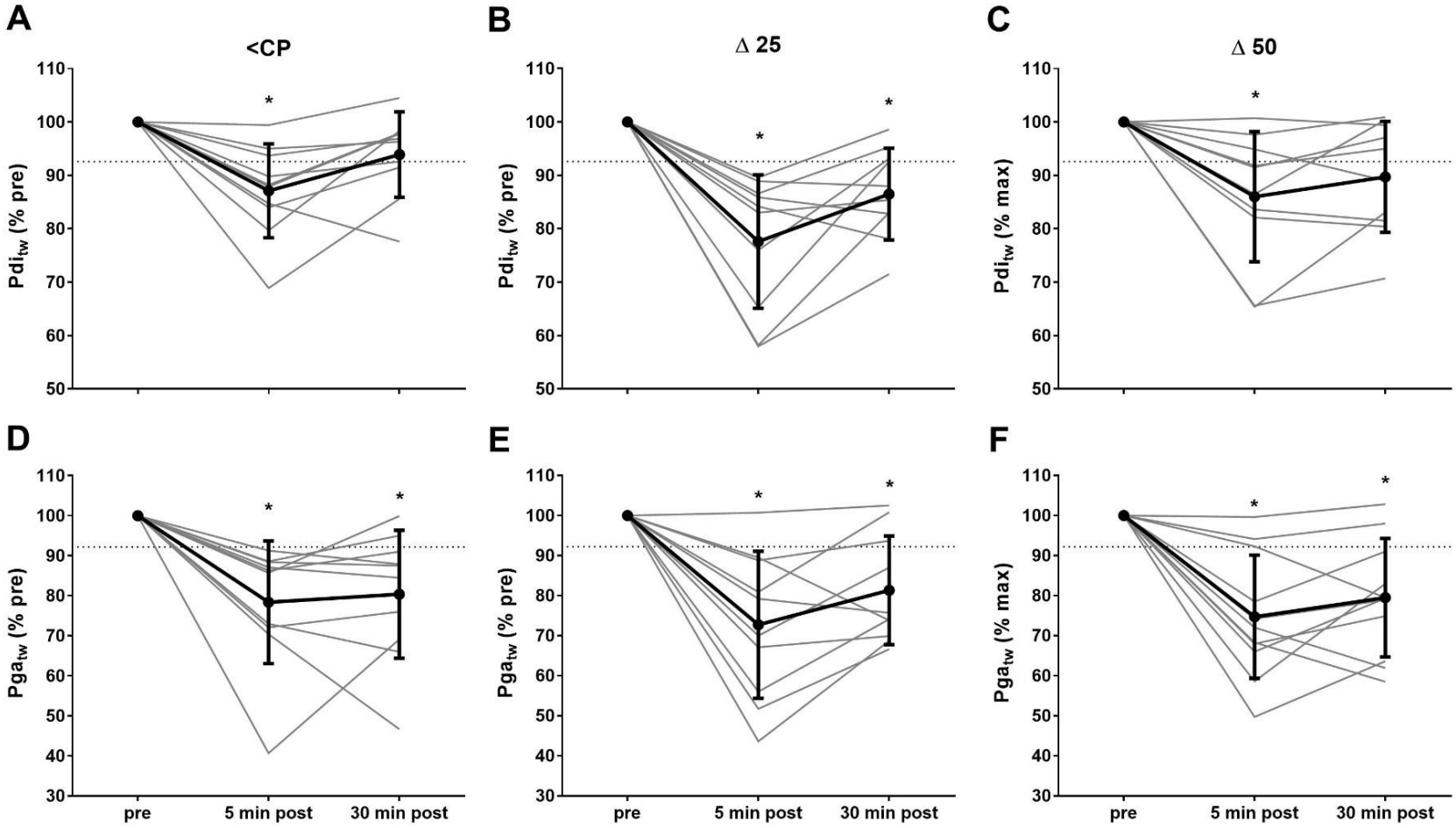


Figure 7.6. Exercise-induced respiratory muscle fatigue. A-C, transdiaphragmatic twitch pressure (Pdi_{tw}) and D-F, gastric twitch pressure (Pga_{tw}) before, ~5 and ~30 min after exercise at <CP (A), Δ25 (B) and Δ50 (C). *Significantly different to pre-exercise ($P < 0.05$); †significantly different to ~5 min post-exercise.

Table 7.1. Mechanical twitch characteristics and volitional muscle function before and (~5 min) after exercise.

	<CP		Δ 25		Δ 50	
Twitch Characteristic	Pre-exercise	Post-exercise	Pre-exercise	Post-exercise	Pre-exercise	Post-exercise
Pdi_{tw}, cmH_2O	39.4 ± 9.7	34.3 ± 9.2†	44.3 ± 12.6	34.3 ± 12.0†	41.3 ± 10.1	35.1 ± 8.4*
CT, ms	117 ± 5	106 ± 7†	115 ± 6	105 ± 6†	114 ± 8	106 ± 7†
MRPD/Pdi_{tw}, s/cm	17.6 ± 1.4	19.4 ± 1.3*	18.0 ± 1.3	20.0 ± 1.4†	18.2 ± 2.0	19.9 ± 2.5†
$RT_{0.5}$, ms	68 ± 10	54 ± 8†	67 ± 6	56 ± 9†	66 ± 11	56 ± 8*
MRR/Pdi_{tw}, s/cm	9.9 ± 1.6	12.8 ± 2.8*	10.2 ± 1.0	12.2 ± 2.2*	10.1 ± 2.1	12.0 ± 2.0*
End-expiratory Poes, cmH_2O	−4.1 ± 1.1	−5.3 ± 1.0	−4.4 ± 1.5	−5.3 ± 2.0	−4.7 ± 0.7	−5.3 ± 1.0
End-expiratory Pdi, cmH_2O	18.4 ± 4.2	19.2 ± 7.4	18.5 ± 5.0	15.8 ± 5.1*	18.5 ± 3.8	16.5 ± 5.2
Voluntary Activation, %	91 ± 4	88 ± 5	90 ± 7	86 ± 7	89 ± 6	87 ± 7
MIP, cmH_2O	141 ± 28	127 ± 24*	142 ± 29	130 ± 25*	145 ± 37	139 ± 37
Pga_{tw}, cmH_2O	54.1 ± 35.3	43.9 ± 33.0†	51.6 ± 36.8	39.3 ± 33.5†	53.4 ± 38.0	42.9 ± 36.8†
CT, ms	112 ± 19	109 ± 20	112 ± 17	108 ± 15	117 ± 20	109 ± 14
MRPD/Pga_{tw}, s/cm	17.1 ± 4.0	17.7 ± 3.3	16.9 ± 3.1	16.6 ± 2.3	15.8 ± 3.2	17.3 ± 3.0
$RT_{0.5}$, ms	127 ± 34	101 ± 26†	111 ± 25	85 ± 21†	120 ± 25	108 ± 39
MRR/Pga_{tw}, s/cm	5.2 ± 1.9	6.8 ± 2.4†	5.8 ± 1.1	7.2 ± 1.9†	5.5 ± 1.0	6.6 ± 1.3*
End-expiratory Poes, cmH_2O	−4.6 ± 2.5	−6.0 ± 2.4	−4.7 ± 1.5	−6.4 ± 2.5*	−5.0 ± 1.5	−7.1 ± 2.2*
End-expiratory Pga, cmH_2O	15.5 ± 3.4	12.9 ± 9.9	16.6 ± 6.6	11.5 ± 6.2†	16.2 ± 5.6	11.4 ± 4.8
Voluntary Activation, %	65 ± 15	61 ± 24	64 ± 13	56 ± 7*	60 ± 18	53 ± 15
MEP, cmH_2O	166 ± 36	148 ± 31†	170 ± 38	147 ± 28†	167 ± 40	150 ± 40*

Values are means ± SD. Pdi_{tw}, transdiaphagmatic twitch pressure; CT, contraction time; MRPD, maximal rate of pressure development; $RT_{0.5}$, one-half relaxation time; MRR, maximal relaxation rate; Poes, oesophageal pressure; Pdi_{MAX}, maximal transdiaphramatic pressure (Müeller manoeuvre) MIP, maximal inspiratory pressure; Pga_{tw}, gastric twitch pressure; Pga_{MAX}, maximal gastric pressure (Valsalva manoeuvre); MEP, maximal expiratory pressure. *P < 0.05 and †P < 0.01, significantly different to pre-exercise value. Note, statistical analyses include 30 min recovery comparisons (data not shown). For gastric voluntary activation, n = 7 due to 3 subjects exceeding the maximum range of the pressure transducer.

Table 7.2. Electrical twitch characteristics before and (~5 min) after exercise.

	<CP		Δ 25		Δ 50	
Twitch Characteristic	Pre-exercise	Post-exercise	Pre-exercise	Post-exercise	Pre-exercise	Post-exercise
DIA M-Wave Amplitude, mV	1.7 ± 1.0	1.8 ± 1.2	2.2 ± 1.2	2.2 ± 1.2	1.8 ± 1.0	2.2 ± 1.2
DIA M-Wave Duration, ms	30.3 ± 4.7	30.6 ± 4.2	30.6 ± 4.5	31.5 ± 5.0	30.9 ± 6.8	29.4 ± 6.5
DIA M-Wave Area, $mV \cdot ms^{-1}$	10.1 ± 5.7	11.0 ± 6.4	11.3 ± 5.8	12.2 ± 5.8	10.0 ± 6.1	12.4 ± 7.0
RA M-Wave Amplitude, mV	2.6 ± 1.6	2.6 ± 1.5	3.0 ± 2.1	3.0 ± 2.2	2.9 ± 2.0	2.8 ± 1.9
RA M-Wave Duration, ms	23.3 ± 5.3	23.2 ± 6.1	22.8 ± 5.3	22.2 ± 5.5	22.9 ± 6.8	22.3 ± 6.5
RA M-Wave Area, $mV \cdot ms^{-1}$	13.3 ± 7.7	13.2 ± 7.1	15.0 ± 10.3	13.9 ± 9.5	13.8 ± 9.0	13.9 ± 9.4

Values are means ± SD. DIA, diaphragm EMG (n = 8); RA, rectus abdominis EMG (n = 10). *P < 0.05 and †P < 0.01, significantly different to pre-exercise value. Note, statistical analyses include 30 min recovery comparisons (data not shown).

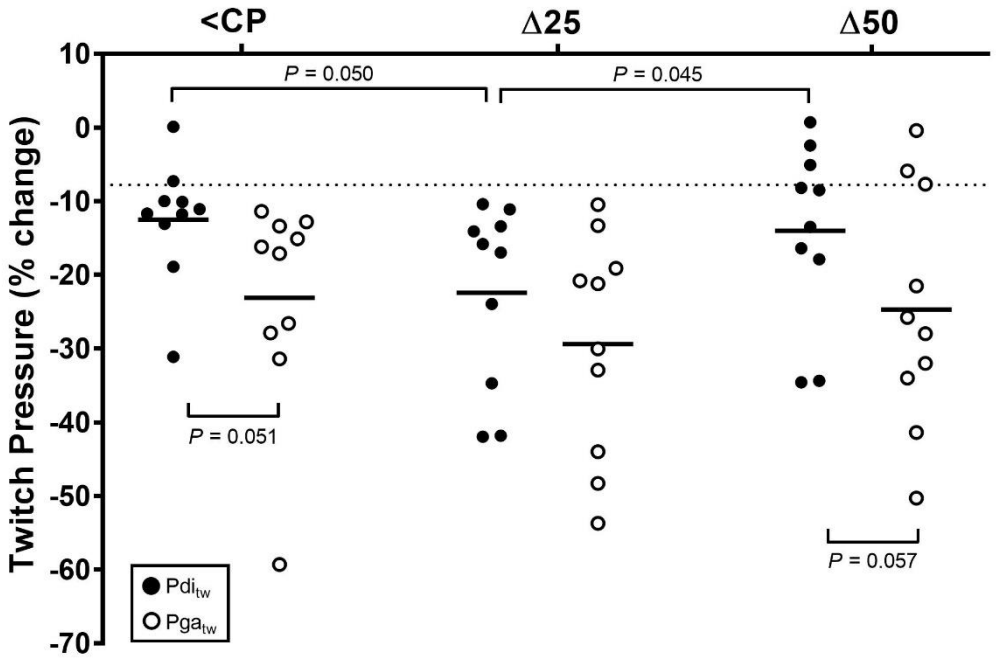


Figure 7.7. A comparison of the magnitude of exercise-induced inspiratory (Pdi_{tw}, black symbols) and expiratory muscle fatigue (Pga_{tw}, open symbols) across trials (<CP vs. Δ25 vs. Δ50) and between muscle groups (inspiratory vs. expiratory muscle).

7.4. DISCUSSION

This study investigated the effect of exercise intensity and tolerable exercise duration on the development of inspiratory and expiratory abdominal muscle fatigue. The major findings were that 1) inspiratory and expiratory muscle fatigue occurred in response to exhaustive heavy-intensity exercise (−13 ± 8% and −23 ± 15%; T_{LIM} ~35 min) and short- (−14 ± 12% and −25 ± 16%; T_{LIM} ~5 min) and long-duration (−22 ± 12% and −29 ± 15%; T_{LIM} ~10 min) severe-intensity exercise; 2) the magnitude of exercise-induced inspiratory muscle fatigue was less when exercise was performed at the same intensity but for a shorter tolerable duration, or at a lower intensity but for a considerably longer duration in comparison to prolonged severe-intensity exercise; 3) there was no effect of exercise intensity and tolerable duration on the magnitude of exercise-induced expiratory muscle fatigue; and 4) the magnitude of expiratory muscle fatigue tended to be greater than inspiratory muscle fatigue in response to both heavy-intensity and short-duration severe-intensity exercise. These

data support the theory that the magnitude of exercise-induced inspiratory muscle fatigue is a function of diaphragmatic work history (cumulative work and power of breathing) and the degree of competition for blood flow between the diaphragm and the locomotor muscles, which is dependent on exercise intensity. In contrast, the absence of an effect of exercise intensity or tolerable duration on the magnitude of exercise-induced expiratory muscle fatigue suggests that the fatigue threshold may be lower for the expiratory muscles, which may relate to the less fatigue-resistant phenotype and/or additional non-ventilatory roles of the expiratory muscles during exercise.

7.4.1. Normalisation of Exercise Intensity

Previous studies investigating the development of exercise-induced respiratory muscle fatigue have prescribed exercise intensity as a percentage of $\dot{V}O_{2max}$ or P_{peak}. Such normalisation procedures may fail to induce uniform physiological responses among participants (Katch et al., 1978), as there may be drastic between-subject differences in the relative point at which physiological thresholds occur as a percentage of peak values (Poole et al., 1988). Indeed, lactate threshold and CP can vary between individuals from 40-85% and 50-95% of $\dot{V}O_{2max}$, respectively (Murgatroyd et al., 2014, Iannetta et al., 2020). Therefore accurate prescription of exercise intensity by use of a % of $\dot{V}O_{2peak}$ or P_{peak} may be problematic, particularly when power outputs are set close to such physiological thresholds, because subjects may be exercising in different intensity domains in the same study. For example, prescribing exercise at 75-85% of $\dot{V}O_{2max}$ has a significant chance of heavy-to-severe intensity 'domain overlap' (Iannetta et al., 2020), the physiological responses to which differ markedly. It currently remains unclear whether such variability in exercise responses contributes to the development (or absence) of respiratory muscle fatigue in previous studies that have prescribed exercise as a percentage of $\dot{V}O_{2peak}$ or P_{peak}. Indeed, in response to high-intensity exercise the prevalence of diaphragmatic

fatigue may be as low as 42-64% (Mador and Dahuja, 1996, Guenette et al., 2010), but no clear relationships exist between the presence of fatigue and subject characteristics or ventilatory responses. In the present study exercise intensity was prescribed by direct estimation of CP, the critical threshold for determination of heavy- vs. severe-intensity exercise. The cardiopulmonary and metabolic responses (Figure 7.1) were consistent with exercise in the heavy- (5% <CP) and severe-intensity domains (Δ25 and Δ50). In addition, estimates of CP were validated via modelling of the power-time relationship following repeated constant-power tests in a subset of subjects (Jones et al., 2010) (*see* Chapter 4.2).

