Limitations to exercise tolerance in health and disease

Matthew John Davies

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

October 2017

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

Acknowledgements

First and foremost I am sincerely grateful to my primary supervisor Dr Carrie Ferguson, your guidance, encouragement and enthusiasm for me and for our research has been unwavering throughout. Put simply, I would never have got here without you. Additionally, to Dr Al Benson and Prof Karen Birch, your support and patience throughout has been invaluable. It has been a fantastic experience and a pleasure to work alongside three such brilliant minds.

Thank you to all the study participants who donated both their time and effort. These studies would not have been possible without your whole-hearted involvement.

To all my fellow postgraduates who have provided support and entertainment throughout this experience, it has been great fun getting to know you as researchers and more importantly, friends. I would especially like to thank Gemma Lyall, Nick Renwick, Jack Garnham and Jen Airlie who I have shared this PhD journey with, you made the good times great, and the occasional bad moments less so.

To the wonderfully welcoming team over at the Cardiac Research Facility; Klaus Witte, John Gierula, Maria Paton and Judith Lowry, the work at the hospital could never of happened without you.

Special thanks go to Sarah, you have been my rock during this experience. Thank you for your patience and for putting up with me, but most importantly for your unwavering love and support throughout. Finally, thank you to my family, your love, support and encouragement have got me to where I am today.

Abstract

Exercise tolerance, the ability to sustain an exercise task, is a key determinant of performance, morbidity, mortality and quality of life. However, the fatigue mechanisms that underpin exercise tolerance remain poorly understood.

The aim of this thesis was to determine: 1) the origins of fatigue causing the limit of tolerance (LoT) during whole-body dynamic exercise in which $\dot{V}O_{2max}$ is attained, and 2) how this is altered by the task demands and in the presence of chronic heart failure (CHF). To assess this, maximum voluntary isokinetic cycling power was measured before, during and instantaneously at LoT of exercise, and compared to the task demands. To provide additional insight these power data were supplemented by gas exchange and electromyography measures.

First, a series of ramp-incremental exercise tests were performed, using different ramp-incrementation rates to change power demand at LoT. Next, the power-tolerable duration relationship was used to investigate the effect of altering the power demand during constant-power exercise. In both studies, reducing power demands, and as a consequence slowing the rate of energy utilisation and metabolite build-up, caused a significant reserve in maximal voluntary power at LoT to become increasingly evident.

A reduced exercise tolerance is a cardinal symptom of CHF, the consequence of fatigue and/or dyspnoeic sensations during exercise. At the LoT in CHF, the magnitude of difference between the maximal voluntary isokinetic power and task demands was different between individuals, suggesting this measurement may distinguish between individuals for which either the exercising muscles, or mechanisms proximal to this, are ultimately limiting exercise.

These data demonstrate that the origins of task failure at $\dot{V}O_{2max}$ can be altered, depending on the exercise task and health status. In future it is hoped these data can inform development of targeted strategies, aimed at increasing exercise tolerance, and as a consequence enhancing quality of life.

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Abbreviations

$\%HR_{peak}$ – percent peak heart rate

$\%\dot{V}O_{2max}$ – percent maximal oxygen uptake

$\%WR_{peak}$ – percent peak work rate

$\Delta\dot{V}O_2$ – change in rate of oxygen uptake

ΔWR – change in work rate

$\tau\dot{V}O_2$ – time constant of the rate of oxygen uptake

ADP – adenosine diphosphate

ATP – adenosine triphosphate

Ca^{2+} – calcium ions

CaO_2 – arterial content of oxygen

CHF – chronic heart failure

CNS – central nervous system

CO_2 – carbon dioxide

COPD – chronic obstructive pulmonary disease

CoV – coefficient of variation

CP – critical power

CvO_2 – venous content of oxygen

DM – diabetes mellitus

ECG – electrocardiogram

EMG – electromyography

$FADH_2$ – flavin adenine dinucleotide

H^+ – hydrogen ions

HR_{peak} – peak heart rate

K^+ – potassium ions

La^- – lactate concentration

LoT – limit of tolerance

LT – lactate threshold

LVEF – left ventricular ejection fraction

MVC – maximal voluntary contraction

$m\dot{V}O_2$ – muscle oxygen consumption

N_2 – nitrogen

Na^+ – sodium ions

NADH – nicotinamide adenine dinucleotide

O_2 – oxygen

P-T_{lim} relationship – power-tolerable duration relationship

P_{HYP} – peak hyperbolic power

Pi – inorganic phosphate

P_{ISO} – isokinetic power

Baseline P_{ISO} – isokinetic power at baseline

LoT P_{ISO} – isokinetic power at the limit of tolerance

P/O – oxygen cost of ATP re-synthesis

P/W – phosphate cost of force production

$\dot{Q}$ - cardiac output

RER – respiratory exchange ratio

RER_{peak} – peak respiratory exchange ratio

$RI\text{-}Flywheel_{peak}$ – peak power at the limit of tolerance of ramp-incremental test

RI10 – 10 W.min ramp-incremental test

RI25 – 25 W.min ramp-incremental test

RI50 – 50 W.min ramp-incremental test

RIT – ramp incremental test

ROS – reactive oxygen species

RPE – rating of perceived exertion

SR – sarcoplasmic reticulum

T_{lim} – time to the limit of tolerance

TMS – transcranial magnetic stimulation

VCO_{2peak} – peak carbon dioxide output

$\dot{V}_E/\dot{V}O_2$ – ventilatory equivalent of oxygen

$\dot{V}_E/\dot{V}CO_2$ – ventilatory equivalent of carbon dioxide

$\dot{V}_{Epeak}$ – peak ventilation

$\dot{V}O_2$ – rate of oxygen uptake

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

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

$\dot{V}O_{2SC}$ – slow component of oxygen uptake

WR – work rate

WR6 – work rate 6 min

WR12 – work rate 12 min

WR_{peak} – peak work rate

Professional Activities

Abstracts arising from this thesis

Davies MJ, Benson AP, Birch KM, Rossiter HB, Ferguson C. Power reserve at the limit of ramp-incremental exercise is affected by incrementation rate. *The FASEB Journal*, **30** (Supplement 1), pp.761-768. Experimental Biology Annual Meeting, San Diego, 2016.

Oral presentations of work from this thesis

Power reserve at the limit of ramp-incremental exercise is affected by incrementation rate. Internationally invited symposium entitled: *From Joseph Priestley's "dephlogisticated air" to magnetic resonance spectroscopy: A rich tradition of physiology in Leeds*. San Diego State University. 2016. (Chapter 3).

Mechanisms of exercise intolerance: power reserve at $\dot{V}O_{2max}$ is dependent on exercise duration. *University of Leeds and LABioMed International research collaboration symposium*. Leeds. 2016. (Chapter 4).

The role of central fatigue in limiting exercise tolerance in heart failure: the heart vs. head conundrum. *Multidisciplinary Cardiovascular Research Centre (MCRC) Retreat*. Lake District. 2017. (Chapter 5).

Other professional activities during PhD study

Manuscripts

Davies MJ, Benson AP, Cannon DT, Marwood S, Kemp GJ, Rossiter HB, Ferguson C. (2017). Dissociating external power from intramuscular exercise intensity during intermittent bilateral knee-extension in humans. *The Journal of Physiology* (*Epub ahead of print*) doi: 10.1113/JP274589.

Abstracts

Davies MJ, Lyall GK, Berry CK, Birch KM, Benson AP, Ferguson C. Predicting individual oxygen uptake responses to interval exercise in humans. *Proc Physiol Soc* **34**. The Physiological Society Annual Meeting, Cardiff, 2015.

Oral Presentations

Quantifying responses to interval training using cardiopulmonary exercise testing and magnetic resonance spectroscopy. *Multidisciplinary Cardiovascular Research Centre (MCRC) Retreat*. Lake District. 2015.

Chapter 1 Literature Review

1.1 Background

The ability to tolerate exercise, including activities of daily living, is related to the effectiveness of oxygen (O_2) transport and utilisation mechanisms, and thus effective integration of pulmonary, cardiovascular and neuromuscular systems. The ability to rapidly adapt and perform aerobic metabolism at high rates to meet the energy requirements of the task reduces the reliance on substrate-level phosphorylation energy systems, preventing the build-up of fatigue and preserving the ability of the skeletal muscles to produce force. When the demands of the exercise drive these systems to their maximal, the overall capacity of these integrated systems is measured as maximal oxygen uptake ($\dot{V}O_{2max}$), and is closely associated with the limit of exercise tolerance (LoT), occurring at different power outputs depending on the individual (Figure 1.1).

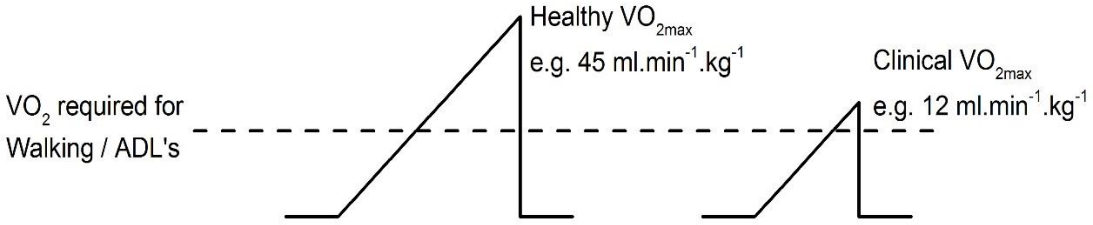

Figure 1.1: Schematic of a maximal ramp-incremental exercise test performed by two individuals, one healthy and one from a clinical population. The dashed line indicates an example $\dot{V}O_2$ requirement for walking and activities of daily living (ADL's), which is not different for these two individuals. However, this $\dot{V}O_2$ requirement occurs at a very different relative % of $\dot{V}O_{2max}$, highlighting how these activities are much more difficult for the clinical patient.

Therefore, delaying the attainment of the LoT via effective integration of these multiple physiological systems results in a greater exercise capacity. Conversley, a limitation in any of these systems will cause a reduction in exercise tolerance. Due to this association, an individuals exercise capacity has been shown in long term studies to be a strong predictor of all-cause mortality in both health, and chronic diseases (Figure 1.2; Myers et al., 2002).

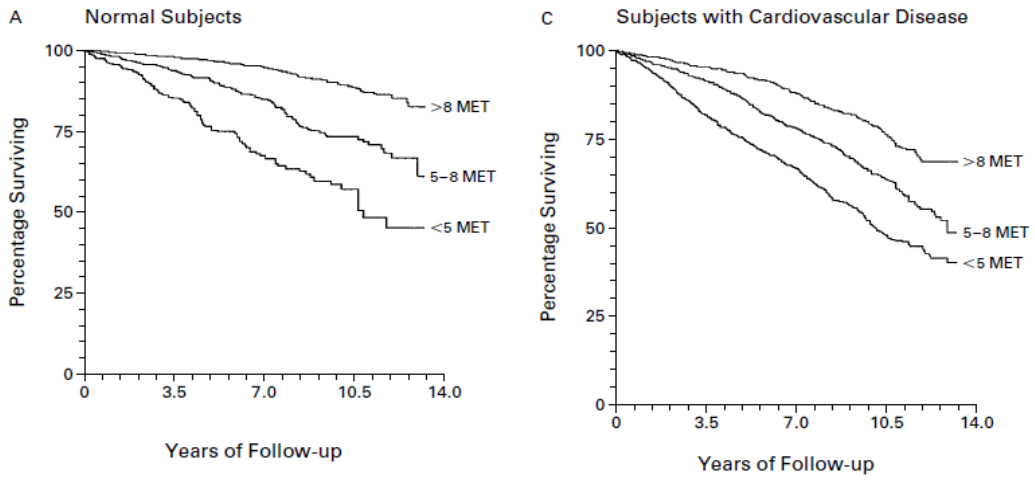

Figure 1.2: Survival curves for healthy individuals (left panel) and individuals with cardiovascular disease (right panel), stratified according to peak exercise capacity. 2534 healthy and 3679 cardiovascular disease individuals were included in the analysis (Myers *et al.*, 2002).

Fatigue, an exercise-induced reduction in power that is reversible with rest, is the consequence of mechanisms related to activation of skeletal muscle and within the exercising muscle itself, and is intimately linked to exercise tolerance (Amann, 2011). The deleterious effects of fatigue accumulation reduce the ability of the neuromuscular system to effectively generate the required force for continuation of the task, eventually resulting in the LoT being attained. Due to the implications for exercise tolerance and thus quality of life, fatigue and its physiological origins have been the subject a significant body of research. A

better understanding of exercise tolerance and the origins of the fatigue mechanisms that ultimately limit this are essential for improving exercise performance and quality of life in both health and disease. Nevertheless, a complete and definitive list of physiological processes that underpin fatigue during exercise and eventually result in the attainment of LoT, at $\dot{V}O_{2max}$, remains elusive.

1.2 Exercise energetics and oxygen uptake responses during exercise

The energy required to generate skeletal muscle contractions that allow for force production and continuation of exercise is produced by the hydrolysis of ATP. However, the intramuscular store of ATP is small (~6 mMol/kg wet weight) and therefore only capable of fuelling exercise for a few seconds (Wasserman and Whipp, 1975). Consequently, ATP needs to be re-synthesised from ADP and inorganic phosphate (Pi) via either aerobic or anaerobic energy systems. The proportional contribution from the different energy systems is a consequence of both the intensity and duration of the exercise (Morton and Hodgson, 1996), with the vast majority of ATP re-synthesis during sustained exercise provided by the most sustainable source of energy; aerobic metabolism, in the form of oxidative phosphorylation (Brooks, 2012). During oxidative phosphorylation an electrochemical gradient is formed across the inner mitochondrial membrane, fuelled by energy from the transfer of electrons down the electron transport chain (ETC) from NADH and $FADH_2$ (generated during glycolysis and the triboxylic acid cycle) to molecular O_2. The flow of H^+ down the electrochemical gradient created by the ETC is then used to re-synthesise ATP from ADP and Pi, using the enzyme ATPase. As O_2 is the final electron acceptor in this process, and intramuscular O_2 stores are small, the rate of aerobic metabolism can be

accurately determined by measuring the rate of muscle oxygen consumption ($m\dot{V}O_2$) (Wasserman and Whipp, 1975).

Direct measurement of $m\dot{V}O_2$, although complex, can be determined during exercise in humans via the Fick principle:

$$m\dot{V}O_2 = \dot{Q} \cdot (CaO_2 - CvO_2).$$

Equation 1.1

However, in order to perform these measures, a direct measurement of blood flow to the exercising muscle ($\dot{Q}$) is required (e.g. using a thermodilution technique), along with simultaneous measurement of the O_2 content of both arterial blood and venous effluent (CaO_2 and CvO_2, respectively) from the exercising muscle, requiring the placement of multiple catheters. The invasive nature of these experiments means they are very complex to complete (e.g. Andersen and Saltin, 1985, Poole *et al.,* 1991; Grassi *et al.,* 1996; Bangsbo *et al.,* 2000). As a result, a simpler, less invasive way of determining muscle O_2 consumption during exercise is necessary for this to be of routine use. The ability to accurately and continuously measure oxygen uptake measured at the mouth – pulmonary oxygen uptake ($\dot{V}O_2$) – was therefore a key development in the field of exercise physiology. Facilitated by the development of rapidly responding gas analysers and advances in computer technology, research was able to move on from using periodic collection of gas exchange with Douglas bags, to systems that enabled breath by breath measures to be calculated online and then stored for viewing and analysis at a later date (Whipp and Wasserman, 1972; Beaver et al., 1973). The algorithms from these original systems have since been refined (e.g. Beaver et al., 1981) and breath by breath gas exchange systems are now commonplace, using pulmonary $\dot{V}O_2$ as a proxy for $m\dot{V}O_2$, thus providing a

simple non-invasive way of measuring the metabolic response to the increase in energy demand that occurs during exercise.

The temporal profile of the $\dot{V}O_2$ response is dependent on the intensity of the exercise (Whipp, 1996; Ozyener *et al.,* 2001). Consequently, characterisation of the specific responses to exercise of different intensities is necessary to induce a common response across individuals and understand the bioenergetics of a specific exercise bout. Several methods are used throughout the literature to define exercise intensity: %maximal heart rate, %peak power and most commonly % $\dot{V}O_{2max}$, all of which can be derived from a maximal ramp-incremental exercise test. However, using a single variable to determine exercise intensity is problematic, as the parameters that induce a common physiological stress profiles during exercise (i.e. intensity demarcators) can be highly variable among different individuals. The implications of this have been presented by Rossiter (2011; Figure 1.3), where two male participants with the same $\dot{V}O_{2max}$ (3.15 $L \cdot min^{-1}$) exercised at a constant power of 215 W (85 %$\dot{V}O_{2max}$). While participant A could sustain this power for ~10 min before $\dot{V}O_{2max}$ and subsequently the LoT was attained, participant B was able to complete the 30 min protocol, and attain a submaximal steady-state $\dot{V}O_2$ (Figure 1.3; Rossiter, 2011). Clearly participant A was exercising at a higher intensity than participant B, thus prescription of exercise using a single parameter (e.g. %$\dot{V}O_{2max}$) can be inappropriate. Consequently, appropriate exercise intensity characterisation that provides responses that are reliably manifest across all individuals, independent of health and fitness level, are paramount.

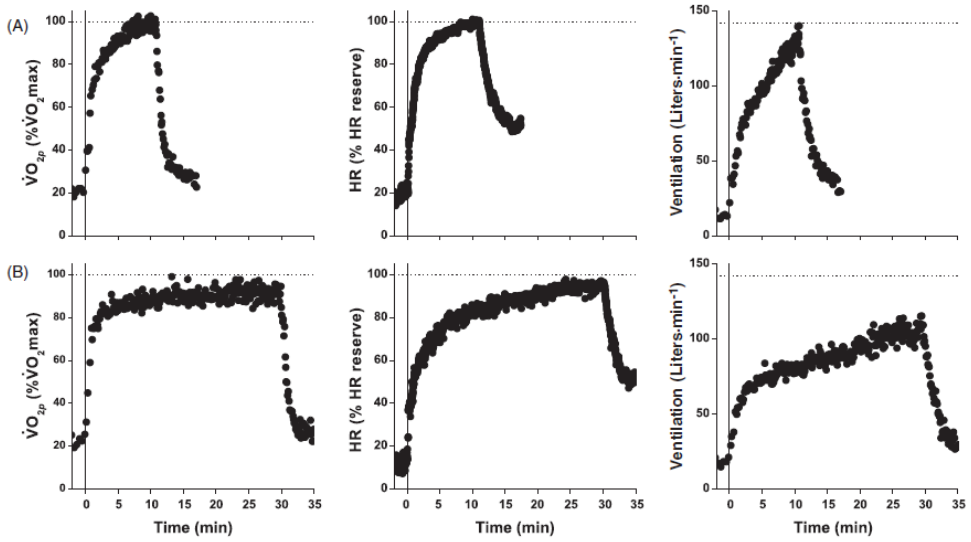

Figure 1.3: Pulmonary oxygen uptake ($\dot{V}O_2$), heart rate (HR) and ventilation ($\dot{V}_E$) responses of two individuals with the same $\dot{V}O_{2max}$ to a constant-power of 215 W (85 % $\dot{V}O_{2max}$ for both). Participant A could only sustain the work rate for ~ 10 min, whereas participant B was able to attain a steady-state and complete the entire 30 min protocol. Dotted lines indicate maximum for each variable. Figure from Rossiter (2011).

1.3 Classification of exercise intensity: exercise intensity domains

An alternative characterisation of exercise intensity that incorporates the profiles of pulmonary $\dot{V}O_2$, supplemented by blood lactate measurements, has been proposed by Whipp (1996). This characterisation consists of four exercise intensity "domains"; moderate, heavy, very heavy and severe. Robust, reliable and consistent prescription of exercise intensity based on these four domains has been shown across different exercise modalities such as: cycling (Ozyener *et al.,* 2001), running (Carter *et al.,* 2002), swimming (Demarie *et al.,* 2001) and knee-extension exercise (Koga *et al.,* 2005).

1.3.1 Moderate intensity exercise

Moderate intensity exercise is defined as any exercise during which there is no sustained metabolic acidosis (Whipp, 1996). The $\dot{V}O_2$ profile in this intensity domain is considered the “fundamental” response and is characterised as tri-phasic (Whipp *et al.,* 1982) (Figure 1.4). This 3-phase response is a consequence of $\dot{V}O_2$ being measured at the mouth, which causes a temporal dissociation at exercise onset between the change in venous O_2 content at the working muscles (that occur immediately), and when these changes are expressed at the lung - termed the limb-lung transit delay (~15-20 s in healthy individuals (Whipp and Ward 1982; Barstow *et al.,* 1990)). However, an increase in $\dot{V}O_2$ is still observed at exercise onset (Figure 1.4). This initial increase in $\dot{V}O_2$ is the result of increased pulmonary blood flow, due to increased cardiac output and venous return at exercise onset. Consequently, this phase I of the $\dot{V}O_2$ response has been termed the cardiodynamic phase (Whipp *et al.,* 1982) (Figure 1.4).

Following this initial cardiodynamic phase, phase II of the $\dot{V}O_2$ response has been shown to be a consequence of venous blood arriving at the lung from the exercising muscles (Barstow *et al.,* 1990; Grassi *et al.,* 1996). Despite the phase I temporal dissociation, phase II kinetics of pulmonary $\dot{V}O_2$ closely reflect $m\dot{V}O_2$ dynamics, with $\dot{V}O_2$ shown to reflect $m\dot{V}O_2$ to within 10 %; validated by simultaneous measurements of muscle and pulmonary $\dot{V}O_2$ (Grassi *et al.,* 1996) and computational modelling (Barstow *et al.,* 1990; Benson *et al.,* 2013). This phase II $\dot{V}O_2$ response, where $\dot{V}O_2$ is increasing toward the new metabolic requirement, consequent to changes in power output demand, is characterised by an exponential function (Figure 1.4).

The magnitude of the steady-state increase in $\dot{V}O_2$ is determined by the change in external power, with a relatively constant increase in $\dot{V}O_2$ per unit increase of WR ($\Delta\dot{V}O_2$ / ΔWR - "functional gain") between individuals of different age, sex and health status (~ 10 ml·min^{-1}·W^{-1} (Wasserman & Whipp, 1975)). The rate at which $\dot{V}O_2$ responds to this change in power and reaches the new metabolic requirement, is defined by the $\dot{V}O_2$ time constant ($\tau\dot{V}O_2$). Due to the relatively slow and finite $\dot{V}O_2$ response to the instantaneous change in energy demand that accompanies a step-change in power, there is a difference between $\dot{V}O_2$ and the $\dot{V}O_2$ required, termed the "oxygen deficit" (Figure 1.4).

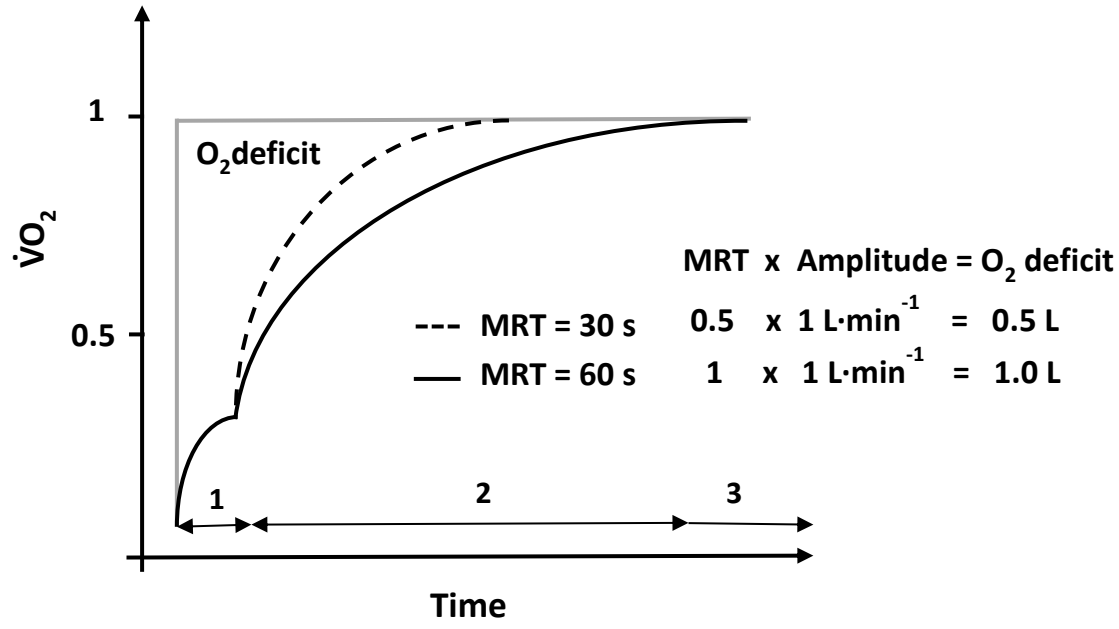

Figure 1.4: Schematic representation of the $\dot{V}O_2$ response to a step increase in work rate. For the solid line example, the three different phases of the $\dot{V}O_2$ response are delineated by the arrows. The schematic also shows how speeding the $\dot{V}O_2$ kinetics and therefore reducing the mean response time (MRT; the product of phase 1 and 2) to 30 s (dashed line) reduces the magnitude of the oxygen deficit. The step increase in ATP demand is outlined by the grey lines.

This O_2 deficit requires that the short fall in energy requirement must be supplemented by anaerobic energy sources, to buffer the increase in rate of ATP resynthesis and prevent any fall in the small ATP stores within the muscle (Chance *et al.,* 1985). Initially, ATP is derived from phosphocreatine (PCr), a finite and high energy store within the skeletal muscle. This PCr system provides an immediate but short-lived energy buffer, re-synthesising ATP via the creatine kinase reaction, a reversible, anaerobic and primarily cytosolic reaction. In addition, anaerobic glycolysis provides another anaerobic energy pathway to rapidly re-synthesise ATP, metabolising blood glucose and muscle glycogen as a high-energy store via ten enzymatically controlled reactions.

Although substrate-level phosphorylation is activated during moderate intensity exercise, this is not to the extent that the negative intramuscular consequences associated with these metabolic pathways can occur. At higher exercise intensities an increased reliance on these unsustainable finite energy stores causes an accumulation of the metabolites Pi (from PCr hydrolysis) and H^+ (from anaerobic glycolysis) that are related to muscle fatigue (discussed in sections; 1.3.3 and 1.5.2). The rate at which oxidative phosphorylation adapts to meet the energy demands (i.e. $\tau\dot{V}O_2$) determines the level of reliance on these anaerobic ATP processes (i.e. size of O_2 deficit; Figure 1.4) and therefore, has implications for the duration the exercise can be sustained (Murgatroyd *et al.,* 2011). Accordingly, $\dot{V}O_2$ kinetics are a strong indicator of health; with fast $\tau\dot{V}O_2$ and thus, small O_2 deficit found in athletes (Koppo *et al.,* 2004) and conversely, slowed $\dot{V}O_2$ kinetics have been observed in the elderly (Babcock *et al.,* 1994) in addition to heart failure (Sietsema *et al.,* 1994) and COPD (Nery *et al.,* 1982).

