Physiological mechanisms limiting exercise tolerance: a multi-disciplinary approach

Matthew Richard Chadwick

Submitted in accordance with the requirements for the degree of Doctor of Philosophy

The University of Leeds

Faculty of Biological Sciences

School of Biomedical Sciences

March 2021

The candidate confirms that the work submitted is his own, except where work which has formed part of jointly-authored publications has been included. The contribution of the candidate and the other authors to this work has been explicitly indicated where appropriate. The candidate confirms that appropriate credit has been given within the thesis where reference has been made to the work of others.

Acknowledgements

I extend my warmest thanks to everyone who has contributed to this doctoral work in any way, no matter how small, including each and every participant (all of whom participated in the studies for no reward other than my gratitude).

In particular, I would like to thank my supervisor Dr Carrie Ferguson for her assistance throughout. Your dedication and commitment have never been anything but absolute. Additionally, I am grateful to Drs Al Benson and Bryan Taylor for their advice and highly valued contributions.

I have been fortunate to work alongside fantastic colleagues who have helped me personally and professionally. I would especially like to thank Tim Hardy, Molly Baldwin, Matt Davies, and Saïd Ibeggazene for their contributions in the lab and conversations in the pub.

I am also grateful to Lara for her patience and understanding throughout, particularly during the prolonged periods of working from home.

Finally, I would like to say a special thanks to my family. I hope this PhD provides some recompense for your sacrifice. Thank you for everything.

Doctoral work outcomes

Published manuscripts

Hardy TA, **Chadwick MR**, Davies MJ (2019). Mechanisms of improved exercise capacity following respiratory muscle training in athletes with cervical spinal cord injury. *J Physiol*, *597*(23): 5531-5532.

Published abstracts

Chadwick MR, Hardy TA, Taylor BJ, Ferguson C (2020). Inspiratory Muscle Fatigue Is Not Different In Response To Long Vs. Short-duration High-intensity Exercise: 1501 Board# 95 May 28 10:30 AM-12:00 PM. *Med & Sci in Sport & Exerc*, *52*(7), p.394.

Hardy TA, **Chadwick MR**, Ferguson C, Taylor BJ (2020). The Effect Of Exercise Intensity On The Development Of Diaphragm And Expiratory Abdominal Muscle Fatigue: 2985 May 29 1:15 PM-1:30 PM. *Med & Sci in Sport & Exerc*, *52*(7), pp.830-831.

Cross TJ, Hardy TA, Isautier J, Kelley E, **Chadwick MR**, Johnson BD, Taylor BJ (2020). Using Critical Power To Predict Ramp Incremental Cycling Performance: Three Parameters Are Better Than Two: 1337 May 28 9:30 AM-9:45 AM. *Med & Sci in Sport & Exerc*, *52*(7), pp.351-352.

Published comments

Ferguson C, **Chadwick MR**, Baldwin MM (2019). Comments on CrossTalk 43: Exercise training intensity is/is not more important than volume to promote increases in human skeletal muscle mitochondrial content, Does the relationship between exercise intensity and tolerance preclude an answer? *J Physiol*, pp 1-2.

Oral presentations

Europhysiology 2018, The Physiological Society, London UK.
Exercise-induced fatigue is a consequence of exercise intensity and is independent of task power.

BBSRC White Rose Annual Symposium, 2019, University of Sheffield.
Understanding the mechanisms that limit exercise tolerance: a multi-disciplinary approach.

Poster presentations

Chadwick MR, Hardy TA, Taylor BJ, Ferguson C, 2020. Mechanisms of intolerance for long- and short-duration high-intensity exercise: a possible role for the inspiratory rib cage muscles. Integrative Physiology of Exercise, American Physiological Society (virtual).

Professional Placements

Three-month placement at the Human Performance Centre of University of Leeds.

Abstract

Poor exercise tolerance (the inability to sustain physical tasks) is indicative of morbidity, mortality, and low quality of life. The aim of this thesis was to elucidate the determinants of exercise tolerance, which remain incompletely understood. The primary outcome measure was maximal voluntary isokinetic power (P_{iso}), from which locomotor neuromuscular fatigue (exercise-induced reduction in P_{iso}) and 'power reserve' (difference between task power and P_{iso} at intolerance) were quantified.

First, a potential association between power reserve magnitude and severity of inspiratory muscle fatigue following short- and long-duration exercise was investigated. Inspiratory rib cage muscle and diaphragm fatigue were inferred from reductions in oesophageal ($Poes_{tw}$) and gastric (Pga_{tw}) twitch pressures, respectively, in response to cervical magnetic stimulation. Global inspiratory muscle fatigue was assessed via transdiaphragmatic pressure (Pdi_{tw}; difference between Pga_{tw} and $Poes_{tw}$). Inter-trial differences (Δ) in power reserve were not related to ΔPdi_{tw} (r=−0.47; P=0.17) or ΔPga_{tw} (r=0.53; P=0.11), but were negatively associated with $\Delta Poes_{tw}$ (r=0.76, P<0.01), suggesting that a larger power reserve results from a greater contribution to intolerance from rib cage muscle fatigue.

Next, whether task power influences locomotor neuromuscular fatigue was assessed. Compared to constant-power exercise, intermittent exercise allows higher powers to be performed without increasing intensity (metabolic stress). Motor unit recruitment and non-oxidative energy provision were

greater during intermittent than intensity-matched constant-power exercise (both $P<0.05$), but the reduction in P_{iso} was not different between protocols (−13±6% vs. −14±6%, respectively; $P=0.69$).

Finally, adaptations to intensity-matched intermittent and constant-power protocols were investigated. Preliminary findings indicated that training-induced increases in P_{iso} and $\dot{V}O_{2peak}$ were not different between protocols (both $P>0.05$). However, there was a greater increase in lactate threshold ($P=0.02$) and 'large' additional increase in exercise tolerance (effect size=2.16) following intermittent compared with constant-power training.

Overall, the investigations presented herein may aid optimisation of exercise training strategies to improve exercise tolerance.

Contents

List of tables

List of figures

Abbreviations

ADP: adenosine diphosphate

ATP: adenosine triphosphate

COPD: chronic obstructive pulmonary disease

CP: critical power

EMG: electromyography

FVC: forced vital capacity

H^+: hydrogen ions

Hb: haemoglobin

LT: lactate threshold

Mb: myoglobin

MIP: maximal inspiratory pressure

MVC: maximal voluntary contraction

MVV: maximal voluntary ventilation

PAV: proportional assist ventilation

PCr: phosphocreatine

Pdi_{tw}: transdiaphragmatic twitch pressure

Pga_{tw}: gastric twitch pressure

Pi: inorganic phosphate

P_{iso}: peak isokinetic power

$Poes_{tw}$: oesophageal twitch pressure

RIT: ramp-incremental test

RIT_{peak}: peak ramp-incremental power

RMS: root mean square

RPE: rating of perceived exertion

Tlim: time to intolerance/tolerable duration

$Tlim_{LONG}$: tolerable durations of 6 minutes or more

$Tlim_{SHORT}$: tolerable durations of less than 5 minutes

TSI: tissue saturation index

$\dot{V}CO_2$: rate of carbon dioxide output

$\dot{V}_E$: minute ventilation

$\dot{V}O_2$: rate of pulmonary oxygen uptake

$\dot{V}O_{2max}$: maximal rate of oxygen uptake

$\dot{V}O_{2peak}$: peak rate of oxygen uptake

Δ locomotor fatigue/MIP/ Pdi_{tw}/Pga_{tw}/$Poes_{tw}$/power reserve: difference between the changes in these values in response to $Tlim_{LONG}$ and $Tlim_{SHORT}$ trials

Δ25: power 25 % of the difference between CP and RIT_{peak}

Δ40: $\dot{V}O_2$ 40% of the difference between that at LT and $\dot{V}O_{2peak}$

1: Literature review and thesis background

1.1 Introduction

The ability to sustain an exercise task is a highly variable characteristic, spanning from athletes capable of exceptional feats of endurance to populations with chronic diseases who struggle to perform activities of daily living. In addition to a reduction in quality of life, poor exercise tolerance is also a powerful predictor of morbidity and all-cause mortality in healthy and patient populations (Morris *et al.*, 1991; Blair *et al.*, 1995; Myers *et al.*, 2002), demonstrating its cardinal importance to overall wellbeing.

The tolerability of exercise is dependent on the ability to deliver and utilise oxygen (O_2) at the necessary rate, which can be assessed via measurement of pulmonary O_2 uptake ($\dot{V}O_2$). An adequate O_2 supply facilitates the sustainable provision of adenosine triphosphate (ATP) for muscular work via oxidative phosphorylation. To transport O_2 from the atmosphere to cytochrome c oxidase - the terminal complex (IV) of the electron transport chain - requires effective integration of the pulmonary, cardiovascular, and neuromuscular systems (Hawley *et al.*, 2014). As a result, exercise tolerance can be increased or decreased by changes in function at multiple physiological sites within any of these systems. When the rate of oxidative phosphorylation is inadequate for the energy demands of the task, substrate-level phosphorylation is required to meet the shortfall, causing the accumulation of metabolites that are associated with impaired muscle function (Allen, Lamb and Westerblad, 2008) and inhibited central motor

drive (Amann, 2011). These effects manifest as a temporary reduction in the maximal power-generating capacity of the active muscles, termed exercise-induced fatigue, which is an fundamental determinant of exercise tolerance (Gandevia, 2001).

Importantly, exercise-induced fatigue resistance, and consequently exercise tolerance, can be enhanced through appropriate stimuli. Exercise training can improve function at multiple sites of the oxygen transport and utilisation chain, promoting improvements in the pulmonary and cardiovascular systems, and inducing metabolic and morphological adaptations in skeletal muscle. Due to the implications of low exercise tolerance, elucidating the underlying mechanisms of fatigue development and identifying more effective exercise training strategies to increase exercise tolerance represent important research areas.

1.2 The bioenergetics of exercise

For a skeletal muscle to contract and generate force, it requires energy. The chemical energy source for all endergonic reactions in the muscle, such as actin-myosin interactions, sequestering calcium into the sarcoplasmic reticulum, and exchange of Na^+-K^+ at the plasma membrane, is adenosine triphosphate (ATP). However, there is only a small intramuscular storage capacity for ATP (~5-6 $mMol \cdot kg^{-1}$ wet weight, which corresponds to less than 1 kcal per kg of muscle; Harris, Hultman and Nordesjö, 1974; Kemp, Meyerspeer and Moser, 2007; Brooks, 2012), with the consequence that ATP must be re-synthesised from adenosine diphosphate (ADP) and inorganic phosphate (Pi) for exercise to be sustained for more than a matter

of seconds (Barclay, 2017; Hargreaves and Spriet, 2020). The energy demands of exercise can be very diverse, requiring rapid rates of ATP turnover for short durations or more moderate but prolonged rates of ATP demand. Therefore, there are three energy systems that use distinct metabolic pathways to resynthesise ATP. While every energy system contributes to energy provision during all forms of exercise, the relative contribution from each is dependent on the demands of the specific task and determines the intensity and tolerability of the exercise (Baker, McCormick and Robergs, 2010).

Despite intramuscular ATP being an immediate energy source, muscle ATP concentration is largely maintained during exercise, with only modest decreases present even after maximal efforts (Allen *et al.*, 1997; Rossiter *et al.*, 2002; Baker, McCormick and Robergs, 2010). As a result, phosphocreatine (PCr) and adenylate kinase (myokinase) act as the immediate energy sources at exercise onset (Brooks, 2012). The intramuscular storage capacity of PCr is approximately four times greater than that of ATP (Kemp, Meyerspeer and Moser, 2007), but is still limited and can be used at a rapid rate (~45 kcal·min^{-1}) with the result that PCr stores are exhausted within 10-15 seconds during strenuous exercise (Brooks, 2012). Consequently, the PCr energy system can immediately supply energy at the onset of exercise but cannot support more than brief muscular activity if acting as the predominant energy source. Estimates of the capacity of adenylate kinase vary but are smaller than for ATP and PCr, with likely less than 1 kcal per 30 kg of human muscle (Brooks, 2012).

Therefore, energy provision must be derived from other systems to sustain exercise.

Within seconds of the onset of exercise, significant rates of ATP can be derived from anaerobic glycolysis of blood glucose and muscle glycogen, producing lactate (Jacobs *et al.*, 1983; Conley, Kushmerick and Jubrias, 1998; Parolin *et al.*, 1999; Walsh *et al.*, 2008). Indeed, during a 6-second maximal effort, the contribution of anaerobic glycolysis to ATP resynthesis is equivalent to that of PCr (Gaitanos *et al.*, 1993). This rapid response is because, like PCr, glycolysis is controlled by a feedforward mechanism activated by muscle contraction (Parolin *et al.*, 1999). However, while greater than PCr, the capacity to generate ATP via glycolysis is also finite (Barclay, 2017). This limitation on ATP supply is likely due to the inhibitory effects of nonmitochondrial energy provision, such as H^+ accumulation, rather than substrate availability (Barclay, 2017; Hargreaves and Spriet, 2020). As a result of this finite capacity, anaerobic glycolysis may contribute to ATP resynthesis during maximal exercise for up to 40 seconds (Brooks, 2012).

Due to the high rates of ATP turnover, the majority of the ATP supply for short bouts of maximal exercise is derived from the substrate-level phosphorylation of PCr hydrolysis and anaerobic glycolysis (McCartney *et al.*, 1986; Putman *et al.*, 1995). However, as substrate-level processes perturb the metabolic milieu and the total ATP production available from these systems combined is relatively small, an additional source of ATP supply is required to support prolonged contractile activity (see Figure 1.1 for

the relative contributions of the three energy systems during maximal exercise).

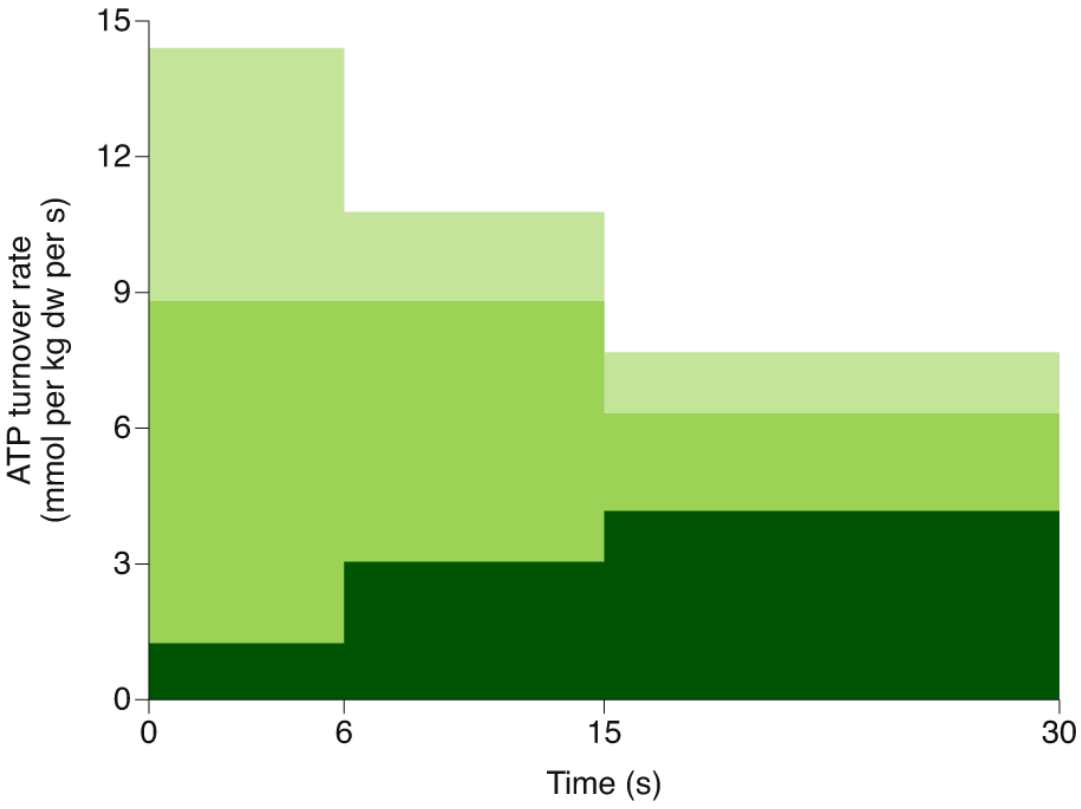

Figure 1.1: Contribution from PCr (light green), glycolysis (medium green), and oxidative phosphorylation (dark green) to ATP turnover during maximal exercise. From Hargreaves and Spriet (2020) using data from Parolin *et al*., (1999).

When exercise time reaches ~1 minute, the predominant energy pathway shifts from substrate-level phosphorylation to oxidative phosphorylation (Medbø and Tabata, 1989). This energy system is also activated at the onset of exercise, but the rate of response of oxidative phosphorylation to the change in the rate of ATP demand is considerably slower than that of substrate-level processes. These differing response times exist because oxidative phosphorylation is regulated by a feedback, rather than

feedforward, mechanism (Chance and Williams, 1956; Jeneson *et al.*, 1996). Consequently, while overall ATP supply for exercise lasting more than several minutes is provided almost exclusively by aerobic pathways (Wells, Selvadurai and Tein, 2009), energy provision from anaerobic sources (predominantly PCr) is still required at exercise onset to compensate for the slow response of oxidative metabolism (termed the O_2 deficit).

While the maximal rate of ATP resynthesis via oxidative phosphorylation is lower than that of substrate-level phosphorylation, the capacity to produce ATP from carbohydrate and fat metabolism is immense and, equally importantly, does not challenge intramuscular homeostasis. These properties of oxidative phosphorylation mean that systemic responses ($\dot{V}O_2$, heart rate, blood lactate concentration) plateau during relatively low rates of work so that tasks can be sustained for several hours by trained athletes with no severe impairment in physiological function (Davies and Thompson, 1986) and for up to 6 hours by recreationally active men before exercise is voluntarily terminated (Black *et al.*, 2017). Indeed, with enough substrate and under suitable environmental conditions, oxidative phosphorylation theoretically allows relatively low rates of work to be sustained almost indefinitely (Brooks, 2012).

1.3 Work rate (power output) and exercise intensity

Work rate and exercise intensity are both measures of the strenuousness of an exercise task. However, while these factors are intrinsically related, they are not conceptually equivalent. The work rate is the rate of chemical to kinetic energy transfer occurring in the exercising muscles and is measured

in Watts, where 1 Watt equals 1 Joule per second. When applied to cycling, work rate is interchangeably referred to as ‘power’ or ‘power output’. Due to the commonality of cycling in the scientific literature and the exclusive use of cycling as the exercise modality in the studies presented in Chapters 3, 4, and 5, these terms predominant throughout this thesis.

Whereas power output is an absolute value, the intensity describes the physiological stress incurred during exercise and reflects the relative difficulty for an individual to perform a task. It is not the power output *per se* that determines the intensity, but the relative contribution of each energy system to meet the rate of ATP demand. Consequently, linear increases in power output do not cause linear increases in intensity. All tasks, incorporating the entire range of power outputs achievable by the working muscles, can be classified into four domains, demarcated by physiological thresholds of energy provision, with common profiles of $\dot{V}O_2$ and blood lactate concentration (see Figure 1.2; Whipp, Ward and Wasserman, 1986; Rossiter, 2011).

1.3.1 Classification of exercise intensity into domains

Moderate-intensity exercise encompasses all power outputs below the lactate threshold (LT) and is characterised by $\dot{V}O_2$ quickly reaching a plateau (typically within 3 minutes in healthy individuals; Whipp and Wasserman, 1972; Henson, Poole and Whipp, 1989; Ozyener *et al.*, 2001) and no sustained metabolic acidosis. The increase in $\dot{V}O_2$ per unit increase in power output (functional gain) is ~9-11 $mL \cdot min^{-1} \cdot W^{-1}$, regardless of sex and training status (Hansen *et al.*, 1987; Henson, Poole and Whipp, 1989).

Heavy-intensity exercise denotes exercise at metabolic rates between LT and, for cycling, critical power (CP, the asymptote of the power-duration relationship), the latter representing the highest power output at which energy provision can be achieved through oxidative phosphorylation alone (Poole *et al.*, 2016). In this domain, the plateau in $\dot{V}O_2$ is delayed compared to moderate-intensity exercise, taking 10-20 minutes to be established (Poole *et al.*, 1988; Ozyener *et al.*, 2001), by a $\dot{V}O_2$ slow component that increases the O_2 cost of exercise above that observed during moderate-intensity exercise. This inefficiency originates primarily in the exercising muscles via changes in the ATP cost of power production and the O_2 cost of ATP resynthesis (Poole *et al.*, 1991; Cannon *et al.*, 2014), and drives the functional gain for heavy-intensity exercise to ~12 $mL \cdot min^{-1} \cdot W^{-1}$ (Ozyener *et al.*, 2001). Another consequence of the altered work demands during heavy-intensity exercise is a sustained metabolic acidosis but, similarly to $\dot{V}O_2$, this reaches a plateau in time.

When power output exceeds CP, $\dot{V}O_2$, and blood lactate concentration rise inexorably until the task is terminated. Exercise in this domain, described as very heavy intensity, requires continued contributions to energy provision from anaerobic glycolysis and cannot be sustained for long periods. The further the power output exceeds CP, the shorter the task can be sustained, with the tolerable duration of very heavy-intensity exercise characterised by the hyperbolic power-duration relationship (Poole *et al.*, 2016). The highest $\dot{V}O_2$ reached during a single bout of very heavy-intensity exercise is termed $\dot{V}O_{2peak}$ and can be verified as the maximal $\dot{V}O_2$ ($\dot{V}O_{2max}$) when an individual's values do not differ between very heavy-intensity tests performed

at different power outputs, as this demonstrates that no change in power output can drive $\dot{V}O_2$ higher (Rossiter, Kowalchuk and Whipp, 2006; Poole and Jones, 2017). In healthy individuals, $\dot{V}O_{2peak}$ derived from a single test typically provides a robust measure of $\dot{V}O_{2max}$ (Rossiter, Kowalchuk and Whipp, 2006; Murias, Pogliaghi and Paterson, 2018; Wagner *et al.*, 2021).

A final domain that has been described is the ‘extreme intensity’ domain, which denotes power ouputs above CP that become intolerable before $\dot{V}O_2$ reaches $\dot{V}O_{2max}$ (Rossiter, 2011). This domain is a modification of the upper domain in the original schema proposed by Whipp, called severe intensity, which incorporated all work rates with associated metabolic rates that exceed $\dot{V}O_{2max}$ from exercise onset (i.e. $\dot{V}O_2$ projects above $\dot{V}O_{2max}$, see Figure 1.2; Rossiter, 2011).

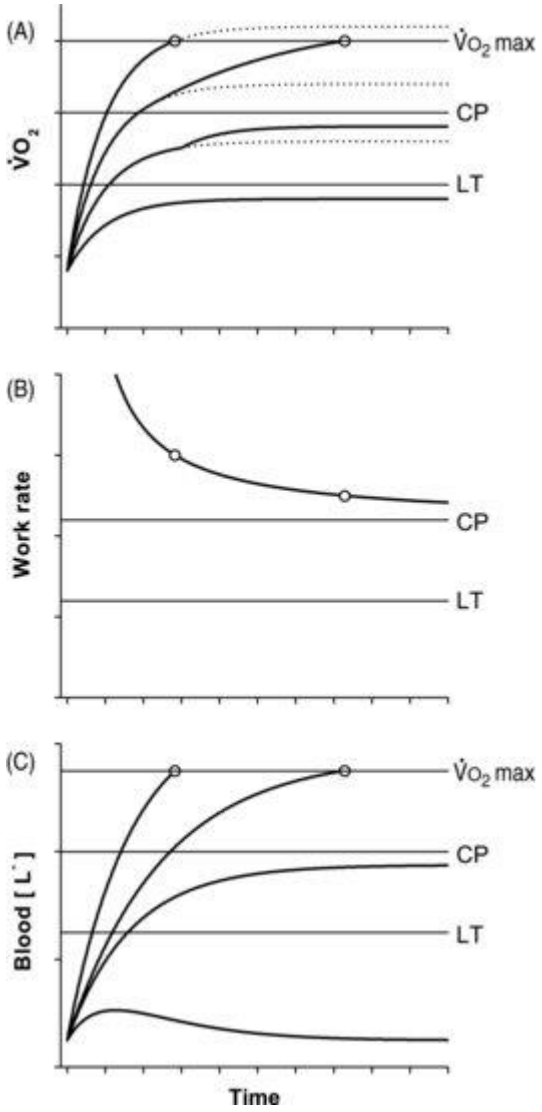

Figure 1.2: Schematic of the dynamics of $\dot{V}O_2$ (A) and blood lactate concentration (C) in relation to the tolerable duration of exercise at a constant power output (B). Based on common profiles of these physiological variables, exercise intensity can be categorised into four domains: moderate, heavy, very heavy, and severe (or extreme in the modified schema, which is not displayed here). The profile of $\dot{V}O_2$ and blood lactate concentration in each domain are presented. Circles indicate intolerance. Taken from Rossiter (2011).

1.3.2 Normalising exercise intensity

When investigating exercise interventions in a research context, it is essential to accurately assign the task power for an exercise protocol to normalise the intensity. The metabolic strain of an exercise task is a

fundamental determinant of the acute and chronic physiological responses to exercise, and incorrectly assigning task power may confound study outcomes. To normalise the intensity of exercise, the physical capabilities of the individual must be characterised, and subsequent exercise tests performed with an appropriate task power for the work and recovery durations selected. Commonly, exercise intensity is prescribed as a percentage of an individual's maximum for a selected variable, typically obtained from a ramp-incremental test (RIT), such as heart rate, $\dot{V}O_{2max}$, or the peak power achieved during the RIT (RIT_{peak}). However, this method can be unreliable because the intensity domain thresholds are not fixed relative to a peak value. For example, of two individuals exercising at the same power output and percentage of $\dot{V}O_{2peak}$ (%$\dot{V}O_2$), one can reach $\dot{V}O_{2peak}$ and intolerance in ~10 minutes (very heavy-intensity exercise) whereas the other achieves a submaximal steady-state in $\dot{V}O_2$ and can complete 30 minutes without reaching intolerance (heavy-intensity exercise; Rossiter, 2011).

The optimal way to accurately prescribe exercise intensity is to determine individualised values for LT and CP to ensure the assigned task power is within the desired intensity domain. A measure of LT can be obtained from a single RIT (Beaver, Wasserman and Whipp, 1986); however, determining CP usually requires several exercise tests performed at a constant power to intolerance. To avoid this considerable participant burden, an RIT can be modified by addition of a three-minute maximal effort, which can be used independently to estimate CP (Vanhatalo, Doust and Burnley, 2007), performed immediately after reaching intolerance of the ramp-incremental phase (a 'ramp-sprint'; Murgatroyd *et al.*, 2014). As the ramp-incremental

phase largely depletes *W′* (Morton *et al.*, 1997), the mean power output during the three-minute maximal effort (‘sprint power’) approximates CP (Murgatroyd *et al.*, 2014). This simple modification allows the accurate prescription of heavy and very heavy-intensity exercise tasks from a single-visit and was utilised in Chapter 3 of this thesis. However, the efficacy of this protocol has only been demonstrated in young, healthy males, and the high levels of motivation required to sustain maximal exercise for three minutes, having already completed a RIT to intolerance, may make the test unsuitable for individuals unaccustomed to intense physical demands. Therefore, different approaches to exercise prescription may be required to ensure testing is appropriate for the population of interest.

An alternative method of normalising exercise intensity is percentage delta (Δ), which accounts for the position of LT relative to $\dot{V}O_{2peak}$ (Casaburi *et al.*, 1987; Roston *et al.*, 1987) and has been shown to increase the consistency of the inter-individual physiological responses compared to $\%\dot{V}O_{2peak}$ (Lansley *et al.*, 2011). The Δ concept can be easily applied using the power outputs that coincide with LT and $\dot{V}O_{2peak}$ during an RIT. An alternative, albeit more complex, method is to use the $\dot{V}O_2$ that corresponds to LT and $\dot{V}O_{2peak}$ to determine a target $\dot{V}O_2$. Using assumptions of functional gain, the power output that evokes this $\dot{V}O_2$ when performed continuously can then be calculated. The advantage of this second method is that, whereas peak power achieved during an RIT is higher when the rate of increase of power output is steeper (e.g. peak power is greater when the ramp rate is 50 $W \cdot min^{-1}$ compared to 20 $W \cdot min^{-1}$; Morton, 2011), $\dot{V}O_{2peak}$ in response to ramp-incremental exercise is consistent regardless of ramp rate (Davis *et*

al., 1982) . As a result, the task power determined from a fixed Δ will depend on the RIT protocol employed when relative to RIT_{peak} but not $\dot{V}O_{2peak}$. Therefore, when CP was not measured, a $\Delta\dot{V}O_2$ approach was taken in Chapters 4 and 5 of this thesis for prescribing power outputs.

1.3.3 Dissociating power output from intensity

Continuous exercise describes any exercise bout performed without recovery or rest periods, such as a time-trial or a laboratory-based test performed at a constant power. For continuous exercise of a fixed duration or volume of work, intensity and power output are associated in the sense that the intensity of exercise can only be changed by performing a different power output. However, this relationship between power output and intensity can be dissociated during intermittent exercise, defined as periods of work alternating with periods of rest or recovery, so that the systemic (e.g. $\dot{V}O_2$ and blood lactate concentration) and intramuscular (e.g. P_i concentration and pH) homeostatic challenge for a given power output is reduced (Astrand *et al.*, 1960; Turner *et al.*, 2006; Davies *et al.*, 2017). This reduction in metabolic perturbation allows a greater volume of work at a power output exceeding CP to be performed during intermittent than constant-power exercise (Chidnok *et al.*, 2012).

The magnitude of this dissociation between power output and intensity is dependent on the duration of the work and recovery phases, and is greatest when the work phases are short (McCrudden, Keir and Belfry, 2017) and recovery phases are long (Chidnok *et al.*, 2013). To illustrate, a power output constituting very heavy-intensity exercise when performed continuously can

be reduced to moderate intensity when performed intermittently with work phase durations of 16 seconds (Davies *et al.*, 2017). This effect occurs due to an almost fourfold increase in the contribution to mean ATP turnover rate from PCr breakdown (Figure 1.3), the stores of which can support short work phases before being replenished during recovery. As such, the effective capacity of PCr is much greater during intermittent than constant-power exercise (Davies *et al.*, 2017).

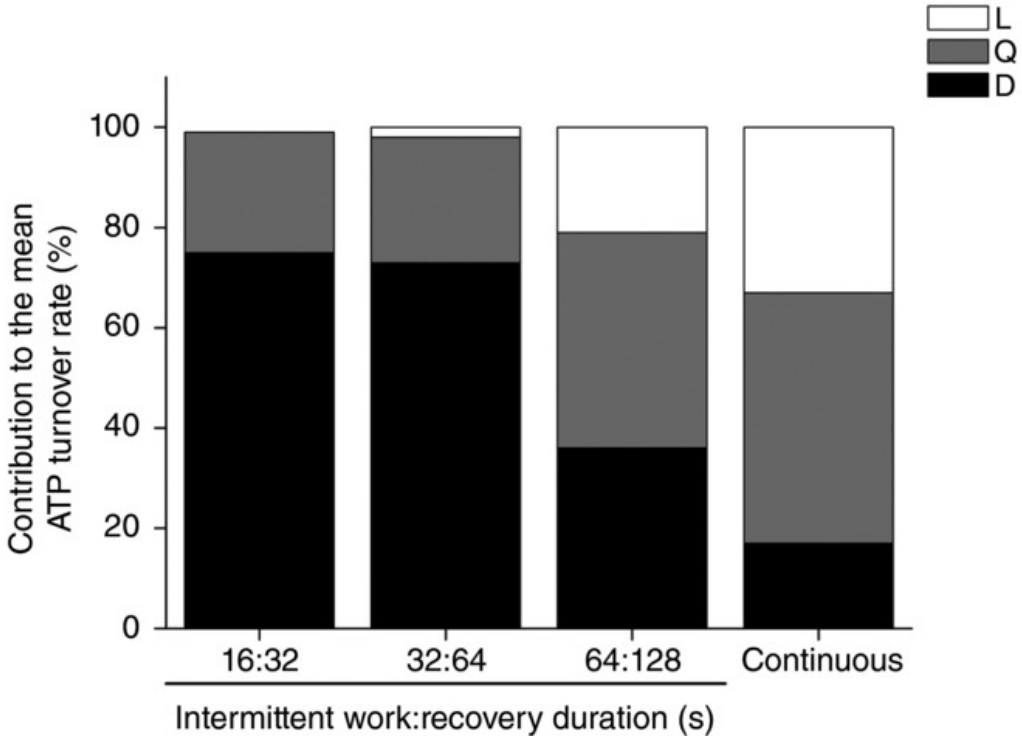

Figure 1.3: Contributions to mean ATP turnover rate from phosphocreatine breakdown (D), oxidative phosphorylation (Q), and anaerobic glycolysis (L) during different exercise tasks performed at the same power output (110 % of peak ramp-incremental power). From Davies *et al*., (2017).

Due to the increase in exercise tolerance and the ability to accumulate greater volumes of work at higher power outputs, the adaptive response to intermittent exercise training has gained considerable attention (e.g. Laursen

and Jenkins, 2002; Gibala and McGee, 2008; Gaesser and Angadi, 2011; Gillen and Gibala, 2018) and is discussed in Section 1.7.1 of this thesis.

1.4 Aetiology of exercise-induced fatigue

Exercise-induced neuromuscular fatigue can be broadly classified into two components with disparate origins that, together, encompass all the processes involved in muscle contraction. Central components of fatigue, defined as any disruption in function occurring proximal to the neuromuscular junction, and peripheral components of fatigue, which refer to disruptions occurring distal to the neuromuscular junction (Gandevia, 2001). However, this classification does not describe two dichotomous systems acting independently but an integrated system with two distinct mechanisms.

To initiate muscle contraction, efferent signals originating from the motor cortex in the brain must transit through neural pathways in the spinal cord to the neuromuscular junction and trigger the contractile apparatus of the muscle. Any inhibition of central nervous system function, which reflects the ability to activate the muscle, is considered a central component of neuromuscular fatigue. Central changes can be further subdivided into reductions in motor cortical output, referred to as supraspinal factors, or signal propagation such as decreased motor neurone excitability (Gandevia, 2001).

Peripheral factors of neuromuscular fatigue refer to a reduction in force output of the contractile apparatus of the muscle in response to a given level of activation. A myriad of factors may contribute to this impairment of muscle

function, such as K^+ accumulation in the T-system that causes membrane depolarisation and subsequent inactivation of the voltage sensor of excitation-contraction coupling (Fitts and Balog, 1996; Ferreira Gregorio *et al*., 2017). Furthermore, depletion of glycogen localised within the myofibrils inhibits the release of calcium ions (Ca^{2+}) from the sarcoplasmic reticulum in response to action potential discharge, thereby disrupting excitation-contraction coupling (Ørtenblad *et al*., 2011; Ørtenblad, Westerblad and Nielsen, 2013).

A cardinal feature of impaired muscle function is the accumulation of fatigue-related metabolites, such as hydrogen ions (H^+) and inorganic phosphate (Pi), which independently and synergistically interfere with cellular processes. Metabolite-induced disruptions include directly inhibiting force and power of the cross-bridge and decreasing myofibrillar Ca^{2+} sensitivity (Allen *et al*., 2008; Sundberg and Fitts, 2019). In particular, accumulation of Pi has a pronounced effect on Ca^{2+} handling by precipitating with Ca^{2+} inside the sarcoplasmic reticulum, thereby preventing Ca^{2+} release from this organelle (Ferreira *et al*., 2020). These metabolites also activate type III and IV afferent fibres, which attenuate central motor drive to the working muscles, potentially to constrain the metabolic perturbation (Amann *et al.*, 2011; Blain *et al.*, 2016). Therefore, fatigue-related metabolites contribute to peripheral and central factors of neuromuscular fatigue. The magnitude of the contribution from peripheral and central factors is dependent on the demands of the exercise task and can be independently evaluated using appropriate experimental techniques.

1.4.1 Assessing exercise-induced fatigue

An essential component of the interpretation of exercise-induced fatigue is the methodology of fatigue assessment. The most prominent and frequently used method is artificial stimulation, which can take the form of electrical or magnetic stimulation and can be applied to the muscle, peripheral nerve, or motor cortex to generate muscle contractions (Gandevia, 2001; Millet *et al.*, 2012). Artificial stimulation has the advantage of being a non-volitional measure, which prevents the assessment of fatigue being influenced by the participants' motivation or effort, and therefore provides a robust measure of the true condition of the muscle. Additionally, artificial stimulation can be combined with a maximal voluntary contraction (MVC), known as the interpolated twitch technique, to provide specific information regarding the contributions of central and peripherals mechanisms to the overall fatigue (Shield and Zhou, 2004). As such, in addition to quantifying the magnitude of fatigue, artificial stimulation can allow mechanistic insight into the aetiology of the fatigue.

However, there are fundamental limitations with artificial stimulation, particularly when assessing fatigue resulting from whole-body exercise. The complexity of the motor unit recruitment pattern when performing whole-body exercise cannot be replicated using artificial stimulation, with the consequence that artificial stimulation can only be used to assess isometric or isokinetic single-joint tasks. This presents methodological and theoretical issues. One such issue is that the exercise protocol and neuromuscular assessment is typically performed using different equipment, introducing a

delay between exercise cessation and fatigue characterisation. While this transition is performed as quickly as possible, a delay of ~1-5 minutes, depending on the protocol, is common and may allow considerable recovery of neuromuscular function (Figure 1.4: (Sargeant and Dolan, 1987). Moreover, the rate of recovery of neuromuscular fatigue depends on the mechanism impaired, with central factors of fatigue recovering faster than peripheral factors (Carroll, Taylor and Gandevia, 2017). Therefore, assessment of neuromuscular fatigue following whole-body exercise via artificial stimulation may underestimate the true magnitude of fatigue and inaccurately reflect the relative contribution of central and peripheral components.

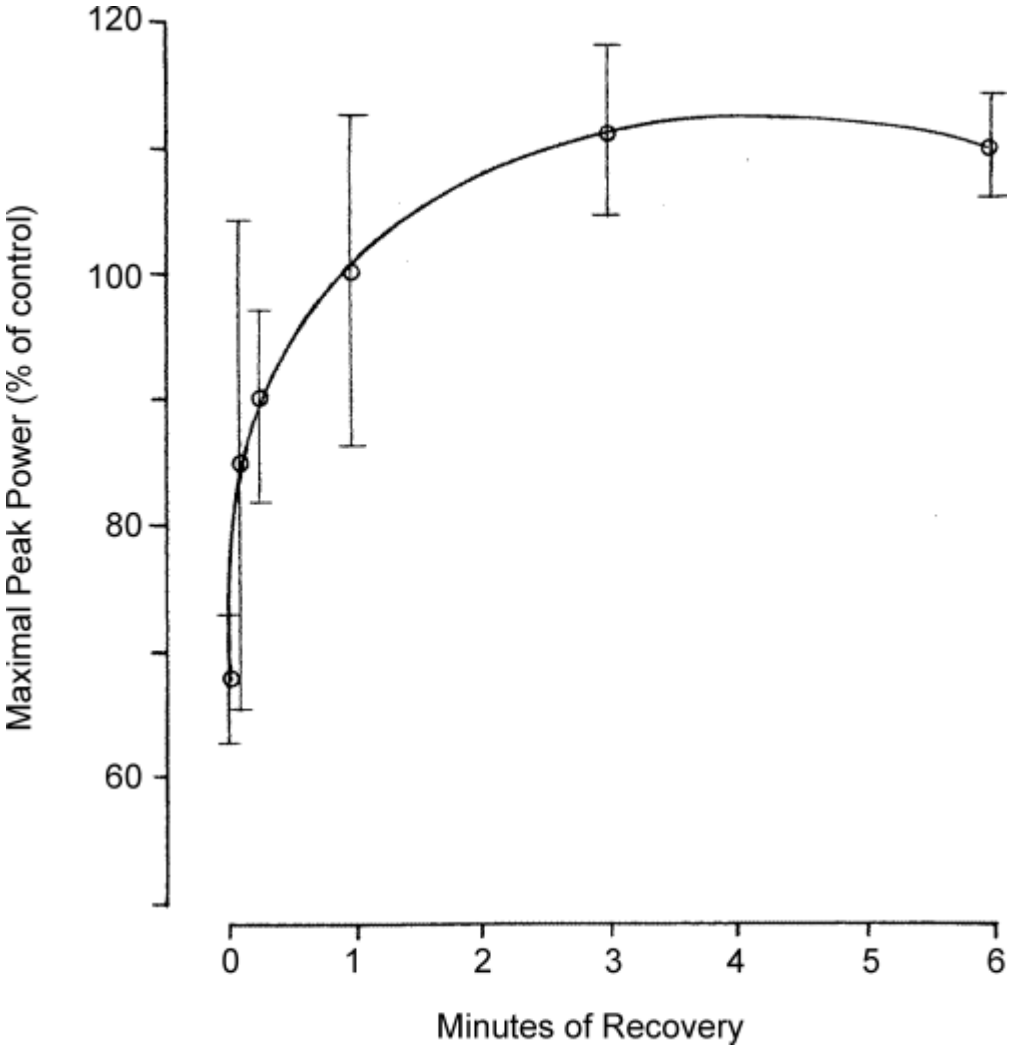

Figure 1.4: Rate of recovery in maximal power-generating capacity after 6 minutes exercising at 90 %$\dot{V}O_{2max}$, adapted from Sargeant and Bolan (1987). Data presented are mean ± SD from four subjects and indicate that maximal power may be fully recovered within 2 minutes. There is an increase above baseline maximal power that may be due to muscle potentiation secondary to increases in muscle temperature. From Sargeant (2007).

More recently, a customised ergometer was designed to eliminate delays in fatigue assessment (Temesi *et al.*, 2017). In addition to dynamic bilateral cycling, the pedals of this ergometer can be locked instantly so that isometric force, generated voluntarily and from artificial stimulation, can be measured without delay.

While overcoming the issue of delayed fatigue assessment, this innovative method suffers from another limitation of artificial stimulation techniques. The neuromuscular assessment of single-joint tasks can quantify and characterise neuromuscular fatigue but are not task specific. Therefore, single-joint fatigue assessments cannot determine the reduction in the capacity to perform the dynamic task, and as such inform whether neuromuscular fatigue is imposing a functional limitation on the task i.e. fatigue has developed to the extent that the working muscles are no longer able to produce the power required to continue the task (Ferguson *et al.*, 2016b). Consequently, isometric fatigue assessments cannot directly inform of the mechanisms limiting dynamic exercise.

Alternative methods to artificial stimulation for assessing neuromuscular fatigue are volitional assessments of muscle function. The primary limitation of such techniques is that these measures are confounded by submaximal efforts. However, when participants are motivated and familiarised with the protocol, these measures are highly reproducible. Throughout this thesis, locomotor neuromuscular fatigue is assessed using changes in peak power output during maximal isokinetic cycling. This methodology allows fatigue assessment to be performed within 1–2 seconds of exercise cessation, minimising the influence of recovery on the results, as well as providing a task-specific measure of muscle function relative to the demands of the task. In addition, isokinetic cycling allows the assessment of peak power production to be cadence-controlled (Cannon *et al.*, 2011; Coelho *et al.*, 2015), preventing the results being confounded by the force-velocity relationship that exists in fatigued and unfatigued muscles (Sargeant, 2007).

A limitation of quantifying locomotor neuromuscular fatigue using isokinetic cycling rather than artificial stimulation is that central and peripheral components of fatigue cannot be determined. To address this issue, the pre- to post-exercise change in muscle activation during maximal isokinetic cycling can be assessed, where possible, using electromyography (EMG). While not allowing quantification of the relative contributions of central and peripheral components of fatigue, this technique may provide insight into the fatigue origins. An exercise-induced reduction in muscle activation may signify a reduction in central motor outflow, whereas a reduction in maximal voluntary power output without a decrease in muscle activation suggests attenuated function of the contractile apparatus.

1.4.2 Task-specificity of exercise-induced locomotor neuromuscular fatigue

The severity of locomotor neuromuscular fatigue is dependent on the intensity and duration of exercise. Moderate-intensity exercise sustained for up to 8 minutes does not typically reduce voluntary power output (Cannon *et al.*, 2011); however, when prolonged for 4–5 hours, exercise in this domain reduces MVC of the knee extensors by 18–28 % (Davies and Thompson, 1986; Lepers *et al.*, 2002; Place *et al.*, 2004). The magnitude of the reduction in MVC of the knee extensors tends be greater with increasing exercise duration, before reaching a plateau after ~1000 hours (Millet and Lepers, 2004; Millet, 2011; Brownstein, Millet and Thomas, 2021), potentially due to the pacing strategy required over such extreme distances (Saugy *et al.*, 2013). Reductions in voluntary power output manifest after only 3 minutes of heavy-intensity exercise, with even greater reductions present after the

same duration of very heavy-intensity exercise (Cannon *et al*., 2011). During very heavy-intensity exercise, neuromuscular locomotor fatigue develops progressively with pseudo-exponential kinetics (Swisher *et al.*, 2019), corresponding with the dynamics of fatigue-related metabolite accumulation (Jones *et al.*, 2008). These similar time courses indicate a mechanistic link between the magnitude of fatigue and metabolite concentration, which may underpin the intensity-dependence of exercise-induced fatigue.

It is not only the *severity*, but also the *mechanism* of locomotor neuromuscular fatigue that is dependent on exercise intensity. The metabolic perturbation (i.e. reductions in pH and [PCr] concomitant with increases in blood lactate concentration) during whole-body exercise is progressively exacerbated at the limit of moderate, heavy and very heavy-intensity exercise, respectively (Black *et al*., 2017), consistent with a greater reduction in electrically-evoked twitch force of the quadriceps following very heavy compared to heavy-intensity exercise (Thomas *et al.*, 2016). A similar trend is observed for isolated muscle mass tasks, with target forces below a relative threshold associated with more limited metabolic perturbations (Newham and Cady, 1990; Jones *et al.*, 2008). As such, the contribution from peripheral factors to neuromuscular fatigue increases with increasing exercise intensity. However, when the task demands exceed the critical threshold for the specific task (i.e. critical power or critical force) for whole-body or isolated muscle mass exercise, consistent metabolic perturbations are present at intolerance regardless of the tolerable duration associated with the power output or the pattern of work (Burnley *et al.*, 2010; Vanhatalo *et al.*, 2010; Black *et al.*, 2017).

Conversely, central factors of fatigue are greater in response to lower power outputs sustained for longer. For example, there was no reduction in electrically evoked twitch force of the quadriceps after 4 hours of continuous running despite MVC decreasing by 25 %, suggesting that the ability to activate the muscle rather than the response of the contractile apparatus to neural input was responsible for the decrease in maximal voluntary force (Davies and Thompson, 1986). This initial demonstration of the importance of reduced voluntary activation in response to prolonged exercise has since been consistently replicated (Millet *et al.*, 2002; Place *et al.*, 2004; Martin *et al.*, 2010; Temesi *et al.*, 2014; Millet, Martin and Temesi, 2018). The increased contribution from central factors to neuromuscular fatigue, which occurs in isolated muscle mass and whole-body tasks, applies to lower intensity domains and lower power outputs that are tolerable for longer durations but in the same intensity domain (Burnley, Vanhatalo and Jones, 2012; Thomas *et al.*, 2016).

The limitations to exercise are also dependent on the task demands. During isometric knee-extensor exercise, neuromuscular fatigue develops to the extent that the working muscles cannot generate more torque than the task requires at the limit of exercise, regardless of the target torque and tolerable duration of the task (Burnley *et al*., 2012). Fatigue assessments using maximal isokinetic cycling have demonstrated that this also occurs during some whole-body tasks, when maximal power production of the working muscles at the limit of exercise is no greater than the power required to continue the task (Ferguson *et al.*, 2016b); unpublished data from our laboratory), or only modestly exceeds this (Coelho *et al*., 2015). However, at

the limit of some whole-body tasks the working muscles are still able to greatly exceed task power i.e. there is a 'power reserve' (Staiano *et al.*, 2018; Davies *et al.*, 2021). While the use of different methodologies may provide conflicting outcomes over whether a power reserve is present at the limit of specific tasks (Marcora and Staiano, 2010; Burnley, 2010; Allen and Westerblad, 2010; Morales-Alamo *et al.*, 2015; Ferguson *et al.*, 2016a), it is apparent that the magnitude of the power reserve at exercise intolerance increases as the power output of the task decreases and the tolerable duration increases (Davies *et al.*, 2021). At present, the mechanisms responsible for this dissociation between functional capacity of the working muscles and the inability to continue the task are unclear. A fundamental aspect of dynamic whole-body exercise is that cardiopulmonary systems encroach upon their respective limits (maximum ventilation and cardiac output), a factor that is absent during isometric single-joint exercise when no reserve is present (Esposito *et al.*, 2010). Therefore, the mechanism causing a power reserve at the limit of long-duration whole-body exercise may be situated outside the neuromuscular system.

1.5 Exercise-induced inspiratory muscle fatigue

Exercise-induced inspiratory muscle fatigue is dependent on the level of inspiratory muscle work imposed by the task and the duration for which this is sustained, with the magnitude and prevalence of fatigue highest in response to exercise evoking at least 80-85 %$\dot{V}O_{2peak}$ (Johnson *et al.*, 1993). During maximal ramp-incremental exercise, when the time spent above this 80-85 %$\dot{V}O_{2peak}$ threshold is only ~5 minutes, the reduction in

transdiaphragmatic twitch pressure (Pdi_{tw}) is less than that observed during high-intensity exercise with tolerable durations that approach or exceed 10 minutes (−15 % vs. −23–32 %; Romer *et al.*, 2007b; Johnson *et al.*, 1993; Babcock *et al.*, 1998). This finding is supported by preliminary data from our laboratory, which demonstrates that global inspiratory muscle fatigue in response to constant-power exercise is greater when the tolerable duration is ~10 minutes compared to ~5 minutes.

Using proportional assist ventilation (PAV) to partially unload the inspiratory muscles and reduce the normal work of breathing does not increase the tolerable duration of maximal ramp-incremental exercise, suggesting that the magnitude of global inspiratory muscle fatigue incurred does not limit the task (Romer *et al.*, 2007b). However, $\dot{V}O_{2peak}$ is reduced by ~10 % during constant-power exercise with a tolerable duration of ~10 minutes by unloading the inspiratory muscles, and the development of inspiratory muscle fatigue is prevented (Babcock *et al.*, 2002). Moreover, constant-power exercise with a tolerable duration of ~9 minutes under control conditions is improved by ~14% by reducing inspiratory muscle work via PAV (Harms *et al.*, 2000).

These data imply that the magnitude of inspiratory muscle fatigue indicates the contribution of the inspiratory muscles to exercise intolerance, and that the inspiratory muscles only limit exercise in cases when Pdi_{tw} is decreased by ~20 % or more. Therefore, the contribution of the inspiratory muscles to the limitation of very heavy-intensity exercise likely increases as tolerable

duration becomes longer and the cumulative work of breathing becomes greater, as these conditions lead to greater inspiratory muscle fatigue.

No fatigue is induced when the inspiratory work during high-intensity exercise is mimicked in otherwise resting individuals, suggesting that the interaction between inspiratory and locomotor muscle fatigue during high-intensity exercise is the result of a competition for blood flow between the exercising muscles in the absence of sufficient cardiac output (Babcock *et al.*, 1995). Accumulation of metabolites within the fatiguing inspiratory muscles causes an increase in sympathetic outflow to systemic circulation, which increases vasoconstriction in, among other areas, the locomotor muscles (Harms *et al.*, 1997; Dempsey *et al.*, 2002; Dempsey *et al.*, 2006). This reduction in locomotor muscle perfusion compromises O_2 delivery and consequently impairs locomotor muscle performance (Harms *et al.*, 1997; Romer and Polkey, 2008). To illustrate, reducing the force output of the inspiratory muscles by 50-60 % during a constant-power trial with a tolerable duration of ~13 minutes under control conditions attenuated the reduction in quadriceps twitch force by almost 30 % (Romer *et al.*, 2006). Therefore, the greater inspiratory muscle fatigue in response to long-duration compared to short-duration high-intensity exercise may trigger a sympathetically-mediated metaboreflex response that compromises locomotor blood flow.

Another potential consequence of inspiratory muscle fatigue is a greater perception of effort. As the diaphragm fatigues, the relative contribution of this primary muscle of inspiration to ventilation declines, thereby increasing recruitment of the accessory inspiratory and expiratory muscles to meet the

ventilatory demands (Johnson *et al.*, 1993; Babcock *et al.*, 1996; Aliverti *et al.*, 1997). This increase in neural drive in response to diaphragm fatigue appears to be specific to the accessory inspiratory muscles as preceding diaphragm fatigue does not increase electrical activity of the diaphragm during CO_2 rebreathing (Luo *et al.*, 2001).

Postulated to occur through corollary discharge as a feedforward mechanism, whereby efference copies of central motor command are received by sensory areas of the brain (Enoka and Stuart, 1992; Abbiss *et al.*, 2015), motor unit recruitment (assessed by EMG) is associated with rating of perceived exertion (Lagally *et al.*, 2002; Duncan, Al-Nakeeb and Scurr, 2006; de Morree, Klein and Marcora, 2012). Therefore, progressive recruitment of accessory inspiratory and expiratory muscles likely causes a rising perception of dyspnoea and/or other unpleasant sensations concomitant with increasing central motor drive. Further, the increasingly active accessory muscles likely fatigue as well, eliciting further increases in central motor drive to maintain ventilation. This reciprocating cause and effect between fatigue and central motor drive may continue until the overall sensation associated with meeting the ventilatory demands becomes intolerable and exercise intensity must be reduced or the task terminated.

Additionally, inspiratory muscle fatigue may increase perception of effort by influencing neural drive to the locomotor muscles. As intramuscular perturbations develop during exercise, central motor drive to the locomotor muscles increases to evoke the required power output from the functionally-declining contractile machinery (Shinohara and Moritani, 1992; Vanhatalo *et*

al., 2011; Thomas *et al.*, 2016; Black *et al.*, 2017). The action of a metaboreflex secondary to inspiratory muscle fatigue may accelerate the development of locomotor muscle fatigue and necessitate further increases in neural drive to the locomotor muscles, which may underlie the respective increase and decrease in limb discomfort reported by increasing or decreasing the force output of the inspiratory muscles (Romer *et al.*, 2006).

The consequences of inspiratory muscle fatigue on exercise performance are multifarious and the extent to which inspiratory muscle fatigue limits exercise, and the mechanisms responsible, appear to be dependent on the nature of the task. However, more research is required to establish the relationship between inspiratory muscle fatigue, locomotor fatigue, and exercise limitation.

1.6 Mechanisms of exercise limitation

While the development of fatigue in the locomotor neuromuscular system is widely agreed to contribute to the exercise limitation, the precise mechanism that precipitates exercise intolerance remains debated and varies with the demands of the task (e.g. isolated muscle compared to whole-body exercise or a marathon compared to a sprint) and population (e.g. young compared to older individuals or healthy compared to diseased populations).

The traditional model of exercise limitation presents ‘man as machine’, originating from pioneering work in the 1920s, with maximal exercise limited by a metabolite-induced inhibition of contractile function of the working muscles (Hill and Lupton, 1923). This arises due to an insufficient ability to

meet the bodily demands for ATP resynthesis through aerobic energy transfer. The reliance on anaerobic energy provision causes an intramuscular metabolic perturbation that impairs the ability of the contractile apparatus to produce power. The declining function of the active motor units decreases the efficiency of contractions (Zoladz *et al.*, 2008; Vanhatalo *et al.*, 2011) and may necessitate recruitment of larger, fatigue-susceptible motor units, further dissociating the rates of ATP demand from oxidative ATP supply and increasing O_2 demand (Shinohara and Moritani, 1992; Breese *et al.*, 2014). During heavy-intensity exercise, the rising ATP requirement eventually stabilises at a rate that can be met solely through aerobic energy transfer, allowing exercise to be sustained. However, no such plateau in ATP demand occurs during very heavy-intensity exercise. Inefficiency of the active motor units progressively rises throughout the task and, when possible, triggers recruitment of additional motor units to contribute to the work output, indicated by increases in EMG amplitude (Shinohara and Moritani, 1992; Vanhatalo *et al.*, 2011; Thomas *et al.*, 2016; Black *et al.*, 2017). The inexorable rise in ATP demand causes continued energy provision from substrate-level phosphorylation, initiating a ‘fatigue cascade’ (Figure 1.5; Murgatroyd and Wylde, 2011). This process continues until $\dot{V}O_{2peak}$ is reached, at which point, or shortly thereafter, the working muscles can no longer produce the power/force required to continue the task.