7.4.2. Factors Affecting the Development of Respiratory Muscle Fatigue

It is well established that exhaustive high-intensity whole body exercise elicits significant fatigue of the diaphragm and the expiratory abdominal muscles (Taylor et al., 2006, Johnson et al., 1993, Guenette et al., 2010, Taylor and Romer, 2008). For example, Guenette et al. (2010) reported a ~13% (women) to ~20% (men) reduction in Pdi_{tw} from before to after high-intensity cycling tolerated for ~11 to 14 min. Similarly, Taylor and Romer (2008) reported a ~27% reduction in Pga_{tw} pre- to post-exercise at 90% of P_{peak} that was tolerated for ~10 min. In the present study, there was a highly comparable reduction in Pdi_{tw} (22 ± 12%) in response to long-duration severe-intensity exercise (Δ25; T_{LIM} ~10 min). However, there were differences in the magnitude of exercise-induced respiratory fatigue for the diaphragm, but not the expiratory abdominal muscles. *So the question becomes, why does exercise intensity and tolerable duration play a role in the magnitude of exercise-induced diaphragmatic, but not expiratory abdominal muscle fatigue?*

7.4.2.1. Exercise Intensity and Inspiratory Muscle Fatigue

To the authors knowledge, this is the first study to directly and systematically assess the effect of exercise intensity domain on the development and magnitude of

inspiratory muscle fatigue. The finding that the magnitude of inspiratory muscle fatigue was blunted in response to heavy- vs. prolonged severe-intensity constant-power cycling (−14% vs. −22%, Figure 7.7) supports the assertion that exercise intensity influences the magnitude of inspiratory muscle fatigue. For example, Johnson et al. (1993) reported that during a combination of cycling and treadmill running, $\dot{V}O_2$ was negatively associated with the diaphragmatic fatigue index (r = −0.67), and that the likelihood of developing fatigue increased at 'exercise intensities' exceeding 85% of $\dot{V}O_{2peak}$. Similarly, it has been reported that the pre- to post-exercise reduction in Pdi_{tw} is exacerbated during higher intensity time-trial exercise (15 min TT: −24 ± 8% vs. 30 min TT: −20 ± 9%) (Wuthrich et al., 2014a).

Why is inspiratory muscle fatigue blunted in response to lower intensity exercise? It has been suggested that the normally-occurring power of breathing during 15 minutes of heavy-intensity cycling (~75% of $\dot{V}O_{2peak}$) is not of sufficient degree to engender a competition for cardiac output between the locomotor and respiratory muscles, and as such to trigger the initiation of a respiratory muscle metaboreflex (Wetter et al., 1999). In response to the heavy-intensity (<CP) trial of the present study V_E, $\dot{V}O_{2peak}$ and PTP_{di} were significantly lower than for severe-intensity exercise (Δ25 and Δ50) (Figure 7.2 and 7.3), likely contributing to a lower diaphragmatic power. This reduction in the diaphragm force output (i.e. PTP) and the degree of the competition for cardiac output between the locomotor and respiratory muscles may have combined to 'preserve' diaphragmatic blood flow resulting in a blunted development of inspiratory muscle fatigue. However, despite the submaximal ventilatory response, inspiratory muscle fatigue was still *present* in 70% of subjects performing heavy-intensity exercise and in subjects with an average $\dot{V}O_2$ across the entire trial of as low as 75% of $\dot{V}O_{2peak}$. Therefore, the present data suggest that the O_2 uptake threshold proposed for the development of diaphragm fatigue may be lower than previously reported (i.e. 85% of $\dot{V}O_{2peak}$) (Johnson et al., 1993). Similarly,

although the validity of volitional generation of maximal mouth pressures can be affected by the influence of subject motivation, previous data demonstrate a group mean decrease in MIP and mouth twitch pressure of 16-19% in response to long-distance endurance running events, that elicit an average $\dot{V}O_2$ that is likely to be substantially lower than 85% of $\dot{V}O_{2peak}$ (Wuthrich et al., 2014b, Loke et al., 1982).

Could a competition for blood flow between the respiratory and locomotor muscles occur by the termination of heavy-intensity exercise?

It has been previously suggested that the ventilatory demands of heavy-intensity exercise are insufficient to elicit a competition for blood flow between the respiratory and locomotor muscles (Wetter et al., 1999). Therefore, the development of inspiratory muscle fatigue observed during heavy-intensity exercise in the present study may not be due to localised diaphragm ischemia secondary to a limitation in blood flow and O_2 delivery. However, in the study by (Wetter et al., 1999), exercise was performed for only 15 minutes, which equates to less than 50% of the T_{LIM} of the present heavy-intensity trial. It is possible that when heavy-intensity exercise is performed for a prolonged duration or to *exhaustion,* there is a progressive increase in ventilatory demand (*and the associated work and O_2 cost of breathing*) and locomotor muscle recruitment, both of which may conspire to elicit a competition for blood flow during the latter stages of such exercise (i.e. after the initial 15 min). Indeed, between the 15th and final minute of exercise of the <CP trial in the present study, there was an increase in V_E, PTP_{di} and EMG_{RMS} of the quadriceps muscles ($\dot{V}_E$: 21 L·min^{-1}; PTP_{di}: 95 cmH_2O; EMG_{RMS}: 9%) (Figure 7.2 and Figure 7.3). In addition, the thermoregulatory requirements of heavy-intensity exercise (<CP) may lead to significant cardiac drift; a progressive rise in heart rate secondary to a sweat-induced loss in blood volume. In the present study, heart rate progressively increased during the <CP trial attaining near-maximal values at exercise intolerance (Figure 7.2). In combination with the increased ventilatory and locomotor requirements during

the latter portion of the <CP trial, such cardiac drift suggests that the may be less of a ‘reserve’ in cardiac output, which could favour a competition for blood flow between the locomotor and respiratory muscles.

Why does inspiratory muscle fatigue occur in response to heavy-intensity exercise if cardiac output is not ‘limiting’?

Alternatively, rather than a drastic metabolic perturbation in the diaphragm (e.g. accumulation of disruptive metabolites and depletion of phosphocreatine), it is possible that the development of inspiratory muscle fatigue in response to heavy-intensity exercise was caused by a combination of centrally- and peripherally-mediated neuromuscular mechanisms. Indeed, voluntary activation of the quadriceps muscle group declines progressively with increasing tolerable exercise duration, and is of a greater magnitude in response to heavy- vs. severe-intensity cycling (−9% vs. −3%) (Thomas et al., 2016). Similarly, corticospinal excitability is attenuated in response to heavy-intensity, but not severe-intensity, cycling (Ansdell et al., 2020). In contrast, the present data suggests that there was no substantial central component of inspiratory muscle fatigue as diaphragm voluntary activation was unchanged in response to all exercise trials (Table 7.1) which is in agreement with the lack of change in voluntary activation recorded from cervically-stimulated mouth twitch pressure following ultra-marathon events (Wuthrich et al., 2014b). The development of neuromuscular fatigue in the heavy domain may instead have been caused by an impairment to excitation-contraction coupling secondary to the depletion of intramuscular glycogen stores (Ortenblad et al., 2013), and/or the accumulation of reactive oxygen species (ROS) and extracellular K^+ (Place et al., 2010). However, previous reports suggest that during maximal exercise in normoxic conditions diaphragm muscle glycogen levels are remarkably well preserved, compared to the substantial reduction in muscle glycogen content observed in the locomotor muscles (Fregosi and Dempsey, 1986). The similar diaphragm M-wave

characteristics pre- to post-exercise also indicates that action potential transmission to the T-tubule system was likely unaffected by exercise trials, and that extracellular K^+ accumulation may not have been 'limiting' (Table 7.1). In contrast, the deleterious effects of ROS accumulation on Ca^{2+} sensitivity and excitation-contraction coupling appear to be more potent as exercise duration increases (Place et al., 2010). Moreover, during heavy-intensity exercise lasting for 30 minutes, antioxidant supplementation with N-acetylcysteine abolishes the development of inspiratory muscle fatigue (Kelly et al., 2009), suggesting that oxidative stress may be a leading contributing factor to the development of inspiratory muscle fatigue during prolonged exercise. The development of inspiratory muscle fatigue without a substantial localised metabolic perturbation could theoretically explain the absence of a change in locomotor blood flow or vascular resistance during heavy-intensity exercise (Wetter et al., 1999). Indeed, it is hypothesised that the accumulation of fatigue associated metabolites and the consequent activation of group III and IV phrenic afferents is the primary stimulus for the activation of a respiratory muscle metaboreflex and the associated sympathetically mediated vasoconstriction and attenuation of blood flow and O_2 delivery (e.g. Dempsey et al., 2006).

7.4.2.2. Tolerable Exercise Duration and Inspiratory Muscle Fatigue

In response to short-duration severe-intensity exercise (Δ50; T_{LIM} ~5 min) inspiratory muscle fatigue was less prevalent (70% vs. 100% of subjects) and blunted in magnitude in comparison to prolonged severe-intensity exercise (Δ25; T_{LIM} ~10 min) (Figure 7.7). These data support the hypothesis that the cumulative force output of the diaphragm influences the magnitude of exercise-induced inspiratory muscle fatigue. Indeed, $\dot{V}O_2$ was sustained at more than 85% of $\dot{V}O_{2peak}$ for ~4 min longer and cumulative PTP_{di} was ~3700 cmH_2O higher during exercise at Δ25 vs. Δ50 (Figure 7.3). However, the findings that inspiratory muscle fatigue was still *present* in response to just ~5 minutes of constant-power exercise contrast to inconsistent

reductions in Pdi_{tw} in response to exercise protocols with a very-short period of substantially increased ventilatory work (e.g. ramp incremental exercise) (Romer et al., 2007). Indeed, in the study by Romer et al. (2007) it was suggested that diaphragmatic fatigue was not present, despite a significant reduction in Pdi_{tw} in response to high-frequency stimulations (50-100 Hz; high-frequency fatigue), and a reduction in 1 Hz Pdi_{tw} amplitude that closely approximated significance (~12%; P = 0.051). More recently, it has been demonstrated that inspiratory muscle fatigue (−24 ± 6% Pdi_{tw}) is present in 75% of subjects after only 6 minutes of submaximal constant-power exercise (75% of T_{LIM}) (Archiza et al., 2018). Therefore, in combination with previous literature, the present data suggest that short-duration severe-intensity exercise (T_{LIM}) is sufficient to evoke inspiratory muscle fatigue, albeit of a lower magnitude and consistency in comparison to prolonged severe-intensity exercise. The somewhat divergent study conclusions to previous literature is likely due to a greater between-subject variability in the development of inspiratory muscle fatigue with shorter periods of very-high respiratory work during exercise, and differences in exercise modality and protocol.

7.4.2.3. Effect of Exercise Intensity and Tolerable Exercise Duration on the Development of Expiratory Abdominal Muscle Fatigue

To the authors' knowledge, this is the first report that expiratory abdominal muscle fatigue is present and of a similar magnitude across a range of exercise intensities and durations, characterised by a 23% to 29% reduction in the Pga_{tw} response to magnetic nerve stimulation of the thoracic nerve roots. This magnitude of expiratory muscle fatigue is within the range of values (15-33%) previously reported in response to cycling at >85% $\dot{V}O_{2max}$ and comparable to reductions in MEP (20-28%) following long-distance (≥42 km) running events (Loke et al., 1982, Wuthrich et al., 2014b, Tiller et al., 2019, Taylor et al., 2006, Verges et al., 2006). In contrast to the diaphragm, the similar magnitude of fatigue across trials is somewhat surprising

considering the vastly different cumulative and average PTP_{ga} (Figure 7.3 and 7.4). Moreover, the magnitude of expiratory muscle fatigue tended to be greater than the magnitude of inspiratory muscle fatigue during heavy-intensity and short-duration severe-intensity exercise (Figure 7.7), suggesting that the threshold of the cumulative force output and the competition for blood flow may be lower for the expiratory muscles versus the diaphragm. These differences may be in part explained by phenotypical and morphological differences between the inspiratory and expiratory muscles, and the consequent effect on their resistance to fatigue. For example, the expiratory muscles possess a lower but more variable proportion of type 1 muscle fibres (30-60% vs. 50-55%) (Keens et al., 1978, Haggmark and Thorstensson, 1979), a reduced oxidative capacity (Uribe et al., 1992), and a lower metabolic efficiency in comparison to the diaphragm (Dodd et al., 1988, Robertson et al., 1977). This muscle phenotype likely explains the ~40% shorter endurance time in response to fatiguing expiratory vs inspiratory resistive loading (*see Chapter 5 and Chapter 6*), and the greater magnitude of fatigue in response to a series of sustained maximal contractions (Gandevia et al., 1983). Moreover, in addition to contributing substantially to the increase in ventilation by augmenting expiratory airflow and expanding tidal volume secondary to a decrease in end-expiratory lung volume (Sharratt et al., 1987, Henke et al., 1988, Sheel and Romer, 2012), the abdominal muscles perform important non-ventilatory roles during cycling. For example, tonic expiratory muscle activity serves to maintain body posture, stabilise the torso (Celli et al., 1988, Abraham et al., 2002, Urquhart et al., 2005) and reduces abdominal compliance providing an 'abdominal fulcrum' which enhances diaphragmatic contractility (Abdallah et al., 2017). These additional non-ventilatory roles likely contribute to the development of expiratory abdominal muscle fatigue in response to upper-body arm-crank exercise with a relatively modest ventilatory response (99 ± 19 $L \cdot min^{-1}$) (Tiller et al., 2017) and may explain the inconclusive evidence of a

relationship between expiratory work and the magnitude of fatigue (Taylor et al., 2006, Verges et al., 2006, Tiller et al., 2017).

7.4.3. Considerations

In order to assess inspiratory and expiratory muscle function, there was a slight delay in the measurement of neuromuscular function post-exercise (5-10 min), which have caused a slight underestimation of the magnitude of neuromuscular fatigue. Indeed, in response to repetitive high-intensity single-limb contractions, single twitch force of the quadriceps recovers by ~20% from immediately post to 2 minute after exercise (Froyd et al., 2013). However, in the present study the twitch order was kept constant within subjects so that any delay was standardised, and the presence of fatigue in all trials demonstrates that this protocol did not affect the ability to detect significant differences. In response to severe-intensity trials there was a small but significant reduction in Poes at the initiation of expiratory stimulations (Table 7.1), which may reflect a small change in lung volume. However, the more negative Poes indicates that, if anything, lung volume would be closer to TLC, eliciting a more optimal length-tension relationship for the expiratory muscles. This minor change in lung volume was consistent across trials, but may have theoretically lead to a slight underestimation of the magnitude of expiratory muscle fatigue.

7.4.4. Conclusions

Exhaustive heavy-intensity as well as short- (~5 min) and long-duration (~10 min) severe-intensity exercise elicits inspiratory and expiratory abdominal muscle fatigue. The findings that the magnitude of inspiratory muscle fatigue was greater in response to long-duration severe-intensity exercise versus heavy-intensity and short-duration severe-intensity exercise support the idea that exercise-induced diaphragm fatigue is a function of work history (cumulative force output and power of breathing) and a competition for blood flow with the locomotor muscles. By contrast, the magnitude of

expiratory muscle fatigue was unaffected by exercise intensity or tolerable duration, which may be due to a less fatigue-resistant phenotype and/or additional non-ventilatory roles of the expiratory muscles during exercise.

CHAPTER 8
The Time Course of Exercise-induced Inspiratory and Expiratory Abdominal Muscle Fatigue

8.1. INTERIM SUMMARY

In the previous Chapter, the effect of exercise intensity and tolerable duration (T_{LIM}) on the development of exercise-induced respiratory muscle fatigue was investigated. In this Chapter, the temporal characteristics of the development of inspiratory and expiratory muscle fatigue are considered. That is, this Chapter will examine when exercise-induced inspiratory and expiratory muscle fatigue develops *during* long-duration severe-intensity exercise, and how the magnitude of such fatigue progresses with increasing exercise time.

8.2. INTRODUCTION

Few studies have examined the time-course of the development of exercise-induced respiratory muscle fatigue *during* exercise. Previously, Johnson et al. (1993) speculated that diaphragmatic muscle fatigue may occur relatively early during exercise, and that a reflex inhibition of further diaphragm recruitment may explain the diminished magnitude of fatigue in individuals who minimised the diaphragmatic contribution to ventilatory work. Indeed, it is well established that, particularly in healthy males, the diaphragmatic contribution to inspiratory pressure generation diminishes as exercise progresses (Johnson et al., 1993, Guenette et al., 2010, Archiza et al., 2018). This allows the diaphragm to contribute primarily to inspiratory airflow generation while the recruitment of accessory respiratory musculature meets the pressure requirements of the progressive hyperpnoea (Aliverti et al., 1997).

In the first reported attempt to quantify the onset of diaphragmatic fatigue *during* exercise, Kabitz et al. (2008) reported that diaphragm ‘strength’, as measured by the

diaphragm twitch pressure (Pdi_{tw}) response to anterior bilateral phrenic nerve stimulation, actually progressively increased with increasing exercise time, and that diaphragmatic fatigue only manifested *after* and not *during* exercise. However, such study findings have been questioned owing to methodological considerations and limitations such as: 1) the exercise trial not being performed to volitional exhaustion; 2) a failure to account for muscle potentiation; and 3) frequent pausing of the exercise trial that likely compromised exercise economy and the assessment of neuromuscular function. In a subsequent study by the same research group, exercise was prescribed at >85% $\dot{V}O_{2max}$ to exhaustion, and Pdi_{tw} was post-hoc corrected for changing lung volumes during exercise. It was reported that diaphragmatic fatigue actually occurred relatively early during exercise (~2/3 of the tolerable duration) with no further decline in contractility as exercise progressed (Walker et al., 2011). In contrast, Archiza et al. (2018) reported that the magnitude of inspiratory muscle fatigue, determined as a pre- to post-exercise reduction in Pdi_{tw} in response to cervical magnetic nerve stimulation (CMS), increased progressively with exercise time (i.e. as a percentage of tolerable duration) in association with the increasing cumulative work of breathing (WoB). It is possible that the discrepancy in study findings is due to the failure of Walker et al. (2011) to account for differences in muscle potentiation of the diaphragm during exercise. Indeed, it is likely that the diaphragm becomes progressively potentiated as the cumulative force output and absolute WoB increase over time, which could theoretically lead to a progressive underestimation of the magnitude of diaphragmatic fatigue as exercise duration increases. Alternatively, it is possible that the diaphragm becomes fatigued relatively early during exercise, and that the further decrease in global inspiratory muscle fatigue during exercise in studies that have used CMS (i.e. Archiza et al., 2018) are caused by the recruitment and consequent fatigue of the accessory inspiratory ribcage muscles. Indeed, CMS of the phrenic nerve roots activates both the diaphragm and the accessory inspiratory muscles acting on the ribcage (e.g.

scalenes, sternocleidomastoid, external intercostal); as such, CMS can be used to discriminate between fatigue of the diaphragm and the accessory inspiratory ribcage muscles (Similowski et al., 1998). However, to date no study has attempted to discern the progression of exercise-induced diaphragmatic vs. accessory inspiratory ribcage muscle fatigue.