Within the moderate-intensity domain, the required steady-state in $\dot{V}O_2$ is achieved within 2-3 min, meaning all energy is provided aerobically, and termed phase III of the $\dot{V}O_2$ response (Figure 1.4). As the $\dot{V}O_2$ response remains below lactate threshold (LT; with no increase in blood La), exercise in this intensity domain is sustainable for long periods with modest effort, where eventual exercise intolerance occurs due to other factors such as energy provision, temperature and/or hydration status (Fukuba and Whipp, 1999).

1.3.2 Heavy intensity exercise

As the power demand increases above that associated with LT, a sustained metabolic acidosis occurs as a consequence of an increased reliance on anaerobic glycolysis, reflected in a sustained increase in blood La^- that will eventually stabilise (Whipp, 1996) (as $\dot{V}O_2$ stabilizes, meaning all the energy can be provided by sustainable, aerobic metabolism). Additionally, above LT there is delayed attainment of a steady-state $\dot{V}O_2$, as a consequence of an additional phase superimposed on the fundamental $\dot{V}O_2$ response. This additional phase is slow to develop and will drive the $\dot{V}O_2$ profile to a steady state value above that expected from the fundamental $\dot{V}O_2$/WR relationship. As a result of this “slow component”, in addition to its delayed onset (~2-3 min (Barstow and Mole, 1991), the attainment of this (greater than projected) steady-state $\dot{V}O_2$ is delayed ~10-15 min, causing a larger O_2 deficit and as a consequence, greater reliance on anaerobic energy systems (Poole *et al.,* 1991; Ozyener *et al.,* 2001).

While the fundamental phase of the $\dot{V}O_2$ response ($\Delta\dot{V}O_2/\Delta WR$ and $\tau\dot{V}O_2$) is not different from those expressed in the moderate domain, this additional $\dot{V}O_2$ slow component ($\dot{V}O_{2SC}$) causes the overall $\Delta\dot{V}O_2/\Delta WR$ during heavy intensity exercise to increase to ~12 $ml \cdot min^{-1} \cdot W^{-1}$, reflecting a greater O_2 cost of

performing the work rate than predicted from the moderate intensity response (Ozyener *et al.,* 2001).

The $\dot{V}O_{2SC}$ has achieved much attention in the literature regarding its origins. Numerous factors such as increased temperature, catecholamines and metabolic acidosis have been proposed but their contributions appear minor (Koga *et al.,* 1997; Gaesser *et al.,* 1994; Poole, 1994). Consequently, the overriding hypothesis remains that the majority (~80-90 %) of the $\dot{V}O_{2SC}$ originates from within the exercising muscles. This was first determined by Poole *et al.* (1991) who compartmentalised the origins of the $\dot{V}O_{2SC}$ using thermodilution techniques to directly measure blood flow and $\dot{V}O_2$, and later supported by the observation of an intramuscular PCr slow component associated with that of $\dot{V}O_2$ by Rossiter *et al.* (2002). It is suggested that during this higher intensity exercise, there is a requirement of increased motor unit recruitment to maintain force, as a result of muscle fatigue (Whipp, 1994). This results in an increased reliance on inefficient type II muscle fibres (Coyle *et al.,* 1992; Shinohara and Moritani, 1992) and consequently both a greater ATP cost of force production and/or O_2 cost of ATP resythesis (Cannon *et al.,* 2014).

The origins of the remaining ~10-20 %, although not definitive, have been suggested to be a consequence of the O_2 cost of the “rest of the body” such as the unmeasured work of auxiliary muscles (e.g. the arms for stabilisation during cycling) and ventilatory work (Aaron *et al.,* 1992; Jones *et al.,* 2011). Despite the debate of its exact origins, the importance of the $\dot{V}O_{2SC}$ remains; driving an increase in $\dot{V}O_2$ above that expected due to a reduced skeletal muscle efficiency, causing an increased perception of effort, with implications for exercise tolerance (Whipp, 1994).

1.3.3 Very heavy intensity exercise

As power increases further, blood La^- and $\dot{V}O_2$ (driven by the $\dot{V}O_{2SC}$) are unable to reach a steady-state, instead rising inexorably until the LoT is attained (Whipp, 1996), typically at or shortly after $\dot{V}O_{2max}$ (Coats *et al.,* 2003). The highest power at which a steady-state $\dot{V}O_2$, La and H^+ can be attained has been termed critical power (CP). Thus, this CP is the upper boundary of heavy intensity exercise, and is therefore the delineating variable between heavy (sustainable) and very-heavy (unsustainable) intensity exercise (Whipp, 1996). For work rates performed above CP, a well-established relationship has been characterised that describes a hyperbolic association between power and the duration the exercise can be sustained; the power-tolerable duration ($P\text{-}T_{lim}$) relationship (Moritani *et al.,* 1981; Poole *et al.,* 1988).

1.3.3.1 Power-tolerable duration relationship

This relationship for supra-CP constant-power exercise between the work rate performed and T_{lim} has been demonstrated consistently across different exercise modalities to be defined by a hyperbolic function (e.g. cycling; Moritani *et al.,* 1981; Poole *et al.,* 1988; running; Hughson *et al.,* 1984; knee-extension; Jones *et al.,* 2008 and swimming; Wakayoshi *et al.,* 1992):

$$T_{lim} = W' / (P - CP)$$

Equation 1.2

where CP is the asymptote and W' is the curvature constant parameter of the hyperbola (Monod and Scherrer, 1965) (Figure 1.5). This mathematical model predicts that once CP is exceeded, only a fixed amount of work can be performed before the LoT is then attained, represented by W' (Figure 1.5) (Moritani *et al.,* 1981; Hill, 1993; Fukuba *et al.,* 2003). As a result, the physiological mechanisms that W' represents is an area of particular interest. Initially, it was surmised that

W' is equivalent to an individual's anaerobic work capacity (analogous to the O_2 deficit), comprising a finite intramuscular energy store (PCr, glycogen and stored O_2) that once depleted causes exercise cessation (Monod & Scherrer, 1965; Moritani *et al.,* 1981; Hill, 1993). Alternatively, it has been suggested that W' actually represents a finite amount of fatigue metabolite build up (e.g. H^+, Pi and K^+) that can be tolerated before LoT is reached (Coates *et al.,* 2003; Fukuba *et al.,* 2003). This build-up of fatigue metabolites is the consequence of an increased contribution of substrate-level phosphorylation to energy demand in high-intensity exercise, with fatigue metabolites shown to impair actin-myosin cross-bridge formation and consequently force production (Vollestad, 1997; Westerblad *et al.,* 2002; Allen *et al.,* 2008) (specific peripheral fatigue mechanisms discussed later; 1.5.2).

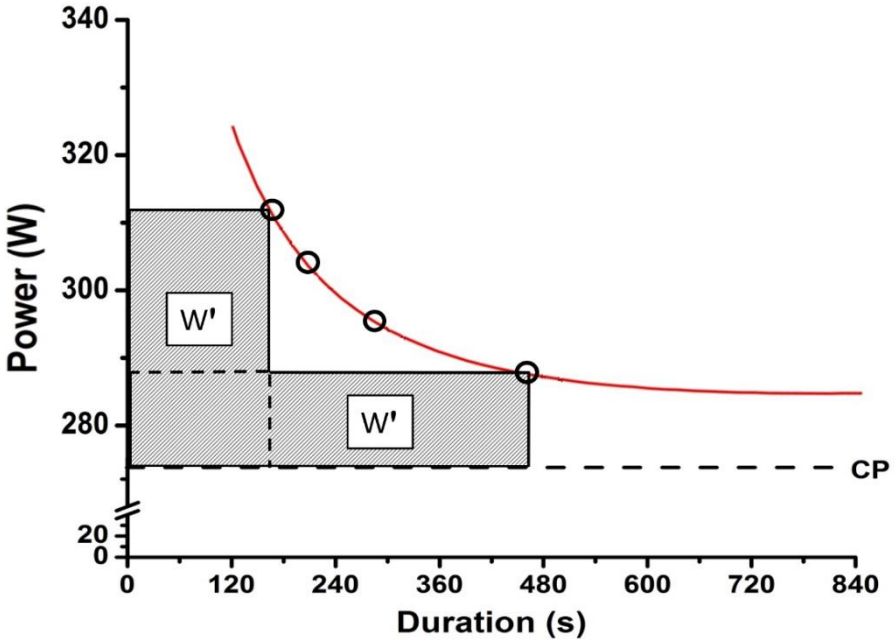

Figure 1.5: A schematic representation of the hyperbolic power-tolerable duration relationship for exercise above critical power (CP). The 4 points denote the tolerable duration for the 4 selected work rates, with these fit by a hyperbolic function (red line). Also shown is the fixed amount of work that can be completed for all work rates above CP (W'). Note this W' does not change with changing power demand and exercise duration.

A physiological cascade has been proposed by Murgatroyd and Wylde (2011) as a schematic that links the $\dot{V}O_2$ response with the fatigue responses and links the processes described above during supra-CP (i.e. very-heavy intensity) exercise that eventually culminates in the LoT. Depletion of energy stores and accumulation of fatigue metabolites (i.e. W' depletion) results in skeletal muscle fatigue and the requirement for recruitment of additional, less efficient muscle fibres (Murgatroyd *et al.,* 2011). As a consequence there is both an increased energy and O_2 cost of exercise manifest as the $\dot{V}O_{2sc}$ (Cannon *et al.,* 2014), driving the $\dot{V}O_2$ response to $\dot{V}O_{2max}$ (Whipp, 1994), closely followed by intolerance when these reach their respective limits (Murgatroyd and Wylde, 2011; Figure 1.6).

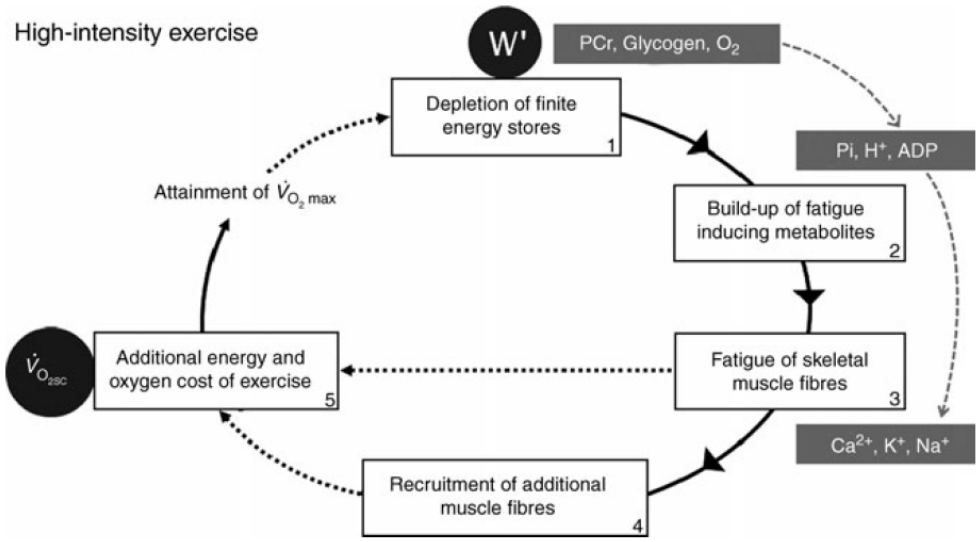

Figure 1.6: The proposed cascade of events that ultimately lead to the limit of tolerance being attained in high-intensity exercise. Known mechanisms are denoted by solid arrows, with proposed mechanisms denoted by dotted arrows. Schema proposed by Murgatroyd and Wylde, 2011.

The ability to accurately predict the tolerable duration of a specific power through characterisation of the P-T_{lim} relationship has implications for both investigations

into the effect of exercise task on the fatigue mechanisms limiting exercise (e.g. Vanhatalo *et al.,* 2010; Burnley *et al.,* 2012), and for quantifying the magnitude of improvement from interventions (Whipp and Ward, 2009). However, an important caveat that needs to be considered for the current work is that although the P-T_{lim} relationship can predict exercise tolerance over a range of exercise durations; 2-30 min (Poole *et al.,* 1988; Hill, 1993; Fukuba and Whipp, 1999), it is unlikely to extend to exercise outside these durations, and exercise where $\dot{V}O_{2max}$ is not attained. This is due to confounding factors such as limitations of mechanical force (during very high power - short duration exercise) and substrate provision, thermoregulation and/or hydration status (during longer exercise) (Fukuba and Whipp, 1999). Consequently, the investigations presented here are limited to exercise in which $\dot{V}O_{2max}$ is attained, and therefore any inferences regarding very short (< 2 min) or longer (> 30 min) exercise tasks without the attainment $\dot{V}O_{2max}$, should be approached with care and are not discussed in detail throughout.

1.3.4 Severe intensity exercise

Within the intensity domains described by Whipp (1996), severe intensity exercise is demarcated by $\dot{V}O_{2max}$, where any power that has a $\dot{V}O_2$ requirement (based on 10 $ml \cdot min^{-1} \cdot W^{-1}$) above an individual's $\dot{V}O_{2max}$ is characterised as within this domain. During severe exercise $\dot{V}O_2$ accelerates rapidly, attaining $\dot{V}O_{2max}$ and subsequently the LoT. The speed of this increase causes the presence of the $\dot{V}O_{2SC}$ to be precluded, causing the $\dot{V}O_2$ profile to revert to a simple (phase II) mono-exponential once again. This lack of $\dot{V}O_{2SC}$ is primarily due to the $\dot{V}O_{2SC}$ being of delayed onset (or indiscernible for 1-3 min), therefore not given time to manifest in the short tolerable exercise duration (Ozyener *et al.,* 2001).

A summary of the $\dot{V}O_2$ profile responses to the different exercise intensity domains and the physiological parameters which provide the boundaries between these, outlined by Whipp (1996), are summarised in Figure 1.7.

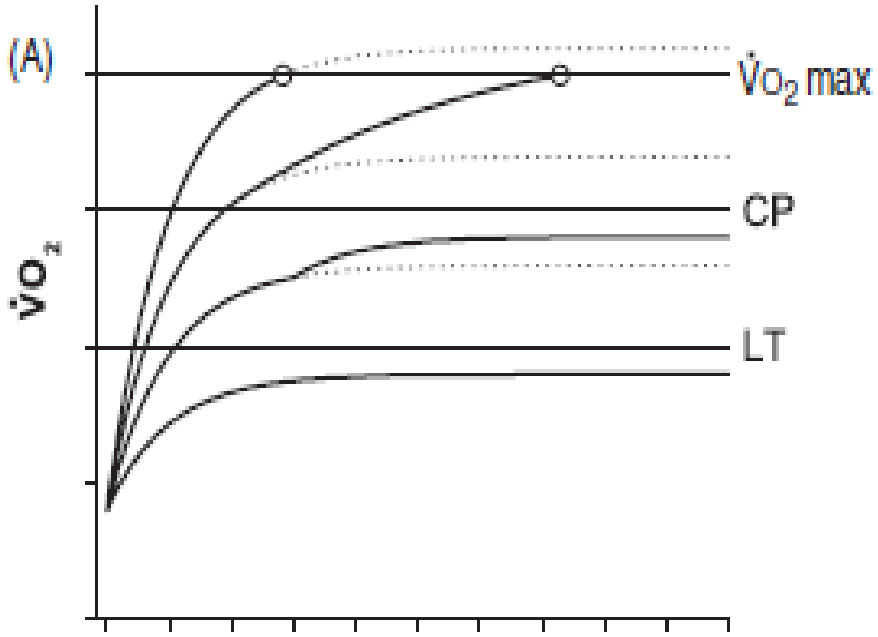

Figure 1.7: A summary of the $\dot{V}O_2$ response to exercise within the 4 proposed intensity domains. Also shown are the parameters that demarcate these domains; Lactate threshold (LT), critical power (CP) and maximal oxygen uptake ($\dot{V}O_{2max}$). The dotted lines represent the predicted $\dot{V}O_2$ response, based on sub-LT $\dot{V}O_2$-work rate relationship. The limit of tolerance is denoted by the open circles. Figure from Rossiter, 2011.

While a slightly alternative exercise intensity construct has been proposed by Hill *et al.* (2002), whereby the highest exercise intensity domain (termed extreme) is defined by whether or not the exercise duration is long enough to attain $\dot{V}O_{2max}$, for simplicity in this thesis, the original intensity domains outlined by Whipp (1996) will be used henceforth.

1.4 Ramp-incremental exercise

The ramp-incremental exercise test spans an individual's metabolic range, progressively challenging both motor unit recruitment and metabolism. As a consequence, this exercise test provides the ability to estimate most of the metabolic thresholds (apart from CP) that demarcate exercise intensity domains (Wasserman *et al.,* 1987). At the onset of the ramp-incremental test there is a delay in in pulmonary $\dot{V}O_2$ due to the limb-lung transit delay, similar to constant-power exercise (Whipp *et al.,* 1982; Whipp and Ward, 1982). This delay between the changing power demand and the pulmonary $\dot{V}O_2$ response at exercise onset, added to the kinetics of the $\dot{V}O_2$ response, are described by the mean response time. After this initial increase, the $\dot{V}O_2$ response progresses as a linear function, determined by the rate of the linear increase in power and the functional gain; 10 $ml \cdot min^{-1} \cdot W^{-1}$. As a result a steady-state in $\dot{V}O_2$ is not attained and $\dot{V}O_2$ continually rises, making $\dot{V}O_2$ kinetics difficult to determine from this test, determined instead as the mean response time (consisting of phase I and II of the $\dot{V}O_2$ response) (Rossiter, 2011). The $\dot{V}O_2$ response will continue to rise with the power demand until $\dot{V}O_{2max}$ is reached and shortly after, the LoT is attained. An additional benefit with this exercise test is that the duration can be altered depending on the individual by changing the rate of work rate increment in order to optimise the exercise duration; 8-12 min (Buchfuhrer *et al.,* 1983). This duration allows for adequate data to be collected for estimation of the desired variables, but not extending the duration too far so that external factors such as motivation have an influence on the responses measured (Buchfuhrer *et al.,* 1983).

1.5 Exercise tolerance and fatigue

As discussed, the attainment of the LoT (both ramp-incremental and supra-CP constant-power exercise) is intimately linked to the development of fatigue. However, “fatigue” is an all-encompassing term that can refer to a range of different mechanisms that may occur in parallel and at multiple sites. While a lot of these will be touched on, the scope of this thesis is primarily focused on neuromuscular fatigue during exercise. Neuromuscular fatigue is defined as any fatigue within the motor pathway; from the motor cortex, via the spinal cord and motorneurones to the exercising skeletal muscles, with the consequences of this fatigue a reduction in the ability of the working muscles to produce force, reversible with rest (Amann, 2011). While the specific origins are still debated, there is general agreement that neuromuscular fatigue mechanisms can be categorised as either central or peripheral in origin (Summarised in Figure 1.8). However, the integration and acute plasticity of these different mechanisms remains not well understood.

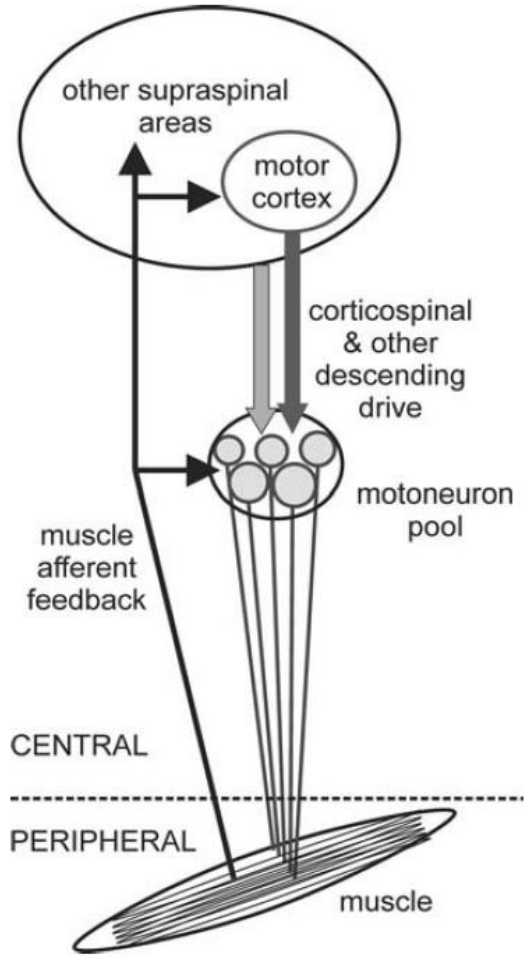

Figure 1.8: A schematic representation summary of the origins of neuromuscular fatigue; both central and peripheral fatigue mechanisms (Taylor *et al.*, 2016).

1.5.1 Central Fatigue

Central fatigue is defined as any fatigue that originates proximal to the neuromuscular junction. To produce the force required to perform a task, efferent signals are relayed from the central nervous system (CNS) to the exercising muscles, activating the motor neurone and signalling for muscle contraction. Therefore, any alterations within the CNS that reduces this central motor drive to the exercising muscles, or reduce its effectiveness, can contribute to any loss in force production (Gandevia, 2001). However, there remains multiple sources within the CNS that have been postulated to result in central fatigue.

The efferent signals that eventually result in muscle contractions and movement originate from within the motor cortex in the brain. Any reduction in these efferent signals from the motor cortex is termed supra-spinal or cortical fatigue (Taylor and Gandevia, 2001). This cortical fatigue may be a consequence of alterations in the concentrations of certain neurotransmitters within the brain. These neurotransmitters, primarily serotonin, dopamine and noradrenaline play a role in communications between neurones both in the brain and in more distal neuronal pathways (Taylor *et al.,* 2016). Serotonin has been suggested to play role in the development of fatigue due to its influences on mood and lethargy (Romanowski and Grabiec, 1974). However, when increasing levels of serotonin, while some authors have seen a reduction in exercise performance and increased perception of effort (Wilson and Maughan, 1992; Marvin *et al.,* 1997; Struder *et al.,* 1998), this has not been corroborated by others (Pannier *et al.,* 1995; Meeusen *et al.,* 2001; Roelands *et al.,* 2009).

There have been similar findings with dopamine and noradrenaline, with some authors reporting negative effects on performance with drug-induced alterations

in neurotransmitter concentrations (Roelands *et al.,* 2008a) and others unable to replicate this (Piacentini *et al.,* 2002). Overall it seems that altering the levels of neurotransmitters using drug interventions has varied results, therefore while uncertain, the role of neurotransmitters cannot be definitively ruled as a potential mechanisms contributing to cortical fatigue during exercise in normal temperatures.

Despite the uncertainty surrounding the mechanisms, cortical fatigue seems to be a well-founded phenomenon. When comparing the ability of the motor cortex to voluntarily recruit the exercising muscle maximally, to when external stimulation of the motor cortex is employed, a reduced force has been found during and immediately after exercise (Gandevia *et al.,* 1996; Gruet *et al.,* 2014; Jubeau *et al.,* 2014), suggesting a reduced capacity of the motor cortex to drive the muscle maximally. However, one of the key distinctions that cannot be discerned using current methodologies is whether cortical output is reduced, or whether the same cortical output has become less effective due to changes distal to the motor cortex, such as reduced motorneurone excitability (Taylor and Gandevia, 2001).

The motorneurone and the muscle fibres it innervates are defined together as a motor unit. Activation of these motor units culminates in the generation of force. Motor units are recruited and de-recruited depending on the exercise task demands in order to maintain the force required, with these recruited in size order; Hennemans's size principle (Henneman and Mendell, 1981). Once recruited, the firing rates of action potentials from the motor neurones down the axon to the muscle fibre can modulate the force production further (Heckman and Enoka, 2012). The excitability of all motor units together (termed the

motorneurone pool), depends on the balance between excitation, that is increased with muscle activation, and inhibitory mechanisms increased by exercise induced fatigue (Weavil *et al.,* 2016). Reduced excitation manifests as a reduced firing rate of the motorneurones and fewer action potentials reaching the muscle fibre, causing reduced fibre recruitment and less force to be produced. The underlying mechanisms of this reduced firing rate are yet to be definitively determined, although the repetitive action of motorneurones has been suggested to make it less excitable therefore requiring greater neural drive, possibly as a consequence of changes to the intrinsic properties of the motorneurones (Johnson *et al.,* 2004).

Another key determinant thought to influence the firing rate, and central fatigue as a whole, are small diameter group III (thinly myelinated) and group IV (unmyelinated) muscle afferents, sensitive to mechanical and metabolic stimuli, respectively. The signalling from these muscle afferents back to the CNS are hypothesised to induce inhibition that reduces excitability. While the specific site of action (spinal or cortical) remains unclear, it is generally agreed that increased feedback from these afferents about the state of the exercising muscle during fatiguing exercise is responsible, at least in part for the reduction in output from the motornuerones (Taylor *et al.,* 2016). The influence of this afferent feedback has been demonstrated using a pharmacological agents to block feedback during exercise. In the presence of afferent blockade, a 8.9 % and 9 % greater EMG signal was present during a 5 km time trial (Amann *et al.,* 2008) and at the LoT (Amann *et al.,* 2011), respectively, suggesting increased muscle recruitment. Consequently, fatigue within the muscles was 13 % greater after a 5 km time trial (Amann *et al.,* 2009) and 10 % greater at exhaustion (Amann *et*

al., 2011) compared with placebo, emphasising the potential importance of these muscle afferents in regulating fatigue.

While the focus of this afferent feedback is generally centred around fatigue and the negative consequences being relayed to the CNS, it must be remembered these muscle afferents are also responsible for the feedback that increases ventilation and vasodilation as required (Kaufman and Forster, 1996). This increased O_2 delivery to the exercising muscles instigated by afferent feedback may actually attenuate certain aspects of muscle fatigue (Amann *et al.,* 2011). Therefore, when afferent feedback is pharmacologically blocked this will inhibit these responses, evidenced by a reduced ventilation response reported in the presence of afferent blockade compared to placebo (158 vs. 151 $ml.min^{-1}$; Amann *et al.,* 2009), and potentially confounding these results to an extent.

1.5.2 Peripheral fatigue

Peripheral fatigue refers to any fatigue that occurs distal to the neuromuscular junction, within the skeletal muscle. As discussed previously, during high-intensity exercise there is a large demand for energy that cannot be met solely by aerobic metabolism and therefore, there is an increased reliance on anaerobic energy sources. The consequence of this is that by using PCr and glycolysis energy pathways, metabolites are produced, namely H^+ and Pi. This fact is magnified as the exercise continues as larger, fast twitch (type II) muscle fibres are recruited which are less oxidatively efficient and more fatigable, therefore causing these metabolic disturbances to be even greater (Grassi *et al.,* 2015). Both Pi and H^+ have been repeatedly associated with the development of peripheral fatigue and the consequent reductions in force production from the contractile apparatus, despite maximal activation (Allen *et al.,* 2008), with this

implicated in the ultimate attainment of LoT for supra-CP exercise (Murgatroyd and Wylde, 2011). This is supported by findings that both Pi accumulation and reductions in pH (as a result of increased H^+) develop to the same level at task failure, independent of exercise duration and power demand (Vanhatalo *et al.,* 2010; Burnley *et al.,* 2010; Chidnok *et al.,* 2013b). Additionally, these consistent levels of metabolite build-up are accompanied by the same levels of overall peripheral fatigue at the LoT (Burnley *et al.,* 2012), although this remains disputed (Froyd *et al.,* 2016).