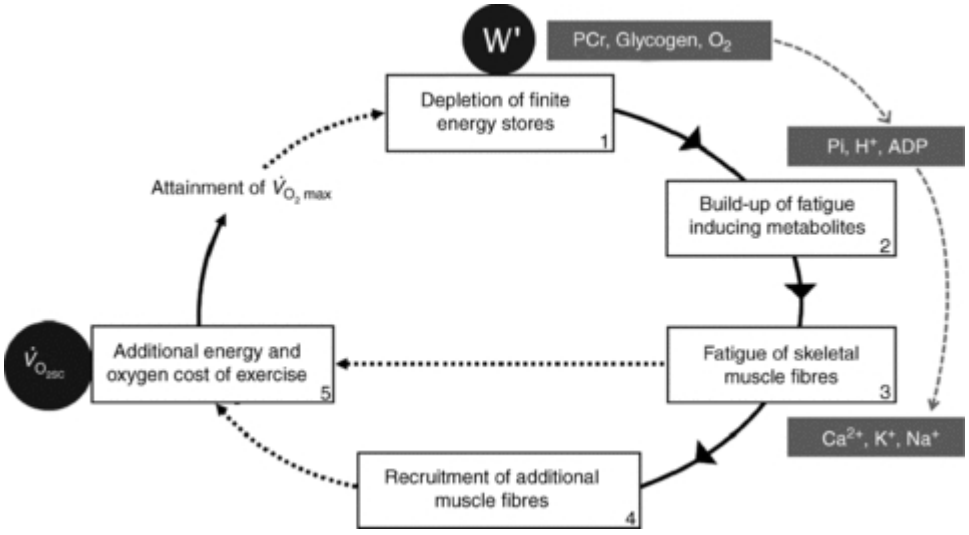

Figure 1.5: A schematic demonstrating the cascade of events causing progressive fatigue and ultimately intolerance during very heavy-intensity exercise. Solid and dotted arrows denote proven and proposed links, respectively. From Murgatroyd and Wylde (2011).

A more recent modification of this model incorporates a fundamental role for group III and IV muscle afferents. In addition to regulating ventilatory and circulatory responses to exercise to increase O_2 delivery (Amann *et al.*, 2010), these nerve fibres have been implicated in the inhibition of muscle activity. The mechanism proposed is the accumulation of fatigue-associated metabolites in the working muscles stimulates these sensory neurons, which in turn attenuate central motor drive and motoneuron excitability (Amann and Dempsey, 2008; Amann *et al.*, 2008; Amann, 2011; Amann *et al.*, 2020). Pharmacological blockade of group III/IV afferent feedback resulted in increased EMG of the quadriceps and power output during the first half of a 5 km time trial and increased peripheral fatigue post-exercise (Amann *et al.*, 2009). However, attenuating somatosensory feedback from the locomotor muscles did not improve performance as power output was 11 % lower during the second half of the time trial (Amann *et al.*, 2009). Therefore, group

III/IV muscle afferents may optimise exercise performance by restricting central motor drive, thereby moderating fatigue development and maintaining muscle function.

Moreover, this regulatory mechanism has been postulated to restrict metabolic perturbation to a 'critical threshold', which limits the extent of the damage incurred (Amann *et al.*, 2006; Amann, 2011). Similar metabolic perturbations (Burnley *et al.*, 2010; Vanhatalo *et al.*, 2010; Black *et al.*, 2017) and reductions in artificially-stimulated quadriceps twitch force at intolerance of exercise under different conditions support this notion (Amann *et al.*, 2006; Romer *et al.*, 2007a; Gagnon *et al.*, 2009; Amann *et al.*, 2009), with computer simulated time courses of muscle metabolism suggesting that muscle work terminates when a peak P_i concentration is reached (Korzeniewski and Rossiter, 2020). Further, inhibiting afferent feedback prior to exercise has been reported to lead to long-lasting and severe impairments in muscle function, such as difficulties with ambulation, which do not occur under control conditions (Amann *et al.*, 2009). This observation suggests that the increase in peripheral fatigue induced had harmful consequences. Doubt has been cast on the existence of a 'critical threshold' as different magnitudes of peripheral fatigue have been found at intolerance of different trials (e.g. (Froyd *et al.*, 2016; Thomas *et al.*, 2016). However, this does not necessarily preclude such a threshold as other mechanisms may have limited the task prior to the threshold being reached.

Recently, it has been suggested that exercise is not universally limited by a peripheral critical threshold of the working locomotor muscles, but a 'sensory

tolerance limit' determined by overall sensory information (Hureau, Romer and Amann, 2018; Thomas, Goodall and Howatson, 2018). Under this construct, exercise performance is modulated by total corollary discharge associated with central motor outflow and global afferent feedback, where an increased disruption in a specific system (e.g. pulmonary system) would result in intolerance ensuing with a reduced disruption in another system (e.g. neuromuscular system; Figure 1.6; Hureau, Romer and Amann, 2018; Thomas, Goodall and Howatson, 2018). Such a mechanism does not dismiss the well-founded role of group III/IV muscle afferents in exercise performance but would provide a reason for different magnitudes of peripheral locomotor fatigue occurring at intolerance of exercise tasks with different tolerable durations (Thomas *et al.*, 2016). While such models acknowledge the integral role of central fatigue processes in limiting exercise, they align with the traditional model in considering peripheral factors as the origin of exercise limitation.

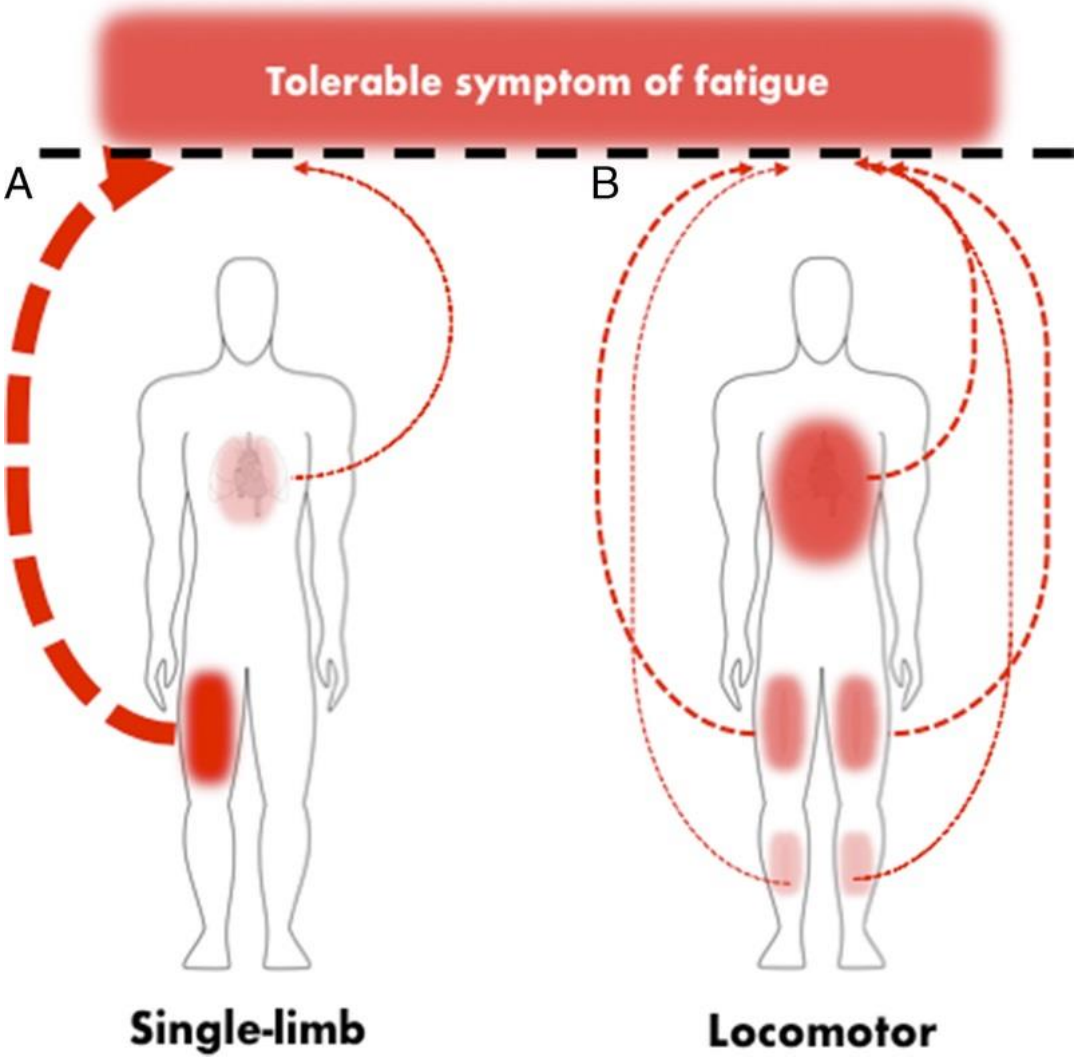

Figure 1.6: Graphic of how the contributions to exercise intolerance may be specific to the task. The size of the contribution is indicated by the thickness of the arrow. During single-limb exercise, the symptom of fatigue is predominantly due to a localised sensation in the muscle mass involved in the task (A). However, when a greater muscle mass is recruited during whole-body exercise, the demands on the pulmonary and cardiovascular systems is increased, with a corresponding contribution from these systems to the sensation of fatigue (B). Consequently, locomotor fatigue at intolerance is greater in response to the single-limb task compared to the whole-body (locomotor) task. Taken from Thomas *et al*., (2018).

The similar but simpler pyschobiological model contends that exercise termination is purely determined by sense of effort and entirely voluntary (Marcora, 2010; Pageaux, 2014). It is proposed that sensory information

from afferent and efferent sources combine to determine the perceived exertion of performing a specific exercise task. When this exertion becomes intolerable, the individual makes a conscious decision to disengage from the task. Support for this mechanism is provided by findings showing that, at intolerance, the rating of perceived exertion is close to maximal but that, in some instances, the locomotor muscles are capable of generating more power than is required to continue the task (Staiano *et al.*, 2018; Davies *et al.*, 2021). Modifying motivational factors such as music (Barwood *et al.*, 2009; Lim *et al.*, 2009) and monetary reward (Cabanac, 1986) can also induce ergogenic effects, independently of changes in physiological function. The psychobiological model fundamentally aligns with the notion of a sensory tolerance limit but emphasises the influence of efferent outflow on the perception of effort and denotes the signal to stop exercise as originating in the brain.

The most controversial model is the Central Governor, which incorporates a role for sensory feedback information alongside anticipatory feedforward control, placing the brain as the ultimate exercise determinant (Noakes, 2000; St Clair Gibson and Noakes, 2004). This sophisticated and complex protective mechanism is postulated to operate unconsciously to prevent catastrophic system failure, such as myocardial ischemia (Noakes, 2012). The model's proponents suggest that maximal cardiopulmonary values, such as $\dot{V}O_{2max}$ and cardiac output, do not represent the true capacity of each system but the maximum permitted by some moderating function within the brain. This theory has been challenged over the paucity of evidence that cardiopulmonary function is constrained by central nervous command rather

than the inherent functional ceiling of the rate of O_2 delivery and utilisation (Shephard, 2009). Indeed, EMG of the locomotor muscles can increase even when $\dot{V}O_{2max}$ has been reached, suggesting that in such instances greater neuronal output is incapable of driving increases in $\dot{V}O_2$ (Brink-Elfegoun *et al.*, 2007).

However, aspects of this model are logical and consistent with data. The anticipatory component provides an explanation for the familiar pacing strategy that culminates with a surge in performance (St Clair Gibson *et al.*, 2006; Tucker, Lambert and Noakes, 2006). Moreover, the self-selected reduction in power output when exercising in the heat (Tucker *et al.*, 2004; Tucker *et al.*, 2006) is a thermoregulatory response and demonstrates that exercise performance can be limited by factors outside cardiopulmonary function and neuromuscular fatigue. Although deaths from exertional heat-related illnesses can occur, the rarity of these cases (Nelson *et al.*, 2011) demonstrates the efficacy of thermoregulatory processes. Whether the reduction in power output to prevent increases in core temperature is voluntary or involuntary is yet to be determined, but the premise of exercise performance being constrained by feedforward control to maintain homeostasis is supported. The Central Governor Model remains controversial, particularly over assertions that a cardiopulmonary ‘reserve’ exists at maximal exercise (Shephard, 2009; Ekblom, 2009), but recent discussions show a growing acceptance that no model of exercise limitation should exclude the brain.

While a unifying theory of exercise limitation is still debated, it is likely that elements of all these models contribute to exercise intolerance. The specific mechanism may be dependent on the inherent physiology of the population and the demands of the exercise task, with the limitation of a heart failure patient performing a cardiopulmonary exercise test different to that of an elite marathon runner competing in a desert race.

1.7 Skeletal muscle adaptations to exercise

Skeletal muscle exhibits remarkable plasticity in response to altered demands, adapting metabolic and morphological properties to improve function. Importantly, the exact adaptation is dependent on the nature of the stimulus, which is composed of the degree of mechanical loading and duration for which the stimulus is imposed. Therefore, exercise training is typically separated into endurance (or aerobic) training, which refers to low-force contractions that are sustained for a prolonged period (up to several hours), and strength (or resistance) training, which comprises high-force contractions that can only be sustained for a small number of repetitions.

Endurance training primarily causes increases in the body's ability to transport and utilise oxygen for aerobic energy transfer, which minimises homeostatic disturbances and thereby enhances exercise tolerance (Bassett and Howley, 2000; Jones and Carter, 2000). Oxygen delivery can be improved by increases in cardiac output secondary to increases in plasma and red blood cell volume (Sawka *et al.*, 2000; Bonne *et al.*, 2014; Montero and Lundby, 2018) and cardiac structure and function (Baggish *et al.*, 2008), whereas extraction and utilisation can be enhanced by greater capillarity

(Andersen and Henriksson, 1977; Ingjer, 1979) and mitochondrial biogenesis (Holloszy, 1967; Holloszy and Coyle, 1984; Jacobs *et al.*, 2013). The intensity, frequency, and duration of training bouts determine the magnitude of the improvement in global aerobic function, with a greater homeostatic challenge providing a greater stimulus for adaptation (Wenger and Bell, 1986; Fiorenza *et al.*, 2018). As such, improvements in functional exercise capacity, assessed by 6-minute walk distance, and health-related quality of life has been reported to be greater in patients who develop fatigue during exercise training (Burtin *et al.*, 2012).

In contrast to endurance training, the adaptations associated with strength training relate primarily to increasing the maximal force- and power-generating capacity of the muscle via morphological, such as increases in muscle cross-sectional area arising through hypertrophy (Housh *et al.*, 1992; Abe *et al.*, 2000), and neurological changes, including improvements in muscle coordination during movement (Rutherford and Jones, 1986; Gabriel, Kamen and Frost, 2006; Folland and Williams, 2007). Consequently, strength training is an effective countermeasure to functional impairments caused by muscle weakness, such as sarcopenia (Roth, Ferrell and Hurley, 2000; Aagaard *et al.*, 2010), and therefore may be of particular importance to older populations due to the age-related decline in muscle strength and mass (Bassey *et al.*, 1992; Doherty, 2003). This impairment in muscle function, attributed predominantly to atrophy of type II muscle fibres (Nilwik *et al.*, 2013), is associated with physical disability (Visser *et al.*, 2005; Hairi *et al.*, 2010) and therefore strength training represents a fundamental approach to support healthy ageing.

While this categorisation of endurance and strength training broadly defines the different adaptations to disparate stimuli, exercise training does not exist as two independent forms but rather on a spectrum defined by the extremes of force and duration. It is established that simultaneous endurance and strength training attenuates the adaptation to either form of training performed in isolation; an ‘interference effect’ potentially caused by antagonistic action of the respective signalling cascades (Hickson, 1980; Hawley, 2009; Fyfe, Bishop and Stepto, 2014). However, while the magnitude of the adaptations may be smaller, some overlap in responses to endurance and strength training exists. For example, endurance training can induce muscle hypertrophy, with increases in muscle size between 5 and 12 % reported after a 12-week endurance training programme (Harber *et al.*, 2009; Harber *et al.*, 2012; Konopka *et al.*, 2010). This finding demonstrates that, although strength training may be superior (Grgic *et al.*, 2019), the low-force contractions inherent to endurance exercise are sufficient to drive changes in muscle morphology.

Conversely, strength training can enhance exercise tolerance in highly-trained athletes, potentially through improvements in parameters such as work economy or neuromuscular efficiency (Støren *et al.*, 2008; Rønnestad and Mujika, 2014), and can improve cardiovascular health to a similar extent as continuous endurance training in obese adults (Schjerve *et al.*, 2008). Consistent with these findings, passive movement exercise, which causes increases in blood flow and muscle tissue stretch, promotes endothelial cell proliferation and an angiogenic response (Hellsten *et al.*, 2008; Høier *et al.*, 2010), suggesting that the mechanical stimulus of movement drives vascular

adaptation independent of a metabolic stimulus. The implication of these findings is that stimuli associated with strength training may confer unique enhancements in aerobic function, and therefore the optimal training strategy to improve exercise tolerance may involve maximising the mechanical and metabolic stimulus provided by the task. This premise has generated considerable investigation into the efficacy of intermittent exercise training, which may offer an approach to combine components of endurance and strength training (MacInnis and Gibala, 2017).

1.7.1 Comparison of adaptations in response to continuous and intermittent exercise

As discussed earlier, intermittent exercise attenuates the metabolic stress associated with a given power output performed continuously (Astrand *et al.*, 1960; Turner *et al.*, 2006; Davies *et al.*, 2017). By extension, a higher power output can be performed for a given metabolic stress, increasing the mechanical strain imposed on the muscle. Additionally, the contribution to mean ATP turnover rate from PCr, which is associated with short, explosive tasks (Gastin, 2001), is greater during intermittent than continuous exercise (Davies *et al.*, 2017). As a result, intermittent exercise may provide a stimulus resembling endurance and strength training. In support of this notion, intermittent training has been reported to evoke comparable increases in $\dot{V}O_{2peak}$ to endurance training, despite constituting a lower volume of work, concomitant with increases in peak power output that were absent following endurance training (Tanisho and Hirakawa, 2009). This hybrid stimulus may have an additive effect on adaptation, giving intermittent exercise an inherent advantage over other forms of training.

In support of this notion, comparisons of interventions have found that intermittent training can induce superior adaptations, such as improvements in $\dot{V}O_{2peak}$ and endothelial function, compared to volume-matched constant-power exercise in different populations (Rognmo *et al.*, 2004; Wisløff *et al.*, 2007; Schjerve *et al.*, 2008; Donelli da Silveira *et al.*, 2020). However, it is common for such studies to implement an intermittent training protocol of higher intensity than the constant-power protocol with which it is compared. Accordingly, the typical terminology used in the literature is 'moderate-intensity continuous training' (maintaining a constant power below LT) and 'high-intensity interval training' (repeated bouts of near-maximal efforts, e.g. ≥80 % of maximal heart rate; MacInnis and Gibala, 2017). Moreover, high-intensity interval training constituting repeated bouts of maximal-effort exercise, commonly referred to as 'sprint-interval exercise', has been increasingly implemented in training studies (MacInnis and Gibala, 2017). As performing constant-power exercise at higher intensities causes greater training-induced adaptations (Wenger and Bell, 1986; Kemi *et al.*, 2005; O'Donovan *et al.*, 2005), the superior effects attributed to intermittent exercise may be due to the higher intensity rather than the pulsatile nature of the protocol.

It should be noted that several studies have reported comparable improvements in $\dot{V}O_{2peak}$ following continuous and intermittent exercise training, despite the intensity of the intermittent protocol exceeding that of the continuous protocol (Burgomaster *et al.*, 2008; Cocks *et al.*, 2013; Iellamo *et al.*, 2013; Gillen *et al.*, 2016). A common feature of these studies was that a lower volume of work was prescribed for the intermittent protocol

than the continuous protocol, suggesting that increases in training volume can compensate for reductions in intensity, and *vice versa*, and that maximising either will induce an optimal response. However, despite these similar improvements in global aerobic function, the mechanisms underlying the training effect of increases in intensity and volume may differ. As evidence for this, intermittent training has been reported to cause an increase in maximal cardiac output that was absent following work-matched continuous training at a lower intensity, but the improvement in oxygen extraction was not different between interventions (Daussin *et al.*, 2007). These findings suggest that intensity drives changes in central determinants of oxygen transport, whereas volume is associated with peripheral adaptations that enhance oxygen utilisation. Moreover, increases in mitochondrial respiration may require high exercise intensities, whereas increases in mitochondrial content are more effectively induced by greater training volume (Granata, Jamnick and Bishop, 2018a; Granata, Jamnick and Bishop, 2018b). This topic remains debated (MacInnis, Skelly and Gibala, 2019; Bishop, Botella and Granata, 2019) but these findings suggest that intensity and volume may be independent contributors to exercise-induced improvements in aerobic function (Figure 1.7). Therefore, consequent to the ability to accumulate a higher volume of supra-CP work (Chidnok *et al.*, 2012), intermittent exercise training may possess intrinsic benefits to continuous exercise training. However, the adaptive influence of the higher power output and increased energy provision from PCr during intermittent exercise is typically not considered.

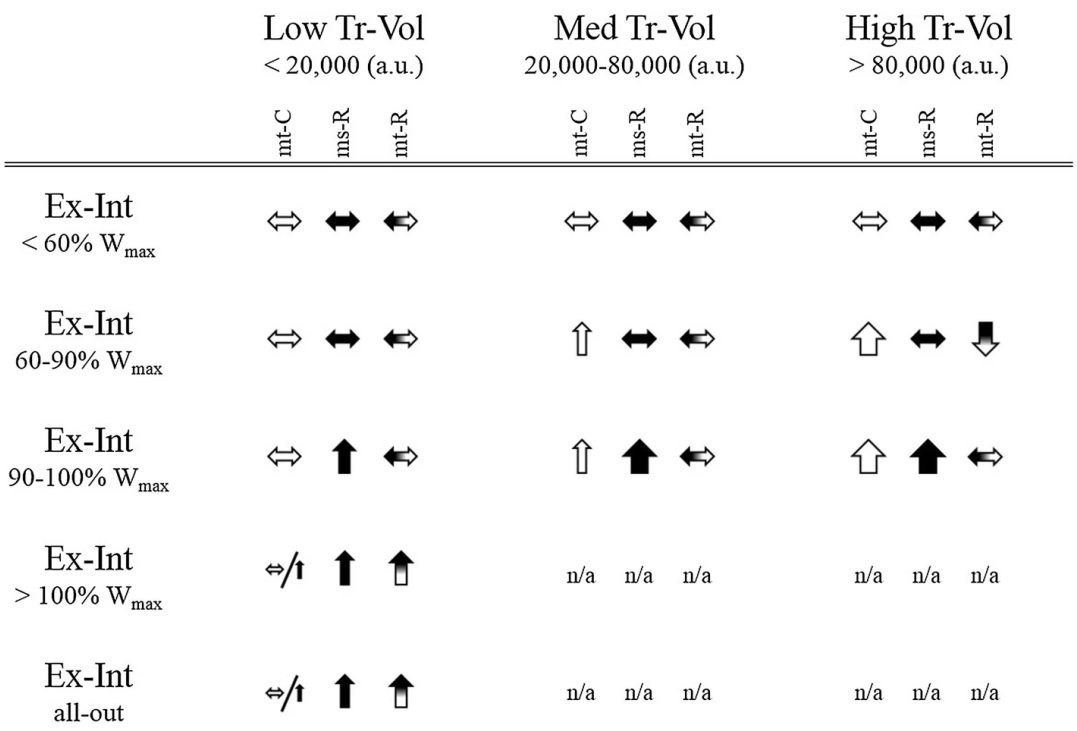

Figure 1.7: Schematic of the training-induced changes in mitochondrial content (mt-C; unfilled arrows), mass-specific mitochondrial respiration (ms-R; filled arrows), and mitochondrial specific respiration (mt-R; half-filled arrows). Exercise intensity (Ex-Int) is presented relative to the maximal rate of work during a standard incremental test (W_{max}). Training volume (Tr-Vol) is calculated as the product of the intensity, duration of work in a single session and number of sessions per week. Arrows represent a decrease (downward), no change (horizontal), or an increase (upward), with the thickness indicating the magnitude of the change. More than one arrow is presented where the outcome is equivocal. From Granata *et al*., 2018b.

To address the confounding effect of differing intensities and volume, Cochran *et al*., (2014) implemented exercise protocols with a fixed volume of work performed intermittently (4 bouts of 30 seconds) or continuously as a maximal effort. In support of an intensity-matched effect, rating of perceived exertion and blood lactate concentration after exercise were not different between protocols (Cochran *et al.*, 2014). To investigate whether the stimulus to induce mitochondrial biogenesis differed between the intermittent and continuous exercise protocols, the activation of signalling

cascades associated with mitochondrial biogenesis was assessed. Specifically, changes in phosphorylation of p38 mitogen-activated protein kinase (MAPK) and acetyl-CoA carboxylase (ACC), and mRNA expression of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) were quantified. In response to a single bout of each protocol, activation of these signalling cascades was not different between groups, indicating that the stimulus to induce mitochondrial adaptations was not influenced by the pulsatile nature of the intermittent protocol. Moreover, when performed three times per week for six weeks, the continuous protocol improved $\dot{V}O_{2peak}$, demonstrating that an intermittent stimulus is not a prerequisite for an exercise protocol to be an effective method of enhancing aerobic function (Cochran *et al.*, 2014).

However, using an intermittent protocol with short work phases and a high number of work-recovery transitions (30 bouts of 1 minute) increased phosphorylation of kinases associated with mitochondrial biogenesis to a greater extent than a constant-power protocol at an equivalent $\%\dot{V}O_{2peak}$ (Combes *et al.*, 2015). Moreover, while continuous exercise training induced an increase in $\dot{V}O_{2peak}$, maximal activity of citrate synthase and content of proteins representative of the complexes of the electron transport chain were unchanged (Cochran *et al*., 2014), which is in contrast to the increases observed following maximal intermittent exercise (Burgomaster *et al*., 2008). These findings suggest that an intermittent pattern of work may be an independent factor of the exercise stimulus that can be manipulated to enhance training outcomes, potentially due to an increase in the mechanical stimulus or change in bioenergetics. However, as there was no direct

comparison between continuous and intermittent interventions (Cochran *et al*., 2014), further research is required to confirm this effect. Additionally, the training effect of intermittent work patterns on other performance parameters, such as peak power output, remains unexplored. Therefore, a focus of this thesis was to investigate the acute (Chapter 4) and chronic (Chapter 5) responses to intensity- and work-matched constant-power and intermittent exercise to elucidate the effect of the mechanical and bioenergetic differences caused by the different work patterns.

1.7.2 Change in $\dot{V}O_{2max}$ limitation with training

In general, the pulmonary system has the capacity to ventilate at rates in excess of that required, even at maximal exercise, and mechanical constraints on hyperpnoea is not a limiting factor in exercise performance. The primary limitation to $\dot{V}O_{2max}$ in sedentary individuals is oxygen extraction capacity of the muscle (Roca *et al.*, 1989). However, while exercise promotes profound adaptations in this peripheral capacity, the lung is remarkably unresponsive (McKenzie, 2012). The respiratory muscles adapt specifically and reversibly to mechanical and metabolic stimuli like any other skeletal muscle (Romer and McConnell, 2003; Gransee, Mantilla and Sieck, 2012), but there are no discernible structural changes in lung parenchyma, airways or the chest wall following exercise training (McKenzie, 2012), despite $\dot{V}_E$ during maximal exercise being almost twofold greater in trained compared to untrained individuals (Johnson, Saupe and Dempsey, 1992). As a consequence, highly-trained individuals often encroach upon the

ventilatory limits of the pulmonary system during exercise, which may contribute to $\dot{V}O_{2peak}$ limitation (Johnson, Saupe and Dempsey, 1992).

Moreover, adaptations of the cardiovascular system, such as increases in plasma volume and capillary to fibre ratio, can increase $\dot{V}O_{2max}$ by enhancing oxygen delivery (Hellsten and Nyberg, 2015). However, a training-induced increase of 19 % in leg blood flow has been demonstrated to be inferior to the 34 % increase in oxygen diffusing capacity, indicating that the capacity of the cardiovascular system to adapt may be far outstripped by the capacity to increase oxygen extraction (Roca *et al.*, 1992).

As a result of the different adaptability of the pulmonary, cardiovascular, and neuromuscular systems, exercise training transposes the limitation of $\dot{V}O_{2max}$ from peripheral extraction to central delivery of oxygen. The relative inability to enhance central oxygen delivery imposes a ceiling on $\dot{V}O_{2max}$ so that, when exercise training is sustained chronically, $\dot{V}O_{2max}$ will eventually plateau (Martin *et al.*, 1986; Jones, 1998; Legaz Arrese *et al.*, 2005). However, increases in exercise tolerance may still be possible for trained individuals through improvements in submaximal factors, such as LT and economy of work (Martin *et al.*, 1986; Acevedo and Goldfarb, 1989; Denadai *et al.*, 2006; Iaia and Bangsbo, 2010; Lundby and Robach, 2015).

1.7.3 Monitoring changes in exercise performance

To accurately evaluate the efficacy of an intervention to enhance cardiopulmonary function, it is imperative to appropriately quantify changes in relevant variables. The principal approaches to achieve this are to directly

assess performance in an exercise test and measure parameters of physiological function that are associated with exercise tolerance (Bassett and Howley, 2000; Jones and Carter, 2000). While a variety of tests exist, the most utilised is an RIT, which allows $\dot{V}O_{2peak}$, LT, and work efficiency to be determined from a single test (Whipp *et al.*, 1981). Peak power achieved during a ramp-incremental test (RIT_{peak}) can also be used as an index of exercise performance. This test is particularly useful for initial evaluation as an appropriate ramp incrementation rate can be designated even when little is known about the training status of the individual. However, as W′ is more rapidly depleted with every increase in power output, large improvements in system function can be reflected as only relatively small increases in RIT_{peak} (Porszasz *et al.*, 2005).

An alternative measure of exercise performance, which is more responsive to changes in interventional efficacy than RIT_{peak}, is the tolerable duration of a continuous very heavy-intensity exercise task (Oga *et al.*, 2000; Porszasz *et al.*, 2005; Palange *et al.*, 2007). A drawback of using this measure is that an RIT must be performed first to determine an appropriate power output, thereby increasing participant burden. Commonly, power output is assigned based on a fixed percentage of RIT_{peak}, but this strategy has limitations. First, RIT_{peak} and any fixed percentage thereof is dependent on ramp incrementation rate, so that faster rates of increase in power output will result in higher peak power outputs and shorter tolerable durations. Second, a fixed percentage of RIT_{peak} does not consider the assigned power output relative to the individual power-duration profile. As substantial individual differences in CP and W′ exist, performing a fixed percentage of RIT_{peak} as a continuous

power output may lead to large variations in tolerable duration (Casaburi and Porszasz, 2009; Whipp and Ward, 2009). This presents an issue because, due to the hyperbolic shape of the power-duration relationship, the magnitude of improvement in exercise tolerance is dependent on the pre-intervention tolerable duration (Casaburi, 2005; Whipp and Ward, 2009). As such, assigning power outputs based on a fixed percentage of RIT_{peak} may compromise statistical analysis and interpretation of intervention efficacy (Casaburi and Porszasz, 2009; Whipp and Ward, 2009; van der Vaart *et al.*, 2014).

A further complication with variations in tolerable duration is that the mechanism of intolerance may differ between individuals, with exercise at power outputs yielding shorter tolerable durations predominantly limited by neuromuscular locomotor fatigue but those allowing exercise to be sustained for longer limited by other mechanisms (Davies *et al*., 2021). Therefore, improvements in tolerable duration in response to a training intervention may differ between individuals as a function of the pre-intervention tolerable duration rather than differences in system adaptation. As a result of the changeable nature of exercise limitation, it is recommended that pre-intervention tolerable durations are not only similar but should be between three and eight minutes (Puente-Maestu *et al.*, 2016). The reason for this time window is that it allows cardiopulmonary variables to reach their limit, which may not occur in very short or long trials, and exercise is typically limited by the integrated functioning of cardiopulmonary and neuromuscular systems, whereas longer trials may be limited by perceptual factors such as motivation (ATS/ACCP, 2003; Palange *et al.*, 2007). Accordingly, all pre-

intervention constant-power exercise tests used in Chapter 5 of this thesis were assigned power outputs that were predicted to cause intolerance in approximately 6 minutes, using a modified algorithm based on ramp-incremental exercise responses (van der Vaart *et al.*, 2014).

Direct assessment of exercise performance, such as tolerable duration of a given power output, can be supported by measures of physiological function, which may also provide insight into the exercise limitation. As exercise tolerance is primarily determined by the rate at which ATP can be resynthesised via oxidative phosphorylation (Jones and Killian, 2000), measures of aerobic function are important markers of intervention efficacy. Probably the most widely measured is $\dot{V}O_{2peak}$, which is associated with endurance performance (Saltin and Astrand, 1967; Sjödin and Svedenhag, 1985; Billat *et al.*, 2001) and health-related outcomes (Joyner and Green, 2009; Kodama *et al.*, 2009; Lee *et al.*, 2010). Typically, $\dot{V}O_{2peak}$ is responsive to exercise training, with the magnitude of the increase dependent on the initial training status (Wenger and Bell, 1986), genetics (Bouchard *et al.*, 2011) and specific training intervention implemented (i.e. training intensity and volume; Wenger and Bell, 1986; Montero and Lundby, 2017).

Given a sufficient stimulus, increases in $\dot{V}O_{2peak}$ can be considerable after only a relatively short training period, such as 23 % reported after 9 weeks of intensive training in a recreationally active cohort (Hickson *et al.*, 1981). Indeed, the majority of this increases occurred within the first 3 weeks (Hickson *et al.*, 1981). As it reflects the integrated ability of the body to deliver and utilise oxygen, $\dot{V}O_{2peak}$ provides a valuable index of maximal aerobic

function that can be used to monitor changes in cardiopulmonary function. However, while allowing clear distinction between trained and untrained individuals, performance can vary appreciably between individuals with equal $\dot{V}O_{2peak}$ (Coyle *et al.*, 1988; Coyle *et al.*, 1991). Moreover, training-induced changes in $\dot{V}O_{2peak}$ are not consistently related to changes in aerobic exercise performance (Vollaard *et al.*, 2009), demonstrating the need to simultaneously assess other determinants of performance, such as LT and economy (Bassett and Howley, 2000; Jones and Carter, 2000; Joyner and Coyle, 2008).

Combining maximal and submaximal parameters of cardiopulmonary function, such as $\dot{V}O_{2peak}$ and LT, respectively, comprises a more holistic approach to monitoring aerobic function, and may allow insight into the adaptations underlying any observed changes in exercise tolerance.

1.8 Aims and objectives

The overarching aim of this thesis was to characterise how the demands of the task influence the acute and chronic physiological responses to different exercise protocols in young, healthy individuals. A focus throughout was the development of locomotor neuromuscular fatigue, and the parameters of the exercise task and physiological consequences that determine this.

The three specific aims established were as follows:

1. To determine whether the larger locomotor power reserve at the limit of long-duration compared to short-duration very heavy-intensity exercise is related to the greater inspiratory muscle fatigue induced.

2. To investigate whether the higher power output during intermittent compared to intensity-matched constant-power exercise influences 1) the physiological or perceptual responses during the task; and 2) the magnitude of the resulting locomotor neuromuscular fatigue.

3. To determine whether intermittent exercise is a more effective training strategy than intensity-matched constant-power exercise.

2: General Methods

This chapter details the fundamental experimental procedures used in multiple studies presented in this thesis. Methods used only in specific studies, such as assessment of inspiratory muscle function in Chapter 3, are described in the relevant chapters.

2.1 Participants and ethical approval

Prior to engaging in the research studies, participants received a written information sheet detailing the experimental procedures involved and provided written informed consent. All participants were screened using a health and activity questionnaire to check eligibility and identify contraindications to exercise. For all studies, participants were aged between 18 and 45 years and were excluded based on the following criteria: a history of cardiovascular, respiratory, or musculoskeletal disease, asthma, recent illness or injury, regular smoker within the previous 6 months, pregnancy, or a family history of sudden cardiac death. Additionally, when oesophageal or gastric pressure in response to magnetic stimulation was assessed (Chapter 3), individuals with metal implants, allergies to local anaesthetic, recent nasal trauma (e.g. broken nose), or a viral illness within 2 weeks previously were excluded. Participants were able to withdraw from all research studies at any point without explanation.

All experimental procedures and supporting documentation, such as information sheets and recruitment posters, were approved by the Faculty of Biological Sciences Research Ethics Committee, University of Leeds.

2.2 Control of external factors

Changes in certain physiological and behavioural aspects had the potential to influence participant exercise performance and metabolic responses from one day to the next, which may have confounded the findings of the studies. Therefore, repeat visits for each participant were conducted at a similar time of day (within 2 hours) to minimise changes due to circadian rhythm and were separated by at least 48 hours to prevent the rigours of an exercise protocol affecting performance in the subsequent exercise protocol. Additionally, participants were required to abstain from food for 3 hours, caffeine for 12 hours, and alcohol and strenuous exercise for 24 hours immediately prior to the laboratory visit. To prevent changes due to environmental conditions, all exercise protocols were performed in a temperature-controlled laboratory.

2.3 Cycle Ergometry

All exercise protocols were performed on an electromagnetically-braked upright cycle ergometer (Excalibur Sport PFM, Lode BV, Groningen, The Netherlands), with the resistance applied to the flywheel determined by programming the associated computer software (Lode Ergometer Manager, Lode BV, Groningen, The Netherlands). When a specific power output, interchangeably referred to as power or work rate (sum product of torque and angular velocity), was programmed, the ergometer was accurate to within 2 W for power outputs below 100 W and within 2 % of power outputs between 100 and 1500 W, with these power outputs capable of being maintained between 25 and 180 $rev \cdot min^{-1}$ (manufacturer's specifications).

The positions of the handlebars and saddle were digitally recorded during the first visit for each participant and replicated on future visits to prevent biomechanical inconsistencies influencing exercise performance. The ergometer was capable of instantaneously switching between three distinct modes: cadence-independent (hyperbolic), cadence-dependent (linear), and cadence-controlled (isokinetic).

2.3.1 Cadence-independent (hyperbolic) mode

To maintain a specific power output, the ergometer can adjust the electromagnetic braking force to correspond with changes in cadence via a closed-loop feedback system. In this cadence-independent (hyperbolic) mode, the braking force is reduced when cadence is increased and *vice versa* so that the programmed power output is maintained. However, as this feedback system responds to changes in cadence, there is a delay in the adjustment in braking force that causes pedal-by-pedal power output to rise above and fall below the programmed power output. The magnitude of this 'natural fluctuation' is dependent on the individual and has important implications for the interpretation of the mechanism of exercise limitation (please see Section 2.3.6 for more information). The hyperbolic mode was used during all ramp-incremental, constant-power, and intermittent exercise protocols.

2.3.2 Cadence-dependent (linear) mode

Rather than adjusting the electromagnetic braking force according to cadence, in cadence-dependent (linear) mode the ergometer applies a fixed braking force determined by a 'linear factor'. Consequently, an increase or

decrease in cadence results in a corresponding increase or decrease in power output, the magnitude of which is dependent on the linear factor. The linear mode was used exclusively as part of a ‘ramp-sprint’ protocol in Chapter 3, where more detail is provided.

2.3.3 Cadence-controlled (isokinetic) mode

The final of the three ergometer modes used in this thesis, cadence-controlled (isokinetic) mode, adjusts electromagnetic braking force using a feedback mechanism to constrain cycling cadence to the programmed value, which was selected as 80 rev·min^{-1} for all bouts of isokinetic cycling performed in the studies presented herein. During cycling in this mode, torque is measured at the left and right crank using three strain gauges able to measure force up to 2000 N, with a resolution of ≤ 0.5 N and uncertainty ≤ 3 % (manufacturer’s specifications). Prior to every exercise protocol, a zero-force calibration of the strain gauges was performed with the right and left cranks perpendicular to the floor to negate the effect of gravity. Angular velocity (rad·s^{-1}) of the crank was measured simultaneously with torque using three optical sensors sampling in series, with an uncertainty ≤ 1 % (manufacturer’s specifications). Torque and angular velocity, and therefore crank power, were measured every 2 ° of rotation during cycling, with an angular measurement uncertainty of 1 % (manufacturer’s specifications).

2.3.4 Peak isokinetic cycling power

Excluding training sessions in Chapter 5, all exercise protocols were preceded by a series of five short (~6 second) variable-effort bouts of isokinetic cycling to determine peak isokinetic power (P_{iso}) in an unfatigued

state. Isokinetic measurements were used to control for the velocity dependence of power output (Sargeant, Hoinville and Young, 1981) and performed at 80 rev·min^{-1} as this approximates the optimal value for power generation in a fatigued state (Beelen and Sargeant, 1991), allowing valid comparison between unfatigued and fatigued values, and is close to the typical self-selected cadence of healthy individuals during high-intensity cycling. The first four efforts were performed at increasing efforts (~25, ~50, ~75, ~100 % of maximal effort) and separated by 30 seconds of recovery cycling (20 W in hyperbolic mode), with the fifth effort constituting a further maximal effort, performed after a minimum of 2 minutes of recovery, to support that a true maximal effort had been provided. When these maximal-effort values were judged by visual inspection to have differed, a third effort was performed after a minimum of a further 2 minutes of recovery. Pre-exercise P_{iso} was then calculated as the mean of the maximal efforts (within-day and between-day test-retest coefficient of variation was < 3 % and < 4 %, respectively, for all studies). Due to the increase in peak power output that can be achieved by performing prior exercise (Sargeant and Dolan, 1987), the submaximal efforts acted as a task-specific warm-up to optimise power output during the maximal efforts.

Between the variable-effort isokinetic cycling bouts, participants were instructed to maintain a cadence of ~75 rev·min^{-1}. This cadence minimised the time taken to reach the target isokinetic cadence while preventing the large braking force that is applied at the initiation of the isokinetic phase, which would inhibit the pedal stroke and therefore power output, when cadence exceeds target isokinetic cadence.

At the end of every exercise protocol, except for training sessions, the ergometer was instantaneously switched from hyperbolic to isokinetic mode and a final ~6 second maximal isokinetic bout was performed to measure P_{iso}. When exercise was submaximal, the ergometer was programmed to automatically switch modes after completion of the assigned exercise time. As during pre-exercise assessment of P_{iso}, participants were instructed to cycle at a consistent cadence of ~75 rev·min^{-1} prior to the isokinetic bout and accelerate to 80 rev·min^{-1} once the ergometer was in isokinetic mode. During maximal exercise, the modes were manually switched by the experimenter immediately after cadence fell below 60 rev·min^{-1}. As cadence at this point was considerably lower than the isokinetic cadence, all flywheel resistance was removed by the ergometer, allowing cadence to be rapidly increased to 80 rev·min^{-1} and limiting the delay between intolerance and P_{iso} assessment to, typically, ~1 second.

2.3.5 Power output analysis

Power output was calculated for each pedal stroke in hyperbolic and isokinetic mode by taking a mean of all 180 power values collected over the entire 360 ° pedal rotation. Left and right crank power were sampled, recorded, and calculated independently, then summed to generate total power for each pedal stroke. This allowed power output during the task to be compared to target power output on a pedal-by-pedal stroke basis. This method was also used when determining peak isokinetic power (P_{iso}) during a maximal isokinetic effort, which was calculated as the mean of the highest three pedal strokes constrained to 80 ± 1 rev·min^{-1} during the isokinetic

phase. Baseline P_{iso} was calculated as the mean P_{iso} of the two maximal isokinetic efforts performed prior to exercise (Davies *et al*., 2021).

2.3.6 Natural fluctuation in power output

A cycling task may only be sustained while participants can produce the necessary power. During constant-power exercise, the power output set by the ergometer is unchanging and the mean power output is equal to this. However, due to slight inconsistencies in motor unit recruitment intrinsic to muscle activity, the pedal-by-pedal stroke power output deviates from the programmed power throughout the task by an individual-specific magnitude (laboratory observations suggest that it is typically smaller in trained cyclists). This 'natural fluctuation', quantified for each individual as the 95 % prediction bands around the local mean for the entire test, is a fundamental consideration when determining whether locomotor neuromuscular fatigue is limiting exercise.

The power-generating capacity of the locomotor muscles at intolerance may exceed the power requirement of the task, but not the upper boundary of natural fluctuation. In such instances, the ability to exceed the power requirement of the task may not be sufficient to compensate for the pedal strokes that fall below the power requirement as part of the natural cycling pattern. Consequently, while isolated pedal strokes may still meet or even exceed the power requirement, *mean* power output falls below the threshold and the task is unsustainable.

2.3.7 Locomotor neuromuscular fatigue and power reserve

In all studies, the magnitude of exercise-induced locomotor neuromuscular fatigue was calculated as the difference between baseline and end-exercise P_{iso}. The magnitude of the power reserve was calculated as the difference between the target (programmed) power during hyperbolic cycling and end-exercise P_{iso}. In addition to statistical significance, a power reserve was only considered to be present when P_{iso} exceeded the upper boundary of the individual natural fluctuation (Davies *et al*., 2021).

2.4 Exercise protocols

Ramp-incremental exercise, during which power output is increased as a linear function of time, was performed during the first visit in all studies to determine key physiological variables (i.e. LT and $\dot{V}O_{2peak}$) that informed subsequent exercise prescription. Constant-power exercise, during which an unchanging power output was performed throughout, was also used during every study, either for a pre-determined time or until intolerance. Details of the specific exercise protocols used in the studies are provided in the relevant chapters.

During all maximal exercise protocols, the point of intolerance was defined as cadence falling below 60 rev·min^{-1} despite strong vocal encouragement. This threshold cadence was selected as healthy participants typically cycle between 70 and 90 rev·min^{-1} during high-intensity cycling (laboratory observation), and therefore 60 rev·min^{-1} provides a suitable limit to demonstrate that the individual is no longer able to produce the required

power output while allowing sufficient error for a temporary drop in cadence to be recovered. The tolerable duration of constant-power exercise was defined as the time between exercise onset and the point at which cadence fell below 60 rev·min^{-1} and the peak ramp-incremental power (RIT_{peak}) was defined as the highest power output achieved before cadence fell below 60 rev·min^{-1}.

2.5 Pulmonary gas exchange

2.5.1 Principles and application

At the onset of exercise, pulmonary and cellular gas exchange is upregulated to increase the O_2 flux from the atmosphere to cytochrome c oxidase - the terminal complex of the mitochondrial electron transport chain - and clearance of CO_2 generated by mitochondrial respiration and buffering processes (Wasserman, Van Kessel and Burton, 1967; Wasserman, 1978; Rossiter, 2011). Aside from differences due to body O_2 and CO_2 storage and vascular transport delays between tissues and lungs (Whipp, 2007; Rossiter, 2011), pulmonary gas exchange measures of O_2 ($\dot{V}O_2$) and CO_2 ($\dot{V}CO_2$) provide acceptable non-invasive estimates of muscle O_2 utilisation and CO_2 production that can be used to evaluate aerobic function and characterise exercise (Grassi *et al.*, 1996).

2.5.2 Equipment and measures

Excluding training sessions conducted in Chapter 5, breath-by-breath pulmonary gas exchange and ventilation was assessed during all exercise protocols using a gas exchange analysis system (Medgraphics Ultima Series, Medical Graphics Corporation, St Paul, MN, USA). To sample gases,

a bidirectional Pitot tube ‘preVent’ flow sensor with a dead space of 39 mL was connected to an umbilical from the analyser, then inserted into a mouthpiece through which the participant breathed. The participant wore a nose clip while pulmonary gas exchange was being measured to direct all inspired and expired gases through the mouthpiece. Analysis of pulmonary gas content was performed by an electrochemical O_2 sensor, in which the magnitude of the voltage generated across a zirconium dioxide element is dependent on the difference in the partial pressure of O_2 between pulmonary gas and atmospheric air, and a non-dispersive infrared CO_2 sensor, which quantifies CO_2 content via relative light absorption. Due to the transit required for gas samples to reach the O_2 and CO_2 sensors, combined with the additional analysis time, flow is calculated prior to relative gas content. Therefore, online time alignment of all measures was performed relative to the start and end of each breath as determined by the flow sensor.

The flow, O_2, and CO_2 sensors were calibrated prior to each exercise protocol. Atmospheric air pressure, temperature, and humidity were recorded to correct inspired volumes relative to the greater warmth and humidity of expired gases. To calibrate the flow sensor, five ‘inspired’ and five ‘expired’ air flows from a 3 L syringe (Hans Rudolph, Kansas City, MO, USA) were generated at flows rates spanning the physiological range from rest to maximal exercise (0.2 – 6 $L \cdot s^{-1}$). This calibration procedure was repeated until mean ‘inspired’ and ‘expired’ volumes were between 2.99 and 3.01 L, with a standard deviation of ≤ 0.02 L, and remains valid for 12 hours (manufacturer’s specifications). Calibration of the O_2 and CO_2 sensors was performed by sampling two gas mixtures with known concentrations of O_2

and CO_2 that represent the extremes of the physiological range at rest and maximal exercise (O_2: 21 and 12 %; CO_2: 0 and 5 %). Upon completion of every exercise protocol, the two calibration gas mixtures were sampled again to verify that the initial calibration had remained stable throughout the exercise protocol.

2.5.3 Data analysis

Pulmonary gas exchange data can be affected by coughs, swallows, or other disruptions to normal breathing pattern, generating breath-by-breath values that are not reflective of the true underlying physiological response (Lamarra *et al.*, 1987). Therefore, the data was initially processed by removing all values that lay outside the 99 % prediction limits fitted around the local mean, thereby excluding erroneous values from subsequent analysis. This range was sufficient to identify outliers while ensuring that no responses reflective of the true underlying physiological response were removed (Lamarra *et al.*, 1987).

2.5.4 Peak oxygen uptake ($\dot{V}O_{2peak}$) determination

During all tests performed to intolerance, peak oxygen uptake during the task ($\dot{V}O_{2peak}$) was determined as a 12-breath mean, which was chosen as the smallest number of values required to acceptably minimise breath-by-breath noise (Bowen *et al.*, 2012). To ensure the peak value was recorded, the highest of 8 rolling means moving backwards from the final breath was taken as $\dot{V}O_{2peak}$. When exercise was performed to intolerance at different power outputs, $\dot{V}O_{2peak}$ could be verified as maximal oxygen uptake ($\dot{V}O_{2max}$;

Rossiter, Kowalchuk and Whipp, 2006; Poole and Jones, 2017), although $\dot{V}O_{2peak}$ during ramp-incremental exercise performed to intolerance in ~10-15 minutes is typically not different from $\dot{V}O_{2max}$ in healthy individuals (Day *et al.*, 2003). The procedure to determine $\dot{V}O_{2peak}$ was also used to determine peak values in other pulmonary gas exchange variables and hear rate.

2.5.5 Lactate threshold (LT)

Pulmonary gas exchange measures during ramp-incremental exercise were used to noninvasively measure lactate threshold (LT). To minimise the risk of a pseudo-threshold, which may arise through CO_2 storage secondary to hyperventilation, ramp-incremental exercise was only initiated when the respiratory exchange ratio (RER) was stable between 0.7 and 0.9 (Ozcelik, Ward and Whipp, 1999). After editing to remove outliers, breath-by-breath data from the incremental phase was isolated. The V-slope method was used to identify LT as the inflection point in $\dot{V}CO_2/\dot{V}O_2$, which is a consequence of non-metabolic CO_2 production from bicarbonate buffering of the sustained acidosis (Beaver, Wasserman and Whipp, 1986). The $\dot{V}O_2$ at LT was then corroborated by a simultaneous increase in the ventilatory equivalent for O_2 ($\dot{V}_E/\dot{V}O_2$) with no increase in the ventilatory equivalent for CO_2 ($\dot{V}_E/\dot{V}CO_2$), concomitant with an increase in end tidal partial pressure of O_2 ($PETO_2$) but not end tidal partial pressure of CO_2 ($PETCO_2$).

Estimates were provided independently by three researchers, with LT calculated as the mean when all values were within 200 ml. In the rare cases when only two of the values were within 200 ml, a mean of these was taken as LT and the third value was discarded.

2.5.6 Electrocardiography (ECG)

Throughout all exercise protocols, a 12-lead electrocardiography (ECG) system (Mortara Instrument; Milwaukee, WI, USA) was used to continuously monitor electrical activity of the heart. All researchers were able to identify primary ECG abnormalities, such as S-T segment depression/elevation, although no exercise protocols had to be terminated due to any related issues. This 12-lead ECG system was also used to measure heart rate from the R-R interval, recorded ‘breath-by-breath’ (to align with pulmonary gas exchange data) on the gas analysis system.

2.6 Electromyography (EMG)

2.6.1 Principles and application

Skeletal muscles are composed of multiple muscle fibres that can be stimulated by motor neurons stemming from the spinal cord (Kuo and Ehrlich, 2015). The axon of a single motor neuron innervates multiple muscle fibres so that an action potential generated by a motor neuron causes simultaneous propagation of action potentials along all these innervated fibres, initiating calcium ion release into the intracellular space and ultimately activation of the contractile apparatus (Heckman and Enoka, 2012). Due to this synchronous response to motor output, the motor neuron and all the muscle fibres innervated by its axon are called a motor unit (Heckman and Enoka, 2012). The action potentials traversing the sarcolemma create extracellular currents that combine to form a field potential that can be detected by electrodes on the surface of the skin (Kazamel and Warren, 2017). As all motor unit action potentials detectable by the electrode are

superimposed to create a single signal (called an interference pattern), the amplitude of the signal is dependent on the number of motor units recruited during the contraction (Farina, Merletti and Enoka, 2004; Farina, Merletti and Enoka, 2014). Therefore, surface EMG is typically unable to isolate single motor unit activity and provides a measure of global muscle activation (Farina, Merletti and Enoka, 2004).

However, there are factors associated with surface EMG that compromise interpretation of the data. First, as the action potential depends on the number and characteristics of the fibres within each motor unit, the amplitude of a surface action potential is only partially due to motor unit size (Keenan *et al.*, 2006; Farina *et al.*, 2010), creating a dissociation between signal amplitude and neural drive. Second, motor neurons do not discharge in unison, causing negative and positive phases of action potentials to overlap. The result of this interference is amplitude cancellation, with the absolute amplitude of surface EMG less than the motor output from the spinal cord (Day and Hulliger, 2001; Farina *et al.*, 2010). Amplitude cancellation increases at higher levels of motor drive, likely due to the increase in action potential discharge that can cause interference, and causes particular suppression of low-threshold motor unit activity (Farina *et al.*, 2008; Farina *et al.*, 2010). However, simulations indicate that the effect of amplitude cancellation can be reduced to below 5 % when EMG amplitude is normalised to maximal values (Keenan *et al.*, 2005). Therefore, surface EMG was used in this thesis to assess muscle activity relative to maximal effort and to quantify exercise-induced reductions in the ability to maximally activate the muscle.

2.6.2 Equipment and application

Electrical activity of the locomotor muscles was assessed using surface electromyography (EMG) in Chapter 3 (Trigno Avanti, Delsys Inc., Natick, MA, USA) and Chapter 4 (Telemyo 2400T G2, Noraxon USA Inc., Scottsdale, AZ, USA), with different systems used due to equipment availability. No EMG data was collected for the study presented in Chapter 5 due to equipment failure. The vastus lateralis and vastus medialis were assessed in Chapter 3, with these supplemented by the rectus femoris, biceps femoris, and gastrocnemius lateralis in Chapter 4.

Electrode positioning adhered to the following guidelines provided by the European concerted action project Surface Electromyography for the Noninvasive Assessment of Muscles (SENIAM): vastus lateralis, 66 % of the distance from anterior superior iliac spine to the lateral side of the patella; vastus medialis, 80 % of the distance from the anterior spina iliac superior and the joint space in front of the anterior border of the medial ligament; rectus femoris, 50 % of the distance from the anterior spina iliac superior to the superior part of the patella; biceps femoris, 50 % of the distance from the ischial tuberosity and the lateral epicondyle of the tibia; gastrocnemius lateralis, 33 % of the distance from the head of the fibula and the heel. Once the position had been established, electrodes were placed over the muscle belly and in line with the orientation of the muscle of interest.

To optimise signal quality and minimise baseline signal noise, the skin under each muscle site was shaved to improve adherence, and abraded and cleaned with an alcohol wipe (70 % isopropyl alcohol) to remove surface skin

cells that may compromise conductance (Nuprep, Weaver and Company, Aurora, CO, USA).

2.6.3 Data analysis

Due to the different equipment used, data collection and analysis varied and therefore details are presented in the relevant chapters. However, the same process was used in Chapters 3 and 4 to evaluate exercise-induced changes in neural drive to the locomotor muscles, which involved collecting EMG data during maximal isokinetic cycling pre- and post-exercise. After initial processing, the three contractions matching the pedal strokes used to determine P_{iso} were identified and the peak EMG amplitude determined for each of the muscles investigated for each pedal stroke. The peak EMG amplitude for each of the muscles was then summed to provide a single value for each pedal stroke, and a mean of the three pedal strokes was subsequently calculated to determine pre- and post-exercise EMG amplitude. All post-exercise EMG amplitudes were normalised to the pre-exercise value (Coelho *et al.*, 2015). In addition to minimising the effect of amplitude cancellation, normalisation limits the influence of daily variations in skin temperature, hydration, conductance, or slight difference in electrode placement, and inter-individual differences such as the thickness of the subcutaneous layer (Dimitrov *et al.*, 2002).