In contrast to the diaphragm, the time-course of the development of exercise-induced expiratory muscle fatigue has not been investigated. As detailed previously in this thesis, the expiratory muscles of the ventrolateral abdominal wall are functionally less fatigue-resistant in comparison to the diaphragm. Indeed, when compared to the diaphragm, the expiratory muscles exhibit a lower but more variable proportion of type I muscle fibres (Keens et al., 1978, Haggmark and Thorstensson, 1979), a decreased oxidative capacity (Uribe et al., 1992), and a reduced metabolic efficiency (Dodd et al., 1988). In addition to contributing substantially to the hyperpnoea of exercise, the expiratory muscles also perform important non-ventilatory roles. Tonic expiratory muscle activity serves to maintain body posture, stabilise the torso, and lower abdominal compliance to augment diaphragmatic contractility by acting as an 'abdominal fulcrum' (Dempsey et al., 1996, Abdallah et al., 2017). These phenotypical and functional differences likely play a role in the shortened time to task failure for expiratory compared to inspiratory muscle resistive loading (*see* Chapters 5 and 6), and the development of a similar magnitude of exercise-induced expiratory muscle fatigue in response to different exercise intensities and tolerable durations (*see* Chapter 7). It could be hypothesised that this greater 'fatigability' of the expiratory vs. the inspiratory muscles could lead to an earlier development of exercise-induced expiratory vs. inspiratory muscle fatigue during severe-intensity exercise. However, as yet this hypothesis has not been empirically investigated.

The aim of the present study was to compare the time-course or 'temporality' of exercise-induced inspiratory and expiratory muscle fatigue during long-duration (~10 min) severe-intensity exercise in healthy males and females. It was hypothesised that: 1) exercise-induced expiratory muscle fatigue would occur at a significantly earlier time-point than inspiratory muscle fatigue; 2) inspiratory and expiratory muscle contractility would decrease significantly with increasing exercise time; and 3) the magnitude of exercise-induced diaphragmatic fatigue would not be different across time points whereas accessory inspiratory ribcage muscle fatigue would increase progressively as a function of exercise time.

8.3. METHODS

8.3.1. Experimental Overview

Subjects who participated in Chapter 7 of this thesis were invited back to the laboratory for two additional visits (*see* Appendix II for full subject demographics) . At these visits, the participants performed constant-power cycle exercise for: 1) 50%; or 2) 75% of the tolerable duration (T_{LIM}) of exercise performed at ~25% of the difference (Δ) between critical power (CP) and peak power (P_{peak}) (*determined in* Chapter 7) (Figure 5.1). The order of these two visits was randomised between the participants. Contractile function of the inspiratory and expiratory muscles was assessed before and after severe-intensity exercise performed to exhaustion (100% T_{LIM}; from Chapter 7) and such exercise terminated at 50% and 75% of T_{LIM}; this allowed for the determination of time-course of the development of respiratory muscle fatigue during prolonged severe-intensity exercise.

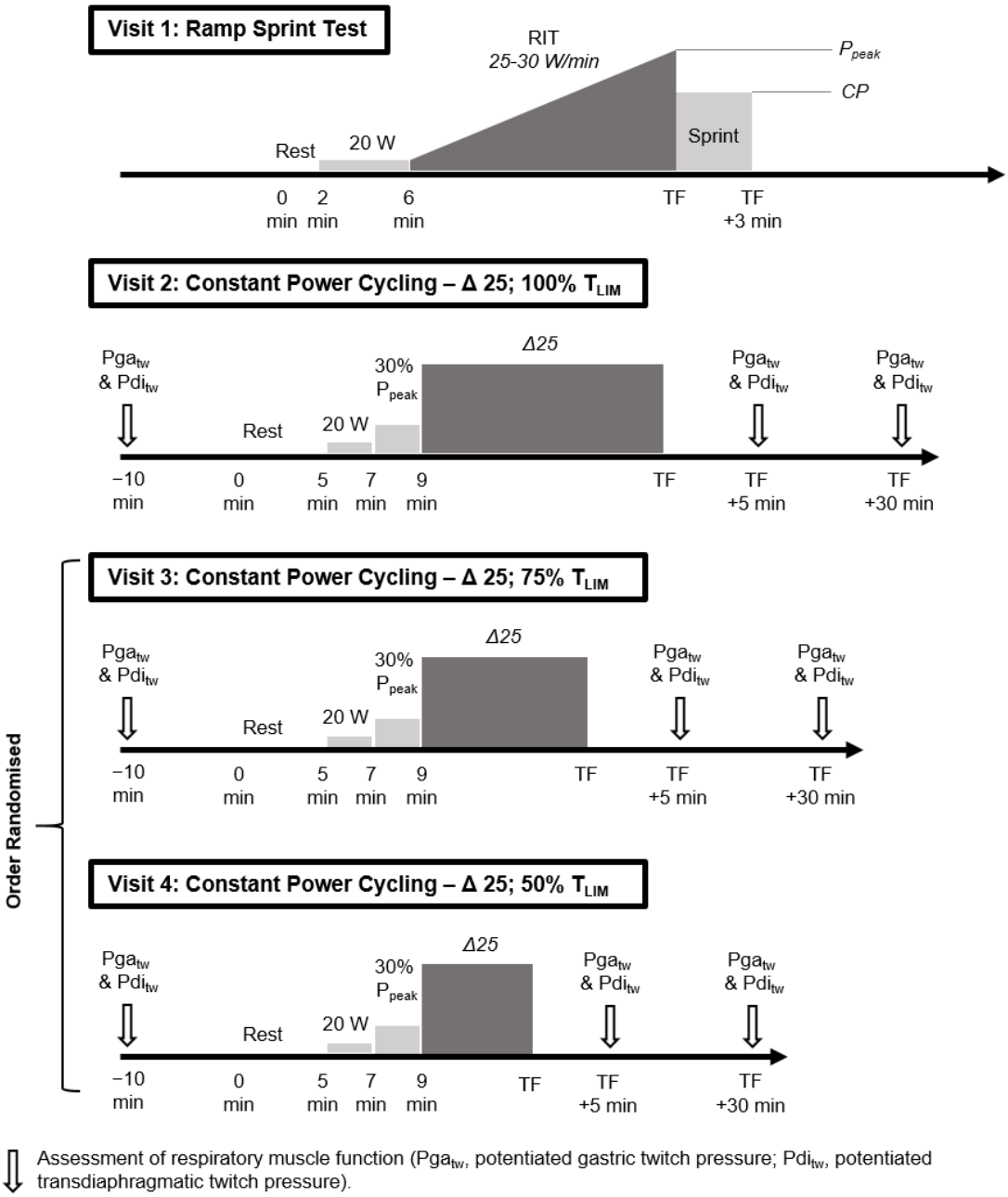


Figure 8.1. Experimental exercise protocols. Visits 1 and 2 refer to exercise trials performed in Chapter 7. RIT, ramp incremental test; TF, task failure; P_{peak}; peak power; CP, critical power; Pga_{tw}, gastric twitch pressure; Pdi_{tw}, transdiaphragmatic twitch pressure.

8.3.2. Additional Twitch Analysis

In addition to activation of the diaphragm, cervical magnetic stimulation (CMS) evokes a contraction of several accessory inspiratory muscles that act to stiffen the ribcage, resulting in an increased $Poes_{tw}$ amplitude in comparison to anterior bilateral phrenic nerve stimulation (Laghi et al., 1996). As such, CMS allows for discrimination between the relative contributions of diaphragm and ribcage muscle fatigue to global

inspiratory muscle fatigue (Similowski et al., 1998). Briefly, 1-Hz CMS evokes a measurable gastric (Pga_{twCMS}), oesophageal ($Poes_{twCMS}$), and as such transdiaphragmatic (Pdi_{tw}) twitch pressure response. A reduction in Pdi_{tw} and Pga_{twCMS} without a decrease in the $Poes_{twCMS}/Pga_{twCMS}$ ratio is suggestive of predominant diaphragm fatigue. Alternatively, a reduction in Pdi_{tw} and the $Poes_{twCMS}/Pga_{twCMS}$ ratio with preserved Pga_{twCMS} is indicative of predominant ribcage muscle fatigue (Similowski et al., 1998). In the present study, the fatigue ratio (i.e. the pre- to post-exercise change in: $Poes_{twCMS}/Pga_{twCMS}$) was used to determine the predominance of diaphragm or inspiratory accessory ribcage muscle fatigue; a fatigue ratio of <1 was indicative of predominant diaphragm fatigue whereas a fatigue ratio of ≥1 was indicative of predominant inspiratory ribcage muscle fatigue. To be clear, however, predominant diaphragm fatigue across trials *does not* preclude an increasing contribution from the accessory ribcage muscles to the overall reduction in Pdi_{tw}. As such, the relative change in $Poes_{twCMS}$ and Pga_{twCMS} were also compared across exercise trials.

Additionally, to gain insight into exercise-induced fatigue of the accessory expiratory ribcage muscles, we inspected the $Poes_{tw}$ response to thoracic nerve stimulation ($Poes_{twTMS}$). Although we are unaware of this analysis being used in any previous study, it was hypothesised that a positive $Poes_{twTMS}$ response would primarily reflect contraction of the accessory expiratory ribcage muscles (i.e. internal intercostal, triangularis sterni). Indeed, the expiratory internal intercostal muscles of the ribcage are innervated from the intercostal nerve originating in the thoracic region of the spinal cord (~T4-T11) (De Troyer et al., 2005, Sears, 1964). In the present study, a substantial positive $Poes_{twTMS}$ response was observed in all subjects, similar to the increase in pleural pressure exhibited in anaesthetised canines following electrical tetanic stimulation of the triangularis sterni or in humans adopting an expiratory

ribcage breathing technique by avoiding contraction of the abdominal muscles (De Troyer and Ninane, 1986, De Troyer et al., 1987).

8.3.3. Statistical Analysis

All data are means ± SD. Statistical analysis was performed in IBM SPSS Statistics version 26. One-way repeated measures ANOVA were performed to assess differences in exercise responses between the final minute of each trial (i.e. 50% vs. 75% vs. 100% of T_{LIM}). Differences in end-exercise perceptual responses were assessed using a Friedman's ANOVA, and Spearman's rank T-tests were performed with a Bonferroni adjustment to identify where differences between tests occurred. For Pdi_{tw} and Pga_{tw}, a two-way repeated measures ANOVA was performed to determine whether any interaction was present between exercise trial (50%, 75% and 100% of T_{LIM}) and time points (pre, 5- and 30 minutes after exercise). In the case of a significant interaction, a one-way ANOVA with a Bonferroni pairwise comparison was performed for the percent change in Pdi_{tw} and Pga_{tw} of each exercise trial from pre- to post-exercise of Pdi_{tw} and Pga_{tw}. Differences in the percent pre- to post exercise change in: $Poes_{twCMS}$, Pga_{twCMS}, $Poes_{twCMS}/Pga_{twCMS}$, and $Poes_{twTMS}$ were also assessed using a one-way repeated measures ANOVA with a Bonferroni adjustment. The acceptable type I error was set at $P < 0.05$.

8.4. RESULTS

8.4.1. Physiological Responses to Constant-Power Exercise Trials

8.4.1.1. Cardiopulmonary, metabolic, and perceptual responses

The metabolic, ventilatory and perceptual responses to the exercise trials are presented in Table 8.1. Subjects cycled at 234 ± 53 W (Δ25) for 10.2 ± 2.6 min (100% T_{LIM}), 7.7 ± 1.9 min (75% T_{LIM}), and 5.1 ± 1.3 min (50% T_{LIM}). In response to the 100% T_{LIM} trial, $\dot{V}O_2$ and $\dot{V}_E$ increased rapidly before a slower more gradual rise towards exercise intolerance (Figure 7.2). Both $\dot{V}O_{2peak}$ and end-exercise $\dot{V}_E$ were significantly

higher during 75% and 100% vs. 50% T_{LIM}, but were not different between 75% and 100% T_{LIM} (Table 8.1). As exercise duration progressed during 100% T_{LIM}, a more tachypneic breathing pattern was observed, characterised by an increase in f_R and a decrease in V_T (Figure 7.2). There was a significant increase in end-exercise f_R as a function of exercise duration (Table 8.1). In contrast, end-exercise V_T was significantly lower at end-exercise of 100% vs. 50% and 75% T_{LIM} trials (Table 8.1). End-exercise T_I and T_E decreased progressively as a function of exercise duration (Table 8.1). There was a progressive increase in heart rate and blood lactate concentration during all exercise trials. At end-exercise, peak heart rate was greater in 100% vs. 50% T_{LIM} but not different between 100% and 75% T_{LIM} (Table 8.1). Similarly, peak blood lactate concentration was greater in 100% vs. 50% T_{LIM} but not different in 100% compared to 75% T_{LIM} (Table 8.1). Breathing discomfort and leg discomfort increased progressively over time during 100% T_{LIM} (Figure 7.2). End-exercise breathing discomfort was higher during 100% vs. 50% T_{LIM} and to a lesser extent vs. 75% T_{LIM} (Table 8.1). All subjects reported maximal leg discomfort at end-exercise for 100% T_{LIM} (10 points), which was significantly greater than during 75% and 50% T_{LIM} (Table 8.1).

8.4.1.2. Respiratory Muscle Pressure Generation

During the 100% T_{LIM} trial PTP_{di}, PTP_{oes}, and PTP_{ga} increased progressively as a function of exercise time (Figure 7.3). End-exercise PTP_{di} was not different between trials (Table 8.2). In contrast, PTP_{oes} was greater at end-exercise of 100% vs. 50% T_{LIM}, and to a lesser extent vs. 75% T_{LIM} (Table 8.1). End-exercise PTP_{ga} was greater during 100% vs. 50% T_{LIM}, but not different to 75% T_{LIM} (Table 8.1). As expected, cumulative PTP_{di} was greater in 100% vs. 50% T_{LIM}, and to a lesser extent, 75% of T_{LIM} (Figure 8.2). Similarly, cumulative PTP_{ga} was greater during 100% vs. 50%, and to a lesser degree, vs. 75% of T_{LIM} (Figure 8.2).

Table 8.1. Ventilatory, metabolic, and perceptual exercise responses for the final minute of exercise.

	50% T_{LIM}	75% T_{LIM}	100% T_{LIM}
$\dot{V}_E$, $L \cdot min^{-1}$	118 ± 19	132 ± 24*	142 ± 30*
f_R, $breaths \cdot min^{-1}$	43 ± 5	50 ± 4*	60 ± 8*†
V_T, litres	2.79 ± 0.63	2.70 ± 0.52	2.38 ± 0.42*†
T_I, s	0.56 ± 0.08	0.49 ± 0.06*	0.41 ± 0.07*†
T_E, s	0.88 ± 0.10	0.75 ± 0.07*	0.62 ± 0.08*†
T_I/T_{TOT}, s	0.39 ± 0.02	0.39 ± 0.02	0.39 ± 0.02
T_E/T_{TOT}, s	0.61 ± 0.02	0.61 ± 0.02	0.61 ± 0.02
PTP_{di}, $cmH_2O \cdot s \cdot min^{-1}$	614 ± 142	724 ± 208	753 ± 169
PTP_{oes}, $cmH_2O \cdot s \cdot min^{-1}$	817 ± 158	966 ± 202*	1189 ± 325*†
PTP_{di}/PTP_{oes}	0.76 ± 0.15	0.74 ± 0.18	0.66 ± 0.16
PTP_{ga}, $cmH_2O \cdot s \cdot min^{-1}$	344 ± 159	446 ± 212	536 ± 247*
$\dot{V}O_{2peak}$, $L \cdot min^{-1}$	3.77 ± 0.74	4.01 ± 0.85*	4.04 ± 0.88*
$\dot{V}O_{2peak}$, % RIT $\dot{V}O_{2peak}$	91.5 ± 5.0	97.0 ± 5.0*	97.5 ± 3.9*
$\dot{V}CO_2$, $L \cdot min^{-1}$	4.15 ± 0.90	4.31 ± 0.98	4.25 ± 0.90
$P_{ET}CO_2$, mmHg	31.0 ± 4.4	28.0 ± 3.7*	26.7 ± 4.1*
Heart Rate, $beats \cdot min^{-1}$	174 ± 10	179 ± 8	182 ± 9*
Blood Lactate, $mmol \cdot L^{-1}$	8.8 ± 3.0	11.3 ± 3.0*	11.6 ± 3.4*
Breathing Discomfort, points	6.2 ± 1.3	7.9 ± 1.1*	9.6 ± 0.7*†
Leg Discomfort, points	7.4 ± 1.0	8.5 ± 1.1	10.0 ± 0.0*†

Values are means ± SD for 10 subjects (blood lactate, $n = 8$). $\dot{V}_E$, minute ventilation; f_R, respiratory frequency; V_T, tidal volume, $\dot{V}O_2$, oxygen uptake; $P_{ET}CO_2$, end-tidal pressure of carbon dioxide. *$P < 0.05$ vs. 50% T_{LIM}; †$P < 0.05$ vs. 75% T_{LIM}.