Initially it was deemed that the build-up of lactate and the accompanying muscle acidosis was the primary mechanism of contractile inhibition during exercise, due its close temporal relationship with decreases in force (Allen *et al.,* 2008). However, it is becoming evident that the effect of H^+ was potentially overstated and the consequences on force production are in fact less than first thought. This is supported by the poor correlation found between changes in pH and contractile function in skinned muscle fibres (Westerblad *et al.,* 1997). Additionally, force production has been shown to recover much quicker than pH at task failure, displaying that pH is clearly not solely responsible for the decreases in force production (Sahlin and Ren, 1989). Consequently, while it cannot be questioned that a consequence of high-intensity exercise is a reduced intramuscular pH, the direct influences of this on force production, while still present, may not be as significant as initially proposed (Westerblad, 2016).

The accumulation of Pi as a consequence of the creatine kinase reaction has gained much focus in the literature (Allen *et al.,* 2008). It is now believed that this build-up of Pi may have a greater influence on force than the metabolic acidosis (Westerblad *et al.,* 2002). Various mechanisms have been shown in support of

Pi inhibition of cross-bridge interactions. By independently increasing Pi concentration in skinned muscle fibres, it has been shown to reduce the sensitivity of Ca^{2+} binding to troponin C (Martyn and Gordon 1992). Additionally, it has been found that increased Pi may inhibit the release of Ca^{2+} from the SR by inhibiting the ryanodine receptors and/or effecting the amount of Ca^{2+} available for release from the SR by entering the SR and combining with Ca^{2+} (Allen and Westerblad, 2001). Both these mechanisms will ultimately reduce the availability of Ca^{2+} and as a consequence, reduce the number of actin-myosin cross-bridges that can be formed, reducing the force the muscle produces.

Another avenue of investigation has been the effects of exercise on action potential propagation. Specifically, it has been shown that repeated high-intensity muscle contractions that require increased action potential rate, result in a net K^+ efflux and thus, accumulation of extracellular K^+ (Clausen, 2003). These alterations in concentrations of Na and K^+ during exercise will affect the electrochemical gradients and could cause failure of excitation, and consequently reduced force production (Cairns *et al.,* 2017).

Together the accumulation of these fatigue metabolites (H^+, Pi and K^+) are a large contributing factor in the development of peripheral fatigue and the accompanying loss of force during exercise (Allen *et al.,* 2008). However, these metabolites may play an additional role in fatigue development via the muscle afferents discussed above, with increases in these metabolites potentially supplying the stimulus for an increase in afferent feedback to the CNS from the exercising muscle that causes an increased neural drive and ultimately, central fatigue (Amann *et al.,* 2009; Amann, 2011).

1.6 Traditional methods of neuromuscular fatigue assessment

To investigate central and peripheral fatigue limitations to exercise, external stimulation to induce the maximal potential of the muscle is a technique that has consistently been used in the literature. This removes the volitional aspect of fatigue to ensure a true reflection of the state of the exercise muscle and in turn removes any overestimations of fatigue that are actually attributable to a participants lack of effort or motivation immediately post-exercise (Cairns *et al.,* 2005). External stimulation research generally involves stimulating the muscle, peripheral nerve or motor cortex using either electrical or magnetic stimulators before and after a bout of fatiguing exercise. When this stimulation is coupled with maximal voluntary contractions of the desired muscle group, this can provide information about the development of both central and peripheral fatigue.

Electrical stimulation involves stimulating either the muscle belly with surface electrodes or the peripheral nerve using a small cathode inserted over the nerve. Prior to exercise a stimulus that provides maximal innervation of the entire muscle under investigation must be determined. This is achieved using a ramp protocol until a plateau in force generating capacity is reached, with this frequency then used post-exercise (Edwards *et al.,* 1977). As mentioned, this external stimulation enables the maximal twitch force of the muscle to be measured, with changes in force from pre to post-exercise providing measurements of fatigue that has occurred within the exercising muscle (i.e. peripheral fatigue) (Lepers *et al.,* 2002). However, electrical stimulation induces discomfort and/or pain, with the origins of pain dependent on the technique used. Surface muscle electrical stimulation has been suggested to also stimulate the nerve endings in the skin and thus, cause pain (Man *et al.,* 2004). Alternatively,

direct nerve stimulation requires insertion of the cathode into the musculature, which is a potential source of discomfort. Furthermore, electrical stimulation independent of method of delivery, has been reported to induce varying degrees of pain in certain circumstances such as; tetanic stimulation and certain muscle groups e.g.; diaphragm or within certain groups; the elderly or clinical populations (Man *et al.,* 2004). Despite this, electrical stimulation does seem to be well tolerated in young, healthy individuals who are familiarised to the techniques (e.g. Verges *et al.,* 2009).

The alternative method of stimulation uses a rapidly changing magnetic field to produce a weak electrical current capable of exciting the underlying neural tissue of the desired site (Man *et al.,* 2004). The benefit of this method of stimulation is that it is well tolerated in all groups, leading to it being widely used in groups such as the elderly and COPD patients (Man *et al.,* 2003). While magnetic stimulation uses muscle stimulation such as that used with electrical methods, crucially this stimulation occurs at a deeper level, avoiding the nerve endings in the skin associated with pain (Bustamante *et al.,* 2010). However, there are technical limitations with this technique. The power that can be generated by these magnetic stimulators is significantly less than their electrical counterparts and as a consequence, performing maximal contractions in larger muscle groups and/or larger individuals can be unachievable (Hamnegard *et al.,* 2004). A suggested solution to this issue is to use two stimulators in tandem, although this may be limited by practicality and cost. An additional limitation of magnetic stimulation is the wider field of stimulation provided by the apparatus compared to the more direct electrical methods which, although making the administration easier for experimenters, can result in the co-activation of nearby muscles (Gandevia, 2001). As a consequence, a reduced twitch may be measured (interpreted as

increased fatigue), when in fact this is the result of other muscles not being measured, unintentionally receiving part of the stimulation.

Independently, both of these techniques can provide reliable information on the reduction in twitch force and thus development of peripheral fatigue after a bout of exercise. Verges *et al.* (2009) found that measurements of force and M wave characteristics did not differ across the two stimulation methods in the quadriceps muscle in both the fresh and fatigued state, therefore providing equally robust fatigue assessment. However, external stimulation applications can be extended further when combined with maximal voluntary contractions (MVC), termed the interpolated twitch technique (Figure 1.9). This technique involves superimposing the force an individual can produce voluntarily over the force produced when the same muscle is maximally innervated by stimulation. The difference between these (i.e. how much lower MVC is than stimulated force) gives a measure of the amount of available muscle fibres that are not being voluntary innervated. When measured before and after exercise, a reduction in this voluntary activation gives an indicator of the magnitude of central fatigue (Merton, 1954). Consequently, combining the reductions in twitch force measured with reductions in this voluntary activation enables the interpolated twitch technique to give measures of both central and peripheral fatigue (Figure 1.9.) (e.g. Burnley *et al.,* 2012).

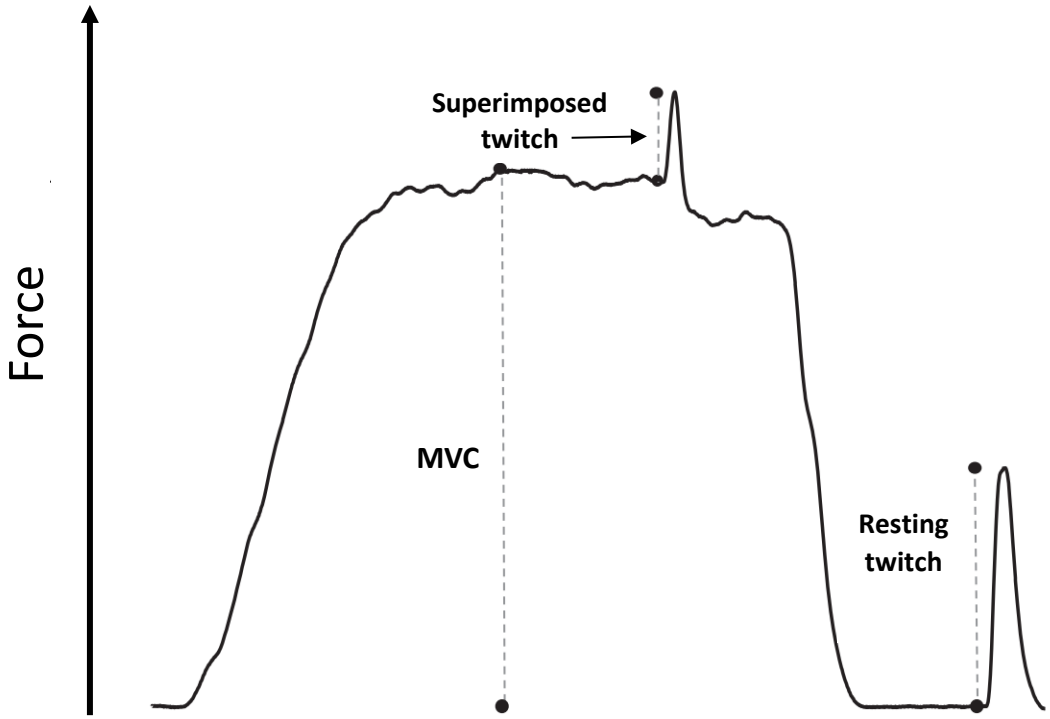

Figure 1.9: A representative example of the interpolated twitch technique. This protocol consists of superimposing a maximal external stimulation, over a maximal voluntary contraction (MVC). Post-exercise changes in the "resting twitch" are considered peripheral fatigue and changes in the "superimposed twitch" are attributed to reduced voluntary activation (i.e. central fatigue). Therefore, this protocol allows determination of both peripheral and central fatigue mechanisms. Adapted from Maffeuletti *et al.* (2016).

Specific methodological limitations to this technique such as; sensitivity during maximal contractions, intermuscular differences, synergistic/antagonistic activation and the effects of joint angle are of considerable debate in the literature (de Haan *et al.,* 2009; Taylor, 2009). However, there remains one overriding limitation with this technique, in that the impairment in voluntary activation could be occurring anywhere proximal to the site of stimulation, so there is no information about where within the CNS the impairment is present (Twomey *et al.,* 2017). Transcranial magnetic stimulation (TMS), the process of stimulating the motor cortex within the brain may provide insight into this. This technique uses a magnetic field to cause an electrical impulse within the motor cortex,

which in turn stimulates the neuronal tissue and causes increased descending efferent cortical activity (Gandevia, 2001). As a consequence, it has been found that when TMS is performed during a MVC, the force that is produced is greater than without TMS, suggesting sub-optimal cortical output is at least in part limiting force during the MVC (Taylor and Gandevia, 2001). Therefore, when performed after exercise this can provide information regarding the origins of central fatigue by isolating the supra-spinal contributions.

As it uses magnetic stimulation, TMS is well tolerated and can be widely used in all populations (Man *et al.,* 2004), although this method does have its own limitations. TMS will only increase the force of an MVC; although it may produce a measurable output, it cannot produce maximal force from relaxed muscle, so MVCs must be used as the control protocol. However, possibly the main limitation of TMS is the lack of specificity; a single desired muscle is not being directly stimulated, therefore by stimulating the motor cortex, this may induce a response in many muscles throughout the body, including the antagonist of the muscle of interest, potentially reducing the force produced (Taylor *et al.,* 2000). Finally, although the motor cortex is the site of stimulation, when TMS is used alone, reduced voluntary activation after exercise cannot definitely be determined as a consequence of reduced cortical output, as inhibition at the spinal level may play a significant and unmeasured role (Taylor and Gandevia, 2001).

A key technique used throughout fatigue literature, independent of the stimulation method is electromyography (EMG), either surface or fine wire. EMG provides a measure of the electrical activity of the motor units within the muscle of interest. The overall EMG signal is a composite of the number and discharge

rate of the motor units (Gonzalez-Izal *et al.,* 2012). Consequently, a larger EMG signal is associated with greater muscle recruitment and larger force production. However, the direct relationship between motor unit action potentials and the number of muscle fibre action potentials that will contribute to the EMG signal is uncertain (Enoka and Duchateau, 2015). Consequently, some authors believe the EMG amplitude does not provide a direct measure of neural drive (Farina *et al.,* 2010). This issue is compounded by the presence of fatigue (Dideriksen *et al.,* 2011) and changes in muscle length during dynamic contractions (Farina, 2006). Additionally, whether specific changes such as fibre type and motor unit recruitment can be determined using EMG remains debated (Vinzenz VonTscharner and Nigg 2008; Farina, 2008). Despite these potential limitations, EMG activity amplitude can still provide a useful tool that gives an indication of alterations in overall neural activation of the muscles (e.g. Coelho *et al.,* 2015).

This array of different external stimulation techniques, used independently or in junction, has enabled researchers to differentiate between the magnitude of central and peripheral fatigue at LoT, in addition to measuring the dynamic accumulation of fatigue during exercise.

1.6.1 Dynamics of central and peripheral fatigue

Peripheral fatigue has been shown to develop in the early part of exercise, with Froyd *et al.* (2013) reporting a 25 % reduction in peak torque at 40 % exercise duration, with only a further 9 % reduction during the remaining knee-extension exercise task. This early development of peripheral fatigue is most likely the consequence of the beginnings of the fatigue cascade within the musculature (anaerobic metabolism and fatigue metabolite build-up) that occur immediately at the onset of supra-CP exercise, where the O_2 deficit is greatest (Murgatroyd

et al., 2011). Some authors have shown that during exercise to LoT, peripheral fatigue will only accumulate until a fixed level or “critical threshold” at which peripheral fatigue will remain, independent of required force (Burnley *et al.,* 2012), possibly for protective reasons (Amann and Dempsey, 2008). This notion is supported by similar levels of intramuscular PCr depletion, Pi accumulation and pH reduction at the LoT, independent of the imposed power and exercise duration (Vanhatalo *et al.,* 2010; Burnley *et al.,* 2010; Chidnok *et al.,* 2013b). However, other studies have not been able to corroborate this, finding that the magnitude of peripheral fatigue did not reach a common threshold across exercise of different intensity and duration (Christian *et al.,* 2014; Froyd *et al.,* 2016).

Conversely, central fatigue has been shown to develop slowly and be more prevalent later in exercise tasks. Gruet *et al.* (2014) found during isometric exercise that there was no significant central fatigue present until the last quarter of the exercise task, with voluntary activation reducing from 96 % at baseline to 86 %, but only in the final 25 % of the exercise task. This delay in central fatigue is suggested to be a consequence of the CNS reacting to the metabolic perturbations within the skeletal muscle (i.e. peripheral fatigue) (Bigland-Ritchie *et al.,* 1983; Amann *et al.,* 2015).

A consequence of these differing peripheral and central kinetics is that the exercise power output and duration has the potential to influence the relative contributions of these fatigue mechanisms at task failure. It has been displayed during knee-extension exercise that, due to its fast accumulation, shorter, high-power exercise is terminated with greater peripheral fatigue, whereas reducing the exercise intensity and therefore increasing the task duration, caused an

increasing contribution of the slower developing central fatigue mechanisms (Christian *et al.,* 2014; Froyd *et al.,* 2016). Contrary to this, using the P-T_{lim} relationship during isometric contractions Burnley *et al.* (2012) have shown that at LoT of 5 different power outputs (exercise duration ranging 3-18 min), peripheral fatigue in all 5 protocols caused a similar reduction in peak torque of 32-38 %, supporting the “critical threshold” hypothesis. However these authors also demonstrated that as the power demand reduced, the contributions of central fatigue increased from 6 % at LoT of the shortest protocol to 29 % at LoT of the protocol with the longest duration. When the maximal voluntary force was measured at LoT by Burnley *et al.* (2012), importantly it was found that this was not different from the power demand of the task. This shows that during single-joint exercise, the LoT occurs when the skeletal muscle can no longer meet the task demands, however if this remains the case for whole-body dynamic exercise is still unclear.

Overall, while the level of peripheral fatigue at LoT seems inconclusive, the effect of the exercise task (power output and duration) on the development of different fatigue mechanisms seems clear.

1.6.2 Limitation to external stimulation techniques

While the benefits of external stimulation are clear (primarily negating volitional effort), there remains fundamental overarching issues with these techniques when assessing neuromuscular fatigue that are well documented in the literature (Cairns *et al.,* 2005; Twomey *et al.,* 2017). External stimulation techniques are by necessity, performed using either isometric (e.g. Burnley *et al.,* 2012) or dynamic isokinetic (e.g. Froyd *et al.,* 2016) single-joint exercise only (e.g. knee extension), in turn enabling the isolation of changes within the neuromuscular

system. However, due to rapid muscle length and velocity changes, external stimulation to measure maximal force capacity and fatigue development is not possible during whole-body bilateral locomotion, without inducing a delay before assessment (discussed below; 1.8). Therefore, this limits the magnitude of cardiorespiratory strain that can be present using these external stimulation modalities (Twomey *et al.,* 2017). Consequently, these single-joint assessments are limited by this technical issue, and as a result it is difficult to determine if the findings during external stimulation methods actually occur during whole-body exercise, where other systems in addition to the skeletal muscle encroach upon their respective limits (e.g. HR_{max}, $\dot{V}_{Emax}$ and $\dot{V}O_{2max}$). Consequently, this limits the extent to which the principles from single-joint studies can be translated to generalised movement performed in daily life, where large muscle mass is recruited (Duchateau and Enoka, 2011).

1.7 Whole-body dynamic exercise

The increased stress on the cardiovascular and respiratory systems during whole-body exercise will provide additional stimuli that has the potential to alter the overall contributions of different fatigue mechanisms and consequently, the ultimate reason for task failure. The consequences of an increased ventilation during whole-body high-intensity exercise in order to constrain the effects of an increasing metabolic acidosis, results in an increased work of breathing (diaphragm, intercostal and accessory muscle work) (Sidhu *et al.,* 2013; Romer and Polkey, 2008). In turn, this increased work increases the O_2 cost of breathing, with this suggested to be a contributor to the $\dot{V}O_{2sc}$ (Aaron *et al.,* 1992; Jones *et al.,* 2011). Additionally, fatigue within the ventilatory muscles causing this increased O_2 demand, has also been suggested to result in a “steal” of blood

flow from the exercise muscles, via a metaboreflex induced vasoconstriction (Romer and Polkey, 2008). Together these two mechanisms will result in less O_2 delivered to the locomotor exercising muscle and consequently, increased reliance on substrate-level phosphorylation, increasing metabolic stress and the associated consequences within the musculature (i.e. peripheral fatigue) (Burnley and Jones, 2016).

Additionally, during high intensity exercise above respiratory compensation point, the ensuing hyperventilation causes a blunting of the increased cerebral blood flow and consequently, reductions in cerebral oxygenation (Bhambhani *et al.,* 2007; Rooks *et al.,* 2010). This is suggested to reduce motor cortex oxygenation and ultimately muscle recruitment (Rasmussen *et al.,* 2010). However the effect of this on central fatigue and performance remains unknown, with a high tolerance for changes in cerebral oxygenation found by Billaut *et al.* (2010.), suggesting these effects may be small in normoxic conditions.

The fatigue induced by whole-body exercise has also been shown to have an impact on the excitability of the corticospinal pathway. During single-joint exercise to exhaustion, motor pathway excitability (measured by EMG) is actually increased, suggesting that the inhibitory effects of fatigue are outweighed by the facilitation of increased muscle activation (McNeil *et al.,* 2011). However, Weavil *et al.* (2016) has recently shown that this is not the case with whole-body cycling, finding that the inhibition of fatigue outweighs the facilitation, causing no increase in overall excitability.

Perception of effort as a fatigue mechanism may also play a greater role in attaining the LoT during whole-body exercise. It is suggested that the increase in cardiovascular demand and particularly work of breathing (Kearon *et al.,* 1991;

Romer and Polkey, 2008) that accompanies high intensity whole-body exercise will increase the overall perception of effort (Kearon *et al.,* 1991; Jones and Killian, 2000), possibly via afferent feedback mechanisms (Amann *et al.,* 2009). Additionally, as the exercise becomes increasingly difficult, the consequence of fatigue will necessitate an increased central drive to maintain the power required, amplifying the perception of effort further (Hureau *et al.,* 2016). There is potential that this increased overall perception of effort can eventually cause a dissociation between the perceived and the actual demands of the task, causing an individual to stop before the physiological limit is reached.

Although it seems beyond doubt that during whole-body exercise, the increased cardiorespiratory demands will alter the overall contributions of different fatigue mechanisms, due to the complex nature of the CNS and the practical difficulties being faced by researchers with regards to isolating the origins of the fatigue mechanisms during this exercise modality, definitive conclusions are difficult to obtain, with much further research needed.

1.8 Coupling whole-body exercise and external stimulation

As a consequence of these various differences between single-joint and whole body exercise, in order to induce fatigue that is more ecological and therefore more translatable, study designs have been employed within which participants complete a whole-body exercise task, therefore providing the cardiovascular strain that accompanies this, combined with neuromuscular fatigue assessments performed before and after this using external stimulation techniques (e.g. Weavil *et al.,* 2016).

The ability to couple these methods allows not only measures of the level fatigue to be determined at LoT, but can also give additional insight into how the different fatigue mechanisms develops during whole-body exercise. When assessing the dynamics of fatigue, the findings during single-joint exercise described above seem to translate to whole-body exercise. Decorte *et al.* (2012) measured fatigue during constant-power cycling at 80 % peak power output to LoT (27 min 38 s ± 7 min 48 s), dismounting the ergometer for neuromuscular assessment at various time points. These authors found that, as with single-joint exercise, peripheral fatigue developed in the early phase of exercise, reducing peak torque by 34.4 % in the first 40 % of exercise and only developing a further 10.4 % at LoT (Figure 1.10). On the other hand central fatigue developed later in the task and was associated with task failure, with significant reductions in voluntary activation only present at 80 % exercise duration, progressing further from here to a 6.4 % fall at LoT (Figure 1.10). This has been corroborated by findings in similar protocols for running (Place *et al.,* 2004; Ross *et al.,* 2010), in addition to short sprint cycling exercise (Pearcey *et al.,* 2014) and much longer cycling exercise (4-5 hour time trials; Lepers *et al.,* 2002; Jubeau *et al.,* 2014).

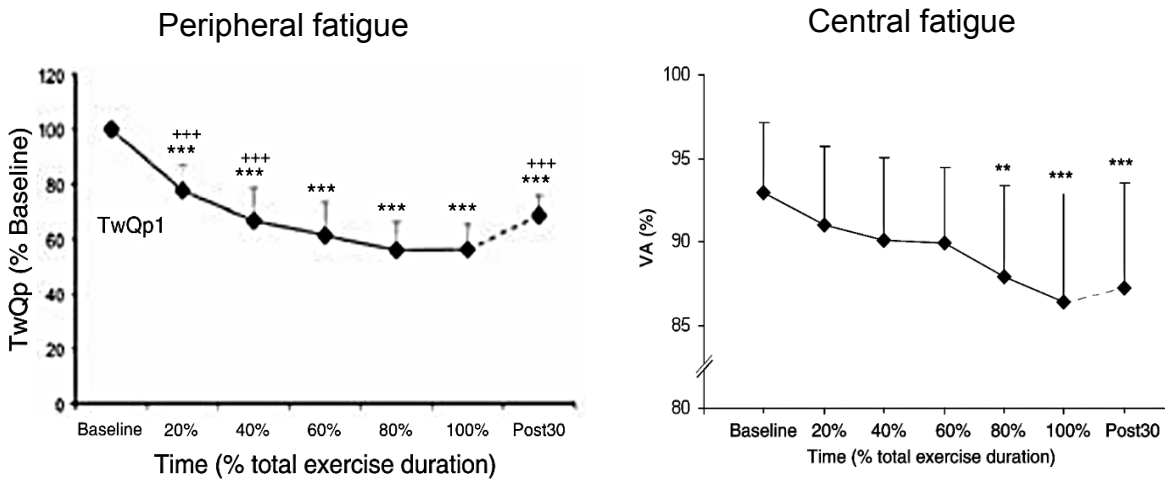

Figure 1.10: The differing kinetics of peripheral and central fatigue during high-intensity cycling exercise to the limit of tolerance. Peripheral fatigue develops during the first 40 % of exercise and remains at this level, whereas central fatigue only develops from 60 % exercise duration onwards to task failure. Figure adapted from Decorte *et al.* (2012).

Moreover, the task dependence of the contributions of central and peripheral fatigue seems to uphold during whole-body exercise. Thomas *et al.* (2015) found that peripheral fatigue was significantly greater after a 4km cycling time trial (40 % reduced twitch force) than after a 20 or 40 km time trial (31% and 29 % reduction, respectively). Conversely, central fatigue developed significantly further after the 40 km (10 %) and 20 km (11 %) time trials than after the 4 km time trial (7 %). A similar study was performed by Thomas *et al.* (2016), measuring fatigue after 3 different constant-load cycling protocols of differing intensity, causing the LoT to be attained at ~ 3, 11 and 42 min. Peripheral fatigue increased with exercise intensity; 11% (42 min), 16 % (11 min) and 33 % (3 min), while central fatigue increased with exercise duration and reduced intensity; 2.7 % (3 min), 6.3 % (11 min) and 9 % (42 min) (Thomas *et al.,* 2016). Together these studies suggest that high-intensity short duration exercise is associated

with greater peripheral fatigue, whereas lower power output, longer exercise is associated with greater central fatigue contributions.

While the coupling of external stimulation and whole-body exercise addresses the issues of single-joint exercise in part, when the exercise mode is changed from a whole-body fatiguing task to a single-joint assessment, the fatigue measured may not be representative of the neural recruitment pathways that were present during the fatiguing task (Wakeling *et al.,* 2010; Twomey *et al.,* 2017). Another limitation with these studies in which a participant is required to switch ergometers, is the importance of fatigue assessment immediately post task failure, something not achieved in these studies. The delay induced (generally reported as ~ 45 s-2.5 min is required for both the safety of the participants and for accurate positioning on the ergometer, so measurements can be made reliably (Twomey *et al.,* 2017). Nevertheless, rapid intramuscular recovery has been observed post-exercise, with Rossiter *et al.* (2002) displaying PCr recovery kinetics of 47 ± 11 s, in addition to Allen and Westerblad (2010) findings 100 % recovery of force only 10 s post-exercise in isolated mouse muscle. These findings are corroborated in knee-extension exercise, where switching ergometers is not required, with Froyd *et al.* (2013) displaying 78 % of the recovery in twitch force occurred within the first 2 min post exercise. Importantly, this rapid neuromuscular recovery also extends to whole-body exercise, where peak voluntary isokinetic cycling power restored to baseline after only 2 min of recovery (Coelho *et al.,* 2015). Consequently, the delay in assessment of neuromuscular fatigue that occurs from switching ergometer has the potential to allow for significant recovery, that may cause an underestimation of the levels of fatigue induced by the exercise task (Gruet *et al.,* 2014; Jubeau *et al.,* 2014; Froyd *et al.,* 2013), complicated further by the fact that the rate and

origins of neuromuscular recovery can be both task intensity and duration dependent (Carroll *et al.,* 2017).