2.7 Capillary blood sampling

2.7.1 Principles and application

When the rate of aerobic energy transfer to resynthesise ATP is inadequate, contributions are derived from PCr and glycolysis/glycogenolysis, the latter resulting in the formation of lactate that accumulates in the muscle and blood. The rate at which lactate accumulates is dependent on the disparity between the rates of ATP production via oxidative phosphorylation and ATP demand. Therefore, while now recognised as an important substrate for oxidative metabolism, gluconeogenesis, and muscle glycogenesis, rather than a waste product and fatigue facilitator, blood lactate concentration is an effective marker of exercise intensity. No sustained increase in blood lactate concentration is indicative of moderate, a sustained increase reaching a plateau is indicative of heavy, and a progressive increase is indicative of very heavy-intensity exercise (Whipp, Ward and Wasserman, 1986).

2.7.2 Equipment and measures

Samples of capillary blood were taken from the earlobe or fingertip (Chapters 3 and 4, respectively) to determine blood lactate concentration at rest and during exercise. For samples taken from the fingertip, arterialisation was performed prior to blood sampling at rest by submerging the finger in warm water (~45 °C) for a minimum of 5 minutes. The sampling site was disinfected with an alcohol wipe (70 % isopropyl alcohol) and allowed to dry to prevent haemolysis, then punctured with a single-use safety lancet (Safe T Pro Plus, Accu-Chek, Msida, Malta). The first drop of blood was removed to prevent sample contamination from interstitial and intracellular fluid. The

sample was then collected and analysed as described in the relevant chapters.

2.8 Rating of perceived exertion (RPE)

2.8.1 Principles and application

During exercise, sensory feedback from the pulmonary, cardiovascular, and neuromuscular systems is integrated in the brain with feedforward efferent output to generate a conscious sensation that can be interpreted as perceived exertion. The most common method to quantify perceived exertion is to use the Borg RPE Scale (Borg, 1974), an interval scale ranging from 6-20 arbitrary units with specific values given descriptions for reference (e.g. 9 and 15 are equal to ‘very light’ and ‘hard’, respectively). The values were proposed to correspond with heart rate by a factor of 10 (e.g. exertion would be given a rating of 10 when heart rate was 100 beats·min^{-1}), which was intended to aid interpretation of the values acquired. However, it was conceded that this is not valid in all cases as the heart rate corresponding to a specific perceived exertion depends on factors such as age, mode of exercise, and environment (Borg, 1982). Nevertheless, RPE using this scale is associated with physiological markers of stress in a heterogeneous population (Scherr *et al.*, 2013) and the rate of increase in RPE during exercise predicts the time to intolerance (Crewe, Tucker and Noakes, 2008). Therefore, RPE data provides a valuable addition to physiological measures for characterising exercise intensity.

The scale was subsequently modified from an interval scale to a ratio scale, allowing the magnitude of perceived exertion to be quantified relative to

individual-specific lower and upper thresholds (i.e. no exertion and maximal exertion), rather than aligning the sensation to descriptions such as ‘light’ and ‘hard’ (Borg, 1982). An advantage of this approach is that it removes inter-individual variation in the interpretation of the descriptions provided (e.g. of two individuals experiencing the same magnitude of perceived exertion, one may consider it somewhat hard and the very hard). Additionally, the number range of this Borg category-ratio scale was simplified to 0-10 (Borg CR-10 Scale), creating a more intuitive scale and, by abandoning the relationship to heart rate, allowing symptoms other than global perceived exertion to be rated. Indeed, blood lactate concentration and heart rate were found to correlate more closely with ratings of ‘leg exertion’ when acquired using the Borg CR-10 Scale rather than the Visual Analogue Scale, which requires exertion to be rated by marking a certain distance on a line between the two extremes of no exertion and maximal exertion, or magnitude estimation, whereby the magnitude of the sensation is assigned to a number with no boundaries imposed (Neely *et al.*, 1992). The Borg CR-10 Scale has also been used to independently assess dyspnoea and perceived ‘leg fatigue’ during exercise in healthy individuals (Borg *et al.*, 2010), which are the symptoms most associated with exercise intolerance in healthy individuals (Ekblom and Goldbarg, 1971).

2.8.2 Equipment and measures

Ratings of dyspnoea and leg tiredness were acquired using a modified Borg CR-10 Scale so that each numerical value was associated with a verbal description, specific symptoms relating to dyspnoea, and colour to aid

participant understanding of what each value represented. The scale used is presented in Figure 2.1. Participants were instructed to rate the ‘difficulty of their breathing’ and ‘how tired their legs were feeling’ as these questions were considered to best reflect sensations of exertion, rather than other noxious sensations such as pain. To avoid talking, which may affect the pulmonary gas exchange measures, the responses to these questions were communicated by pointing to the appropriate value on the scale. These same questions were used in all studies. The time points at which these data were acquired are detailed in the relevant chapters.

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

Figure 2.1: Modified Borg CR-10 Scale used to acquire perceived ratings of dyspnoea and leg tiredness.

2.9 Near-infrared spectroscopy (NIRS)

2.9.1 Principles and application

The transmission of light through a medium is dependent on the reflectance, scattering and absorption of photons during transit. Reflectance is primarily due to the angle at which the light strikes the surface of the medium; however, scattering and absorption are dependent on the wavelength of light. While the effect of scattering is uniformly reduced as the wavelength increases, absorption is determined by the properties of the light-absorbing molecules (chromophores) within the medium.

Although largely impenetrable to visible light (~400-700 nm) due to the magnitude of scattering and absorption at these wavelengths, skeletal muscle is relatively transparent to NIR light (~700-1000 nm), allowing the amount of light passing through the illuminated tissue to be measured. The main chromophores in skeletal muscle are haemoglobin (Hb), myoglobin (Mb), and cytochrome c oxidase, the terminal complex of the electron transport chain. Absorption of NIR light by Hb and Mb, which are oxygen carriers in the blood and skeletal myocytes respectively, is dependent on whether the iron core of these chromophores is bound to oxygen. The absorption coefficient of deoxygenated Hb and Mb (deoxy[Hb+Mb]) decreases as wavelength increases, whereas the inverse is true for oxygenated Hb and Mb (oxy[Hb+Mb]). Although cytochrome c oxidase has four redox centres, two haem and two copper, which produce optical interference, this chromophore exists in concentrations of less than 5 % compared to Hb and Mb (Balaban, Mootha and Arai, 1996; Davis and

Barstow, 2013) and therefore has a relatively small effect on light attenuation. Consequently, although not possible to discriminate between the independent contribution of Hb and Mb to light attenuation as the NIR absorption spectra are indistinguishable (Mancini *et al.*, 1994; Davis and Barstow, 2013), changes in NIR light absorption by skeletal muscle are considered to primarily reflect the concentration and oxygenated status of Hb and Mb. By measuring the relative changes in different wavelengths of light, NIRS can provide a noninvasive measure of muscle oxygenation.

To accurately measure changes in oxy[Hb+Mb], deoxy[Hb+Mb] and total (oxy[Hb+Mb] + deoxy[Hb+Mb]), skeletal muscle light attenuation must be appropriately quantified. In addition to contributing to light attenuation through dispersion, scattering increases the optical path length from source to detector. Consequently, the Beer-Lambert Law, which describes the attenuation of light, must be modified to account for this interference in photon travel by incorporating the differential path length factor (DPF). Due to the variability in the dispersion of photons caused by scattering, DPF is calculated as the mean path length of light travelling from source to detector. Therefore, changes in skeletal muscle light attenuation from an arbitrary baseline can be quantified using the Modified Beer-Lambert Law as follows:

$$A = \log\left(\frac{Io}{I}\right) = \varepsilon \cdot [C] \cdot L \cdot DPF + G$$

Equation 2.1

where A is attenuation of light; I_o is the incident or source light; I is the recovered light; ε represents the absorption coefficient of the chromophore;

C is the concentration of the chromophore; L is the distance between the source and detector (minimum path length); DPF is the differential path length factor; and G is light attenuation due to scattering (light lost from field of view).

Continuous-wave spectroscopy utilising a light source of constant intensity and a photodiode detector, which together can quantify changes in light attenuation caused by the skeletal muscle, was used in the studies presented in this thesis. An inherent assumption of continuous wave spectroscopy is that light attenuation due to scattering, and consequently the DPF, remains constant throughout the measurement. Therefore changes in light attenuation solely represent changes in oxy[Hb+Mb], deoxy[Hb+Mb] and total (oxy[Hb+Mb] + deoxy[Hb+Mb]) from an arbitrary baseline, providing an estimate of the change in muscle oxidation that reflects the dynamic balance between the rate of O_2 delivery and utilisation (i.e. oxygen extraction). It should be acknowledged that it is unknown whether dehydration and increases in muscle temperature during high-intensity exercise affect scattering, which would influence the NIRS signal.

While continuous-wave spectrometers cannot calculate the true path length of light (L · DPF) and therefore cannot determine the absolute concentration of chromophores in the illuminated muscle, spatially-resolved spectrometers can provide a semi-quantitative measure of muscle oxygenation (expressed as a percentage). This measure, termed tissue saturation index (TSI), is calculated as oxy[Hb+Mb]*100/total[Hb+Mb] and can be directly compared between exercise trials and subjects (Jones *et al.*, 2016). Spatial resolution

involves measuring light attenuation from light sources at multiple distances from the detector, allowing the gradient of light attenuation against distance to be calculated and thereby improving the estimation of light attenuation due to scatter. This technique also increases the contribution of deeper skeletal muscle to the measurement and dampens the influence of more superficial tissues, such as the skin and adipose tissue, enhancing the signal quality (Messere and Roatta, 2013).

2.9.2 Equipment and measures

A continuous-wave spectrometer with spatial resolution (PortaMon, Artinis Medical Systems, Elst, The Netherlands) was used to estimate muscle oxygenation during exercise in Chapters 4 and 5. The spectrometer used had three light emitting diodes located 30, 35, and 40 mm from the photodiode detector, with each source emitting light with wavelengths of 760 and 850 nm. The device was positioned over the muscle belly of the vastus lateralis of the left leg, with the centre ~20 cm from the patella, and affixed parallel with the vertical axis of the upper leg using Transpore tape (Blacknell, Berkshire, UK). The vastus lateralis was investigated due to its significance during cycling and the ability to locate the spectrometer on this muscle without disrupting the cycling motion. The location of the spectrometer was chosen to minimise alterations of the interface between the spectrometer and skin caused by changing muscle length and has been shown to provide measures consistent with more proximal positions during high-intensity cycling (Kennedy *et al.*, 2006). Once secured, the position of the spectrometer was marked with indelible ink to aid replacement during

subsequent visits, and the device and surrounding skin were covered with opaque material to prevent ambient light disrupting the signal. Recordings were initiated with participants at rest so that exercise measures were relative to resting values. The spectrometer was connected to a laptop via Bluetooth for data acquisition (sampled at 10 Hz), analogue-to-digital conversion, and to export data for subsequent analysis.

2.10 Statistical analysis

All statistical analysis was performed using SPSS Statistics 24-26 (SPSS Inc, Chicago, IL). Data for all variables were determined to be from normally distributed populations, as assessed by visual inspection of box plots and histograms and the Shapiro-Wilk test. As rating of perceived exertion scores were associated with written descriptions (e.g. easy, moderate, hard), the data was considered ordinal and therefore was analysed using nonparametric tests. All other variables were analysed using parametric tests. All data are reported as mean ± standard deviation, with statistical significance (acceptable type 1 error) set at $P < 0.05$. Exact P values are reported to 2 decimal places except where multiple values are reported simultaneously.

3: Mechanisms of intolerance for long- and short-duration very heavy-intensity exercise: a possible role for inspiratory muscle fatigue

The data presented in this chapter represent a subset of that collected as part of a larger study conducted in collaboration with another PhD student. Therefore, data that are relevant to the different study components, such as characterisation of exercise performance and global inspiratory muscle fatigue measures, are common to both theses. However, the study component presented in this chapter has unique aims and novel data.

3.1 Introduction

During very heavy-intensity exercise, defined as exercise at a power output that exceeds critical power (CP; the asymptote of the power-duration relationship), fatigue-related metabolites (e.g. ADP, P_i, and H^+) progressively accumulate before reaching a consistent intramuscular concentration, $\dot{V}O_2$ rises inexorably to $\dot{V}O_{2max}$, and the power-generating capacity of the working muscles progressively decreases (development of exercise-induced fatigue; Poole *et al.*, 1988; Jones *et al.*, 2008; Burnley *et al.*, 2010; Vanhatalo *et al.*, 2010; Black *et al.*, 2017; Swisher *et al.*, 2019). By measuring maximal voluntary isokinetic power (P_{iso}) prior to exercise and immediately at intolerance, it is possible to quantify the magnitude of exercise-induced fatigue in a task-specific manner, without the confounding effects of recovery that are inherent to non-volitional techniques (Coelho *et al.*, 2015; Cannon *et al.*, 2016; Ferguson *et al.*, 2016b). Moreover, comparison of P_{iso} at intolerance and task power (power required at the cycle ergometer flywheel

to continue the exercise task) can provide insight into the relative contribution of locomotor neuromuscular fatigue to the mechanism of intolerance (Coelho *et al.*, 2015; Cannon *et al.*, 2016; Ferguson *et al.*, 2016b).

In young healthy individuals, P_{iso} at the limit of short-duration (< 6 minutes) constant-power exercise ($Tlim_{SHORT}$) is not different from task power (unpublished data from our laboratory). This inability to generate more than task power at the limit of $Tlim_{SHORT}$ suggests that neuromuscular fatigue has developed to the extent that task power represents the limit of the power-generating capacity of the locomotor muscles (there is no 'power reserve'). Conversely, at intolerance of longer-duration (~7–15 minutes) constant-power exercise ($Tlim_{LONG}$), locomotor neuromuscular fatigue is present but P_{iso} remains greater than task power, revealing the presence of a power reserve at intolerance (unpublished data from our laboratory; Staiano *et al.*, 2018). A reserve in locomotor power at intolerance intimates a dissociation between perceptual and physiological statuses, with the task perceived as unsustainable prior to physiological limitation by neuromuscular locomotor fatigue. These findings suggest that, while $Tlim_{SHORT}$ is ultimately limited by neuromuscular locomotor fatigue, additional physiological mechanisms likely contribute to intolerance in $Tlim_{LONG}$.

One factor that might influence the contribution of locomotor neuromuscular fatigue to exercise intolerance is inspiratory muscle fatigue, which develops during high-intensity whole-body exercise (> 85 %$\dot{V}O_{2max}$) in spontaneously-breathing, healthy individuals (Johnson *et al.*, 1993). Inspiratory muscle fatigue can be assessed non-volitionally via changes in transdiaphragmatic

twitch pressure (Pdi_{tw}) in response to cervical magnetic stimulation (Similowski *et al.*, 1989). Data from our laboratory, which comprise a subset of the data presented herein and are reported in detail in the PhD thesis by Tim Hardy, showed that constant-power exercise with a tolerable duration of ~5 minutes (i.e. $Tlim_{SHORT}$) induced a reduction in Pdi_{tw} (−14 ± 12 %), indicating that inspiratory muscle fatigue was present. However, when the power output was reduced so that the tolerable duration was ~10 minutes (i.e. $Tlim_{LONG}$), the reduction in Pdi_{tw} was greater (−22 ± 12 %), indicating that inspiratory muscle fatigue was exacerbated (more data is presented in the results of this chapter).

The reduction in Pdi_{tw} following $Tlim_{SHORT}$ was similar to that observed in response to maximal incremental exercise (−15 %). Moreover, high rates of inspiratory muscle work were sustained for a comparably brief time during both protocols (< 5 minutes; Romer *et al.*, 2007b). Reducing the normal inspiratory muscle work during maximal incremental exercise did not enhance performance, suggesting that the inspiratory muscles were not contributory to exercise limitation (Romer *et al.*, 2007b). Due to the similar severity of inspiratory muscle fatigue in response to $Tlim_{SHORT}$ and maximal incremental exercise, it is likely that the inspiratory muscles also do not contribute to limiting $Tlim_{SHORT}$ (at least in healthy individuals). This proposal is consistent with the absence of a power reserve at intolerance of $Tlim_{SHORT}$ and therefore that locomotor neuromuscular fatigue is the predominant limitation to this task.

In contrast, decreasing inspiratory muscle work during constant-power cycling with a tolerable duration of ~9 minutes under normal conditions (i.e. $Tlim_{LONG}$), in response to which inspiratory muscle fatigue is likely exacerbated, increased tolerable duration by 14 % (Harms *et al.*, 2000). This ergogenic effect supports the assertion that the inspiratory muscles can contribute to the exercise limitation during longer tasks, when a greater volume of high rates of inspiratory work is accumulated. Therefore, greater inspiratory muscle fatigue in response to $Tlim_{LONG}$ compared to $Tlim_{SHORT}$ may underly the presence and absence, respectively, of a power reserve at intolerance.

As support for an association between inspiratory and locomotor neuromuscular fatigue, the time taken to complete a cycling time trial is increased and locomotor fatigue attenuated when the inspiratory muscles are fatigued prior to the task (Fulton *et al.*, 2020). This finding suggests that locomotor muscle power output may be limited as a consequence of inspiratory muscle fatigue. Such an integrated system response to whole-body exercise (unlike isolated muscle tasks, when the cardiopulmonary demand does not encroach on the limits of the systems; Thomas, Goodall and Howatson, 2018) could arise as a result of a ‘sensory tolerance limit’ (Gandevia, 2001). This construct proposes that exercise tolerance is determined by global feedforward and feedback sensory information, manifesting as an overall perceived exertion or sense of effort, rather than processes localised exclusively in the skeletal muscle (Hureau, Romer and Amann, 2018).

While the diaphragm is the primary muscle of inspiration, accessory inspiratory muscles that act on the rib cage (scalene muscles, sternocleidomastoid, and intercostal muscles) also contribute to inspiratory muscle work. Indeed, the rib cage muscle contribution to inspiratory pressure generation progressively increases relative to the diaphragm as exercise proceeds (Johnson *et al.*, 1993; Babcock *et al.*, 1996; Aliverti *et al.*, 1997). Using cervical magnetic stimulation, it is possible to discriminate between diaphragm and rib cage muscle fatigue (Similowski *et al.*, 1998). However, it is unclear what the relative contributions of diaphragm and rib cage muscle fatigue are to the greater global inspiratory muscle fatigue following $Tlim_{LONG}$ compared to $Tlim_{SHORT}$. Further, sense of inspiratory effort is correlated with rising rib cage muscle, but not diaphragm, activity (Ward *et al.*, 1988) and global inspiratory muscle and diaphragm fatigue have little or no effect on perceived leg or inspiratory effort (Bradley *et al.*, 1986; Sliwiński *et al.*, 1996). As a power reserve at intolerance is proposed to be the consequence of a dissociation between perceptual and physiological limits, locomotor neuromuscular fatigue may be constrained during $Tlim_{LONG}$ by rib cage muscle fatigue independently of diaphragm fatigue.

Therefore, the aims of this study were to 1) determine the relative contribution of diaphragm and rib cage muscle fatigue to the greater global inspiratory muscle fatigue that occurs in response to $Tlim_{LONG}$ compared to $Tlim_{SHORT}$; and 2) identify whether the magnitude of exercise-induced inspiratory muscle fatigue (global, diaphragmatic or rib cage muscle fatigue) is related to the magnitude of the reserve in locomotor power at intolerance. It was hypothesised that the greater global inspiratory muscle fatigue in

response to $Tlim_{LONG}$ compared to $Tlim_{SHORT}$ would be predominantly due to increased rib cage muscle fatigue, and that the severity of rib cage muscle fatigue, but not diaphragm fatigue, would be associated with the magnitude of the power reserve.

3.2 Methods

3.2.1 Participants and ethical approval

Ten healthy participants (2 females: 8 males; age 25 ± 5 yr; height 176 ± 8 cm; weight 71.9 ± 9.5 kg) volunteered to participate in the study. The exclusion criteria applied is described in Section 2.1. Written informed consent was provided by all participants and all experimental techniques were approved by the University of Leeds Faculty of Biological Sciences Research Ethics Committee (approval ref: BIOSCI 17-016). Pulmonary function was assessed and confirmed within normal limits via percent predicted values of forced vital capacity (FVC; 103 ± 12 %), forced expiratory volume in 1 second (FEV_1; 98 ± 10 %), and maximal voluntary ventilation (MVV; 113 ± 18 %).

3.2.2 Study overview

The study design involved three separate sessions that were each conducted at least 48 hours apart in a temperature-controlled laboratory. Each session was performed at a similar time of day to minimise the influence of circadian rhythm. Subjects were requested to avoid caffeine ingestion for 12 hours, alcohol consumption for 24 hours, and strenuous exercise for 48 hours before each session. During the first visit, participants performed pulmonary function testing and were familiarised with the

magnetic stimulation procedures and volitional test (Mueller manoeuvre) to determine maximal inspiratory pressure (MIP), which were assessed during future visits. During this first visit, participants also performed a 'ramp-sprint' protocol to determine appropriate task powers for the subsequent exercise trials. The second and third visits incorporated constant-power cycling exercise protocols performed to intolerance, with different power outputs prescribed for each so that the tolerable durations would be approximately 5 and 10 minutes. The order of these visits was randomised and counterbalanced. During visits 2 and 3, exercise-induced inspiratory muscle fatigue and locomotor neuromuscular fatigue were determined, alongside quantification of the power reserve (Figure 3.1).

3.2.3 Pulmonary function Testing

Pulmonary function (FVC, FEV_1, and MVV) was assessed in accordance with standard procedures (Miller *et al.*, 2005) using an online spirometer (Medical Graphics D-series, Medgraphics, Medical Graphics Corporation, St Paul, MN, USA). Briefly, the FVC and FEV_1 manoeuvre required a maximal inspiration to total lung capacity, a brief pause at TLC (1-2 seconds), followed by a 'blasted' expiration to residual volume, and completed by another maximal inspiration to total lung capacity. Assessment of MVV required the participant to breathe as rapidly and deeply as possible for 12 s, with an ideal rate of 90 breaths·min^{-1} (Miller *et al.*, 2005). All manoeuvres were demonstrated to the participants before being performed, with verbal feedback and encouragement. The FVC and FEV_1 manoeuvre was performed until three measures within 150 ml were recorded (typically

requiring three to five manoeuvres). Due to the strenuous nature of the assessment, MVV was measured only twice for each participant.

3.2.4 Exercise protocols

All exercise protocols were performed on a computer-controlled electromagnetically-braked cycle ergometer, with the capability of switching instantaneously between cadence-independent (hyperbolic), isokinetic and cadence-dependent (linear) cycling modes (Excalibur Sport PFM; Lode, Groningen, The Netherlands). The power generated by the participant, calculated as the product of torque and angular velocity, was measured at the bottom bracket of the crank every 2° of angular rotation.

3.2.4.1 Ramp-sprint

During the first visit, a modified ramp-sprint protocol was performed (Murgatroyd et al., 2014). This protocol involved (1) a ramp-incremental phase at 25-30 $W \cdot min^{-1}$ (RIT_{rate}) to measure peak ramp-incremental power (RIT_{peak}) and peak oxygen uptake ($\dot{V}O_{2peak}$); (2) a 6-second maximal effort in isokinetic mode, with cadence fixed at 80 $rev \cdot min^{-1}$, at intolerance of the ramp-incremental phase to measure P_{iso} and determine whether a power reserve was present; (3) a maximal effort 3-minute ‘sprint’ in linear mode, during which the flywheel resistance was fixed and power was solely determined by cycling cadence, to measure ‘sprint power’ (an estimate of critical power).

In the sprint phase the flywheel braking resistance applied was determined using a linear factor calculated as:

$$\frac{\text{predicted critical power}}{\text{target cadence}^2}$$

Equation 3.1

where predicted critical power was estimated as the product of body mass in kilograms and a fixed factor dependent on participant characteristics. The fixed factor used was 3 for males (Murgatroyd et al., 2014) and 2.5 for females (determined from pilot work conducted for this thesis). In instances where the participant was highly trained, as identified by a training history questionnaire (e.g. competitive cyclist), the fixed factor was increased by 0.5 (determined by further pilot work conducted for this thesis). Target cadence was set at 80 rev·min^{-1}, which represents the optimal cadence for power generation in fatigued conditions (Beelen and Sargeant, 1991; Elmer *et al.*, 2013). The time taken for power output to stabilise during the sprint phase can depend on the individual (Murgatroyd *et al.*, 2014), and therefore the variability in power output during the sprint phase was assessed for each participant using comparison of 30 second time bins. Using this approach, power output during the first 30 seconds has been shown to be lower than all subsequent time points (Murgatroyd *et al.*, 2014), likely due to the time taken in linear mode to increase cadence from that at intolerance of the ramp-incremental phase (< 50 rev·min^{-1}) to the target cadence (80 rev·min^{-1}). However, in isokinetic mode, which was used to measure P_{iso} between the ramp-incremental and sprint phases, the flywheel is unloaded

when cadence is below the target value. Therefore, in this modified version, cadence can be rapidly increased at the start of the sprint phase until the target value (in this instance 80 rev·min^{-1}) is met, attenuating the delay in reaching the cadence associated with the highest sustainable power output during the linear phase. Therefore, mean power output during the entire duration of the sprint phase performed in linear mode was initially calculated, with any 30-second time-bins that were not within 5 % of this value subsequently discarded and the recalculated value defined as sprint power. Using this technique, the time taken for a plateau in power output to be established ranged from 0 to 90 seconds.

3.2.4.2 Constant-power trials

The constant-power trials, which were performed in a randomised order and counterbalanced between participants, were preceded by a warm-up consisting of 2 minutes of unloaded pedalling and 2 minutes at 30 % of RIT_{peak}, which was below lactate threshold in all participants. The power outputs for the trials were calculated as:

$$Tlim_{SHORT} = RIT_{peak} - 2 \cdot RIT_{rate}$$

Equation 3.2

$$Tlim_{LONG} = CP + 0.25 \cdot (RIT_{peak} - CP)$$

Equation 3.3

Exercise was maintained until the limit of tolerance, defined as an inability to maintain cadence above 60 rev·min^{-1}.

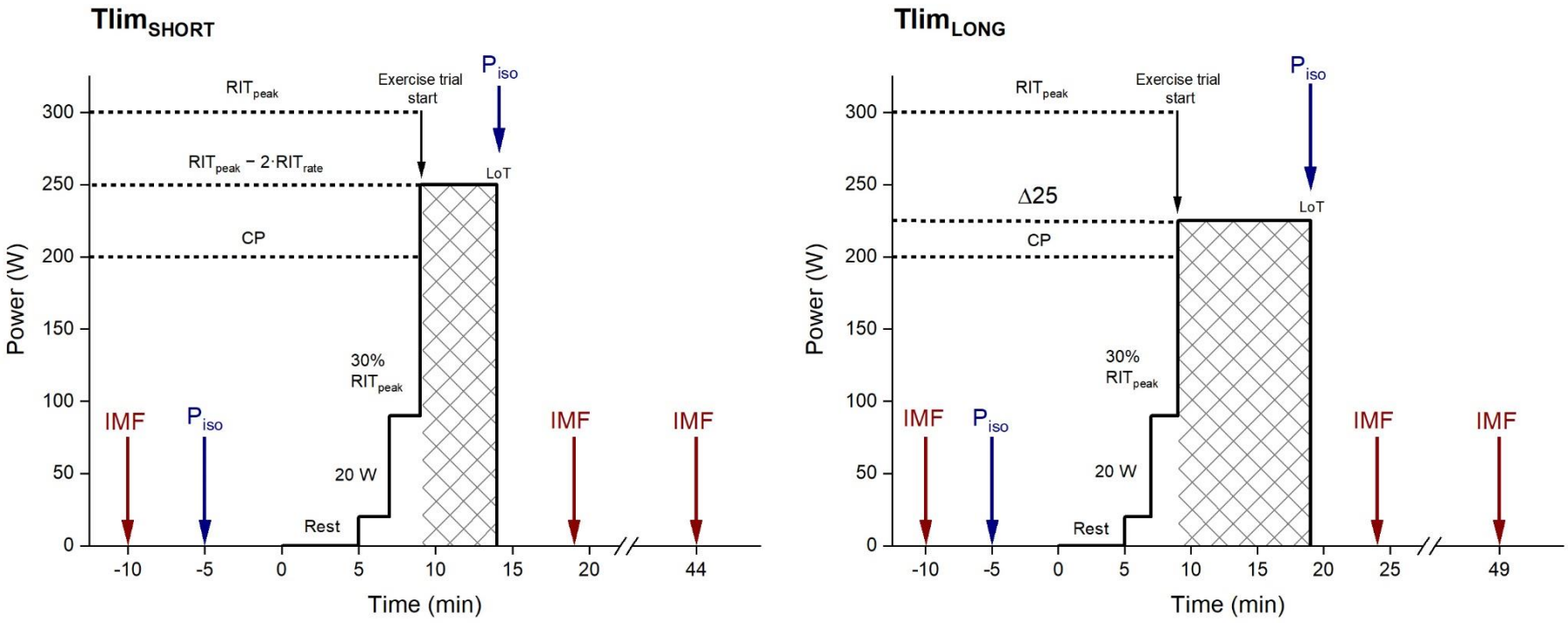

Figure 3.1: Constant-power exercise experimental protocols. RIT_{peak}, peak ramp-incremental power; RIT_{rate}, rate of increase of power during ramp-incremental test; CP, critical power; Δ25, the power 25 % of the difference between CP and RIT_{peak}; IMF, inspiratory muscle function assessment; P_{iso}, peak isokinetic cycling power assessment; LoT; limit of tolerance. All values for power and tolerable duration are provided as representative examples and do not constitute data from the study.

3.2.5 Exercise responses

Breath-by-breath pulmonary gas exchange variables and heart rate (from a 12-lead ECG) were measured throughout all exercise protocols (Medical Graphics D-series, Medgraphics, Medical Graphics Corporation, St Paul, MN, USA). Briefly, the analyser was calibrated prior to each protocol across a range of flow rates using a 3-L syringe and gases of known O_2 and CO_2 concentration. All participants breathed through a mouthpiece while wearing a nose clip. Capillary blood samples were obtained from the earlobe before exercise and at intolerance to determine blood lactate concentration (n = 9; Lactate Pro 2). Perceptual ratings of dyspnoea (‘rate the difficulty of your breathing’) and leg exertion (‘rate how tired your legs are feeling’) were recorded using a modified Borg CR-10 scale accompanied by verbal descriptions (see Section 2.8 for full details of the scale used).

3.2.5.1 Operational lung volumes

To evaluate the prevalence of dynamic lung hyperinflation, defined as an increase in end-expiratory lung volume above functional residual capacity, end-expiratory lung volume was assessed at rest, after the first minute of exercise, and thereafter at 2-minute intervals until intolerance. End-expiratory lung volume was calculated as the difference between FVC at rest and inspiratory capacity, thereby assuming that total lung capacity is constant throughout exercise (Johnson *et al.*, 1999). Each participant was familiarised with the inspiratory capacity manoeuvre prior to the first exercise trial, including a detailed explanation and demonstration. Participants performed multiple inspiratory capacity manoeuvres at rest until these could be consistently

reproduced, as confirmed by visual inspection of changes in volume and Poes. Additional guidance and practice were provided when required. A verbal prompt was delivered to the participants to initiate each inspiratory capacity manoeuvre, which were accompanied by verbal encouragement to maximise participant effort. Two inspiratory capacity manoeuvres were performed at each time point and, when comparable, a mean was calculated. When a manoeuvre was clearly disrupted (e.g. by a cough or a swallow), the corresponding value was discarded. A minimum of six breaths immediately prior to the first inspiratory capacity manoeuvre were analysed and, when appropriate, pneumotachograph drift and changes in breathing pattern were corrected via manual adjustment.

3.2.6 Locomotor neuromuscular fatigue and power reserve

A standardised warm-up was performed before each exercise protocol. This consisted of a minimum of 4 minutes unloaded cycling followed by 3 submaximal isokinetic cycling bouts at variable efforts (~25, ~50, and ~75% maximum effort). Each submaximal effort was performed for 6 seconds and separated by 30 seconds of unloaded cycling (20 W). Subsequently, peak isokinetic cycling power (P_{iso}) was measured in an unfatigued state. For this, participants performed two maximal 6-second efforts, separated by 2 minutes, in the isokinetic cycling mode with cadence constrained to 80 $rev \cdot min^{-1}$. In addition, P_{iso} was measured within 1 second of exercise task termination using the same technique to assess the power-generating capacity of the locomotor muscles at intolerance. The mean of the highest three maximal effort crank revolutions that were constrained to 80 ± 2 $rev \cdot min^{-1}$ during the 6-second

isokinetic phase was then P_{iso}. The magnitude of locomotor fatigue was calculated as the difference in pre- to post-exercise P_{iso}.

To determine whether a reserve in locomotor power was present at intolerance, the difference between post-exercise P_{iso} and the programmed task power was calculated. However, while the mean power produced over the duration of the task is equal to the programmed task power, each pedal stroke has minor variations in muscle recruitment and pedal-by-pedal stroke power fluctuates around the mean, with approximately half of all pedal strokes above and half below the mean. Therefore, a power reserve was only considered to be present when post-exercise P_{iso} exceeded this 'natural fluctuation' in pedal-to-pedal stroke power output. Natural fluctuation was quantified for each constant-power trial as the 95 % prediction bands around the mean of all pedal strokes during the trial.

3.2.7 Electromyography

Surface electromyography (EMG) was recorded for the vastus lateralis and vastus medialis. Locomotor muscle activity was defined as the sum of the EMG signals for these two muscles. Electrode positioning followed Surface Electromyography for the Non-invasive Assessment of Muscle guidelines and sites were marked with indelible ink to assist the accurate replacement of electrodes on subsequent visits. Raw EMG was recorded throughout all isokinetic efforts and the duration of the exercise task. The raw EMG signal was bandpass filtered between 20 and 500 Hz and rectified and smoothed via root mean square (RMS) with a moving time window of 25 ms. Locomotor muscle activity during the isokinetic efforts at baseline and the limit of tolerance was

defined as the mean RMS peak amplitude of the three pedal strokes selected as representative of P_{iso} in that condition. Locomotor muscle activity RMS peak amplitude was also calculated for each pedal stroke during the exercise task, and mean activity for each minute was subsequently calculated to quantify changes in locomotor muscle activity during the task.

3.2.8 Inspiratory pressure measurements

Transdiaphragmatic pressure was calculated as the difference between gastric and oesophageal pressures using balloon-tipped catheters inserted into the lower one-third of the oesophagus and the stomach (Figure 3.2). The exact position within the oesophagus was determined by the 'occlusion' technique (Baydur *et al.*, 1982). Differential pressure transducers Validyne DP15, Northridge, CA), which were calibrated across the standard physiological range using an electromanometer (model M14; Mercury, Glasgow, Scotland), were connected to each catheter. The balloon positioned within the oesophagus was filled with 1 ml of air and, to prevent collapse under the higher pressure, the balloon positioned in the stomach was filled with 2 ml of air.

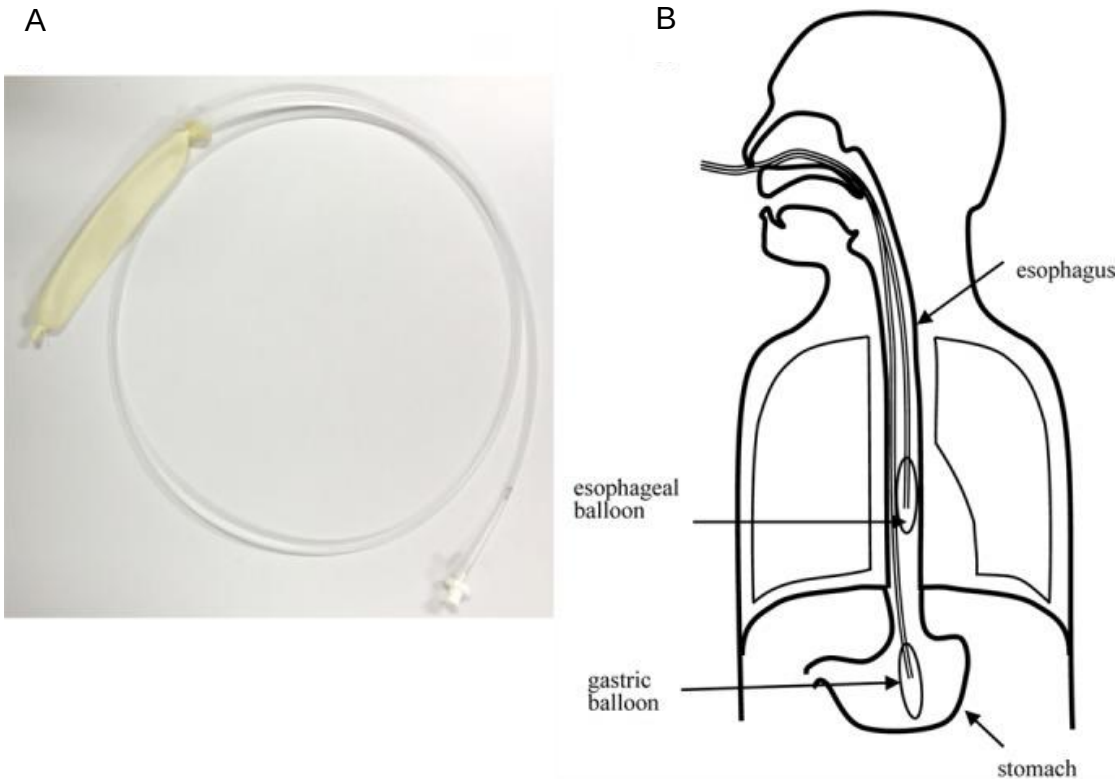

Figure 3.2: The balloon catheter technique for oesophageal and gastric pressure measurements. A: example image of a balloon catheter; B: schematic demonstration of the positioning of the balloon catheters. From Mase *et al.*, (2018).

3.2.9 Magnetic nerve stimulation

3.2.9.1 Principles and application

According to Faraday's Law of electromagnetic induction, exposing a conductor to a changing magnetic field induces a current (Faraday, 1832). In the case of motor neurones, an induced current of sufficient amplitude causes depolarisation of the cell membrane and initiates an action potential, ultimately causing muscle contraction. Therefore, cervical magnetic stimulation of the phrenic nerve roots can be used to obtain nonvolitional measures of peripheral fatigue for the inspiratory muscles. The mechanism of stimulation is equivalent to electrical stimulation, but the mechanism of induction is different.

To reach deep nervous tissue, the current supplied by electrical stimulation must pass through all intervening structures and the loss of current that occurs via dispersion requires the currents flowing in the skin to be greater than that required to depolarise the motor neurone. Therefore, to ensure supramaximality, the electrical stimulus is often large enough to trigger pain by stimulating the sensory nerve endings in the skin. In contrast, a magnetic field can penetrate soft tissue and bone to depolarise deep neurones without inducing high currents in the skin, and therefore without causing pain. Moreover, magnetic fields preferentially activate larger fibres, which are not associated with pain mediation. Consequently, magnetic stimulation has considerable advantages over electrical stimulation in terms of participant discomfort and tolerability of the technique.

The stimulator generates magnetic pulses using a capacitor discharge circuit. An energy-storage capacitor is charged to a high voltage by an external power supply so that, when triggered, current is rapidly discharged from the capacitor into a stimulating copper coil, generating a magnetic field with a strength up to several Teslas.

3.2.9.2 Equipment and measures

Magnetic stimuli were delivered to the phrenic nerve roots via a circular 90-mm coil powered by a magnetic stimulator (Magstim BiStim 2; Magstim, Whitland, Wales). The subjects were sat in a vertical position with the neck flexed to encourage a relaxed posture while permitting maximum contact between the coil and the skin. The coil was placed over the vertebrae column between the 5^{th} (C5) and 7^{th} (C7) cervical vertebrae. The optimal position within this range

was defined as that which evoked the highest transdiaphragmatic twitch pressure and was marked with indelible ink to maximise the consistency of the coil position. Due to the influence of diaphragm muscle length on Pdi_{tw}, all stimulations were performed at functional residual capacity, which was inferred from Poes. To determine whether nerve stimulation using this technique was 'supramaximal' (i.e. the stimulator intensity was greater than that required to fully depolarise the phrenic nerve roots), three single twitches were administered at 50, 60, 70, 80, 85, 90, 95 and 100 % of the maximal power of the stimulator, each separated by 30 seconds. There was a plateau (represented by no significant increase in amplitude as determined by a one-way repeated-measures ANOVA) in Pdi_{tw} between 90 % and 100 % of the maximal power of the stimulator, indicating that phrenic nerve depolarisation was approaching maximality in response to the stimulation procedure.

3.2.10 Inspiratory muscle function assessment

Voluntary maximal inspiratory pressure (MIP) was assessed via a brief (~5 seconds) maximal forced inspiration against an occluded airway (Mueller manoeuvre) at residual volume and a potentiated resting twitch was obtained immediately after the Mueller manoeuvre. This procedure was performed a minimum of six times but, due to a smaller degree of potentiation in response to the first and second Mueller manoeuvre, the first two measurements were typically discarded along with measurements where Poes at the point of stimulation clearly deviated from that at functional residual capacity. Potentiated twitches were used to assess fatigue as postactivation potentiation induced by the exercise task may confound changes in pre- to post-exercise unpotentiated twitch pressure (Laghi, D'Alfonso and Tobin, 1995). Potentiated twitches are

also more sensitive measures of fatigue than unpotentiated twitches and therefore are more valid when the magnitude of fatigue is small (Kufel, Pineda and Mador, 2002), as was expected in the present study due to the short duration of one of the exercise trials. Global inspiratory muscle fatigue was assessed as the pre- to post-exercise reduction in magnetically evoked Pdi_{tw}, calculated as the difference between Pga_{tw} and $Poes_{tw}$. The first post-exercise assessment was performed ~5 minutes after exercise cessation.

Magnetic cervical stimulation induces contractions of the diaphragm and accessory inspiratory muscles that act on the rib cage (scalenes, sternocleidomastoid, and external intercostal muscles). It is possible to discriminate between diaphragm and rib cage muscle fatigue and determine the relative contribution of each to global inspiratory muscle fatigue (reduction in Pdi_{tw}; Similowski *et al.*, 1998). While rib cage muscle fatigue and diaphragm fatigue can both contribute to a reduction in Pdi_{tw}, stimulation of the rib cage muscles acts primarily on Poes rather than Pga, and therefore rib cage muscle fatigue should reduce $Poes_{tw}$ but have no influence on Pga_{tw}. Conversely, stimulation of the diaphragm acts on both Poes and Pga, but due to the action of the rib cage muscles, diaphragm fatigue should result in a greater decrease in Pga_{tw} than $Poes_{tw}$. Therefore, a reduction in Pdi_{tw} associated with a reduction in $Poes_{tw}/Pga_{tw}$ but not Pga_{tw} would indicate predominantly rib cage muscle fatigue, whereas a reduction in Pdi_{tw} associated with a reduction in Pga_{tw} with no reduction in $Poes_{tw}/Pga_{tw}$ would suggest predominantly diaphragm fatigue.

The within-day reproducibility of diaphragm function was determined during a session selected at random. The procedure was performed as described above

and then repeated 30 minutes later, during which time the participants were disconnected from the apparatus and sat at rest on the bench where the procedure was performed. There were no systematic differences in MIP or Pdi_{tw}, and the coefficients of variation for these values were 2.8 % and 3.7 % respectively.

3.2.11 Data capture and analysis

Gastric and oesophageal pressure analogue signals were passed through an amplifier (model 1902; Cambridge Electronic Design, Cambridge, UK) and digitised at a sampling rate of 150 Hz using an analogue-to-digital converter (micro 1401, Cambridge Electronic Design). Pressure and EMG data was captured and subsequently analysed using data acquisition and analysis software (Spike 2 version 8.1; Cambridge Electronic Design).

3.2.12 Statistical analysis

All single value variables (e.g. $\dot{V}O_{2peak}$, peak heart rate) during the constant-power protocols were compared using paired-sample t-tests. Two-way repeated-measures analysis of variance (ANOVA) was used to compare absolute changes in Pdi_{tw} and P_{iso} from pre- to post-exercise and between groups ($Tlim_{SHORT}$ vs. $Tlim_{LONG}$) to determine whether (1) the task induced fatigue and (2) the magnitude was different between protocols. Pearson's correlation coefficient (*r*) was calculated to assess the relationship between exercise-induced changes in inspiratory and locomotor parameters. Considerable individual variation has been reported in the magnitude of exercise-induced inspiratory muscle fatigue (Johnson *et al.*, 1993) and the

power reserve (Hodgson *et al.*, 2018), even under controlled exercise conditions with highly comparable demands. This individual variability may be due to differences in training status, tolerable duration of a task, or pain threshold, among other physiological and perceptual factors. To account for these individual differences, all correlations were performed using inter-trial differences (Δ) for each individual. These Δ values were calculated as the pre- to post-exercise response to $Tlim_{SHORT}$ subtracted from the corresponding value for $Tlim_{LONG}$, where zero would represent an equal pre- to post-exercise change in both trials. As such, this method of analysis did not determine whether the physiological responses to a given task were associated, which could be confounded by individual differences, but whether the change in physiological responses *between* tasks were associated. Inspiratory parameters evaluated were MIP, Pdi_{tw}, Pga_{tw}, and $Poes_{tw}$, all expressed as percent change from baseline. Locomotor parameters were locomotor fatigue (P_{iso} as a % of baseline) and power reserve (W). Moderate and strong correlations were defined as when *r* exceeded 0.4 and 0.7, respectively. Results are expressed as the mean ± the standard deviation. Statistical significance was set at $P < 0.05$ and all statistical analyses were performed using SPSS 26 (SPSS Inc., Chicago, IL).

3.3 Results

3.3.1 Ramp-sprint

Ramp-incremental $\dot{V}O_{2peak}$ was 4.15 ± 0.92 L·min^{-1} (57.1 ± 9.0 ml·kg^{-1}·min^{-1}) and peak power (RIT_{peak}) was 321 ± 57 W, which was not different to P_{iso} at intolerance (306 ± 93 W; P = 0.56). During the plateau phase of the maximum-effort 3-minute bout (i.e. the 'sprint'), cadence was 76 ± 8 rev·min^{-1} and mean power output was 202 ± 55 W. Mean $\dot{V}O_2$ during this phase was lower than $\dot{V}O_{2peak}$ achieved on the ramp (P < 0.01), but was still 96 ± 3 % of $\dot{V}O_{2peak}$, indicating that a maximal effort was maintained.

3.3.2 Constant-power exercise and responses

Selected variables measured at intolerance are displayed in Table 3.1. Task power was higher during $Tlim_{SHORT}$ (263 ± 53 W) than $Tlim_{LONG}$ (234 ± 53 W; P < 0.01) and consequently tolerable duration was shorter (4.9 ± 0.7 minutes vs. 10.2 ± 2.6 minutes, respectively; P < 0.01). Peak values for $\dot{V}CO_2$ and $\dot{V}_E$ were higher at the limit of $Tlim_{SHORT}$ than $Tlim_{LONG}$, with the latter achieved through increases in B_f and V_t (all P < 0.05). Conversely, peak heart rate was higher during $Tlim_{LONG}$ than $Tlim_{SHORT}$ (P = 0.046), although the physiological significance of 3 beats·min^{-1} is equivocal. There was no difference between tasks in $\dot{V}O_{2peak}$ (P = 0.31; confirming $\dot{V}O_{2peak}$ as $\dot{V}O_{2max}$) or end-exercise lactate (P = 0.63), consistent with maximum effort being provided in all tests. Electrical activity of the locomotor muscles was higher during the final minute of the task than the first minute of the task during $Tlim_{SHORT}$ and $Tlim_{LONG}$ (EMG_{RMS} amplitude increased by 27 ± 24 % and 22 ± 22 %, respectively; main effect, P = 0.02), but this change over time was not different between protocols

(interaction effect, P = 0.19). Final ratings of perceived exertion were also not different between $Tlim_{SHORT}$ and $Tlim_{LONG}$, with sensations of dyspnoea (9 ± 1 vs. 10 ± 1, respectively; P = 0.59) and leg tiredness (10 ± 0 vs. 10 ± 0, respectively; P = 0.32) rated as between ‘severe’ and ‘maximal’.

Table 3.1: Peak exercise values

	$Tlim_{SHORT}$	$Tlim_{LONG}$
$\dot{V}O_{2peak}$ ($L \cdot min^{-1}$)	4.08 ± 0.82	4.02 ± 0.86
$\dot{V}CO_2$ ($L \cdot min^{-1}$)	4.79 ± 1.00	4.32 ± 0.90*
B_f ($breaths \cdot min^{-1}$)	66 ± 7	62 ± 7*
V_t (L)	2.5 ± 0.4	2.4 ± 0.1*
$\dot{V}_E$ ($L \cdot min^{-1}$)	157 ± 31	145 ± 29*
Heart rate ($beats \cdot min^{-1}$)	180 ± 8	183 ± 9*
Blood lactate (mM)	12.3 ± 4.3	11.6 ± 3.5

* Significantly different from $Tlim_{SHORT}$ (P < 0.05). Values are means ± S.D. Definitions of abbreviations: $\dot{V}O_2$, oxygen uptake; $\dot{V}CO_2$, carbon dioxide output; B_f, breathing frequency; V_t, tidal volume; V_E, minute ventilation.

3.3.3 Locomotor neuromuscular fatigue and power reserve

Pre-exercise P_{iso} was not different between $Tlim_{SHORT}$ and $Tlim_{LONG}$ (777 ± 162 W vs. 776 ± 172 W, respectively; $P = 0.93$). There was a pre- to post-exercise reduction in P_{iso} following $Tlim_{SHORT}$ and $Tlim_{LONG}$ (−461 ± 192 W vs. −420 ± 226, respectively; main effect, $P < 0.01$); however, this was not different between trials (interaction effect, $P = 0.24$; Figure 3.3).

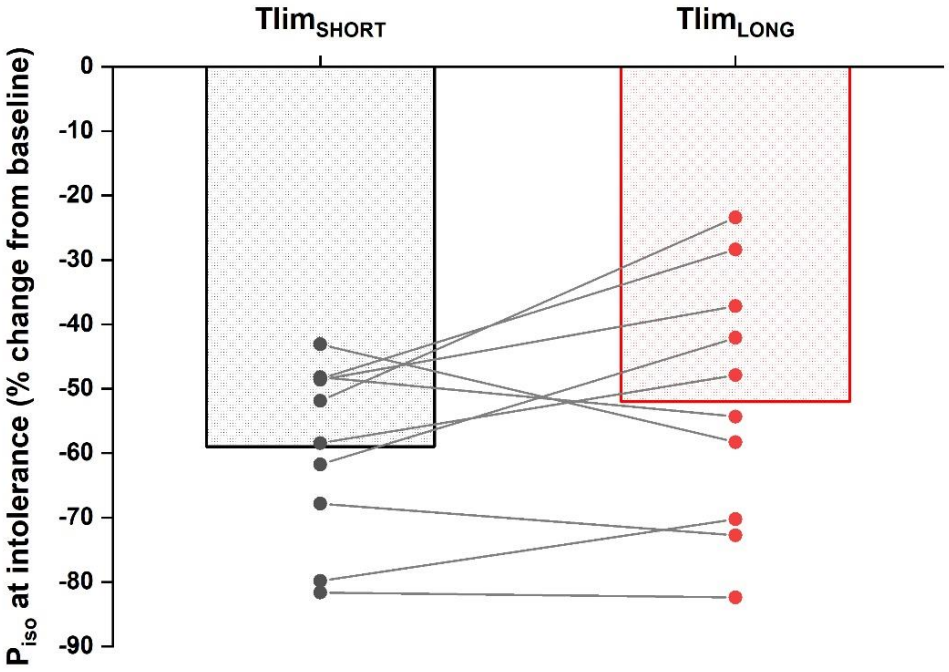

Figure 3.3: Individual and mean pre- to post-exercise reduction in peak isokinetic power at the limit of tolerance of $Tlim_{SHORT}$ and $Tlim_{LONG}$. There was no statistical difference between the groups, suggesting that exercise was terminated in both trials at a similar magnitude of locomotor fatigue.

P_{iso} at intolerance of $Tlim_{SHORT}$ was not different to task power (P = 0.11; i.e. there was no reserve in locomotor power). However, P_{iso} at intolerance of $Tlim_{LONG}$ was greater than task power (P < 0.01; i.e. power reserve was present; Figure 3.4). There was a pre- to post-exercise reduction in peak EMG_{RMS} during P_{iso} assessment following $Tlim_{SHORT}$ (29 ± 16 %) and $Tlim_{LONG}$ (27 ± 20 %; main effect, P < 0.01), but the magnitude of this reduction was also not different between groups (P = 0.79). This finding is indicative of similar reductions in muscle activation and therefore similar contributions from this mechanism to the reduction in P_{iso}.

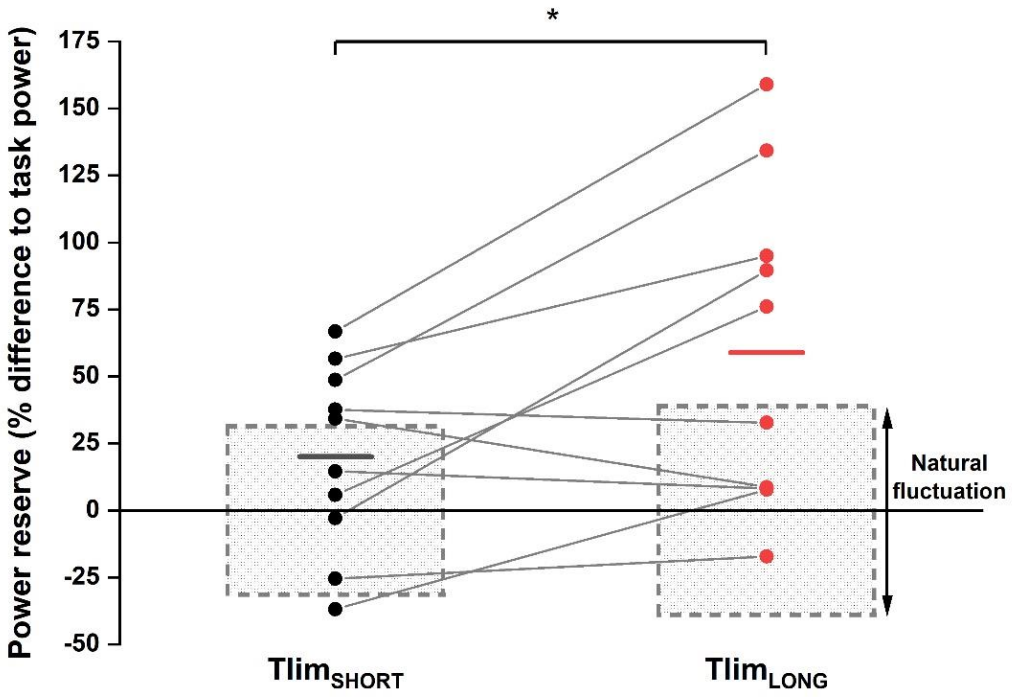

Figure 3.4: Individual responses for peak isokinetic power (P_{iso}) at intolerance of $Tlim_{SHORT}$ and $Tlim_{LONG}$ exercise trials. Thick bars represent mean P_{iso} at intolerance and dashed boxes represent the mean natural fluctuation in pedal force during the trial. Although mean P_{iso} exceeds mean pedal force fluctuation following $Tlim_{LONG}$ but not $Tlim_{SHORT}$, the prevalence of individuals presenting a reserve in locomotor power generation is the same between trials (five in each). * represents a statistical difference in the magnitude of the power reserve between trials (P = 0.04).

3.3.4 Inspiratory muscle fatigue

MIP was not different at baseline between protocols ($Tlim_{SHORT}$: 145 ± 37 cmH_2O vs. $Tlim_{LONG}$: 142 ± 29 cmH_2O; P = 0.64). There was no decrease in MIP pre- to post-exercise in response to $Tlim_{SHORT}$ (−5 ± 7 %; P = 0.09), but there was in response to $Tlim_{LONG}$ (−8 ± 7 %; P < 0.01). Pre- to post-exercise reductions in Pdi_{tw} occurred in response to both protocols but was greater (interaction effect; P = 0.03) following $Tlim_{LONG}$ (−22 ± 12 %; P < 0.01) than $Tlim_{SHORT}$ (−14 ± 12 %; P = 0.03; Figure 3.5).