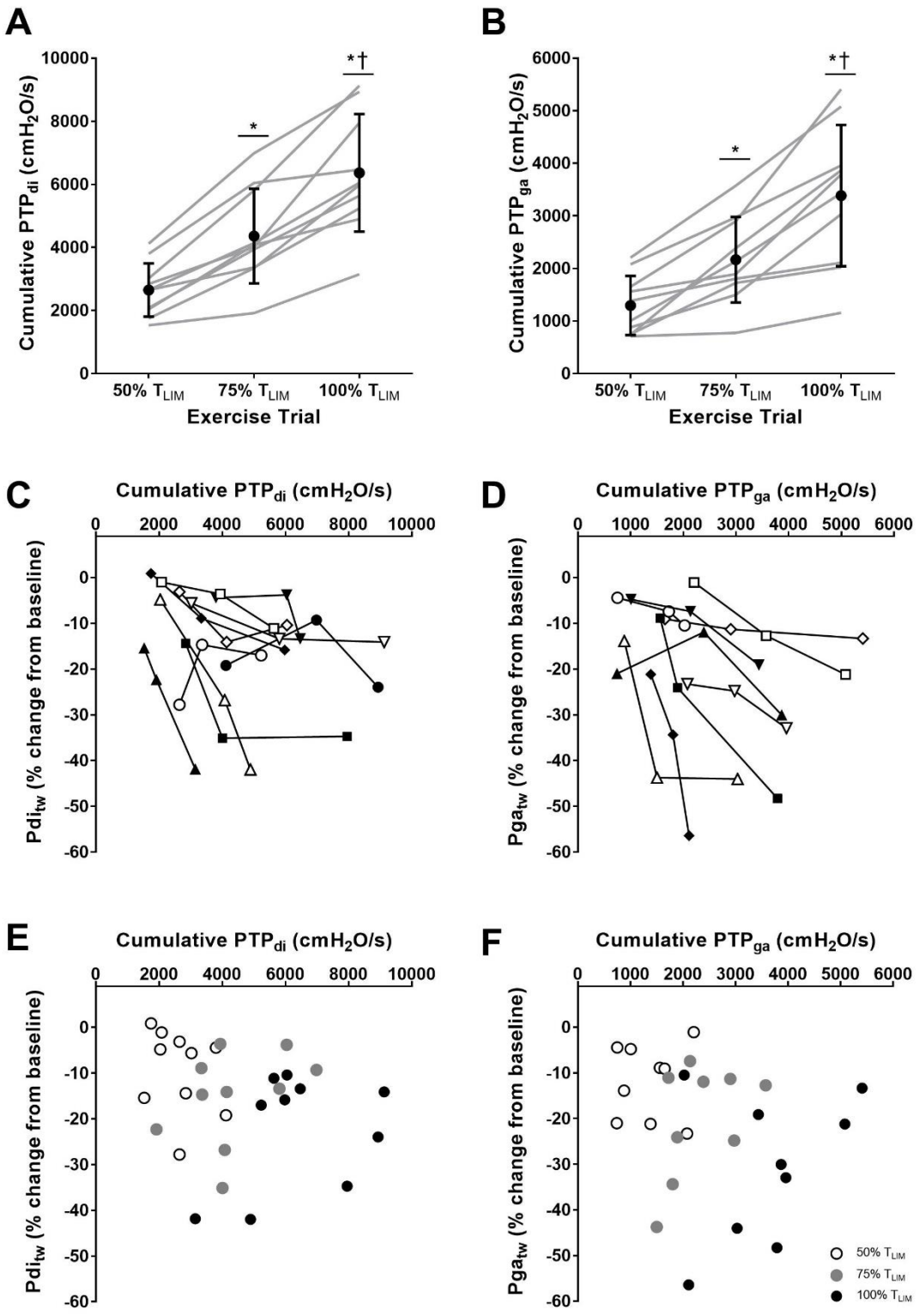


Figure 8.2. Cumulative pressure time product (PTP) across exercise trials. A-B, total cumulative diaphragmatic and gastric PTP in response to all exercise trials; C-D, individual relationships of total cumulative diaphragmatic and gastric PTP vs. exercise-induced diaphragm (Pdi_{tw}) and expiratory muscle fatigue (Pga_{tw}); E-F, comparison of exercise trial to the total cumulative diaphragm and gastric PTP vs. Pdi_{tw} and Pga_{tw} relationship. *Significant difference to 50% T_{LIM} ($P < 0.05$); † significant difference to 75% T_{LIM} ($P < 0.05$).

8.4.2. Exercise-induced Respiratory Muscle Fatigue

The mechanical and electrical responses to magnetic stimulation of the cervical and thoracic nerve roots before and after exercise performed to 100%, 75%, and 50% of T_{LIM} in a representative participant are shown in Figure 8.3 (*panels A and* B).

8.4.2.1. Inspiratory Muscle Fatigue

From before to after exercise, there was a reduction in group mean Pdi_{tw} for 100%, 75%, and 50% T_{LIM} (all $P < 0.05$) (Figure 8.4, *panel A*). Although the pre- to post-exercise reduction in group mean Pdi_{tw} was statistically significant in each of the exercise trials, the reduction in Pdi_{tw} was >2 x CV in 4 subjects in 50% T_{LIM} (40%), 8 subjects in 75% T_{LIM} (80%), and in all subjects in 100% T_{LIM} (100%) (Figure 8.4, *panel A*). There was recovery in Pdi_{tw} amplitude across all 3 trials at 30 min post-exercise, but Pdi_{tw} remained significantly decreased from baseline values in 75% and 100% T_{LIM} ($P < 0.05$).

The group mean magnitude of exercise-induced inspiratory muscle fatigue was greater after 100% (−22.4 ± 12.5%) vs. 50% (−9.5 ± 9.2%; $P = 0.037$) and 75% T_{LIM} (−15.2 ± 10.1%; $P = 0.044$) (Figure 8.4). In response to all exercise trials, the greater decrease in Pga_{twCMS} vs. $Poestw_{CMS}$ (as reflected by a $Poes_{twCMS}/Pga_{twCMS}$ fatigue index of <1) suggests that there was consistently predominant exercise-induced diaphragmatic fatigue (Figure 8.5). However, the pre- to post-exercise change in Pga_{twCMS} was not significantly different across exercise trials and demonstrated a clear plateau from 75% to 100% of T_{LIM} (Figure 8.5, *panel B*). In contrast, the pre- to post-exercise change in $Poes_{twCMS}$ decreased progressively across exercise trials (Figure 8.5, *panel A*). Therefore, although the diaphragmatic contribution to inspiratory muscle fatigue remained 'predominant', these data suggest that the accessory inspiratory ribcage muscles increasingly contributed to the reduction in global inspiratory muscle fatigue as exercise duration progressed.

8.4.2.2. Expiratory muscle fatigue

Due to unacceptable gastric twitch variability in one subject, defined as a Pga_{tw} CV of '>3' times the within-day test-retest CV at any measurement time-point, analyses of Pga_{tw} and associated variables pertains to 9 of the 10 subjects. From before to after exercise, there was a group mean reduction in Pga_{tw} for 100%, 75%, and 50% T_{LIM} (all $P < 0.05$) (Figure 8.4, *panel B*). Although the pre- to post-exercise reduction in group mean Pga_{tw} was statistically significant in all of the exercise trials, the reduction in Pga_{tw} was >2 x CV in 6 subjects in 50% T_{LIM} (67%), 8 subjects in 75% T_{LIM} (89%), and in all 9 subjects in 100% T_{LIM} (100%) (Figure 8.4, *panel B*). There was recovery in Pga_{tw} amplitude across all 3 trials at 30 min post-exercise, but Pga_{tw} remained significantly decreased from baseline values in 75% and 100% T_{LIM} ($P < 0.05$).

The group mean magnitude of exercise-induced expiratory abdominal muscle fatigue (i.e. decrease in Pga_{tw}) was greater after 100% T_{LIM} (−30.3 ± 15.6%) vs. 50% (−11.9 ± 8.2%; $P = 0.008$) and, to a lesser extent, 75% T_{LIM} (−20.0 ± 12.6%; $P = 0.024$) (Figure 8.4 and 8.5, *panel D*). Compared to the change in Pga_{tw}, there was a more gradual progression in the $Poes_{tw}$ response to thoracic nerve stimulation with increasing exercise duration, and the pre- to post-exercise change in $Poes_{twTMS}$ was not different in 50%, 75% and 100% of T_{LIM} (Figure 8.5, *panel D*). The expiratory ribcage muscles may therefore fatigue to a less progressive extent during exercise in comparison to the abdominal expiratory muscles.

8.4.2.3. Inspiratory vs. Expiratory Muscle Fatigue

Due to unacceptable Pga_{tw} variability in one subject, differences in the magnitude of inspiratory vs. expiratory muscle fatigue were compared in $n = 9$. There was no significant difference in the magnitude of inspiratory vs. expiratory muscle fatigue at 50%, 75%, or 100% T_{LIM} (all $P > 0.14$).

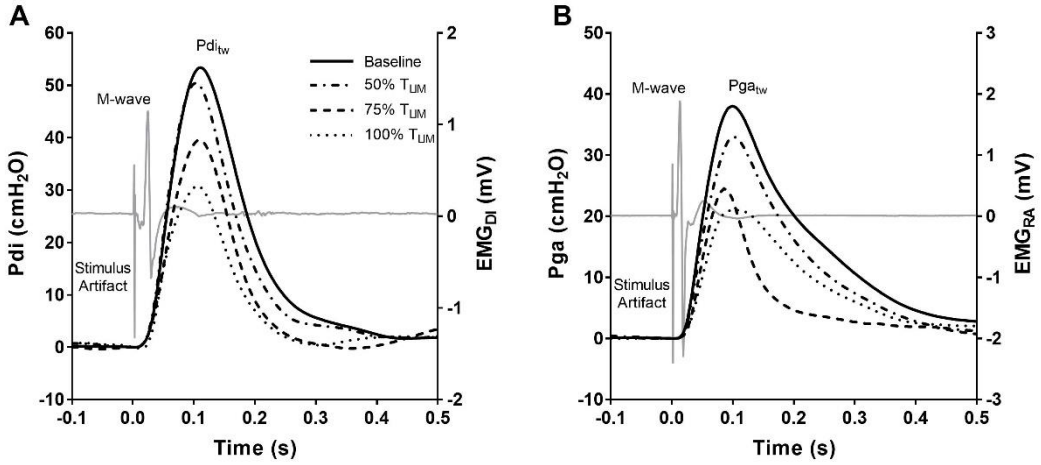


Figure 8.3. Individual representative raw data traces for transdiaphragmatic twitch pressure (Pdi_{tw}) (A) and gastric twitch pressure (Pga_{tw}) (B) from before to ~5 min after exercise trials. Baseline represents ensemble average twitch pressure from all baseline periods. Electromyographic responses for the diaphragm (EMG_{DI}) and rectus abdominis (EMG_{RA}) are also shown.

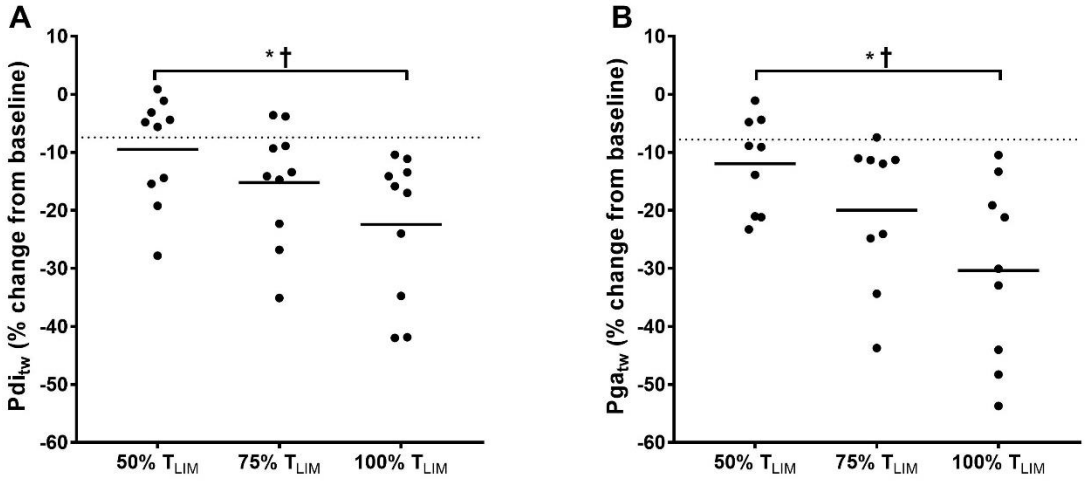


Figure 8.4. A comparison of the magnitude of the pre- to post exercise change in (A) Pdi_{tw} and (B) Pga_{tw} in response to exercise of different durations. *Significant difference between 100% T_{LIM} vs. 50% T_{LIM} ($P < 0.05$); †Significant difference between 100% T_{LIM} and 75% T_{LIM} ($P < 0.05$).

Table 8.2 Mechanical twitch characteristics and volitional muscle function before and (~5 min) after exercise.

	50% T_{LIM}		75% T_{LIM}		100% T_{LIM}	
Twitch Characteristic	Pre-exercise	Post-exercise	Pre-exercise	Post-exercise	Pre-exercise	Post-exercise
Pdi_{tw}, cmH_2O	40.4 ± 11.6	36.9 ± 12.5*	43.1 ± 9.3	36.2 ± 7.2†	44.3 ± 12.6	34.3 ± 12.0†
CT, ms	112 ± 5	106 ± 7†	114 ± 9	106 ± 7†	115 ± 6	105 ± 6†
MRPD/Pdi_{tw}, s/cm	19.0 ± 2.9	19.6 ± 1.9	17.9 ± 0.9	19.9 ± 1.8†	18.0 ± 1.3	20.0 ± 1.4†
$RT_{0.5}$, ms	68 ± 11	58 ± 8†	68 ± 11	57 ± 10†	67 ± 6	56 ± 9†
MRR/Pdi_{tw}, s/cm	10.1 ± 1.7	12.0 ± 1.9†	10.1 ± 1.8	12.2 ± 2.3†	10.2 ± 1.0	12.2 ± 2.2*
End-expiratory Poes, cmH_2O	−4.9 ± 1.9	−5.0 ± 1.7	−4.6 ± 1.7	−5.3 ± 2.0	−4.4 ± 1.5	−5.2 ± 2.0
End-expiratory Pdi, cmH_2O	20.5 ± 7.6	16.6 ± 6.1*	18.9 ± 4.6	20.8 ± 8.7	18.5 ± 5.0	15.8 ± 5.1*
Voluntary Activation, %	88 ± 7	87 ± 7	90 ± 6	88 ± 8	89 ± 8	84 ± 8
MIP, cmH_2O	143 ± 35	140 ± 37	138 ± 28	137 ± 33	142 ± 29	130 ± 25*
Pga_{tw}, cmH_2O	52.1 ± 29.6	45.7 ± 26.8†	51.5 ± 28.0	42.7 ± 27.1†	54.8 ± 36.8	40.7 ± 33.5†
CT, ms	109 ± 14	108 ± 14	108 ± 16	104 ± 15	112 ± 17	108 ± 15
MRPD/Pga_{tw}, s/cm	16.6 ± 3.8	16.7 ± 3.7	16.8 ± 4.2	16.7 ± 3.3	17.1 ± 3.2	16.7 ± 2.4
$RT_{0.5}$, ms	122 ± 17	101 ± 17†	116 ± 23	94 ± 22*	112 ± 26	87 ± 22†
MRR/Pga_{tw}, s/cm	5.4 ± 1.2	6.3 ± 0.8	5.8 ± 1.6	7.0 ± 2.4	5.7 ± 1.1	7.1 ± 2.0*
End-expiratory Poes, cmH_2O	−5.4 ± 1.9	−6.6 ± 2.1	−4.9 ± 1.7	−6.1 ± 2.2*	−4.6 ± 1.6	−6.0 ± 2.6
End-expiratory Pga, cmH_2O	13.7 ± 4.5	9.9 ± 4.0	14.4 ± 4.5	14.1 ± 8.2	15.8 ± 6.5	10.8 ± 6.1*
Voluntary Activation, %	60 ± 16	56 ± 18	62 ± 14	59 ± 16	64 ± 13	56 ± 7*
MEP, cmH_2O	164 ± 43	162 ± 46	175 ± 47	161 ± 43†	170 ± 38	147 ± 28†

Values are means ± SD for 10 subjects for Pdi_{tw} and 9 subjects for Pga_{tw}. Pdi_{tw}, transdiaphragmatic twitch pressure; CT, contraction time; MRPD, maximal rate of pressure development; $RT_{0.5}$, one-half relaxation time; MRR, maximal relaxation rate; Poes, oesophageal pressure; Pdi_{MAX}, maximal transdiaphragmatic pressure (Müeller manoeuvre); MIP, maximal inspiratory pressure; Pga_{tw}, gastric twitch pressure; Pga_{MAX}, maximal gastric pressure (Valsalva manoeuvre); MEP, maximal expiratory pressure. *P < 0.05 and † P < 0.01, significantly different to pre-exercise value. Note, statistical analyses include 30 min recovery comparisons (data not shown). For gastric voluntary activation, n = 8 due to 1 subject exceeding the maximum range of the pressure transducer.