Recently, a novel ergometer was developed by Temesi *et al.* (2017) that attempts to address this issue of delayed fatigue assessment. A cycle ergometer was adapted so that the pedals can be locked immediately in a fixed position, allowing performance of both bilateral dynamic cycling, followed by isometric force measurements of the knee-extensors (Temesi *et al.,* 2017). This development enables stimulation immediately at the LoT of whole-body exercise, therefore negating the issue of recovery prior to fatigue assessment. However, as the authors remark, this method is still limited by the fact force is measured isometrically, after a dynamic fatiguing task, possibly confounding results. So although this is an exciting development, some limitations remain and research where dynamic contractions are performed is warranted.

A technique that potentially provides an insight into this as a possibility was developed by Sidhu *et al.* (2012) for sub-maximal exercise, and then used recently by Black *et al.* (2017) during exercise to LoT. This protocol involves incorporating maximal external stimulation immediately at end-exercise while still sat on the cycle ergometer performing whole-body dynamic cycling exercise. External stimulation of the knee-extensors was triggered automatically at the optimum knee-angle (~30-60 °), to induce a maximal twitch during each desired pedal revolution (Sidhu *et al.,* 2012). However as noted by the authors, this stimulation caused significant disruption to the normal activation pattern during cycling (Sidhu *et al.,* 2012), and was only measured instantaneously at the desired knee angle not throughout the pedal stroke, questioning the extent to which these measurements reflect the neural recruitment patterns during the

exercise task itself. Additionally, force measurements were not possible with this methodology due to the position of the strain gauges on the pedals. Without either MVC's or maximal twitch force measurements, neuromuscular fatigue assessment was limited to alterations in EMG activity. Although Sidhu *et al.* (2012) did couple the femoral nerve stimulation with TMS to provide greater insight into the origins of central fatigue, as mentioned the exercise was submaximal. Therefore, although this protocol does allow for maximal stimulation immediately at LoT without dismounting ergometer, significant limitations still exist that could be addressed in future work.

1.9 Proposed models of the of task failure in whole-body exercise

While it is agreed that an accumulation of fatigue, both peripheral and central will contribute to the attainment of the LoT, there are a number of models that have been proposed in the literature as to how fatigue ultimately causes task failure at the limit of high-intensity exercise, summarised here.

The "traditional" model of fatigue mechanisms has already been discussed throughout this review and is summarised by Murgatroyd and Wyle (2011). Briefly, the high power demand results in increasing metabolic perturbations that will directly affect the ability of the contractile apparatus to produce force. As a result additional, larger and more fatigable motor units are recruited (Coyle *et al.,* 1992; Shinohara and Moritani 1992), resulting in an increased O_2 cost of the exercise (Ozyener *et al.,* 2001). Simultaneously, the fatigue metabolite build-up consequent of anaerobic energy utilisation will inhibit descending drive from the CNS to recruit additional fibres (Allen *et al.,* 2008), as discussed above (section 1.5.2). O_2 demand will continue to increase until $\dot{V}O_{2max}$ is reached, at which

point the only way of continuation of the task is increasing reliance on anaerobic energy sources and recruiting increasingly fatigue-sensitive muscle fibres (Murgatroyd *et al.,* 2011). In turn this will amplify the above processes, eventually causing the LoT to be reached when the neuromuscular system can no longer meet the force demands of the task, consistent with task failure in single-joint exercise (Burnley *et al.,* 2012).

Another model of fatigue is provided by Amann and colleagues (summarised by Amann, 2011), centred around the role of feedback from muscle III and IV afferents to the CNS regarding the metabolic state of the exercising muscles, again discussed briefly elsewhere in this review. These authors suggest that feedback from these afferents provides a feedback loop that ensures muscle recruitment from the CNS, and as a consequence peripheral fatigue, is limited at the highest possible, tolerable limit (or “critical threshold”) that cannot be exceeded for protective reasons (Amann *et al.,* 2014). Evidence for this critical threshold is supplied by a consistent level of peripheral fatigue found after different maximal exercise tasks (Amann and Dempsey, 2008; Burnley *et al.,* 2012). This would seem in agreement with the findings of a consistent level of intramuscular PCr, Pi and pH at LoT of different maximal exercise tasks (Burnley *et al.,* 2010; Vanhatalo *et al.,* 2010; Chidnok *et al.,* 2013b). Additionally, pharmacologically blocking the feedback from these afferents during cycling caused an increased central drive (measured via EMG) and power output above that “allowed” by the CNS in control conditions. As a result greater peripheral fatigue (measured via femoral nerve stimulation) was measured post-exercise, attaining a level beyond the suggested critical threshold (Amann *et al.,* 2009).

While the feedback from group III and IV muscle afferents to the CNS is well-founded and the neural anatomy exists to influence central drive to the exercising muscle, the direct influence of afferents on performance, and the presence of this ultimate critical threshold that limits the continuation of exercise has been questioned. Other research groups have failed to replicate this critical threshold, finding that the level of peripheral fatigue increased throughout exercise (Froyd *et al.,* 2016), in addition to a different magnitude of peripheral fatigue at task failure, dependent on the exercise tasks intensity and duration (Thomas *et al.,* 2015; Christian *et al.,* 2015; Thomas *et al.,* 2016; Froyd *et al.,* 2016).

While these two fatigue models provide peripheral fatigue as the origins of the stimulus of task failure, there remains other models that suggest a more proximal origin of task failure in high-intensity exercise. The presence of a "central governor" that is postulated to provide subconscious feedforward decisions regarding central drive to the exercising muscles has been presented in the literature (Noakes *et al.,* 2005). It is suggested that the neural drive for muscle recruitment is subconsciously limited (possibly by cardiac output) at a level below maximum physiological capacity, to prevent metabolic catastrophe (e.g. myocardial ischemia) (Noakes *et al.,* 2005). This model would suggest that the maximal values regularly measured (e.g. $\dot{V}O_{2max}$ and HR_{max}) are in fact not maximal, just the rate "allowed" by the central governor. However, this theory has been regularly challenged by authors, with the evidence seemingly not supporting its existence (e.g. Ekblom, 2009). When working at maximal capacity (i.e. $\dot{V}O_{2max}$), it has been demonstrated that a further increase in power results in no further increase in $\dot{V}O_2$ or cardiac output (Brink-Elfegoun *et al.,* 2007b; Hawkins *et al.,* 2007), as there would be if a physiological reserve was available. Secondly, Brink-Elfegoun *et al.* (2007a) have reported that when at $\dot{V}O_{2max}$, a

further increase in power did result in an increased muscle activation (measured via EMG), that would not be “allowed” if this was modulated by a central governor.

A final fatigue model that will be discussed here is the psychobiological model. First proposed by Marcora and Staiano (2010), this model suggests that task failure is the consequence of an effort based decision to disengage from the exercise task. Evidence for this is provided by the near-maximal perception of effort reported by participants at task failure (Marcora *et al.,* 2008, 2009), in addition to the reserve in skeletal muscle power generating capacity of approximately 300 % that was found immediately after a cycling task to LoT at 80 % peak power output (Marcora and Staiano, 2010). The findings of this later study however have been disputed, as the power measurement at LoT was performed at a much higher cadence than the final stages of the fatiguing task (~137 vs. ~40 rpm), therefore not accounting for the force-velocity relationship that is present in fresh and fatigued muscle (Elmer *et al.,* 2013; Figure 1.11). Moreover, there were technical limitations to this study such as power being measured at the flywheel instead of the crank, in addition to the 3-4 s delay to switch cycling modes at the LoT that may allow for rapid skeletal muscle recovery (Allen and Westerblad, 2010). Therefore, it has been widely suggested that, contrary to the conclusions of Marcora and Staiano (2010), it was the lack of velocity control and technical limitations that resulted in the increase in power, not a functional physiological reserve due to psychological disengagement (Burnley, 2010; MacIntosh and Fletcher, 2011). Despite these methodological issues, further evidence against this conscious decision to stop is provided by findings that task failure (i.e. an inability to meet required force) can occur before participant’s voluntary terminate the task (Burnley *et al.,* 2012; Amann *et al.,*

2007b). In addition, EMG amplitude remains high, with no decrease at task failure in cycling (Chidnok *et al.,* 2013a), a finding that suggests there has been no decrease in central drive and thus, effort to continue (Burnley and Jones, 2016).

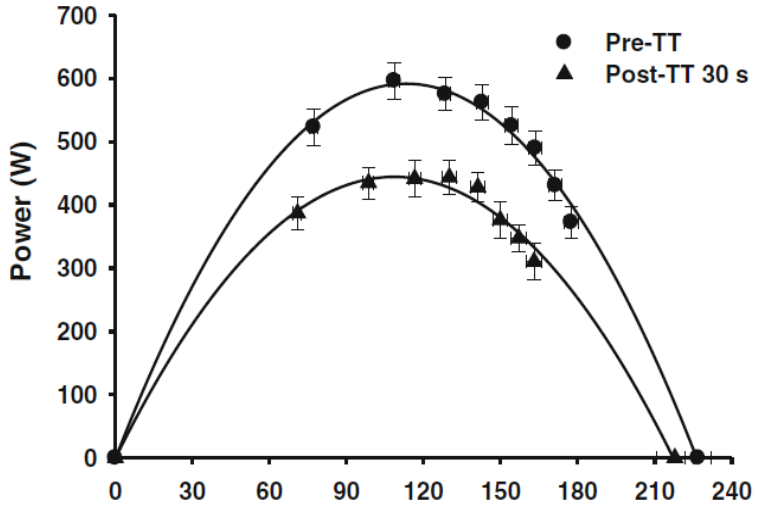

Figure 1.11: The parabolic force-velocity relationship, displayed in a fresh and fatigued state (Elmer *et al.,* 2013).

As outlined here, evidence both supporting and refuting the discussed models of fatigue can be found throughout the literature. As a consequence, the ultimate cause of task failure, especially in whole-body high-intensity exercise, remains widely disputed.

1.10 Isokinetic cycling neuromuscular fatigue assessment during whole-body dynamic exercise

Although criticised in the literature, the protocol implemented by Marcora and Staiano (2010), addressed a number of the limitations associated with traditional neuromuscular fatigue assessment. The protocol used allowed for the assessment of fatigue almost immediately at the LoT of whole-body exercise,

without switching ergometers and thus, muscle recruitment patterns. Additionally, this almost immediate fatigue assessment allowed little time for neuromuscular recovery after task failure. However, as indicated this study has different limitations that confounded the authors conclusions, primarily the lack of velocity control in the fatigue assessment vs. the fatiguing task (~ 40 vs. ~ 137 rpm) (Burnley, 2010; MacIntosh and Fletcher, 2011). Clearly, using isometric or isokinetic contractions such as those performed on isokinetic dynamometers in the external simulation protocols does not have these velocity related issues. Isometric contractions were incorporated into a whole-body ergometer by Temesi *et al.* (2017), however the difference between isometric and dynamic isokinetic contractions, could confound the findings with this ergometer.

Consequently, efforts have been made to combine these isokinetic measurements with whole-body exercise. Sargeant *et al.* (1981) devised an ergometer that allowed the control of cycling cadence using an electric motor driving the cranks at a constant velocity, allowing for isokinetic cycling power to be determined using strain gauges attached to the crank of the ergometer. These authors then used this novel ergometer to investigate the effect of different cycling velocities on peak power output during a 20 s maximal effort, finding an optimum cadence of ~110 rpm. Sergeant and Dolan (1987) then performed a series of experiments displaying the utility of this isokinetic power methodology in quantifying fatigue, whereby increasing either the duration an individual exercised at 98 % $\dot{V}O_{2max}$ or increasing the exercise intensity, caused a greater fall in maximum isokinetic cycling power output post-exercise. The effect of this fatigue was investigated with respect to the power-velocity relationship, finding that fatigue caused a flattening of the hyperbolic relationship, and as a result peak isokinetic cycling power could now be produced at cadences ~80-120 rpm

(Beelen and Sargeant, 1991). The use of this ergometer has also been extended beyond short maximal efforts, with Zoladz *et al.* (2000) using the ergometers velocity control to investigate the optimum cadence for high-intensity exercise, finding that a high cadence (up to 100 rpm) was beneficial in resisting the development of fatigue.

However more recently, the development of electro magnetically braked cycle ergometers has enabled greater accuracy and velocity control during isokinetic cycling. As a consequence a number of researchers have incorporated short (~ 5 s) bouts of maximal isokinetic cycling into exercise protocols, in order to measure the maximal voluntary power generating capacity of the exercising muscles (Cannon *et al.,* 2011; Coelho *et al.,* 2015; Ferguson *et al.,* 2016b; Morales-Alamo *et al.,* 2015; De Souza *et al.,* 2016; Cannon *et al.,* 2016).

The use of isokinetic cycling almost immediately at the LoT of whole-body exercise negates the main concerns highlighted earlier regarding the time for recovery (when switching ergometers) and the use of different modalities (single joint fatigue assessments) (Coelho *et al.,* 2015). Additionally, this enables the assessment of fatigue after exercise during which an individual's physiological maximum (i.e. $\dot{V}O_{2max}$) has been reached (Coelho *et al.,* 2015). The first study to incorporate this isokinetic cycling method into exercise tests that produced the LoT in whole-body exercise to $\dot{V}O_{2max}$ was Coelho *et al.* (2015), using ramp-incremental exercise. These authors found that in a healthy but heterogeneous group (age: 29-79 yr.), contrary to Marcora and Staiano (2010), significant fatigue was present; with power reducing to ~ 55 % baseline, at intolerance. However, this value was still, albeit much less so, significantly above the required power (261 ± 58 vs. 335 ± 88 W; 18 ± 11 % $p < 0.05$). While this difference

implied a small power reserve was present at LoT, the authors suggest this is actually the result of a limited ability to fleetingly increase power output, evidenced by the fluctuations in measured power output during the ramp-incremental phase of the test (Coelho *et al.,* 2015). While the power-required increases as a smooth function, the actual power measured at the crank fluctuated above and below this requirement, becoming more exaggerated near the LoT, and evidencing a short-term ability to increase power output, even near exhaustion (Coelho *et al.,* 2015). Therefore, these authors concluded that the LoT was attained with no physiological reserve in power generating capacity, as power did not exceed this natural fluctuation in crank power. This finding has since been corroborated by Ferguson *et al.* (2016b) finding in endurance athletes that no significant power reserve was present at the LoT of ramp-incremental exercise; 352 ± 58 W vs. 391± 72 W (~111 % required $p > 0.05$). This population was chosen as athletes have been shown to primarily be limited by O_2 delivery (Roca *et al.,* 1992; Mortensen *et al.,* 2008) and therefore, if a power reserve were present, it would likely be most evident in this population, not limited by the exercising muscle (Ferguson *et al.,* 2016b). The finding that no additional power could be produced by the exercising muscle, above that required by the task at LoT, suggests that similarly to single-joint exercise (Burnley *et al.,* 2012), the LoT was ultimately attained due to metabolic changes within the peripheral exercising muscle, causing the contractile apparatus to no longer be able meet the task demand.

However, there being no significant difference between the power required at task failure and the maximal voluntary isokinetic power immediately post-exercise is not a uniform finding in ramp-incremental exercise. Morales-Alamo *et al.* (2015) reported that when using the same methodology, a significant reserve

in power generating capacity was present at LoT (240 W; ~ 83 %). This reserve, in addition to a skeletal muscle metabolic reserve measured with biopsy samples, caused the authors to conclude that the attainment of task failure was the consequence of central fatigue mechanisms, not within the exercising muscles (Morales-Alamo *et al.,* 2015). However, there is an important distinction between the isokinetic powers reported in this study and those reported previously (Coelho *et al.,* 2015; Ferguson *et al.,* 2016b). Morales-Alamo *et al.* (2015) reported instantaneous power, a value immediately at the onset of the maximal effort, that will likely be influenced by the flywheel inertia, consequent of the increasing cadence from LoT, resulting in an increased isokinetic power measurement (Ferguson *et al.,* 2016a), displayed graphically in Figure 1.12. When the data from the entire 10 s isokinetic effort are used, providing both a more relevant comparison to the power required by the task, and physiological measurement of the muscles power capacity, the power is not significantly different from the task requirement (318 ± 55 vs. 290 ± 36 W; ~ 10 % reserve), in line with previous ramp-incremental research (Coelho *et al.,* 2015; Ferguson *et al.,* 2016b). The intramuscular metabolic reserve reported as supporting evidence for this skeletal muscle reserve is likely a consequence of the inherent limitation of biopsy samples, taking only a small area of muscle. It has been shown using magnetic resonance spectroscopy (MRS) that an overall metabolic reserve exists at LoT of ramp-incremental exercise, with the heterogeneity across the muscle suggesting complete depletion of energy is only required in a small area of muscle to cause task failure (Cannon *et al.,* 2013). Additionally, the exercise modality used in this MRS study (knee-extension) has shown no reserve in power generating capacity at LoT (Burnley *et al.,* 2012). Therefore, the metabolic reserve in Morales-Alamo *et al.* (2015) does not provide definitive

evidence of an ability of the skeletal muscle to produce more power than required (Ferguson *et al.,* 2016a).

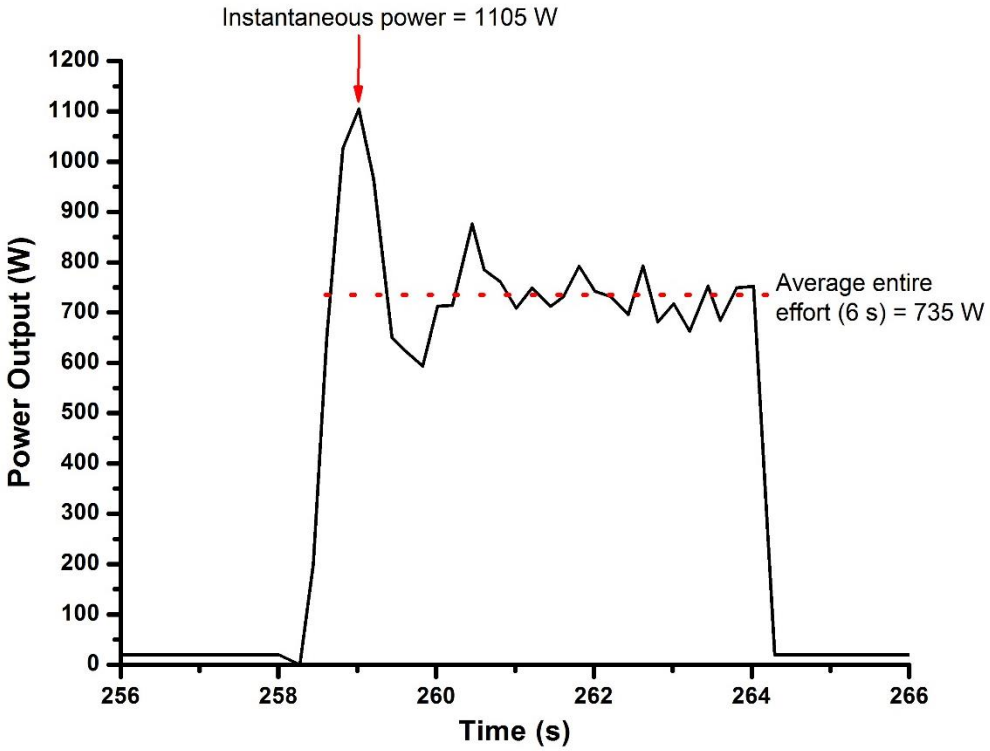

Figure 1.12: A representative example of the influence of flywheel inertia on the measured power generating capacity of the muscles during an unfatigued bout of isokinetic cycling. Power is measured every 2 degrees of angular rotation throughout. Note the difference in reported power if the initial instantaneous power (1105 W) is used compared to the average of the entire 6 s maximal effort (735 W).

A power reserve using this protocol was also found when ramp-incremental exercise was performed in a group of COPD patients (260 %; $p < 0.05$; Cannon *et al.,* 2016). However, this physiologically significant power reserve was suggested to be a consequence of the patients ultimately being limited by dyspnoea arising from their COPD, and not an inability of the exercising muscles to produce power (Cannon *et al.,* 2016). Therefore, it seems in the presence of

COPD the perceptual and physiological limits are dissociated, causing a reserve in skeletal muscle power.

Other studies that are of note here, using this isokinetic cycling mode to investigate the development of muscle fatigue are Cannon *et al.* (2011) and De Souza *et al.* (2016). However, neither of these studies were designed to take participants to the LoT, so with the ultimate cause of task failure not a focus, these studies are not discussed in detail here (although discussion of De Souza *et al.* (2016) can be found in Chapter 4).

A second, important facet to the protocols used by Coelho *et al.* (2015) and to an extent Ferguson *et al.* (2016b), was the use of EMG measurements to give an indication of muscle activity. EMG activity was measured in both studies from five predominant muscles during cycling. While inherent limitations exist with surface EMG measurements (discussed elsewhere; 1.6), it is important to note that the EMG activity in these studies was not used as a direct measure of central drive, as this link between EMG signals and motor output is not certain (Enoka and Duchateau, 2015). It was found that in a fresh state, a linear relationship exists between isokinetic power and the magnitude of EMG signal that was both reliable and dependant on the cycling velocity (Coelho *et al.,* 2015). Using this un-fatigued relationship, the authors devised a way of comparing this to the reduction in isokinetic power and EMG amplitude during the LoT maximal effort, to determine the contributions of “activation fatigue” and “muscle fatigue” to overall performance fatigue (Figure 1.13; Coelho *et al.,* 2015). Although the authors intentionally did not refer to these as central and peripheral fatigue, the relative contributions may provide insight into where this primary fatigue mechanism originates; within the musculature or possibly more proximal.

Findings from both this study and Ferguson *et al.* (2016b) suggest a significant contribution of both activation fatigue and muscle fatigue to overall performance fatigue in standard (8-12 min) duration ramp-incremental exercise.

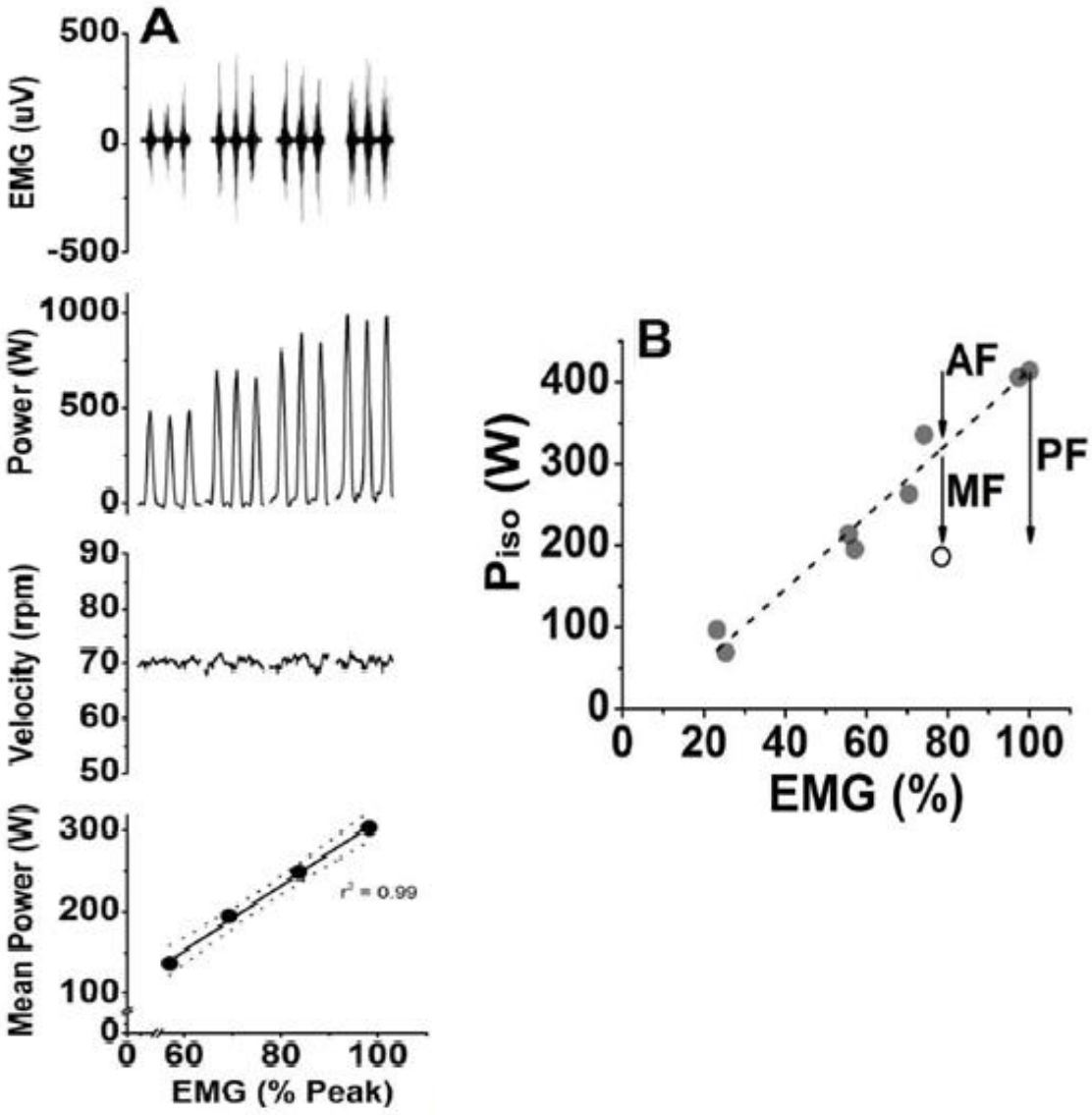

Figure 1.13: A) EMG, Power and velocity responses during 4 isokinetic efforts of 25, 50, 75 and 100 % volitional effort. Also shown is the strongly linear relationship between power and EMG that is present in the fresh state. B) displays how, using the fresh linear relationship, the power and EMG produced during a maximal effort immediately post task failure can potentially differentiate between activation fatigue (AF) and muscle fatigue (MF) contributions to overall performance fatigue (PF). Figures from Coelho *et al.* (2015).

The use of maximal isokinetic cycling efforts clearly has its advantages over other fatigue methods, however there are also limitations to address, the primary of which is the volitional effort required. The lack of external stimulation means these protocols rely on the participant giving a true maximal effort and not being effected by a lack of effort or motivation. To incorporate stimulation techniques into this protocol would be complex (as shown by Black *et al.,* 2016 and Temesi *et al.,* 2017) and negates the simplicity and practical applications of this protocol in a wider context. Nonetheless, if a sub-maximal effort is given by a participant at any point during the test, this may be interpreted as a greater drop in power capacity than is actually present, influencing the findings. This effect can be minimised by comprehensive familiarisation of participants, in addition to good instructions and verbal encouragement throughout the entire protocols. With these procedures in place, maximal isokinetic power has been shown to a reproducible, well tolerated and easily administered measurement of fatigue (CoV; 4 % (Coelho *et al.,* 2015) and 3 % (Ferguson *et al.,* 2016b)). Additional limitations include changes in EMG activity and the short (~1-2 s) delay between task failure and fatigue assessment. While a new EMG-power relationship is determined prior to each test, negating day-to-day changes in EMG signals, changes in conductance during the test as a consequence of alterations in skin temperature, hydration status and preparation could impact the EMG signals measured and thus, the conclusions drawn from these. Finally, the importance of quick fatigue assessment after task failure has been outlined previously. While the delay in these protocols - a consequence of participants having to increase cadence from task failure at ~50 rpm to the isokinetic assessment cadence of 70 or 80 rpm - is significantly shorter than that during other procedures (45 s – 2.5 min to switch ergometers), even these few seconds have the potential to allow

for some neuromuscular recovery that may impact results (Allen and Westerblad, 2010).