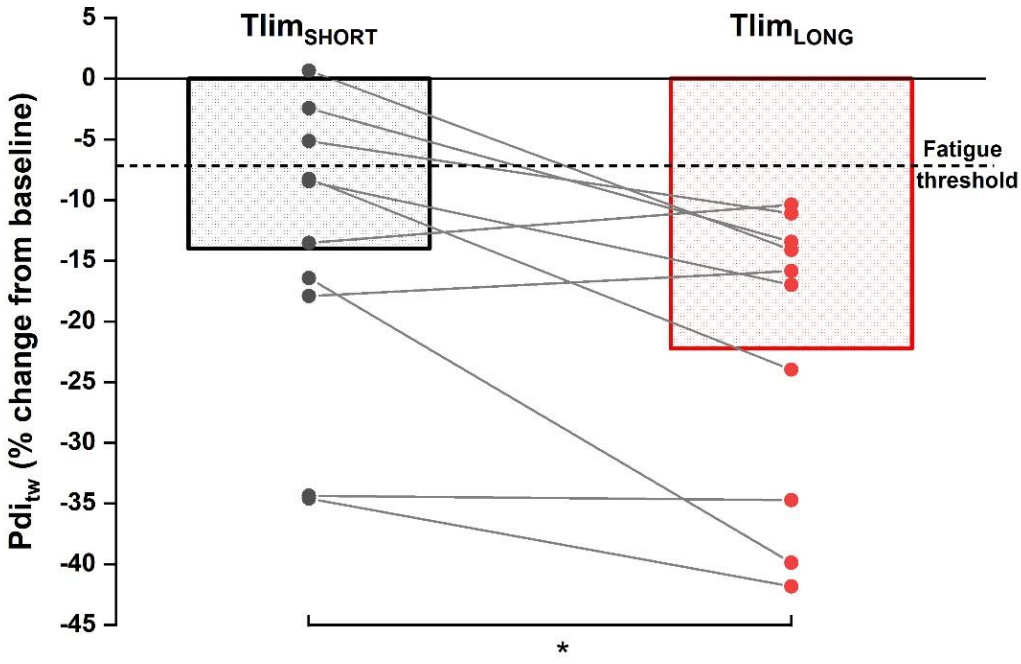

Figure 3.5: Individual responses in the reduction in transdiaphragmatic twitch pressure in response to $Tlim_{SHORT}$ and $Tlim_{LONG}$ exercise trials. Boxes represent mean responses. Dashed line marks twice the coefficient of variation of the measurement, with a change greater than this considered to be indicative of fatigue. * represents a statistical difference between groups.

Post-exercise, Pga_{tw} was reduced from baseline following $Tlim_{SHORT}$ (−25 ± 20 %) and $Tlim_{LONG}$ (−35 ± 13 %). The reduction in Pga_{tw} was greater following

$Tlim_{LONG}$ than $Tlim_{SHORT}$ (P = 0.04). There was also an exercise-induced reduction in $Poes_{tw}$ in response to $Tlim_{SHORT}$ (−9 ± 13 %; P = 0.04) and $Tlim_{LONG}$ (−15 ± 15 %; P = 0.02), but this was not different between trials (P = 0.22). The $Poes_{tw}/Pga_{tw}$ ratio increased pre- to post-exercise in $Tlim_{SHORT}$ (0.8 ± 1.1) and $Tlim_{LONG}$ (0.8 ± 0.8; main effect, P = 0.02), with no difference between trials (P = 0.88), indicating that inspiratory muscle fatigue was predominantly diaphragmatic in origin. Together, these results indicate that the greater global inspiratory muscle fatigue following $Tlim_{LONG}$ was predominantly due to greater diaphragm fatigue and not rib cage muscle fatigue (Figure 3.6). However, it is important to note here that predominant diaphragm fatigue does not equal exclusive diaphragm fatigue and this finding does not preclude the presence of rib cage muscle fatigue.

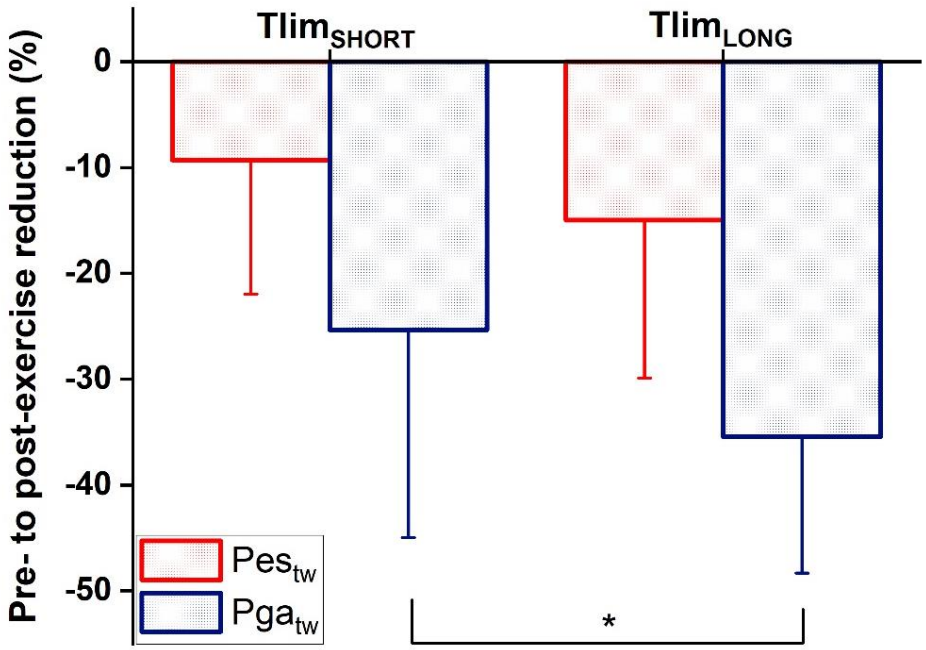

Figure 3.6: Comparison of the exercise-induced reduction in oesophageal twitch pressure ($Poes_{tw}$) and gastric twitch pressure between $Tlim_{SHORT}$ and $Tlim_{LONG}$. * indicates a significant difference between trials (P < 0.05).

3.3.5 Operational lung volumes

One participant was excluded from the analysis of operational lung volumes due to an equipment fault. During $Tlim_{SHORT}$, mean values for end-expiratory lung volume decreased from rest (41 ± 4 %FVC) after 1 minute of exercise (33 ± 5 %FVC; $P = 0.02$) and remained below resting values at intolerance (32 ± 4 %FVC; $P < 0.01$), with no difference between these in-exercise time points ($P = 1.00$). During $Tlim_{LONG}$, there was no difference between rest (41 ± 4 %FVC) and minute 1 of exercise (36 ± 4 %FVC; $P = 0.09$). However, end-expiratory lung volume was decreased by minute 3 of exercise (31 ± 4 %FVC; $P < 0.01$) and remained below resting values after 5 minutes of exercise (30 ± 5 %FVC; $P < 0.01$) and at intolerance (34 ± 4 %FVC; $P = 0.02$). End-expiratory lung volume did not change between minute 3 of exercise and intolerance (all $P > 0.05$). These data indicate that dynamic lung hyperinflation did not occur during either trial.

3.3.6 Relationships between locomotor and inspiratory muscle parameters

There was no relationship between ΔMIP and Δ locomotor fatigue (r = 0.24, P = 0.51) or Δ power reserve (r = 0.09, P = 0.81). While there was a moderate negative correlation, Δ Pdi_{tw} was not related to the Δ locomotor fatigue (r = −0.42, P = 0.22) or Δ power reserve (r = −0.47, P = 0.17; Figure 3.7).

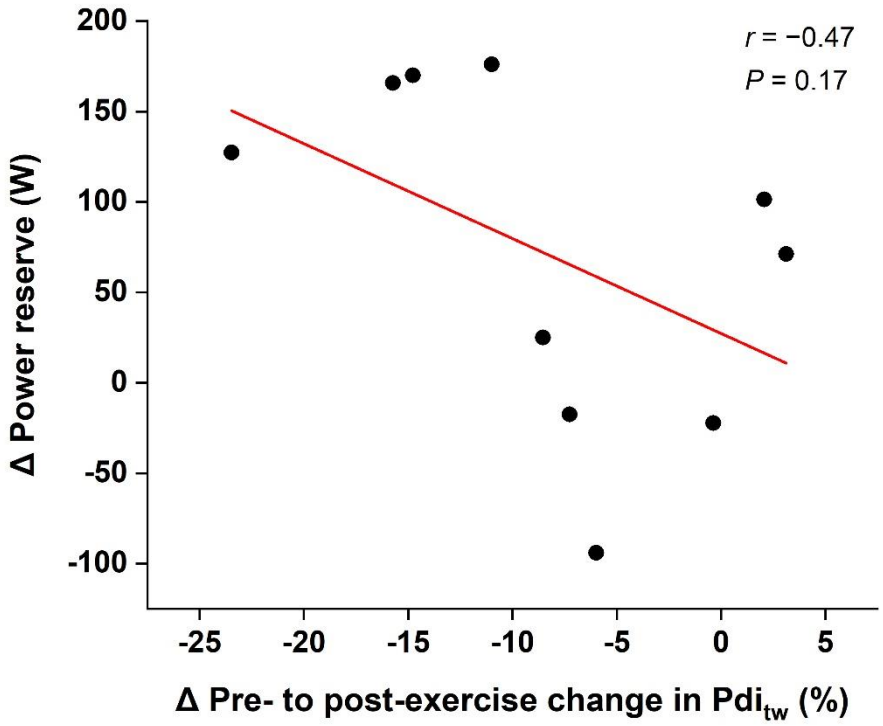

Figure 3.7: Relationship between the inter-trial difference (Δ; $Tlim_{SHORT}$ subtracted from $Tlim_{LONG}$) in power reserve and exercise-induced change in transdiaphragmatic twitch pressure (Pdi_{tw}), which represents global inspiratory muscle fatigue. This relationship was moderately negative but not significant (P = 0.17).

Although there were also moderate, positive correlations between Δ Pga_{tw} and both Δ locomotor fatigue (r = 0.43; P = 0.21) and Δ power reserve (r = 0.53; P = 0.11; Figure 3.8), this was not significant.

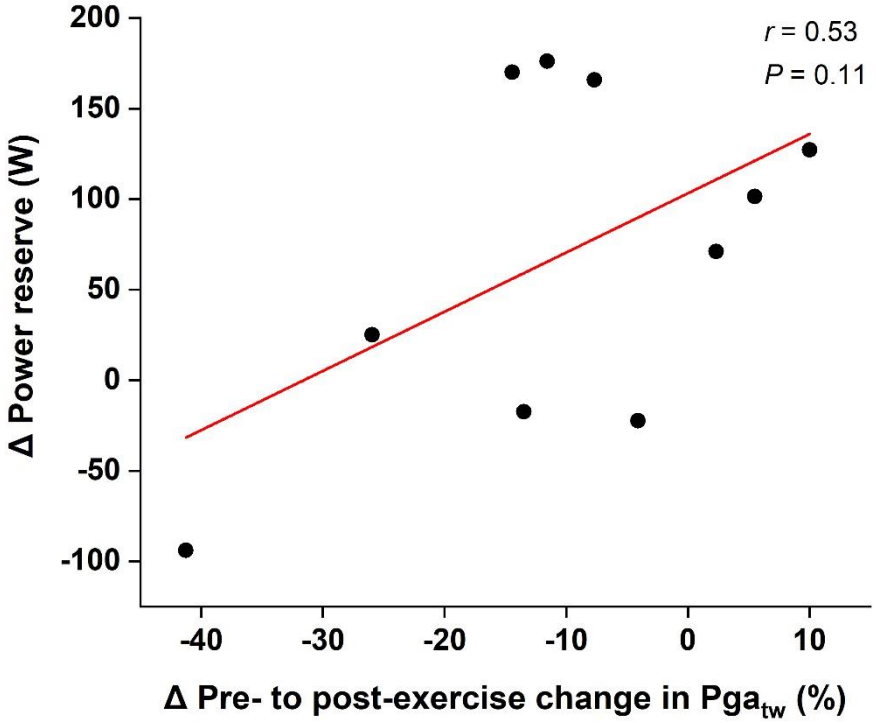

Figure 3.8: Relationship between the inter-trial difference (Δ; $Tlim_{SHORT}$ subtracted from $Tlim_{LONG}$) in power reserve and exercise-induced change in transdiaphragmatic twitch pressure (Pdi_{tw}), which represents global inspiratory muscle fatigue. This relationship was moderately negative but not significant (*P* = 0.11).

However, $\Delta Poes_{tw}$ was significantly negatively correlated with Δ locomotor fatigue (r = −0.72, P = 0.02) and Δ power reserve (r = 0.76, P < 0.01; Figure 3.9). This suggests that a greater reduction in $Poes_{tw}$ was related to a greater power reserve.

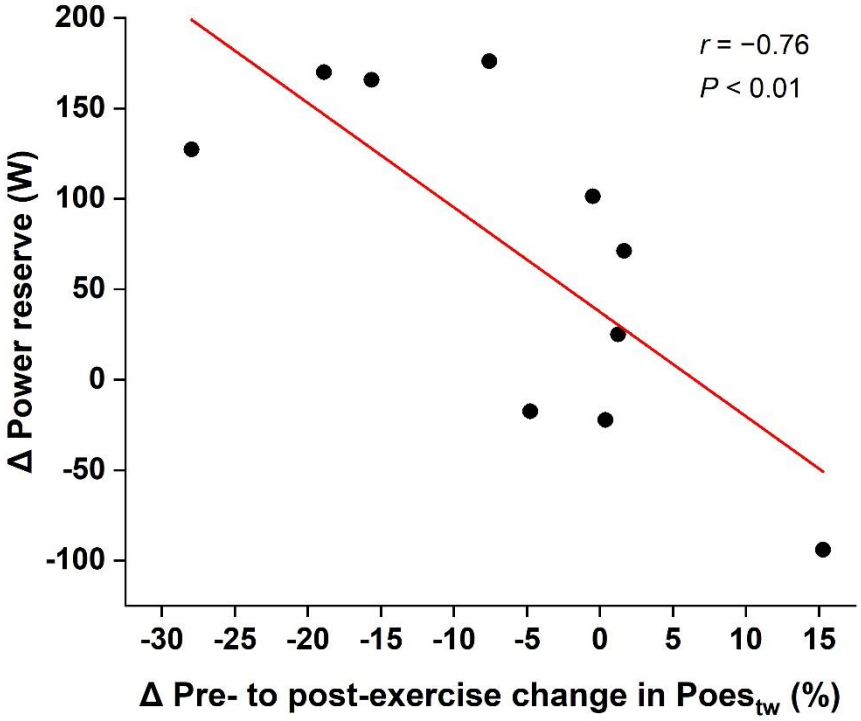

Figure 3.9: Relationship between the inter-trial difference (Δ; $Tlim_{SHORT}$ subtracted from $Tlim_{LONG}$) in power reserve and exercise-induced change in oesophageal twitch pressure ($Poes_{tw}$). This relationship was strongly negative and significant (P < 0.01).

3.4 Discussion

In a subset of data from this study (reported in detail elsewhere) we have previously shown that exercise-induced global inspiratory muscle fatigue (reduction in Pdi_{tw}) is greater in response to $Tlim_{LONG}$ than $Tlim_{SHORT}$. The present study has demonstrated that the greater global inspiratory muscle fatigue in response to $Tlim_{LONG}$ compared to $Tlim_{SHORT}$ is predominantly due to an increase in diaphragm fatigue, rather than rib cage muscle fatigue. However, the inter-trial difference (Δ) in power reserve was not related to either ΔPdi_{tw} (global inspiratory muscle fatigue) or ΔPga_{tw} (reflective of diaphragm fatigue exclusively). Conversely, despite no difference between $Tlim_{LONG}$ and $Tlim_{SHORT}$ in the pre- to post-exercise reduction in $Poes_{tw}$ (reflective of the combined action of the diaphragm and rib cage muscles), $\Delta Poes_{tw}$ was related to Δ power reserve. As no such relationship existed between Δ power reserve and ΔPdi_{tw} or ΔPga_{tw}, the association between Δ power reserve and $\Delta Poes_{tw}$ suggests that a greater power reserve at intolerance is associated with a greater severity of exercise-induced rib cage muscle fatigue, independent of diaphragm fatigue.

3.4.1 Locomotor neuromuscular fatigue and power reserve

Despite different task powers and tolerable durations, the magnitude of locomotor neuromuscular fatigue, quantified by the reduction in P_{iso} from baseline to the limit of tolerance, was not different between trials ($Tlim_{SHORT}$ −58 ± 14 % vs. $Tlim_{LONG}$ −51 ± 20 %). In addition, there was no difference between trials in the pre- to post-exercise reduction in neural activation of the locomotor muscles during maximal isokinetic cycling (peak EMG_{RMS} decreased by 29 ± 16 % and 27 ± 20 % following $Tlim_{SHORT}$ and $Tlim_{LONG}$, respectively). As locomotor

neuromuscular fatigue and the reduction in neural activation were not different between $Tlim_{SHORT}$ and $Tlim_{LONG}$, together these results suggest that the inhibition in the response of the contractile apparatus to neural activation was also not different between trials (Coelho *et al.*, 2015).

However, as the locomotor power reserve is relative to task power, which is lower during $Tlim_{LONG}$ than $Tlim_{SHORT}$, the present study found that, despite no difference in locomotor neuromuscular fatigue, there was a reserve in locomotor power at the limit of $Tlim_{LONG}$ that was absent in $Tlim_{SHORT}$ (see Figure 3.4). This statistical analysis was supported by 95% prediction bands of the pedal force profile during the task. The use of prediction bands allows the maximum power that can be produced at intolerance to be considered relative to the natural fluctuation in pedal-by-pedal power output that occurs during voluntary exercise, a characteristic that is overlooked using statistical analysis alone. This natural fluctuation shows there is consistently a small difference between the target power and the power generated during each pedal stroke (the magnitude of which is quantified by the prediction bands). To meet the target power and sustain the task, pedal strokes are frequently required to exceed task power to compensate for those that fall below, with the consequence that P_{iso} at intolerance must exceed the upper boundary of the prediction bands for a reserve in locomotor power to be considered meaningful.

At the limit of $Tlim_{SHORT}$, P_{iso} was neither statistically greater than task power nor the upper boundary of the natural fluctuation. As such, maximal voluntary power production declines during $Tlim_{SHORT}$ until it is coincident with the power required to continue the task, imposing a direct locomotor limitation to exercise

tolerance. However, despite significant locomotor neuromuscular fatigue, P_{iso} at the limit of $Tlim_{LONG}$ was statistically greater than the target power and exceeded the upper boundary of the prediction bands, confirming that intolerance occurs even though the locomotor muscles can generate more power than is required to continue the task. As further support for these findings, unpublished data from our laboratory found that a reserve in locomotor power was only present when exercise duration exceeded ~6 minutes (range 6-12 minutes), and constant-power exercise with a tolerable duration of ~14 minutes is also terminated with a reserve in locomotor power (Staiano *et al.*, 2018). These data show that locomotor fatigue directly limits $Tlim_{SHORT}$, but cannot be the sole factor that limits $Tlim_{LONG}$, which must be limited by additional physiological mechanisms.

A potential mechanism responsible for this power reserve is a locomotor fatigue threshold determined by metabolite-mediated afferent feedback (Amann, 2011), which is supported by consistent reductions in quadriceps twitch force following cycling trials under different conditions (Amann *et al.*, 2006; Amann and Dempsey, 2008; Romer *et al.*, 2007a; Schäfer, Hayes and Dekerle, 2019). This proposal is a logical extension of the similar metabolite concentration observed at intolerance (Burnley *et al.*, 2010; Vanhatalo *et al.*, 2010; Black *et al.*, 2017) and consistent with the finding in the present study that the magnitude of locomotor neuromuscular fatigue was not different between trials. However, some studies reporting a phenomenon of a critical threshold in voluntary measures of muscle fatigue have been questioned due to the variable individual responses, with the similar group responses seeming to be an artefact of data aggregation (Neyroud, Kayser and Place, 2016). Indeed, the present study

showed consistent individual variability in neuromuscular locomotor fatigue between trials. While the individual responses appear consistent (Figure 3.3), the difference between $Tlim_{LONG}$ and $Tlim_{SHORT}$ in the pre- to post-exercise reduction in P_{iso} was more than 10 % in 7 of the 10 participants. Therefore, termination of whole-body exercise coinciding with a locomotor fatigue threshold remains equivocal.

An alternate explanation for no difference in the magnitude of locomotor fatigue between protocols may be that the relatively similar tolerable durations of the exercise trials made statistical detection of a difference in locomotor fatigue difficult. Indeed, P_{iso} at the limit of tolerance was 41 W higher following $Tlim_{LONG}$ (360 ± 105 W) compared to $Tlim_{SHORT}$ (319 ± 110 W), whereas task power differed by only 27 W between protocols. Data from our lab comparing P_{iso} at the limit of tolerance of four constant-power tasks found that locomotor fatigue was attenuated at the limit of tolerance of a task with a tolerable duration of ~12 minutes (~50% reduction in P_{iso}) compared to tasks during which intolerance was reached in ~3.5-7 minutes (~57-59% reduction in P_{iso}), indicating that a relationship exists between tolerable duration of an exercise task and the magnitude of the locomotor fatigue present at intolerance (unpublished data from our laboratory). This time-dependent response of locomotor neuromuscular fatigue at the point of exercise intolerance suggests that mechanisms external to the locomotor muscles progressively contribute to exercise limitation as the tolerable duration of the task increases, causing exercise termination with a power reserve.

3.4.2 Inspiratory muscle fatigue

Preliminary data from our laboratory (discussed in detail in the PhD thesis by Tim Hardy) demonstrated that global inspiratory muscle fatigue is present following $Tlim_{LONG}$ and $Tlim_{SHORT}$, but is greater in response to $Tlim_{LONG}$ (Pdi_{tw} was reduced pre- to post-exercise by 22 ± 12 % and 14 ± 12 %, respectively). This increased severity of global inspiratory muscle fatigue was present at intolerance despite lower peak values of $\dot{V}CO_2$ and $\dot{V}_E$, demonstrating the critical importance of the combined rate *and* volume of inspiratory muscle work on the inspiratory muscle fatigue response. The present study has extended these findings to show that the $Poes_{tw}/Pga_{tw}$ ratio is increased following $Tlim_{LONG}$ and $Tlim_{SHORT}$. This is suggestive of global inspiratory muscle fatigue being primarily a consequence of diaphragmatic fatigue in both trials. Moreover, the greater reduction in Pga_{tw} (pressure change determined by the diaphragm only) following $Tlim_{LONG}$ compared to $Tlim_{SHORT}$, but no difference between trials in $Poes_{tw}$ (determined by the diaphragm and rib cage muscles), suggests that the greater global inspiratory muscle fatigue in response to $Tlim_{LONG}$ compared to $Tlim_{SHORT}$ is also primarily due to greater diaphragm fatigue.

While the $Poes_{tw}/Pga_{tw}$ ratio can be used to infer that diaphragm fatigue was predominant, this does not necessarily preclude the presence of rib cage muscle fatigue. The reduction in Pga_{tw} indicates that diaphragm fatigue contributed to the reduction in $Poes_{tw}$ observed in response to $Tlim_{SHORT}$ and $Tlim_{LONG}$ but, because both the diaphragm and rib cage muscles influence $Poes_{tw}$, rib cage muscle fatigue may also be contributing. Discriminating between the diaphragm and rib cage muscle fatigue is further complicated by

the unequal contribution of the muscle groups to $Poes_{tw}$, with the diaphragm accounting for ~75 % of the total pressure generated (Similowski et al., 1998), although the exact value is unknown in the present study. However, the magnitude of the difference in the reduction in $Poes_{tw}$ relative to Pga_{tw}, represented by an increase of 0.8 in the $Poes_{tw}/Pga_{tw}$ ratio pre- to post-exercise in both trials, suggests that the contribution of diaphragm fatigue to the global inspiratory muscle fatigue response far exceeded rib cage muscle fatigue. Furthermore, it is possible that the reason the reduction in $Poes_{tw}$ was not different between trials (−15 ± 15 and −9 ± 13 % for $Tlim_{LONG}$ and $Tlim_{SHORT}$, respectively), when the reduction in Pga_{tw} was greater in response to $Tlim_{LONG}$ (−35 ± 13 %) compared to $Tlim_{SHORT}$ (−25 ± 20 %), was that the disparate severities of diaphragm fatigue between trials was counteracted by rib cage muscle function remaining largely preserved, such that the difference in diaphragm fatigue was no longer discernible from the change in $Poes_{tw}$. Collectively, these results suggest that not only was diaphragm fatigue the predominant contributor to global inspiratory muscle fatigue following both trials and exacerbated in response to $Tlim_{LONG}$ compared to $Tlim_{SHORT}$, but that rib cage muscle fatigue was generally modest, if present at all, and not affected by the different exercise trial demands.

These findings are somewhat surprising as recruitment of the accessory rib cage and abdominal muscles progressively increases during high-intensity exercise, concomitant with increases in $\dot{V}_E$ and demands on the diaphragm, primarily to generate the pressure required to displace the rib cage and abdomen (Aliverti *et al.*, 1997). Due to this accessory muscle activity, the load on the diaphragm is reduced and the velocity of contraction is increased,

allowing the diaphragm to act primarily as a flow generator (Aliverti *et al.*, 1997). This would suggest that, contrary to the findings in the present study, rib cage muscle fatigue would be exacerbated in response to $Tlim_{LONG}$ compared to $Tlim_{SHORT}$.

One potential reason for the absence of rib cage muscle fatigue was the task power set for the trials and the consequent tolerable durations. Following cycling time trials lasting 15 and 30 minutes, Pdi_{tw} and the $Poes_{tw}/Pga_{tw}$ ratio were reduced (Wüthrich, Eberle and Spengler, 2014), indicating that rib cage muscle fatigue was the predominant component of the global inspiratory muscle fatigue present. However, these time trials were performed for considerably longer than the ~10 minutes of $Tlim_{LONG}$ in the present study. The task power of the trials in the present study may have been too high, and therefore the tolerable duration too short, to accumulate sufficient inspiratory muscle work to influence the severity of rib cage muscle fatigue. Therefore, this study demonstrates that diaphragm fatigue is the predominant component of global inspiratory muscle fatigue in response to very heavy-intensity exercise when tolerable duration is less than 10 minutes. Additionally, the magnitude of this diaphragm fatigue is greater in response to $Tlim_{LONG}$ than $Tlim_{SHORT}$.

3.4.3 Operational lung volumes

Consistent with previous findings (Henke *et al.*, 1988; Johnson, Saupe and Dempsey, 1992; Mota *et al.*, 1999; Vogiatzis *et al.*, 2005; Guenette *et al.*, 2007), a decrease in end-expiratory lung volume during exercise was observed in the present study. Further, in both $Tlim_{SHORT}$ and $Tlim_{LONG}$, end-expiratory lung volume remained below resting values throughout exercise after the decrease

had been established, with no inflection as intolerance approached. A rise in end-expiratory lung volume (dynamic hyperinflation) increases the elastic work of breathing, requires the inspiratory muscles to contract from a shorter, less-optimal length, and consequently hastens inspiratory muscle fatigue (Roussos *et al.*, 1979). However, a higher operational lung volume allows higher expiratory flow rates (Pellegrino *et al.*, 1993b) and is proposed to be a consequence of afferent feedback from airways undergoing dynamic compression due to expiratory flow limitation (Pellegrino *et al.*, 1993a). Therefore, the absence of dynamic hyperinflation in the present study suggests that expiratory flow limitation was either not present or not severe enough to trigger a reflex increase in operational lung volume and change in breathing pattern. As such, inspiratory muscle fatigue responses to $Tlim_{SHORT}$ and $Tlim_{LONG}$ were unrelated to dynamic hyperinflation and associated changes in lung mechanics.

The finding that end-expiratory lung volume does not increase at maximal exercise agrees with some (Vogiatzis *et al.*, 2005; Guenette *et al.*, 2007) but not all data (Johnson, Saupe and Dempsey, 1992; Mota *et al.*, 1999; Guenette *et al.*, 2007). One factor that may underlie such discrepancies is sex as, unlike other studies, the present study used a mixed-sex cohort. Unfortunately, the small sample size used does not allow men and women to be assessed independently. However, sex differences exist in ventilatory responses to exercise, due to factors such as women having smaller lungs and airways than men, which increases pulmonary flow resistance (Briscoe and Dubois, 1958). Indeed, Guenette *et al*. (2007) found that end-expiratory lung volume increased at maximal exercise in women but not men. As the cohort used in the present

study is predominantly male, the absence of dynamic hyperinflation at maximal exercise is not without precedent. However, this is at odds with studies that have found dynamic hyperinflation in exclusively male participants (Johnson, Saupe and Dempsey, 1992; Mota *et al.*, 1999). Therefore, other factors must also influence the changes in operational lung volume during exercise.

One such factor is participant training status. Endurance training shifts the primary limitation of exercise away from O_2 extraction in untrained individuals (Roca *et al.*, 1989) towards O_2 delivery in highly trained individuals (Roca *et al.*, 1992), with the lungs particularly unresponsive to exercise training (Wagner, 2005). All the participants in the present study were physically active but only one was a trained cyclist (as assessed via self-reported training history; $\dot{V}O_{2peak}$: 74 ml·kg^{-1}·min^{-1}). Indeed, $\dot{V}O_{2peak}$ of the participants in the present study (57 ± 9 ml·kg^{-1}·min^{-1}) was considerably lower than the 73 ± 1 and 72 ± 6 ml·kg^{-1}·min^{-1} reported in previous studies finding an increase in end-expiratory lung volume (Johnson, Saupe and Dempsey, 1992; Mota *et al.*, 1999). This relatively untrained status may have resulted in O_2 extraction becoming limiting prior to O_2 delivery. Consequently, ventilatory load, while high enough to cause inspiratory muscle fatigue, may not have encroached upon the mechanical limits of the pulmonary system (peak $\dot{V}_E$ was 82 ± 8 and 76 ± 10 %MVV during $Tlim_{SHORT}$ and $Tlim_{LONG}$, respectively, compared to 88 % in Mota *et al*. 1999). At these submaximal rates of ventilation, it is unlikely that the severity of expiratory flow limitation, if present at all, would elicit dynamic hyperinflation.

While highly trained men (Johnson, Saupe and Dempsey, 1992; Mota *et al.*, 1999) and women (Guenette *et al.*, 2007) have been shown to exhibit increases

in end-expiratory lung volume close to maximal exercise, this was not found in either untrained men or women (Vogiatzis *et al.*, 2005). Moreover, the highly trained cyclist tested in the present study exhibited the highest peak $\dot{V}_E$ relative to MVV (approaching 100 % in both trials) and the largest increase in end-expiratory lung volume from the previous time point at intolerance (increasing from 29 to 37 %FVC during $Tlim_{SHORT}$ and 26 to 35 %FVC during $Tlim_{LONG}$). Therefore, the relatively untrained status, but unimpaired pulmonary function, of the participants in the present study may underlie the absence of dynamic hyperinflation.

3.4.4 Relationships between inspiratory muscle and locomotor fatigue

Exercise triggers an integrated response from multiple physiological mechanisms that control the respiratory, cardiovascular, and neuromuscular systems. Whole-body exercise requires the recruitment of large muscle mass, causing considerable increases in ventilatory demand and cardiac output that stress the pulmonary and cardiovascular systems. Such interdependence may cause the perturbations in one system to have consequences for the function of another system via a global feedback loop (Hureau, Romer and Amann, 2018; Thomas, Goodall and Howatson, 2018). Indeed, the respiratory system is proposed to be a regulatory component of locomotor function as pre-existing inspiratory muscle fatigue constrains locomotor power output, and consequently locomotor fatigue, during subsequent exercise (Fulton *et al.*, 2020).

During loaded breathing, motor command and the sense of effort increase progressively as inspiratory muscle fatigue develops (Gandevia, Killian and Campbell, 1981; Supinski *et al.*, 1987). Therefore, the corollary and afferent discharge associated with inspiratory muscle work, and the resultant inspiratory muscle fatigue, may be a fundamental contributor to the sensory tolerance limit. Supporting this, exercise tolerance in patients with chronic obstructive pulmonary disease (COPD), a condition characterised by pathophysiological respiratory function, is increased via inhibition of group III/IV sensory afferents by spinal anaesthesia, primarily due to a reduction in ventilatory response and dyspnoeic sensations to exercise (Gagnon *et al.*, 2012). Patients with COPD also exhibit a greater locomotor power reserve at intolerance of maximal incremental exercise compared to age-matched controls, supporting an interaction between the respiratory system and the magnitude of the locomotor power reserve (Cannon *et al.*, 2016).

In contrast to these previous findings, the absence of a relationship between Δ power reserve and either ΔPdi_{tw} or ΔPga_{tw} in the present study suggests that neither global inspiratory muscle nor diaphragm fatigue influence the magnitude of the power reserve at intolerance. However, the significant relationship between $\Delta Poes_{tw}$ and both Δ power reserve (Figure 3.9) and Δ locomotor fatigue, coupled with no such relationship for Pdi_{tw} or Pga_{tw}, indicates that rib cage muscle fatigue, independent of diaphragm fatigue, is negatively associated with these locomotor parameters. Although speculative, this negative correlation suggests that greater rib cage muscle fatigue constrains the development of locomotor neuromuscular fatigue and contributes to the termination of $Tlim_{LONG}$ with a power reserve.

When evaluating the relationships between locomotor neuromuscular fatigue and inspiratory muscle fatigue reported in this study, it is important to consider the time between exercise cessation and measurement collection. While P_{iso} was measured instantaneously at intolerance, inspiratory twitch pressures were obtained 5–10 minutes after exercise cessation due to technical limitations. Therefore, the magnitude of exercise-induced inspiratory muscle fatigue reported in this study likely underestimates the magnitude at intolerance. However, while not possible to measure due to technical limitations, it is unlikely that the rate of recovery of inspiratory muscle function was different between exercise trials and the measurement delay was not of sufficient duration to allow complete recovery. Consequently, inter-trial differences measured 5–10 minutes after exercise cessation are likely to reflect the inter-trial differences at intolerance (i.e. a larger magnitude of inspiratory muscle fatigue 5–10 minutes post exercise is likely to reflect a larger magnitude of fatigue at intolerance). As such, the delay in the measurement of inspiratory muscle fatigue should not compromise the validity of the relationships between locomotor neuromuscular fatigue and inspiratory muscle fatigue presented in this chapter.

3.4.5 Exercise limitation via interaction between inspiratory and locomotor fatigue

The proposed mechanisms by which the inspiratory and locomotor muscles may interact to limit exercise are conflicting. One potential consequence of the interaction between these two systems is that inspiratory muscle fatigue increases locomotor muscle fatigue via a metaboreflex (Romer and Polkey, 2008; Dempsey *et al.*, 2002). Fatigue-related metabolites accumulate in response to elevated levels of diaphragmatic work, which are detected by

metaboreceptors in the diaphragm. The associated afferent feedback triggers sympathetically-mediated vasoconstriction in the locomotor muscles to preserve blood flow to the diaphragm, thereby compromising locomotor perfusion and increasing the rate of locomotor muscle fatigue development. However, this study found no associations to support this interaction (there was no significant correlation between ΔPga_{tw} and Δ power reserve). While a reduction in blood flow to the locomotor muscles would certainly have implications for locomotor muscle fatigue, the respiratory muscle metaboreflex likely accelerates the development, rather than increases the magnitude, of locomotor muscle fatigue induced by the task.

Conversely, greater inspiratory muscle fatigue may attenuate locomotor muscle fatigue at exercise intolerance due to a ‘sensory tolerance limit’ which proposes the summation of sensory information from all systems subjected to homeostatic perturbation determines the tolerability of the task (Hureau, Romer and Amann, 2018; Thomas, Goodall and Howatson, 2018). This concept is supported by the task specificity of fatigue responses, which are dependent on the size of the muscle mass engaged in the task. Reductions in quadriceps twitch force are greater following single-leg knee extensor exercise compared to double-leg knee extension exercise (Rossman *et al.*, 2014) and cycling (Rossman *et al.*, 2012), which both require greater active muscle mass and therefore a higher cardiorespiratory response. These additional sources of afferent discharge contribute to the overall afferent feedback signal, which may constrain locomotor neuromuscular fatigue (Fulton *et al.*, 2020). Such an interaction would agree with the significant negative relationship between $\Delta Poes_{tw}$ and Δ locomotor fatigue found in the present study.

One potential mechanism of the sensory tolerance limit is that afferent feedback from rib cage muscle fatigue directly constrains voluntary activation of the locomotor muscles via inhibition of central motor drive. By constraining locomotor muscle activity in this way, the locomotor muscles may retain the capacity to exceed task power at exercise intolerance. However, the present study found a significant negative correlation between $\Delta Poes_{tw}$ and ΔEMG_{RMS} during maximal isokinetic cycling (−0.73; $P = 0.02$). No such relationship existed for ΔPdi_{tw} or ΔPga_{tw}, suggesting that voluntary activation of the locomotor muscles at intolerance was better maintained with increasing severity of rib cage muscle fatigue. While contrary to the theory that greater afferent feedback arising from rib cage muscle fatigue inhibits central motor drive to the locomotor muscles, this correlation is consistent with the finding that $\Delta Poes_{tw}$ was related to Δ power reserve as it would be incompatible for reductions in muscle activity to be associated with increases in power output, regardless of the perturbation of the metabolic milieu. If impaired voluntary activation of the locomotor muscles contributed to exercise termination with a power reserve, any inhibition of central motor drive would have to be ‘overridden’ during maximal isokinetic cycling at intolerance. Therefore, it is unlikely that rib cage muscle fatigue constrains locomotor muscle fatigue via reducing central motor drive to the locomotor muscles.

Alternatively, the afferent feedback generated by rib cage muscle fatigue may indirectly constrain locomotor neuromuscular fatigue by amplifying sensations of exertion, which have been suggested to be fundamental regulators of exercise performance (Okano *et al.*, 2015). Blocking group III/IV afferents prevents sensory feedback information reaching the central nervous system

and causes reductions in perceived exertion during whole-body exercise (Amann *et al.*, 2010; Sidhu *et al.*, 2014; Amann *et al.*, 2020). This finding suggests that, in addition to inhibiting contractile function directly, intramuscular metabolic perturbations may contribute to exercise limitation via afferent feedback that exacerbates sensations of exertion. The relative contribution from the rib cage muscles to overall noxious sensations may be increased under conditions that induce greater rib cage muscle fatigue, causing intolerable sensations of exertion prior to locomotor neuromuscular fatigue limiting the task. Such a mechanism is consistent with the concept of a sensory fatigue threshold, which constrains homeostatic disruption to a critical level by enforcing disengagement from the task, whether that be voluntary or involuntary (Noakes, St Clair Gibson and Lambert, 2004; Marcora, 2008). When activity of group III/IV afferents are suppressed using an intrathecal opioid analgesic, the development of locomotor fatigue following a 5 km cycling time trial is exacerbated (Amann *et al.*, 2009), implying that the extent to which metabolic perturbations can occur is limited by afferent feedback from sensory pathways. The role of this inhibitory mechanism may be to preserve some level of muscle integrity and prevent severe functional impairments, as subjects reported issues with ambulation and muscle soreness following the task performed with a blockade on afferent feedback. These maladies were not manifest in response to the same exercise task performed under control conditions.

Under this construct of exercise limitation, a relative increase in fatigue in one system would correspond with a proportionate reduction in fatigue in another system, but with no direct suppression of central motor drive. This agrees with the negative relationship between locomotor EMG_{RMS} and $\Delta Poes_{tw}$ in this study,

with voluntary activation of the locomotor muscles remaining highest when rib cage muscle fatigue was most severe. In addition, perceived dyspnoea and leg tiredness were rated as ‘maximal’ at the limit of $Tlim_{LONG}$ and $Tlim_{SHORT}$, suggesting that intolerable sensations of exertion coincided with exercise termination during both trials.

3.4.6 Disparate influences of rib cage muscle and diaphragm fatigue on dyspnoea

A fundamental question arising from this study is why the magnitude of the power reserve is associated specifically with the severity of rib cage muscle fatigue and is independent of diaphragm fatigue. If exercise was limited by a sensory threshold then a potential insight into this conundrum may be gained through the perceptual responses. Like sensations of dyspnoea and leg tiredness, pre to post-exercise reductions in P_{iso} and $Poes_{tw}$ were also not different between trials, suggesting that locomotor neuromuscular and rib cage muscle fatigue may have contributed to perceived exertion. However, the greater reduction in Pdi_{tw} and Pga_{tw} following $Tlim_{LONG}$ compared to $Tlim_{SHORT}$ indicates that diaphragm fatigue did not influence the sensation of dyspnoea, which may underpin the absence of a correlation between either ΔPdi_{tw} or ΔPga_{tw} and Δ power reserve. Global inspiratory muscle fatigue prior to exercise at 90 % of RIT_{peak} has been shown to cause slight but significant increases in dyspnoea, but the relative magnitude of diaphragm and rib cage muscle fatigue was not established (Sliwiński *et al.*, 1996). As such global inspiratory muscle fatigue heightens sensations of dyspnoea, but the importance of the muscle group incurring this fatigue remains unanswered. A dissociation between diaphragm fatigue and the sensory consequences would be consistent with

perceptual responses to inspiratory resistive loading. The severity of inspiratory effort following targeted loading of the diaphragm is independent of diaphragm fatigue, but a strong correlation exists between the rating of inspiratory effort and P_{oes} relative to the maximal P_{oes} for the individual (Bradley *et al.*, 1986). Similarly, the sense of inspiratory effort during loaded breathing is associated with increasing activity of the rib cage muscles, but no such relationship exists for diaphragm activity (Ward *et al.*, 1988). Therefore, it is possible that the power reserve was not associated with exercise-induced reductions in Pdi_{tw} or Pga_{tw} because diaphragm fatigue *per se* did not heighten dyspnoea, and consequently did not contribute to the overall perception of exertion that precipitated exercise termination.

That fatigue of the diaphragm, the principal muscle of inspiration, does not influence the sensation of dyspnoea (including inspiratory effort) is somewhat irreconcilable. One potential reason for this disconnect is that the afferent and efferent innervations differ between the diaphragm and the rib cage muscles. For example, the distribution of spindles and Golgi tendon organs is variable between inspiratory muscle groups (Corda, Voneuler and Lennerstrand, 1965), so the sensory response may be dependent on the specific muscles involved. Limited proprioceptive innervation of the diaphragm may contribute to. In addition, there is no increase in neural respiratory drive to the diaphragm during CO_2 rebreathing in response to diaphragm fatigue, at least of the magnitude of ~20 % reduction in twitch force (Luo *et al.*, 2001). Together, these characteristics provide potential afferent and efferent mechanisms to explain the disproportionately weak influence of diaphragm fatigue on dyspnoea.

Another proposition is that the diaphragm is also a pressure generator for functions such as micturition and defecation (Laporta and Grassino, 1985), and as such reductions in the capacity to generate intra-abdominal pressure (Pga_{tw}) does not contribute to dyspnoea or sensations of exertion during exercise. Consequently, it would be expected that exercise-induced reductions in Pdi_{tw} and Pga_{tw} are not associated with the power reserve. Alternatively, it may be that dyspnoea is driven by a declining ability to generate negative intrathoracic pressure. Indeed, P_{oes} is the main variable associated with the sensation of inspiratory effort during loaded inspiration, regardless of whether this is predominantly generated by the rib cage muscles or the diaphragm (Fitting *et al.*, 1987). Due to the mechanism of ventilatory control whereby the rib cage muscles function primarily as pressure generators so that the diaphragm may act as a flow generator, it is likely that rib cage muscle fatigue would be the predominant source of sensations of dyspnoea, either as a consequence of activity and/or fatigue triggering receptors or via increased central motor drive to maintain sufficient pressure generation.

3.4.7 Conclusions

A reserve in locomotor power, which is not present at the limit of high-intensity constant-power cycling with a duration of ~5 minutes, manifests when the power demand of the task is reduced so that the tolerable exercise duration is increased to ~10 minutes. This reserve in power suggests that locomotor fatigue does not limit the task, and that exercise intolerance is determined by mechanisms independent of the locomotor muscles. However, increases in the magnitude of the reserve in power do not coincide with greater reductions in Pdi_{tw} or Pga_{tw}, indicating that diaphragm fatigue *per se* does not contribute to

the dissociation between the perceived and physical ability to continue the task. However, while speculative, the significant correlation between inter-trial differences in $Poes_{tw}$ and power reserve suggest that greater rib cage muscle fatigue may contribute to exercise intolerance, prior to locomotor neuromuscular fatigue limiting the task, through elevated sensations of dyspnoea and/or exertion.

4: No difference in the fatigue and perceptual responses to intensity-matched constant-power and intermittent exercise

4.1 Introduction

Exercise-induced fatigue is defined as a reduction in the capacity of the skeletal muscles to develop force and/or velocity that is reversible with rest. This fatigue is a consequence of peripheral (attenuated function of processes at or distal to the neuromuscular junction) and/or central (a reduction in voluntary activation) factors (Gandevia, 2001). The magnitude of exercise-induced fatigue can be quantified instantaneously during cycle ergometry using brief (~6 seconds) isokinetic measurements of maximal voluntary power (Cannon *et al.*, 2011; Coelho *et al.*, 2015; Ferguson *et al.*, 2016b). For constant-power exercise, the magnitude of exercise-induced fatigue is related to the accumulation of fatigue-related metabolites and depends on exercise intensity (Cannon *et al.*, 2011). The boundaries of the intensity domains are demarcated by the metabolic rates at the lactate threshold and critical power, with each intensity domain evoking common pulmonary O_2 uptake ($\dot{V}O_2$) and blood acid-base responses that reflect the severity of the metabolic perturbation (Wasserman, Van Kessel and Burton, 1967; Whipp, Ward and Wasserman, 1986; Rossiter, 2011).

Intermittent exercise, which comprises alternating phases of work and active or passive recovery, dissociates the task power from the intramuscular (phosphocreatine – PCr – breakdown, muscle acidification) and systemic responses ($\dot{V}O_2$, heart rate, blood lactate concentration; Astrand *et al.*, 1960; Turner *et al.*, 2006; Chidnok *et al.*, 2013; Davies *et al.*, 2017). Therefore, the metabolic stress can be reduced during intermittent exercise compared to

continuous exercise at the same power output and, when task power output exceeds CP, performing the task intermittently increases exercise tolerance (Astrand *et al.*, 1960; Turner *et al.*, 2006; Chidnok *et al.*, 2013; Davies *et al.*, 2017). The magnitude of the dissociation, which is determined by the duration of the phases of work (Turner *et al.*, 2006; Davies *et al.*, 2017) and recovery (Chidnok *et al.*, 2013), is greatest when work durations are short and can be to the extent that a very heavy constant-power task, which is tolerable for only a few minutes, can be reduced to moderate intensity if the same task power is performed intermittently with short work phases (typically less than ~20 seconds; Turner *et al.*, 2006; Davies *et al.*, 2017).

Repeatedly alternating between work and recovery phases results in an oscillatory $\dot{V}O_2$ response due to the fluctuating rate of ATP demand, and the corresponding underlying $\dot{V}O_2$ kinetics. Critically, it is the peak of the $\dot{V}O_2$ oscillation that determines exercise intensity (Turner *et al.*, 2006; Davies *et al.*, 2017). The $\dot{V}O_2$ oscillation amplitude is dependent on the work and recovery durations, with increasingly shorter durations reducing the oscillation amplitude during exercise despite the same magnitude of change in the rate of ATP demand between task and recovery power (Casaburi *et al.*, 1977). When the work and recovery durations are short (e.g. ~15 seconds), $\dot{V}O_2$ oscillations are typically no greater than the breath-by-breath ‘noise’ inherent in pulmonary gas exchange measurements (SD of breath fluctuations during moderate-intensity exercise is typically ~100–150 $mL \cdot min^{-1}$; Lamarra *et al.*, 1987; Puente-Maestu *et al.*, 2002; Bowen *et al.*, 2012), with the result that the $\dot{V}O_2$ response is indistinguishable to that of constant-power exercise (Belfry *et al.*, 2012a).

Due to the dissociation between task power and the systemic physiological responses, power output is higher during the work phases of intermittent exercise than constant-power exercise for a given $\dot{V}O_2$ (steady-state $\dot{V}O_2$ for constant-power exercise and peak $\dot{V}O_2$ for intermittent exercise). Generating more power would be expected to require a concomitant increase in muscle activity, which may have implications for the perceptual responses to the tasks. An increase in central motor drive to the working muscles is associated with an increase in perceived effort during exercise (Marcora, 2009; de Morree, Klein and Marcora, 2012) suggesting that, despite equivalent systemic and intramuscular stress, the perception of effort may be greater during intermittent compared to intensity-matched constant-power exercise.

Moreover, generating the higher task power during intermittent exercise may cause recruitment of additional motor units with different contractile and metabolic properties. Motor unit recruitment in humans is characterised by the initial recruitment of low-threshold motor units (Henneman, 1957; Henneman, Somjen and Carpenter, 1965b), which are primarily composed of type I (slow-twitch) muscle fibres (McPhedran, Wuerker and Henneman, 1965b). However, as the power generated by the muscle increases, larger, high-force motor units are progressively recruited in accordance with the hierarchical recruitment of motor units described by Henneman's Size Principle (Henneman, 1957; Henneman, Somjen and Carpenter, 1965b). These higher-threshold motor units are composed primarily of type II (A/X; fast-twitch) muscle fibres (McPhedran, Wuerker and Henneman, 1965a), which typically have a lower oxidative and higher glycolytic enzyme activity, coupled with reduced capillary and mitochondrial density, compared to type I fibres (Essén *et al.*, 1975;

Andersen, 1975; Sjøgaard, 1982; Bottinelli and Reggiani, 2000; Schiaffino and Reggiani, 2011; Zierath and Hawley, 2004). Additionally, these muscle fibre types exhibit disparate calcium handling capabilities, including type II fibres having an increased ability to generate larger and faster calcium transients, but a lower calcium sensitivity, than type I fibres (Ruff, 1989; Fink, Stephenson and Williams, 1990). These specialised properties allow type II muscle fibres to contract faster (hence the nomenclature) and generate more force than type I fibres (Ivy *et al.*, 1981; Bottinelli, Schiaffino and Reggiani, 1991; Bottinelli *et al.*, 1996; Widrick *et al.*, 1996), but are associated with an increased accumulation of fatigue-related metabolites (e.g. inorganic phosphate and H^+) and impaired calcium handling (Schiaffino and Reggiani, 2011). Consequently, type II muscle fibres are less resistant to fatigue than type I fibres (Edström and Kugelberg, 1968; Fitts, 1994), and increased activity of the former during intermittent exercise may result in greater exercise-induced fatigue compared to intensity-matched constant-power exercise.

Therefore, the purpose of this study was to compare differences between intensity ($\dot{V}O_2$)-matched constant-power and intermittent exercise in: 1) the perceptual responses to the exercise and 2) the magnitude of fatigue induced by the exercise. It was hypothesised that, in the absence of any difference in $\dot{V}O_2$, perceptual responses (ratings of perceived dypsnoea and leg tiredness) to constant-power and intermittent exercise would not be different, but that differences in power and the associated changes in muscle fibre recruitment would increase the magnitude of locomotor neuromuscular fatigue following intermittent compared to intensity-matched constant-power exercise.

4.2 Methods

4.2.1 Participants and ethical approval

Fifteen healthy participants (5 female, 10 male; age: 23 ± 3 yr; height: 174 ± 9 cm; weight: 70.0 ± 9.0 kg) provided written informed consent after completing a health and physical activity questionnaire to confirm that they had no known disease or other contraindication to high-intensity exercise (please see Section 2.1). All experimental procedures were approved by the University of Leeds Faculty of Biological Sciences Research Ethics Committee (approval ref: BIOSCI 16-015).

4.2.2 Exercise protocols

Participants visited the temperature-controlled laboratory (19-21°C) on five occasions, separated by a minimum of 24 hours. Prior to each test, participants abstained from strenuous exercise (previous 24 hours), alcohol consumption (previous 24 hours), and caffeine (previous 12 hours) and food (previous 3 hours) ingestion. The protocol performed on every visit was comprised of two phases: 1) short (~6 seconds) bouts of isokinetic cycling (80 rev·min^{-1}) at four variable efforts (~25, ~50, ~75, and 100% maximum effort) separated by 30 seconds of unloaded cycling (20 W), repeated after a minimum of three minutes of rest; and 2) one of three exercise tests (ramp-incremental, constant-power or intermittent exercise) immediately (< 1 second) followed by a short (~6 seconds) bout of isokinetic cycling (80 rev·min^{-1}) at 100% effort. Pulmonary gas exchange was measured throughout the second phase of each visit, with EMG recorded during each isokinetic effort.

Each exercise test was preceded by approximately two minutes seated rest on the cycle ergometer and four minutes unloaded cycling. The first exercise test performed was a ramp-incremental test (RIT; 20–25 $W \cdot min^{-1}$) to the limit of tolerance, defined as the point at which cadence fell below 50 $rev \cdot min^{-1}$ despite strong verbal encouragement. This test was used to determine the power outputs for the subsequent exercise tests.

The exercise tests performed on visits 2-5 were either constant-power or intermittent exercise, with both modalities performed in duplicate and in a randomised order. For intermittent exercise, work and recovery durations of 10 seconds were selected to maximise the dissociation between power output and $\dot{V}O_2$ and minimise the oscillations in $\dot{V}O_2$. The test duration for the first of these visits, regardless of protocol, was 30 minutes, with the remaining test matched for total work done in kJ (calculated as the product of task power, where 1 W is equal to 1 $J \cdot s^{-1}$, and duration). The power output for these tests was predicted using a modified computational model of circulatory and pulmonary gas exchange dynamics (Benson, Grassi and Rossiter, 2013) to evoke a pulmonary oxygen uptake ($\dot{V}O_2$) equivalent to 40% of the difference between the lactate threshold (LT) and $\dot{V}O_{2peak}$ (Δ40), which was selected as CP typically occurs between approximately Δ 40 and Δ60 (Roston *et al.*, 1987; Poole *et al.*, 1988; Pringle and Jones, 2002). Pilot testing in 8 participants also identified Δ 40 as the highest target $\dot{V}O_2$ that was consistently tolerated for ~30 minutes. For intermittent protocols, the recovery phases were fixed at 20 W and $\dot{V}O_2$ was calculated to coincide with Δ40 at the peak of the oscillation, as the peak rather than the mean determines the intensity (Turner *et al.*, 2006; Davies *et al.*, 2017). The model was parameterised using individual-specific $\dot{V}O_2$ during unloaded

cycling (measured during the RIT), and typical values for $\dot{V}O_2$ time constant (30 s; Ozyener *et al.*, 2001; McNarry, Kingsley and Lewis, 2012) and total gain (12 $mL \cdot min^{-1} \cdot W^{-1}$; Barstow and Molé, 1991; Ozyener *et al.*, 2001) for healthy, physically active individuals performing heavy-intensity exercise. An iterative approach was used to calculate the power output predicted to evoke the required $\Delta 40$ $\dot{V}O_2$. While the modelled $\dot{V}O_2$ exhibits moderate-intensity exercise kinetics, and therefore has a different temporal profile to $\dot{V}O_2$ recorded during heavy-intensity exercise, the power output required to evoke a specified 'steady-state' $\dot{V}O_2$ during heavy-intensity exercise can be accurately predicted (unpublished data from our laboratory).

All exercise tests were performed in cadence-independent (hyperbolic) mode that was instantaneously switched upon the completion of the exercise test into isokinetic mode. Participants were requested to maintain a consistent self-selected cadence between 75 and 95 $rev \cdot min^{-1}$ throughout hyperbolic cycling and were encouraged to either increase or decrease cadence as appropriate when this deviated by more than 5 $rev \cdot min^{-1}$ from the target. This was to minimise any potential contributions to achieving the required power through changes in the inertial energy stored in the flywheel, particularly during the frequent switching between work and recovery phases during intermittent exercise. Blood samples and RPE were taken at rest and every 5 minutes during constant-power and intermittent tests, with 1 minute of EMG recorded at the same time intervals during exercise. All exercise tests were concluded with ~6 minutes unloaded (20W) cycling.

4.2.3 Equipment and measures

4.2.3.1 Pulmonary gas exchange

Each measure was obtained for all fifteen participants unless otherwise stated. All exercise protocols were performed on a computer-controlled, electromagnetically braked cycle ergometer (Excalibur Sport PFM; Lode, Groningen, The Netherlands). Pulmonary gas concentrations and volumes were measured throughout all exercise tests, with O_2 and CO_2 measured using electrochemical and infrared gas analysers, respectively (Medgraphics Ultima Series, Medical Graphics Corporation, St Paul, MN, USA). Prior to every test, the flow sensor was calibrated across a range of flow rates representative of normal ventilation using a 3-L syringe (Medical Graphics Corporation, St Paul, MN, USA), and the gas analysers were calibrated using certified calibration gases that corresponded to the physiological range of gas concentrations (CO_2: 0% and 5%; O_2: 12% and 21%). These gases were resampled after every test to verify the stability of the calibration of the gas analysers throughout the test. Heart rate (HR) was measured from the R-R interval of a 12-lead ECG (Mortara, Milwaukee, WI, USA).

4.2.3.2 Surface electromyography

Surface electromyography (EMG; Telemyo 2400T G2; Noraxon, Scottsdale, AZ, USA) was recorded at 1500 Hz in five muscles (*vastus lateralis, rectus femoris, vastus lateralis, biceps femoris,* and *lateral gastrocnemius*) of the right leg during all isokinetic cycling bouts and in 1-minute bins at 5-minute intervals during hyperbolic cycling. Information about electrode placement and skin preparation can be found in Section 2.6. Two surface electrodes (Kendall

H93SG, Covedien, Minneapolis, MN, USA) were adhered to the skin over the muscle belly at each site with an inter-electrode distance of ~2 cm. The electrodes were then connected to a transmitter, positioned on the participant's right hip, by wires that were taped to the participant's leg to minimise signal interference from wire motion and distraction of the participant (3M Transpore Surgical Tape, Blacknell, Berkshire, UK). Data was acquired via wireless transmission to a receiver connected to a laptop and analysed using the proprietary software (MyoResearch XP, Noraxon USA Inc, Scottsdale, AZ, USA).