Table 8.3. Electrical twitch characteristics.

	50% T_{LIM}		75% T_{LIM}		100% T_{LIM}	
Twitch Characteristic	Pre-exercise	Post-exercise	Pre-exercise	Post-exercise	Pre-exercise	Post-exercise
Diaphragm M-Wave						
Amplitude, mV	2.2 ± 1.2	2.4 ± 1.3	2.1 ± 1.3	2.2 ± 1.3	2.2 ± 1.2	2.2 ± 1.2
Duration, ms	33.3 ± 6.1	31.0 ± 5.1	30.4 ± 4.5	30.4 ± 4.3	30.7 ± 5.2	30.9 ± 5.2
Area, $mV \cdot ms^{-1}$	14.1 ± 6.1	15.4 ± 7.0	12.7 ± 7.2	13.6 ± 7.5	12.2 ± 5.6	13.0 ± 5.7
Rectus Abdominis M-Wave						
Amplitude, mV	3.1 ± 1.7	3.2 ± 1.7	3.1 ± 1.6	3.1 ± 1.7	3.4 ± 2.1	3.3 ± 2.3
Duration, ms	25.0 ± 5.8	24.5 ± 6.0	23.9 ± 6.8	23.7 ± 5.4	24.5 ± 4.7	24.0 ± 5.6
Area, $mV \cdot ms^{-1}$	15.6 ± 8.1	15.5 ± 8.2	15.2 ± 8.1	15.2 ± 7.8	16.8 ± 10.0	15.4 ± 9.4

Values are means ± SD. Note, diaphragm M-waves were obtained for $n = 7$, rectus abdominis $n = 9$.

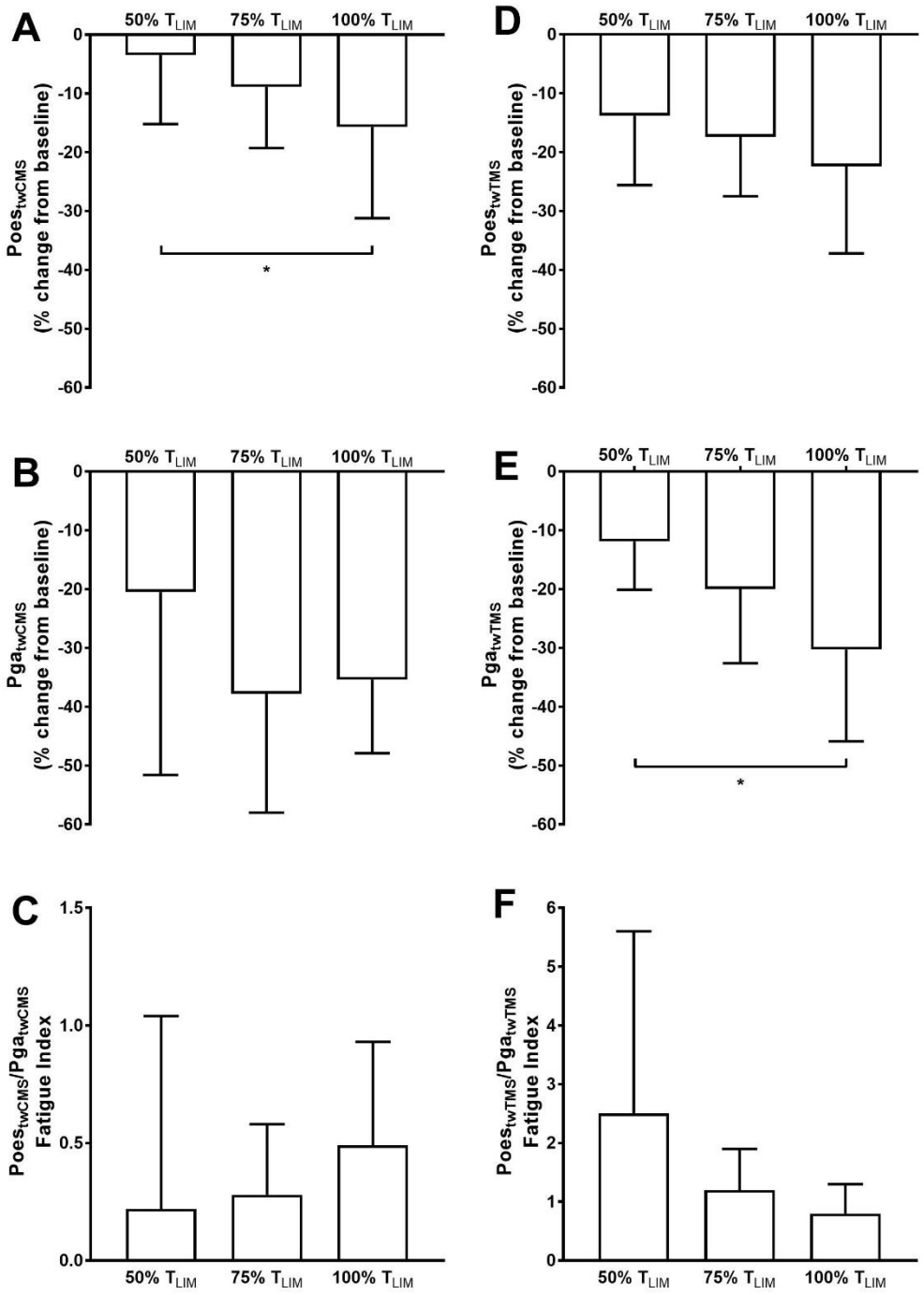


Figure 8.5. A-C, the accessory inspiratory ribcage and diaphragm muscle contribution to global exercise-induced inspiratory muscle fatigue in response to cervical magnetic nerve stimulation (CMS); and D-F, the accessory expiratory ribcage and expiratory abdominal muscle contribution to global exercise-induced expiratory muscle fatigue in response to thoracic magnetic nerve stimulation (TMS). $Poes_{tw}$, oesophageal twitch pressure; Pga_{tw}, gastric twitch pressure. *Significant difference between trials, $P < 0.05$.

8.5. DISCUSSION

8.5.1. Main Findings

The present study investigated the time-course of exercise-induced inspiratory and expiratory abdominal muscle fatigue. The major findings were that: 1) the magnitude and prevalence of inspiratory and expiratory muscle fatigue increased as a function of exercise time (i.e. percentage of tolerable duration); 2) the diaphragm component of global inspiratory muscle fatigue remained relatively stable over time, particularly during the latter phase of exercise, whereas the magnitude of accessory ribcage muscle fatigue increased progressively with exercise time; and 3) exercise-induced expiratory muscle fatigue was more prevalent at an earlier time point during exercise than inspiratory muscle fatigue, potentially due to the less fatigue-resistant phenotype and the additional non-ventilatory roles during exercise.

8.5.2. Time Course of Exercise-Induced Inspiratory Muscle Fatigue

Exercise-induced inspiratory muscle fatigue appears to be a function of both an elevation in the inspiratory power of breathing and a limitation in the availability of blood flow. Indeed, voluntarily mimicking the diaphragmatic ‘work’ incurred during exercise in otherwise resting individuals does not result in diaphragmatic fatigue (Babcock et al., 1995b), and it has been suggested that the reserve in cardiac output was a contributing factor to the absence or lower prevalence of diaphragmatic fatigue in response to heavy-intensity exercise protocols (Wetter et al., 1999, Johnson et al., 1993, Wuthrich et al., 2014a). Importantly, both the intensity of diaphragmatic work and the time for which such work is sustained (i.e. cumulative force history) also appear to be important determinants of the magnitude of exercise-induced diaphragmatic fatigue. For example, diaphragmatic fatigue is abolished in comparison to time-matched control conditions when inspiratory muscle power is reduced via proportional assist ventilation (Babcock et al., 2002). Additionally, previous studies have demonstrated the absence of diaphragmatic fatigue in

response to exercise protocols that engender a high inspiratory power of breathing for only a short time period (i.e. >90% of $\dot{V}O_{2max}$ for <~4 min) (Romer et al., 2007, Levine and Henson, 1988).

The present findings that the prevalence and magnitude of inspiratory muscle fatigue increased in association with the percentage of T_{LIM} (Figure 8.4) further highlight the importance of the duration of elevated inspiratory muscle power. Indeed, although the intensity of final minute diaphragm pressure production (PTP_{di}) was similar across trials, cumulative PTP_{di} increased substantially in relation to exercise time (Table 8.2). The time-course of exercise-induced inspiratory muscle fatigue was also highly comparable to previous findings for severe-intensity constant power exercise. For example, Archiza et al. (2018) reported a progressive impairment in inspiratory muscle contractility during high-intensity constant-power cycling, characterised by a reduction in Pdi_{tw} of −24 ± 6% after 75% of T_{LIM} (~6 min) and −35 ± 12% by exercise intolerance (~7.5 min). Similarly, in the present study the magnitude of global inspiratory muscle fatigue increased from −15 ± 10% at 75% of T_{LIM} (~7.5 min) to −22 ± 12% at exercise intolerance (~10 min) (Figure 8.4). In combination, the present findings provide further evidence that contrasts to the original suggestion that diaphragm ‘strength’ progressively increases during high-intensity exercise, and that impairments in diaphragm contractility are only evident during recovery (Kabitz et al., 2008, Kabitz et al., 2007). As previously highlighted (*see section* 8.2), such differences in the latter studies are likely explained by methodological limitations.

In a subsequent study by the same research group that corrected some of these methodological limitations by prescribing exhaustive constant-power exercise at >85% $\dot{V}O_{2max}$ and correcting for changing lung volumes during exercise, a decline in Pdi_{tw} was reported within the initial two thirds of the exercise bout, but there was no further decrease by exercise intolerance (i.e. fatigue was not progressive) (Walker et

al., 2011). Due to the utilisation of anterior bilateral stimulation of the phrenic nerves in the aforementioned study, which avoids co-contraction of the accessory inspiratory ribcage muscles (Wragg et al., 1994), it was suggested that the magnitude of diaphragmatic fatigue does not increase from relatively early during exercise to intolerance. In the present study we were able to estimate the contribution of the accessory inspiratory ribcage muscles and the diaphragm to the progressive reduction in Pdi_{tw} by measuring the relative change across trials in the pre- to post-exercise change in $Poes_{twCMS}$, Pga_{twCMS}, and the $Poes_{twCMS}/Pga_{twCMS}$ fatigue index (Similowski et al., 1998). There was a similar reduction in Pga_{twCMS} in response to increasing exercise duration, and clear evidence of a plateau from 75% to 100% of T_{LIM} (Figure 8.5, *panel B*). In contrast, the pre- to post-exercise reduction in $Poes_{twCMS}$ increased progressively as a function of exercise time (Figure 8.5, *panel A*). These data are speculatively interpreted as evidence of an early reduction in diaphragm contractility followed by a progressive recruitment and fatigue of the accessory inspiratory ribcage muscles, which would support the potential link between the development of diaphragm fatigue and the change in the breathing pattern observed during exercise. Indeed, previous data suggests that during exercise the relative contribution of the diaphragm to ventilation decreases over time, and that the diaphragm increasingly functions as a flow- rather than a pressure-generator (Aliverti et al., 1997). Moreover, pre-fatigue of the diaphragm elicits an increase in sternocleidomastoid EMG activity during subsequent constant-power exercise, but no change in diaphragm EMG activity in comparison to control conditions (Boyle et al., 2020). From 50% to 100% of T_{LIM} in the present study, PTP_{di} was similar whereas PTP_{oes} increased progressively (Table 8.1). An early but consistent impairment in diaphragm contractility during exercise is also consistent with the relatively rapid decrease and plateau in Pdi_{tw} exhibited in response to volitional hyperpnoea and inspiratory resistive breathing that precedes task failure (Renggli et al., 2008, Laghi et al., 1998, Bellemare and Bigland-Ritchie, 1987, Hamnegard et al., 1996) and early

suggestions that cumulative diaphragm work is not well correlated to the magnitude of diaphragm fatigue during maximal exercise (Johnson et al., 1993).

8.5.3. Consequences of 'Pre-Terminal' Diaphragmatic Fatigue

Although we and others have presented a similar time-course for the development of diaphragm fatigue to the increased accessory inspiratory muscle contribution to exercise hyperpnoea, it remains to be determined whether muscle fatigue *per se* causes the relative derecruitment of the diaphragm during maximal exercise, potentially via a 'reflexive inhibition' that limits further recruitment to prevent a further decline in contractility (Johnson et al., 1993). For example, it is well established that phrenic afferent discharge increases in response to metabolite infusion (lactic acid) into the phrenic artery and in response to electrically-evoked fatiguing contractions of the diaphragm in anaesthetised canines and rats, respectively (Rodman et al., 2003, Hill, 2000). Similarly, it is hypothesised that metabolite-induced stimulation of group III/IV phrenic afferents is responsible for the increase in sympathetic outflow and consequent peripheral vasoconstriction (i.e. respiratory muscle metaboreflex) exhibited in healthy humans performing fatiguing inspiratory resistive loading (Sheel et al., 2001, Smith et al., 2016, St Croix et al., 2000). In anaesthetised cats, an electrically-evoked increase in phrenic afferent discharge modulated ventilatory timing and reduced the number of efferent impulses in the contralateral phrenic nerve, likely via an inhibition of diaphragm motor drive secondary to an effect on the central respiratory controllers (Jammes et al., 1986). Similarly, observations that the electrically-evoked fatigue-induced reduction in diaphragm EMG and increased parasternal EMG activity were abolished by phrenic section have led to the suggestion that phrenic afferent activity has an inhibitory effect on diaphragm motor drive (Supinski et al., 1989). Although speculative, it remains possible that, secondary to metabolite-induced phrenic afferent stimulation, diaphragmatic fatigue *per se* could inhibit further diaphragm recruitment during high-intensity constant-

power exercise as a protective mechanism to prevent ventilatory failure. Although we do not have a direct measure of diaphragm metabolic perturbation in the present study, systemic arterial blood lactate concentration was elevated prior to exercise termination (Table 8.1) and in combination with clear evidence of diaphragmatic fatigue, is suggestive of an accumulation of metabolic by-products in the diaphragm circulation. Indirect support for this mechanism may also be gleaned from recent studies of sex differences in the development exercise-induced diaphragmatic fatigue. Indeed, in response to exhaustive constant-power exercise males exhibited both a greater reduction in the diaphragm contribution to ventilation (PTP_{di}/PTP_{oes}) and an augmented severity of diaphragmatic fatigue in comparison to females (Guenette et al., 2010). Therefore it could be speculated that the more fatigue-resistant female diaphragm is better able to delay or prevent the accumulation of fatigue-associated metabolites and the consequent phrenic afferent stimulation that may lead to inhibition to diaphragm motor drive.

8.5.4. Time Course of Exercise-Induced Expiratory Muscle Fatigue

The development of expiratory abdominal muscle fatigue in response to severe-intensity whole-body exercise is relatively well established (Taylor et al., 2006, Taylor and Romer, 2008, Verges et al., 2006); however, to the authors' knowledge no previous study has measured the time course of its development. In the present study, the magnitude and prevalence of expiratory muscle fatigue increased as a function of exercise time (Figure 8.4). Similarly to the inspiratory muscles, these data demonstrate the importance of the duration of elevated expiratory muscle work (i.e. cumulative force history) in determining the magnitude of exercise-induced expiratory muscle fatigue. Indeed, concomitant with the progressive decrease in Pga_{tw}, cumulative PTP_{ga} increased substantially from 50% to 75% to 100% of T_{LIM} (Figure 8.2). Similarly, previous studies have reported a significant relationship between the cumulative force output of the expiratory muscles and the magnitude of expiratory

muscle fatigue (r = −0.73), in response to severe-intensity constant-power exercise in healthy males (Taylor et al., 2006). Although the author is unaware of any previous data investigating the time-course of expiratory muscle fatigue during exercise, previous studies have measured the progression of expiratory muscle fatigue during submaximal isocapnic hyperpnoea. Indeed, in response to volitional hyperpnoea at ~70% of maximal voluntary ventilation, Renggli et al. (2008) reported a decrease in Pga_{tw} of ~20% after 8 min, that did not further decline by task failure (~25 min). However, unlike submaximal isocapnic hyperpnoea, the expiratory abdominal muscles contribute progressively to the increased demands for ventilation during exercise. Indeed, in the present study end-exercise PTP_{ga} increased by ~200 $cmH_2O \cdot s \cdot min^{-1}$ from 50% to 100% of T_{LIM} and previous data show that EMG activity of the external oblique increases over time in parallel with the time dependent rise in oxygen uptake during constant-power exercise (Abraham et al., 2002). This increased expiratory muscle recruitment in combination with an increasingly limited supply of cardiac output during exercise likely explains the difference in the 'progression' of expiratory muscle fatigue in comparison to rested isocapnic hyperpnoea.