Finally, the functional relevance of the presence/absence of a reserve in isokinetic cycling power at LoT has been questioned by Burnley and Jones (2016). These authors suggest that different recruitment patterns will be used during the short maximal effort at LoT, compared with the recruitment during the fatiguing exercise. However, as muscle recruitment at the LoT should be maximal, and the fatigue assessment is the same modality as the exercise protocol, as well as occurring immediately after LoT with no changes in joint angles, changes in recruitment patterns are unlikely. Even small changes in recruitment that may occur, are of significantly less importance than the changes that will occur when switching from whole-body exercise to a single-joint fatigue assessment (consequences of which have been discussed in section 1.6.2).

Overall while limitations exist, the benefits of using isokinetic cycling; primarily immediate fatigue assessment and whole-body fatiguing exercise, mean this remains a beneficial methodology with which to assess overall neuromuscular fatigue. In addition, this method can be readily added to any cycling exercise protocol, with the assessment easy to implement and well tolerated in populations including the elderly (Coelho *et al.,* 2015) and COPD (Cannon *et al.,* 2016). Isokinetic cycling will be used throughout this thesis in order to aid the investigations into how fatigue is ultimately limiting whole-body maximal exercise in both health and disease.

1.11 Aims and Objectives

The overall aim of this thesis was to determine the origin of the fatigue that ultimately results in the LoT during whole-body exercise. Additionally, how these mechanisms are affected by the task being performed and an individual's health status.

In order to achieve this, 3 studies were completed, with the specific aims of each outlined below:

1) To determine the effect of ramp-incrementation rate on the magnitude of fatigue at task failure, and the presence/absence of a reserve in maximal voluntary power production.

2) To use the power-tolerable duration relationship to a) determine how changing the exercise task affects the level of fatigue at task failure by the presence/absence of a power reserve and b) investigate the development of the fatigue profile during exercise and if this is altered by different exercise protocols.

3) To use the isokinetic cycling methodology in a group of chronic heart failure patients, in order to determine, on an individual basis, where the primary limitation for exercise originated; within the exercising muscles or due to sensations proximal to this, such as dyspnoea.

Chapter 2 General Methods

2.1 Participants

2.1.1 Healthy participants

The studies presented in Chapter 3 and Chapter 4 were carried out in healthy participant groups, with the specific physical characteristics presented in the relevant study chapters. Healthy participants were recruited via word of mouth and the use of posters around the university advertising the research. All participants were screened using a health and activity questionnaire to exclude anyone in whom maximal exercise testing would be unsafe. Exclusion criteria were any individual with a history of cardiovascular, respiratory or musculoskeletal disease. Additionally, anyone with recent illness or injury, asthma, recent smoking (< 6 months) or a family history of sudden cardiac death were also excluded. All participants provided written informed consent before the commencement of any exercise protocols and were free to withdraw at any point without explanation.

2.1.2 Chronic heart failure patients

The study presented in Chapter 5 involved patients with chronic heart failure (CHF) (NYHA I-III, Webber Class A-C). Specific characteristics for this patient group, including diagnosis, heart function as well as physical characteristics are presented in Chapter 5. Patients suitable for the study were identified by their cardiologist while attending the Leeds General Infirmary Heart Failure Clinic. Once identified, patients were then informed of the study and given an information sheet. Participants were then contacted (~7 days later) to discuss any questions regarding the project, with a decision to volunteer to participate or

not then made. Participants had symptomatic but stable CHF due to left ventricular dysfunction and were on optimal medical therapy at the time of testing. Exclusion criteria were ongoing ischemia or arrhythmias, in addition to any contraindication that meant patients were unable to perform an exercise test such as arthritis, musculoskeletal injury or severe chronic obstructive pulmonary disease. All participants provided written informed consent prior to any exercise testing and were free to withdraw at any point without explanation.

2.2 Ergometry

All studies in this thesis were performed on an upright cycle ergometer, with power (i.e. sum of torque * angular velocity, also referred to as work rate or power output) computer-controlled and applied using an electromagnetically braked flywheel (Lode Excalibur Sport PFM, Lode BV, Groningen, NL Figure 2.1). The accuracy of the work rates imposed for this ergometer were within ± 2 % for work rates 100-1500 W and within 2 W for work rates below 100 W, with the ergometer capable of maintaining these between 25-180 rpm (manufacturer's specifications). To ensure these variables were maintained, the cycle ergometer was calibrated using a motor driven calibrator, on a yearly basis (Calibrator 2000, Lode BV, Groningen, NL). Data from the calibration on 11/07/17 are displayed in Figure 2.2. Two different cycling modes were used during the studies presented; cadence independent (hyperbolic) and isokinetic cycling, with details of both outlined below.

Figure 2.1: The cycle ergometer used throughout all the experiments presented; Lode Excalibur Sport PFM.

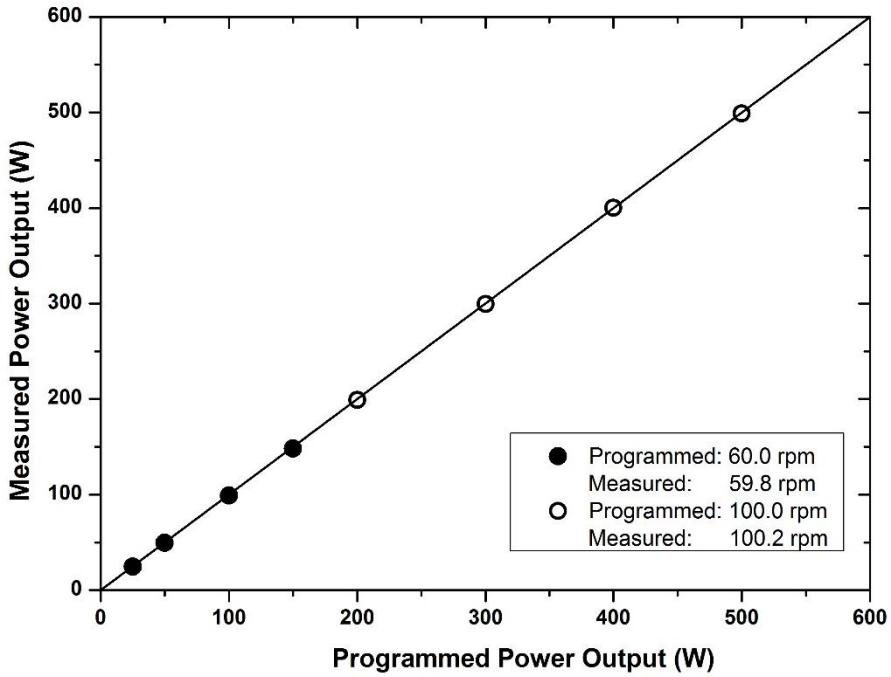

Figure 2.2: Calibration of the cycle ergometer. Actual power output (from the calibrator) is shown as a function of the programmed power output (from the computer). Power was calibrated at 60 and 100 rpm. y = x line also shown.

2.2.1 Cadence-independent cycling

The cadence independent (or hyperbolic) cycling mode enables the work rate imposed on the flywheel of the ergometer to be maintained, independent of changes in cadence. The desired (programmed) work rate during exercise is maintained by a closed-loop feedback system that alters the electromagnetic braking force on the flywheel inversely with changes in cadence on a pedal stroke by pedal stroke basis; i.e. increasing breaking force with decreases in cadence and decreasing force with increased cadence to maintain power. Importantly, this feedback system introduces a natural fluctuation in the power produced by the participant for each pedal stroke vs. what is programmed (the characterisation of which is discussed in detail in section 2.6.2.2.). The cadence-independent mode was used during all ramp-incremental and constant-power tests to control the exercise task.

2.2.2 Isokinetic cycling

A key feature of the cycle ergometer used in this thesis is the ability to switch instantaneously from cadence-independent cycling to isokinetic cycling. The ability to use isokinetic cycling enables velocity independent cycling power to be measured, controlling for the velocity dependence of muscle power (Sargeant *et al.,* 1981). The isokinetic mode on this ergometer uses the electromagnetic flywheel breaking force to constrain cycling cadence to a pre-set level (80 rpm chapter 3 and 4; 70 rpm chapter 5) using a continuous feedback loop. In this mode torque is measured at the left and right crank using a combination of strain gauges capable of measuring a peak force of 2000 N, with a resolution of $\leq$ 0.5 N and uncertainty $\leq$ 3 % (manufacturer's specifications). To ensure consistency, these strain gauges were calibrated against a factory standard on a yearly basis,

with no differences reported. Additionally, a zero force adjustment of the strain gauges were performed prior to every exercise test, with the right crank moved to 180° (left crank at 0°) so that no effect of gravity could confound the zero calibration. Simultaneously, angular velocity (rad.s^{-1}) of the crank was measured using 3 optical sensors sampling in series, with an uncertainty ≤ 1 % (manufacturer's specifications). Maintenance of target cadence during isokinetic cycling was also confirmed *post-hoc,* with error during isokinetic phases never exceeding ± 1 rpm. Both torque and angular velocity were sampled every 2° of rotation (angle measurement uncertainty 1 %; manufacturer's specifications). Thus, crank power was calculated every 2° degrees rotation during isokinetic cycling; torque * angular velocity. As left and right are sampled and processed independently, this allowed independent left and right crank power to be determined for each pedal stroke. Signals from the strain gauges and optical sensors were digitised and stored on a computer using software provided by the manufacturer (Lode Ergometry Manager, Lode BV, Groningen, NL).

2.3 Pulmonary gas exchange

During all exercise tests respired gases were analysed using a gas exchange analysis system (Medgraphics D series, Medgraphics, Medical Graphics Corporation, St Paul, MN, USA). Gases were sampled through a bidirectional preVent flow sensor: range: ± 18 L/s, accuracy ± 3 % or 50 ml, whichever is greater, resistance < 1.5 cm H_2O, deadspace 39 mL, resolution 8.64 mL/s (manufacturer's specifications). The preVent was inserted into a mouthpiece worn by participants and attached to an umbilical which passed respired gases to the O_2 and CO_2 sensors for analysis. O_2 analysis was performed by an O_2 sensor (Zirconia electrochemical fuel cell): range 0-100 %, response time (10-90

%) < 80 ms, accuracy ± 0.03 %. CO_2 analysis was by non-dispersive infrared sensor: range 0-10 %, response time (10-90 %) < 130 ms, accuracy ± 0.1 %. The flow sensor responds faster than the gas analysers, due to the transit-time for gas samples to reach the O_2 and CO_2 analysers and time for the analysis process to occur. Therefore, time alignment of all signals was performed online by determination of the start and end of each breath at the flow sensor, with the gas concentrations then aligned to this, prior to being displayed.

2.3.1 Calibration

Before every exercise test, both the flow sensor and the gas analysers were calibrated according to manufacturer guidelines. First, the current temperature, air pressure and humidity were inputted to the system to account for daily fluctuations. The flow sensor was then calibrated using a 3 L syringe (Hans Rudolph, Kansas City, MO). Ten syringe strokes of varying flow rates (range; 0.2-6 $L.s^{-1}$) were passed over the flow sensor, spanning the physiological range expected at rest and during maximal exercise. This calibration was then verified by passing a further ten syringe strokes over the flow sensor at varying flow rates, with a calibration accepted when the mean volume was 2.99-3.01 L with an SD $\leq$ 0.02 L. Both O_2 and CO_2 sensors were then calibrated using calibration gases of known concentrations, spanning the physiological range (O_2 12 %, CO_2 5 %, Bal N_2 and O_2 21 %, CO_2 0 %, Bal N_2, respectively). Additionally, these gases were sampled pre and post each exercise test to detect any drift and thus, verify the initial calibration.

2.3.2 Heart rate and ECG monitoring

A 12-lead Mortara ECG system was used that allowed for continuous monitoring of participants ECG throughout the exercise session. For healthy participants

monitoring was performed by researchers capable of identifying primary ECG abnormalities in healthy individuals such as S-T segment depression/elevation. With CHF patients, the ECG was continuously monitored by a specialised cardiac physiologist experienced in exercise testing in CHF patients. In both participant groups, if any abnormalities were detected, the test was immediately halted as a precaution and individuals given an adequate cool-down (~6 min) and then both the participant and the ECG trace were monitored, until the cardiac physiologist present confirmed the ECG was normal (at least an additional 6 min). Incidences of ECG abnormalities where the exercise test was stopped early were healthy: 0 and CHF: 2. In all testing, this 12-lead ECG system was also used to record heart rate during exercise.

2.4 Electromyography

In Chapters 3 and 4, surface electromyography (EMG) was used to measure the electrical activity of the skeletal muscle during the isokinetic cycling phases of each exercise protocol. EMG signals were recorded at 1500 Hz using a surface EMG system (Noraxon, Telemyo 2400T G2, Noraxon USA Inc, Scottsdale, AZ, USA). These signals were then transmitted wirelessly to a computer interface receiver and displayed using the proprietary software (Noraxon MyoResearch XP, Noraxon USA Inc, Scottsdale, AZ, USA).

2.4.1 Muscle sites

EMG signals were collected from 5 muscle sites of the right leg only; *gastrocnemius lateralis*, *biceps femoris*, *vastus lateralis*, *rectus femoris* and *vastus medialis*. These 5 sites were chosen as they have been shown to be the dominant muscles used during cycling (Ericson *et al.,* 1986) and have been consistently used for similar protocols in the literature (Coelho *et al.,* 2015;

Ferguson *et al.,* 2016b). The anatomical placements of EMG electrodes followed the Surface Electromyography for the Non-Invasive Assessment of Muscles (SENIAM) recommendations (Trigno Wireless System, Delsys Inc., Boston, MA): *gastrocnemius lateralis*; 1/3rd the distance along the line between the head of the fibula and the calcaneus, *biceps femoris*; half way along the line of the ischial tuberosity and lateral epicondyle of the tibia, *vastus lateralis*; 2/3rd distance from anterior superior iliac spine to the lateral side of the patella, *rectus femoris*; halfway between the anterior superior iliac spine and the superior border of the patella and *vastus medialis*; 8/10^{ths} of the distance on the line between the anterior superior iliac spine to the joint space in front of the anterior border of the medial ligament (Figure 2.3).

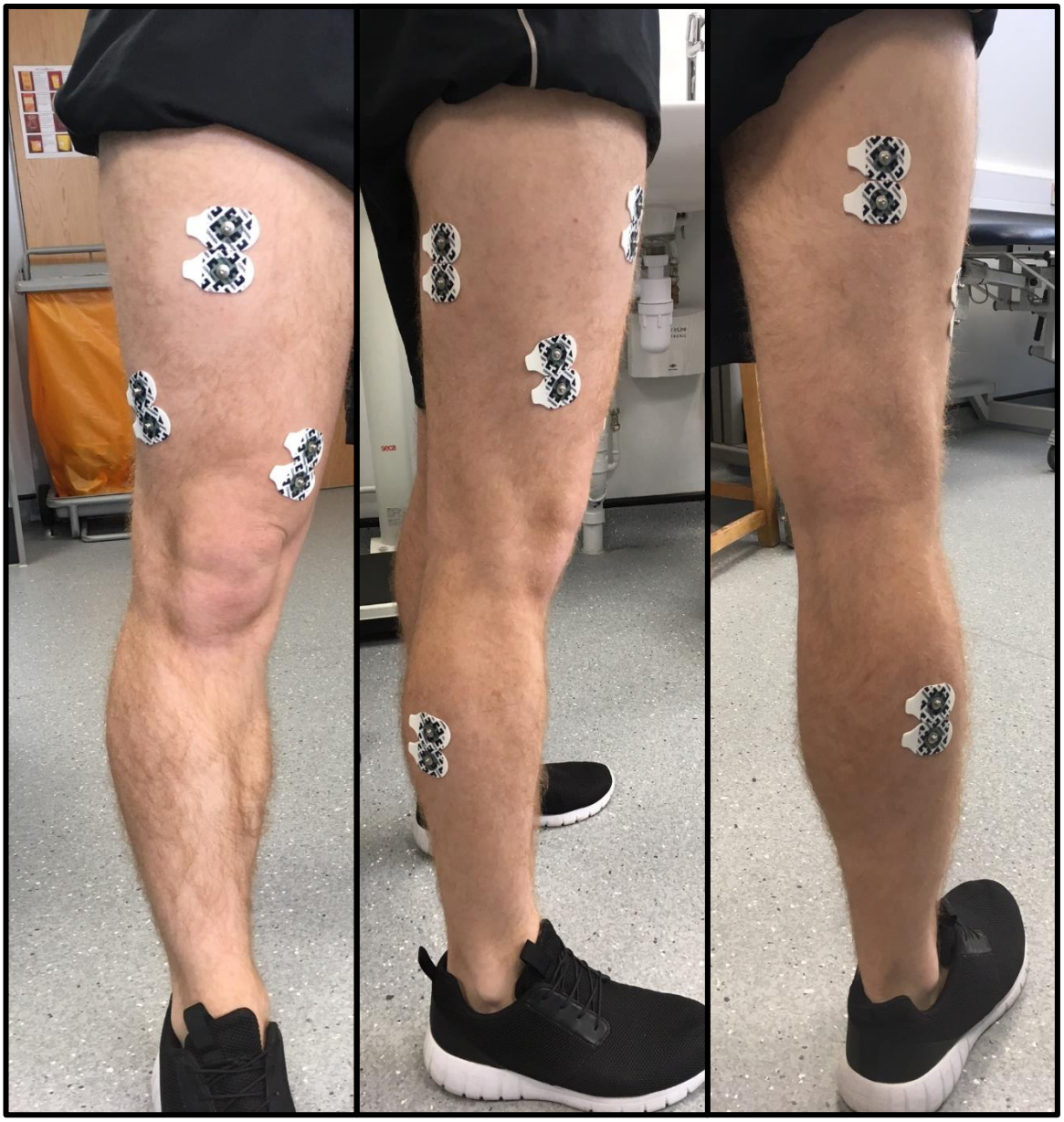

Figure 2.3: EMG electrode placement locations. Only the electrodes are shown and not the wires for clarity as to the anatomical positions.

2.4.2 Skin prep and electrode placement

Multiple measures were taken in order to improve the EMG signal quality and to minimise any noise that may occur. First, hair was removed from the muscle site to improve adhesion of the electrodes and minimise interference. The shaved area was then sterilised using an alcohol wipe (70 % Isopropyl alcohol), and an

abrasive paste used to remove any dead skin cells to improve conductance (Nuprep, Weaver and company, Aurora, CO, USA).

At each muscle site, 2 Kendall surface electrodes (H93SG, Covedien, Minneapolis, MN, USA) were placed over the muscle belly, across the direction of the muscle fibres to maximise signal strength, with an inter-electrode distance of 24 mm. Before participants mounted the cycle ergometer, the signal quality of each site was assessed using voluntary contractions of the associated muscle, with alterations in electrode position made if required, replacing the electrode with an unused one in the process. Finally, wires transmitting the EMG signal from the muscle site to the transmitter, that is placed on the participants right hip, were taped to the participants leg using surgical tape (3M, Bracknell, Berkshire, UK) to minimise interference during cycling and any distraction to the participant.

2.5 Exercise protocols

Specific exercise protocols for each study are outlined in the relevant chapters, however details of exercise protocols used consistently throughout the different studies are described here. All exercise visits were performed in temperature controlled (19 – 21 °C) exercise laboratory either at the University (Chapters 3 and 4) or at the cardiovascular research facility at Leeds General Infirmary (Chapter 5).

It is important to note that all the exercise protocols described in this thesis were designed to attain the limit of exercise tolerance (LoT) within approximately 2-30 min. The use of these durations allows insight into the mechanisms of exercise intolerance, without the effects of contraindicating factors such as mechanical limitations of the high powers required in very short exercise (< 2 min) or the effects of core body temperature, substrate provision and hydration status that

may occur during longer duration exercise (Fukuba and Whipp, 1999). Therefore, the physiological implications of the results discussed here may not hold true during very short or longer exercise, or during protocols that are not designed to attain the LoT/ $\dot{V}O_{2max}$. Additionally, as all exercise was performed in a temperature controlled laboratory at sea level, these protocols may not provide insight into the fatigue mechanisms involved in limiting exercise tolerance at environmental extremes such as with changes in temperature or barometric pressure.

2.5.1 Participant requirements for testing

When repeated visits to the exercise labs were required (Chapters 3 and 4), to reduce the effects of external influences on exercise performance and metabolic measurements, participants were asked to attend each visit at a similar time of day (± 2 hours), with at least 48 hours between visits. Additionally, healthy participants were instructed to abstain from alcohol, drugs and strenuous exercise (24 hours pre-exercise test) as well as ingesting only water and no caffeine or food (3 hours pre-exercise test).

2.5.2 Isokinetic cycling

The cycle ergometer used throughout was capable of switching instantaneously from cadence-independent to isokinetic cycling modes. Short (~6 s) isokinetic efforts at a fixed cadence (80 rpm – healthy, and 70 rpm – CHF) were used in all studies presented here to assess the voluntary power generating capacity of the exercising muscles at different levels of volitional effort (25-100 %).

Due to the velocity dependence of power output (Sergeant *et al.*, 1981) the cadence selected for the isokinetic cycling was vital, ensuring a cadence from which maximal power output can be achieved. In a fresh state maximal isokinetic

cycling power has been shown to be produced at ~110 rpm (Sergeant *et al.,* 1981). However, in this thesis the key power measurements are performed immediately at LoT, in a fatigued state. When fatigued, a flattening of the hyperbolic power-velocity relationship has been shown, where maximal isokinetic cycling power now occurs at ~80-120 rpm (Beelen and Sergeant, 1991; Elmer *et al.,* 2013). Also of importance when selecting our isokinetic cadence was minimising the time it takes for participants to increase cadence from the LoT (50 rpm) to the isokinetic power assessment post-exercise, in order to minimise any chance for rapid neuromuscular recovery. The lowest cadence that also enabled maximal isokinetic cycling power to be achieved in a fatigued state was therefore determined as 80 rpm, with this used throughout all protocols in healthy individuals. This cadence was performed both at baseline and the LoT so as to eliminate the effect of velocity when comparing these discrete measurements.

In chronic heart failure, due to their limited function it was determined that they would not be able to efficiently reach 80 rpm post maximal exercise. While there is a lack of evidence as to the optimum cycling cadence in CHF, Cannon *et al.,* (2016) has performed maximal isokinetic cycling in COPD using a cadence of 70 rpm. Therefore, the cadence for all maximal isokinetic power measurements in CHF was reduced to 70 rpm.

2.5.3 Familiarisation

Isokinetic cycling was a novel exercise modality for participants, therefore familiarisation of how exactly to perform these efforts was paramount. Participants performed multiple, sub-maximal practice isokinetic efforts, until they were comfortable with isokinetic cycling, and were capable of generating

reproducible power output measurements. This familiarisation phase was also important as it allowed participants time to get accustomed to the laboratory environment, the experimenters and the cycle ergometer. This minimised the effects on the data collected of apprehension or excitement that may be present, due to the unfamiliar surroundings. Finally, this phase enabled participants to find the optimum ergometer setup. The saddle height, handlebar height and handle bar distance were all altered to provide maximum comfort during cycling, with these settings then saved and used throughout all subsequent visits.

2.5.4 Pre-exercise isokinetic efforts

A series of short (~6 s) bouts of isokinetic cycling were performed prior to the exercise test in each study presented here. These were performed at a range of volitional efforts depending on the specific study protocol, with all studies including at least one maximal effort (for example, subjects were asked to perform isokinetic bouts at what they perceived to be 25, 50, 75 % of maximal effort, followed by a fourth isokinetic bout at maximal (100 %) effort). Participants were instructed to maintain a cadence of ~5-10 rpm below the isokinetic cadence (70 or 80 rpm) for the 10 s preceding each effort, to minimise the time taken to increase to the isokinetic cadence. These instructions also ensured participants didn't cycle too fast (i.e. > 80 rpm), which would cause a severe braking force to be applied by the ergometer when the isokinetic phase began, immediately slowing cadence and potentially influencing the subsequent effort. More details about the specific isokinetic protocols can be found in the experimental chapters.

2.5.5 Ramp-incremental exercise

A maximal ramp-incremental exercise test (RIT) was the first test performed in all studies. The RIT is the most commonly used exercise test throughout the

literature in both health and disease as this single, simple to implement test can provide numerous physiological variables (e.g. Lactate threshold, $\dot{V}_E/\dot{V}CO_2$, $\dot{V}O_{2peak}$) that can provide predictions of performance, mortality and morbidity, in addition to supporting a clinical diagnosis (Guazzi *et al.,* 2012; Keteyian *et al.,* 2016; Poole and Jones, 2017). The parameters derived from this test also provided a standard from which to design and implement subsequent exercise protocols, on an individual participant basis.

First, participants sat on the cycle ergometer at rest for ~2 min to allow the collection of resting data. Participants were then instructed to begin a warm-up phase of "unloaded" cycling at 50-60 rpm. Both these phases were performed until key pulmonary variables were in a steady state (e.g. respiratory exchange ratio; 0.7-0.9), generally lasting ~ 4-6 min. This was essential as any indications of hyperventilation would have an adverse influence on lactate threshold determination, possibly inducing a pseudothreshold due to its effects on body CO_2 and O_2 storage (Ozcelik *et al.,* 1999).

Once stable pulmonary variables were attained, the work rate was increased as a linear function of time. The ramp-incrementation rate for this initial RIT was 25 $W.min^{-1}$ for healthy participants and 5-15 $W.min^{-1}$ for CHF patients. These specific rates were chosen to ensure the optimum exercise duration was attained (8-12 min), allowing enough time for sufficient data collection to determine all desired variables but not so long as to effect participant motivation (Buchfuhrer *et al.,* 1987). In all RIT protocols, participants were asked to self-select the cadence that was most comfortable and to then increase this gradually during the exercise if and when required (within 60-100 rpm). Verbal cues were given if required.

This increase in work rate continued until participants reached the LoT, defined as when they could no longer maintain a cadence above 50 rpm, despite strong verbal encouragement. At this point, the mode on the ergometer was instantaneously switched to isokinetic and a maximal isokinetic effort was performed (see below, section 2.5.7). Each RIT was completed with a minimum of 6 min active recovery at the unloaded pedalling work rate, again maintaining a cadence 50-60 rpm. A schematic representation of this protocol is displayed in Figure 2.4.