4.2.3.3 Near infrared spectroscopy

A noninvasive measure of muscle tissue oxygenation, tissue saturation index (TSI), was calculated using continuous-wave near-infrared spectroscopy with spatial resolution (NIRS; PortaMon, Artinis Medical Systems, Elst, The Netherlands; n = 14). Three light emitting diodes were located 30, 35, and 40 mm away from the photodiode detector and emitted light at wavelengths of 760 and 850 nm. Changes from an arbitrary baseline in oxygenated and deoxygenated haemoglobin concentration were determined by the degree of light attenuation by the tissue. To discriminate between the effect of scatter and absorption on light attenuation, the gradient of light attenuation against distance was calculated for each wavelength of light. This provided a quantitative measure of muscle oxygenation, TSI, defined as the ratio of oxygenated to total tissue haemoglobin and expressed as percent. The spectrometer was positioned over the muscle belly of the vastus lateralis of the left leg, with the centre ~20 cm from the patella, and affixed parallel with the

vertical axis of the upper leg using Transpore tape (Blacknell, Berkshire, UK). The position was then marked with indelible ink to aid replacement of the device during subsequent sessions. The device and surrounding skin were covered with opaque material to prevent ambient light disrupting the signal. The spectrometer was connected to a laptop via Bluetooth for data acquisition (sampled at 10 Hz), analogue-to-digital conversion, and to export data for subsequent analysis. More information regarding the theoretical underpinnings, practical applications, and limitations of NIRS is in Section 2.9.

4.2.3.4 Blood lactate concentration

Blood lactate concentration was measured via capillary blood samples obtained using a single-use safety lancet (n = 10). Prior to blood collection, the participants' hands were heated in ~45°C water for a minimum of 5 minutes to dilatate the local superficial blood vessels, and the site was cleaned with an alcohol swab and allowed to dry. The puncture site was blotted with tissue to remove the first drops of blood, and approximately 30 µL of arterialised capillary blood was collected in a capillary tube containing an anticoagulant. An automated analyser (GL5, Analox Instruments Ltd, Stourbridge, UK) was used to quantify blood lactate concentration (coefficient of variation 0.75%) and was calibrated prior to analysis using an 8 mM lactate standard provided by the manufacturer.

4.2.3.5 Ratings of perceived exertion

Modified Borg CR-10 scales accompanied by verbal descriptions were used to provide ratings of perceived exertion (RPE) for sensations of dyspnoea ('how

difficult does your breathing feel?') and leg tiredness ('how tired do your legs feel?'). Full details of the scale used are provided in Section 2.8.

4.2.4 Data Analysis

4.2.4.1 Power output

Power produced by the participant at the crank (crank power) was calculated as the product of torque and instantaneous angular velocity and measured every 2° of angular rotation (Cannon *et al.*, 2011; Coelho *et al.*, 2015; Ferguson *et al.*, 2016b). The mean crank power for each complete revolution of the crank was calculated to provide a pedal-by-pedal stroke power profile of each task. Mean crank power for the constant-power protocol was calculated as the mean of the entire task, whereas mean crank power for the intermittent protocol was calculated as the mean of the power output during the 10-second work phases. Power measurements for the left and right crank were determined independently then summed.

To compare crank power to task power during the intermittent protocol, the crank power of every pedal stroke was systematically separated into work or recovery phases using 10-second intervals. The point at which each pedal stroke was completed was designated as the time that it occurred during the phase (the categorisation of pedal strokes into work or recovery was discrete, although some pedal strokes may have overlapped the phase boundary). All pedal strokes from the same phase (work or recovery) were then compiled, arranged into ascending order of time, and a rolling mean with no overlap was

calculated. The number of data points in each rolling mean subset was equal to the number of work/recovery phases of the protocol.

Once the average power profile for the work and recovery phases was determined for each individual, a group mean response was calculated. Crank power was considered to have reached target power when the difference between these was within ‘natural fluctuation’ of crank power during constant-power exercise (95 % prediction bands around the mean of all pedal strokes during the trial). This measure reflects the magnitude by which crank power oscillates above and below target power during constant-power exercise, due to minor differences in motor unit recruitment and flywheel resistance from one pedal stroke to the next.

Peak isokinetic power (P_{iso}) for each maximal effort was calculated as the mean of the three revolutions that produced the highest power and were appropriately constrained at 80 ± 2 rev·min^{-1}. Baseline P_{iso} was calculated as the mean P_{iso} of the two baseline efforts, and post-exercise P_{iso} was determined from the maximal isokinetic effort immediately upon completion of the exercise test. Exercise-induced fatigue was quantified as the pre- to post-exercise reduction in P_{iso}.

4.2.4.2 Pulmonary gas exchange

Breath-by-breath $\dot{V}O_2$ data were examined to exclude erroneous breaths that lay outside the expected physiological responses (>99 % of the local prediction limits) and were likely the result of swallowing or coughing (Lamarra *et al.*, 1987). From the RIT, LT was estimated by three experienced assessors using

the V-slope relationship, which was supported by end-tidal fractions of O_2 and CO_2 and ventilatory equivalents for O_2 and CO_2 ($\dot{V}_E/\dot{V}O_2$ and $\dot{V}_E/\dot{V}CO_2$; Whipp and Agostoni, 2007; Rossiter, 2011). $\dot{V}O_{2peak}$ was calculated as the highest 12-breath mean over the final ~30 seconds prior to the limit of tolerance during the RIT.

4.2.4.3 Endogenous energy provision

Using the computational model of pulmonary and circulatory dynamics from which the power outputs were calculated (Benson, Grassi and Rossiter, 2013), cumulative energy provision from endogenous resources (i.e. those independent of O_2 transfer from the atmosphere; PCr, venous and muscle O_2 stores from haemoglobin and myoglobin, glycolysis and glycogenolysis) was calculated as the summed difference between predicted $\dot{V}O_2$ and predicted steady-state $\dot{V}O_2$ during work phases. Repletion of endogenous energy resources during the recovery phases of intermittent exercise was omitted from the calculation. As such, values represent the total summed contribution from these resources to ATP provision during work phases and represent the energy provision of muscular activity during exercise.

4.2.4.4 Electromyography

The EMG signal for each of the five muscles was rectified, band-pass filtered (20–500 Hz), and smoothed via root mean square (RMS) with a 100 ms moving window and no overlap. Following identification of the three pedal strokes used to determine P_{iso} of each maximal effort, the peak activity of each of the five muscles investigated was summed for each pedal stroke, as the activity of each muscle contributes to generating crank power, and a mean of these three

values calculated. Baseline EMG amplitude was defined as the mean of the EMG amplitude of the two maximal efforts performed prior to exercise and the exercise-induced change in EMG amplitude was calculated as the pre- to post-exercise difference.

4.2.5 Statistical Analysis

Results are presented as means ± SD and statistical significance was set at $P < 0.05$. All statistical analyses were completed using the Statistical Package for the Social Sciences (SPSS 24, SPSS Inc., Chicago, IL, USA). Pulmonary gas exchange, heart rate, and muscle oxygenation responses were separated into 5-minute bins incorporating data from exercise onset to the 20th minute, and the final 5 minutes. The effect of the exercise protocol on changes in all outcome measures during and pre- to post- exercise were assessed with a two-way repeated measures ANOVA, with Bonferroni post hoc analyses performed when appropriate to identify the specific differences. The comparisons of data within an individual protocol were made using a one-way repeated measures ANOVA with Bonferroni post-hoc. Paired t-tests were used to compare target $\dot{V}O_2$ to measured $\dot{V}O_2$ during the final minute of the protocols, target task power to mean task power measured at the crank, and mean task power measured at the crank during the constant-power and work phases of the intermittent protocol. RPE data were initially analysed using the Friedman test to check whether any differences were present. Subsequently, Wilcoxon signed-rank post-hoc analyses with a Bonferroni correction for multiple comparisons were used to identify where differences existed.

4.3 Results

4.3.1 Ramp-incremental exercise responses

The estimated LT was 1.96 ± 0.52 $L \cdot min^{-1}$, with the limit of tolerance occurring at a $\dot{V}O_{2peak}$ of 3.59 ± 0.79 $L \cdot min^{-1}$ and peak power of 300 ± 62 W.

4.3.2 Constant-power and intermittent exercise

Total work done was not different between intermittent and constant-power protocols (288 ± 74 vs. 288 ± 74 kJ; $P = 0.15$), with the consequence that the intermittent exercise protocol duration was longer than the constant-power exercise (32 ± 2 vs. 28 ± 2 minutes; $P < 0.01$). The target power predicted to evoke a $\dot{V}O_2$ of Δ40 during the intermittent work bouts was 75% higher than that of constant-power exercise (298 ± 78 vs. 170 ± 43 W; 98 ± 9 vs. 56 ± 5 $\%RIT_{peak}$, $P < 0.01$). Although mean crank power output exceeded the target power during the constant-power protocol ($P = 0.02$), this was only by 2 W (172 ± 43 vs. 170 ± 43 W), which is within the 2 % error in the accuracy of power outputs between 100 and 1500 W (manufacturer's specifications). This discprepancy is also of minimal physiological significance when considered relative to the pedal stroke-to-pedal stroke fluctuation of 24 ± 8 % in crank power during constant-power exercise (Figure 4.1), which is consistent with previous findings from our laboratory.

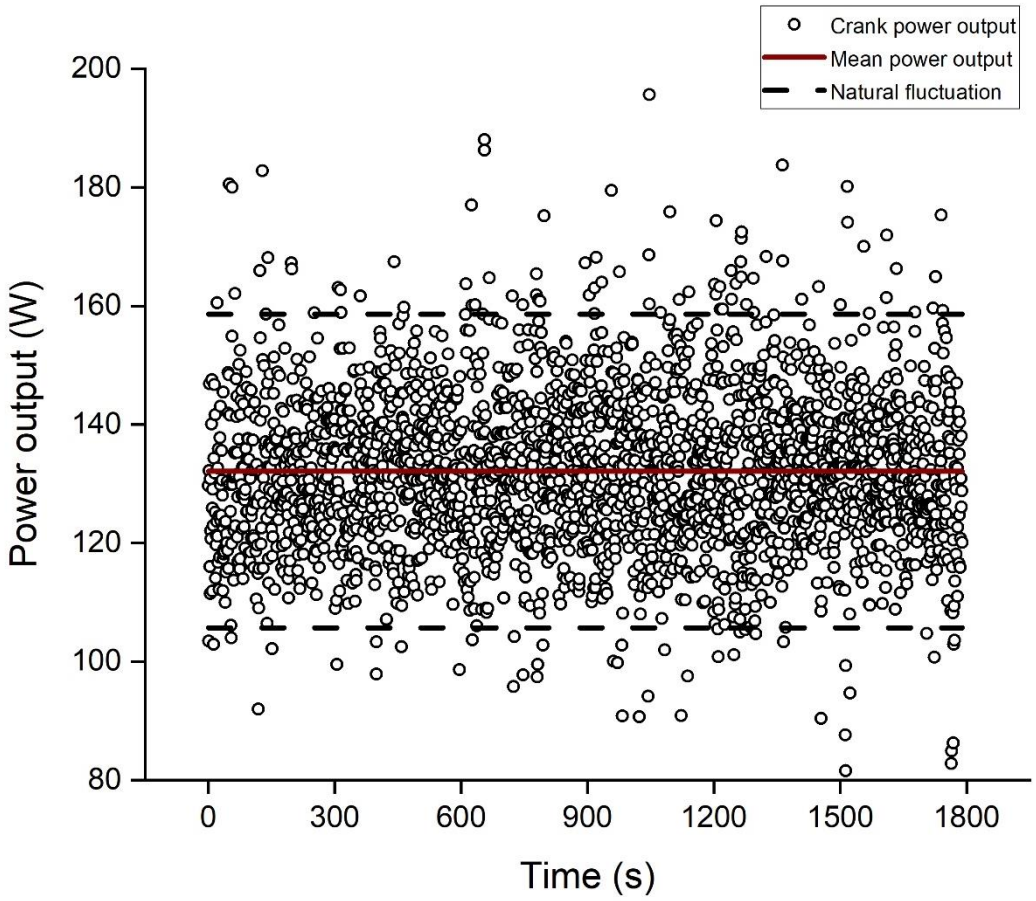

Figure 4.1: A representative example of the variability in pedal stroke crank power during constant-power cycling. Although mean crank power was equivalent to target power (132 W), crank power naturally fluctuated (95 % prediction bands of all pedal strokes) by 20 % of this value (group value 24 ± 8 %).

During the 10-second work phases of the intermittent protocol, mean power output was significantly lower than the task power (253 ± 70 vs. 298 ± 78 W ; $P < 0.01$; Figure 4.2). This was at least partly due to the time required to transition from the 20 W recovery phase power to the task power, which took ~3 seconds to be achieved (Figure 4.2). However, the mean crank power during the work phases of intermittent exercise was still 47 ± 12 % greater than the mean crank power during the constant-power protocol ($P < 0.01$). Furthermore, 74 ± 14 % of the pedal strokes were within the natural fluctuation in crank power that

occurs during constant-power cycling, indicating that the majority of pedal strokes during the work phases of the intermittent protocol were within the acceptable difference between crank and target power (Figure 4.2).

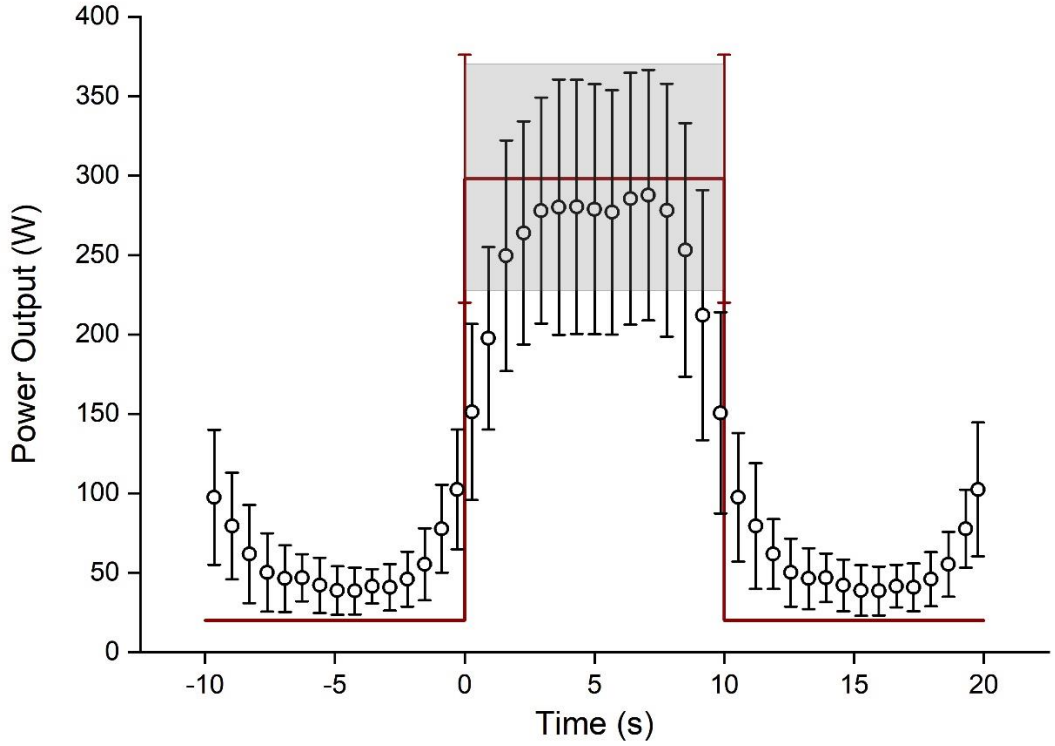

Figure 4.2: Comparison of the target power (red solid line) and crank power (open symbols) during the intermittent exercise protocol (mean ± SD). The natural fluctuation in crank power during constant-power cycling in the same individuals is provided for reference (light grey shaded area).

While cadence was higher during the intermittent protocol than the constant-power protocol (91 ± 9 vs. 84 ± 5 rev·min^{-1}; $P < 0.01$), the target range was adhered to during both protocols. The cumulative energy provision from endogenous resources was greater in response to the intermittent protocol compared to the constant-power protocol (25.24 ± 7.32 vs. 1.20 ± 0.32 L; $P < 0.01$).

4.3.3 Systemic physiological responses

There was a progressive increase in $\dot{V}O_2$ during constant-power and intermittent exercise ($P < 0.05$ for all values compared to previous time point, with the exception of no difference between Minute 20 and final 5 minutes during intermittent exericse; $P = 0.22$). However, the absolute difference between Minutes 10-15 and the final 5 minutes was less than 100 $mL \cdot min^{-1}$ in both protocols, suggesting that, typical of heavy-intensity exercise, a steady-state was achieved after ~10 minutes (Figure 4.3, upper panels). There was no difference between protocols in the change in $\dot{V}O_2$ over time (interaction effect, $P = 0.21$). Mean $\dot{V}O_2$ during the final 5 minutes of constant-power (2.71 ± 0.61 $L \cdot min^{-1}$) and intermittent exercise (2.64 ± 0.54 $L \cdot min^{-1}$) was not different between protocols or to target $\dot{V}O_2$ (2.61 ± 0.61 $L \cdot min^{-1}$; all $P > 0.05$). Similarly, heart rate exhibited an intitial exponental increase before gradually but progressively increasing ($P < 0.05$ at all time points compared to previous time point; Figure 4.3, lower panels), but this change over time was not different between protocols (interaction effect, $P = 0.10$).

Blood lactate concentration increased from rest to Minute 5 during constant-power (1.8 ± 0.5 vs. 3.6 ± 0.7 mM; $P < 0.01$) and intermittent exercise (1.6 ± 0.4 vs. 3.3 ± 1.2 mM; $P = 0.04$) before reaching a steady-state in both protocols ($P > 0.05$ for all further values compared to previous time point). End-exercise blood lactate concentration during constant-power and intermittent exercise was 4.5 ± 1.2 and 4.0 ± 1.1 mM, respectively, and there was no difference between protocols in the change in blood lactate concentration across time (interaction effect; $P = 0.86$).

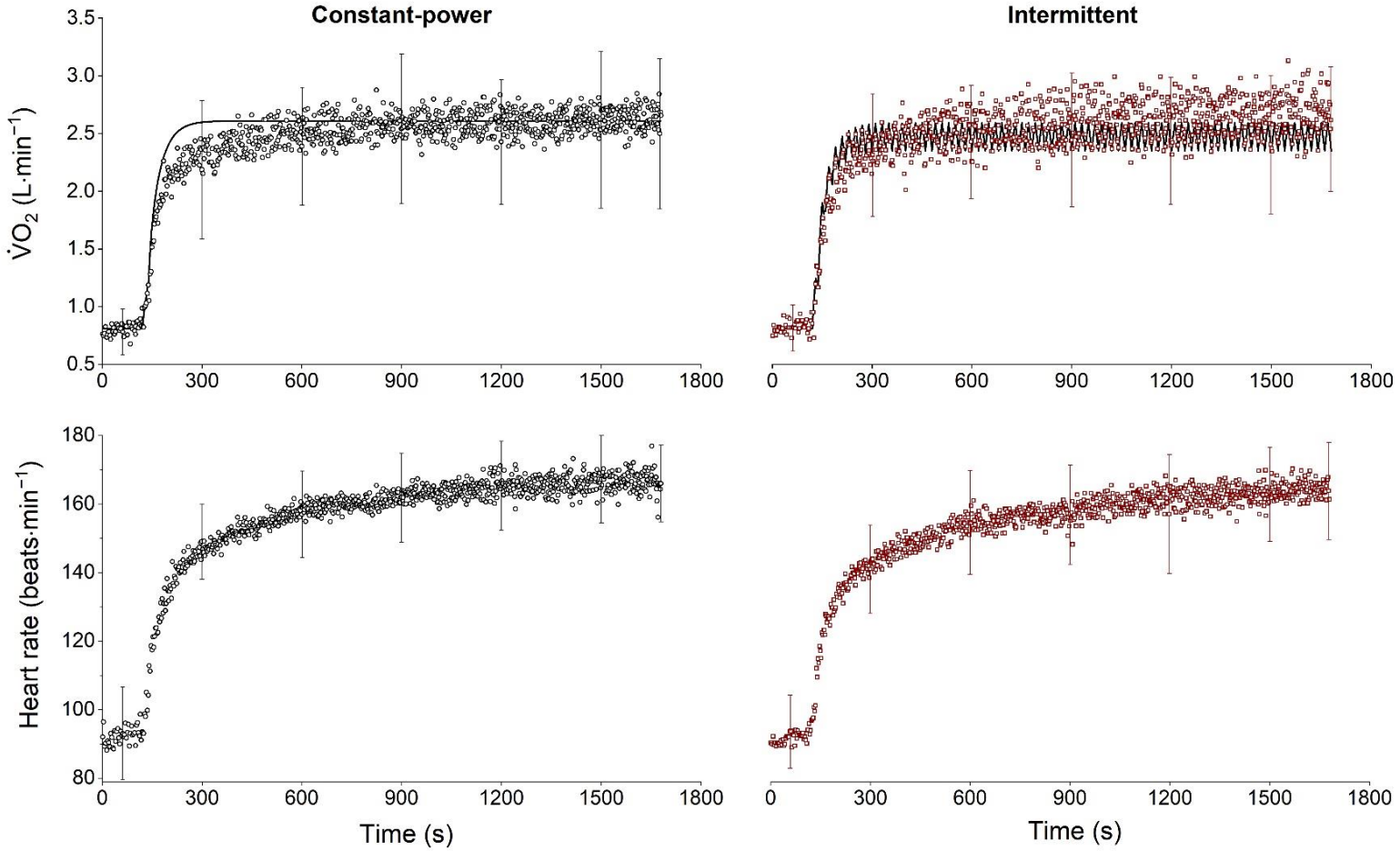

Figure 4.3: Top row: group interpolated modelled (solid lines) and measured $\dot{V}O_2$ (open circles) responses during constant-power (left) and intermittent (right) exercise, prescribed to evoke the same target $\dot{V}O_2$. Bottom row: heart rate during the same constant-power (left) and intermittent (right) exercise. Error bars demonstrate SD for a 1-minute bin every 5 minutes.

4.3.4 Muscle oxygenation and electrical activity

During constant-power and intermittent exercise, tissue saturation index (TSI) decreased progressively from rest to Minute 10 ($P < 0.05$ for each) before reaching a steady-state during constant-power and intermittent exercise (all $P > 0.05$ compared to previous time point; Figure 4.5). The change in TSI across time was not different between protocols (interaction effect, $P = 0.45$), emphasised by the similarity of mean TSI during the final 5 minutes of constant-power and intermittent exercise (60.2 ± 8.8 vs. 60.2 ± 8.7 %, respectively).

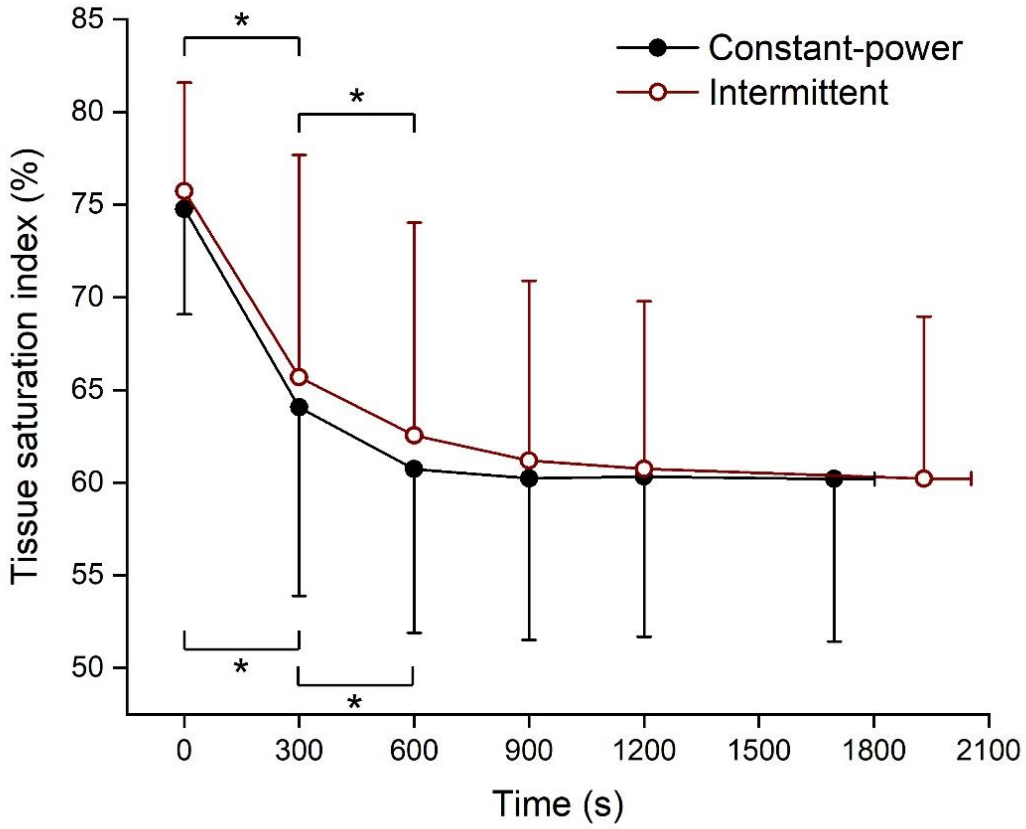

Figure 4.4: Tissue saturation index measured over the vastus lateralis using near-infrared spectroscopy during constant-power (black, filled in symbols) and intermittent exercise (dark red, open symbols). For each protocol, n = 14. * *P* < 0.05.

Locomotor muscle EMG_{RMS} amplitude was higher during the work phases of intermittent exercise than during constant-power exercise at all time points (all $P < 0.01$), with overall mean muscle activity 28 ± 16 % greater during the intermittent protocol. There was an increase in muscle activity over time during constant-power (main effect; $P < 0.01$) and intermittent exercise (main effect; $P = 0.02$). Compared to Minute 5, muscle activity during the final minute was 12 ± 8 % greater for constant-power exercise and 10 ± 14 % greater for intermittent exercise, with the change in muscle activity not different between protocols (interaction effect, $P = 0.63$).

4.3.5 Ratings of perceived exertion

The perceptual responses followed the same trend as $\dot{V}O_2$ and heart rate: a large initial increase from baseline (i.e. ratings of '0') to Minute 5 was followed by incremental increases in the rating of perceived dyspnoea and leg tiredness throughout constant-power and intermittent exercise (Figure 4.4). The only exception to this progressive increase in perceived exertion was no difference in leg tiredness between Minute 20 and the final minute of exercise during the constant-power protocol. Ratings of perceived dyspnoea and leg tiredness were not different between constant-power and intermittent exercise at any time point (all $P > 0.05$), demonstrating that the protocols were closely matched for perceived exertion.

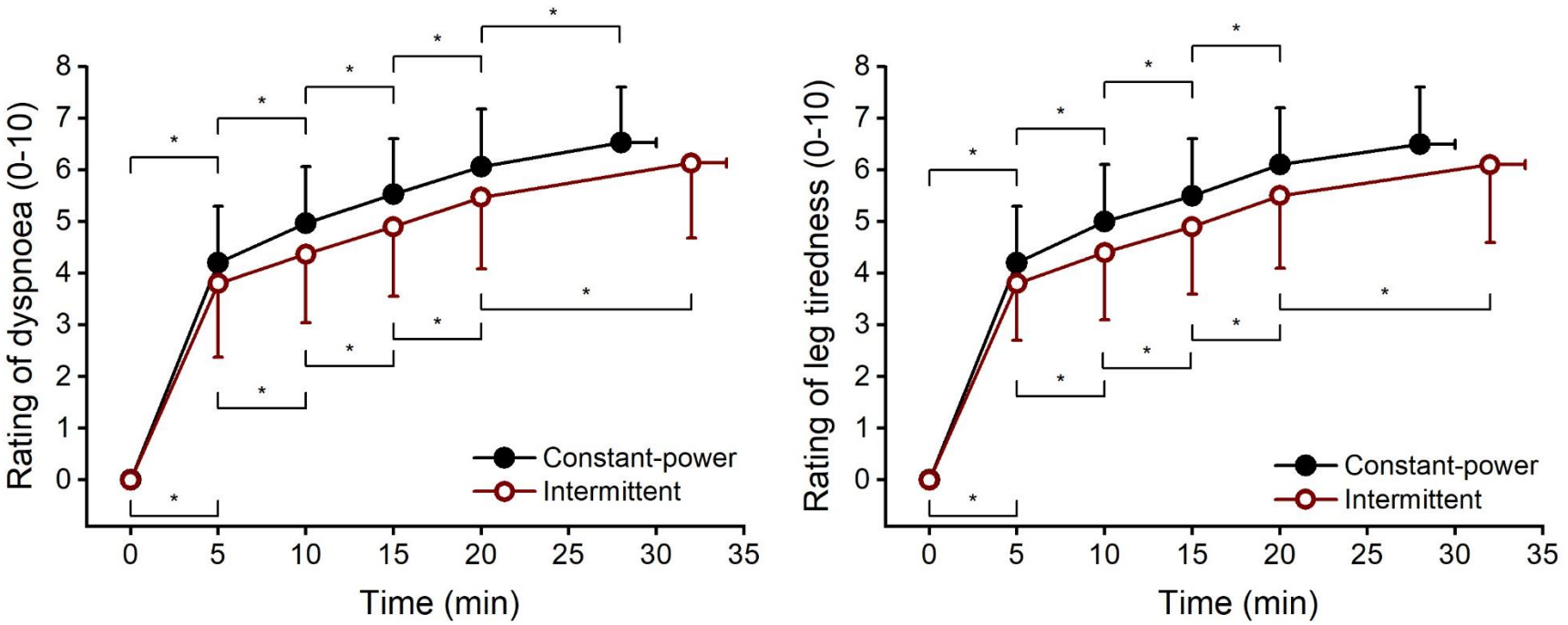

Figure 4.5: Rating of perceived dyspnoea (left panel) and leg tiredness (right panel) during intensity-matched constant-power and intermittent exercise. There was no difference between protocols at any time point. * indicates a significant increase compared to subsequent time point.

4.3.6 Locomotor neuromuscular fatigue

Baseline P_{iso} was not different between constant-power and intermittent protocols (767 ± 195 vs. 771 ± 193 W; P = 0.53). Post-exercise, P_{iso} was reduced following constant-power (−104 ± 51 W) and intermittent exercise (−99 ± 48 W; both P < 0.01 compared to respective baseline values), but the magnitude of the exercise-induced reduction in P_{iso} was not different between protocols (Figure 4.5; interaction effect, P = 0.69). Compared to baseline, peak muscle activity during maximal isokinetic cycling (inferred from EMG_{RMS}) tended to be lower following constant-power (95 ± 14 % of baseline) and intermittent exercise (95 ± 16 % of baseline), but this did not reach statistical significance (main effect, P = 0.06). There was no difference between groups in the exercise-induced reduction in peak muscle activity during maximal isokinetic cycling (interaction effect, P = 0.60).

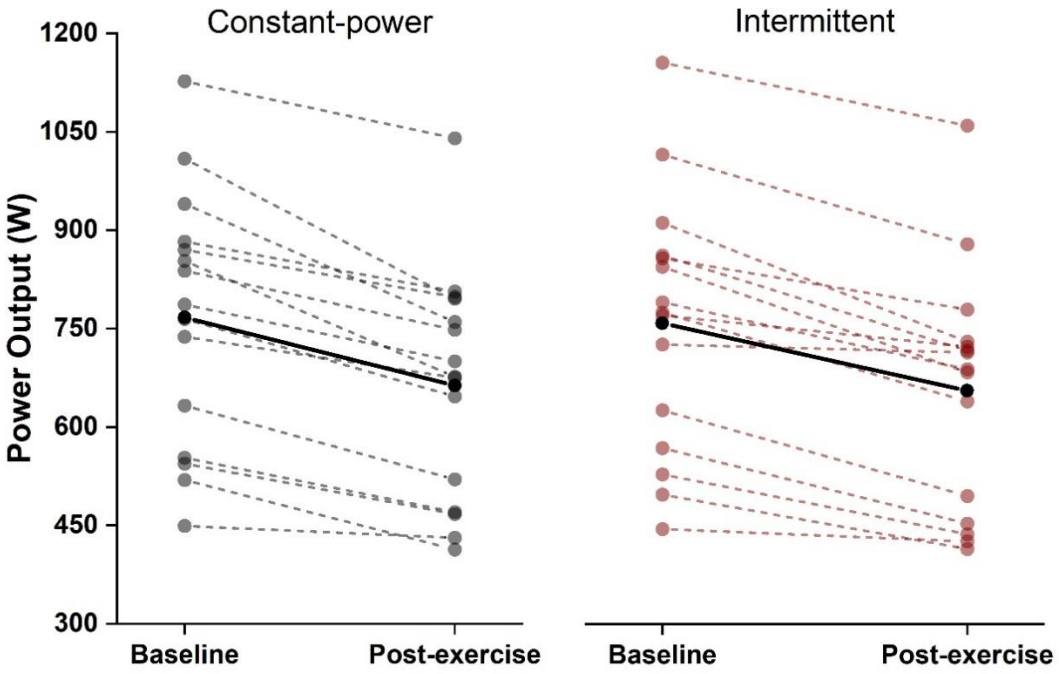

Figure 4.6: Individual (dashed lines) and mean (solid lines) P_{iso} at baseline and following intensity-matched constant-power (left) and intermittent (right) exercise. P_{iso} was reduced in both protocols, but with no difference between constant-power or intermittent responses.

4.4 Discussion

There was no difference in $\dot{V}O_2$, heart rate, or blood lactate concentration during constant-power and intermittent exercise, confirming that these protocols were intensity-matched. Target power was 75 % and mean crank power 47 ± 12 % higher during the work phases of intermittent exercise than constant-power exercise. However, neither perceptions of dyspnoea and leg tiredness, nor the exercise-induced reduction in peak isokinetic cycling power (P_{iso}), were different between protocols. Therefore, this study demonstrates that perceived exertion during exercise and the consequent exercise-induced fatigue are independent of power output and are determined by exercise intensity.

4.4.1 Dissociating exercise intensity from power output

The terms ‘intensity’ and ‘power output’ are often used interchangeably when defining exercise prescription; however, these are physiologically distinct concepts. During intermittent exercise, the intensity (physiological stress) of the exercise is dependent on the interaction between power output (rate of ATP demand), duration, and ratio of work and recovery bouts (Astrand *et al.*, 1960; Margaria *et al.*, 1969; Vogiatzis, Nanas and Roussos, 2002; Turner *et al.*, 2006; Davies *et al.*, 2017; Combes *et al.*, 2018). The present study demonstrates that the power output required to evoke a target $\dot{V}O_2$ during constant-power and intermittent exercise can be robustly predicted in young, healthy individuals using a computational model with assumptions for gain (12 $mL \cdot min^{-1} \cdot W^{-1}$ for heavy-intensity) and $\dot{V}O_2$ time constant (30 seconds). However, the mean programmed power during intermittent exercise in the present study was lower than that of the constant-power exercise (159 W vs. 170 W), which is likely due to the exercise protocols being matched for peak $\dot{V}O_2$ and not mean $\dot{V}O_2$. As such, mean task power alone is not a valid surrogate of intensity, as has been previously suggested (MacInnis and Gibala, 2017).

Recovery periods during intermittent exercise allow the repletion of endogenous energy stores that can contribute to ATP supply during the subsequent work phase, thereby preventing the progressive accumulation of fatigue-related metabolites and attenuating $\dot{V}O_2$ (Belfry *et al.*, 2012b; Belfry *et al.*, 2012a; McCrudden, Keir and Belfry, 2017). Consequently, when intermittent exercise has a fixed power output and work:recovery ratio, exercise intensity is

progressively lowered as the duration of work phases is shortened (Astrand *et al.*, 1960; Turner *et al.*, 2006; Davies *et al.*, 2017). In accordance with this relationship, for the intensity of constant-power and intermittent exercise to remain equivalent, task power of intermittent exercise must increase as the work phases become shorter. Therefore, a 10:10-second work:recovery protocol was used to maximise the difference in task power between intermittent and constant-power intensity ($\dot{V}O_2$)-matched protocols. Consistent with previous studies, there was a large power output-metabolic dissociation during intermittent exercise, in which crank power was 47 ± 12 % greater during the intermittent than the constant-power task, with no differences in the change in $\dot{V}O_2$, heart rate, or blood lactate concentration throughout the exercise. This dissociation between the power output and metabolic responses was also seen in the perceptual responses. There was no difference in the ratings of dyspnoea and leg tiredness throughout the exercise trials, thus perceived exertion was determined by exercise intensity and the associated physiological responses rather than the power demands of the exercise. Therefore, the perceptual responses appear to be independent of differences in central motor drive associated with the increased mechanical loading during the intermittent protocol (inferred from greater EMG_{RMS} amplitude during work phases of intermittent exercise compared to constant-power exercise) and, provided that appropriate work and recovery durations are selected, the task power that can be sustained during intermittent exercise is theoretically limited only by an individual's maximum power-generating capacity.

In the present study, the short work and recovery durations meant that, due to the relatively slow $\dot{V}O_2$ kinetic response relative to changes in the rate of ATP demand, the oscillations in $\dot{V}O_2$ were within the steady-state breath-by-breath noise (Figure 4.3), despite the frequent and large changes in the rate of ATP demand. Therefore, the physiological profiles ($\dot{V}O_2$, $\dot{V}_E$, heart rate, muscle oxygenation) of the constant-power and intermittent exercise protocols were indistinguishable. However, the underlying cellular bioenergetics associated with each protocol are distinct to support the differing patterns of ATP demand. This is demonstrated by the greater cumulative energy provision from endogenous resources, calculated using the same assumptions that allowed target power output to be accurately predicted, during intermittent exercise compared with continuous. Although the energy provision from endogenous resources is associated with the accumulation of fatigue-related metabolites, when work bouts are sufficiently short, repeated contributions to ATP resynthesis from endogenous resources can occur without significant disruption to system stability, such as a reduction in intramuscular pH or accumulation of inorganic phosphate (Davies *et al.*, 2017). This occurs when the work bout is completed prior to PCr being substantially depleted and the proceeding recovery phase allows PCr to be resynthesised, thereby facilitating an increased contribution from PCr to overall ATP supply.

Very short work and recovery durations were utilised during the intermittent protocol to maximise the dissociation between power output and $\dot{V}O_2$, and consequently amplify the difference in power output for each protocol. However,

due to the function of the ergometer, which adjusts flywheel resistance through a feedback loop, the magnitude of the change in power output caused a lag between the initiation of the work phase and achieving task power. Coupled with the brevity of the work and recovery phases, which meant that task power was maintained for only a short time, the power profile exhibited sinusoidal characteristics (Figure 4.2). Consequently, when using this intermittent approach, the magnitude of the dissociation must be balanced against the practical application of the protocol; longer work and recovery durations will minimise the difference between the target power and the mean power produced during the work phase but will reduce the dissociation between power output and intensity. Intermittent exercise with sinusoidal patterns of increasing and decreasing task power allow more gradual changes in power that may permit higher peak powers to be achieved compared to square wave patterns, albeit for much shorter durations (Porszasz *et al.*, 2013). Therefore, further research is required to determine the optimal work:recovery pattern to maximise the muscle stimulus applied during exercise.

4.4.2 Exercise-induced fatigue

Heavy-intensity constant-power and intermittent exercise both induced fatigue, consistent with previous findings showing that fatigue was present after only 3 minutes of heavy-intensity constant-power exercise (Cannon *et al.*, 2011). However, in contrast to the hypothesis, there was no difference in the magnitude of fatigue induced by the protocols (a reduction in P_{iso} of 104 ± 51 W vs. 100 ± 48 W for constant-power and intermittent tasks, respectively). Moreover, there

was no difference between protocols in the aetiology of fatigue, evidenced by no differences in the increase in muscle activity required to maintain task power or the pre- to post-exercise reduction in muscle activity during maximal isokinetic cycling. Henneman's Size Principle dictates that motor units are recruited in order of ascending size, with small motor units recruited first and larger units only recruited when greater forces must be generated (Henneman, 1957; Henneman, Somjen and Carpenter, 1965a; Henneman, Somjen and Carpenter, 1965b). The higher task powers inherent to intermittent exercise increased muscle activity by 28 ± 16 % (inferred from EMG_{RMS}), and may have caused the recruitment of larger motor units that tend to have a greater composition of more fatigue-susceptible type II muscle fibres than smaller motor units (McPhedran, Wuerker and Henneman, 1965a; McPhedran, Wuerker and Henneman, 1965b). Type II, or fast-twitch, muscle fibres have relatively high glycolytic but low oxidative capacity and therefore the sustained activity of these fibres causes lactate accumulation and an intramuscular acidosis, which are associated with fatigue (Allen, Lamb and Westerblad, 2008). Although the relative contribution of type I and II muscle fibres to power production is not known, muscle activity was 28 ± 16 % higher during the intermittent than the constant-power protocol, suggesting that larger motor units were recruited. A possible reason for the absence of greater fatigue, despite the higher power and greater muscle activation of intermittent exercise, is the dampened homeostatic disturbances when work is performed intermittently. Additionally, in contrast to prolonged heavy-intensity constant-power exercise, which is characterised by

low pH after the initial alkalosis, intermittent exercise with short work phases may exhibit acute increases in pH during the work bouts, for which PCr is the predominant energy source. This shift in the status of the intramuscular environment during muscular activity may occur due to the sequestering of H^+ in the Lohmann reaction (ADP + PCr + H^+ ←→ ATP + Cr), causing pH to rise as PCr falls. The influence of this alkalising mechanism is unclear, but it may, in addition to a reduction in Pi accumulation, attenuate the development of fatigue during intermittent exercise comprised of short work bouts. As such, when intermittent exercise is intensity-matched, the higher task powers and likely recruitment of larger motor units (indicated by the higher surface EMG amplitude) does not increase the magnitude of fatigue induced.

4.4.3 Implications for exercise training

4.4.3.1 Adaptation

A key principle of physiological adaptation to exercise is that the magnitude of the adaptation is dependent on the magnitude of the stress imposed by the exercise (Wenger and Bell, 1986; O'Donovan *et al.*, 2005; Kemi *et al.*, 2005; Gibala and McGee, 2008; Fiorenza *et al.*, 2018; Hawley *et al.*, 2018). Consequently, the severity of fatigue developed during an exercise task has been proposed to indicate the efficacy of the protocol as a training intervention. Chronic obstructive pulmonary disease patients who develop fatigue during exercise training sessions demonstrate superior improvements in exercise performance and quality of life to those who do not develop fatigue (Burtin *et al.*, 2012). Therefore, the similar fatigue responses to intensity-matched

constant-power and intermittent exercise suggest comparable efficacies as training interventions. However, the disparate bioenergetics of constant-power and intermittent exercise may promote the activation of different signalling cascades and consequently metabolic adaptations, such as PCr recovery kinetics (which can be speeded after only two weeks of interval training; Forbes et al., 2008) or $\dot{V}O_2$ kinetics due to the repeated stimulus to increase $\dot{V}O_2$. Therefore, specific adaptations in physiological function may be targeted by selecting the appropriate training strategy.

Furthermore, the mechanical-metabolic dissociation during intermittent exercise can be exploited to maintain the mechanical loading of the working locomotor muscles at a lower exercise intensity (or increase mechanical loading of the working locomotor muscles at the same intensity) compared to constant-power exercise. The mechanical load placed on skeletal muscle is a fundamental component of exercise training, evidenced by resistance training (Tesch, 1988; Egan and Zierath, 2013) and muscle overload interventions such as synergist ablation (Terena *et al.*, 2017), and acts as an adaptive stimulus independently of metabolic challenges. Such training effects are powerful combatants of age-related declines in muscle performance caused by atrophy (sarcopenia) and the resultant functional impairments (e.g. risk of falls; Hunter, McCarthy and Bamman, 2004; Papa, Dong and Hassan, 2017; Martín Del Campo Cervantes, Habacuc Macías Cervantes and Monroy Torres, 2019). In addition to enhancing muscle contractile properties such as force and power-generating capacity, this mechanical component can drive vascular adaptations, evidenced by passive

limb ‘exercise’ promoting angiogenesis in the absence of any metabolic stimulus (Hellsten *et al.*, 2008; Høier *et al.*, 2010), and therefore may also confer training effects that enhance exercise tolerance secondary to increases in oxidative function.

In addition to the higher power, the oscillatory nature of intermittent exercise, which is typically associated with large transient changes in muscle oxygenation and $\dot{V}O_2$ amplitude during work and recovery phases, has been proposed to be a key aspect of the stimulus provided by exercise (Cochran *et al.*, 2014; Combes *et al.*, 2015), and may independently influence outcomes with training when comparing intensity-matched constant-power and intermittent exercise.

Consequently, intermittent exercise may have intrinsic advantages over traditional constant-power exercise when applied as a training strategy, particularly for specific patient populations with ventilatory limitations or skeletal muscle impairments that constrain constant-power exercise (Vogiatzis, Nanas and Roussos, 2002; Wisløff *et al.*, 2007; Porszasz *et al.*, 2013). Despite no increase in fatigue, the bioenergetic differences due to the greater cumulative energy provision from endogenous resources and higher muscle power output generated during intermittent exercise may translate into additional and/or superior adaptations to training compared to intensity-matched constant-power exercise.

4.4.3.2 Adherence

As intensity-matched intermittent and constant-power exercise elicit the same magnitude of fatigue, in addition to indistinguishable perceptual responses, the higher power output during intermittent exercise does not reduce exercise tolerance and therefore is unlikely to decrease adherence to exercise training. However, a final consideration regarding the practical application of these protocols as training strategies is that the intermittent protocol requires a slightly greater time commitment to perform the same volume of work (~13%). This is contrary to the prevailing viewpoint that intermittent is a more time-efficient modality of exercise than constant-power (Skelly *et al.*, 2014; Gillen and Gibala, 2018), as a greater volume of work can be accumulated in a shorter space of time, which is only true in cases when intermittent exercise is performed at a higher intensity than constant-power exercise. Due to 'lack of time' being commonly cited as a reason for low exercise participation (Stutts, 2002; Trost *et al.*, 2002; Kimm *et al.*, 2006), the time commitment must be considered when applying intensity-matched intermittent exercise as a training strategy. However, it is unlikely that the relatively small increase in exercise duration would act as a deterrent should the exercise modality confer appreciable long-term benefits.

4.4.4 Conclusion

Increasing the task power demands of intensity-matched exercise using intermittent exercise has no effect on the physiological, perceptual, or fatigue responses. These data indicate that the magnitude of fatigue induced following

exercise is independent of the task power and is driven by the metabolic responses that determine exercise intensity. However, the increase in task power and cumulative energy provision from endogenous resources during intermittent exercise, with no consequent increase in the magnitude of exercise-induced fatigue, may improve chronic adaptation without reducing the tolerability of the exercise training.

5: Intermittent exercise training may confer superior improvements in exercise tolerance to intensity-matched constant-power training

The data presented in this chapter was collected as part of a larger study conducted in collaboration with another PhD student. Therefore, elements of the data that are relevant to the different study components overlap, such as ramp-incremental exercise responses. However, the study component presented in this chapter has unique aims and novel data.

Due to restrictions relating to COVID-19, data collection for this study was not completed and consequently all findings presented are preliminary.

5.1 Introduction

Exercise training is a potent stimulus to induce improvements in physiological function, with the adaptation conferred dependent on the specific training stimulus imposed (Coffey and Hawley, 2007; Coffey and Hawley, 2017). Training comprising isolated muscular contractions with a high external load relative to MVC is associated with increases in strength via neural adaptations, such as motor unit synchronisation, and morphological adaptations, such as muscle hypertrophy (Sale, 1988; Gabriel, Kamen and Frost, 2006; Folland and Williams, 2007; Suchomel *et al.*, 2018). Conversely, whole-body endurance exercise promotes mitochondrial biogenesis and other enhancements in O_2 delivery and utilisation that are related to improvements in aerobic parameters of fitness, such as lactate threshold (LT) and $\dot{V}O_{2peak}$, and exercise tolerance

(Holloszy, 1967; Holloszy and Booth, 1976; Holloszy and Coyle, 1984; Jones and Carter, 2000). However, intermittent exercise, with characteristics somewhere between these two extremes, is widely considered to be an effective alternative training strategy to traditional endurance training, inducing a range of similar physiological adaptations relating to aerobic parameters (Billat, 2001a; Billat, 2001b; Laursen and Jenkins, 2002; Gibala and McGee, 2008).

Typically, the work phases of intermittent exercise are performed at either maximal effort (sometimes referred to as ‘all-out’ efforts or sprint interval training) or predetermined power outputs that elicit responses close to $\dot{V}O_{2peak}$. When applied as a chronic stimulus, these intermittent protocols induce comparable or even superior physiological adaptations to more conventional constant-power exercise, during which power output is maintained continuously throughout the task (Rognmo *et al.*, 2004; Gibala *et al.*, 2006; Helgerud *et al.*, 2007; Wisløff *et al.*, 2007; Burgomaster *et al.*, 2008). The adaptive response to exercise is influenced by the volume of work (Wenger and Bell, 1986; Granata, Jamnick and Bishop, 2018b), intensity (Wenger and Bell, 1986; O'Donovan *et al.*, 2005), and severity of fatigue induced (Burtin *et al.*, 2012), which in turn are determined by the interaction of power output, work and recovery durations, and number of work phases performed. However, whether the physiological characteristics associated with an intermittent pattern of work are fundamental components of the stimulus of intermittent exercise, and promote adaptation independently of other factors, is unclear.

A fundamental consequence of performing exercise intermittently is a shift in the bioenergetic profile compared to intensity-matched constant-power exercise. Including regular recovery phases during exercise allows a greater contribution to energy provision from phosphocreatine (PCr), which can be repeatedly resynthesised during the interspersed recovery periods when the rate of ATP demand is low. For example, energy provision from PCr, which has a limited capacity, can be fourfold greater during intermittent exercise with work durations of less than 30 seconds compared to constant-power exercise at an equivalent power output (Davies *et al.*, 2017). This contrasting PCr turnover between exercise protocols may stimulate different signalling pathways, or the same pathways to different extents, thereby inducing unique adaptations (Cochran *et al.*, 2014).

A further effect of intermittent exercise is that, due to the dissociation between power output and intensity (Turner *et al.*, 2006; Davies *et al.*, 2017), higher power outputs can be performed with no increase in metabolic stress or noxious sensations compared to constant-power exercise. Generating the higher power outputs inherent to intensity-matched intermittent exercise increases global muscle activity during work phases (Chapter 4), likely causing greater recruitment of high-threshold motor units. Although this change in motor unit activity did not increase the fatigue induced by the protocol (Chapter 4), performed chronically it may cause adaptations specific to high-threshold motor units or improvements in global factors, such as motor unit synchronisation (Sale, 1988; Suchomel *et al.*, 2018). In turn, these adaptations may translate

into superior improvements in power-generating capacity and/or exercise tolerance, secondary to increased fatigue resistance of high-threshold motor units, compared to constant-power exercise training. Additionally, the greater power outputs may increase the mechanical stimulus applied to the myofibrils, such as cell deformation, which has been shown to promote endothelial cell proliferation and angiogenesis independently of a metabolic stimulus (Hellsten *et al.*, 2008; Høier *et al.*, 2010).

Due to these characteristics, the alternating pattern of work and recovery during intermittent exercise may be an important aspect of the training stimulus. When performed by healthy but untrained individuals three days per week for six weeks, four 30-second bouts of maximal exercise increased $\dot{V}O_{2peak}$ and maximal activity of citrate synthase (Burgomaster *et al.*, 2008), a marker of mitochondria content (Larsen *et al.*, 2012), as well as protein content of glucose transporter type 4 (GLUT4) and monocarboxylate transporters (MCT; Burgomaster *et al.*, 2007), which regulate lactate and H^+ exchange in the muscle (Halestrap, 2013). However, when the same volume of work was performed as quickly as possible in a maximal continuous bout, at the same frequency of three days per week for six weeks, the increase in $\dot{V}O_{2peak}$ was comparable (both ~6 %) but all other metabolic adaptations were absent (Cochran *et al.*, 2014). This greater adaptive response to intermittent training may translate into superior improvements in exercise performance compared to constant-power exercise matched for intensity and volume of work; however, this has not been directly compared.

Therefore, the aim of this study was to determine whether intermittent exercise training improves peak power output, parameters of aerobic function, muscle fatigue resistance, and/or exercise tolerance more than constant-power exercise training matched for relative intensity and volume of work. As demonstrated in Chapter 4, intensity-matched constant-power and intermittent exercise can be prescribed using a modified computational model of circulatory and pulmonary gas exchange dynamics (Benson, Grassi and Rossiter, 2013). Despite the similar systemic physiological responses, global muscle activity and the summed energy provision from endogenous resources were greater during the intermittent protocol. Consequently, these exercise protocols provide an ideal model to investigate the influence of the intermittent work pattern, independently of intensity or volume of work, on the training response.

It was hypothesised that the increase in exercise tolerance and fatigue resistance (reduction in peak power in response to a training session) and peak power production, would be greater following intermittent exercise training compared to intensity-matched constant-power exercise training, but that there would be no difference between groups in the change in $\dot{V}O_{2peak}$.

5.2 Methods

5.2.1 Participants and ethical approval

Ten healthy individuals (5 females, 5 males; age: 27 ± 4 years; height: 171 ± 5 cm; weight: 67.0 ±10.8 kg) volunteered, and provided informed consent, to participate in the study. Participants were habitually active but did not participate

in structured cycling training. All participants were screened prior to exercise testing to confirm their suitability for the study in relation to the exclusion criteria detailed in Section 2.1. All experimental procedures were approved by the University of Leeds Faculty of Biological Sciences Research Ethics Committee (approval ref: BIOSCI 17-021).

5.2.2 Study design

This study required 16 visits to the laboratory over 7-8 weeks and participants were randomised into either the intermittent training or constant-power training group. To assess pre- to post-training changes in markers of aerobic function (LT, $\dot{V}O_{2max}$) and exercise tolerance, a maximal ramp-incremental test (RIT) and very heavy-intensity constant-power test (exercise tolerance test) were performed prior to and following completion of the training period. Peak isokinetic power (P_{iso}) was also measured pre- and post-training to assess changes in maximal voluntary power-generating capacity. Training consisted of twice weekly heavy-intensity exercise sessions for six weeks, with the power output increased by 5 % every two weeks to provide progression and maintain the relative intensity of the session. To characterise the training stimulus, pulmonary gas exchange, tissue saturation index, and locomotor neuromuscular fatigue were recorded (EMG was not recorded due to an equipment failure) during the first and final training sessions (training characterisation sessions). To compare responses to the same external demands, the final training session was performed at the same power output as the first training session, regardless of training progression.

Pre- and post-training tests and the first and final training sessions were performed at a similar time of day to minimise effects relating to circadian rhythm (Racinais *et al.*, 2005; Racinais *et al.*, 2010; Fernandes *et al.*, 2014) and were separated by at least 24 hours. Participants were asked to maintain their normal diet and exercise regime throughout the study to minimise confounding effects from external factors. Additionally, in the time immediately preceding the test, participants were asked to avoid performing strenuous exercise and consuming alcohol (24 hours), or ingesting caffeine (12 hours), food or drink (besides water; 2 hours) to prevent such factors influencing the test outcomes. Training sessions were not subject to the same conditions and were performed at the participants' convenience.

5.2.3 Exercise protocols

5.2.3.1 Exercise testing

Prior to and at the limit of all maximal exercise tests, peak isokinetic power (P_{iso}) was measured during an ~6-second maximal effort with cadence constrained to 80 rev·min^{-1}. Baseline assessment of P_{iso} was preceded by three variable effort (~25, ~50, and ~75% of maximum) cycling bouts to act as a warm-up, (re)familiarise the participant with the sensation of isokinetic cycling, and facilitate attainment of the true maximal P_{iso}. The limit of tolerance was defined as when the participant could no longer maintain a cadence above 50 rev·min^{-1} despite strong vocal encouragement, at which point the ergometer was instantaneously switched from hyperbolic to isokinetic mode and the participant

was instructed to perform a final ~6-second maximal isokinetic cycling effort to determine whether a power reserve – the ability to generate more power at intolerance than was required to continue the task – was present. This was performed to determine whether exercise was predominantly limited by locomotor neuromuscular fatigue and whether the limitation changed pre- to post-training. Each test was immediately preceded by 2 minutes of rest on the ergometer followed by a 4- to 6-minute warm-up of unloaded (20 W) pedalling, and the test concluded with an ~4-minute cool-down of unloaded pedalling.