In the present study, the $Poes_{tw}$ response to thoracic nerve stimulation was inspected to gain insight into the relative progression of accessory expiratory ribcage muscle fatigue during exercise. The author is unaware of any previous study that has used this technique, but it is well established that the expiratory intercostal muscles are innervated from the intercostal nerves in the thoracic region of the spinal cord (~T4-T11) (De Troyer et al., 2005), and a highly positive $Poes_{tw}$ response was observed in response to thoracic nerve stimulation in the present study. Interestingly, this measure of accessory expiratory ribcage muscle fatigue demonstrated substantially less 'progression' during exercise in comparison to the magnitude of expiratory abdominal muscle fatigue, and $Poes_{twTMS}$ was not significantly different at 50%, 75%

or 100% of T_{LIM} (Figure 8.5, *panel D*). Such findings may relate to the specific recruitment pattern of the expiratory muscles during exercise. In otherwise resting individuals exposed to hyperoxic hypercapnia, the expiratory contribution to the increased ventilatory demand is primarily and foremost mediated by activity of the transversus abdominis, prior to the gradual recruitment of the more superficial and accessory expiratory abdominal muscles (De Troyer et al., 1990, Abe et al., 1996). In contrast, during high-intensity constant-power exercise the more superficial accessory expiratory muscles are recruited almost immediately, most likely to assist the maintenance of posture and to maximise locomotor performance (Abraham et al., 2002). Therefore, the early and non-progressive fatigue of the expiratory accessory muscles may relate to the performance of additional non-ventilatory functions.

8.5.5. Differences in Exercise-Induced Expiratory vs. Inspiratory Muscle Fatigue

In the present study, it was hypothesised that based on a lower resistance to fatigue and the additional non-ventilatory functions performed during exercise (Gandevia et al., 1983, Urquhart et al., 2005), the expiratory abdominal muscles would fatigue earlier and to a greater extent during exercise than in comparison to the inspiratory muscles. Despite the absence of a significant difference in the magnitude of expiratory vs. inspiratory muscle fatigue between trials, expiratory muscle contractility was impaired in the majority of subjects (67%) after only ~5 min of submaximal exercise (50% T_{LIM}) compared to ~7.5 min (75% T_{LIM}) for the inspiratory muscles (Figure 8.4). Therefore, expiratory muscle fatigue was more prevalent at an earlier time point during exercise in comparison to inspiratory muscle fatigue. These findings are highly comparable to fatiguing resistive loading protocols, whereby the ability to generate maximal pressure falls at a substantially faster rate for the expiratory vs. inspiratory muscles (Gandevia et al., 1983). Similarly, in this thesis the group mean time to task failure was considerably shorter (~45%) in response to expiratory vs.

inspiratory resistive loaded breathing, presumably due to the augmented rate of fatigue (*see* Chapters 5 and 6).

8.5.6. Implications

It is well established that the development of inspiratory and expiratory abdominal muscle fatigue may limit exercise tolerance in healthy individuals primarily via an increased perception of dyspnoea and/or the activation of a respiratory muscle metaboreflex (Romer and Polkey, 2008). Our findings that the development of inspiratory and expiratory muscle fatigue occur prior to exercise tolerance, at ~75% and 50% of T_{LIM} respectively, have important implications for interventional studies aiming to delay or attenuate the development of respiratory muscle in order to improve exercise performance. For example, previous studies suggest that inspiratory muscle training attenuates the cardiovascular response associated with the inspiratory muscle metaboreflex and blunts the impairment in peripheral locomotor fatigue (Witt et al., 2007, McConnell and Lomax, 2006). Similarly, improvements in exercise performance following inspiratory muscle training have been associated with a decreased magnitude of inspiratory muscle fatigue at exercise intolerance (Romer et al., 2002). However, in addition to an attenuation of its magnitude, it remains to be empirically determined whether respiratory muscle training is able to *delay* the progression of exercise-induced respiratory muscle fatigue, and to postpone the occurrence of task-limiting fatigue-induced cardiovascular or dyspnoegenic effects. Due to the earlier development of expiratory muscle fatigue during exercise, and the well-documented expiratory contribution to a respiratory muscle metaboreflex (*see section* 2.6.2), the data presented in this thesis highlight the need for future studies investigating the effect of expiratory muscle training on exercise tolerance in healthy individuals. In addition, heart failure and chronic obstructive pulmonary disease patients exhibit respiratory muscle weakness, and during exercise a heightened perception of dyspnoea, a greater prevalence of

ventilatory constraint and an increased power of breathing for a given ventilation (Cross et al., 2012, Johnson et al., 2000, Meyer et al., 2001, Ferrari et al., 1997). Therefore, it remains possible that the development of exercise-induced respiratory muscle fatigue occurs more abruptly for such patients in comparison to healthy individuals, although this hypothesis is yet to be investigated.

8.5.7. Conclusion

Expiratory and inspiratory muscle contractility are impaired relatively early during severe-intensity constant-power exercise, and the magnitude of inspiratory and expiratory abdominal muscle fatigue increases progressively towards exercise intolerance. From additional analyses of the transdiaphragmatic twitch response, it is proposed that the magnitude of diaphragmatic fatigue remains at a similar magnitude from early during exercise to exercise intolerance and that the progressive reduction in global inspiratory muscle fatigue is caused by the recruitment and consequent fatigue of the accessory inspiratory ribcage muscles, a mechanism that speculatively may serve to limit further impairments in diaphragm function. There was a greater prevalence of expiratory vs. inspiratory muscle fatigue early during exercise, which is consistent with the less fatigue-resistant phenotype of the expiratory abdominal muscles, and the early recruitment of accessory expiratory ribcage muscles to aid non-ventilatory functions.

CHAPTER 9
General Discussion

This chapter will summarise the main findings of the present thesis. Within the context of the existing literature, it will highlight the important considerations and critically discuss the key implications of the presented data, and will suggest future research directions.

9.1. SUMMARY OF FINDINGS

9.1.1. The Cardiovascular Consequences of Fatiguing Expiratory and Inspiratory Muscle Work in Healthy Otherwise Resting Humans

The original aim of Chapters 5 and 6 of this thesis was to compare and contrast the time-course and magnitude of the cardiovascular response to fatiguing expiratory vs. inspiratory muscle work in the resting human. Indeed, previous evidence had suggested that inspiratory resistive loaded breathing (IRL) performed until task failure in otherwise resting individuals evokes a metabolically-induced sympathoexcitation, characterised by a time-dependent increase in muscle sympathetic nerve activity (MSNA), mean arterial pressure (MAP), and limb vascular resistance (LVR), and a reduction in limb blood flow ($\dot{Q}_L$) (Croix et al., 2000, Sheel et al., 2001, Smith et al., 2016). It was reported that fatiguing expiratory resistive loading (ERL) also induced a time-dependent increase in MSNA and MAP, but the effects on vascular resistance and blood flow in the peripheral limb were unknown (Derchak et al., 2002). In this thesis, despite the development of respiratory muscle fatigue and a somewhat time-dependent increase in MAP, there was no reduction in group mean $\dot{Q}_L$ or increase in group mean LVR in response to ERL *or* IRL, even when specifically targeting the expiratory abdominal muscles or the diaphragm, respectively. However, upon further analyses, it was clear that there was a cyclical within-breath fluctuation $\dot{Q}_L$ in response to ERL, with alternating periods of high antegrade $\dot{Q}_L$ (*i.e. positive or forward flow*) during resisted expirations and high retrograde $\dot{Q}_L$ (*i.e. negative or*

backward flow) during unresisted inspirations. It is speculated that the mechanical effect(s) of generating very-high intra-abdominal pressures may have superseded the underlying metabolically-induced peripheral vasoconstriction.

9.1.2. The Effect of Exercise Intensity and Tolerable Duration on the Development of Inspiratory and Expiratory Abdominal Muscle Fatigue

The primary aim of Chapter 7 was to compare and contrast the prevalence and magnitude of exercise-induced inspiratory and expiratory abdominal muscle fatigue. Using magnetic stimulation of the cervical and thoracic nerve roots and interrogation of the transdiaphragmatic and gastric twitch pressure response, respectively, the magnitude of exercise-induced inspiratory and expiratory muscle fatigue was assessed in response to exhaustive heavy-intensity exercise and short- (~5 min) and long-duration (~10 min) severe-intensity exercise. Exercise-induced inspiratory muscle fatigue was evident in response to all exercise trials, but was of a greater magnitude in response to prolonged severe-intensity exercise, demonstrating the importance of the cumulative work and power of breathing and the degree of the competition for cardiac output. In contrast, exercise-induced expiratory muscle fatigue was of a substantial but similar magnitude across all exercise trials. These differing inspiratory vs. expiratory responses are likely explained by the less fatigue-resistant phenotype and/or the additional non-ventilatory roles of the expiratory muscles during exercise.

9.1.3. The Time-course of the Development of Exercise-induced Inspiratory and Expiratory Muscle Fatigue

The aim of Chapter 8 was to compare and contrast the time-course (*or 'temporality'*) of the development of exercise-induced inspiratory and expiratory muscle fatigue. Because it is technically very challenging to accurately and reliably assesses respiratory muscle function via magnetic stimulation of the cervical and thoracic

nerve roots *during* exercise due to methodological constraints (e.g. controlling for lung volume), fatigue was measured in response to three separate trials that were terminated at 50%, 75% and 100% of prolonged severe-intensity exercise time. Exercise-induced expiratory muscle fatigue was more prevalent at an earlier time point than inspiratory muscle fatigue during exercise (50% vs. 75% T_{LIM}), but the magnitude of both expiratory and inspiratory muscle fatigue increased towards exercise intolerance. Based on the methods of Similowski et al. (1998), the oesophageal ($Poes_{tw}$) and gastric (Pga_{tw}) twitch components of the cervically-stimulated transdiaphragmatic twitch (Pdi_{tw}) response were also inspected to gain further insight into the relative contribution of accessory inspiratory ribcage muscle fatigue vs. diaphragm muscle fatigue as exercise duration progressed. The magnitude of exercise-induced diaphragmatic fatigue was similar in response to increasing exercise duration (i.e. percentage of T_{LIM}), and there was a clear plateau in the impairment in diaphragm contractility from 75% to 100% of T_{LIM}. This pattern of diaphragmatic fatigue was mirrored by the temporality of 'derecruitment' of the diaphragm during exercise (i.e. decrease in inspiratory PTP_{di}/PTP_{oes}). In contrast, fatigue of the accessory inspiratory ribcage muscles was more progressive, increasing from 50% to 75% T_{LIM}, and further still to 100% T_{LIM}. This response likely reflects the progressive and increased ribcage contribution to ventilation during exercise. In combination with previous research, it was speculated that diaphragm fatigue *per se* may be a potential mechanism that triggers its own 'reflexive inhibition', thereby reducing the diaphragm contribution to pressure generation. In support of this mechanism, previous data show that an electrically-evoked increase in phrenic afferent discharge modulates ventilatory timing in anesthetised cats, potentially via an inhibition of diaphragm motor drive secondary to an effect on the central respiratory controllers (Jammes et al., 1986).

9.2. CONSIDERATIONS FOR THE ACTIVATION OF A RESPIRATORY MUSCLE METABOREFLEX

9.2.1. Factors Affecting the Development of Inspiratory and Expiratory Abdominal Muscle fatigue

The development of exercise-induced inspiratory muscle fatigue appears to be caused by a high and sustained power of breathing and a limitation in the supply of blood flow to the diaphragm. For example, unloading the inspiratory power of breathing during severe-intensity exercise abolishes the development of exercise-induced diaphragmatic fatigue (Babcock et al., 2002). Similarly, the development of diaphragmatic fatigue does not occur when the breathing pattern ($\int Pdi \cdot f_R$) observed during exercise is mimicked at rest, most likely because of an adequate supply of blood flow to the diaphragm in the absence of competition from the exercising locomotor muscles (Babcock et al., 1995b). Moreover, when a high diaphragm force output is sustained for only a short duration (e.g. ramp incremental exercise), exercise-induced diaphragmatic fatigue does not develop (Romer et al., 2007). The data from this thesis support the importance of the cumulative diaphragmatic force output, the power of breathing, and the availability of cardiac output on the presence and severity of exercise-induced inspiratory muscle fatigue. Indeed, it was demonstrated that the magnitude of inspiratory muscle fatigue was greatest in response to prolonged severe-intensity exercise, whereby the power of breathing is elevated for a considerable time-period and cardiac output is limited due to the locomotor muscle blood flow demand (*see* Figure 9.1). Based on the linear relationship between cardiac output and $\dot{V}O_2$ (Saltin and Calbet, 2006), cardiac output is likely similarly limited during short-duration severe-intensity exercise; however, the shorter time spent at an elevated power of breathing (<5 min) may result in less time available for the metabolites associated with diaphragm ischemia to accumulate, eliciting a lower magnitude and prevalence of exercise-induced inspiratory muscle fatigue (*see* Figure 9.1). In contrast, during heavy-intensity

exercise, the cumulative diaphragm force output is very-high but the lower power of breathing and the absence of a competition for cardiac output between the locomotor and respiratory muscles (Wetter et al., 1999) results in a blunted magnitude and lower consistency of inspiratory muscle fatigue (*see* Figure 9.1).

By comparison, far less is known about the factors contributing to the development of exercise-induced expiratory muscle fatigue. To date no study has measured the presence and/or magnitude of expiratory muscle fatigue in response to exercise whereby the expiratory power of breathing has been manipulated, or in response to mimicking the expiratory breathing pattern exhibited during exercise ($\int Pga \cdot f_R$) in otherwise resting individuals. Therefore, the importance of the expiratory power of breathing and the competition for cardiac output for the development of expiratory muscle fatigue is less well understood. The findings in Chapter 7 of this thesis suggest that the fatigue threshold is lower for expiratory vs. the inspiratory muscles; indeed the magnitude of exercise-induced expiratory vs. inspiratory muscle fatigue tended to be higher in response to heavy-intensity and short-duration severe-intensity exercise. Therefore, in order to develop fatigue during exercise, the expiratory muscles may require only a relatively short time period (i.e. <5 min) at a very high power of breathing, or a sustained duration at a moderate power of breathing (>30 min) (*see* Figure 9.1). This lower 'fatigue threshold' may be explained by the lower resistance to fatigue and/or the additional non-ventilatory roles performed by the expiratory abdominal muscles during exercise (Gandevia et al., 1983, Uribe et al., 1992, Cresswell et al., 1992, Abraham et al., 2002). Moreover, the responsiveness of the expiratory abdominal arterioles to increased sympathetic motor-outflow remains unknown. It is possible that expiratory muscle arterioles are more responsive than the diaphragm to adrenergic stimuli (e.g. more similar to peripheral skeletal muscle), and therefore are less able to maintain blood flow when global sympathetic outflow is increased.

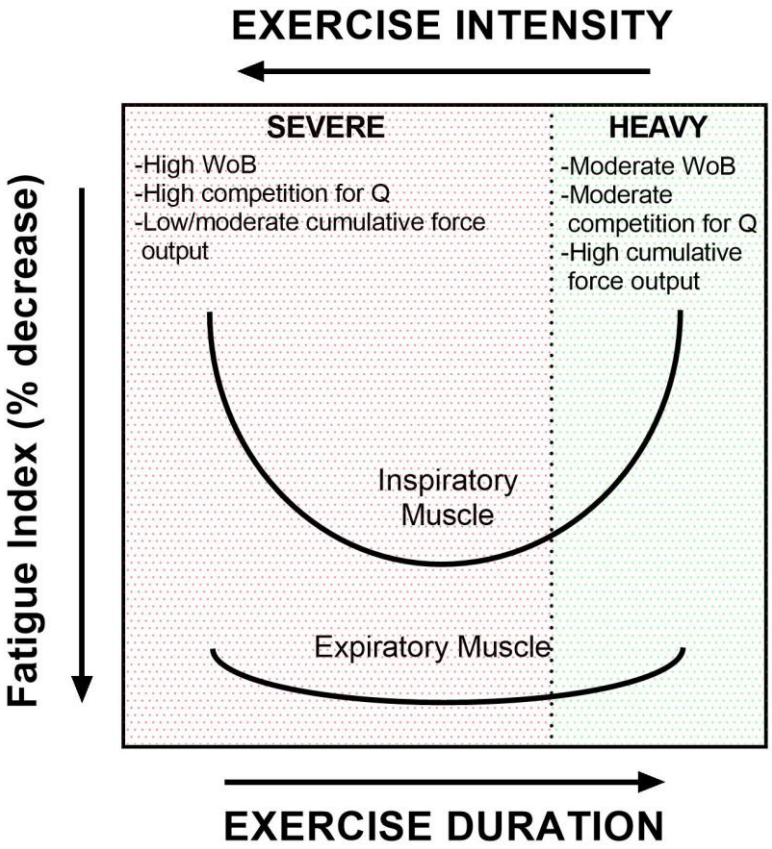


Figure 9.1. A schematic demonstrating the role of exercise intensity and tolerable duration on the development of exercise-induced inspiratory and expiratory abdominal muscle fatigue. WoB, work of breathing; Q, cardiac output.