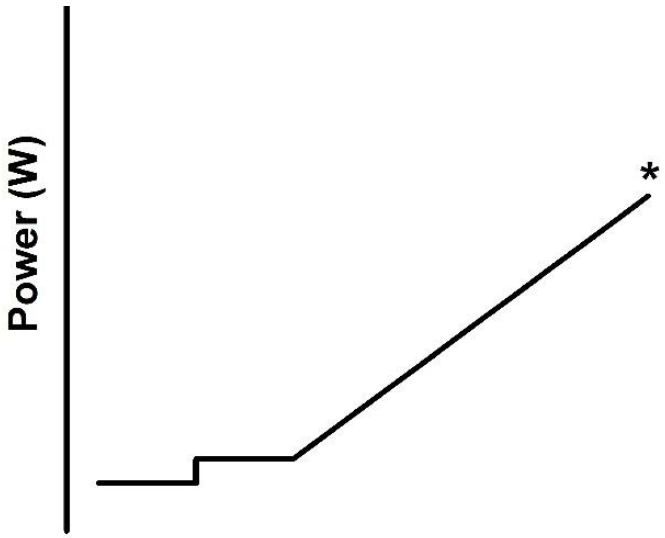

Figure 2.4: Schematic of the ramp-incremental exercise tests. *Denotes the limit of tolerance, which was followed by a maximal isokinetic effort and then at least 6 min of active recovery.

2.5.6 Constant-power exercise

These tests involved a step wise increase in work rate as opposed to the gradual linear increase in the RIT. For constant-power tests, following the same rest and warm-up phases as the RIT, power was instantaneously (rate of 1000 $W.s^{-1}$) increased to the target power, pre-determined using the peak power output from the RIT. Due to the significant and sudden increase in flywheel resistance as the work rate is imposed, participants were informed ~10 s before the beginning of

the test phase, giving the opportunity to increase cadence to between 70-100 rpm, overcoming the initial increase in resistance. Participants were then instructed to continue the exercise until the LoT was attained, again defined as the point at which participants could no longer maintain a cadence above 50 rpm, despite strong verbal encouragement to do so. The duration of this was also pre-determined, and designed so the LoT was reached between 2-15 min (see section 4.2.3.3 for explanation). As with the RIT, at LoT the mode on the ergometer was then switched and a short, maximal voluntary isokinetic effort was performed (described below, section 2.5.7). Following this, the work rate was reduced back to unloaded pedalling for a period of active recovery, lasting a minimum of 6 min at a cadence of 50-60 rpm. A schematic representation of this protocol is displayed in Figure 2.5.

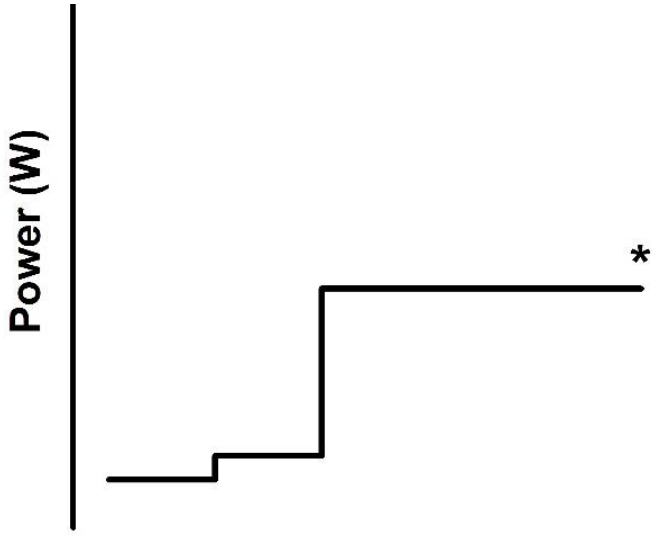

Figure 2.5: Schematic of the constant-power exercise tests. *Denotes the limit of tolerance, which was followed by a maximal isokinetic effort and then at least 6 min of active recovery.

2.5.7 Limit of tolerance isokinetic effort

In order to measure the exercise induced reduction in voluntary power, and investigate the mechanisms limiting exercise tolerance, all tests were followed

instantaneously by a final isokinetic effort to measure the power generating capacity of the skeletal muscles. Once the LoT was attained (defined as a cadence below 50 rpm), the mode was switched to isokinetic, causing flywheel resistance to immediately fall to zero (due to cadence being below the isokinetic cadence; 70 or 80 rpm). Participants were then instructed to increase cadence to the isokinetic level as quickly as possibly (generally ≤ 1 s) where this final maximal isokinetic effort was performed (Figure 2.6).

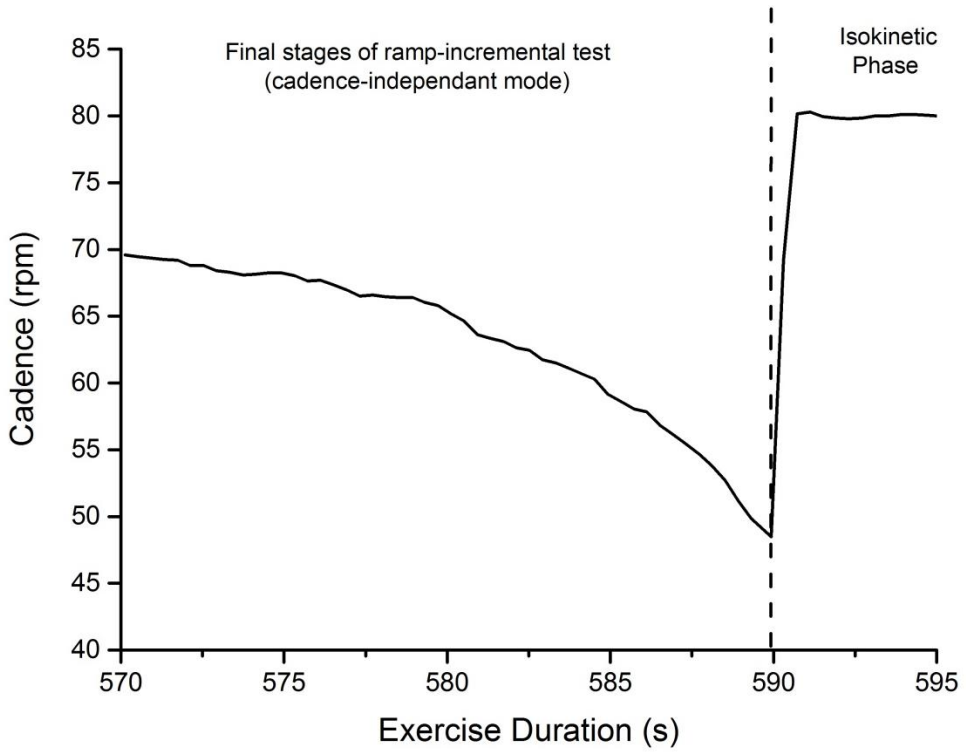

Figure 2.6: A representative example of the cadence profile in the final stages of a ramp-incremental exercise test, followed instantaneously (≤ 1 s) by a maximal isokinetic effort (constrained at 80 rpm).The moment the modes on the cycle ergometer are switched is displayed by the dashed line.

2.6 Data analysis

Data analyses of both pulmonary gas exchange and power measurements were all performed using a combination of OriginPro 8.6 (OriginLab Corporation, Northampton, MA, USA) and Microsoft Excel (Microsoft Corporation, Redmond, WA, USA). EMG data analysis was performed using proprietary Noraxon software; myoresearch XP software (Noraxon U.S.A Inc, Scottsdale, AZ, USA).

2.6.1 Pulmonary gas exchange

2.6.1.1 Data editing

To collect pulmonary gas exchange data, participants were required to breathe through a mouthpiece with a nose clip also in place. The presence of these apparatus has the potential to effect an individual's natural breathing patterns. As a consequence, occasional coughs, swallows or sighs can occur, causing breath-by-breath outliers that can distort the interpretation of the true underlying physiological response (Lamarra *et al.,* 1987). Therefore, data editing to delete these erroneous "breaths" was the first stage of analysis, before any interpretation of these data could be made. Editing the gas exchange data involved fitting 99 % prediction limits to the local mean of $\dot{V}O_2$ data, and deleting any point that lay outside of these (Figure 2.7). This method provided enough caution so that only errant breaths were deleted and not any of the true underlying physiological response (Whipp and Rossiter, 2005).

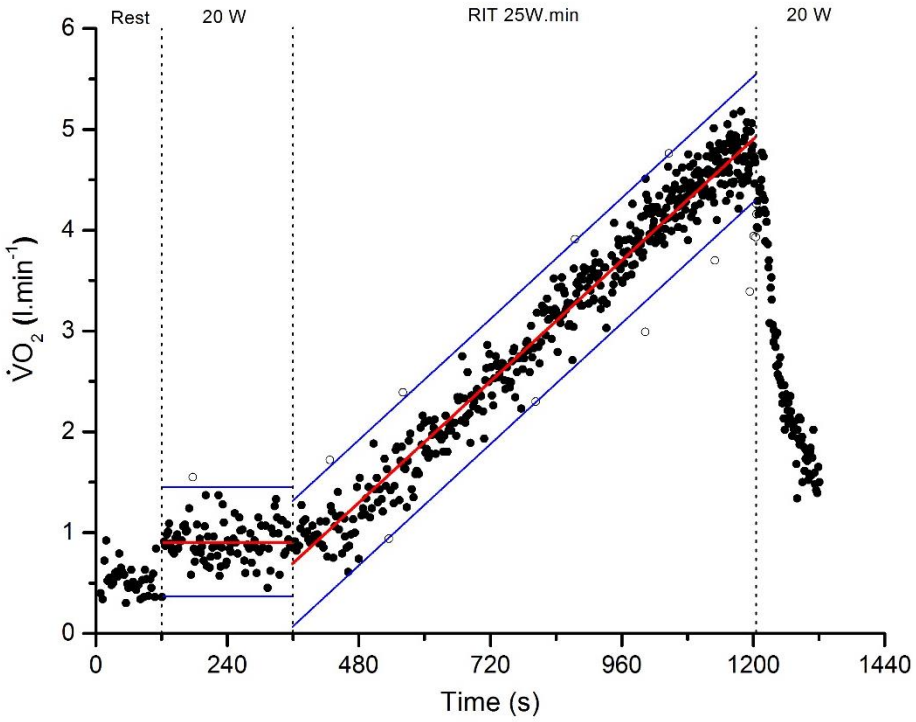

Figure 2.7: A representative example of the $\dot{V}O_2$ response to a standard ramp-incremental exercise test on which the data editing procedures have been implemented. The mean fit of the data (red lines) and 99 % prediction bands (blue lines) are displayed. Note all data points outside of the prediction bands have been deleted from the edited data set (closed circles), so are now displayed as open circles only. Dashed lines also indicate the different phases of the exercise test.

2.6.1.2 Lactate Threshold

Lactate threshold (LT) was determined non-invasively from the RIT to add to the characterisation of participants. After editing procedures, breath-by-breath data from the ramp phase only of the RIT were isolated and $\dot{V}CO_2$ was plotted against $\dot{V}O_2$. The V-slope method was then used to determine LT as the inflection point at which there was a greater increase in $\dot{V}CO_2$ than $\dot{V}O_2$, as a consequence of the excess CO_2 produced from bicarbonate buffering of a metabolic acidosis caused by hydrogen ions (Beaver *et al.,* 1986). The $\dot{V}O_2$ estimated from the V slope method was then corroborated by an increase in the ventilatory equivalent for oxygen ($\dot{V}_E/\dot{V}O_2$) without a similar increase in the ventilatory equivalent for

CO_2 ($\dot{V}_E/\dot{V}CO_2$), in addition to an increase in end tidal partial pressure of oxygen ($PETO_2$) and not end tidal partial pressure of CO_2 ($PETCO_2$). An example non-invasive LT determination is shown in Figure 2.8.

This process was performed by 3 independent researchers to ensure accuracy. If the 3 values were within 200 ml, a mean was taken as the LT; however, if they were outside of this, the values were discussed by the 3 researchers and an appropriate LT was determined.

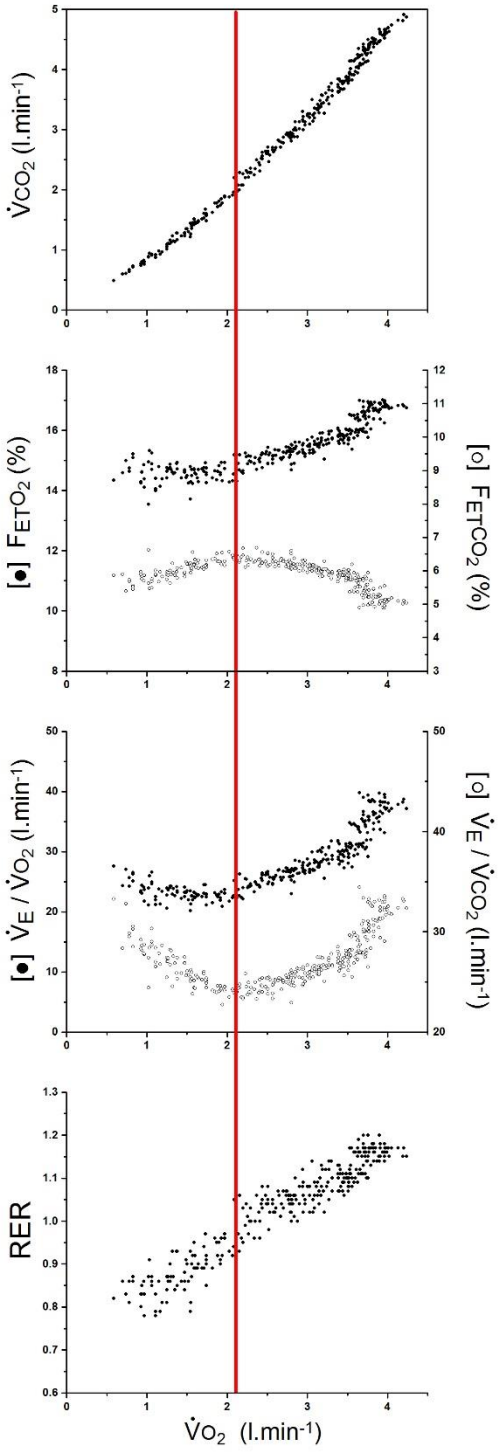

Figure 2.8: A representative example of the data used to estimate non-invasive lactate threshold (LT). Shown is the typical gas exchange responses during the isolated ramp-incremental phase of the ramp-incremental test on the cycle ergometer. The estimated LT for this test is indicated by the solid red line.

2.6.1.3 Maximum oxygen uptake

To determine $\dot{V}O_{2peak}$ for each exercise test, a 12 breath average was taken from the end of the exercise phase of the protocol. This was done 8 times, working backwards as a rolling average from the point of the LoT to find the highest 12 breath average. By using 12 breaths, this minimised the effect of breath-by-breath noise while still providing enough data to produce an accurate $\dot{V}O_{2peak}$ (Bowen *et al.,* 2012a). Where multiple maximal exercise tests were performed (Chapters 3 and 4) $\dot{V}O_{2peak}$ could be validated and therefore confirmed as a participants maximal oxygen uptake ($\dot{V}O_{2max}$) (Poole and Jones, 2017). Although it has been shown that an individual's $\dot{V}O_{2peak}$ of a RIT is not likely different from their $\dot{V}O_{2max}$ (Day *et al.,* 2003) without confirmation (Chapter 5 only), $\dot{V}O_2$ at the limit of tolerance was termed $\dot{V}O_{2peak}$. The same 12 breath average determined for $\dot{V}O_{2peak}$ was used for all maximal pulmonary gas exchange variables and for HR_{max} determination.

2.6.2 Power measurements

The peak power attained at the LoT of the RIT (when cycling in the hyperbolic mode; RI-Flywheel$_{peak}$) was determined using the ramp rate and the duration of the ramp phase of the exercise as follows:

$$\text{RI-Flywheel}_{\text{peak}} = \frac{\text{exercise duration}}{60} \times \text{ramp rate} + \text{WR}_{\text{UP}}$$

Equation 2.1

where exercise duration is in seconds, ramp rate is in W.min^{-1}, and WR_{UP} is the unloaded pedalling work rate (W). Although this was the flywheel work rate "required" at the limit of tolerance, the "actual" power being produced at the crank during the final 3 pedal strokes before the LoT (50 rpm) was also assessed (termed hyperbolic power at LoT; P_{HYP} LoT). These measurements were made

in the hyperbolic mode on a pedal stoke by pedal stroke bases using the strain gauges (torque) and optical sensors (angular velocity), as described above. Measuring P_{HYP} LoT was designed so as to confirm that, although participants could no longer meet the power demanded by the task, they were still providing a maximal effort up to LoT, and so no recovery could occur before the maximal isokinetic phase.

2.6.2.1 Isokinetic Data

For each isokinetic phase, first a mean of the 180 power measurements (1 every 2° of rotation) for each isokinetic pedal stroke was taken. As there was no systematic difference between left and right crank power ($p > 0.05$), these were then summed to provide a single power output for each pedal stroke. By taking a mean of each pedal stroke and a sum of the left and right pedals this allowed comparisons with the flywheel power to be made, as this is also how participants meet the flywheel power requirement during exercise. The first 3 pedal strokes correctly constrained at the desired cadence were selected for each isokinetic effort, with a mean of these reported as the power produced during that effort (P_{ISO}). This process is shown in its entirety for the 3 isolated pedal strokes for both a maximal effort baseline P_{ISO} and LoT P_{ISO} (Figure 2.9), to display the difference in power output during these 2 efforts.

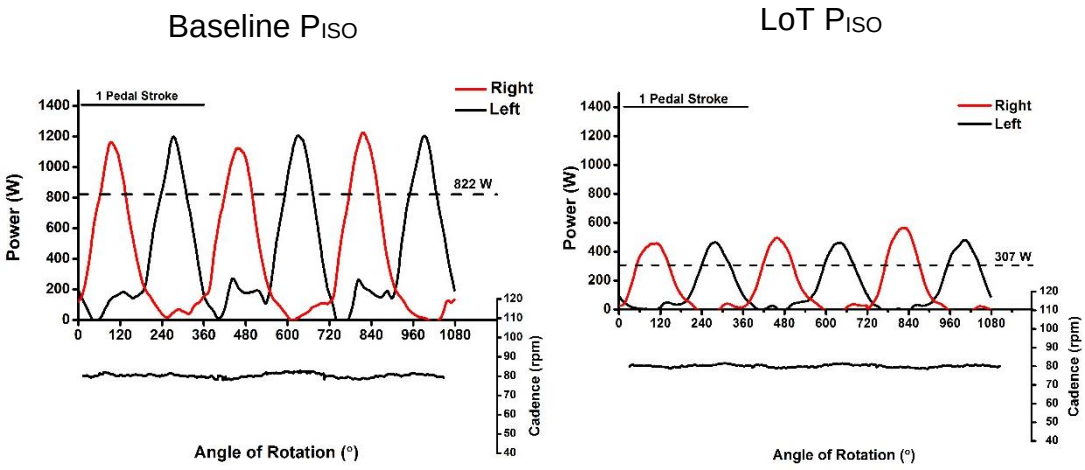

Figure 2.9: Representative participant maximal voluntary effort isokinetic effort at baseline (Baseline P_{ISO}) and the limit of tolerance (LoT P_{ISO}). Displayed are the first 3 pedal strokes constrained at 80 rpm for each phase, with left and right power shown independently and a mean of the 3 isolated pedal strokes displayed (dashed line).

From the isokinetic power measurements, the following variables were then calculated for each exercise test (Figure 2.10):

$$\text{Power Reserve} = \text{P}_{\text{ISO}} - \text{Task requirement at LoT}$$

Equation 2.2

$$\text{Performance Fatigue} = \text{Baseline P}_{\text{ISO}} - \text{LoT P}_{\text{ISO}}$$

Equation 2.3

$$\text{Power Accessed} = \frac{\text{RI-Flywheel}_{\text{peak}}}{\text{Baseline P}_{\text{ISO}}} \times 100$$

Equation 2.4

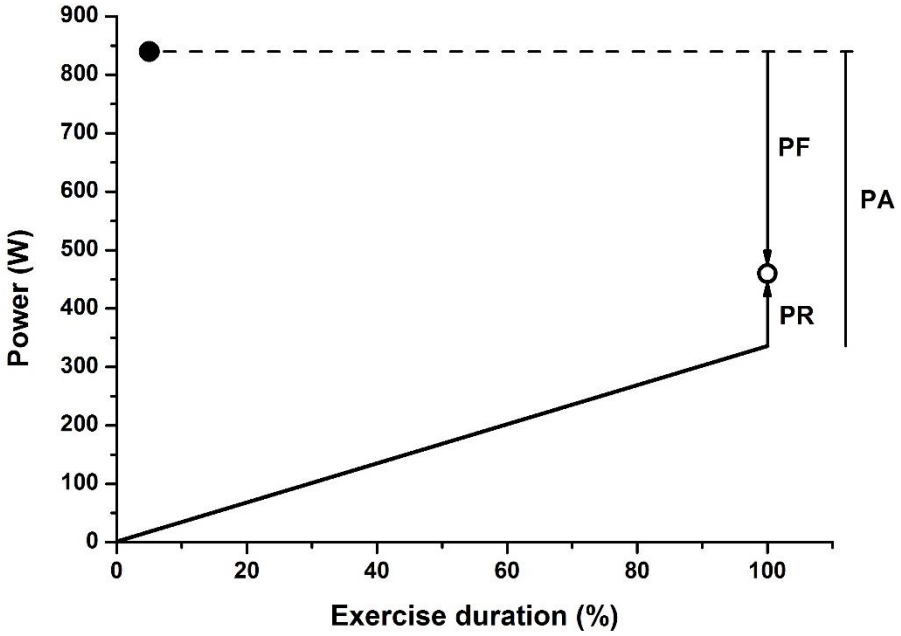

Figure 2.10: Schematic of performance fatigue (PF), power reserve (PR) and power accessed (PA) calculations. Baseline isokinetic power (closed circle and dashed line) and limit of tolerance isokinetic power (open circle) are shown. Also, the task required power during, in this case a ramp-incremental test, is displayed (solid line).

2.6.2.2 Natural fluctuation in power

In order to continue any exercise task, participants must produce the power required at the flywheel, set by the ergometer. However, although during the ramp-incremental or constant-power tests mean power produced will match that required, the pedal-by-pedal stroke power output will fluctuate around this task requirement (Figure 2.11 A). It was possible to make these measurements in the cadence-independent mode at a resolution of 2 ° using the same method as during the isokinetic phases (section 2.5.2). The natural fluctuation was deemed to be accurately represented by the 95 % prediction bands around the local mean for the whole exercise test (Figure 2.11 B). This natural fluctuation in crank power was determined for each individual and a mean of the participants then reported alongside relevant data to provide context.

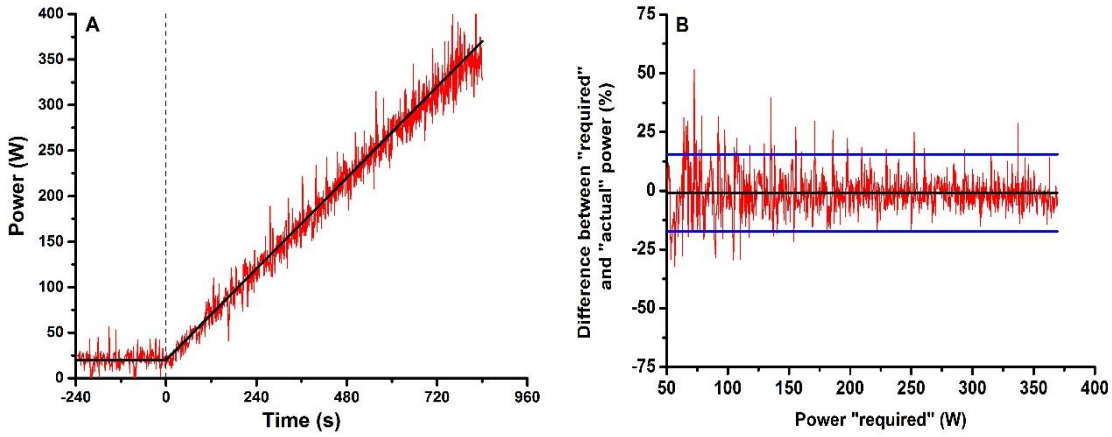

Figure 2.11: An example of the natural fluctuation in crank power and characterisation of this response. A; the natural fluctuation during a ramp-incremental test (red line) around the required flywheel power (solid black line). Exercise onset is also shown (dashed black line). B; the % difference of the same test between the required power of the task and the actual power measured at the crank. Displayed are the 95 % prediction bands used to characterise the natural fluctuation of each test. Note the mean (sold black line) is zero, showing over the entire task the power required was produced by the participant.

2.6.3 EMG data

EMG data were recorded during the isokinetic efforts in Chapters 3 and 4. Initial post-processing included rectification of the EMG signals, followed by the application of a band-pass filter (10-500 Hz) to remove erroneous signals outside these boundaries, with the data then smoothed via a root-mean-square, with a moving window of 100 ms (Coelho *et al.,* 2015). EMG data processing is displayed in Figure 2.12. After initial processing, the 3 contractions matching the 3 pedal strokes used for isokinetic power measurements were isolated, with peak EMG activity determined for each of the 5 muscles measured. The 5 muscles were then summed to provide a single EMG datum for each isokinetic effort, matching the power data. All data are reported relative to the maximal voluntary isokinetic effort EMG activity for that day, limiting the effects of daily variations in skin temperature, hydration, preparation and conductance, providing a normalisation for each test (Coelho *et al.,* 2015).

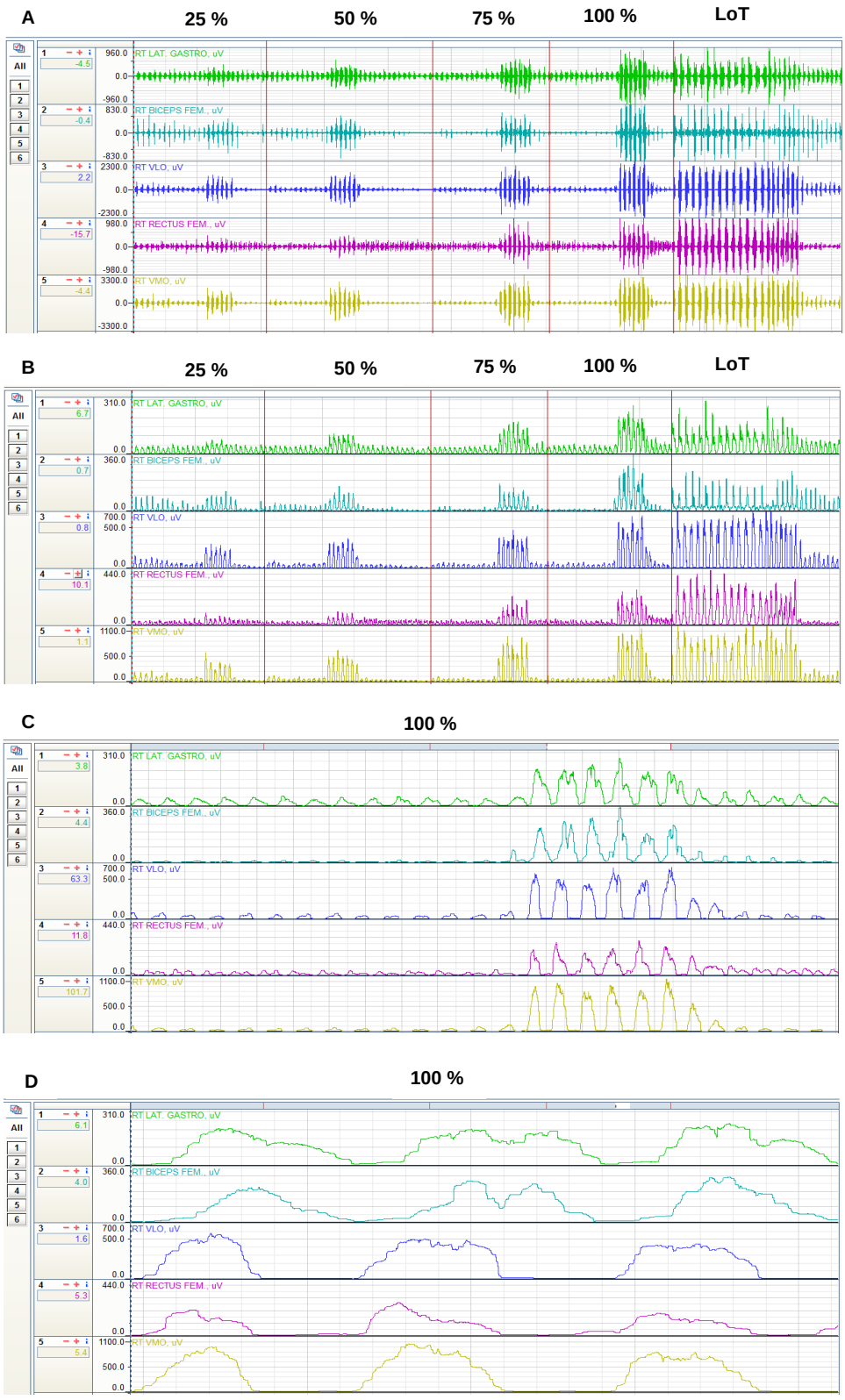

Figure 2.12: Details of the EMG analysis process. A; Raw EMG data from the 4 pre-exercise efforts at 25, 50, 75 and 100 % effort as well as the 100 % effort at limit of tolerance (LoT). B; EMG data after initial processing of rectification, filtering and smoothing processes. C; the 100 % unfatigued effort has been isolated. D; the 3 contractions have now been isolated from this effort, after which a peak EMG amplitude was then taken for each of these 5 muscles and summed to give the EMG signal for each effort.