The first test performed by each participant was an RIT with an incrementation rate of 20 and 25 $W \cdot min^{-1}$ for females and males respectively. This test was used to determine non-invasive lactate threshold (LT), $\dot{V}O_{2peak}$, and peak incremental power. The second test performed was a constant-power test continued to intolerance, with the power output set as the peak incremental power less two times the incrementation rate, which was selected to elicit a tolerable duration (Tlim) of ~5 minutes (van der Vaart *et al.*, 2014). These two tests were repeated post-training in the same sequence to prevent order effects influencing post-training outcomes.

5.2.3.2 Exercise training

All participants commenced training within 7 days of completing the baseline testing. All training protocols began and concluded with ~4 minutes of unloaded pedalling. The intensity-matched constant-power and intermittent protocols were prescribed based on the research presented in Chapter 4. Briefly, power outputs were calculated using a modified computational model of circulatory and

pulmonary dynamics (Benson et al., 2013), which incorporated each individual's measured $\dot{V}O_2$ during unloaded pedalling (measured from the RIT) and gain of 12 $mL \cdot min \cdot W^{-1}$ (assumed based on previously reported values for heavy-intensity exercise; Barstow and Molé, 1991; Ozyener *et al.*, 2001) to evoke a $\dot{V}O_2$ that was 40% of the difference between LT and $\dot{V}O_{2peak}$. In Chapter 4, analysis of the pedal force measurements during intermittent exercise with work phases of 10 seconds revealed that the transition from unloaded pedalling to the target power output takes ~3 seconds and there is a decrease in power output at the end of the phase in anticipation of flywheel unloading, with the result that only ~60 % of the phase is at the target power output. Therefore, the duration of the work and recovery phases of the interval protocol used in this study were 15 seconds to increase the proportion of the work phase performed at the target power output, while still maintaining the large dissociation between the power output and the intensity ($\dot{V}O_2$). Power outputs were calculated for both protocols for each participant, with the duration of the constant-power protocol fixed to 30 minutes and the interval protocol matched for total work. Each participant then performed the protocol from the group to which they were randomly assigned.

5.2.4 Equipment and measurements

All procedures were as previously reported in this thesis, and so are briefly reviewed. A computer-controlled electromagnetically braked cycle ergometer was used during all exercise protocols. Following completion of baseline P_{iso} measurement, pulmonary gas exchange was measured throughout all

subsequent components of pre- and post-training tests and the first and final training session. Participants breathed through a mouthpiece while wearing a nose clip, with gas concentrations and volumes recorded by a breath-by-breath analyser (MedGraphics Ultima Series; Medical Graphics Corporation, St Paul, MN, USA) that was calibrated and independently verified as previously described in Section 2.5. A 12-lead ECG was used to measure heart rate from the R-R interval, which was recorded over the same time period as pulmonary gas exchange and simultaneously with each breath.

5.2.4.1 Near-infrared spectroscopy (NIRS)

Tissue saturation index (TSI), a noninvasive measure of muscle tissue oxygenation, was calculated using continuous-wave near-infrared spectroscopy with spatial resolution (NIRS; PortaMon, Artinis Medical Systems, Elst, The Netherlands). The device settings and measuring procedures were as reported in Chapter 4. Briefly, the spectrometer was affixed over the muscle belly of the vastus lateralis of the left leg, with the center ~20 cm from the patella and the device parallel with the vertical axis of the upper leg. This position was marked with indelible ink to aid replacement of the device during subsequent sessions. Opaque material was used to cover the device and surrounding skin to prevent ambient light disrupting the signal. The spectrometer was connected to a laptop via Bluetooth for data acquisition (sampled at 10 Hz), analogue-to-digital conversion, and to export data for subsequent analysis. Information regarding the theoretical underpinnings, practical applications, and limitations of NIRS is in Section 2.9.

5.2.4.2 Monitoring training progression

Numeric scales ranging from 0-10 accompanied by verbal descriptions were used to obtain ratings of perceived exertion (RPE) for sensations of dyspnoea (‘rate the difficulty of your breathing’) and leg tiredness (‘rate how tired your legs are feeling’) every 5 minutes during all training sessions. Excluding the training characterisation sessions (first and final training sessions), heart rate was measured during all training sessions using a chest-strap monitor (Polar RS800CX, Polar Electro, Finland), with the output in 5-second epochs, as a further evaluation of training progression.

5.2.5 Data analysis

Data analysis procedures for all pedal force measurements (used to derive P_{iso} and measures of fatigue) and pulmonary gas exchange variables ($\dot{V}O_2$ editing, LT estimation, $\dot{V}O_{2peak}$ determination) were as reported previously in this thesis. The procedures are briefly summarised below for convenience, but more details can be found in Chapters 2 and 4.

5.2.5.1 Power output

Power produced by the participant at the crank (crank power) was measured every 2° of angular rotation and subsequently calculated as the product of torque and instantaneous angular velocity. The mean crank power for each complete revolution of the crank was calculated to provide a pedal-by-pedal stroke power profile of each task. Mean crank power for the constant-power

protocol was calculated as the mean pedal-by-pedal crank power of the entire task. Mean crank power for the intermittent protocol was calculated as the mean of the power output during all 15-second work phases, excluding recovery phases.

To compare crank power to target power during the intermittent protocol, the crank power of every pedal stroke was systematically separated into work or recovery phases using 15-second intervals. All pedal strokes from the same phase (work or recovery) were then compiled and arranged into ascending order of time. Based on the number of work/recovery phases performed, a rolling mean with no overlap was calculated to generate the mean pedal stroke power output profile for each phase.

After the mean work and recovery power profile was determined for each individual, a group mean response was calculated. Crank power was considered to have reached target power when the difference between these was within ‘natural fluctuation’ of crank power during constant-power exercise (95% prediction bands around the mean of all pedal strokes during the trial). This measure reflects the oscillation in crank power around target power during constant-power exercise, which occurs due to natural variability in motor unit recruitment and adjustments in flywheel resistance from one pedal stroke to the next.

Peak isokinetic power (P_{iso}) was calculated for each maximal isokinetic effort as the mean of the three revolutions that produced the highest power and were

appropriately constrained at 80 ± 2 rev·min^{-1}. Exercise-induced fatigue was quantified as the pre- to post-exercise reduction in P_{iso}. The magnitude of the power reserve was calculated as the difference between post-exercise P_{iso} and target power.

5.2.5.2 Pulmonary gas exchange

Breath-by-breath $\dot{V}O_2$ data that lay outside the expected physiological responses (>99% of the local prediction limits), and therefore were likely the result of swallowing or coughing (Lamarra *et al.*, 1987), were removed. From the RIT, LT was estimated by three experienced assessors using the V-slope relationship, end-tidal fractions of O_2 and CO_2, and ventilatory equivalents for O_2 and CO_2 ($\dot{V}_E/\dot{V}O_2$ and $\dot{V}_E/\dot{V}CO_2$). For the RIT and exercise tolerance test, $\dot{V}O_{2peak}$ was calculated as the highest 12-breath mean over the final ~30 seconds prior to intolerance. End-exercise values for pulmonary gas exchange and heart rate during training characterisation sessions, during which a metabolic steady-state would be reached, were calculated as the mean of the final 5 minutes.

5.2.6 Statistical analysis

All statistical analyses were performed using SPSS 26 (SPSS Inc., Chicago, IL). The required sample size was estimated to detect differences between training groups in the change in exercise tolerance, quantified as the change in Tlim of a task with a pre-training Tlim of ~300 seconds (exercise tolerance test). Based on the data collected in Chapter 3 and published data from other groups (van

der Vaart *et al.*, 2014), it was estimated that the mean and standard deviation would be 300 and 42 seconds, respectively. Limited information is available regarding the minimal clinically important difference for exercise tolerance but, based on data in COPD patients, an increase of 60 seconds, equivalent to an increase of 22 % compared to baseline, has been proposed as a marker of physiological significance (Puente-Maestu *et al.*, 2009; Puente-Maestu *et al.*, 2016). As the exercise tolerance test in the present study was predicted to elicit a Tlim of ~300 seconds, an absolute increase of 60 seconds would approximate a relative increase of 22 %. Therefore, an additional 60-second increase in Tlim compared to the comparative training protocol was deemed to be physiologically relevant. Setting an alpha value of 0.05 and power of 0.8, it was calculated that a sample size of 16 (8 in each group) was required. As detailed previously, this was not feasible due to restrictions relating to COVID-19. In addition, data from the participant who was unable to complete the intermittent exercise protocol was excluded from group analysis, as this could not be considered an equivalent training stimulus. Therefore, there were five participants in both the constant-power and intermittent training groups in the final analyses for peak power output, Tlim of the exercise tolerance test, and responses during characterisation sessions.

Restrictions relating to COVID-19 also prevented two individuals (one in each training group) performing the post-training RIT. Consequently, there were four participants in the constant-power and intermittent training groups included in the final analyses of LT, $\dot{V}O_{2peak}$, and RIT_{peak}. All continuous variables were

analysed using a two-way mixed analysis of variance (ANOVA), with training status (two levels: pre-training and post-training) as the within-subjects factor and training intervention (two levels: constant-power and intermittent exercise) as the between-subjects factor. Discrete data (i.e. ratings of perceived exertion) were analysed using the Mann-Whitney U test to identify differences between interventions and pre- to post-training, with the Friedman test to identify time-dependent differences. Where main effects of time occurred, subsequent Wilcoxon signed-rank tests were performed but, to reduce the loss of power through multiple comparisons and therefore type II error, only between Minute 5, Minute 15, and the final minute of exercise. Standardised mean differences were used to compare the magnitude of the change following each intervention (effect size; ES) and were calculated as:

$$ES = \frac{(\mu_{C,post} - \mu_{C,pre}) - (\mu_{I,post} - \mu_{I,pre})}{\left(\frac{SD_{C,pre} + SD_{I,pre}}{2}\right)}$$

Equation 5.1

where μ is the mean and *I* and *C* are the intermittent ('treatment') and constant-power ('control') exercise interventions, respectively (Faraone, 2008; Morris, 2008). For main effects of training, ES was calculated as:

$$ES = \frac{\left(\frac{(\mu_{C,post} - \mu_{C,pre}) + (\mu_{I,post} - \mu_{I,pre})}{2}\right)}{\left(\frac{SD_{C,pre} + SD_{I,pre}}{2}\right)}$$

Equation 5.2

Appropriate thresholds for medium (between 0.5 and 0.79) and large (0.8 and above) effects when the expected effect size is not available were set as recommended (Cohen, 2013). An ES below 0.5 was considered negligible.

5.3 Results

5.3.1 Pre-training exercise characteristics

All pre-training parameters of exercise performance are reported in Table 5.2. As measured during ramp-incremental exercise, $\dot{V}O_{2peak}$ was 2.92 ± 0.73 $L \cdot min^{-1}$ (48 ± 9 $ml \cdot kg \cdot min^{-1}$) in the constant-power group and 2.95 ± 0.81 $L \cdot min^{-1}$ (44 ± 10 $ml \cdot kg \cdot min^{-1}$) in the intermittent group. The estimated LT was 1.54 ± 0.26 and 1.56 ± 0.33 $L \cdot min^{-1}$ in the constant-power and intermittent training group, respectively. These values were not different between groups at baseline (both $P > 0.05$) and were subsequently used to calculate the power outputs for the training protocols.

5.3.2 Exercise training stimulus

Training adherence was 100 % in the constant-power exercise group and 97 % in the intermittent group as a result of two subjects missing one session each. The parameters of exercise training measured during pre-training characterisation sessions are shown in Table 5.1. End-exercise $\dot{V}O_2$ was significantly higher than target (Δ40) $\dot{V}O_2$ during the pre-training characterisation sessions for both constant-power and intermittent exercise (main effect, $P < 0.01$). However, this disparity was only ~200 $mL \cdot min^{-1}$ in both groups, which is similar to the breath-by-breath ‘noise’ of $\dot{V}O_2$ measurements

(the standard deviation of the breath fluctuations during moderate-intensity upright cycling is ~100–150 mL·min^{-1}; Lamarra *et al.*, 1987; Puente-Maestu *et al.*, 2002; Bowen *et al.*, 2012). Therefore, the difference between target and measured $\dot{V}O_2$ was considered of minimal physiological significance and of no consequence to the intensity-matched premise.

Table 5.1: Exercise training protocol characteristics

	Constant-power training	Intermittent training
Duration (min)	30.00 ± 0.0	35.4 ± 0.9*
Mean power output (% P_{iso})	20 ± 3	30 ± 3*
Target $\dot{V}O_2$ (L·min^{-1})	2.28 ± 0.57	2.04 ± 0.49
End $\dot{V}O_2$ (L·min^{-1})	2.47 ± 0.64	2.26 ± 0.44
End $\dot{V}O_2$ (% of peak)	78 ± 2	81 ± 9
End heart rate (bpm)	174 ± 4	187 ± 15
End heart rate (% of peak)	92 ± 2	96 ± 5
End dyspnoea (0-10)	7 ± 1	8 ± 2
End leg tiredness (0-10)	7 ± 1	8 ± 2

Mean power output is reported as the mean crank (measured) power output as a percentage of P_{iso} in an unfatigued state. For the intermittent protocol, this value reflects power output during the work phases only. * $P < 0.05$ compared to constant-power training.

Natural fluctuation in power output during the pre-training constant-power characterisation session was 28 ± 9 % and measured power output was 101 ± 1 % of the target power output. The power profile of the intermittent protocol

during the pre-training characterisation session is displayed in Figure 5.1. Mean power output during the work phase was lower than the target power ($P < 0.01$). However, this mean was 87 ± 4 % of the target power and 85 ± 9 of the pedal strokes performed during the work phase were within the individual natural fluctuation during constant-power exercise. These data demonstrate that, although not as tightly regulated as during constant-power exercise, power output during intermittent exercise was controlled within reasonable limits of the programmed power by the ergometer, and therefore participants were performing the work as intended during both protocols.

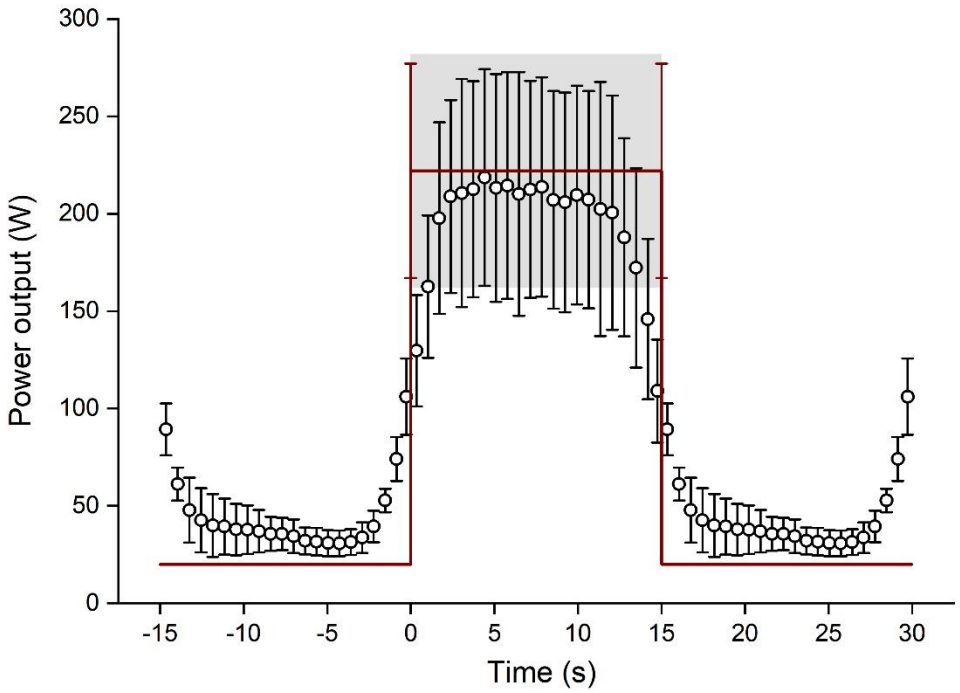

Figure 5.1: Comparison of the target power (red solid line) and crank power (open symbols) during the intermittent exercise protocol (mean ± SD). The natural fluctuation in crank power during constant-power cycling in the same individuals is provided for reference (light grey shaded area). Pedal strokes within natural fluctuation were considered to be within an acceptable range of the target.

5.3.3 Training characterisation responses

There was an exercise-induced reduction in P_{iso} in response to the characterisation session pre- and post-training (main effect, both $P < 0.01$), which was not different between groups (interaction effect, both $P > 0.05$). However, there was a 'medium' difference in the mean reduction in response to the pre-training characterisation session (ES = −0.66), suggesting that neuromuscular fatigue was greater following intermittent exercise. Training attenuated exercise-induced fatigue (main effect, $P < 0.01$). Although not reaching statistical significance, this attenuation tended to be greater following intermittent exercise training ($P = 0.07$), which was supported by a 'large' difference in this effect between interventions (ES = 0.92; Figure 5.2).

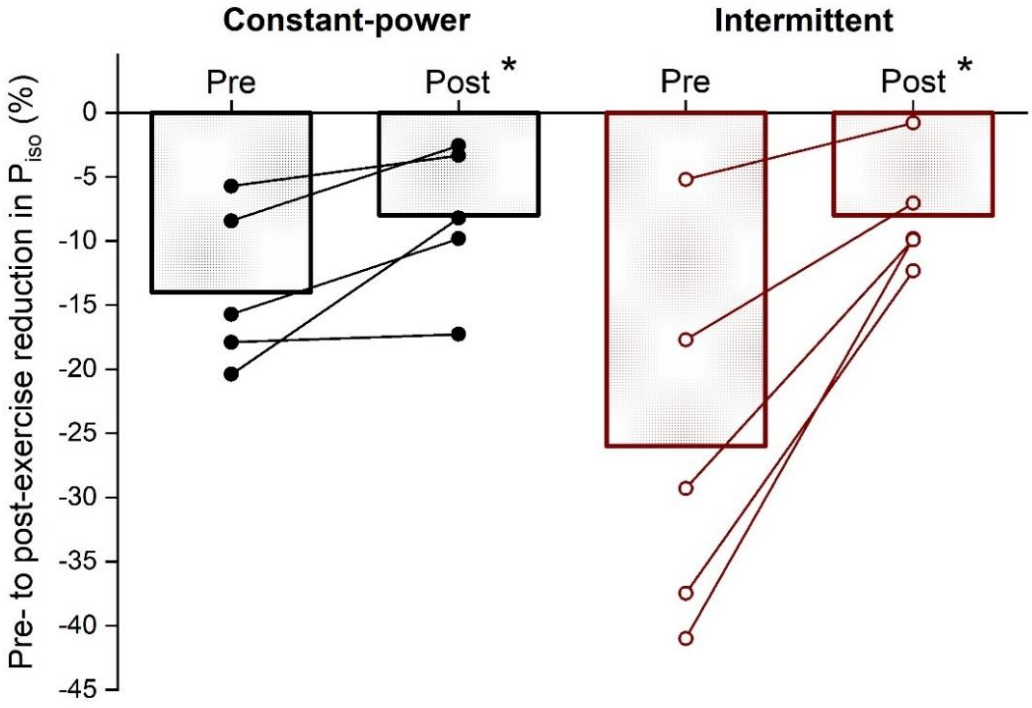

Figure 5.2: Exercise-induced reduction in peak isokinetic power (P_{iso}) following intensity-matched constant-power and intermittent exercise, before and after 6 weeks of the respective training. Symbols represent individual data and boxes represent mean responses. * $P < 0.05$ (main effect of training).

Pre-training, there was a decrease in muscle oxygenation between unloaded cycling and Minute 5 of the characterisation session for constant-power and intermittent exercise ($P < 0.01$), with no further change between time points (all $P > 0.05$). Changes in muscle oxygenation during the characterisation session were not different between constant-power and intermittent exercise prior to training (interaction effect, $P = 0.59$). Post-training, muscle oxygenation also decreased between unloaded cycling and Minute 5 of exercise ($P = 0.01$) before reaching a plateau ($P > 0.05$ between all further time points). There was no difference in the change in muscle oxygenation over time between constant-power and intermittent exercise during the post-training characterisation session ($P = 0.69$; Figure 5.3). As a result of there being no difference between time points from Minute 5 to the final 5 minutes of exercise, pre- to post-training differences were analysed using a mean of this period. Training did not change the magnitude of reduction in muscle oxygenation during the characterisation session (main effect, $P = 0.11$; ES = −0.33; Figure 5.3).

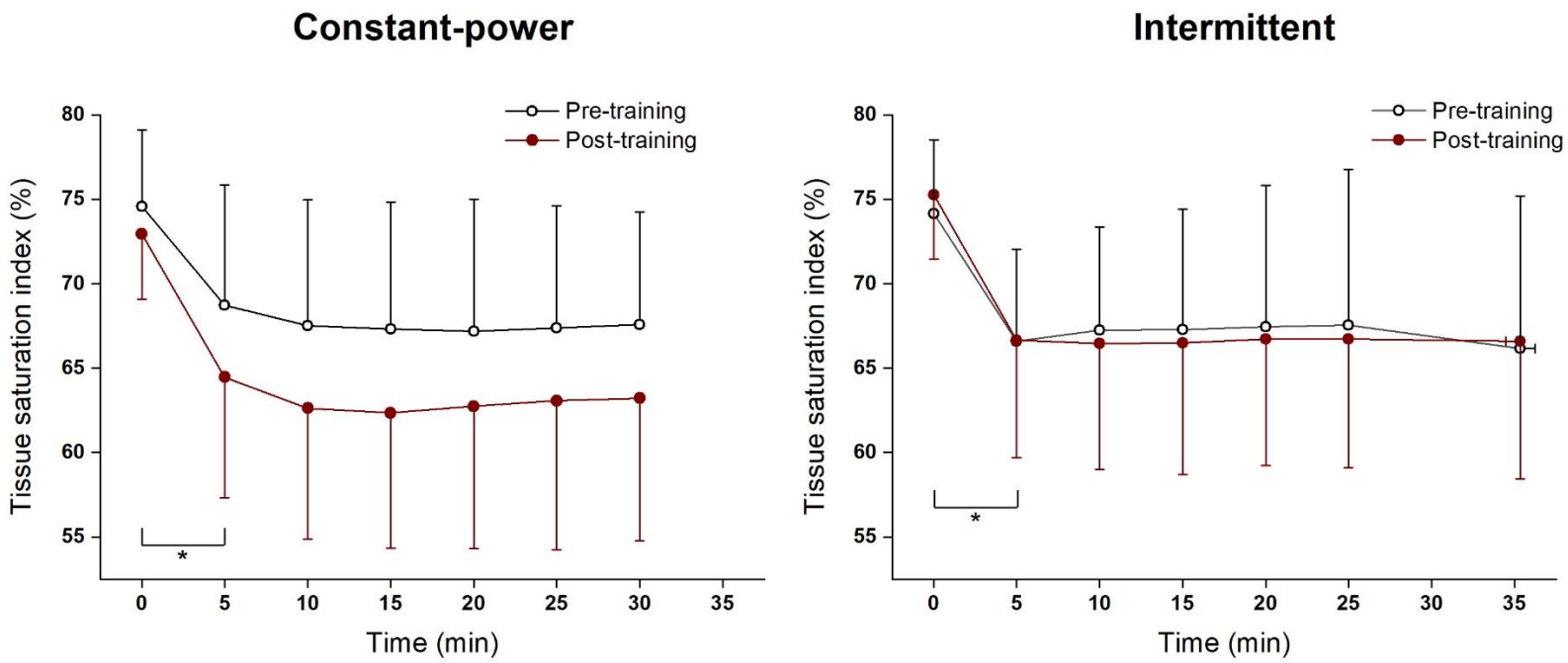

Figure 5.3: Changes in muscle oxygenation, as assessed via tissue saturation index measured using near-infrared spectroscopy, during intensity-matched constant-power and intermittent exercise before and after 6 weeks of the respective training. Values are mean ± SD. * *P* < 0.05 (main effect of time but no difference between groups).

During pre- and post-training characterisation sessions, perceived dyspnoea and leg tiredness were time-dependent (main effect, $P < 0.01$), increasing progressively between Minute 5, Minute 15, and the final minute of exercise (all $P < 0.05$), except for no change in leg tiredness between Minute 15 and the final minute of exercise post-training (although this approached significance; $P = 0.06$). There were also no differences between constant-power and intermittent characterisation sessions, pre- or post-training, in ratings of perceived dyspnoea or leg tiredness at any time point (all $P > 0.05$).

Pre- and post-training ratings of dyspnoea and leg tiredness during constant-power and intermittent exercise are presented in Figure 5.4. Ratings of dyspnoea in the final minute of exercise were lower during the post- than the pre-training characterisation session ($P = 0.03$), with a trend towards a greater reduction in response to intermittent training ($P = 0.056$; ES = −1.36). There were no other differences in ratings of perceived dyspnoea pre- to post-training at any other time point (all $P > 0.05$). Compared to pre-training, perceived leg tiredness was not reduced post-training at minutes 5 or 10 (both $P > 0.05$) but was reduced at every subsequent time point (all $P < 0.05$). The difference in attenuation of leg tiredness was not statistically significant between interventions at any time point (all $P > 0.05$), but the additional reduction in response to intermittent compared to constant-power exercise training was quantified as ‘large’ from Minute 15 onwards (ES ≥ 0.80 at every time point).

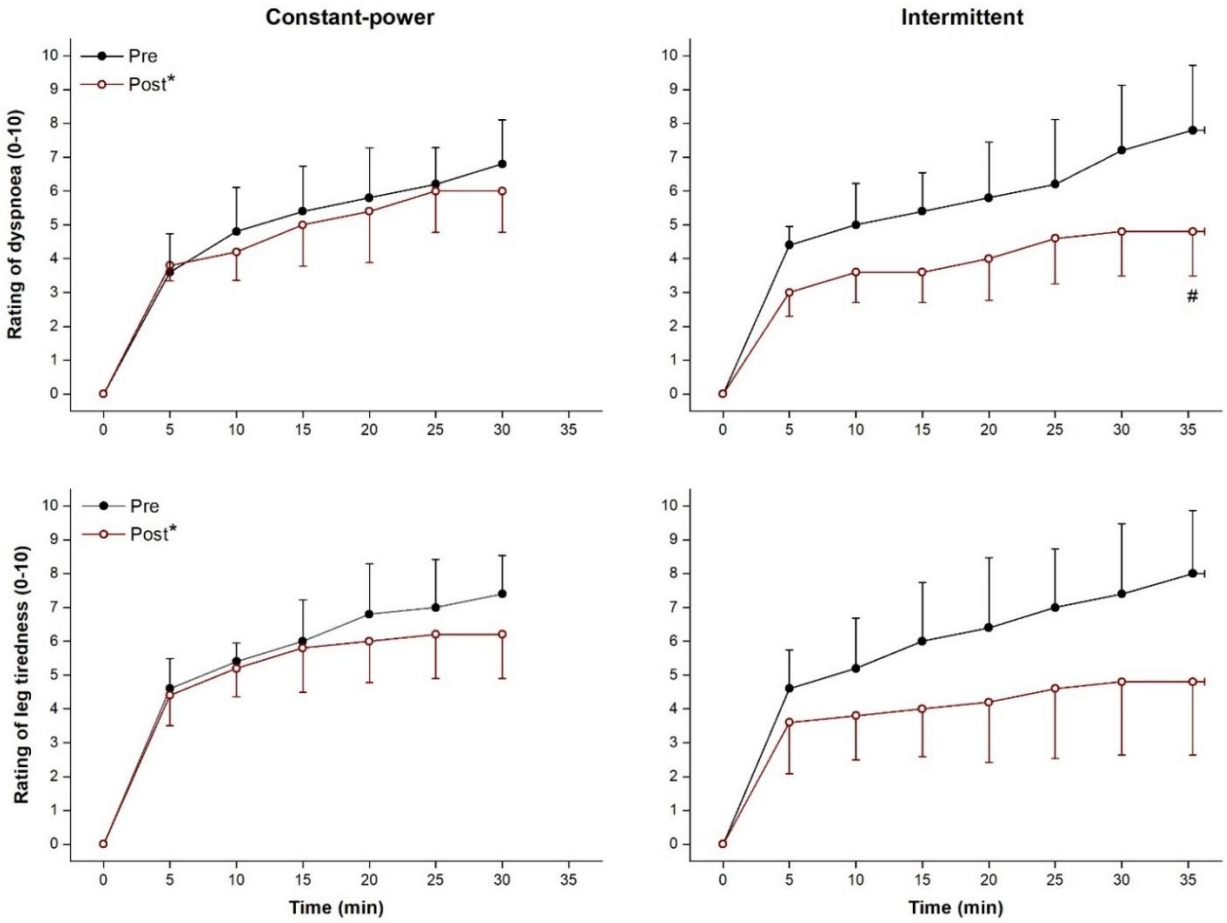

Figure 5.4: Ratings of dyspnoea and leg tiredness during intensity-matched constant-power and intermittent exercise, before and after 6 weeks of respective training. Values are mean ± SD. * *P* < 0.05 (main effect of training). # *P* = 0.056 (interaction effect between training interventions in the pre- to post-training rating of dyspnoea during the final minute of exercise).

5.3.4 Effects of training on parameters of exercise performance

Pre- and post-training values for all measures are presented in Table 5.2. RIT_{peak} increased in response to training (constant power: 5.0 ± 6.4 %, intermittent: 10 ± 2.1 %; main effect of training, $P < 0.01$). However, the change in peak power output was not different between interventions ($P = 0.26$; ES = 0.21). There was no power reserve at the limit of ramp-incremental exercise pre- (constant-power: 13 ± 56 W; intermittent: 19 ± 25 W; both $P > 0.05$) or post-training (constant-power: 11 ± 49 W; intermittent: 0 ± 48 W; both $P > 0.05$). These data indicate that ramp-incremental exercise was predominantly limited by locomotor neuromuscular fatigue pre- and post-training, but that the development of this fatigue was attenuated post-training. Absolute and relative $\dot{V}O_{2peak}$ increased in response to training (main effect of training, both $P < 0.01$) with no difference between interventions (interaction effect, $P = 0.29$ and 0.28, respectively). There was an increase in LT in response to training (main effect of training, $P < 0.01$), however the increase was greater following the intermittent than the constant-power intervention (interaction effect, $P = 0.02$; ES = 0.68).

The Tlim of the exercise tolerance test increased in response to training (main effect of training, $P < 0.01$). The constant-power exercise intervention increased Tlim by 75 ± 52 % and the intermittent exercise intervention by 91 ± 54 %. This improvement in exercise performance was not statistically different between trials (interaction effect, $P = 0.63$). However, the additional increase in Tlim following intermittent compared to constant-power training was ‘large’ (ES = 2.16).

Constant-power and intermittent training increased absolute P_{iso} by 5 ± 2 % and 2 ± 4 %, respectively (main effect of training, $P < 0.01$), with no difference between groups (interaction effect, $P = 0.10$; ES = −0.12). Relative P_{iso} ($W \cdot kg^{-1}$) increased pre- to post-training by 5 ± 2 % and 3 ± 5 % following constant-power and intermittent training, respectively (main effect of training, $P < 0.01$), also with no difference between groups ($P = 0.28$; ES = −0.13).

Pre-training, P_{iso} at the limit of the exercise tolerance test exceeded task power (constant-power: 293 ± 73 W vs. 232 ± 70 W; intermittent: 250 ± 101 W vs. 199 ± 52 W; main effect, $P < 0.01$), so that a power reserve was statistically present in both training groups. However, group mean P_{iso} did not exceed task power by more than natural fluctuation (constant-power: 61 ± 34 W vs. 55 ± 18 W; intermittent: 51 ± 50 W vs. 54 ± 18 W; main effect, $P = 0.91$), with P_{iso} at intolerance exceeding the upper boundary of natural fluctuation in only 3 of the 10 participants. This indicates that the power reserve was statistically but not physiologically significant. There was no difference in the magnitude of this reserve between groups (interaction effect, $P = 0.72$).

Post-training, P_{iso} at the limit of the exercise tolerance test was also greater than task power (constant-power: 347 ± 93 W vs. 232 ± 70 W; intermittent: 274 ± 66 W vs. 199 ± 52 W; main effect, $P < 0.01$), with no difference between groups (interaction, $P = 0.41$). While the magnitude of the reserve was not statistically greater than natural fluctuation (constant-power: 116 ± 90 W vs. 49 ± 21 W; intermittent: 75 ± 56 W vs. 61 ± 30 W; main effect, $P = 0.12$), the power reserve was greater than natural fluctuation in 7 of the 10 participants. Together, this data would suggest that the reserve was physiologically significant. In support, despite the power reserve not increasing pre- to post-

exercise statistically (main effect, P = 0.23), there was a ‘large’ effect of training (ES = 0.95) that suggests power reserve was greater post-training compared to pre-training. There was also a ‘medium’ effect indicating that this increase in power reserve was greater following constant-power than intermittent exercise training (ES = −0.76).

Table 5.2: Parameters of exercise performance pre- and post-6 weeks of training

	Constant-power		Intermittent	
	Pre-training	Post-training	Pre-training	Post-training
P_{iso} (W)	768 ± 237	810 ± 236	660 ± 199	674 ± 205
P_{iso} (W·kg^{-1})	9.9 ± 2.1	10.2 ± 2.1	11.6 ± 2.7	12.2 ± 2.7
RIT_{peak} (W)	252 ± 52	265 ± 61	252 ± 60	277 ± 68
$\dot{V}O_{2peak}$ (L·min^{-1})	2.92 ± 0.73	3.11 ± 0.71	2.95 ± 0.81	3.26 ± 0.84
$\dot{V}O_{2peak}$ (L·min^{-1}·kg^{-1})	47.6 ± 8.5	50.7 ± 7.8	44.1 ± 9.9	49.1 ± 9.7
LT (L·min^{-1})	1.54 ± 0.26	1.59 ± 0.29	1.56 ± 0.33	1.84 ± 0.28*
Tlim (s)	308 ± 23	533 ± 139	307 ± 22	581 ± 151

P_{iso}, peak isokinetic power; RIT_{peak}, peak ramp-incremental power; LT, lactate threshold; Tlim, tolerable duration. There was a main effect of training for all values (P < 0.05). * denotes an interaction effect, P < 0.05, indicating a greater increase following intermittent compared to constant-power exercise training.

5.4 Discussion

The primary aim of the present study was to investigate whether intermittent exercise is a superior training intervention to intensity-matched constant-power exercise due to the pulsatile rate of ATP demand, which influences the bioenergetics and allows greater power outputs to be performed without

increasing the metabolic stress. The suspension of research within the University due to COVID-19 prevented the necessary sample size being achieved. Therefore, there is a risk of type II error and these preliminary results must be interpreted with a degree of caution.

While aware of the limitations of the sample size, the present study suggests that intensity-matched intermittent exercise training does not confer additional increases in peak power generating capacity or $\dot{V}O_{2peak}$ but may induce additional increases in fatigue resistance and exercise tolerance. A potential reason for these superior improvements in exercise performance is a greater increase in LT in response to intermittent exercise training compared to intensity-matched constant-power exercise training.

5.4.1 Training stimulus

Consistent with data presented in Chapter 4 of this thesis, the constant-power and intermittent exercise protocols used in this study were matched for relative total work done and intensity, as confirmed by no difference between protocols in $\dot{V}O_2$, heart rate, muscle oxygenation, neuromuscular fatigue, or perceptual measures. However, the pattern of work was varied such that the power output performed during the work phases of the intermittent protocol was greater than during the constant-power protocol, secondary to an increased contribution to energy provision from PCr during the intermittent protocol. Additionally, the intermittent nature of the protocol provided large, rapid changes in the rate of ATP demand that were absent during the constant-power protocol. Therefore, differences between adaptations to constant-power and intermittent exercise training found in the present study are

independent of volume of work and intensity and relate to inherent differences in the stimuli provided by constant-power and intermittent exercise.

5.4.2 Changes in parameters of exercise performance

5.4.2.1 $\dot{V}O_{2peak}$

The 5-10 % increase in $\dot{V}O_{2peak}$ in response to constant-power and intermittent exercise training was comparable with previously reported values using a variety of interventions (e.g. (Burgomaster *et al.*, 2008; Tjønna *et al.*, 2013; Cochran *et al.*, 2014; Astorino *et al.*, 2017; Belfry, Paterson and Thomas, 2020) and demonstrates that the exercise training protocols prescribed were effective strategies for increasing $\dot{V}O_{2peak}$. Moreover, it was found that the constant-power and intermittent training protocols were equally effective at increasing $\dot{V}O_{2peak}$, suggesting that the physiological characteristics associated with intermittent exercise provide no additional stimulus for improvements in this parameter. This finding is consistent with multiple previous comparisons between intermittent and constant-power exercise training, which found no difference between protocols in the increase in $\dot{V}O_{2peak}$ (Burgomaster *et al.*, 2008; Cocks *et al.*, 2013; Tjønna *et al.*, 2013; Belfry, Paterson and Thomas, 2020). As further support, maximal continuous (power output decreased over time but there were no recovery phases) exercise lasting less than 10 minutes, performed 3 times per week for 6 weeks, increased $\dot{V}O_{2peak}$ by 6 % (Cochran *et al.*, 2014), indicating that an intermittent exercise profile is not required to evoke significant increases in $\dot{V}O_{2peak}$.

An almost identical intermittent exercise protocol to that used in the present study (work:recovery durations of 15:15 seconds), only running not cycling, has been found to increase $\dot{V}O_{2peak}$ more than moderate-intensity constant-speed exercise (Helgerud *et al.*, 2007). However, this increase in $\dot{V}O_{2peak}$ was not different to another intermittent protocol used in the same study, which was performed at equivalent work and recovery heart rates but with fewer work-recovery transitions (4 bouts of 4 minutes). Therefore, it is likely that the greater improvement in $\dot{V}O_{2peak}$ in response to intermittent compared to constant-speed exercise training was due to the higher exercise intensity, rather than the intermittent profile in and of itself. Indeed, several previous findings of superior adaptations following intermittent compared to constant-power exercise training can be at least partly explained by the higher intensity of the intermittent protocol (Rognmo *et al.*, 2004; Helgerud *et al.*, 2007; Wisløff *et al.*, 2007; Wang *et al.*, 2017; Donelli da Silveira *et al.*, 2020; MacInnis *et al.*, 2017); a difference that may arise due to intensity being quantified as the mean rather than the peak metabolic stress (MacInnis and Gibala, 2017).

The findings from the present study suggest that intermittent exercise training confers no additional increase in $\dot{V}O_{2peak}$ compared to intensity-matched constant-power exercise training and therefore that increases in $\dot{V}O_{2peak}$ in response to exercise training are determined primarily by exercise intensity and/or other factors such as volume of work (Gormley *et al.*, 2008; Huang *et al.*, 2016; Montero and Lundby, 2017).

5.4.2.2 Locomotor neuromuscular fatigue resistance and exercise tolerance

Although not statistically significant, there were ‘large’ effect sizes towards greater increases in muscle fatigue resistance and exercise tolerance following intermittent compared to constant-power exercise training. This greater training effect following intermittent exercise cannot be due to changes in $\dot{V}O_{2peak}$, which was not different between groups (as discussed above), and therefore other mechanisms must underlie this response.

One factor that may provide insight into this superior performance enhancement is LT, a fundamental parameter of exercise performance (Coyle *et al.*, 1988; Coyle *et al.*, 1991; Sjödin and Svedenhag, 1985; Jones and Carter, 2000), as a greater increase in LT was found following intermittent than constant-power training. LT is typically considered a key parameter of endurance exercise performance rather than high-intensity exercise performance, with the latter performed at power outputs much greater than that associated with LT. However, an increase in LT represents a reduced rate of blood lactate accumulation at a given power output and an increased contribution to ATP turnover from oxidative phosphorylation per unit time (Jones and Carter, 2000). At power outputs above CP, such an adaptation would result in a reduced rate of depletion of the finite anaerobic energy stores. Therefore, the increase in LT may contribute to the increase in exercise tolerance reported in this study, despite the relatively high task power and short duration of the protocol. An increase in LT may occur either through a reduced rate of lactate production at a given power output (Favier *et al.*, 1986; MacRae *et al.*, 1992), which may have been achieved via intermittent

exercise promoting greater increases in mitochondrial biogenesis (Combes *et al.*, 2015), or a greater rate of lactate clearance (Donovan and Brooks, 1983; MacRae *et al.*, 1992).

Monocarboxylate transporters (MCT) 1 and 4 are key regulators of lactate and H^+ translocation from the muscle to the blood and an elevated density of these membrane transporter proteins is associated with an increased lactate and H^+ release from muscle during exercise (Bonen *et al.*, 1998; Dubouchaud *et al.*, 2000; Juel, 2001). Exercise training can increase MCT1 and MCT 4 expression, but the mechanisms of regulation have not been established and may depend on the specific exercise demands (Bonen *et al.*, 1998; Pilegaard *et al.*, 1999; Juel, 2006). The content of MCT1 and MCT4 in human skeletal muscle has been shown to increase in response to six weeks of maximal intermittent exercise (Burgomaster *et al.*, 2007) but not continuous exercise (Cochran *et al.*, 2014), suggesting that an intermittent pattern of work is an important component of this adaptive stimulus. A larger increase in MCT1 and MCT4 expression following intermittent training in the present study would reduce lactate accumulation during exercise and contribute to the greater increase in LT. Moreover, an elevated density of these membrane proteins would upregulate removal of H^+, which is implicated in skeletal muscle fatigue (Allen, Lamb and Westerblad, 2008), thereby enhancing muscle fatigue resistance.

Another characteristic that may contribute to the superior training effect of intermittent exercise is the greater mechanical stimulus applied to the locomotor muscles. Passive limb ‘exercise’, which elevates blood flow and causes mechanical stretch of the tissue, promotes endothelial cell proliferation

and initiates capillarisation in skeletal muscle (Hellsten *et al.*, 2008; Høier *et al.*, 2010). An intermittent pattern of work may affect blood flow via changes in the muscle pump and/or cause greater tissue deformation due to the higher power generation during the work phases, thereby promoting greater vascular adaptations than constant-power exercise.

The findings from the present study suggest that the physiological characteristics associated with intermittent exercise are important stimuli for enhancing exercise tolerance and muscle fatiguability, secondary to an increase in LT. While it is possible to speculate on the mechanisms responsible, more research is required to elucidate the regulatory processes underlying this response.

5.4.2.3 Peak power output

It was proposed that the higher neural drive (reported in Chapter 4) and likely greater recruitment of high-threshold motor units during intermittent exercise would drive larger increases in maximal power-generating capacity. However, contrary to the hypothesis, the increase in P_{iso} following intermittent exercise training (2 %) was not different to that following constant-power exercise training (5 %). Indeed, despite the relatively low power demands, the individual increases in P_{iso} following constant-power exercise training were modest but consistent. Although the intermittent protocol maximised the dissociation between power output and intensity, the power outputs were likely still not high enough relative to peak power output (30 % of P_{iso}) to augment increases in maximal power-generating capacity. In fact, the greater recruitment of high-order motor units required to meet the higher power

demands may have caused the active fibres to shift towards a less glycolytic, more oxidative (and therefore fatigue-resistant) phenotype, which has been reported to occur in response to high-intensity cycling training (Simoneau *et al.*, 1985).

In addition to MCT density, intermittent exercise training has been found to increase maximal activity of citrate synthase (a marker of mitochondrial biogenesis) and protein content of GLUT4 (a glucose transporter; Burgomaster *et al.*, 2007); changes which are absent in response to continuous exercise (Cochran *et al.*, 2014). Expression of MCT1 and GLUT4 and activity of citrate synthase are all associated with the proportion of type I muscle fibres (Jansson and Sylvén, 1985; Daugaard *et al.*, 2000; Juel, 2001), as is LT (Ivy *et al.*, 1980; Sjödin and Jacobs, 1981; Coyle *et al.*, 1991), which increased more in the present study in response to intermittent compared to constant-power exercise training. Such a phenotypic transformation in the high-threshold motor units recruited during intermittent exercise would increase oxidative capacity of the active fibres, but at the cost of blunting the increase in peak power generation.

Endurance exercise training has been reported to stimulate morphological changes, such as hypertrophy (Harber *et al.*, 2009; Harber *et al.*, 2012; Konopka and Harber, 2014), but this is not a universal finding (Farup *et al.*, 2012) and may not occur within the relatively short training period used in the present study. A second and more likely reason for the increase in peak power output following constant-power exercise is neural adaptation. Highly-trained cyclists exhibit different patterns of leg muscle recruitment to novices, likely as a consequence of a learning effect arising from the repetition of movement

(Chapman *et al.*, 2008). These neural adaptations may change synchronisation and coordination of muscle activation during cycling to optimise the pedal stroke (Chapman *et al.*, 2009), thereby increasing peak power. However, it was beyond the scope of the present study to assess such factors.

The findings presented suggest that the higher power outputs inherent to intermittent exercise do not augment the modest but robust increases in peak power output that occur following constant-power endurance training. Moreover, increased recruitment of high-threshold motor units during exercise training with relatively high work volumes may cause the active fibres to shift to a more oxidative phenotype. Therefore, the number of work phases and target power of intermittent exercise training must be carefully considered when concurrent increases in peak power output and endurance performance are desired.

5.4.3 Optimisation of intermittent exercise protocol

In the previous chapter of this thesis, an intermittent protocol with work and recovery durations of 10 seconds was used as the dissociation between power output and intensity is greatest when the work durations are short. It was found that the mean power output during the work phases of this intermittent protocol was 85 ± 6 % of the target. This was partly due to the mechanics of the ergometer, with ~3 seconds required to transition from unloaded to task power, and a reduction in power near the end of the phase, likely an anticipatory response by the participants to prevent an increase in cadence when the flywheel was unloaded for the recovery phase.

In response to this finding, which indicated that the demands of the task where below what was intended, the work and recovery durations of the intermittent protocol used in this study were increased to 15 seconds. It was reasoned that the delay in transitioning from 20 W to target power would remain constant as the same ergometer would be used and target power would be only negligibly reduced (< 2 %) by this change in work phase duration. Moreover, it was assumed that the time spent below task power at the end of the work phase would also be unchanged, as the power differential between the work and recovery phases would be equivalent, and therefore sudden changes in cadence between phases no more likely. Accordingly, it was hypothesised that the mean power output of the work phase more be a greater percentage of target power as these times spent below target power would be proportionately lower when the work phase is longer. However, mean power output during the work phase using the 15:15 second intermittent protocol was 87 ± 4 %, only a marginal increase compared to the 10:10 second protocol.

There were striking similarities between the power profiles of the protocols, with two pedal strokes at the start and end of the work phase below natural fluctuation regardless of the work phase duration. Consequently, the number of pedal strokes performed within natural fluctuation was greater during the 15:15 second protocol (85 ± 9 %) compared to the 10:10 second protocol (74 ± 14 %). However, this did not translate into a substantial increase in mean power output as a percentage of target. The exact reason for this is unclear but, while both protocols exhibit a precipitous drop in power output immediately prior to transitioning to the recovery phase, there is a gradual decline in power output during the second half of the work phase of the 15:15

second protocol (Figure 5.1) that is absent during the 10:10 second protocol (please see previous chapter). These results suggest that the stimulus provided by each protocol is comparable.

However, the inertia of the rotating flywheel contributes to power generation during the early part of the work phase, reducing the demands on the locomotor muscles. While the exact contribution of this inertial component was not quantified, it likely supported only the first few pedal strokes, after which the power would have to be generated entirely by the locomotor muscles. As such, the relative contribution to overall power output from inertia of the flywheel would be less during a longer work phase, suggesting that the 15:15 second protocol may provide a greater stimulus to the locomotor muscles than the 10:10 second protocol. Conversely, if an intermittent pattern of work is a fundamental stimulus for adaptation to exercise then the 10:10 second protocol may be superior to the 15:15 second protocol as more recovery to work transitions are performed in a given time period. Therefore, further research is required to determine the optimal intermittent protocol to maximise the stimulus provided by the task.

5.4.4 Conclusion

Increases in peak power production and $\dot{V}O_{2peak}$ are similar following intensity-matched constant-power and intermittent training. However, the increase in lactate threshold was greater in response to intermittent compared to intensity-matched constant-power exercise. Moreover, there were large effect sizes towards greater improvements in muscle fatigue resistance and exercise tolerance following the intermittent protocol, which may reach

statistical significance given appropriate statistical power. Therefore, the physiological characteristics associated with intermittent exercise may be an important adaptive stimulus, independent of exercise intensity or volume of work. Further research is required to corroborate these findings and elucidate the mechanisms underlying the superior improvements in exercise performance conferred by intermittent exercise.

6: General Discussion

The physiological mechanisms that limit exercise are dependent on the population and the task, but the relative contributions of the different mechanisms to a given task are not fully described. Additionally, knowledge of the factors determining exercise-induced adaptation remains incomplete. While it is well established that exercise intensity and volume of work influence the adaptive response to exercise, whether the transition between different power outputs, thereby altering the rate of ATP turnover and motor unit recruitment patterns, is an independent adaptive stimulus has received little attention (please see Section 1.7.1). Together, these gaps in understanding impede the ability to optimise training strategies so that increases in exercise tolerance may be maximised.

To investigate the physiological mechanisms of exercise limitation, a multi-disciplinary approach incorporating computational modelling, and measures of locomotor neuromuscular fatigue and inspiratory muscle fatigue was used. The studies presented in this thesis were conducted to elucidate the interaction between locomotor neuromuscular fatigue and inspiratory muscle fatigue, the influence of different work patterns on locomotor neuromuscular fatigue, and to determine whether changes in work patterns can augment the adaptive response to exercise, thereby engendering superior improvements in exercise tolerance. The key findings are summarised below, with considerations and future directions subsequently discussed.

6.1 Thesis summary

The first study presented in this thesis investigated whether there was an association between the severity of inspiratory muscle fatigue and the magnitude by which task power (the programmed power of the ergometer) could be exceeded at the limit of very heavy-intensity exercise (i.e. the 'power reserve'). Peak isokinetic power (P_{iso}) during a maximal voluntary effort was not different to task power at the limit of exercise with a tolerable duration (Tlim) of ~5 minutes ($Tlim_{SHORT}$), demonstrating that there was no power reserve. Conversely, P_{iso} at intolerance of exercise with a Tlim of ~10 minutes ($Tlim_{LONG}$) exceeded task power, showing that a power reserve was present. These findings were consistent with research showing that the magnitude of the power reserve is greater when Tlim is longer (Davies *et al*., 2021).

Inspiratory muscle fatigue was measured using changes in oesophageal and gastric twitch pressure in response to cervical magnetic stimulation ($Poes_{tw}$ and Pga_{tw}, respectively). Global inspiratory muscle fatigue was represented by the reduction in transdiaphragmatic twitch pressure (Pdi_{tw}), calculated as the difference between $Poes_{tw}$ and Pga_{tw}, and inspiratory rib cage muscle and diaphragm fatigue were inferred from $Poes_{tw}$ and Pga_{tw}, respectively. Preliminary data from our laboratory demonstrated that global inspiratory muscle fatigue was greater following $Tlim_{LONG}$ than $Tlim_{SHORT}$. The study presented in Chapter 3 extended this finding to show that the greater global inspiratory muscle fatigue in response to $Tlim_{LONG}$ compared to $Tlim_{SHORT}$ was predominantly the result of increased diaphragm fatigue, rather than rib cage muscle fatigue. However, inter-trial differences (the differences in the individual responses to $Tlim_{SHORT}$ and $Tlim_{LONG}$; Δ) in power reserve were not

associated with ΔPdi_{tw} or ΔPga_{tw}. Despite no difference between $Tlim_{SHORT}$ and $Tlim_{LONG}$ in exercise-induced reduction in $Poes_{tw}$, $\Delta Poes_{tw}$ was related to Δ power reserve. This specific combination of relationships between variables suggests that global inspiratory muscle and diaphragm fatigue are not underlying factors of the power reserve but, while speculative, that an increased severity of rib cage muscle fatigue may be associated with a larger power reserve. It is proposed that rib cage muscle fatigue may contribute to dyspnoea or other noxious sensations, which precipitate exercise intolerance prior to exercise becoming limited by locomotor neuromuscular fatigue.

The next study sought to determine whether the pattern of work demands during intensity-matched exercise influenced the severity of locomotor neuromuscular fatigue induced. Using a modified computational model of pulmonary and circulatory dynamics, power outputs were calculated for heavy-intensity constant-power and intermittent exercise protocols so that the steady-state $\dot{V}O_2$ during the constant-power protocol would equal the peak $\dot{V}O_2$ during the work phase of the intermittent protocol (i.e. the protocols were intensity-matched). As intermittent exercise dissociates the power output-intensity relationship of constant-power exercise, higher power outputs can be performed during intermittent than intensity-matched constant-power exercise. The extent of this dissociation increases as the duration of the work phase decreases. Therefore, to maximise the difference in power output between the two protocols, an intermittent protocol with work:recovery durations of 10 seconds was used. It was found that power output during the work phases of this intermittent protocol were ~75 % greater than during constant-power exercise and the modelled bioenergetics showed that the

cumulative energy provision from endogenous sources was ~20-fold greater during the intermittent than the constant-power protocol. However, systemic physiological responses ($\dot{V}O_2$, heart rate, and blood lactate concentration), sensations of dyspnoea and leg tiredness, and locomotor neuromuscular fatigue were not different between protocols.

The findings from this second study generated the research question for the third study: do the differences in power output and bioenergetics during intermittent exercise confer independent adaptive stimuli and therefore increase the adaptive response to exercise without increasing physiological or perceptual stress? The same computational model was used to match the intensities of constant-power and intermittent exercise. An intermittent protocol with work:recovery durations of 15 seconds was used instead of the 10:10-second protocol used in the second study to increase the time spent at the programmed power output of the work phase (please see Section 5.4.3). Training consisted of twice-weekly exercise sessions for six weeks, with participants randomised into either the constant-power training group or the intermittent training group. Due to restrictions on research relating to the COVID-19 pandemic, data collection was prematurely ended and only preliminary findings were obtained. The available data indicated that there was no difference between intensity-matched constant-power and intermittent exercise training in the increase in $\dot{V}O_{2peak}$ or peak power production. However, the increase in lactate threshold was greater in response to the intermittent protocol and, while not statistically significant, large effect sizes suggested that the increase in exercise tolerance and locomotor neuromuscular fatigue resistance were also greater following intermittent training. These findings suggest that an intermittent pattern of work is an

independent adaptive stimulus during exercise, potentially promoting greater aerobic adaptations specific to the locomotor muscles.

6.2 Study considerations

It is important to note that the findings of the studies presented in this thesis may be specific to the population investigated. All studies used young, healthy participants with $\dot{V}O_{2peak}$ values typically between 40 and 60 mL·min·kg^{-1}. The mechanisms of intolerance during specific tasks have been shown to vary between different populations, such as the presence of a power reserve at intolerance of ramp-incremental exercise in COPD (Cannon *et al.*, 2016) that is absent in endurance-trained individuals (Ferguson *et al.*, 2016b). Therefore, the acute and chronic responses to the specific exercise protocols used may only apply to young, healthy individuals who are recreationally active. Moreover, it should be noted that the findings and interpretations represent the typical responses within the population, and that individual heterogeneity in the exercise responses was present even within this relatively homogenous population. For example, some individuals exhibited a power reserve at the limit of short-duration maximal exercise whereas others did not at the limit of long-duration maximal exercise, in contrast to the expected response, despite no clear differences in characteristics between these individuals.

A key methodological consideration is the isokinetic technique used to assess locomotor neuromuscular fatigue. While this technique has numerous advantages over stimulation techniques, such as the task-specificity and minimal delay between exercise cessation and measurement acquirement – which minimises the influence of recovery on the measurement – the cardinal

limitation with this technique is its dependence on volitional effort. It was beyond experimental control to ensure or verify that a maximal effort was provided during the manoeuvre and, consequently, the findings reported have the potential to be affected by submaximal efforts.

However, all reasonable measures were taken to evoke a maximal effort from the participant in each instance, including impressing upon the participant the importance of giving a maximal effort, familiarisation with the technique, and strong vocal encouragement from the experimenters. Maximal voluntary power output is also highly reproducible in an unfatigued state, as evidenced by the low coefficient of variation for baseline P_{iso} reported in this thesis (< 3 %), supporting the notion that individual-specific maximal efforts can be reliably replicated. As further support for the validity of volitional measures, individuals with a power reserve at the limit of ramp-incremental exercise exhibit greater peripheral muscle fatigue, as assessed via electrical stimulation, than those without a power reserve (Hodgson et al., 2018).

It should also be noted that all investigations of the mechanisms of exercise limitation rely on participants providing a maximal effort during an exercise task to intolerance. Therefore, the assumption that participants have provided a true maximal effort is inherent to all such studies. Ultimately, exercise is volitional and therefore volitional measures, either in isolation or supported by non-volitional measures, provide valuable insights into the mechanisms of limitation. For example, by measuring P_{iso} immediately after exercise cessation, it is possible to determine if there is a reserve in locomotor muscle power at intolerance, which indicates whether the task is predominantly limited by the locomotor muscles or other physiological mechanisms.