9.2.2. Is Respiratory Muscle Fatigue Essential for the Activation of a Respiratory Muscle Metaboreflex?

The sympathetically-mediated cardiovascular consequences of a high power of breathing have traditionally been attributed to the development of respiratory muscle fatigue (Dempsey et al., 2006). Indeed, the concept of a respiratory muscle metaboreflex is that fatigue-induced metabolic perturbation is responsible for the increased group III/IV respiratory muscle afferent firing rate, and the consequent decrease in locomotor limb blood flow and the rise in limb vascular resistance secondary to an increase in sympathetic outflow. In support of this mechanism, it is generally accepted that in response to IRL in otherwise resting individuals, there is a 'time-dependency' in the sympathetically-mediated cardiovascular responses (~2-3

min) thought to reflect the time taken for fatigue and metabolite accumulation to occur (Sheel et al., 2001, Smith et al., 2016). However, it remains doubtful that respiratory muscle fatigue (and particularly inspiratory muscle fatigue) develops at such a fast rate. Indeed, in response to 6 minutes of brief sustained maximal diaphragmatic contractions performed at an even higher TTI than previous IRL protocols (0.50 vs. 0.42) (Sheel et al., 2001), the ability to volitionally generate maximal inspiratory pressure is remarkably well preserved (i.e. 92% of baseline values, indicative of no inspiratory muscle fatigue) (Gandevia et al., 1983). Similarly, the alterations in limb locomotor blood flow and vascular resistance that are observed during near-maximal exercise (>95% $\dot{V}O_{2max}$) when the power of breathing is reduced or increased, occur rapidly (i.e. within 2.5 min) (Harms et al., 1997). From previous data and findings from this thesis, it is highly likely that such cardiovascular responses occur prior to the development of substantial inspiratory muscle fatigue. Indeed, in agreement with data presented by Archiza et al. (2018), diaphragmatic fatigue was not evident until ~75% of the tolerable exercise duration of prolonged severe-intensity exercise in this thesis (i.e. ~7.5 min), and was present but only to a relatively modest degree (−14 ± 12%) in response to exhaustive shorter duration severe-intensity exercise (5 min). Therefore, in combination with previous findings the data from this thesis suggests that the development of respiratory muscle fatigue may not be essential for the activation of a respiratory muscle metaboreflex.

9.2.3. Does a respiratory muscle blood flow ‘steal’ actually occur?

It has previously been hypothesised that activation of a respiratory muscle metaboreflex elicits a redirection of blood flow (i.e. a ‘blood flow steal’) from the locomotor muscles towards the respiratory muscles during maximal exercise, potentially as a protective mechanism to prevent further respiratory muscle fatigue (Seals, 2001). Owing to the complex anatomical arrangement and extensive vascular network, direct measurement of respiratory muscle blood flow *in vivo* in humans is

difficult. However, several studies have now indirectly assessed blood flow in the accessory respiratory muscles via near-infrared spectroscopy in combination with indocyanine green dye (NIRS + ICG). Using this technique, Vogiatzis et al. (2009) measured intercostal and quadriceps muscle blood flow in response to incremental cycling exercise and rested volitional isocapnic hyperpnoea at the same ventilation and power of breathing as observed during exercise. Interestingly, despite a progressive increase in intercostal muscle blood flow in line with ventilation and the power of breathing during isocapnic hyperpnoea, intercostal and quadriceps muscle blood flow plateaued at ~60% of peak power output during incremental exercise, and decreased from ~80% peak power towards end-exercise (Figure 9.2) (Vogiatzis et al., 2009). These data suggest that blood flow during maximal exercise is not preferentially redirected towards the intercostal muscles; instead when cardiac output approaches maximum capacity the circulatory system is unable to meet the demands of both the respiratory and locomotor muscles, potentially due to the requirement to maintain blood pressure at an adequate level. Moreover, evidence suggests that during exercise in hypoxic conditions the respiratory muscles are not able to access a greater share of total blood flow, despite a higher demand to meet the O_2 requirements. For example, Vogiatzis et al. (2008) reported that in response to exercise matched for tidal volume, respiratory frequency and ventilatory load in hypoxia (F_IO_2 = 0.13) vs. normoxia, intercostal muscle blood flow was not different (54 ± 9 vs. 50 ± 6 ml·[100 ml·min^{-1}]·min^{-1}), despite a greater magnitude of diaphragmatic fatigue (−33 ± 5% vs. −26 ± 4%). Thus, despite the similar energy requirement, blood flow to the intercostal muscles did not compensate for the reduction in O_2 content. Additionally, the similar kinetics of the tissue O_2 saturation and tissue haemoglobin concentration in the intercostal and quadriceps muscles during maximal exercise at sea level vs. at 4350 m altitude (i.e. conditions favouring a competition for cardiac output), suggest that the respiratory muscles do not preferentially access a greater proportion of blood flow when cardiac output and O_2

supply are limited (de Bisschop et al., 2014). However, in a more recent study using NIRS + ICG to measure respiratory and quadriceps blood flow, it was reported that in response to severe-intensity constant-power exercise reducing the inspiratory power of breathing caused an increase in locomotor blood flow and a decrease in sternocleidomastoid blood flow; conversely increasing the inspiratory power of breathing elicited a decrease in quadriceps blood flow and an increase in sternocleidomastoid blood flow (Dominelli et al., 2017). Therefore, there are conflicting data regarding the ability of the respiratory muscles to preferentially compete for a greater proportion of cardiac output than the locomotor muscles during exercise conditions promoting a substantial increase in respiratory muscle power. It is possible that there may be some asynchrony in the distribution of blood flow within the respiratory muscles (e.g. sternocleidomastoid vs. scalenes vs. intercostal muscles). Indeed, although the second order arterioles of the diaphragm are less responsive to adrenergic stimuli compared to peripheral skeletal muscle (Aaker and Laughlin, 2002), the responsiveness of the accessory respiratory musculature to sympathetic stimuli is unknown.

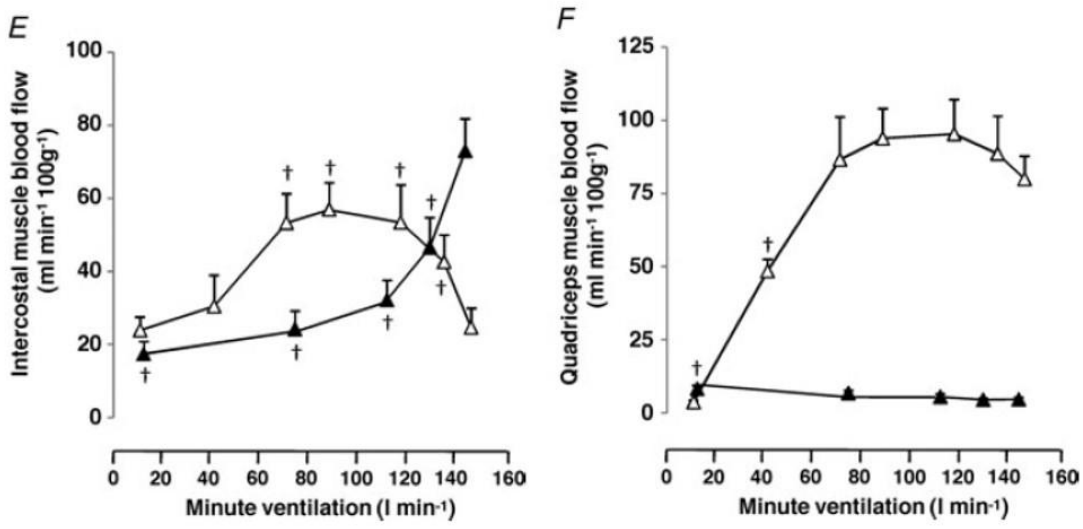


Figure 9.2. The intercostal (E) and quadriceps (F) muscle blood flow responses to incremental exercise (white symbols) and isocapnic hyperpnoea (black symbols). Figure adapted from (Vogiatzis et al., 2009).

9.3. EXERCISE-INDUCED RESPIRATORY MUSCLE FATIGUE IN CLINICAL POPULATIONS

In Chapter 7 of this thesis, it was demonstrated that respiratory muscle fatigue develops in response to a lower exercise intensity than previously hypothesised (i.e. <85% of $\dot{V}O_{2max}$). These findings may have important implications for clinical populations who exhibit respiratory muscle weakness, an increased power of breathing for a given ventilation, and an increased prevalence of expiratory flow limitation, which may predispose such individuals to the development of exercise-induced respiratory muscle fatigue at relatively low exercise intensities. For example, maximal inspiratory and expiratory pressure generation at the mouth may be up to 50% lower in heart failure (HF) and chronic obstructive pulmonary disease (COPD) patients in comparison to healthy age-matched control subjects (Hammond et al., 1990, Meyer et al., 2001, Gosselink et al., 1996, Ferrari et al., 1997). During exercise, HF and COPD patients exhibit an augmented and inefficient ventilatory response, frequently develop expiratory flow limitation and/or dynamic hyperinflation, and the overall power of breathing is vastly increased versus healthy individuals (Johnson et al., 2000, Cross et al., 2012).

9.3.1. Evidence for the Development of Exercise-induced Respiratory Muscle Fatigue

Several studies have reported the absence of diaphragm fatigue in HF or COPD patients in response to incremental exercise (Dayer et al., 2006, Mancini et al., 1992, Hopkinson et al., 2010, Polkey et al., 1995), or exhaustive constant-power exercise at 60-70% of peak power (Kufel et al., 2002a, Mador et al., 2000). However, some previous studies have used non-potentiated twitches to detect the development of respiratory muscle fatigue in combination with short recovery periods (10-20 minutes post-exercise); therefore the lower sensitivity to detect fatigue and the potential for residual exercise-induced potentiation may have led to an underestimation of the

magnitude of fatigue (Kufel et al., 2002b). For example, in the study by Mador et al. (2000), cervically-stimulated Pdi_{tw} actually increased following exercise in 6/12 patients with COPD, suggesting that diaphragm muscle potentiation incurred from the ventilatory response to exercise may have still been present at the post-exercise assessment of Pdi_{tw}. Additionally, the absence of respiratory muscle fatigue in such previous studies may relate to the exercise intensity and modality prescribed. Indeed, the findings in this thesis support previous suggestions that diaphragmatic fatigue is augmented during prolonged severe- vs. heavy-intensity exercise (*see* Chapter 7) and that the magnitude and prevalence of inspiratory muscle fatigue progresses with increased tolerable exercise durations (*see* Chapter 8). Therefore, similarly to healthy individuals, the absence of respiratory muscle fatigue in HF and COPD patients in previous studies may relate to the short time spent at a high power of breathing during incremental exercise (Romer et al., 2007), and the lower intensity of respiratory muscle work and less intense competition for cardiac output exhibited during heavy-intensity exercise. Indeed, in the only study to have performed severe-intensity constant-power exercise (80% of peak power output) in COPD patients, Bachasson et al. (2013) reported a 31 ± 15% reduction in Pdi_{tw} from pre- to post-exercise. To date, only two studies have measured the development of exercise-induced expiratory abdominal muscle fatigue objectively in HF or COPD patients. Hopkinson et al. (2010) reported a significant reduction in Pga_{tw} in response to incremental exercise in COPD patients (51.3 ± 27.1 vs. 47.4 ± 25.2 cmH_2O), despite the absence of diaphragmatic fatigue, although only 8/15 patients exhibited a reduction of >10% in Pga_{tw}. These findings are consistent with the increased susceptibility of the expiratory muscles to fatigue at lower exercise intensities (*see* Chapter 7) and with a shorter period at an elevated power of breathing (*see* Chapter 8) in comparison to the diaphragm. In contrast, in response to severe-intensity constant-power exercise in COPD patients, group mean Pga_{tw} was not different to pre-exercise values, and a decrease in Pga_{tw} of >10% occurred in only ~25% of subjects (Bachasson et al.,

2013). Therefore, although there is some limited evidence supporting the presence of exercise-induced respiratory muscle fatigue in HF and COPD patients, the balance of previous data remains inconclusive, most likely due to methodological considerations of several previous studies including the prescription of exercise modality and the protocol used to assess respiratory muscle function. Importantly, respiratory muscle weakness in patients is associated with poor exercise capacity and a worse prognosis (Meyer et al., 2001); this effect may be at least in part mediated by a greater/more abrupt development of exercise-induced respiratory muscle fatigue, and the associated consequent cardiovascular effects (i.e. activation of a respiratory muscle metaboreflex). Therefore, it is recommended that future studies assess the presence/absence of exercise-induced respiratory muscle fatigue in patient populations by prescribing prolonged severe-intensity constant-power exercise, and using sensitive objective measures of respiratory muscle function (i.e. the potentiated twitch response to stimulation of the phrenic or thoracic nerve roots).

9.3.2. Evidence that Exercise-induced Respiratory Muscle Fatigue may limit Exercise Tolerance in Clinical Populations

The high degree of inter-subject variability in the development of diaphragmatic and expiratory muscle fatigue in HF and COPD may relate to the specific breathing pattern adopted during exercise. Indeed, the percent reduction in Pdi_{tw} and Pga_{tw} is negatively correlated to the change in IC during exercise in COPD patients (Bachasson et al., 2013), suggesting that the magnitude of diaphragm and expiratory abdominal muscle fatigue is greater in those individuals who exhibit a lesser degree of dynamic hyperinflation. Although the mechanisms remain unclear, it has been suggested that dynamic hyperinflation could be a protective mechanism, ameliorating the development of diaphragm fatigue by shortening the operating length of the diaphragm (Clanton et al., 1993). Moreover, terminal end-expiratory lung volume (EELV) in HF patients' is negatively associated with post-exercise leg fatigue;

therefore individuals with less dynamic hyperinflation exhibit more locomotor muscle fatigue (Butcher et al., 2009). It could be speculated that patients who were not able to limit the rise in EELV via increased abdominal muscle recruitment were subject to increased sensations of dyspnoea, secondary to accessory respiratory muscle recruitment and consequent distortion of the chest wall, decreased mechanical efficiency of breathing, and an increased respiratory muscle metabolic demand (Grimby et al., 1976, Dodd et al., 1988, Sheel and Romer, 2012). Such increased sensations of dyspnoea may contribute to earlier exercise termination in these patients, preventing the accumulation of work required to elicit the development of respiratory and locomotor muscle fatigue. In support of this, in response to heliox administration, which attenuates the development of ventilatory constraint by lowering turbulent airflow and airway resistance (Sheel and Romer, 2012), patients who exhibited a greater magnitude of dynamic hyperinflation in room air conditions were more likely to lower end-inspiratory lung volume and increase exercise tolerance, which resulted in an augmented severity of locomotor muscle fatigue (absent in these patients during room air exercise) (Butcher et al., 2009). As such, it remains possible that at least in some patients, exercise may be terminated before respiratory muscle fatigue develops, limited by intolerable sensations of dyspnoea secondary to the development of dynamic hyperinflation.

9.4. THESIS CONSIDERATIONS

9.4.1. Cervical vs. Bilateral Phrenic Nerve Stimulation

In this thesis, the transdiaphragmatic twitch pressure response (Pdi_{tw}) to cervical magnetic nerve stimulation (CMS) was used to objectively assess diaphragm and inspiratory ribcage muscle contractility before and after IRL and exhaustive whole-body exercise. One concern with using CMS rather than bilateral electrical or magnetic stimulation of the phrenic nerve is that due to coactivation of accessory ribcage muscles the diaphragm is not isolated (Laghi et al., 1996), and the likelihood

of supramaximality is reduced. Despite this concern, the Pdi_{tw} response to CMS in the present thesis was consistently supramaximal across subjects (*see section* 4.3); this finding is consistent several other previous works (Archiza et al., 2018, Welch et al., 2018b, Ramsook et al., 2020). Moreover, that using CMS allowed for the recruitment and objective assessment of inspiratory ribcage muscle function was considered as a strength of the present research. Indeed, using CMS enabled discrimination between the development and progression diaphragmatic vs. accessory inspiratory ribcage muscle fatigue during exercise (Chapter 8). This technique therefore facilitated the novel findings of this thesis that exercise-induced diaphragmatic fatigue occurs relatively early, but remains at a similar magnitude as exercise time progresses, whereas the magnitude of accessory inspiratory ribcage fatigue increases progressively as a function of exercise duration, likely due to progressive accessory muscle recruitment to meet the ventilatory requirements of exercise as diaphragm recruitment is inhibited (Aliverti et al., 1997).

9.4.2. Considerations of Resistive Loaded Breathing Trials. Does ERL and IRL Mimic Exercise Hyperpnoea?

The primary aim of Chapters 5 & 6 was to investigate the cardiovascular consequences of fatiguing expiratory and inspiratory muscle work, respectively, in healthy humans who were otherwise at rest. Therefore, it was critical that the experimental approach adopted allowed for isolation of inspiratory or expiratory muscle fatigue on sympathetically-induced changes in peripheral limb vasomotor tone, blood flow and vascular resistance. It is well established that simply mimicking the hyperpnoea of exercise while otherwise at rest *does not* elicit fatigue of the respiratory muscles (Babcock et al., 1995b). Indeed, likely due to the absence of a competition for available cardiac output in otherwise resting individuals, it has been shown the diaphragm pressure time integral during voluntary hyperpnoea must be elevated to approximately twofold greater than observed during exercise in order to

evoke diaphragmatic fatigue (Babcock et al., 1995b). Such fatiguing hyperpnoea has been used as an experimental model to determine effect of respiratory muscle fatigue on subsequent exercise tolerance (Martin et al., 1982); however, voluntary hyperpnoea results in simultaneous inspiratory and expiratory muscle fatigue, and as such was not suitable to address the present research questions of this thesis.