2.6.4 Power-EMG relationship

Coelho *et al.* (2015) identified that there is a linear relationship between isokinetic cycling power and EMG activity, which can be used to provide an insight into potential fatigue mechanisms. This same methodology was employed for each exercise test in Chapter 3.

First, the power and EMG data acquired during the initial baseline pre-exercise isokinetic efforts were plotted against each other. This unfatigued series of efforts were then characterised with a linear function. This relationship could then be used to estimate the contributions of activation and muscle fatigue to the overall fatigue present.

The power and EMG data acquired from the maximal isokinetic effort performed at LoT was added to this fresh power-EMG relationship. Performance fatigue was determined as the fall in maximal isokinetic power from baseline to the LoT effort (for calculation see section 2.6.2). Then, using the linear fit of the unfatigued relationship, it was possible to determine the contributions of activation fatigue (i.e. the power equivalent drop in EMG activity) and muscle fatigue (i.e. the drop in power below that expected for the EMG activity present). These calculations are shown for a representative subject in Figure 2.13.

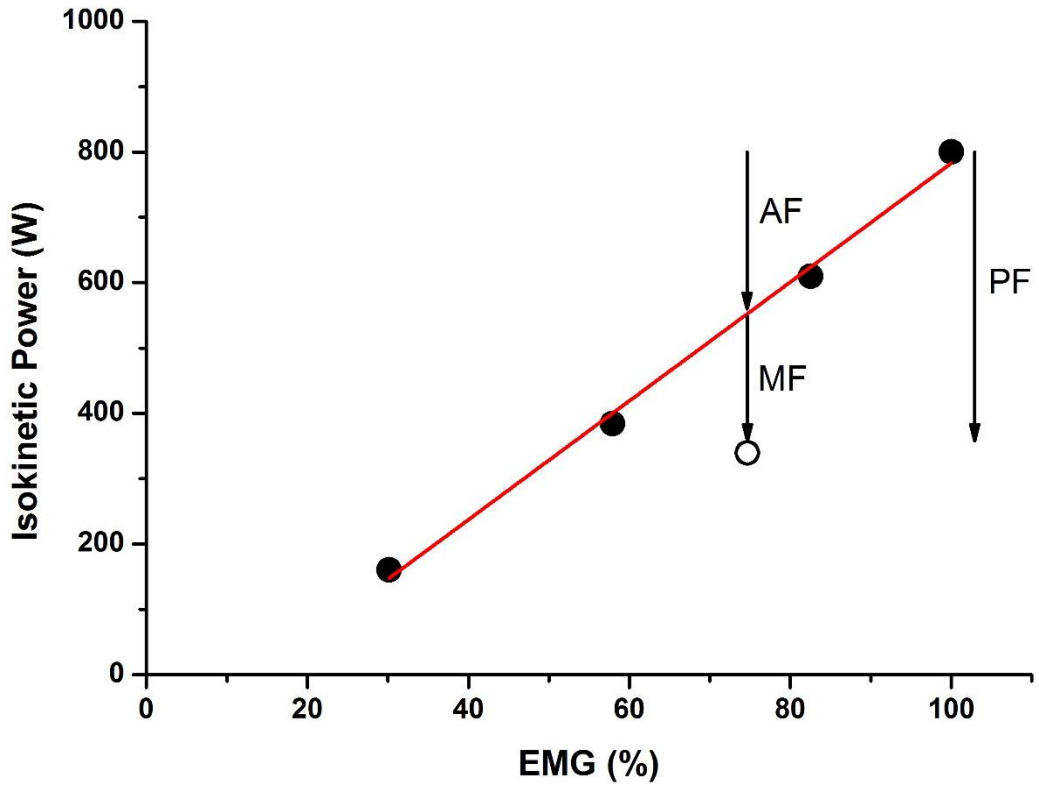

Figure 2.13: A representative isokinetic power-EMG relationship. The calculations of contributions to overall performance fatigue (PF) of activation fatigue (AF) and muscle fatigue (MF) are shown. The unfatigued efforts of 25, 50, 75 and 100 % volitional effort are displayed (closed circles) alongside the LoT maximal effort (open circle). The unfatigued linear relationship between power-EMG is also displayed (red line).

2.7 Statistics

All statistical analysis was performed in OriginPro 8.6 (OriginLab Corporation, Northampton, MA, USA). Initially, all data were assessed for normality using the Sharipo-Wilk method, with all variables for all studies found to be from a normally distributed population. The specific statistical processes that were implemented following this are outlined in the methods sections of the relevant experimental chapters. Data are reported as mean ± SD, with significance level set at 0.05 throughout all studies presented here.

Chapter 3 Power reserve at the limit of ramp-incremental exercise is dependent on incrementation rate

3.1 Introduction

During whole-body dynamic exercise such as cycling or running, integration of pulmonary, cardiovascular and neuromuscular systems enable the power required by the task to be maintained by ensuring O_2 can be supplied and utilised at a rate that meets the demands of the exercise. However, when the energy (ATP) demands of the exercise task exceed the integrated capacity of these systems to meet the ATP requirement through aerobic metabolism, reliance on anaerobic energy systems occurs, resulting in the development of peripheral and central fatigue, together contributing to progressively limiting the power producing capacity of the exercise muscles, eventually causing task failure. The longer an individual can delay this task failure, the greater their exercise tolerance, which remains an important predictor for quality of life and all-cause mortality (Myers *et al.,* 2002). Although the development of fatigue has been purported as the cause of task failure, how central and peripheral fatigue mechanisms combine to cause intolerance, especially in whole-body dynamic exercise, remains poorly understood.

Central fatigue comprises any mechanisms from within the central nervous system that ultimately causes a reduction in the ability to recruit the exercising muscle and, therefore reduces force production (Gruet *et al.,* 2014). Peripheral fatigue consists of mechanisms within the exercising muscle that inhibit excitation-contraction coupling and actin-myosin cross-bridge formation, reducing force production (Westerblad *et al.,* 2002; Allen *et al.,* 2008).

Consequently, in the presence of central fatigue, external stimulation to maximally activate the muscle will increase force, whereas with peripheral fatigue present the muscle has no capacity to increase force production, even when maximally stimulated (Twomey *et al.,* 2017).

It has been shown during a wide range of exercise tasks, that both central and peripheral fatigue contribute to the fatigue accumulated at the limit of exercise tolerance (LoT) (Burnley *et al.,* 2012; Decorte *et al.,* 2012). Of particular interest is whether, at the LoT, task failure occurs when the voluntarily evocable maximal power matches or exceeds the power required by the task; i.e. is a "power reserve" present at the LoT? (Ferguson *et al.,* 2016b). This may provide information about whether the sensations of fatigue and the physiological limits are coupled at task failure (i.e. no reserve; suggesting primarily peripheral fatigue) or dissociated (i.e. reserve present; suggesting primarily central fatigue).

Using isometric knee-extension exercise, Burnley *et al.* (2012) found that voluntarily evocable maximal power at task failure was not different from the task requirement. However, there is little pulmonary and cardiovascular strain during single joint exercise (Sidhu *et al.,* 2013), and therefore fatigue is isolated to the peripheral level. Consequently, the power not exceeding the task requirement (i.e. no power reserve) may be expected. Recently, a protocol developed by Coelho *et al.* (2015) has utilised short (~5 s) maximal voluntary isokinetic efforts on a cycle ergometer to measure velocity-independent peak isokinetic power (P_{ISO}) before and immediately (≤ 1 s) after a ramp-incremental exercise test (RIT) to LoT. Importantly, unlike knee-extension, using whole-body exercise enabled the attainment of $\dot{V}O_{2max}$, increasing the utility of this protocol. At LoT (and $\dot{V}O_{2max}$) a small power reserve was observed (~118 % task requirement).

However, this was attributed to be the result of a small capacity to increase power, evidenced by the large swings in instantaneous power production during the RIT (Coelho *et al.,* 2015) (see section 2.6.2.2 for explanation). This protocol has since been applied by Ferguson *et al.* (2016b) in endurance athletes. This population was chosen as athletes are generally predominantly limited by O_2 delivery not the exercising muscle (Roca *et al.,* 1992; Mortensen *et al.,* 2008) and therefore, if a power reserve were present, it would likely be most evident in this population. It was found that LoT P_{ISO} was not significantly different (~ 111 %) from the RIT task requirement. The findings using whole-body exercise (Coelho *et al.,* 2015; Ferguson *et al.,* 2016b) support the findings in single joint exercise (Burnley *et al.,* 2012; Bigland-Ritchie *et al.,* 1983) and suggest that the LoT occurs primarily due to fatigue mechanisms within the exercising muscles (i.e. peripheral fatigue).

To date, the only exercise protocols that have incorporated this isokinetic cycling methodology are ramp-incremental tests of a standard duration (Coelho *et al.,* 2015; Morales-Alamo *et al.,* 2015; Ferguson *et al.,* 2016b), designed to produce optimal ramp durations of ~8-12 min (Buchfuhrer *et al.,* 1983). However, the dynamics of fatigue, both central and peripheral, have been shown to vary depending on the task performed; more specifically the exercise intensity and exercise duration (Froyd *et al.,* 2016; Thomas *et al.,* 2016; Burnley *et al.,* 2012). The first fatigue mechanisms to develop during exercise are within the skeletal muscle, with some authors reporting the presence of a “critical threshold” of peripheral fatigue at which it will not increase further (Burnley *et al.,* 2012; Vanhatalo *et al.,* 2010; Cannon *et al.,* 2011), possibly determined by the CNS via afferent feedback, for protective reasons (Amann *et al.,* 2009). However, this remains debated, as others have measured peripheral fatigue increasing

throughout exercise up to the LoT (Froyd *et al.,* 2016; Christian *et al.,* 2014; Thomas *et al.,* 2016). In contrast, central fatigue has been found to be relatively slower to develop during exercise (Decorte *et al.,* 2012; Pearcey *et al.,* 2014; Thomas *et al.,* 2016), possibly as the CNS reacts to the increasing effects of peripheral fatigue by increasing neural drive (Bigland-Ritchie *et al.,* 1983; Amann *et al.,* 2009). Consequently, the magnitude of central fatigue is greater with reduced intensity and increased exercise duration (Froyd *et al.,* 2016; Thomas *et al.,* 2015; Burnley *et al.,* 2012).

If peripheral fatigue is in fact not different, then at task failure, this would suggest that alterations in fatigue contributions due to changes in the exercise task are limited to changes in the magnitude of central fatigue present. Increasing the contributions of central fatigue may alter the sense of effort, amplifying the sensations of fatigue that may ultimately cause a dissociation of the perceived fatigue stimulus from the actual physiological strain (Jones and Killian 2000; Hureau *et al.,* 2016). This increased perception of effort may cause task failure to be attained before the maximum capacity of the muscles has been achieved. How the effect of these alterations in fatigue contributions translates to changes in the maximum voluntary evocable power at task failure is unclear. Similarly, whether there is no power reserve at LoT (suggesting the perceived and physiological limits are coupled, as after “optimal duration” ramp-incremental exercise) or whether changes in the task demands results in a power reserve being present, remains to be determined.

Thus, the aim of this study was to determine the effect of altering the exercise task, by altering the ramp-incrementation rate, on the magnitude of fatigue at task failure and the presence or absence of a reserve in maximal voluntary power capacity. It was hypothesised that during short and optimal duration incrementation rates no power reserve would be present, but by extending the exercise duration and reducing the power required at LoT, this would increase the contributions of central fatigue resulting in the presence of a power reserve at task failure.

3.2 Methods

3.2.1 Ethical Approval

This study and all the procedures involved were approved by the Faculty of Biological Sciences Ethics Committee, University of Leeds. Written informed consent was obtained from all eligible volunteers prior to their involvement in the study, with participants free to withdraw at any point and without explanation.

3.2.2 Participants

Twelve healthy, habitually active and non-smoking participants volunteered to partake in this study (9 male and 3 female; Age 25 ± 5 yr; Height 175 ± 7 cm; Weight 73 ± 9 kg). Each participant was screened before any exercise was performed using a health and activity questionnaire. Screening participants ensured that anyone for which high-intensity exercise may be unsafe was excluded. Exclusion criteria consisted of anyone with a history of cardiovascular, respiratory or musculoskeletal disease, in addition to individuals with asthma, a family history of sudden cardiac death or recent illness or injury.

3.2.3 Exercise Protocols

All exercise tests were performed on an electromagnetically braked, computer-controlled upright cycle ergometer (Excalibur Sport PFM, Lode BV, Groningen, NL). Each participant completed 3 visits to the temperature controlled laboratory (19 – 21 °C), separated by at least 48 hours. Each visit consisted of two separate parts; 1) a series of short, varied effort bouts of isokinetic cycling and 2) a ramp-incremental exercise test to LoT, followed immediately by a further single bout of maximal isokinetic cycling (Figure 3.1).

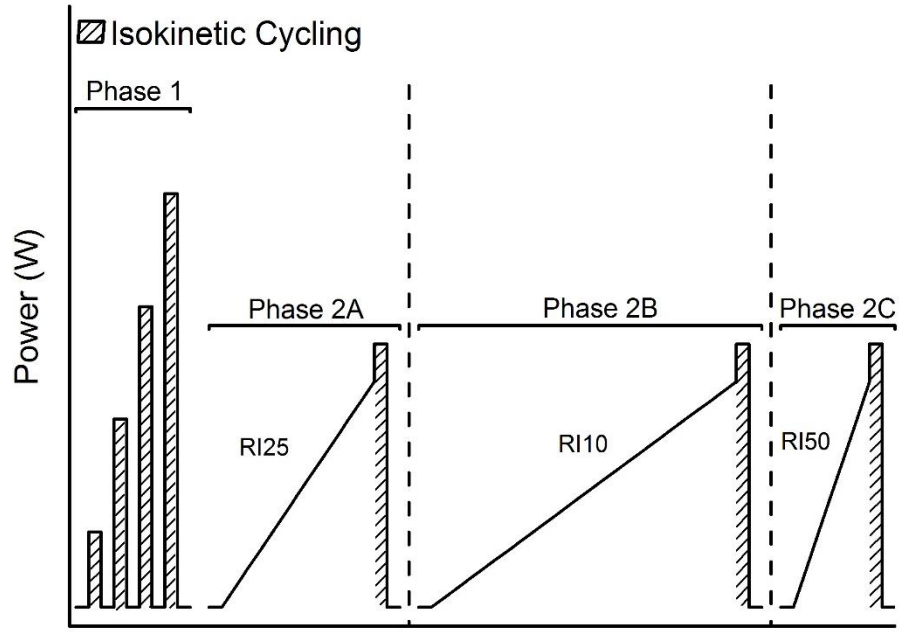

Figure 3.1: Schematic representation of the 2 phases of each of the 3 visits performed by each participant. Hashed bar = short (~6 s) isokinetic cycling. Part 1: 4 varied effort (25-100 %) bouts of isokinetic cycling and Part 2: ramp-incremental exercise test to attain the limit of tolerance: 25 W.min^{-1} (2A), 10 W.min^{-1} (2B), 50 W.min^{-1} (2C), followed immediately by a final maximal isokinetic bout of cycling. Dashed vertical lines indicate that each of the different ramp-incremental tests were performed on a different day, separated by at least 48 hours.

Phase 1 of each visit required the participant to perform 4 separate bouts of isokinetic cycling at what the participant perceived to be 25, 50, 75 % of volitional effort, with a final isokinetic bout at 100 % effort, with isokinetic velocity constrained to 80 rpm by the electromagnetic braking system of the flywheel. Each bout lasted ~6 s and was preceded by 30 s of cycling at 20 W.

Phase 2 of each visit started with the participant sat on the ergometer at rest for 2 min, followed by ~ 4-6 min of cycling at a low work rate, analogous to unloaded pedalling (20 W). After the warm-up, the work rate was then increased as a smooth function of time at either: 10, 25 or 50 W.min^{-1}, designed to alter the duration of the exercise task, and ramp-incremental power at task failure. Each

of these 3 exercise protocols were performed to the LoT, defined as the point at which the participant could no longer maintain a cadence above 50 rpm, despite strong verbal encouragement. At the LoT, the ergometer was instantaneously switched back to the isokinetic mode, causing flywheel resistance to temporarily fall to zero (as cadence was below 80 rpm), and participants were instructed to increase cadence to 80 rpm as quickly as possible (≤ 1 s), where a final bout of ~6 s maximal effort isokinetic cycling was performed. All protocols were then followed by 4-6 min of active recovery at 20 W.

3.2.4 Measurements

The isokinetic mode on the cycle ergometer was used to measure velocity-independent isokinetic power (P_{ISO}) at the crank of the ergometer; calculated as the product of torque (Nm) and angular velocity ($Rad.s^{-1}$), and measured every 2º of angular rotation. Details on the cycle ergometer specifications, calibration, error allowance, and detailed explanation of the calculations can be found in section 2.2.

Additionally, during each of these brief bouts of isokinetic cycling, surface electromyography (EMG) (Noraxon TeleMyo 2400T G2, Noraxon USA Inc, Scottsdale, AZ, USA) was used to measure the electrical activity of 5 muscles in the right leg: *gastrocnemius lateralis*, *biceps femoris*, *vastus lateralis*, *rectus femoris*, *vastus medialis*. Before attachment of the electrodes, the skin was prepared to maximise the signal by shaving excess hair, using an abrasive gel to remove dead skin cells and sterilising with an alcohol wipe. EMG data were recorded at 1500 Hz. More details on anatomical positioning, skin preparation and EMG measurements can be found in section 2.4.

Respired gases were measured during phase 2 of each visit only. Participants breathed through a mouthpiece while wearing a nose clip, with gases sampled through a preVent flow sensor and then analysed by a breath-by-breath system (MedGraphics D-Series, Medgraphics, Medical Graphics Corporation, St Paul, MN, USA). Prior to the exercise test, the flow sensor was calibrated using a 3.0 L syringe, across a range of flow rates and the O_2 and CO_2 analysers were calibrated using gases of known concentrations, independently verified before and after each test. More details on the breath-by-breath analysis system and standard calibration procedures are found in section 2.3. A 12 lead ECG was also attached to each participant throughout the exercise for measurement of heart rate.

3.2.5 Data Analysis

Mean left and right P_{ISO} were determined independently and then summed to determine P_{ISO} for each pedal stroke. To characterise each isokinetic cycling phase, the first 3 pedal strokes correctly constrained at 80 rpm were selected and a mean of these 3 reported as P_{ISO}. Performance fatigue, the magnitude of a power reserve, power accessed and the natural fluctuation in power were then determined for each test. For more details on all the specific power calculations, see section 2.6.2.

Initially, raw EMG data was rectified, band-pass filtered and smoothed via root-mean-square with a 100 ms moving window, with no overlap. For each isokinetic phase, the 3 contractions that corresponded to the 3 pedal strokes selected for P_{ISO} data were then isolated. The peak muscle activity of each contraction for the individual muscles was found, with the 5 muscles then summed for each pedal stroke. Then as with P_{ISO} data, a mean of the 3 pedal strokes was reported.

The unfatigued, 4 pre-exercise P_{ISO} and EMG measurements were then plotted and the relationship between these characterised by a linear function. These data, along with the P_{ISO} and EMG during LoT isokinetic phase were then used to investigate the contributions of activation and muscle fatigue to overall performance fatigue. Detailed explanation of this relationship and how these contributions were calculated can be found in section 2.6.4.

Breath-by-breath data was initially edited to remove errant breaths due to coughs, swallows and sighs and then analysed to determine non-invasive lactate threshold and maximum pulmonary variables (e.g. $\dot{V}O_{2peak}$) for each test. $\dot{V}O_{2peak}$ values were confirmed as $\dot{V}O_{2max}$ by comparing the values attained across the 3 visits with different end-exercise work rates (Rossiter *et al.,* 2006; Poole and Jones, 2017). For detailed explanation of these procedures, see section 2.6.1.

3.2.6 Statistics

One-way repeated measures ANOVA was used to compare 3 different ramp-incremental tests. Pulmonary gas exchange data (e.g. $\dot{V}O_{2peak}$, HR_{peak}) and power measurements were then compared across the 3 protocols using Bonferroni *post-hoc* tests. For P_{ISO} data comparisons within each incrementation rate Dunnett *post-hoc* was used (RI-$Flywheel_{peak}$ control level). The coefficient of variation (CoV) was also calculated for the 3 baseline maximal effort P_{ISO}.

3.3 Results

As the rate of incrementation slowed, RI-Flywheel$_{peak}$ decreased ($p < 0.05$) while time to the limit of tolerance (T_{lim}) increased ($p < 0.05$). Despite this, $\dot{V}O_{2peak}$ was not different across the 3 protocols ($p > 0.05$), confirming $\dot{V}O_{2max}$ was achieved in all protocols. In line with previous research, increasing the incrementation rate increased $\dot{V}CO_{2peak}$, and as a consequence RER_{peak} (both $p < 0.05$). A small but significant difference was also found with HR_{peak} reaching a value slightly lower in RI50 than the other protocols. All gas exchange variables for the group are shown in Table 3.1.

Table 3.1: Group responses to ramp-incremental tests to the limit of tolerance at incrementation rates of 50, 25 and 10 W.min^{-1}.

	RI50	RI25	RI10
T_{lim} (s)	411 ± 58[2,3]	732 ± 93[1,3]	1531 ± 288[1,2]
RI-Flywheel$_{peak}$ (W)	361 ± 48[2,3]	323 ± 39[1,3]	275 ± 38[1,2]
LT (L·min^{-1})	1.85 ± 0.20	1.90 ± 0.19	1.95 ± 0.30
$\dot{V}O_{2peak}$ (L·min^{-1})	3.94 ± 0.67	4.09 ± 0.65	3.93 ± 0.67
$\dot{V}CO_{2peak}$ (L·min^{-1})	4.85 ± 0.70[2,3]	4.61 ± 0.74[1,3]	4.09 ± 0.62[1,2]
RER_{peak}	1.24 ± 0.11[2,3]	1.13 ± 0.13[1,3]	1.05 ± 0.08[1,2]
$\dot{V}_{Epeak}$ (L·min^{-1})	170 ± 29	167 ± 30	163 ± 28
HR_{peak} (bpm)	183 ± 10[2,3]	190 ± 10	189 ± 10

Values are mean ± SD. T_{LIM}, time to the limit of tolerance; LT, Lactate threshold. All variables compared with ANOVA and bonferroni post-hoc: [1] significantly different from RI50; [2] significantly different from RI25; [3] significantly different from RI10.

3.3.1 Power data

All P_{ISO} measurements were constrained at the desired cadence, confirmed by *post hoc* analysis (RI50, RI25, RI10); baseline P_{ISO}; 80.3 ± 0.2, 80.5 ± 0.2, 80.3 ± 0.2 rpm; LoT P_{ISO}; 80.4 ± 0.2, 80.3 ± 0.2, 80.3 ± 0.2 rpm. Maximal baseline P_{ISO} was not different across the 3 visits ($p > 0.05$, CoV; 4.2 %), confirming the reproducibility of these measurements. LoT P_{ISO} was significantly reduced compared to baseline P_{ISO} in all 3 protocols ($p < 0.05$; Table 3.2), showing substantial fatigue was present. However, performance fatigue was significantly less at LoT of the RI10 than at the LoT of both RI50 and RI25 ($p < 0.05$; Table 3.2). A representative participants power (RI-Flywheel resistance, baseline P_{ISO} and LoT P_{ISO}) and $\dot{V}O_2$ responses for the 3 protocols are shown in Figure 3.2.

Table 3.2: Group power output responses for ramp-incremental exercise of incrementation rates of 50, 25 and 10 W.min^{-1}.

	RI50	RI25	RI10
Baseline P_{ISO} (W)	725 ± 132[1]	751 ± 161[1]	723 ± 141[1]
RI-Flywheel$_{peak}$ (W)	361 ± 48[b,c]	323 ± 39[a,c]	275 ± 38[a,b]
P_{HYP} LoT (W)	299 ± 42[1,c]	268 ± 35[c]	248 ± 34[a,b]
LoT P_{ISO} (W)	346 ± 43	353 ± 45	392 ± 69[1,a,b]
Performance Fatigue (W)	379 ± 127	399 ± 145	331 ± 124[a,b]
Power Accessed (%)	51 ± 6[b,c]	44 ± 7[a,c]	39 ± 5[a,b]

Values are mean ± SD. P_{ISO}, isokinetic power, shown at 100 % effort baseline and the limit of tolerance (LoT). P_{HYP} is the power produced during the last 3 hyperbolic mode pedal strokes before LoT was attained. Comparisons within each protocol (Baseline P_{ISO}, P_{HYP} LoT, LoT P_{ISO}); ANOVA with Dunnett post-hoc, [1] significantly different from RI-Flywheel$_{peak}$ control level. Comparisons between the 3 protocols; ANOVA with Bonferroni post-hoc, [a] significantly different from RI50; [b] significantly different from RI25; [c] significantly different from RI10.