A final consideration of the techniques used in this thesis is what constitutes a ‘power reserve’. In this regard, it is imperative to account for the differential in pedal stroke power output during the task, termed natural fluctuation (see Figure 4.1). In this thesis, a power reserve was only considered to be present when P_{iso} was statistically greater than the target power *and* exceeded the upper boundary of natural fluctuation. This second criterion was included because mean pedal stroke power, not peak pedal stroke power, must equal or exceed the target power for the task to remain sustainable. As such, P_{iso} exceeding target power does not necessarily indicate that the locomotor muscles were capable of sustaining target power at intolerance. However, if P_{iso} exceeds the upper boundary of natural fluctuation, it is likely that the locomotor muscles could generate a mean power output equal to or greater than target power.

When determining whether a power reserve is present at intolerance, using statistical testing alone may leave data interpretation vulnerable to type 1 error because natural fluctuation is not included in these calculations. For example, there was no statistical difference between P_{iso} at intolerance and target power of $Tlim_{SHORT}$ in Chapter 3; however, P_{iso} at intolerance was significantly greater than target power of an identical exercise protocol in Chapter 5. If the presence of a reserve was dependent on statistical differences alone, these data would provide confounding outcomes. However, as P_{iso} at intolerance did not exceed the upper boundary of natural fluctuation in pedal stroke power in either protocol, the interpretation presented in this thesis was that a reserve was not present in either case.

6.3 Future directions

Based on the findings presented in this thesis, the following investigations are proposed for future research.

6.3.1 The interaction between inspiratory rib cage muscle fatigue and locomotor neuromuscular fatigue

The findings presented in Chapter 3 suggest that a larger power reserve is associated with an increased severity of rib cage muscle fatigue. Speculatively, this relationship may be due to an increased contribution from the rib cage muscles to the global noxious sensations, which would cause a sensory tolerance limit to engender intolerance prior to locomotor neuromuscular fatigue limiting the task (Hureau, Romer and Amann, 2018; Thomas, Goodall and Howatson, 2018). However, this postulation is based solely on correlational evidence. Future research should focus on implementing interventions to establish whether this relationship denotes cause and effect.

One intervention that could be used to elucidate whether rib cage muscle fatigue influences the magnitude of the power reserve at intolerance is to unload the inspiratory muscles during exercise. This can be achieved experimentally via inspiring a gas less dense than air (typically an oxygen and helium mixture known as Heliox) or proportional-assist ventilation, which is a ventilatory assistance mechanism that generates a synchronised pressure in proportion to the physiological pressure generated by the individual (Poon and Ward, 1986; Younes *et al.*, 1987). Inspiratory muscle unloading has been shown to prevent diaphragm fatigue (Babcock *et al.*, 2002), increase leg blood

flow (Babcock *et al.*, 2002), and consequently enhance exercise tolerance to specific tasks (Powers *et al.*, 1986; Harms *et al.*, 2000). In addition, the reduction in inspiratory muscle work and consequent reduction in diaphragm fatigue likely also attenuates the progressive recruitment of the inspiratory rib cage muscles during exercise (Johnson *et al.*, 1993; Babcock *et al*., 1996; Aliverti *et al.*, 1997). However, it remains unknown whether a power reserve at intolerance of a task under control conditions can be eliminated by unloading the inspiratory muscles, indicating a cause and effect relationship between inspiratory muscle work and the magnitude of the power reserve. A limitation with using inspiratory muscle unloading for this purpose is that the technique has multifarious physiological implications, rather than selectively targeting rib cage muscle function. Therefore, if inspiratory muscle unloading does reduce the power reserve, the effect could not be attributed solely to attenuation of rib cage muscle fatigue.

An alternative, and essentially opposite, approach to inspiratory muscle unloading is to fatigue the inspiratory muscles prior to exercise. Importantly, the diaphragm and rib cage muscles can be selectively fatigued via voluntary uncoupling of the other (Fitting *et al.*, 1988; Similowski *et al.*, 1998), allowing the effects of rib cage muscle fatigue to be independently investigated and compared to the effects of diaphragm fatigue. Based on the results from Chapter 3, it would be expected that, while both rib cage muscle and diaphragm fatigue induced prior to the task would reduce Tlim, the magnitude of the power reserve would be increased in response to prior rib cage muscle fatigue but unchanged in response to prior diaphragm fatigue.

6.3.2 The adaptive response to intermittent compared to constant-power exercise training

The work presented in Chapter 5 demonstrated that intermittent exercise training increased LT more than intensity-matched constant-power training and, while not statistically significant, there were large effect sizes towards greater muscle fatigue resistance and increases in exercise tolerance. As reported earlier, research restrictions prevented the intended sample size being achieved. Therefore, initial investigation should focus on confirming or refuting the preliminary findings from this study indicating that intermittent exercise confers superior training effects to intensity-matched constant-power exercise.

If these findings are corroborated, research should next turn to elucidating the mechanisms of action for these superior training effects. As discussed in Chapter 5, factors of interest include control of lactate production and clearance, particularly through monocarboxylate transporters (MCT) 1 and 4, the density of which have been reported to increase in response to intermittent (Burgomaster *et al.*, 2007) but not constant-power training (Cochran *et al.*, 2014). However, other factors that were not apparent from the techniques used in this thesis (particularly those that can be assessed using muscle biopsy analysis) may also be contributory to the augmented training effect and merit investigation.

A final future direction proposed here is the application of intermittent exercise training with short work and recovery durations to different populations, particularly older individuals. Ageing is associated with sarcopenia, a syndrome characterised by the progressive loss of muscle mass and in

particular atrophy of type II muscle fibres, which is associated with functional decline, such as a diminished ability to generate power, and compromised quality of life (Deschenes, 2004; Santilli *et al.*, 2014; Wilkinson, Piasecki and Atherton, 2018; Larsson *et al.*, 2019). Therefore, it is paramount that older individuals engage in strength training to promote neural and morphological adaptations that enhance power-generating capacity (Lopez *et al.*, 2018; Fragala *et al.*, 2019). While the findings in Chapter 5 indicate that the higher power output during intermittent exercise does not augment the increase in peak power output compared to constant-power exercise training, the difference in muscle fibre characteristics between younger and older populations may cause divergent acute and chronic responses to the exercise stimulus.

An investigation into the acute and chronic responses to intensity-matched constant-power and intermittent exercise in a cohort aged over 55 years was intended to be performed for inclusion in this thesis, but only a single participant was able to complete the acute exercise bouts before restrictions to research curtailed data collection. Therefore, applying this intermittent exercise paradigm to older populations remains unexplored and represents a pertinent future direction based on the findings presented in this thesis.

6.3.3 Can the specific limitation to exercise be identified and targeted to enhance exercise tolerance?

The overarching aim of the studies presented in this thesis was to further understand the physiological mechanisms that limit exercise and, ultimately, develop interventions to overcome these limitations and enhance exercise tolerance. Exercise limitation is a multifaceted phenomenon and the relative

contribution of each mechanism to intolerance is specific to the task and population. The ability to determine and then target the predominant limitation with a tailored training programme has the potential to augment increases in exercise tolerance compared to a more generalised approach. For example, improvements in exercise tolerance in response to respiratory muscle training are greater for longer tasks (Bailey *et al.*, 2010; Illi *et al.*, 2012), consistent with the postulate that the relative contribution to exercise limitation from the respiratory system increases as exercise duration increases. Conversely, intermittent protocols, which dissociate the intensity-power output relationship of constant-power exercise and thereby allow higher power outputs to be achieved, may specifically target adaptations in the locomotor muscles. Consequently, intermittent exercise may be a particularly effective training intervention to improve exercise tolerance of short-duration tasks (as indicated by the findings of Chapter 5), which are predominantly limited by locomotor neuromuscular fatigue (Davies *et al*., 2021).

It is hoped that the studies presented in this thesis will aid prescription of training interventions to effectively target the predominant exercise limitation (Figure 6.1). If successful, this individualised approach would not only augment improvements in exercise tolerance but concomitantly maximise improvements in associated outcomes, such as quality of life and risk of morbidity and mortality, and therefore have profound implications for health and wellbeing.

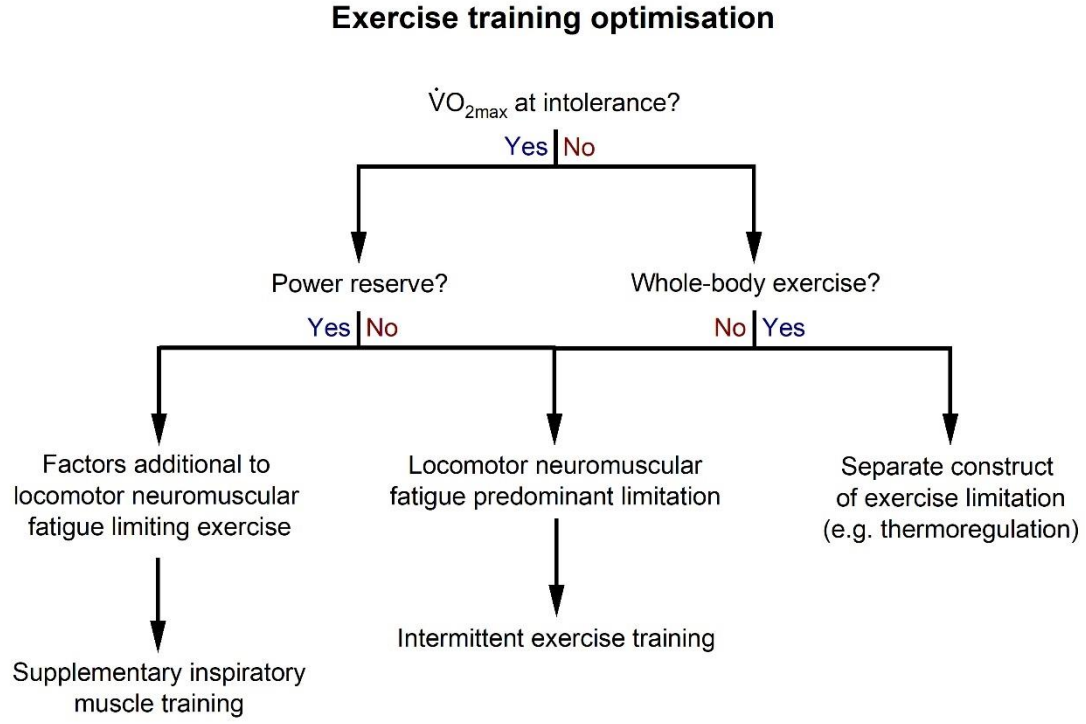

Figure 6.1: Flow chart to show how the predominant mechanism of exercise limitation may be identified and then targeted via appropriate prescription of training interventions. It is important to note that these interventions are suggested to support, not replace, other forms of training. This targeted approach is proposed to increase the efficacy of training so that the greatest improvement in exercise tolerance may be achieved.

References

Aagaard, P., Suetta, C., Caserotti, P., Magnusson, S. P. and Kjaer, M. (2010) 'Role of the nervous system in sarcopenia and muscle atrophy with aging: strength training as a countermeasure', *Scand J Med Sci Sports,* 20(1), pp. 49-64.

Abbiss, C. R., Peiffer, J. J., Meeusen, R. and Skorski, S. (2015) 'Role of Ratings of Perceived Exertion during Self-Paced Exercise: What are We Actually Measuring?', *Sports Med,* 45(9), pp. 1235-1243.

Abe, T., DeHoyos, D. V., Pollock, M. L. and Garzarella, L. (2000) 'Time course for strength and muscle thickness changes following upper and lower body resistance training in men and women', *Eur J Appl Physiol,* 81(3), pp. 174-80.

Acevedo, E. O. and Goldfarb, A. H. (1989) 'Increased training intensity effects on plasma lactate, ventilatory threshold, and endurance', *Med Sci Sports Exerc,* 21(5), pp. 563-8.

Aliverti, A., Cala, S. J., Duranti, R., Ferrigno, G., Kenyon, C. M., Pedotti, A., Scano, G., Sliwinski, P., Macklem, P. T. and Yan, S. (1997) 'Human respiratory muscle actions and control during exercise', *J Appl Physiol (1985),* 83(4), pp. 1256-69.

Allen, D. and Westerblad, H. (2010) 'What limits exercise during high-intensity aerobic exercise?', *Eur J Appl Physiol,* 110(3), pp. 661-2; author reply 663-4.

Allen, D. G., Lamb, G. D. and Westerblad, H. (2008) 'Skeletal muscle fatigue: cellular mechanisms', *Physiol Rev,* 88(1), pp. 287-332.

Allen, P. S., Matheson, G. O., Zhu, G., Gheorgiu, D., Dunlop, R. S., Falconer, T., Stanley, C. and Hochachka, P. W. (1997) 'Simultaneous 31P MRS of the soleus and gastrocnemius in Sherpas during graded calf muscle exercise', *Am J Physiol,* 273(3 Pt 2), pp. R999-1007.

Amann, M. (2011) 'Central and peripheral fatigue: interaction during cycling exercise in humans', *Med Sci Sports Exerc,* 43(11), pp. 2039-45.

Amann, M., Blain, G. M., Proctor, L. T., Sebranek, J. J., Pegelow, D. F. and Dempsey, J. A. (2010) 'Group III and IV muscle afferents contribute to ventilatory and cardiovascular response to rhythmic exercise in humans', *J Appl Physiol (1985),* 109(4), pp. 966-76.

Amann, M., Blain, G. M., Proctor, L. T., Sebranek, J. J., Pegelow, D. F. and Dempsey, J. A. (2011) 'Implications of group III and IV muscle afferents for high-intensity endurance exercise performance in humans', *J Physiol,* 589(Pt 21), pp. 5299-309.

Amann, M. and Dempsey, J. A. (2008) 'Locomotor muscle fatigue modifies central motor drive in healthy humans and imposes a limitation to exercise performance', *J Physiol,* 586(1), pp. 161-73.

Amann, M., Eldridge, M. W., Lovering, A. T., Stickland, M. K., Pegelow, D. F. and Dempsey, J. A. (2006) 'Arterial oxygenation influences central motor output and exercise performance via effects on peripheral locomotor muscle fatigue in humans', *J Physiol,* 575(Pt 3), pp. 937-52.

Amann, M., Proctor, L. T., Sebranek, J. J., Eldridge, M. W., Pegelow, D. F. and Dempsey, J. A. (2008) 'Somatosensory feedback from the limbs exerts inhibitory influences on central neural drive during whole body endurance exercise', *J Appl Physiol (1985),* 105(6), pp. 1714-24.

Amann, M., Proctor, L. T., Sebranek, J. J., Pegelow, D. F. and Dempsey, J. A. (2009) 'Opioid-mediated muscle afferents inhibit central motor drive and limit peripheral muscle fatigue development in humans', *J Physiol,* 587(1), pp. 271-83.

Amann, M., Wan, H. Y., Thurston, T. S., Georgescu, V. P. and Weavil, J. C. (2020) 'On the Influence of Group III/IV Muscle Afferent Feedback on Endurance Exercise Performance', *Exerc Sport Sci Rev,* 48(4), pp. 209-216.

Andersen, P. (1975) 'Capillary density in skeletal muscle of man', *Acta Physiol Scand,* 95(2), pp. 203-5.

Andersen, P. and Henriksson, J. (1977) 'Capillary supply of the quadriceps femoris muscle of man: adaptive response to exercise', *J Physiol,* 270(3), pp. 677-90.

Astorino, T. A., Edmunds, R. M., Clark, A., King, L., Gallant, R. A., Namm, S., Fischer, A. and Wood, K. M. (2017) 'High-Intensity Interval Training Increases Cardiac Output and V˙O2max', *Med Sci Sports Exerc,* 49(2), pp. 265-273.

Astrand, I., Astrand, P. O., Christensen, E. H. and Hedman, R. (1960) 'Intermittent muscular work', *Acta Physiol Scand,* 48, pp. 448-53.

ATS/ACCP (2003) 'Use of exercise testing in the evaluation of interventional efficacy: an official ERS statement', *Am J Respir Crit Care Med,* 167(2), pp. 211-77.

Babcock, M. A., Pegelow, D. F., Harms, C. A. and Dempsey, J. A. (2002) 'Effects of respiratory muscle unloading on exercise-induced diaphragm fatigue', *J Appl Physiol (1985),* 93(1), pp. 201-6.

Babcock, M. A., Pegelow, D. F., Johnson, B. D. and Dempsey, J. A. (1996) 'Aerobic fitness effects on exercise-induced low-frequency diaphragm fatigue', *J Appl Physiol (1985),* 81(5), pp. 2156-64.

Babcock, M. A., Pegelow, D. F., McClaran, S. R., Suman, O. E. and Dempsey, J. A. (1995) 'Contribution of diaphragmatic power output to exercise-induced diaphragm fatigue', *J Appl Physiol (1985),* 78(5), pp. 1710-9.

Babcock, M. A., Pegelow, D. F., Taha, B. H. and Dempsey, J. A. (1998) 'High frequency diaphragmatic fatigue detected with paired stimuli in humans', *Med Sci Sports Exerc,* 30(4), pp. 506-11.

Baggish, A. L., Wang, F., Weiner, R. B., Elinoff, J. M., Tournoux, F., Boland, A., Picard, M. H., Hutter, A. M. and Wood, M. J. (2008) 'Training-specific changes in cardiac structure and function: a prospective and longitudinal assessment of competitive athletes', *J Appl Physiol (1985),* 104(4), pp. 1121-8.

Bailey, S. J., Romer, L. M., Kelly, J., Wilkerson, D. P., DiMenna, F. J. and Jones, A. M. (2010) 'Inspiratory muscle training enhances pulmonary O_2 uptake kinetics and high-intensity exercise tolerance in humans', *J Appl Physiol (1985),* 109(2), pp. 457-68.

Baker, J. S., McCormick, M. C. and Robergs, R. A. (2010) 'Interaction among Skeletal Muscle Metabolic Energy Systems during Intense Exercise', *J Nutr Metab,* 2010, pp. 905612.

Balaban, R. S., Mootha, V. K. and Arai, A. (1996) 'Spectroscopic determination of cytochrome c oxidase content in tissues containing myoglobin or hemoglobin', *Anal Biochem,* 237(2), pp. 274-8.

Barclay, C. J. (2017) 'Energy demand and supply in human skeletal muscle', *J Muscle Res Cell Motil,* 38(2), pp. 143-155.

Barstow, T. J. and Molé, P. A. (1991) 'Linear and nonlinear characteristics of oxygen uptake kinetics during heavy exercise', *J Appl Physiol (1985),* 71(6), pp. 2099-106.

Barwood, M. J., Weston, N. J., Thelwell, R. and Page, J. (2009) 'A motivational music and video intervention improves high-intensity exercise performance', *J Sports Sci Med,* 8(3), pp. 435-42.

Bassett, D. R. and Howley, E. T. (2000) 'Limiting factors for maximum oxygen uptake and determinants of endurance performance', *Med Sci Sports Exerc,* 32(1), pp. 70-84.

Bassey, E. J., Fiatarone, M. A., O'Neill, E. F., Kelly, M., Evans, W. J. and Lipsitz, L. A. (1992) 'Leg extensor power and functional performance in very old men and women', *Clin Sci (Lond),* 82(3), pp. 321-7.

Baydur, A., Behrakis, P. K., Zin, W. A., Jaeger, M. and Milic-Emili, J. (1982) 'A simple method for assessing the validity of the esophageal balloon technique', *Am Rev Respir Dis,* 126(5), pp. 788-91.

Beaver, W. L., Wasserman, K. and Whipp, B. J. (1986) 'A new method for detecting anaerobic threshold by gas exchange', *J Appl Physiol (1985),* 60(6), pp. 2020-7.

Beelen, A. and Sargeant, A. J. (1991) 'Effect of fatigue on maximal power output at different contraction velocities in humans', *J Appl Physiol (1985),* 71(6), pp. 2332-7.

Belfry, G. R., Paterson, D. H., Murias, J. M. and Thomas, S. G. (2012a) 'The effects of short recovery duration on VO2 and muscle deoxygenation during intermittent exercise', *Eur J Appl Physiol,* 112(5), pp. 1907-15.

Belfry, G. R., Paterson, D. H. and Thomas, S. G. (2020) 'High-Intensity 10-s Work: 5-s Recovery Intermittent Training Improves Anaerobic and Aerobic Performances', *Res Q Exerc Sport,* 91(4), pp. 640-651.

Belfry, G. R., Raymer, G. H., Marsh, G. D., Paterson, D. H., Thompson, R. T. and Thomas, S. G. (2012b) 'Muscle metabolic status and acid-base balance during 10-s work:5-s recovery intermittent and continuous exercise', *J Appl Physiol (1985),* 113(3), pp. 410-7.

Benson, A. P., Grassi, B. and Rossiter, H. B. (2013) 'A validated model of oxygen uptake and circulatory dynamic interactions at exercise onset in humans', *J Appl Physiol (1985),* 115(5), pp. 743-55.

Billat, L. V. (2001a) 'Interval training for performance: a scientific and empirical practice. Special recommendations for middle- and long-distance running. Part I: aerobic interval training', *Sports Med,* 31(1), pp. 13-31.

Billat, L. V. (2001b) 'Interval training for performance: a scientific and empirical practice. Special recommendations for middle- and long-distance running. Part II: anaerobic interval training', *Sports Med,* 31(2), pp. 75-90.

Billat, V. L., Demarle, A., Slawinski, J., Paiva, M. and Koralsztein, J. P. (2001) 'Physical and training characteristics of top-class marathon runners', *Med Sci Sports Exerc,* 33(12), pp. 2089-97.

Bishop, D. J., Botella, J. and Granata, C. (2019) 'CrossTalk opposing view: Exercise training volume is more important than training intensity to promote increases in mitochondrial content', *J Physiol,* 597(16), pp. 4115-4118.

Black, M. I., Jones, A. M., Blackwell, J. R., Bailey, S. J., Wylie, L. J., McDonagh, S. T., Thompson, C., Kelly, J., Sumners, P., Mileva, K. N., Bowtell, J. L. and Vanhatalo, A. (2017) 'Muscle metabolic and neuromuscular

determinants of fatigue during cycling in different exercise intensity domains', *J Appl Physiol (1985),* 122(3), pp. 446-459.

Blain, G. M., Mangum, T. S., Sidhu, S. K., Weavil, J. C., Hureau, T. J., Jessop, J. E., Bledsoe, A. D., Richardson, R. S. and Amann, M. (2016) 'Group III/IV muscle afferents limit the intramuscular metabolic perturbation during whole body exercise in humans', *J Physiol,* 594(18), pp. 5303-15.

Blair, S. N., Kohl, H. W., Barlow, C. E., Paffenbarger, R. S., Gibbons, L. W. and Macera, C. A. (1995) 'Changes in physical fitness and all-cause mortality. A prospective study of healthy and unhealthy men', *JAMA,* 273(14), pp. 1093-8.

Bonen, A., McCullagh, K. J., Putman, C. T., Hultman, E., Jones, N. L. and Heigenhauser, G. J. (1998) 'Short-term training increases human muscle MCT1 and femoral venous lactate in relation to muscle lactate', *Am J Physiol,* 274(1), pp. E102-7.

Bonne, T. C., Doucende, G., Flück, D., Jacobs, R. A., Nordsborg, N. B., Robach, P., Walther, G. and Lundby, C. (2014) 'Phlebotomy eliminates the maximal cardiac output response to six weeks of exercise training', *Am J Physiol Regul Integr Comp Physiol,* 306(10), pp. R752-60.

Borg, E., Borg, G., Larsson, K., Letzter, M. and Sundblad, B. M. (2010) 'An index for breathlessness and leg fatigue', *Scand J Med Sci Sports,* 20(4), pp. 644-50.

Borg, G. A. (1974) 'Perceived exertion', *Exerc Sport Sci Rev,* 2, pp. 131-53.

Borg, G. A. (1982) 'Psychophysical bases of perceived exertion', *Med Sci Sports Exerc,* 14(5), pp. 377-81.

Bottinelli, R., Canepari, M., Pellegrino, M. A. and Reggiani, C. (1996) 'Force-velocity properties of human skeletal muscle fibres: myosin heavy chain isoform and temperature dependence', *J Physiol,* 495 (Pt 2), pp. 573-86.

Bottinelli, R. and Reggiani, C. (2000) 'Human skeletal muscle fibres: molecular and functional diversity', *Prog Biophys Mol Biol,* 73(2-4), pp. 195-262.

Bottinelli, R., Schiaffino, S. and Reggiani, C. (1991) 'Force-velocity relations and myosin heavy chain isoform compositions of skinned fibres from rat skeletal muscle', *J Physiol,* 437, pp. 655-72.

Bouchard, C., Sarzynski, M. A., Rice, T. K., Kraus, W. E., Church, T. S., Sung, Y. J., Rao, D. C. and Rankinen, T. (2011) 'Genomic predictors of the maximal O_2 uptake response to standardized exercise training programs', *J Appl Physiol (1985),* 110(5), pp. 1160-70.

Bowen, T. S., Cannon, D. T., Begg, G., Baliga, V., Witte, K. K. and Rossiter, H. B. (2012) 'A novel cardiopulmonary exercise test protocol and criterion to determine maximal oxygen uptake in chronic heart failure', *J Appl Physiol (1985),* 113(3), pp. 451-8.

Bradley, T. D., Chartrand, D. A., Fitting, J. W., Killian, K. J. and Grassino, A. (1986) 'The relation of inspiratory effort sensation to fatiguing patterns of the diaphragm', *Am Rev Respir Dis,* 134(6), pp. 1119-24.

Breese, B. C., Barker, A. R., Armstrong, N., Fulford, J. and Williams, C. A. (2014) 'Influence of thigh activation on the VO_2 slow component in boys and men', *Eur J Appl Physiol,* 114(11), pp. 2309-19.

Brink-Elfegoun, T., Kaijser, L., Gustafsson, T. and Ekblom, B. (2007) 'Maximal oxygen uptake is not limited by a central nervous system governor', *J Appl Physiol (1985),* 102(2), pp. 781-6.

Briscoe, W. A. and Dubois, A. B. (1958) 'The relationship between airway resistance, airway conductance and lung volume in subjects of different age and body size', *J Clin Invest,* 37(9), pp. 1279-85.

Brooks, G. A. (2012) 'Bioenergetics of exercising humans', *Compr Physiol,* 2(1), pp. 537-62.

Brownstein, C. G., Millet, G. Y. and Thomas, K. (2021) 'Neuromuscular responses to fatiguing locomotor exercise', *Acta Physiol (Oxf),* 231(2), pp. e13533.

Burgomaster, K. A., Cermak, N. M., Phillips, S. M., Benton, C. R., Bonen, A. and Gibala, M. J. (2007) 'Divergent response of metabolite transport proteins in human skeletal muscle after sprint interval training and detraining', *Am J Physiol Regul Integr Comp Physiol,* 292(5), pp. R1970-6.

Burgomaster, K. A., Howarth, K. R., Phillips, S. M., Rakobowchuk, M., Macdonald, M. J., McGee, S. L. and Gibala, M. J. (2008) 'Similar metabolic adaptations during exercise after low volume sprint interval and traditional endurance training in humans', *J Physiol,* 586(1), pp. 151-60.

Burnley, M. (2010) 'The limit to exercise tolerance in humans: validity compromised by failing to account for the power-velocity relationship', *Eur J Appl Physiol,* 109(6), pp. 1225-6.

Burnley, M., Vanhatalo, A., Fulford, J. and Jones, A. M. (2010) 'Similar metabolic perturbations during all-out and constant force exhaustive exercise in humans: a (31)P magnetic resonance spectroscopy study', *Exp Physiol,* 95(7), pp. 798-807.

Burnley, M., Vanhatalo, A. and Jones, A. M. (2012) 'Distinct profiles of neuromuscular fatigue during muscle contractions below and above the critical torque in humans', *J Appl Physiol (1985),* 113(2), pp. 215-23.

Burtin, C., Saey, D., Saglam, M., Langer, D., Gosselink, R., Janssens, W., Decramer, M., Maltais, F. and Troosters, T. (2012) 'Effectiveness of exercise training in patients with COPD: the role of muscle fatigue', *Eur Respir J,* 40(2), pp. 338-44.

Cabanac, M. (1986) 'Money versus pain: experimental study of a conflict in humans', *J Exp Anal Behav,* 46(1), pp. 37-44.

Cannon, D. T., Bimson, W. E., Hampson, S. A., Bowen, T. S., Murgatroyd, S. R., Marwood, S., Kemp, G. J. and Rossiter, H. B. (2014) 'Skeletal muscle ATP turnover by 31P magnetic resonance spectroscopy during moderate and heavy bilateral knee extension', *J Physiol,* 592(23), pp. 5287-300.

Cannon, D. T., Coelho, A. C., Cao, R., Cheng, A., Porszasz, J., Casaburi, R. and Rossiter, H. B. (2016) 'Skeletal muscle power and fatigue at the tolerable limit of ramp-incremental exercise in COPD', *J Appl Physiol (1985),* 121(6), pp. 1365-1373.

Cannon, D. T., White, A. C., Andriano, M. F., Kolkhorst, F. W. and Rossiter, H. B. (2011) 'Skeletal muscle fatigue precedes the slow component of oxygen uptake kinetics during exercise in humans', *J Physiol,* 589(Pt 3), pp. 727-39.

Carroll, T. J., Taylor, J. L. and Gandevia, S. C. (2017) 'Recovery of central and peripheral neuromuscular fatigue after exercise', *J Appl Physiol (1985),* 122(5), pp. 1068-1076.

Casaburi, R. (2005) 'Factors determining constant work rate exercise tolerance in COPD and their role in dictating the minimal clinically important difference in response to interventions', *COPD,* 2(1), pp. 131-6.

Casaburi, R. and Porszasz, J. (2009) 'Constant work rate exercise testing: a tricky measure of exercise tolerance', *COPD,* 6(5), pp. 317-9.

Casaburi, R., Storer, T. W., Ben-Dov, I. and Wasserman, K. (1987) 'Effect of endurance training on possible determinants of VO2 during heavy exercise', *J Appl Physiol (1985),* 62(1), pp. 199-207.

Casaburi, R., Whipp, B. J., Wasserman, K., Beaver, W. L. and Koyal, S. N. (1977) 'Ventilatory and gas exchange dynamics in response to sinusoidal work', *J Appl Physiol Respir Environ Exerc Physiol,* 42(2), pp. 300-1.

Chance, B. and Williams, G. R. (1956) 'The respiratory chain and oxidative phosphorylation', *Adv Enzymol Relat Subj Biochem,* 17, pp. 65-134.

Chapman, A., Vicenzino, B., Blanch, P. and Hodges, P. (2009) 'Do differences in muscle recruitment between novice and elite cyclists reflect different movement patterns or less skilled muscle recruitment?', *J Sci Med Sport,* 12(1), pp. 31-4.

Chapman, A. R., Vicenzino, B., Blanch, P. and Hodges, P. W. (2008) 'Patterns of leg muscle recruitment vary between novice and highly trained cyclists', *J Electromyogr Kinesiol,* 18(3), pp. 359-71.

Chidnok, W., Dimenna, F. J., Bailey, S. J., Vanhatalo, A., Morton, R. H., Wilkerson, D. P. and Jones, A. M. (2012) 'Exercise tolerance in intermittent cycling: application of the critical power concept', *Med Sci Sports Exerc,* 44(5), pp. 966-76.

Chidnok, W., DiMenna, F. J., Fulford, J., Bailey, S. J., Skiba, P. F., Vanhatalo, A. and Jones, A. M. (2013) 'Muscle metabolic responses during high-intensity intermittent exercise measured by (31)P-MRS: relationship to the critical power concept', *Am J Physiol Regul Integr Comp Physiol,* 305(9), pp. R1085-92.

Cochran, A. J., Percival, M. E., Tricarico, S., Little, J. P., Cermak, N., Gillen, J. B., Tarnopolsky, M. A. and Gibala, M. J. (2014) 'Intermittent and continuous high-intensity exercise training induce similar acute but different chronic muscle adaptations', *Exp Physiol,* 99(5), pp. 782-91.

Cocks, M., Shaw, C. S., Shepherd, S. O., Fisher, J. P., Ranasinghe, A. M., Barker, T. A., Tipton, K. D. and Wagenmakers, A. J. (2013) 'Sprint interval and endurance training are equally effective in increasing muscle microvascular density and eNOS content in sedentary males', *J Physiol,* 591(3), pp. 641-56.

Coelho, A. C., Cannon, D. T., Cao, R., Porszasz, J., Casaburi, R., Knorst, M. M. and Rossiter, H. B. (2015) 'Instantaneous quantification of skeletal muscle activation, power production, and fatigue during cycle ergometry', *J Appl Physiol (1985),* 118(5), pp. 646-54.

Coffey, V. G. and Hawley, J. A. (2007) 'The molecular bases of training adaptation', *Sports Med,* 37(9), pp. 737-63.

Coffey, V. G. and Hawley, J. A. (2017) 'Concurrent exercise training: do opposites distract?', *J Physiol,* 595(9), pp. 2883-2896.

Cohen, J. (2013) *Statistical power analysis for the behavioral sciences.* Academic press.

Combes, A., Dekerle, J., Bougault, V. and Daussin, F. N. (2018) 'Physiological comparison of intensity-controlled, isocaloric intermittent and continuous exercise', *Eur J Sport Sci,* 18(10), pp. 1368-1375.

Combes, A., Dekerle, J., Webborn, N., Watt, P., Bougault, V. and Daussin, F. N. (2015) 'Exercise-induced metabolic fluctuations influence AMPK, p38-MAPK and CaMKII phosphorylation in human skeletal muscle', *Physiol Rep,* 3(9).

Conley, K. E., Kushmerick, M. J. and Jubrias, S. A. (1998) 'Glycolysis is independent of oxygenation state in stimulated human skeletal muscle in vivo', *J Physiol,* 511 (Pt 3), pp. 935-45.

Corda, M., Voneuler, C. and Lennerstrand, G. (1965) 'Proprioceptive Innervation of the Diaphragm', *J Physiol,* 178, pp. 161-77.

Coyle, E. F., Coggan, A. R., Hopper, M. K. and Walters, T. J. (1988) 'Determinants of endurance in well-trained cyclists', *J Appl Physiol (1985),* 64(6), pp. 2622-30.

Coyle, E. F., Feltner, M. E., Kautz, S. A., Hamilton, M. T., Montain, S. J., Baylor, A. M., Abraham, L. D. and Petrek, G. W. (1991) 'Physiological and biomechanical factors associated with elite endurance cycling performance', *Med Sci Sports Exerc,* 23(1), pp. 93-107.

Crewe, H., Tucker, R. and Noakes, T. D. (2008) 'The rate of increase in rating of perceived exertion predicts the duration of exercise to fatigue at a fixed power output in different environmental conditions', *Eur J Appl Physiol,* 103(5), pp. 569-77.

Daugaard, J. R., Nielsen, J. N., Kristiansen, S., Andersen, J. L., Hargreaves, M. and Richter, E. A. (2000) 'Fiber type-specific expression of GLUT4 in human skeletal muscle: influence of exercise training', *Diabetes,* 49(7), pp. 1092-5.

Daussin, F. N., Ponsot, E., Dufour, S. P., Lonsdorfer-Wolf, E., Doutreleau, S., Geny, B., Piquard, F. and Richard, R. (2007) 'Improvement of VO_{2max} by cardiac output and oxygen extraction adaptation during intermittent versus continuous endurance training', *Eur J Appl Physiol,* 101(3), pp. 377-83.

Davies, C. T. and Thompson, M. W. (1986) 'Physiological responses to prolonged exercise in ultramarathon athletes', *J Appl Physiol (1985),* 61(2), pp. 611-7.

Davies, M. J., Benson, A. P., Cannon, D. T., Marwood, S., Kemp, G. J., Rossiter, H. B. and Ferguson, C. (2017) 'Dissociating external power from intramuscular exercise intensity during intermittent bilateral knee-extension in humans', *J Physiol,* 595(21), pp. 6673-6686.

Davies, M. J., Lyall, G. K., Benson, A. P., Cannon, D. T., Birch, K. M., Rossiter, H. B. and Ferguson, C. (2021) 'Power Reserve at Intolerance in Ramp-

Incremental Exercise Is Dependent on Incrementation Rate', *Medicine & Science in Sports & Exercise,* Publish Ahead of Print.

Davis, J. A., Whipp, B. J., Lamarra, N., Huntsman, D. J., Frank, M. H. and Wasserman, K. (1982) 'Effect of ramp slope on determination of aerobic parameters from the ramp exercise test', *Med Sci Sports Exerc,* 14(5), pp. 339-43.

Davis, M. L. and Barstow, T. J. (2013) 'Estimated contribution of hemoglobin and myoglobin to near infrared spectroscopy', *Respir Physiol Neurobiol,* 186(2), pp. 180-7.

Day, J. R., Rossiter, H. B., Coats, E. M., Skasick, A. and Whipp, B. J. (2003) 'The maximally attainable VO2 during exercise in humans: the peak vs. maximum issue', *J Appl Physiol (1985),* 95(5), pp. 1901-7.

Day, S. J. and Hulliger, M. (2001) 'Experimental simulation of cat electromyogram: evidence for algebraic summation of motor-unit action-potential trains', *J Neurophysiol,* 86(5), pp. 2144-58.

de Morree, H. M., Klein, C. and Marcora, S. M. (2012) 'Perception of effort reflects central motor command during movement execution', *Psychophysiology,* 49(9), pp. 1242-53.

Dempsey, J. A., Romer, L., Rodman, J., Miller, J. and Smith, C. (2006) 'Consequences of exercise-induced respiratory muscle work', *Respir Physiol Neurobiol,* 151(2-3), pp. 242-50.

Dempsey, J. A., Sheel, A. W., St Croix, C. M. and Morgan, B. J. (2002) 'Respiratory influences on sympathetic vasomotor outflow in humans', *Respir Physiol Neurobiol,* 130(1), pp. 3-20.

Denadai, B. S., Ortiz, M. J., Greco, C. C. and de Mello, M. T. (2006) 'Interval training at 95% and 100% of the velocity at VO2 max: effects on aerobic physiological indexes and running performance', *Appl Physiol Nutr Metab,* 31(6), pp. 737-43.

Deschenes, M. R. (2004) 'Effects of aging on muscle fibre type and size', *Sports Med,* 34(12), pp. 809-24.

Dimitrov, G. V., Disselhorst-Klug, C., Dimitrova, N. A., Trachterna, A. and Rau, G. (2002) 'The presence of unknown layer of skin and fat is an obstacle to a correct estimation of the motor unit size from surface detected potentials', *Electromyogr Clin Neurophysiol,* 42(4), pp. 231-41.

Doherty, T. J. (2003) 'Invited review: Aging and sarcopenia', *J Appl Physiol (1985),* 95(4), pp. 1717-27.

Donelli da Silveira, A., Beust de Lima, J., da Silva Piardi, D., Dos Santos Macedo, D., Zanini, M., Nery, R., Laukkanen, J. A. and Stein, R. (2020) 'High-intensity interval training is effective and superior to moderate continuous training in patients with heart failure with preserved ejection fraction: A randomized clinical trial', *Eur J Prev Cardiol,* 27(16), pp. 1733-1743.

Donovan, C. M. and Brooks, G. A. (1983) 'Endurance training affects lactate clearance, not lactate production', *Am J Physiol,* 244(1), pp. E83-92.

Dubouchaud, H., Butterfield, G. E., Wolfel, E. E., Bergman, B. C. and Brooks, G. A. (2000) 'Endurance training, expression, and physiology of LDH, MCT1, and MCT4 in human skeletal muscle', *Am J Physiol Endocrinol Metab,* 278(4), pp. E571-9.

Duncan, M. J., Al-Nakeeb, Y. and Scurr, J. (2006) 'Perceived exertion is related to muscle activity during leg extension exercise', *Res Sports Med,* 14(3), pp. 179-89.

Edström, L. and Kugelberg, E. (1968) 'Histochemical composition, distribution of fibres and fatiguability of single motor units. Anterior tibial muscle of the rat', *J Neurol Neurosurg Psychiatry,* 31(5), pp. 424-33.

Egan, B. and Zierath, J. R. (2013) 'Exercise metabolism and the molecular regulation of skeletal muscle adaptation', *Cell Metab,* 17(2), pp. 162-84.

Ekblom, B. (2009) 'Counterpoint: maximal oxygen uptake is not limited by a central nervous system governor', *J Appl Physiol (1985),* 106(1), pp. 339-41; discussion 341-2.

Ekblom, B. and Goldbarg, A. N. (1971) 'The influence of physical training and other factors on the subjective rating of perceived exertion', *Acta Physiol Scand,* 83(3), pp. 399-406.

Elmer, S. J., Amann, M., McDaniel, J., Martin, D. T. and Martin, J. C. (2013) 'Fatigue is specific to working muscles: no cross-over with single-leg cycling in trained cyclists', *Eur J Appl Physiol,* 113(2), pp. 479-88.

Enoka, R. M. and Stuart, D. G. (1992) 'Neurobiology of muscle fatigue', *J Appl Physiol (1985),* 72(5), pp. 1631-48.

Esposito, F., Mathieu-Costello, O., Shabetai, R., Wagner, P. D. and Richardson, R. S. (2010) 'Limited maximal exercise capacity in patients with chronic heart failure: partitioning the contributors', *J Am Coll Cardiol,* 55(18), pp. 1945-54.

Essén, B., Jansson, E., Henriksson, J., Taylor, A. W. and Saltin, B. (1975) 'Metabolic characteristics of fibre types in human skeletal muscle', *Acta Physiol Scand,* 95(2), pp. 153-65.

Faraday, M. (1832) 'V. Experimental researches in electricity', *Philosophical transactions of the Royal Society of London,* (122), pp. 125-162.

Faraone, S. V. (2008) 'Interpreting estimates of treatment effects: implications for managed care', *P T,* 33(12), pp. 700-11.

Farina, D., Cescon, C., Negro, F. and Enoka, R. M. (2008) 'Amplitude cancellation of motor-unit action potentials in the surface electromyogram can be estimated with spike-triggered averaging', *J Neurophysiol,* 100(1), pp. 431-40.

Farina, D., Holobar, A., Merletti, R. and Enoka, R. M. (2010) 'Decoding the neural drive to muscles from the surface electromyogram', *Clin Neurophysiol,* 121(10), pp. 1616-23.

Farina, D., Merletti, R. and Enoka, R. M. (2004) 'The extraction of neural strategies from the surface EMG', *J Appl Physiol (1985),* 96(4), pp. 1486-95.

Farina, D., Merletti, R. and Enoka, R. M. (2014) 'The extraction of neural strategies from the surface EMG: an update', *J Appl Physiol (1985),* 117(11), pp. 1215-30.

Farup, J., Kjølhede, T., Sørensen, H., Dalgas, U., Møller, A. B., Vestergaard, P. F., Ringgaard, S., Bojsen-Møller, J. and Vissing, K. (2012) 'Muscle morphological and strength adaptations to endurance vs. resistance training', *J Strength Cond Res,* 26(2), pp. 398-407.

Favier, R. J., Constable, S. H., Chen, M. and Holloszy, J. O. (1986) 'Endurance exercise training reduces lactate production', *J Appl Physiol (1985),* 61(3), pp. 885-9.

Ferguson, C., Cannon, D. T., Wylde, L. A., Benson, A. P. and Rossiter, H. B. (2016a) 'Power-Velocity and Power-Efficiency Implications in the Limitation of Ramp Incremental Cycle Ergometry: Reply to Morales-Alamo et al', *J Appl Physiol (1985),* 120(4), pp. 477.

Ferguson, C., Wylde, L. A., Benson, A. P., Cannon, D. T. and Rossiter, H. B. (2016b) 'No reserve in isokinetic cycling power at intolerance during ramp incremental exercise in endurance-trained men', *J Appl Physiol (1985),* 120(1), pp. 70-7.

Fernandes, A. L., Lopes-Silva, J. P., Bertuzzi, R., Casarini, D. E., Arita, D. Y., Bishop, D. J. and Lima-Silva, A. E. (2014) 'Effect of time of day on performance, hormonal and metabolic response during a 1000-M cycling time trial', *PLoS One,* 9(10), pp. e109954.

Ferreira Gregorio J., Pequera G., Manno C., Ríos E. and Brum G. (2017) 'The voltage sensor of excitation-contraction coupling in mammals: Inactivation and interaction with Ca^{2+}' *J Gen Physiol* 149(11), pp. 1041–1058.

Ferreira, J. J., Pequera, G., Launikonis, B. S., Ríos, E., and Brum, G. (2021) 'A chloride channel blocker prevents the suppression by inorganic phosphate of the cytosolic calcium signals that control muscle contraction'. *J Physiol*, *599*(1), pp. 157-170.

Fink, R. H., Stephenson, D. G. and Williams, D. A. (1990) 'Physiological properties of skinned fibres from normal and dystrophic (Duchenne) human muscle activated by Ca2+ and Sr2+', *J Physiol,* 420, pp. 337-53.

Fiorenza, M., Gunnarsson, T. P., Hostrup, M., Iaia, F. M., Schena, F., Pilegaard, H. and Bangsbo, J. (2018) 'Metabolic stress-dependent regulation of the mitochondrial biogenic molecular response to high-intensity exercise in human skeletal muscle', *J Physiol,* 596(14), pp. 2823-2840.

Fitting, J. W., Bradley, T. D., Easton, P. A., Lincoln, M. J., Goldman, M. D. and Grassino, A. (1988) 'Dissociation between diaphragmatic and rib cage muscle fatigue', *J Appl Physiol (1985),* 64(3), pp. 959-65.

Fitting, J. W., Chartrand, D. A., Bradley, T. D., Killian, K. J. and Grassino, A. (1987) 'Effect of thoracoabdominal breathing patterns on inspiratory effort sensation', *J Appl Physiol (1985),* 62(4), pp. 1665-70.

Fitts, R. H. (1994) 'Cellular mechanisms of muscle fatigue', *Physiol Rev,* 74(1), pp. 49-94.

Fitts, R. H. and Balog, E. M. (1996) 'Effect of intracellular and extracellular ion changes on E–C coupling and skeletal muscle fatigue', *Acta Physiol Scand*, *156*(3), pp. 169-181.

Folland, J. P. and Williams, A. G. (2007) 'The adaptations to strength training : morphological and neurological contributions to increased strength', *Sports Med,* 37(2), pp. 145-68.

Fragala, M. S., Cadore, E. L., Dorgo, S., Izquierdo, M., Kraemer, W. J., Peterson, M. D. and Ryan, E. D. (2019) 'Resistance Training for Older Adults: Position Statement From the National Strength and Conditioning Association', *J Strength Cond Res,* 33(8), pp. 2019-2052.

Froyd, C., Beltrami, F. G., Millet, G. Y. and Noakes, T. D. (2016) 'Central Regulation and Neuromuscular Fatigue during Exercise of Different Durations', *Med Sci Sports Exerc,* 48(6), pp. 1024-32.

Fulton, T. J., Baranauskas, M. N., Paris, H. L., Koceja, D. M., Mickleborough, T. D. and Chapman, R. F. (2020) 'Respiratory Muscle Fatigue Alters Cycling

Performance and Locomotor Muscle Fatigue', *Med Sci Sports Exerc,* 52(11), pp. 2380-2389.

Fyfe, J. J., Bishop, D. J., and Stepto, N. K. (2014) 'Interference between concurrent resistance and endurance exercise: molecular bases and the role of individual training variables' *Sports Med*, *44*(6), pp. 743-762.

Gabriel, D. A., Kamen, G. and Frost, G. (2006) 'Neural adaptations to resistive exercise: mechanisms and recommendations for training practices', *Sports Med,* 36(2), pp. 133-49.

Gaesser, G. A. and Angadi, S. S. (2011) 'High-intensity interval training for health and fitness: can less be more?', *J Appl Physiol (1985),* 111(6), pp. 1540-1.

Gagnon, P., Bussières, J. S., Ribeiro, F., Gagnon, S. L., Saey, D., Gagné, N., Provencher, S. and Maltais, F. (2012) 'Influences of spinal anesthesia on exercise tolerance in patients with chronic obstructive pulmonary disease', *Am J Respir Crit Care Med,* 186(7), pp. 606-15.

Gagnon, P., Saey, D., Vivodtzev, I., Laviolette, L., Mainguy, V., Milot, J., Provencher, S. and Maltais, F. (2009) 'Impact of preinduced quadriceps fatigue on exercise response in chronic obstructive pulmonary disease and healthy subjects', *J Appl Physiol (1985),* 107(3), pp. 832-40.

Gaitanos, G. C., Williams, C., Boobis, L. H. and Brooks, S. (1993) 'Human muscle metabolism during intermittent maximal exercise', *J Appl Physiol (1985),* 75(2), pp. 712-9.

Gandevia, S. C. (2001) 'Spinal and supraspinal factors in human muscle fatigue', *Physiol Rev,* 81(4), pp. 1725-89.

Gandevia, S. C., Killian, K. J. and Campbell, E. J. (1981) 'The effect of respiratory muscle fatigue on respiratory sensations', *Clin Sci (Lond),* 60(4), pp. 463-6.

Gastin, P. B. (2001) 'Energy system interaction and relative contribution during maximal exercise', *Sports Med,* 31(10), pp. 725-41.

Gibala, M. J., Little, J. P., van Essen, M., Wilkin, G. P., Burgomaster, K. A., Safdar, A., Raha, S. and Tarnopolsky, M. A. (2006) 'Short-term sprint interval versus traditional endurance training: similar initial adaptations in human skeletal muscle and exercise performance', *J Physiol,* 575(Pt 3), pp. 901-11.

Gibala, M. J. and McGee, S. L. (2008) 'Metabolic adaptations to short-term high-intensity interval training: a little pain for a lot of gain?', *Exerc Sport Sci Rev,* 36(2), pp. 58-63.

Gillen, J. B. and Gibala, M. J. (2018) 'Interval training: a time-efficient exercise strategy to improve cardiometabolic health', *Appl Physiol Nutr Metab,* 43(10), pp. iii-iv.

Gillen, J. B., Martin, B. J., MacInnis, M. J., Skelly, L. E., Tarnopolsky, M. A. and Gibala, M. J. (2016) 'Twelve Weeks of Sprint Interval Training Improves Indices of Cardiometabolic Health Similar to Traditional Endurance Training despite a Five-Fold Lower Exercise Volume and Time Commitment', *PLoS One,* 11(4), pp. e0154075.

Gormley, S. E., Swain, D. P., High, R., Spina, R. J., Dowling, E. A., Kotipalli, U. S. and Gandrakota, R. (2008) 'Effect of intensity of aerobic training on VO2max', *Med Sci Sports Exerc,* 40(7), pp. 1336-43.

Granata, C., Jamnick, N. A. and Bishop, D. J. (2018a) 'Principles of Exercise Prescription, and How They Influence Exercise-Induced Changes of Transcription Factors and Other Regulators of Mitochondrial Biogenesis', *Sports Med,* 48(7), pp. 1541-1559.

Granata, C., Jamnick, N. A. and Bishop, D. J. (2018b) 'Training-Induced Changes in Mitochondrial Content and Respiratory Function in Human Skeletal Muscle', *Sports Med,* 48(8), pp. 1809-1828.

Gransee, H. M., Mantilla, C. B. and Sieck, G. C. (2012) 'Respiratory muscle plasticity', *Compr Physiol,* 2(2), pp. 1441-62.

Grassi, B., Poole, D. C., Richardson, R. S., Knight, D. R., Erickson, B. K. and Wagner, P. D. (1996) 'Muscle O2 uptake kinetics in humans: implications for metabolic control', *J Appl Physiol (1985),* 80(3), pp. 988-98.

Grgic, J., Mcllvenna, L. C., Fyfe, J. J., Sabol, F., Bishop, D. J., Schoenfeld, B. J. and Pedisic, Z. (2019) 'Does Aerobic Training Promote the Same Skeletal Muscle Hypertrophy as Resistance Training? A Systematic Review and Meta-Analysis', *Sports Med,* 49(2), pp. 233-254.

Guenette, J. A., Witt, J. D., McKenzie, D. C., Road, J. D. and Sheel, A. W. (2007) 'Respiratory mechanics during exercise in endurance-trained men and women', *J Physiol,* 581(Pt 3), pp. 1309-22.

Hairi, N. N., Cumming, R. G., Naganathan, V., Handelsman, D. J., Le Couteur, D. G., Creasey, H., Waite, L. M., Seibel, M. J. and Sambrook, P. N. (2010) 'Loss of muscle strength, mass (sarcopenia), and quality (specific force) and its relationship with functional limitation and physical disability: the Concord Health and Ageing in Men Project', *J Am Geriatr Soc,* 58(11), pp. 2055-62.

Halestrap, A. P. (2013) 'Monocarboxylic acid transport', *Compr Physiol,* 3(4), pp. 1611-43.

Hansen, J. E., Sue, D. Y., Oren, A. and Wasserman, K. (1987) 'Relation of oxygen uptake to work rate in normal men and men with circulatory disorders', *Am J Cardiol,* 59(6), pp. 669-74.

Harber, M. P., Konopka, A. R., Douglass, M. D., Minchev, K., Kaminsky, L. A., Trappe, T. A. and Trappe, S. (2009) 'Aerobic exercise training improves whole muscle and single myofiber size and function in older women', *Am J Physiol Regul Integr Comp Physiol,* 297(5), pp. R1452-9.

Harber, M. P., Konopka, A. R., Undem, M. K., Hinkley, J. M., Minchev, K., Kaminsky, L. A., Trappe, T. A. and Trappe, S. (2012) 'Aerobic exercise training induces skeletal muscle hypertrophy and age-dependent adaptations in myofiber function in young and older men', *J Appl Physiol (1985),* 113(9), pp. 1495-504.

Hargreaves, M. and Spriet, L. L. (2020) 'Skeletal muscle energy metabolism during exercise', *Nat Metab,* 2(9), pp. 817-828.

Harms, C. A., Babcock, M. A., McClaran, S. R., Pegelow, D. F., Nickele, G. A., Nelson, W. B. and Dempsey, J. A. (1997) 'Respiratory muscle work compromises leg blood flow during maximal exercise', *J Appl Physiol (1985),* 82(5), pp. 1573-83.

Harms, C. A., Wetter, T. J., St Croix, C. M., Pegelow, D. F. and Dempsey, J. A. (2000) 'Effects of respiratory muscle work on exercise performance', *J Appl Physiol (1985),* 89(1), pp. 131-8.

Harris, R. C., Hultman, E. and Nordesjö, L. O. (1974) 'Glycogen, glycolytic intermediates and high-energy phosphates determined in biopsy samples of musculus quadriceps femoris of man at rest. Methods and variance of values', *Scand J Clin Lab Invest,* 33(2), pp. 109-20.

Hawley, J. A. (2009) 'Molecular responses to strength and endurance training: are they incompatible?' *Appl Physiol Nutr Metab*, *34*(3), pp. 355-361.

Hawley, J. A., Hargreaves, M., Joyner, M. J. and Zierath, J. R. (2014) 'Integrative biology of exercise', *Cell,* 159(4), pp. 738-49.

Hawley, J. A., Lundby, C., Cotter, J. D. and Burke, L. M. (2018) 'Maximizing Cellular Adaptation to Endurance Exercise in Skeletal Muscle', *Cell Metab,* 27(5), pp. 962-976.

Heckman, C. J. and Enoka, R. M. (2012) 'Motor unit', *Compr Physiol,* 2(4), pp. 2629-82.

Helgerud, J., Høydal, K., Wang, E., Karlsen, T., Berg, P., Bjerkaas, M., Simonsen, T., Helgesen, C., Hjorth, N., Bach, R. and Hoff, J. (2007) 'Aerobic

high-intensity intervals improve VO2max more than moderate training', *Med Sci Sports Exerc,* 39(4), pp. 665-71.

Hellsten, Y. and Nyberg, M. (2015) 'Cardiovascular Adaptations to Exercise Training', *Compr Physiol,* 6(1), pp. 1-32.

Hellsten, Y., Rufener, N., Nielsen, J. J., Høier, B., Krustrup, P. and Bangsbo, J. (2008) 'Passive leg movement enhances interstitial VEGF protein, endothelial cell proliferation, and eNOS mRNA content in human skeletal muscle', *Am J Physiol Regul Integr Comp Physiol,* 294(3), pp. R975-82.

Henke, K. G., Sharratt, M., Pegelow, D. and Dempsey, J. A. (1988) 'Regulation of end-expiratory lung volume during exercise', *J Appl Physiol (1985),* 64(1), pp. 135-46.

Henneman, E. (1957) 'Relation between size of neurons and their susceptibility to discharge', *Science,* 126(3287), pp. 1345-7.

Henneman, E., Somjen, G. and Carpenter, D. O. (1965a) 'Excitability and inhibitability of motoneurons of different sizes', *J Neurophysiol,* 28(3), pp. 599-620.

Henneman, E., Somjen, G. and Carpenter, D. O. (1965b) 'FUNCTIONAL SIGNIFICANCE OF CELL SIZE IN SPINAL MOTONEURONS', *J Neurophysiol,* 28, pp. 560-80.

Henson, L. C., Poole, D. C. and Whipp, B. J. (1989) 'Fitness as a determinant of oxygen uptake response to constant-load exercise', *Eur J Appl Physiol Occup Physiol,* 59(1-2), pp. 21-8.

Hickson, R. C. (1980) 'Interference of strength development by simultaneously training for strength and endurance' *Eur J Appl Physiol Occup Physiol*, *45*(2), pp. 255-263.