Accordingly, IRL and ERL protocols were used to specifically elicit fatigue of the inspiratory and the expiratory muscles, respectively. One concern with this approach is that the demands placed on the inspiratory and expiratory muscles during IRL and ERL may not be representative of the normally occurring demands placed on these muscles during exercise. Indeed, peak expiratory gastric (~120 cmH_2O) and inspiratory oesophageal pressures (~−80 cmH_2O) during ERL and IRL, respectively, far exceeded values typically observed during maximal exercise in healthy individuals (+30 and −30 cmH_2O). Therefore, a limitation of the experiments in Chapters 5 & 6 is the requirement of very-high intrathoracic and intra-abdominal pressures which likely evoked consequent mechanically-induced haemodynamic effects in the resting limb far in excess of that observed during exercise (*see section* 5.4.4 and 6.5.3). As such, caution is urged when considering how the mechanical effects of ERL and IRL on cardiovascular function detailed in this thesis may impact whole-body exercise. Despite this concern, the degree of inspiratory and expiratory muscle fatigue elicited by IRL and ERL, respectively, appears to be similar to, and representative of, the magnitude of exercise-induced inspiratory and expiratory muscle fatigue (Johnson et al., 1993, Guenette et al., 2010, Taylor et al., 2006, Taylor and Romer, 2008).

9.4.3. Measurement of Cardiac Output

The measurement of cardiac output in this thesis would have been advantageous to facilitate the interpretation of the availability of cardiac output and the consequent state of competition for blood flow between the respiratory and locomotor muscles

during exercise and resistive loaded breathing tasks. In the present thesis, finger photoplethysmography was used to estimate stroke volume and cardiac output via the Modelflow algorithm (Wesseling et al., 1993). However, owing to methodological limitations of the Modelflow methodology used to derive the aortic pressure waveform during substantial alterations in intrathoracic pressure (Bogert and van Lieshout, 2005), this data was excluded from the analysis. While a measure of cardiac output would have been advantageous, the lack of such a measure did not impact the overall findings of the research.

9.5. IMPLICATIONS AND FUTURE DIRECTIONS

9.5.1. Does Exercise-induced Respiratory Muscle Fatigue Limit Heavy-intensity and Short-duration Severe-intensity Exercise?

Evidence suggests that the development of respiratory muscle fatigue contributes to the limitation of severe-intensity exercise performance. For example, pre-fatigue of the inspiratory or expiratory muscles impairs severe-intensity exercise performance by 14-33% (Taylor and Romer, 2008, Mador and Acevedo, 1991a, Welch et al., 2018a, Wuthrich et al., 2013). Similarly, reducing the inspiratory power of breathing during severe-intensity exercise abolishes diaphragmatic fatigue (Babcock et al., 2002) and prolongs exercise time by ~15% (Harms et al., 2000). Although such exercise-induced respiratory muscle fatigue could limit exercise by exaggerating the haemodynamic cardiopulmonary interaction or increasing the sensation of dyspnoea (*see section* 2.6), the preponderance of evidence suggests that fatigue may induce a sympathetically-mediated peripheral vasoconstriction that augments the development of locomotor muscle fatigue by attenuating locomotor limb blood flow and O_2 delivery (i.e. via a respiratory muscle metaboreflex).

A novel finding of this thesis was that both inspiratory and expiratory muscle fatigue occurred in response to heavy-intensity exercise, and very-short duration severe-

intensity exercise. These findings therefore raise the possibility that the performance of heavy-intensity exercise and short-duration severe-intensity exercise may also be limited by the development of inspiratory and/or expiratory muscle fatigue. However, to date no study has measured the influence respiratory muscle fatigue and/or power on exercise performance time for such trials (i.e. by pre-fatiguing or manipulating the power of breathing during exercise). Interestingly, in response to near-maximal exercise (>95% of $\dot{V}O_{2max}$) lasting for only 2.5 min, reducing the inspiratory power of breathing elicited an increase in locomotor blood flow (Harms et al., 1997), suggesting that the normally-occurring power of breathing constrains locomotor blood flow during short-duration severe-intensity exercise (i.e. via activation of a respiratory muscle metaboreflex), although the data from this thesis would question whether fatigue could develop so quickly during exercise. Similarly, reducing the inspiratory power of breathing during exercise at ~80% $\dot{V}O_{2max}$ (heavy-intensity) results in a decrease in muscle sympathetic nerve burst frequency compared to control conditions, suggesting that the normally-occurring ventilatory work of heavy exercise contributes to the rise in sympathetic outflow (Dominelli et al., 2019); whether it is the development of fatigue *per se* or a mechanical consequence of manipulating intrathoracic pressure remains to be determined. Indeed, it has been suggested that a fatigue-induced metaboreflex may not develop during the initial 15 minutes of constant-power exercise at ~75% of $\dot{V}O_{2peak}$, because of the absence of a competition for available cardiac output between the respiratory and locomotor muscles (Wetter et al., 1999). In combination with the data from this thesis, such findings may suggest that the genesis of exercise-induced respiratory muscle fatigue during heavy-intensity exercise is caused in the absence of a localised metabolic perturbation of a sufficient magnitude to incite a sympathetic efferent response. If this were the case, then respiratory muscle fatigue would not be likely to limit exercise performance, at least via a cardiovascular effect. Alternatively, it is possible that a fatigue-induced competition for cardiac output only becomes evident during the latter

stages of heavy-intensity exercise (i.e. >15 min) due to the increased force output of the respiratory muscles and greater locomotor muscle recruitment. In such conditions, respiratory muscle fatigue would be expected to limit exercise performance. Because the findings from Chapter 7 of this thesis demonstrate that the magnitude of expiratory vs. inspiratory muscle fatigue tended to be of a greater magnitude in response to heavy and short-duration severe-intensity exercise, it is possible that unloading expiratory muscle work during such exercise trials would have an even greater effect on locomotor blood flow, and potentially exercise performance.

9.5.2. Does Diaphragm Fatigue cause a Reflexive Inhibition of Diaphragm Activation?

The data from Chapter 8 of this thesis suggest that diaphragmatic fatigue develops relatively early during prolonged severe-intensity exercise (i.e. ~50-75% of the tolerable duration), and does not increase in magnitude towards exercise intolerance. Interestingly, the development of diaphragmatic fatigue occurs at a similar time-point to its 'derecruitment', whereby the increasing demands for pressure generation are fulfilled by the recruitment of the accessory inspiratory muscles (i.e. PTP_{di}/PTP_{oes} decreases). It was speculated that diaphragm fatigue *per se* may be the critical trigger for such a reflexive inhibition of diaphragm activation. Indeed, previous data also suggest that female participants exhibit a lower magnitude of diaphragmatic fatigue and also a smaller change in PTP_{di}/PTP_{oes}, and therefore less diaphragm 'derecruitment' than males during severe-intensity exercise (Guenette et al., 2010). However, it remains unclear if diaphragmatic fatigue directly causes or simply shares a similar time-course to its own reflexive inhibition. One potential method to gain further insight into the relationship between diaphragm fatigue and recruitment would be to experimentally manipulate the time-course of the development of diaphragmatic fatigue, and assess the potential effect on the time-course of changes in accessory

muscle recruitment. For example, if diaphragm fatigue does indeed evoke its own derecruitment, a period of inspiratory muscle training would be hypothesised to delay the onset of fatigue and equally post-pone the increased contribution of the accessory muscles to pressure generation. Similarly, it could be hypothesised that blocking phrenic afferent feedback via intrathecal opioid injection (e.g. fentanyl) would reduce any inhibition of diaphragm activation thereby preventing its derecruitment during exercise, and also evoking a greater magnitude of exercise-induced diaphragmatic fatigue in comparison to control conditions.

9.5.3.Expiratory Muscle Training, an Underutilised Ergogenic Resource?

A key finding from this thesis is that, compared to the diaphragm, the expiratory abdominal muscles exhibit a greater susceptibility to exercise-induced fatigue. It is relatively well established that the development of expiratory muscle fatigue limits exercise tolerance (Verges et al., 2007, Taylor and Romer, 2008), most likely via a fatigue-induced attenuation in locomotor blood flow and O_2 delivery and a hastening of the development of locomotor muscle fatigue (i.e. activation of a respiratory muscle metaboreflex) and/or an increased perception of dyspnoea. However, despite numerous investigations into the ergogenic benefit of inspiratory muscle training (IMT) (Illi et al., 2012, HajGhanbari et al., 2013), few studies have explored the utility of expiratory muscle training (EMT) as a strategy to enhance exercise performance. It is highly likely that strengthening the expiratory muscles via EMT, similarly to the ergogenic effects of IMT (Romer et al., 2002, McConnell and Lomax, 2006, Witt et al., 2007, Chiappa et al., 2008), would delay the onset of expiratory muscle fatigue and attenuate the development of locomotor muscle fatigue secondary to a blunting of the respiratory muscle metaboreflex. Moreover, a decreased fractional utilisation (secondary to increased maximal expiratory tension generating capacity) and a lesser magnitude of expiratory muscle fatigue would be expected to improve exercise tolerance by alleviating the perception of dyspnoea during exercise (Taylor and

Romer, 2008, Romer and Polkey, 2008, Suzuki et al., 1995). Therefore, with respect to the findings from this thesis it could be hypothesised that EMT would provide a greater ergogenic stimulus than IMT in for healthy individuals or athletes performing short-duration severe-intensity exercise (~5 min) or prolonged endurance tasks (>30 min).

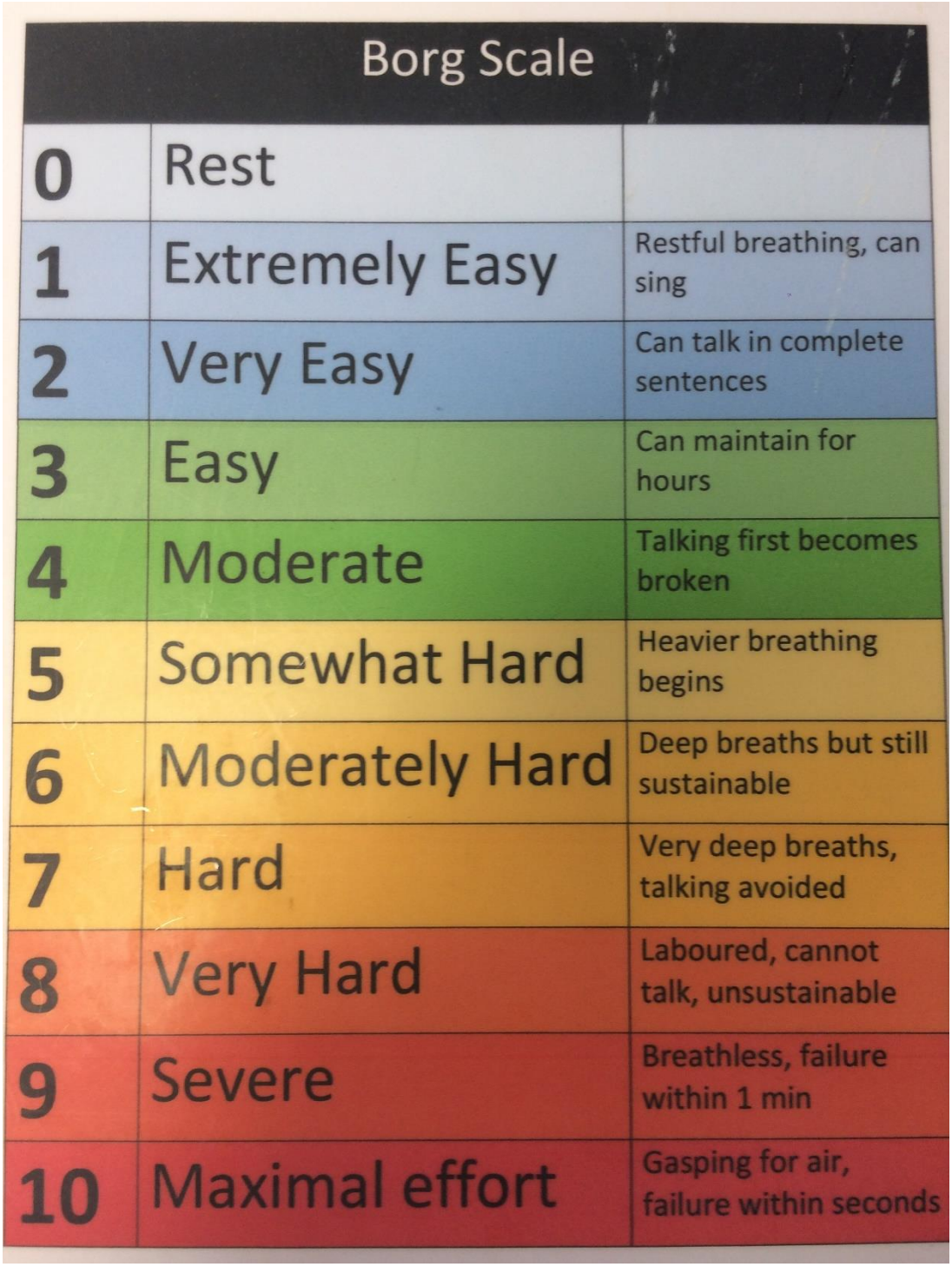
The use of EMT in chronic disease populations may also exert a particularly potent ergogenic benefit. For example, CHF and COPD patients demonstrate expiratory muscle weakness (MEP of ~50% lower vs. age-matched controls) (Hammond et al., 1990, Ferrari et al., 1997), a high prevalence of expiratory flow limitation (Johnson et al., 2000), and a vastly increased (approximately fivefold higher) expiratory resistive power of breathing for a given ventilation during exercise vs. age-matched controls (Cross et al., 2012). Additionally, unlike the diaphragm in CHF which undergoes a shift towards a more oxidative phenotype in response to chronically increased work (Tikunov et al., 1997, De Sousa et al., 2001), there is no evidence to suggest that the expiratory muscles undergo morphologic adaptations to sustain the increased work required during exercise. Therefore, the exaggerated imbalance between expiratory demand vs. capacity during exercise, and the findings of this thesis of a high susceptibility to expiratory muscle fatigue across exercise intensity domains, suggest that EMT could be particularly beneficial for improving exercise tolerance in chronic disease populations, even at relatively low exercise intensities. Indeed, in COPD patients EMT has been shown to increase six minute walk test performance (13-19%) and to evoke clinically meaningful reductions in the perception of dyspnoea during exercise (Weiner et al., 2003b, Mota et al., 2007).

Further investigation is also warranted into whether EMT and IMT can be combined to elicit superior ergogenic benefits in both healthy individuals and chronic disease populations, theoretically by delaying the onset of both inspiratory and expiratory

muscle fatigue during exercise. Indeed, combining IMT and EMT performed independently (i.e. morning vs. afternoon sessions) or concurrently by targeting inspiratory and expiratory pressures during simultaneous breaths (Wells et al., 2005) has been shown to increase both inspiratory and expiratory muscle strength. However, it remains unclear whether combining IMT and EMT evokes greater improvements in exercise tolerance than either training strategy alone (Weiner et al., 2003a).

APPENDIX I

Modified Borg Scale



Borg Scale		
0	Rest	
1	Extremely Easy	Restful breathing, can sing
2	Very Easy	Can talk in complete sentences
3	Easy	Can maintain for hours
4	Moderate	Talking first becomes broken
5	Somewhat Hard	Heavier breathing begins
6	Moderately Hard	Deep breaths but still sustainable
7	Hard	Very deep breaths, talking avoided
8	Very Hard	Laboured, cannot talk, unsustainable
9	Severe	Breathless, failure within 1 min
10	Maximal effort	Gasping for air, failure within seconds

APPENDIX II
Table for Subjects

Subject	Sex	Age (yr)	Height (m)	Body Mass (kg)	$\dot{V}O_{2peak}$ ($ml \cdot kg^{-1} \cdot min^{-1}$)
Chapters 5 & 6					
1	M	25	1.79	70.4	–
2	F	30	1.60	60.0	–
3	F	23	1.72	62.6	–
4	M	34	1.75	84.0	–
5	M	22	1.83	74.5	–
6	M	23	1.75	63.9	–
7	M	23	1.80	93.5	–
8	M	27	1.78	81.0	–
9	M	26	1.73	73.0	–
10	M	21	1.83	77.5	–
11	M	21	1.79	71.6	–
MEAN	–	25	1.76	73.8	–
SD	–	4	0.06	9.9	–
Chapters 7 & 8					
1	M	26	1.79	68.6	53.4
2	M	36	1.74	88.3	62.2
3	M	25	1.77	62.8	60.8
4	M	24	1.75	74.1	54.9
5	F	21	1.64	57.0	41.9
6	M	28	1.89	86.0	49.3
7	M	26	1.78	71.9	73.7
8	M	18	1.86	77.0	63.5
9	F	24	1.75	64.0	52.5
10	M	23	1.75	73.6	59.0
MEAN	–	25	1.77	72.3	57.1
SD	–	5	0.07	9.9	8.8

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