Hickson, R. C., Hagberg, J. M., Ehsani, A. A. and Holloszy, J. O. (1981) 'Time course of the adaptive responses of aerobic power and heart rate to training', *Med Sci Sports Exerc,* 13(1), pp. 17-20.

Hill, A. V. and Lupton, H. (1923) 'Muscular exercise, lactic acid, and the supply and utilization of oxygen', *QJM: An International Journal of Medicine,* (62), pp. 135-171.

Hodgson, M. D., Keir, D. A., Copithorne, D. B., Rice, C. L. and Kowalchuk, J. M. (2018) 'Power reserve following ramp-incremental cycling to exhaustion: implications for muscle fatigue and function', *J Appl Physiol (1985),* 125(2), pp. 304-312.

Holloszy, J. O. (1967) 'Biochemical adaptations in muscle. Effects of exercise on mitochondrial oxygen uptake and respiratory enzyme activity in skeletal muscle', *J Biol Chem,* 242(9), pp. 2278-82.

Holloszy, J. O. and Booth, F. W. (1976) 'Biochemical adaptations to endurance exercise in muscle', *Annu Rev Physiol,* 38, pp. 273-91.

Holloszy, J. O. and Coyle, E. F. (1984) 'Adaptations of skeletal muscle to endurance exercise and their metabolic consequences', *J Appl Physiol Respir Environ Exerc Physiol,* 56(4), pp. 831-8.

Housh, D. J., Housh, T. J., Johnson, G. O. and Chu, W. K. (1992) 'Hypertrophic response to unilateral concentric isokinetic resistance training', *J Appl Physiol (1985),* 73(1), pp. 65-70.

Huang, G., Wang, R., Chen, P., Huang, S. C., Donnelly, J. E. and Mehlferber, J. P. (2016) 'Dose-response relationship of cardiorespiratory fitness adaptation to controlled endurance training in sedentary older adults', *Eur J Prev Cardiol,* 23(5), pp. 518-29.

Hunter, G. R., McCarthy, J. P. and Bamman, M. M. (2004) 'Effects of resistance training on older adults', *Sports Med,* 34(5), pp. 329-48.

Hureau, T. J., Romer, L. M. and Amann, M. (2018) 'The 'sensory tolerance limit': A hypothetical construct determining exercise performance?', *Eur J Sport Sci,* 18(1), pp. 13-24.

Høier, B., Rufener, N., Bojsen-Møller, J., Bangsbo, J. and Hellsten, Y. (2010) 'The effect of passive movement training on angiogenic factors and capillary growth in human skeletal muscle', *J Physiol,* 588(Pt 19), pp. 3833-45.

Iaia, F. M. and Bangsbo, J. (2010) 'Speed endurance training is a powerful stimulus for physiological adaptations and performance improvements of athletes', *Scand J Med Sci Sports,* 20 Suppl 2, pp. 11-23.

Iellamo, F., Manzi, V., Caminiti, G., Vitale, C., Castagna, C., Massaro, M., Franchini, A., Rosano, G. and Volterrani, M. (2013) 'Matched dose interval and continuous exercise training induce similar cardiorespiratory and metabolic adaptations in patients with heart failure', *Int J Cardiol,* 167(6), pp. 2561-5.

Illi, S. K., Held, U., Frank, I. and Spengler, C. M. (2012) 'Effect of respiratory muscle training on exercise performance in healthy individuals: a systematic review and meta-analysis', *Sports Med,* 42(8), pp. 707-24.

Ingjer, F. (1979) 'Effects of endurance training on muscle fibre ATP-ase activity, capillary supply and mitochondrial content in man', *J Physiol,* 294, pp. 419-32.

Ivy, J. L., Withers, R. T., Brose, G., Maxwell, B. D. and Costill, D. L. (1981) 'Isokinetic contractile properties of the quadriceps with relation to fiber type', *Eur J Appl Physiol Occup Physiol,* 47(3), pp. 247-55.

Ivy, J. L., Withers, R. T., Van Handel, P. J., Elger, D. H. and Costill, D. L. (1980) 'Muscle respiratory capacity and fiber type as determinants of the lactate threshold', *J Appl Physiol Respir Environ Exerc Physiol,* 48(3), pp. 523-7.

Jacobs, I., Tesch, P. A., Bar-Or, O., Karlsson, J. and Dotan, R. (1983) 'Lactate in human skeletal muscle after 10 and 30 s of supramaximal exercise', *J Appl Physiol Respir Environ Exerc Physiol,* 55(2), pp. 365-7.

Jacobs, R. A., Flück, D., Bonne, T. C., Bürgi, S., Christensen, P. M., Toigo, M. and Lundby, C. (2013) 'Improvements in exercise performance with high-intensity interval training coincide with an increase in skeletal muscle mitochondrial content and function', *J Appl Physiol (1985),* 115(6), pp. 785-93.

Jansson, E. and Sylvén, C. (1985) 'Creatine kinase MB and citrate synthase in type I and type II muscle fibres in trained and untrained men', *Eur J Appl Physiol Occup Physiol,* 54(2), pp. 207-9.

Jeneson, J. A., Wiseman, R. W., Westerhoff, H. V. and Kushmerick, M. J. (1996) 'The signal transduction function for oxidative phosphorylation is at least second order in ADP', *J Biol Chem,* 271(45), pp. 27995-8.

Johnson, B. D., Babcock, M. A., Suman, O. E. and Dempsey, J. A. (1993) 'Exercise-induced diaphragmatic fatigue in healthy humans', *J Physiol,* 460, pp. 385-405.

Johnson, B. D., Saupe, K. W. and Dempsey, J. A. (1992) 'Mechanical constraints on exercise hyperpnea in endurance athletes', *J Appl Physiol (1985),* 73(3), pp. 874-86.

Johnson, B. D., Weisman, I. M., Zeballos, R. J. and Beck, K. C. (1999) 'Emerging concepts in the evaluation of ventilatory limitation during exercise: the exercise tidal flow-volume loop', *Chest,* 116(2), pp. 488-503.

Jones, A. M. (1998) 'A five year physiological case study of an Olympic runner', *Br J Sports Med,* 32(1), pp. 39-43.

Jones, A. M. and Carter, H. (2000) 'The effect of endurance training on parameters of aerobic fitness', *Sports Med,* 29(6), pp. 373-86.

Jones, A. M., Wilkerson, D. P., DiMenna, F., Fulford, J. and Poole, D. C. (2008) 'Muscle metabolic responses to exercise above and below the "critical

power'' assessed using 31P-MRS', *Am J Physiol Regul Integr Comp Physiol,* 294(2), pp. R585-93.

Jones, N. L. and Killian, K. J. (2000) 'Exercise limitation in health and disease', *N Engl J Med,* 343(9), pp. 632-41.

Jones, S., Chiesa, S. T., Chaturvedi, N. and Hughes, A. D. (2016) 'Recent developments in near-infrared spectroscopy (NIRS) for the assessment of local skeletal muscle microvascular function and capacity to utilise oxygen', *Artery Res,* 16, pp. 25-33.

Joyner, M. J. and Coyle, E. F. (2008) 'Endurance exercise performance: the physiology of champions', *J Physiol,* 586(1), pp. 35-44.

Joyner, M. J. and Green, D. J. (2009) 'Exercise protects the cardiovascular system: effects beyond traditional risk factors', *J Physiol,* 587(Pt 23), pp. 5551-8.

Juel, C. (2001) 'Current aspects of lactate exchange: lactate/H+ transport in human skeletal muscle', *Eur J Appl Physiol,* 86(1), pp. 12-6.

Juel, C. (2006) 'Training-induced changes in membrane transport proteins of human skeletal muscle', *Eur J Appl Physiol,* 96(6), pp. 627-35.

Kazamel, M. and Warren, P. P. (2017) 'History of electromyography and nerve conduction studies: A tribute to the founding fathers', *J Clin Neurosci,* 43, pp. 54-60.

Keenan, K. G., Farina, D., Maluf, K. S., Merletti, R. and Enoka, R. M. (2005) 'Influence of amplitude cancellation on the simulated surface electromyogram', *J Appl Physiol (1985),* 98(1), pp. 120-31.

Keenan, K. G., Farina, D., Merletti, R. and Enoka, R. M. (2006) 'Influence of motor unit properties on the size of the simulated evoked surface EMG potential', *Exp Brain Res,* 169(1), pp. 37-49.

Kemi, O. J., Haram, P. M., Loennechen, J. P., Osnes, J. B., Skomedal, T., Wisløff, U. and Ellingsen, Ø. (2005) 'Moderate vs. high exercise intensity: differential effects on aerobic fitness, cardiomyocyte contractility, and endothelial function', *Cardiovasc Res,* 67(1), pp. 161-72.

Kemp, G. J., Meyerspeer, M. and Moser, E. (2007) 'Absolute quantification of phosphorus metabolite concentrations in human muscle in vivo by 31P MRS: a quantitative review', *NMR Biomed,* 20(6), pp. 555-65.

Kennedy, M. D., Haykowsky, M. J., Boliek, C. A., Esch, B. T., Scott, J. M. and Warburton, D. E. (2006) 'Regional muscle oxygenation differences in vastus lateralis during different modes of incremental exercise', *Dyn Med,* 5, pp. 8.

Kimm, S. Y., Glynn, N. W., McMahon, R. P., Voorhees, C. C., Striegel-Moore, R. H. and Daniels, S. R. (2006) 'Self-perceived barriers to activity participation among sedentary adolescent girls', *Med Sci Sports Exerc,* 38(3), pp. 534-40.

Kodama, S., Saito, K., Tanaka, S., Maki, M., Yachi, Y., Asumi, M., Sugawara, A., Totsuka, K., Shimano, H., Ohashi, Y., Yamada, N. and Sone, H. (2009) 'Cardiorespiratory fitness as a quantitative predictor of all-cause mortality and cardiovascular events in healthy men and women: a meta-analysis', *JAMA,* 301(19), pp. 2024-35.

Konopka, A. R., Douglass, M. D., Kaminsky, L. A., Jemiolo, B., Trappe, T. A., Trappe, S. and Harber, M. P. (2010) 'Molecular adaptations to aerobic exercise training in skeletal muscle of older women', *J Gerontol A Biol Sci Med Sci,* 65(11), pp. 1201-7.

Konopka, A. R. and Harber, M. P. (2014) 'Skeletal muscle hypertrophy after aerobic exercise training', *Exerc Sport Sci Rev,* 42(2), pp. 53-61.

Korzeniewski, B. and Rossiter, H. B. (2020) 'Exceeding a "critical" muscle P_i: implications for VO_2 and metabolite slow components, muscle fatigue and the power-duration relationship', *Eur J Appl Physiol,* 120(7), pp. 1609-1619.

Kufel, T. J., Pineda, L. A. and Mador, M. J. (2002) 'Comparison of potentiated and unpotentiated twitches as an index of muscle fatigue', *Muscle Nerve,* 25(3), pp. 438-44.

Kuo, I. Y. and Ehrlich, B. E. (2015) 'Signaling in muscle contraction', *Cold Spring Harb Perspect Biol,* 7(2), pp. a006023.

Lagally, K. M., Robertson, R. J., Gallagher, K. I., Goss, F. L., Jakicic, J. M., Lephart, S. M., McCaw, S. T. and Goodpaster, B. (2002) 'Perceived exertion, electromyography, and blood lactate during acute bouts of resistance exercise', *Med Sci Sports Exerc,* 34(3), pp. 552-9; discussion 560.

Laghi, F., D'Alfonso, N. and Tobin, M. J. (1995) 'Pattern of recovery from diaphragmatic fatigue over 24 hours', *J Appl Physiol (1985),* 79(2), pp. 539-46.

Lamarra, N., Whipp, B. J., Ward, S. A. and Wasserman, K. (1987) 'Effect of interbreath fluctuations on characterizing exercise gas exchange kinetics', *J Appl Physiol (1985),* 62(5), pp. 2003-12.

Lansley, K. E., Dimenna, F. J., Bailey, S. J. and Jones, A. M. (2011) 'A 'new' method to normalise exercise intensity', *Int J Sports Med,* 32(7), pp. 535-41.

Laporta, D. and Grassino, A. (1985) 'Assessment of transdiaphragmatic pressure in humans', *J Appl Physiol (1985),* 58(5), pp. 1469-76.

Larsen, S., Nielsen, J., Hansen, C. N., Nielsen, L. B., Wibrand, F., Stride, N., Schroder, H. D., Boushel, R., Helge, J. W., Dela, F. and Hey-Mogensen, M. (2012) 'Biomarkers of mitochondrial content in skeletal muscle of healthy young human subjects', *J Physiol,* 590(14), pp. 3349-60.

Larsson, L., Degens, H., Li, M., Salviati, L., Lee, Y. I., Thompson, W., Kirkland, J. L. and Sandri, M. (2019) 'Sarcopenia: Aging-Related Loss of Muscle Mass and Function', *Physiol Rev,* 99(1), pp. 427-511.

Laursen, P. B. and Jenkins, D. G. (2002) 'The scientific basis for high-intensity interval training: optimising training programmes and maximising performance in highly trained endurance athletes', *Sports Med,* 32(1), pp. 53-73.

Lee, D. C., Artero, E. G., Sui, X. and Blair, S. N. (2010) 'Mortality trends in the general population: the importance of cardiorespiratory fitness', *J Psychopharmacol,* 24(4 Suppl), pp. 27-35.

Legaz Arrese, A., Serrano Ostáriz, E., Jcasajús Mallén, J. A. and Munguía Izquierdo, D. (2005) 'The changes in running performance and maximal oxygen uptake after long-term training in elite athletes', *J Sports Med Phys Fitness,* 45(4), pp. 435-40.

Lepers, R., Maffiuletti, N. A., Rochette, L., Brugniaux, J. and Millet, G. Y. (2002) 'Neuromuscular fatigue during a long-duration cycling exercise', *J Appl Physiol (1985),* 92(4), pp. 1487-93.

Lim, H. B., Atkinson, G., Karageorghis, C. I., Eubank, M. R. and Eubank, M. M. (2009) 'Effects of differentiated music on cycling time trial', *Int J Sports Med,* 30(6), pp. 435-42.

Lopez, P., Pinto, R. S., Radaelli, R., Rech, A., Grazioli, R., Izquierdo, M. and Cadore, E. L. (2018) 'Benefits of resistance training in physically frail elderly: a systematic review', *Aging Clin Exp Res,* 30(8), pp. 889-899.

Lundby, C. and Robach, P. (2015) 'Performance Enhancement: What Are the Physiological Limits?', *Physiology (Bethesda),* 30(4), pp. 282-92.

Luo, Y. M., Hart, N., Mustfa, N., Lyall, R. A., Polkey, M. I. and Moxham, J. (2001) 'Effect of diaphragm fatigue on neural respiratory drive', *J Appl Physiol (1985),* 90(5), pp. 1691-9.

MacInnis, M. J. and Gibala, M. J. (2017) 'Physiological adaptations to interval training and the role of exercise intensity', *J Physiol,* 595(9), pp. 2915-2930.

MacInnis, M. J., Skelly, L. E. and Gibala, M. J. (2019) 'CrossTalk proposal: Exercise training intensity is more important than volume to promote increases in human skeletal muscle mitochondrial content', *J Physiol,* 597(16), pp. 4111-4113.

MacInnis, M. J., Zacharewicz, E., Martin, B. J., Haikalis, M. E., Skelly, L. E., Tarnopolsky, M. A., Murphy, R. M. and Gibala, M. J. (2017) 'Superior mitochondrial adaptations in human skeletal muscle after interval compared to continuous single-leg cycling matched for total work', *J Physiol,* 595(9), pp. 2955-2968.

MacRae, H. S., Dennis, S. C., Bosch, A. N. and Noakes, T. D. (1992) 'Effects of training on lactate production and removal during progressive exercise in humans', *J Appl Physiol (1985),* 72(5), pp. 1649-56.

Mancini, D. M., Bolinger, L., Li, H., Kendrick, K., Chance, B. and Wilson, J. R. (1994) 'Validation of near-infrared spectroscopy in humans', *J Appl Physiol (1985),* 77(6), pp. 2740-7.

Marcora, S. (2009) 'Perception of effort during exercise is independent of afferent feedback from skeletal muscles, heart, and lungs', *J Appl Physiol (1985),* 106(6), pp. 2060-2.

Marcora, S. (2010) 'Counterpoint: Afferent feedback from fatigued locomotor muscles is not an important determinant of endurance exercise performance', *J Appl Physiol (1985),* 108(2), pp. 454-6; discussion 456-7.

Marcora, S. M. (2008) 'Do we really need a central governor to explain brain regulation of exercise performance?', *Eur J Appl Physiol,* 104(5), pp. 929-31; author reply 933-5.

Marcora, S. M. and Staiano, W. (2010) 'The limit to exercise tolerance in humans: mind over muscle?', *Eur J Appl Physiol,* 109(4), pp. 763-70.

Margaria, R., Oliva, R. D., Di Prampero, P. E. and Cerretelli, P. (1969) 'Energy utilization in intermittent exercise of supramaximal intensity', *J Appl Physiol,* 26(6), pp. 752-6.

Martin, D. E., Vroon, D. H., May, D. F. and Pilbeam, S. P. (1986) 'Physiological Changes in Elite Male Distance Runners Training for Olympic Competition', *Phys Sportsmed,* 14(1), pp. 152-206.

Martin, V., Kerhervé, H., Messonnier, L. A., Banfi, J. C., Geyssant, A., Bonnefoy, R., Féasson, L. and Millet, G. Y. (2010) 'Central and peripheral contributions to neuromuscular fatigue induced by a 24-h treadmill run', *J Appl Physiol (1985),* 108(5), pp. 1224-33.

Martín Del Campo Cervantes, J., Habacuc Macías Cervantes, M. and Monroy Torres, R. (2019) 'Effect of a Resistance Training Program on Sarcopenia and Functionality of the Older Adults Living in a Nursing Home', *J Nutr Health Aging,* 23(9), pp. 829-836.

Mase, K., Yamamoto, K., Murakami, S., Kihara, K., Matsushita, K., Nozoe, M. and Takashima, S. (2018) 'Changes in ventilation mechanics during expiratory rib cage compression in healthy males', *J Phys Ther Sci,* 30(6), pp. 820-824.

McCartney, N., Spriet, L. L., Heigenhauser, G. J., Kowalchuk, J. M., Sutton, J. R. and Jones, N. L. (1986) 'Muscle power and metabolism in maximal intermittent exercise', *J Appl Physiol (1985),* 60(4), pp. 1164-9.

McCrudden, M. C., Keir, D. A. and Belfry, G. R. (2017) 'The effects of short work vs. longer work periods within intermittent exercise on $\dot{V}O_{2p}$ kinetics, muscle deoxygenation, and energy system contribution', *J Appl Physiol (1985),* 122(6), pp. 1435-1444.

McKenzie, D. C. (2012) 'Respiratory physiology: adaptations to high-level exercise', *Br J Sports Med,* 46(6), pp. 381-4.

McNarry, M. A., Kingsley, M. I. and Lewis, M. J. (2012) 'Influence of exercise intensity on pulmonary oxygen uptake kinetics in young and late middle-aged adults', *Am J Physiol Regul Integr Comp Physiol,* 303(8), pp. R791-8.

McPhedran, A. M., Wuerker, R. B. and Henneman, E. (1965a) 'Properties of motor units in a heterogeneous pale muscle (m. gastrocnemius) of the cat', *J Neurophysiol,* 28, pp. 85-99.

McPhedran, A. M., Wuerker, R. B. and Henneman, E. (1965b) 'Properties of motor units in a homogeneous red muscle (soleus) of the cat', *J Neurophysiol,* 28, pp. 71-84.

Medbø, J. I. and Tabata, I. (1989) 'Relative importance of aerobic and anaerobic energy release during short-lasting exhausting bicycle exercise', *J Appl Physiol (1985),* 67(5), pp. 1881-6.

Messere, A. and Roatta, S. (2013) 'Influence of cutaneous and muscular circulation on spatially resolved versus standard Beer-Lambert near-infrared spectroscopy', *Physiol Rep,* 1(7), pp. e00179.

Miller, M. R., Hankinson, J., Brusasco, V., Burgos, F., Casaburi, R., Coates, A., Crapo, R., Enright, P., van der Grinten, C. P., Gustafsson, P., Jensen, R., Johnson, D. C., MacIntyre, N., McKay, R., Navajas, D., Pedersen, O. F., Pellegrino, R., Viegi, G., Wanger, J. and Force, A. E. T. (2005) 'Standardisation of spirometry', *Eur Respir J,* 26(2), pp. 319-38.

Millet, G. Y. (2011) 'Can neuromuscular fatigue explain running strategies and performance in ultra-marathons?: the flush model', *Sports Med,* 41(6), pp. 489-506.

Millet, G. Y., Bachasson, D., Temesi, J., Wuyam, B., Féasson, L., Vergès, S. and Lévy, P. (2012) 'Potential interests and limits of magnetic and electrical stimulation techniques to assess neuromuscular fatigue', *Neuromuscul Disord,* 22 Suppl 3, pp. S181-6.

Millet, G. Y. and Lepers, R. (2004) 'Alterations of neuromuscular function after prolonged running, cycling and skiing exercises', *Sports Med,* 34(2), pp. 105-16.

Millet, G. Y., Lepers, R., Maffiuletti, N. A., Babault, N., Martin, V. and Lattier, G. (2002) 'Alterations of neuromuscular function after an ultramarathon', *J Appl Physiol (1985),* 92(2), pp. 486-92.

Millet, G. Y., Martin, V. and Temesi, J. (2018) 'The role of the nervous system in neuromuscular fatigue induced by ultra-endurance exercise', *Appl Physiol Nutr Metab,* 43(11), pp. 1151-1157.

Montero, D. and Lundby, C. (2017) 'Refuting the myth of non-response to exercise training: 'non-responders' do respond to higher dose of training', *J Physiol,* 595(11), pp. 3377-3387.

Montero, D. and Lundby, C. (2018) 'Regulation of Red Blood Cell Volume with Exercise Training', *Compr Physiol,* 9(1), pp. 149-164.

Morales-Alamo, D., Losa-Reyna, J., Torres-Peralta, R., Martin-Rincon, M., Perez-Valera, M., Curtelin, D., Ponce-González, J. G., Santana, A. and Calbet, J. A. (2015) 'What limits performance during whole-body incremental exercise to exhaustion in humans?', *J Physiol,* 593(20), pp. 4631-48.

Morris, C. K., Ueshima, K., Kawaguchi, T., Hideg, A. and Froelicher, V. F. (1991) 'The prognostic value of exercise capacity: a review of the literature', *Am Heart J,* 122(5), pp. 1423-31.

Morris, S. B. (2008) 'Estimating effect sizes from pretest-posttest-control group designs', *Organizational research methods,* 11(2), pp. 364-386.

Morton, R. H. (2011) 'Why peak power is higher at the end of steeper ramps: an explanation based on the "critical power" concept', *J Sports Sci,* 29(3), pp. 307-9.

Morton, R. H., Green, S., Bishop, D. and Jenkins, D. G. (1997) 'Ramp and constant power trials produce equivalent critical power estimates', *Med Sci Sports Exerc,* 29(6), pp. 833-6.

Mota, S., Casan, P., Drobnic, F., Giner, J., Ruiz, O., Sanchis, J. and Milic-Emili, J. (1999) 'Expiratory flow limitation during exercise in competition cyclists', *J Appl Physiol (1985),* 86(2), pp. 611-6.

Murgatroyd, S. R. and Wylde, L. A. (2011) 'The power-duration relationship of high-intensity exercise: from mathematical parameters to physiological mechanisms', *J Physiol,* 589(Pt 10), pp. 2443-5.

Murgatroyd, S. R., Wylde, L. A., Cannon, D. T., Ward, S. A. and Rossiter, H. B. (2014) 'A 'ramp-sprint' protocol to characterise indices of aerobic function and exercise intensity domains in a single laboratory test', *Eur J Appl Physiol,* 114(9), pp. 1863-74.

Murias, J. M., Pogliaghi, S. and Paterson, D. H. (2018) 'Measurement of a True VO_{2max} during a Ramp Incremental Test Is Not Confirmed by a Verification Phase', *Front Physiol,* 9, pp. 143.

Myers, J., Prakash, M., Froelicher, V., Do, D., Partington, S. and Atwood, J. E. (2002) 'Exercise capacity and mortality among men referred for exercise testing', *N Engl J Med,* 346(11), pp. 793-801.

Neely, G., Ljunggren, G., Sylvén, C. and Borg, G. (1992) 'Comparison between the Visual Analogue Scale (VAS) and the Category Ratio Scale (CR-10) for the evaluation of leg exertion', *Int J Sports Med,* 13(2), pp. 133-6.

Nelson, N. G., Collins, C. L., Comstock, R. D. and McKenzie, L. B. (2011) 'Exertional heat-related injuries treated in emergency departments in the U.S., 1997-2006', *Am J Prev Med,* 40(1), pp. 54-60.

Newham, D. J. and Cady, E. B. (1990) 'A 31P study of fatigue and metabolism in human skeletal muscle with voluntary, intermittent contractions at different forces', *NMR Biomed,* 3(5), pp. 211-9.

Neyroud, D., Kayser, B. and Place, N. (2016) 'Are There Critical Fatigue Thresholds? Aggregated vs. Individual Data', *Front Physiol,* 7, pp. 376.

Nilwik, R., Snijders, T., Leenders, M., Groen, B. B., van Kranenburg, J., Verdijk, L. B. and van Loon, L. J. (2013) 'The decline in skeletal muscle mass with aging is mainly attributed to a reduction in type II muscle fiber size', *Exp Gerontol,* 48(5), pp. 492-8.

Noakes, T. D. (2000) 'Physiological models to understand exercise fatigue and the adaptations that predict or enhance athletic performance', *Scand J Med Sci Sports,* 10(3), pp. 123-45.

Noakes, T. D. (2012) 'Fatigue is a Brain-Derived Emotion that Regulates the Exercise Behavior to Ensure the Protection of Whole Body Homeostasis', *Front Physiol,* 3, pp. 82.

Noakes, T. D., St Clair Gibson, A. and Lambert, E. V. (2004) 'From catastrophe to complexity: a novel model of integrative central neural regulation of effort and fatigue during exercise in humans', *Br J Sports Med,* 38(4), pp. 511-4.

O'Donovan, G., Owen, A., Bird, S. R., Kearney, E. M., Nevill, A. M., Jones, D. W. and Woolf-May, K. (2005) 'Changes in cardiorespiratory fitness and coronary heart disease risk factors following 24 wk of moderate- or high-intensity exercise of equal energy cost', *J Appl Physiol (1985),* 98(5), pp. 1619-25.

Oga, T., Nishimura, K., Tsukino, M., Hajiro, T., Ikeda, A. and Izumi, T. (2000) 'The effects of oxitropium bromide on exercise performance in patients with stable chronic obstructive pulmonary disease. A comparison of three different exercise tests', *Am J Respir Crit Care Med,* 161(6), pp. 1897-901.

Okano, A. H., Fontes, E. B., Montenegro, R. A., Farinatti, P. e. T., Cyrino, E. S., Li, L. M., Bikson, M. and Noakes, T. D. (2015) 'Brain stimulation modulates the autonomic nervous system, rating of perceived exertion and performance during maximal exercise', *Br J Sports Med,* 49(18), pp. 1213-8.

Ørtenblad, N., Nielsen, J., Saltin, B., and Holmberg, H. C. (2011) 'Role of glycogen availability in sarcoplasmic reticulum Ca2+ kinetics in human skeletal muscle', *J Physiol*, *589*(3), pp. 711-725.

Ørtenblad, N., Westerblad, H., and Nielsen, J. (2013) 'Muscle glycogen stores and fatigue' *J Physiol*, *591*(18), pp. 4405-4413.

Ozcelik, O., Ward, S. A. and Whipp, B. J. (1999) 'Effect of altered body CO2 stores on pulmonary gas exchange dynamics during incremental exercise in humans', *Exp Physiol,* 84(5), pp. 999-1011.

Ozyener, F., Rossiter, H. B., Ward, S. A. and Whipp, B. J. (2001) 'Influence of exercise intensity on the on- and off-transient kinetics of pulmonary oxygen uptake in humans', *J Physiol,* 533(Pt 3), pp. 891-902.

Pageaux, B. (2014) 'The psychobiological model of endurance performance: an effort-based decision-making theory to explain self-paced endurance performance', *Sports Med,* 44(9), pp. 1319-20.

Palange, P., Ward, S. A., Carlsen, K. H., Casaburi, R., Gallagher, C. G., Gosselink, R., O'Donnell, D. E., Puente-Maestu, L., Schols, A. M., Singh, S., Whipp, B. J. and Force, E. T. (2007) 'Recommendations on the use of exercise testing in clinical practice', *Eur Respir J,* 29(1), pp. 185-209.

Papa, E. V., Dong, X. and Hassan, M. (2017) 'Resistance training for activity limitations in older adults with skeletal muscle function deficits: a systematic review', *Clin Interv Aging,* 12, pp. 955-961.

Parolin, M. L., Chesley, A., Matsos, M. P., Spriet, L. L., Jones, N. L. and Heigenhauser, G. J. (1999) 'Regulation of skeletal muscle glycogen phosphorylase and PDH during maximal intermittent exercise', *Am J Physiol,* 277(5), pp. E890-900.

Pellegrino, R., Brusasco, V., Rodarte, J. R. and Babb, T. G. (1993a) 'Expiratory flow limitation and regulation of end-expiratory lung volume during exercise', *J Appl Physiol (1985),* 74(5), pp. 2552-8.

Pellegrino, R., Violante, B., Nava, S., Rampulla, C., Brusasco, V. and Rodarte, J. R. (1993b) 'Expiratory airflow limitation and hyperinflation during methacholine-induced bronchoconstriction', *J Appl Physiol (1985),* 75(4), pp. 1720-7.

Pilegaard, H., Domino, K., Noland, T., Juel, C., Hellsten, Y., Halestrap, A. P. and Bangsbo, J. (1999) 'Effect of high-intensity exercise training on lactate/H+ transport capacity in human skeletal muscle', *Am J Physiol,* 276(2), pp. E255-61.

Place, N., Lepers, R., Deley, G. and Millet, G. Y. (2004) 'Time course of neuromuscular alterations during a prolonged running exercise', *Med Sci Sports Exerc,* 36(8), pp. 1347-56.

Poole, D. C., Burnley, M., Vanhatalo, A., Rossiter, H. B. and Jones, A. M. (2016) 'Critical Power: An Important Fatigue Threshold in Exercise Physiology', *Med Sci Sports Exerc,* 48(11), pp. 2320-2334.

Poole, D. C. and Jones, A. M. (2017) 'Measurement of the maximum oxygen uptake $\dot{V}o_{2max:}$ $\dot{V}o_{2peak}$ is no longer acceptable', *J Appl Physiol (1985),* 122(4), pp. 997-1002.

Poole, D. C., Schaffartzik, W., Knight, D. R., Derion, T., Kennedy, B., Guy, H. J., Prediletto, R. and Wagner, P. D. (1991) 'Contribution of exercising legs to the slow component of oxygen uptake kinetics in humans', *J Appl Physiol (1985),* 71(4), pp. 1245-60.

Poole, D. C., Ward, S. A., Gardner, G. W. and Whipp, B. J. (1988) 'Metabolic and respiratory profile of the upper limit for prolonged exercise in man', *Ergonomics,* 31(9), pp. 1265-79.

Poon, C. S. and Ward, S. A. (1986) 'A device to provide respiratory-mechanical unloading', *IEEE Trans Biomed Eng,* 33(3), pp. 361-5.

Porszasz, J., Emtner, M., Goto, S., Somfay, A., Whipp, B. J. and Casaburi, R. (2005) 'Exercise training decreases ventilatory requirements and exercise-induced hyperinflation at submaximal intensities in patients with COPD', *Chest,* 128(4), pp. 2025-34.

Porszasz, J., Rambod, M., van der Vaart, H., Rossiter, H. B., Ma, S., Kiledjian, R. and Casaburi, R. (2013) 'Sinusoidal high-intensity exercise does not elicit ventilatory limitation in chronic obstructive pulmonary disease', *Exp Physiol,* 98(6), pp. 1102-14.

Powers, S. K., Jacques, M., Richard, R. and Beadle, R. E. (1986) 'Effects of breathing a normoxic He-O2 gas mixture on exercise tolerance and VO2 max', *Int J Sports Med,* 7(4), pp. 217-21.

Pringle, J. S. and Jones, A. M. (2002) 'Maximal lactate steady state, critical power and EMG during cycling', *Eur J Appl Physiol,* 88(3), pp. 214-26.

Puente-Maestu, L., Abad, M. J., Godoy, R., Pérez-Parra, J. M., Cubillo, J. M. and Whipp, B. J. (2002) 'Breath-by-breath fluctuations of pulmonary gas exchange and ventilation in COPD patients', *Eur J Appl Physiol,* 87(6), pp. 535-41.

Puente-Maestu, L., Palange, P., Casaburi, R., Laveneziana, P., Maltais, F., Neder, J. A., O'Donnell, D. E., Onorati, P., Porszasz, J., Rabinovich, R., Rossiter, H. B., Singh, S., Troosters, T. and Ward, S. (2016) 'Use of exercise testing in the evaluation of interventional efficacy: an official ERS statement', *Eur Respir J,* 47(2), pp. 429-60.

Puente-Maestu, L., Villar, F., de Miguel, J., Stringer, W. W., Sanz, P., Sanz, M. L., de Pedro, J. G. and Martínez-Abad, Y. (2009) 'Clinical relevance of constant power exercise duration changes in COPD', *Eur Respir J,* 34(2), pp. 340-5.

Putman, C. T., Jones, N. L., Lands, L. C., Bragg, T. M., Hollidge-Horvat, M. G. and Heigenhauser, G. J. (1995) 'Skeletal muscle pyruvate dehydrogenase activity during maximal exercise in humans', *Am J Physiol,* 269(3 Pt 1), pp. E458-68.

Racinais, S., Connes, P., Bishop, D., Blonc, S. and Hue, O. (2005) 'Morning versus evening power output and repeated-sprint ability', *Chronobiol Int,* 22(6), pp. 1029-39.

Racinais, S., Perrey, S., Denis, R. and Bishop, D. (2010) 'Maximal power, but not fatigability, is greater during repeated sprints performed in the afternoon', *Chronobiol Int,* 27(4), pp. 855-64.

Roca, J., Agusti, A. G., Alonso, A., Poole, D. C., Viegas, C., Barbera, J. A., Rodriguez-Roisin, R., Ferrer, A. and Wagner, P. D. (1992) 'Effects of training on muscle O2 transport at VO2max', *J Appl Physiol (1985),* 73(3), pp. 1067-76.

Roca, J., Hogan, M. C., Story, D., Bebout, D. E., Haab, P., Gonzalez, R., Ueno, O. and Wagner, P. D. (1989) 'Evidence for tissue diffusion limitation of VO2max in normal humans', *J Appl Physiol (1985),* 67(1), pp. 291-9.

Rognmo, Ø., Hetland, E., Helgerud, J., Hoff, J. and Slørdahl, S. A. (2004) 'High intensity aerobic interval exercise is superior to moderate intensity exercise for increasing aerobic capacity in patients with coronary artery disease', *Eur J Cardiovasc Prev Rehabil,* 11(3), pp. 216-22.

Romer, L. M., Haverkamp, H. C., Amann, M., Lovering, A. T., Pegelow, D. F. and Dempsey, J. A. (2007a) 'Effect of acute severe hypoxia on peripheral fatigue and endurance capacity in healthy humans', *Am J Physiol Regul Integr Comp Physiol,* 292(1), pp. R598-606.

Romer, L. M., Lovering, A. T., Haverkamp, H. C., Pegelow, D. F. and Dempsey, J. A. (2006) 'Effect of inspiratory muscle work on peripheral fatigue of locomotor muscles in healthy humans', *J Physiol,* 571(Pt 2), pp. 425-39.

Romer, L. M. and McConnell, A. K. (2003) 'Specificity and reversibility of inspiratory muscle training', *Med Sci Sports Exerc,* 35(2), pp. 237-44.

Romer, L. M., Miller, J. D., Haverkamp, H. C., Pegelow, D. F. and Dempsey, J. A. (2007b) 'Inspiratory muscles do not limit maximal incremental exercise performance in healthy subjects', *Respir Physiol Neurobiol,* 156(3), pp. 353-61.

Romer, L. M. and Polkey, M. I. (2008) 'Exercise-induced respiratory muscle fatigue: implications for performance', *J Appl Physiol (1985),* 104(3), pp. 879-88.

Rossiter, H. B. (2011) 'Exercise: Kinetic considerations for gas exchange', *Compr Physiol,* 1(1), pp. 203-44.

Rossiter, H. B., Kowalchuk, J. M. and Whipp, B. J. (2006) 'A test to establish maximum O2 uptake despite no plateau in the O2 uptake response to ramp incremental exercise', *J Appl Physiol (1985),* 100(3), pp. 764-70.

Rossiter, H. B., Ward, S. A., Howe, F. A., Kowalchuk, J. M., Griffiths, J. R. and Whipp, B. J. (2002) 'Dynamics of intramuscular 31P-MRS P(i) peak splitting and the slow components of PCr and O2 uptake during exercise', *J Appl Physiol (1985),* 93(6), pp. 2059-69.

Rossman, M. J., Garten, R. S., Venturelli, M., Amann, M. and Richardson, R. S. (2014) 'The role of active muscle mass in determining the magnitude of peripheral fatigue during dynamic exercise', *Am J Physiol Regul Integr Comp Physiol,* 306(12), pp. R934-40.

Rossman, M. J., Venturelli, M., McDaniel, J., Amann, M. and Richardson, R. S. (2012) 'Muscle mass and peripheral fatigue: a potential role for afferent feedback?', *Acta Physiol (Oxf),* 206(4), pp. 242-50.

Roston, W. L., Whipp, B. J., Davis, J. A., Cunningham, D. A., Effros, R. M. and Wasserman, K. (1987) 'Oxygen uptake kinetics and lactate concentration during exercise in humans', *Am Rev Respir Dis,* 135(5), pp. 1080-4.

Roth, S. M., Ferrell, R. F. and Hurley, B. F. (2000) 'Strength training for the prevention and treatment of sarcopenia', *J Nutr Health Aging,* 4(3), pp. 143-55.

Roussos, C., Fixley, M., Gross, D. and Macklem, P. T. (1979) 'Fatigue of inspiratory muscles and their synergic behavior', *J Appl Physiol Respir Environ Exerc Physiol,* 46(5), pp. 897-904.

Ruff, R. L. (1989) 'Calcium sensitivity of fast- and slow-twitch human muscle fibers', *Muscle Nerve,* 12(1), pp. 32-7.

Rutherford, O. M. and Jones, D. A. (1986) 'The role of learning and coordination in strength training', *Eur J Appl Physiol Occup Physiol,* 55(1), pp. 100-5.

Rønnestad, B. R. and Mujika, I. (2014) 'Optimizing strength training for running and cycling endurance performance: A review', *Scand J Med Sci Sports,* 24(4), pp. 603-12.

Sale, D. G. (1988) 'Neural adaptation to resistance training', *Med Sci Sports Exerc,* 20(5 Suppl), pp. S135-45.

Saltin, B. and Astrand, P. O. (1967) 'Maximal oxygen uptake in athletes', *J Appl Physiol,* 23(3), pp. 353-8.

Santilli, V., Bernetti, A., Mangone, M. and Paoloni, M. (2014) 'Clinical definition of sarcopenia', *Clin Cases Miner Bone Metab,* 11(3), pp. 177-80.

Sargeant, A. J. (2007) 'Structural and functional determinants of human muscle power', *Exp Physiol,* 92(2), pp. 323-31.

Sargeant, A. J. and Dolan, P. (1987) 'Effect of prior exercise on maximal short-term power output in humans', *J Appl Physiol (1985),* 63(4), pp. 1475-80.

Sargeant, A. J., Hoinville, E. and Young, A. (1981) 'Maximum leg force and power output during short-term dynamic exercise', *J Appl Physiol Respir Environ Exerc Physiol,* 51(5), pp. 1175-82.

Saugy, J., Place, N., Millet, G. Y., Degache, F., Schena, F. and Millet, G. P. (2013) 'Alterations of Neuromuscular Function after the World's Most Challenging Mountain Ultra-Marathon', *PLoS One,* 8(6), pp. e65596.

Sawka, M. N., Convertino, V. A., Eichner, E. R., Schnieder, S. M. and Young, A. J. (2000) 'Blood volume: importance and adaptations to exercise training, environmental stresses, and trauma/sickness', *Med Sci Sports Exerc,* 32(2), pp. 332-48.

Scherr, J., Wolfarth, B., Christle, J. W., Pressler, A., Wagenpfeil, S. and Halle, M. (2013) 'Associations between Borg's rating of perceived exertion and physiological measures of exercise intensity', *Eur J Appl Physiol,* 113(1), pp. 147-55.

Schiaffino, S. and Reggiani, C. (2011) 'Fiber types in mammalian skeletal muscles', *Physiol Rev,* 91(4), pp. 1447-531.

Schjerve, I. E., Tyldum, G. A., Tjønna, A. E., Stølen, T., Loennechen, J. P., Hansen, H. E., Haram, P. M., Heinrich, G., Bye, A., Najjar, S. M., Smith, G. L., Slørdahl, S. A., Kemi, O. J. and Wisløff, U. (2008) 'Both aerobic endurance and strength training programmes improve cardiovascular health in obese adults', *Clin Sci (Lond),* 115(9), pp. 283-93.

Schäfer, L. U., Hayes, M. and Dekerle, J. (2019) 'The magnitude of neuromuscular fatigue is not intensity dependent when cycling above critical power but relates to aerobic and anaerobic capacities', *Exp Physiol,* 104(2), pp. 209-219.

Shephard, R. J. (2009) 'Is it time to retire the 'central governor'?', *Sports Med,* 39(9), pp. 709-21.

Shield, A. and Zhou, S. (2004) 'Assessing voluntary muscle activation with the twitch interpolation technique', *Sports Med,* 34(4), pp. 253-67.

Shinohara, M. and Moritani, T. (1992) 'Increase in neuromuscular activity and oxygen uptake during heavy exercise', *Ann Physiol Anthropol,* 11(3), pp. 257-62.

Sidhu, S. K., Weavil, J. C., Venturelli, M., Garten, R. S., Rossman, M. J., Richardson, R. S., Gmelch, B. S., Morgan, D. E. and Amann, M. (2014) 'Spinal μ-opioid receptor-sensitive lower limb muscle afferents determine corticospinal responsiveness and promote central fatigue in upper limb muscle', *J Physiol,* 592(22), pp. 5011-24.

Similowski, T., Fleury, B., Launois, S., Cathala, H. P., Bouche, P. and Derenne, J. P. (1989) 'Cervical magnetic stimulation: a new painless method for bilateral phrenic nerve stimulation in conscious humans', *J Appl Physiol (1985),* 67(4), pp. 1311-8.

Similowski, T., Straus, C., Attali, V., Duguet, A. and Derenne, J. P. (1998) 'Cervical magnetic stimulation as a method to discriminate between diaphragm and rib cage muscle fatigue', *J Appl Physiol (1985),* 84(5), pp. 1692-700.

Simoneau, J. A., Lortie, G., Boulay, M. R., Marcotte, M., Thibault, M. C. and Bouchard, C. (1985) 'Human skeletal muscle fiber type alteration with high-intensity intermittent training', *Eur J Appl Physiol Occup Physiol,* 54(3), pp. 250-3.

Sjödin, B. and Jacobs, I. (1981) 'Onset of blood lactate accumulation and marathon running performance', *Int J Sports Med,* 2(1), pp. 23-6.

Sjödin, B. and Svedenhag, J. (1985) 'Applied physiology of marathon running', *Sports Med,* 2(2), pp. 83-99.

Sjøgaard, G. (1982) 'Capillary supply and cross-sectional area of slow and fast twitch muscle fibres in man', *Histochemistry,* 76(4), pp. 547-55.

Skelly, L. E., Andrews, P. C., Gillen, J. B., Martin, B. J., Percival, M. E. and Gibala, M. J. (2014) 'High-intensity interval exercise induces 24-h energy expenditure similar to traditional endurance exercise despite reduced time commitment', *Appl Physiol Nutr Metab,* 39(7), pp. 845-8.

Sliwiński, P., Yan, S., Gauthier, A. P. and Macklem, P. T. (1996) 'Influence of global inspiratory muscle fatigue on breathing during exercise', *J Appl Physiol (1985),* 80(4), pp. 1270-8.

St Clair Gibson, A., Lambert, E. V., Rauch, L. H., Tucker, R., Baden, D. A., Foster, C. and Noakes, T. D. (2006) 'The role of information processing between the brain and peripheral physiological systems in pacing and perception of effort', *Sports Med,* 36(8), pp. 705-22.

St Clair Gibson, A. and Noakes, T. D. (2004) 'Evidence for complex system integration and dynamic neural regulation of skeletal muscle recruitment during exercise in humans', *Br J Sports Med,* 38(6), pp. 797-806.

Staiano, W., Bosio, A., de Morree, H. M., Rampinini, E. and Marcora, S. (2018) 'The cardinal exercise stopper: Muscle fatigue, muscle pain or perception of effort?', *Prog Brain Res,* 240, pp. 175-200.

Stutts, W. C. (2002) 'Physical activity determinants in adults. Perceived benefits, barriers, and self efficacy', *AAOHN J,* 50(11), pp. 499-507.

Støren, O., Helgerud, J., Støa, E. M. and Hoff, J. (2008) 'Maximal strength training improves running economy in distance runners', *Med Sci Sports Exerc,* 40(6), pp. 1087-92.

Suchomel, T. J., Nimphius, S., Bellon, C. R. and Stone, M. H. (2018) 'The Importance of Muscular Strength: Training Considerations', *Sports Med,* 48(4), pp. 765-785.

Sundberg, C. W., and Fitts, R. H. (2019) 'Bioenergetic basis of skeletal muscle fatigue' *Curr Opin Physiol, 10*, pp. 118-127.

Supinski, G. S., Clary, S. J., Bark, H. and Kelsen, S. G. (1987) 'Effect of inspiratory muscle fatigue on perception of effort during loaded breathing', *J Appl Physiol (1985),* 62(1), pp. 300-7.

Swisher, A. R., Koehn, B., Yong, S., Cunha, J., Ferguson, C. and Cannon, D. T. (2019) 'Dynamics of Locomotor Fatigue during Supra-critical Power Exercise', *Med Sci Sports Exerc,* 51(8), pp. 1720-1726.

Tanisho, K. and Hirakawa, K. (2009) 'Training effects on endurance capacity in maximal intermittent exercise: comparison between continuous and interval training', *J Strength Cond Res,* 23(8), pp. 2405-10.

Temesi, J., Mattioni Maturana, F., Peyrard, A., Piucco, T., Murias, J. M. and Millet, G. Y. (2017) 'The relationship between oxygen uptake kinetics and neuromuscular fatigue in high-intensity cycling exercise', *Eur J Appl Physiol,* 117(5), pp. 969-978.

Temesi, J., Rupp, T., Martin, V., Arnal, P. J., Féasson, L., Verges, S. and Millet, G. Y. (2014) 'Central fatigue assessed by transcranial magnetic stimulation in ultratrail running', *Med Sci Sports Exerc,* 46(6), pp. 1166-75.

Terena, S. M., Fernandes, K. P., Bussadori, S. K., Deana, A. M. and Mesquita-Ferrari, R. A. (2017) 'Systematic review of the synergist muscle ablation model for compensatory hypertrophy', *Rev Assoc Med Bras (1992),* 63(2), pp. 164-172.

Tesch, P. A. (1988) 'Skeletal muscle adaptations consequent to long-term heavy resistance exercise', *Med Sci Sports Exerc,* 20(5 Suppl), pp. S132-4.

Thomas, K., Elmeua, M., Howatson, G. and Goodall, S. (2016) 'Intensity-Dependent Contribution of Neuromuscular Fatigue after Constant-Load Cycling', *Med Sci Sports Exerc,* 48(9), pp. 1751-60.

Thomas, K., Goodall, S. and Howatson, G. (2018) 'Performance Fatigability Is Not Regulated to A Peripheral Critical Threshold', *Exerc Sport Sci Rev,* 46(4), pp. 240-246.

Tjønna, A. E., Leinan, I. M., Bartnes, A. T., Jenssen, B. M., Gibala, M. J., Winett, R. A. and Wisløff, U. (2013) 'Low- and high-volume of intensive endurance training significantly improves maximal oxygen uptake after 10-weeks of training in healthy men', *PLoS One,* 8(5), pp. e65382.

Trost, S. G., Owen, N., Bauman, A. E., Sallis, J. F. and Brown, W. (2002) 'Correlates of adults' participation in physical activity: review and update', *Med Sci Sports Exerc,* 34(12), pp. 1996-2001.

Tucker, R., Lambert, M. I. and Noakes, T. D. (2006) 'An analysis of pacing strategies during men's world-record performances in track athletics', *Int J Sports Physiol Perform,* 1(3), pp. 233-45.

Tucker, R., Marle, T., Lambert, E. V. and Noakes, T. D. (2006) 'The rate of heat storage mediates an anticipatory reduction in exercise intensity during cycling at a fixed rating of perceived exertion', *J Physiol,* 574(Pt 3), pp. 905-15.

Tucker, R., Rauch, L., Harley, Y. X. and Noakes, T. D. (2004) 'Impaired exercise performance in the heat is associated with an anticipatory reduction in skeletal muscle recruitment', *Pflugers Arch,* 448(4), pp. 422-30.

Turner, A. P., Cathcart, A. J., Parker, M. E., Butterworth, C., Wilson, J. and Ward, S. A. (2006) 'Oxygen uptake and muscle desaturation kinetics during intermittent cycling', *Med Sci Sports Exerc,* 38(3), pp. 492-503.

van der Vaart, H., Murgatroyd, S. R., Rossiter, H. B., Chen, C., Casaburi, R. and Porszasz, J. (2014) 'Selecting constant work rates for endurance testing in COPD: the role of the power-duration relationship', *COPD,* 11(3), pp. 267-76.

Vanhatalo, A., Doust, J. H. and Burnley, M. (2007) 'Determination of critical power using a 3-min all-out cycling test', *Med Sci Sports Exerc,* 39(3), pp. 548-55.

Vanhatalo, A., Fulford, J., DiMenna, F. J. and Jones, A. M. (2010) 'Influence of hyperoxia on muscle metabolic responses and the power-duration relationship during severe-intensity exercise in humans: a 31P magnetic resonance spectroscopy study', *Exp Physiol,* 95(4), pp. 528-40.

Vanhatalo, A., Poole, D. C., DiMenna, F. J., Bailey, S. J. and Jones, A. M. (2011) 'Muscle fiber recruitment and the slow component of O2 uptake: constant work rate vs. all-out sprint exercise', *Am J Physiol Regul Integr Comp Physiol,* 300(3), pp. R700-7.

Visser, M., Goodpaster, B. H., Kritchevsky, S. B., Newman, A. B., Nevitt, M., Rubin, S. M., Simonsick, E. M. and Harris, T. B. (2005) 'Muscle mass, muscle strength, and muscle fat infiltration as predictors of incident mobility limitations in well-functioning older persons', *J Gerontol A Biol Sci Med Sci,* 60(3), pp. 324-33.

Vogiatzis, I., Aliverti, A., Golemati, S., Georgiadou, O., Lomauro, A., Kosmas, E., Kastanakis, E. and Roussos, C. (2005) 'Respiratory kinematics by

optoelectronic plethysmography during exercise in men and women', *Eur J Appl Physiol,* 93(5-6), pp. 581-7.

Vogiatzis, I., Nanas, S. and Roussos, C. (2002) 'Interval training as an alternative modality to continuous exercise in patients with COPD', *Eur Respir J,* 20(1), pp. 12-9.

Vollaard, N. B., Constantin-Teodosiu, D., Fredriksson, K., Rooyackers, O., Jansson, E., Greenhaff, P. L., Timmons, J. A. and Sundberg, C. J. (2009) 'Systematic analysis of adaptations in aerobic capacity and submaximal energy metabolism provides a unique insight into determinants of human aerobic performance', *J Appl Physiol (1985),* 106(5), pp. 1479-86.

Wagner, J., Niemeyer, M., Infanger, D., Hinrichs, T., Guerra, C., Klenk, C., Königstein, K., Cajochen, C., Schmidt-Trucksäss, A. and Knaier, R. (2021) 'Verification-phase tests show low reliability and add little value in determining VO_{2max} in young trained adults', *PLoS One,* 16(1), pp. e0245306.

Wagner, P. D. (2005) 'Why doesn't exercise grow the lungs when other factors do?', *Exerc Sport Sci Rev,* 33(1), pp. 3-8.

Walsh, B., Stary, C. M., Howlett, R. A., Kelley, K. M. and Hogan, M. C. (2008) 'Glycolytic activation at the onset of contractions in isolated Xenopus laevis single myofibres', *Exp Physiol,* 93(9), pp. 1076-84.

Wang, N., Liu, Y., Ma, Y. and Wen, D. (2017) 'High-intensity interval versus moderate-intensity continuous training: Superior metabolic benefits in diet-induced obesity mice', *Life Sci,* 191, pp. 122-131.

Ward, M. E., Eidelman, D., Stubbing, D. G., Bellemare, F. and Macklem, P. T. (1988) 'Respiratory sensation and pattern of respiratory muscle activation during diaphragm fatigue', *J Appl Physiol (1985),* 65(5), pp. 2181-9.

Wasserman, K. (1978) 'Breathing during exercise', *N Engl J Med,* 298(14), pp. 780-5.

Wasserman, K., Van Kessel, A. L. and Burton, G. G. (1967) 'Interaction of physiological mechanisms during exercise', *J Appl Physiol,* 22(1), pp. 71-85.

Wells, G. D., Selvadurai, H. and Tein, I. (2009) 'Bioenergetic provision of energy for muscular activity', *Paediatr Respir Rev,* 10(3), pp. 83-90.

Wenger, H. A. and Bell, G. J. (1986) 'The interactions of intensity, frequency and duration of exercise training in altering cardiorespiratory fitness', *Sports Med,* 3(5), pp. 346-56.

Whipp, B. J. (2007) 'Physiological mechanisms dissociating pulmonary CO2 and O2 exchange dynamics during exercise in humans', *Exp Physiol,* 92(2), pp. 347-55.

Whipp, B. J. and Agostoni, P. (2007) 'Noninvasive estimation of the lactate threshold in a subject with dissociated ventilatory and pulmonary gas exchange indices: a case report', *Chest,* 132(6), pp. 1994-7.

Whipp, B. J., Davis, J. A., Torres, F. and Wasserman, K. (1981) 'A test to determine parameters of aerobic function during exercise', *J Appl Physiol Respir Environ Exerc Physiol,* 50(1), pp. 217-21.

Whipp, B. J. and Ward, S. A. (2009) 'Quantifying intervention-related improvements in exercise tolerance', *Eur Respir J,* 33(6), pp. 1254-60.

Whipp, B. J., Ward, S. A. and Wasserman, K. (1986) 'Respiratory markers of the anaerobic threshold', *Adv Cardiol,* 35, pp. 47-64.

Whipp, B. J. and Wasserman, K. (1972) 'Oxygen uptake kinetics for various intensities of constant-load work', *J Appl Physiol,* 33(3), pp. 351-6.

Widrick, J. J., Trappe, S. W., Costill, D. L. and Fitts, R. H. (1996) 'Force-velocity and force-power properties of single muscle fibers from elite master runners and sedentary men', *Am J Physiol,* 271(2 Pt 1), pp. C676-83.

Wilkinson, D. J., Piasecki, M. and Atherton, P. J. (2018) 'The age-related loss of skeletal muscle mass and function: Measurement and physiology of muscle fibre atrophy and muscle fibre loss in humans', *Ageing Res Rev,* 47, pp. 123-132.

Wisløff, U., Støylen, A., Loennechen, J. P., Bruvold, M., Rognmo, Ø., Haram, P. M., Tjønna, A. E., Helgerud, J., Slørdahl, S. A., Lee, S. J., Videm, V., Bye, A., Smith, G. L., Najjar, S. M., Ellingsen, Ø. and Skjaerpe, T. (2007) 'Superior cardiovascular effect of aerobic interval training versus moderate continuous training in heart failure patients: a randomized study', *Circulation,* 115(24), pp. 3086-94.

Wüthrich, T. U., Eberle, E. C. and Spengler, C. M. (2014) 'Locomotor and diaphragm muscle fatigue in endurance athletes performing time-trials of different durations', *Eur J Appl Physiol,* 114(8), pp. 1619-33.

Younes, M., Bilan, D., Jung, D. and Kroker, H. (1987) 'An apparatus for altering the mechanical load of the respiratory system', *J Appl Physiol (1985),* 62(6), pp. 2491-9.

Zierath, J. R. and Hawley, J. A. (2004) 'Skeletal muscle fiber type: influence on contractile and metabolic properties', *PLoS Biol,* 2(10), pp. e348.

Zoladz, J. A., Gladden, L. B., Hogan, M. C., Nieckarz, Z. and Grassi, B. (2008) 'Progressive recruitment of muscle fibers is not necessary for the slow component of VO2 kinetics', *J Appl Physiol (1985),* 105(2), pp. 575-80.