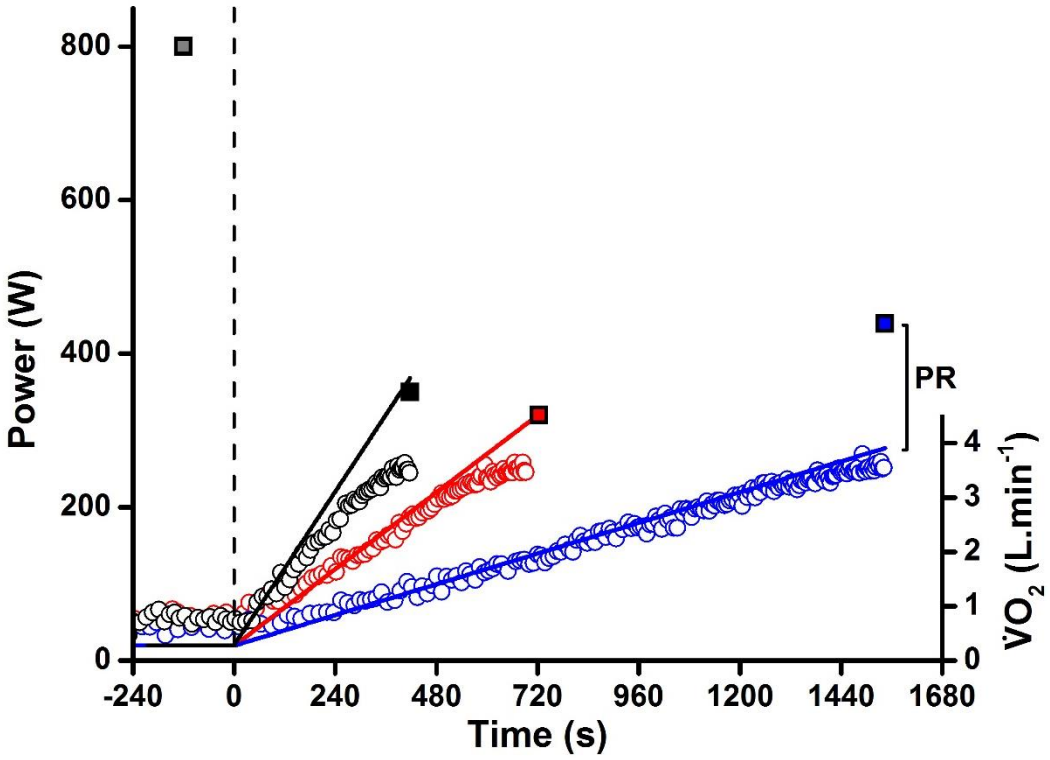

Figure 3.2: A representative participants $\dot{V}O_2$ and isokinetic power (P_{ISO}) responses to the 3 different incrementation rates. $\dot{V}O_2$; RI50 (○), RI25 (○), RI10 (○). Baseline P_{ISO} (3 protocol mean) (■). LoT P_{ISO}; RI50 (■), RI25 (■), RI10 (■). The required work rate (RI-Flywheel$_{peak}$) for each test is shown (solid lines of matching colour), as well as the ramp-incrementation phase onset (dashed line). Note the power reserve (PR) at LoT of the RI10 protocol only.

LoT P_{ISO} was not different from RI-Flywheel$_{peak}$ immediately post RI50 and RI25 ($p > 0.05$) (i.e. no power reserve was present), however LoT P_{ISO} was significantly greater than RI-Flywheel$_{peak}$ post RI10 ($p < 0.05$) (i.e. a significant power reserve present) (Figure 3.3). While there was a significant effect of gender on both baseline P_{ISO} and RI-Flywheel$_{peak}$, with males able to produce greater absolute power than females ($p < 0.05$), this did not translate to an effect of gender on the magnitude of the power reserve at LoT in any of the 3 protocols ($p > 0.05$).

The 95 % CI of the fluctuation in crank power during the RIT was -64 to 64 W (± 20 %) from the task requirement (imposed work rate). This natural fluctuation in

power is displayed on Figure 3.3 to give the power reserves context (i.e. was P_{ISO} above the natural fluctuation – see section 2.6.2.2 for graphical representation and further discussion).

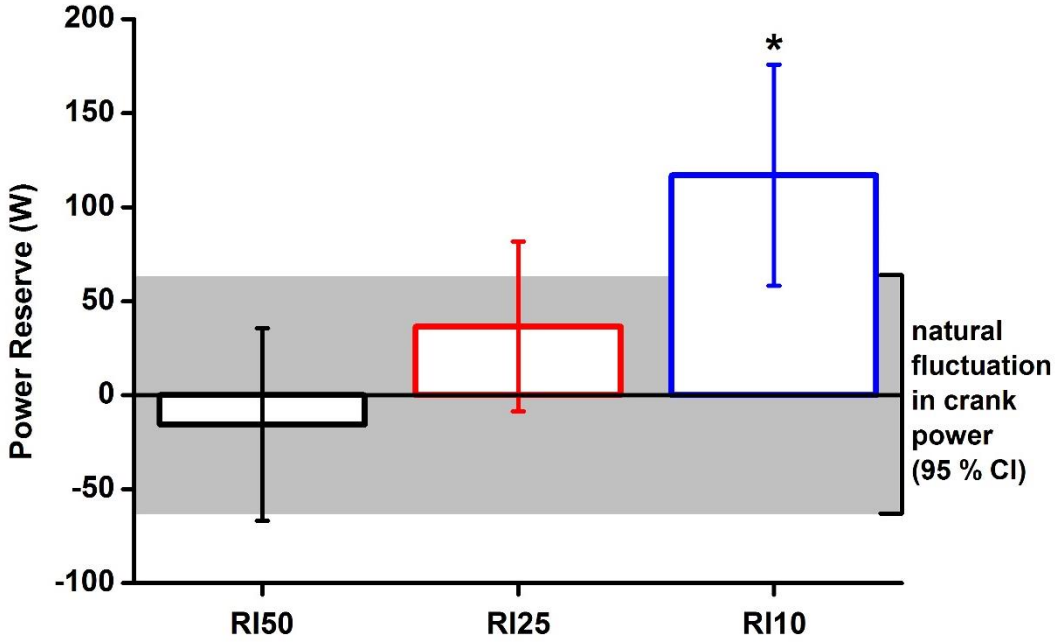

Figure 3.3: Mean power reserve ± SD (W) at the LoT of the 3 different incrementation rates. RI50 (black), RI25 (red) and RI10 (blue). For physiological context, the natural fluctuation in crank power during the RI test (calculated as 95% confidence intervals) is also shown (grey rectangle - mean from 12 participants across 3 protocols; ± 64 W. * LoT P_{ISO} significantly greater than RI-Flywheel$_{peak}$.

3.3.2 Contributions of Activation fatigue and Muscle fatigue

As previously reported (Coelho *et al.,* 2015), the unfatigued P_{ISO}-EMG relationship was found to be strongly linear prior to all exercise tests; mean of all protocols r^2 = 0.93 ± 0.08. The contributions of activation fatigue and muscle fatigue to overall performance fatigue, assessed using this P_{ISO}-EMG relationship are displayed in Figure 3.4. Activation fatigue increased as the incrementation rate was reduced, and was therefore greater at LoT of RI10 than RI50 ($p < 0.05$; Figure 3.4). Conversely, muscle fatigue was found to decrease

with the reduced incrementation rate, and as a consequence was less at LoT of RI10 than RI50 ($p < 0.05$; Figure 3.4).

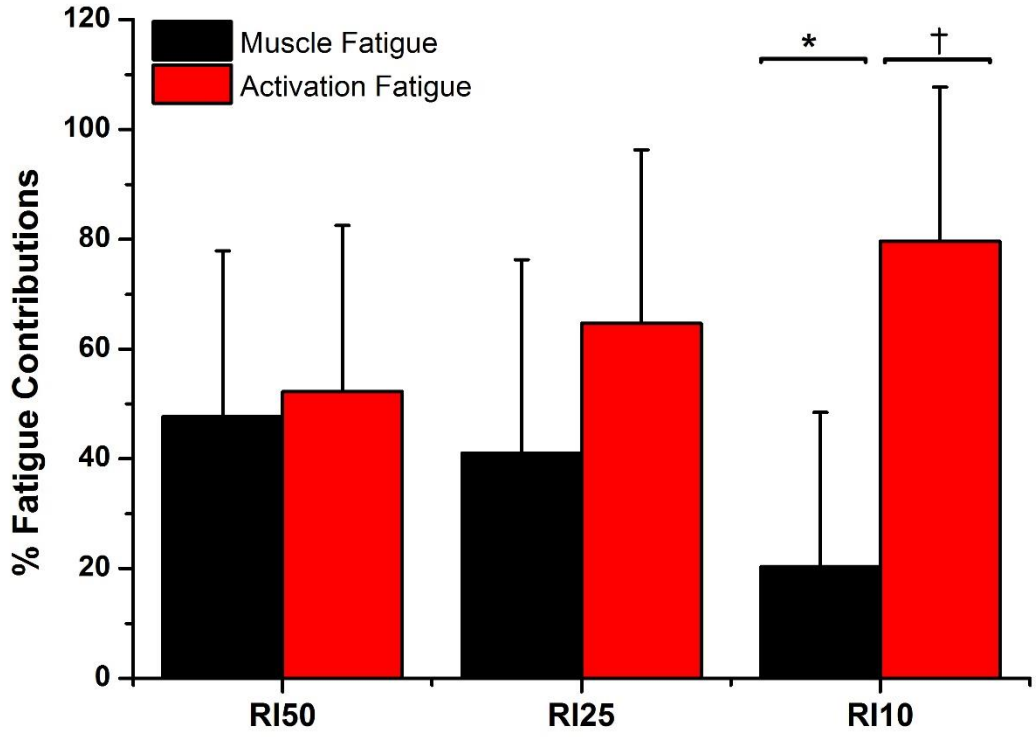

Figure 3.4: Mean (± SD) relative contributions of muscle fatigue and activation fatigue to overall performance fatigue for each of the 3 protocols; RI50, RI25, RI10. Means compared with one-way repeated measures ANOVA, with Bonferroni post-hoc. * Muscle fatigue significantly different from RI50. † Activation fatigue significantly different from RI50.

3.3.3 Alternative EMG analysis

The use of the P_{ISO}-EMG relationship to determine activation and muscle fatigue contributions had only previously been used in ramp-incremental exercise that did not produce a power reserve at LoT. However, when performance fatigue was significantly reduced, and a significant power reserve was present (namely RI10), this caused an increase in LoT P_{ISO} above that when no power reserve was present, with the result being a LoT P_{ISO}-EMG data point in some individuals plotted either on or above the unfatigued linear fit and not below it (Figure 3.5).

Consequently, using this method would interpret this data as 100 % of performance fatigue originating from activation fatigue; Figure 3.5. Evidently, this interpretation is physiologically incorrect, as significant peripheral muscle fatigue is present at LoT of whole-body cycling exercise (DeCorte *et al.,* 2012; Thomas *et al.,* 2016), including ramp-incremental protocols (Hopkinson *et al.,* 2013).

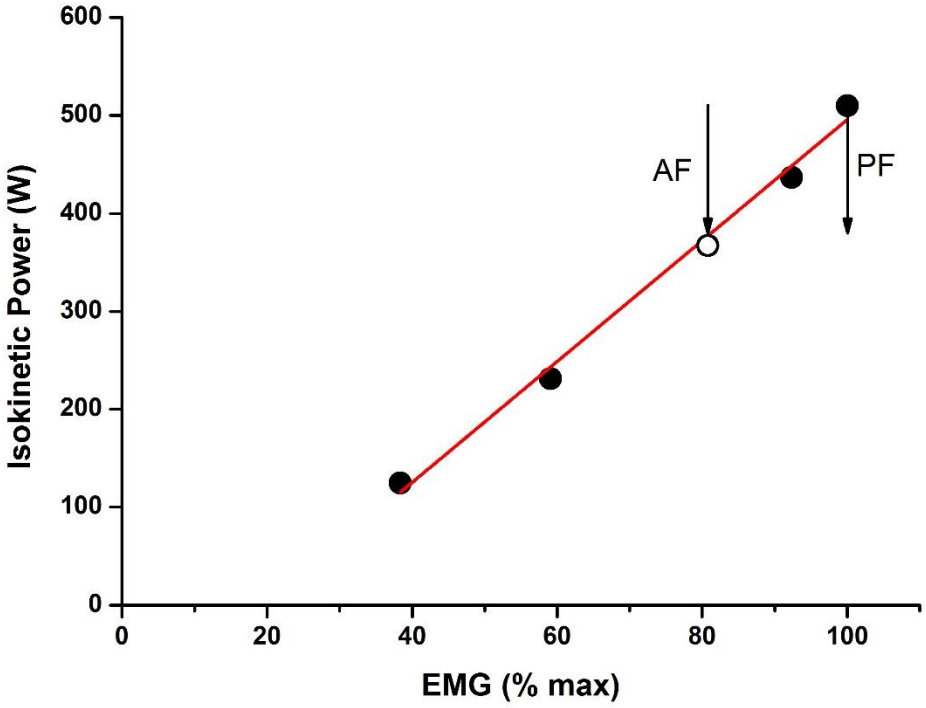

Figure 3.5: A representative participant's isokinetic power-EMG relationship where a significant power reserve is present at LoT. Due to the significantly reduced magnitude of performance fatigue (PF), the LoT data point sits on the linear relationship, which using this analysis suggests fatigue is 100% from activation fatigue (AF).

Consequently, alternative EMG analysis was performed on these data, with the EMG measured during the LoT isokinetic effort reported as a percent of maximal baseline EMG. At LoT of RI50 the EMG signal had reduced to 79 ± 12 $\%_{max}$. Although the drop in EMG was greater at LoT of RI25; 73 ± 14 $\%_{max}$ and RI10; 72 ± 11 $\%_{max}$, this difference was not significant ($p > 0.05$).

3.4 Discussion

The main finding of this study was that at the LoT of whole-body ramp-incremental cycle ergometry to $\dot{V}O_{2max}$, the origin of the fatigue that results in task failure can be altered depending on the ramp-incremental task, in young healthy participants. At LoT of RI50 and RI25 the physiological capacity to generate power limited the continuation of exercise and there was no power reserve. Conversely, with a slower incrementation rate (RI10), ramp-incremental power at task failure, and overall fatigue were reduced, combining to bring about task failure before the voluntary limits for power production were attained, resulting in a significant power reserve.

3.4.1 Limitations to ramp-incremental exercise

At the limit of tolerance of RI25 exercise, no significant reserve in maximal voluntary power generating capacity was found (30 ± 42 W; 10 ± 14 %; $p > 0.05$). This supports previous research using isokinetic efforts to measure maximal power after ramp-incremental exercise of similar incrementation rate and thus, power demands and duration (Ferguson *et al.,* 2016b; 12 ± 15 % and Coelho *et al.,* 2015; 18 ± 11 %). This suggests exercise cessation occurred in RI25 when the maximal evocable power could no longer meet the task requirement.

Contrary to this, Morales-Alamo *et al.* (2015) recently found a large reserve in maximal isokinetic cycling power at the LoT of a similar ramp-incremental test. These findings led the authors to conclude that task failure was primarily a consequence of central fatigue mechanisms (Morales-Alamo *et al.,* 2015). Although isokinetic cycling was employed to measure power, the same as in the current study, the use of instantaneous power at the LoT (i.e. the 2° of rotation with the highest power output) is largely responsible for the reserve in power

reported, confounding these findings. When power measurements comparable to the current work are used (the closest reported being a 10 s mean) the participants in this study display LoT P_{ISO} ~110 % of task requirement (Ferguson *et al.,* 2016a), in line with both the data presented here (110 ± 14 %) and previous work (Coelho *et al.,* 2015; Ferguson *et al.,* 2016b).

3.4.2 Limitations to ramp-incremental exercise with a faster incrementation rate

With a faster rate of incrementation (RI50), peak power required at LoT was greater compared to RI25 (361 ± 48 vs. 323 ± 39 W), a consequence of the finite, slow $\dot{V}O_2$ kinetics (Whipp and Ward, 2009). Additionally, tolerable duration was reduced compared with RI25 (411 ± 58 vs. 732 ± 93 s). At LoT, performance fatigue had developed to the extent that there was no significant power reserve at task failure (-16 ± 51 W; -3 ± 15 %; $p > 0.05$). Like RI25, this would suggest that the LoT was attained when the maximal voluntary power that could be generated no longer exceeded the task requirement and the physiological limits for power production had been reached. Thus, while both central and peripheral fatigue were present, these data suggest RI50 and RI25 were primarily limited by fatigue mechanisms within the skeletal muscle, such as increases in the fatigue metabolites Pi and H^+, influences on Ca^{2+} handling, and Na^+ and K^+ alterations, all of which ultimately result in reduced actin-myosin cross-bridge formation and force production (Allen *et al.,* 2008).

3.4.3 Limitation to ramp-incremental exercise with a slower incrementation rate

Importantly, the LoT in this task was attained with no difference in $\dot{V}O_{2peak}$ compared to the other two protocols ($p > 0.05$), confirming maximum effort across all protocols. However, as a consequence of the reduced incrementation

rate, the task duration was extended (1531 ± 288 s) and peak power required at LoT reduced (275 ± 38 W). Additionally, overall performance fatigue (331 ± 124 W) was significantly less than RI25 and RI50 ($p < 0.05$). This, in combination with the reduced power requirement, resulted in a significant reserve in voluntary power generating capacity at LoT (117 ± 59 W; 43 ± 22 %; $p < 0.05$). Also of note, the measured crank power during the final pedal strokes of the exercise before LoT (P_{HYP}) was not different from the task requirement (248 ± 34 vs. 275 ± 38 W; $p > 0.05$), thus although the power required could no longer be met, a maximal effort was still given by participants with every pedal stroke up to the LoT and suggesting no recovery occurred that could confound these results.

The significant power reserve at LoT of RI10 suggests that with the slowest incrementation rate, although significant performance fatigue was present, this was insufficient to limit continuation of the exercise task. Previous literature has found central fatigue to be relatively slower at developing and as a result more prevalent during the latter stages of an exercise task (Decorte *et al.,* 2012; Thomas *et al.,* 2016). It has been postulated this delay is caused as central mechanisms reacts to the growing effects of other fatigue stimuli (e.g. skeletal muscle fatigue), increasing the central motor drive to compensate the loss of force (Bigland-Ritchie *et al.,* 1983; Amann *et al.,* 2009). Consequently, central fatigue has been found to develop to a greater extent in longer duration exercise tasks with a reduced power output demand (such as RI10) (Burnley *et al.,* 2012; Thomas *et al.,* 2016; Froyd *et al.,* 2016). This has the potential to alter the interactions of central and peripheral fatigue mechanisms; the presence of a power reserve within the skeletal muscle seems to suggest a dissociation between the perceived capacity for power production from the actual maximum evocable power, mechanisms that are better coupled in RI25 and RI50.

3.4.4 Potential mechanisms causing the presence of a power reserve

The presence of a skeletal muscle power reserve suggests the primary exercise limitation with a slower incrementation rate does not seem to be within the active muscles. This, combined with the fact that peripheral fatigue has been shown to develop to the same level independent of the exercise task (Amann and Dempsey, 2008; Vanhatalo *et al.,* 2010; Burnley *et al.,* 2012), is consistent with an increased central fatigue contribution to the attainment of task failure in RI10. This increased central contribution, increasing perception of effort, could be modulated by an increased afferent feedback (Amann, 2011). However, if the skeletal muscle perturbations and, therefore afferent feedback from active skeletal muscles are similar at LoT (Vanhatalo *et al.,* 2010; Chidnok *et al.,* 2013b), this suggests increased feedback is coming from elsewhere.

While the peak pulmonary gas exchange variables ($\dot{V}O_{2max}$, $\dot{V}_{Emax}$) are not different across the 3 ramp-incremental exercise protocols, the overall work done by the ventilatory system will be greater in RI10, as the protocol dictates a longer duration (and therefore more breaths) at a higher percent of $\dot{V}O_{2max}$ and $\dot{V}_{Emax}$ (Figure 3.6). An increased ventilatory work has been suggested to cause an increased overall afferent feedback (Hureau *et al.,* 2016), which may in turn cause greater central fatigue development in this protocol. The importance of the perception of ventilatory work has been shown using interventions that are designed to reduce this stimulus. Using a mechanical ventilator to reduce the work of breathing by up to 80 % during high-intensity cycling exercise, attenuated the perceived ratings of effort (Harms *et al.,* 2000; Romer *et al.,* 2006; Amann *et al.,* 2007a), resulting in an improved exercise tolerance (Harms *et al.,* 2000). Furthermore, spinal injections of fentanyl used to suppress these dyspnoeic

sensations and reduce the ventilatory afferent feedback have been shown to reduce ventilation in healthy individuals (Amann *et al.,* 2009), in addition to reducing perception of effort and increasing exercise tolerance COPD patients, whose primary exercise limitation is an increased sensation of breathlessness (Gagnon *et al.,* 2012). However, these intervention studies all switched ergometers to perform single-joint neuromuscular fatigue assessments at LoT. Therefore, the effect of reducing central sensations of dyspnoea on whole-body, maximal evocable power at LoT, and whether these interventions can allow some of the power reserve in RI10 to be accessed, remains unknown.

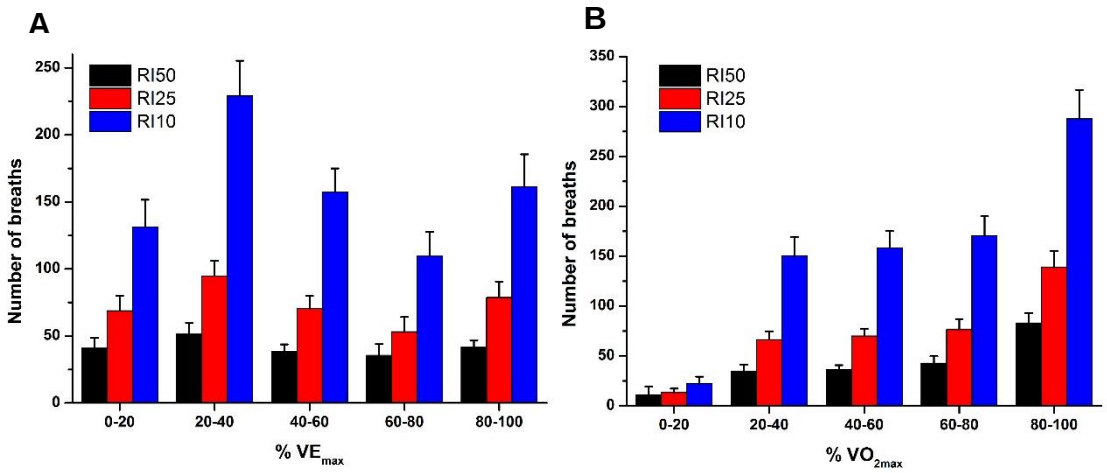

Figure 3.6: The number of breaths performed at a range of % of maximal ventilation (A) and oxygen uptake (B) during each of the 3 ramp-incremental tests.

However, overall afferent feedback during exercise is a culmination of feedback from different mechanisms, the primary origins of which are the exercising muscles (Amann, 2011). This skeletal muscle afferent feedback, a consequence of intramuscular metabolic and mechanical perturbations, may also be playing a role in increasing the central fatigue component. With exercise tasks such as RI10 where the duration is relatively long and LoT is attained at a lower power output, overall skeletal muscle inefficiency, a consequence of recruitment of

progressively inefficient muscle fibres and manifest as the $\dot{V}O_2$ slow component, will be of a greater amplitude compared with shorter, high power exercise (Whipp, 1994; Jones *et al.,* 2011; Grassi *et al.,* 2015). Therefore, while peripheral fatigue itself may be the same at LoT, this greater intramuscular inefficiency may result in increased afferent feedback, potentially increasing the overall feedback and central fatigue further in these protocols. Although origins of afferent feedback such as other organs and inactive muscles may also play a role, these are considered of less significance than the ventilatory and skeletal muscle components (Hureau *et al.,* 2016).

3.4.5 Contributions of activation and muscle fatigue

Using the method developed by Coelho *et al.* (2016), the current study aimed to provide insight into the contributions of activation and muscle fatigue during RIT exercise depending on the ramp-incrementation rate. It was found that, after RI50, muscle fatigue was the primary fatigue contributor, significantly greater than RI10 ($p < 0.05$). However, as the incrementation rate was decreased, and therefore the exercise duration increased and power demand reduced at LoT, activation fatigue became more predominant, to the extent that AF was significantly greater in RI10 than RI50 ($p <0.05$). These findings seem to support previous literature that has measured central and peripheral fatigue at LoT of different exercise tasks (Burnley *et al.,* 2012; Froyd *et al.,* 2016; Thomas *et al.,* 2016). However, limitations were discovered in this methodology when a power reserve was present (see section 3.3.3), confounding to a large extent these findings.

Consequently, the change in maximal EMG amplitude from baseline to LoT was used to compare the different exercise tasks. At LoT of RI25, EMG amplitude

reduced to 73 ± 14 % baseline. Interestingly, when measured during the same isokinetic cycling methodology, endurance athletes were able to reduce EMG further (63 ± 10 % baseline; Ferguson *et al.,* 2016b) and an elderly population less so (81 ± 10 % baseline; Cannon *et al.,* 2016) compared to our young healthy group, suggesting a link between exercise tolerance and an ability to continue exercise in the presence of reduced muscle activation. Although not significantly different, there was a trend for an attenuated fall in EMG at LoT of RI50 compared to both RI25 and RI10, suggesting a smaller activation fatigue in RI50 and greater importance of muscle fatigue in the shorter, higher power exercise, supporting previous work (Froyd *et al.,* 2016; Thomas *et al.,* 2016). Nonetheless, the reduction in EMG at LoT of all 3 protocols shows the important contribution of central fatigue mechanisms to overall performance fatigue.

3.4.6 Additional Considerations

The methodology incorporated in this study relies on maximal participant effort throughout. Unlike when using external stimulation to measure fatigue (e.g. Decorte *et al.,* 2012; Thomas *et al.,* 2016), the use of volitional effort has the potential to influence any findings from these data. However, the use of external stimulation negates the simplicity of the current protocol and more importantly, when incorporated with whole-body exercise, requires switching ergometer at task failure, causing a delay in assessment of fatigue, potentially confounding findings (Froyd *et al.,* 2013; Gruet *et al.,* 2014; Coelho *et al.,* 2015). Nevertheless, this does highlight the need for adequate familiarisation and strong motivational encouragement throughout the exercise. Significant familiarisation was implemented, with good reproducibility of isokinetic power measurements reported here (CoV; 4.2 %) and previously (3 %; Ferguson *et al.,* 2016b), with

the consistent $\dot{V}O_{2peak}$ values and the P_{HYP} LoT data providing additional evidence for this.

Recently, Temesi *et al.* (2017) developed an ergometer that allows external stimulation to be performed immediately at the LoT of cycling exercise by locking the crank arms in place, negating the issues discussed previously. However, as the authors mention themselves, this method is still limited because force is measured isometrically. So, although there are benefits to this ergometer, some limitations remain and more research where dynamic contractions are performed to measure fatigue is warranted.

The use of surface EMG in this study was necessary to maintain the simple, non-invasive protocol intended. However, although this can provide an indication of activation fatigue (Enoka and Duchatuea, 2015; Coelho *et al.,* 2015), EMG is unable to accurately determine the origins of central fatigue within the CNS. Using external stimulation techniques such as coupling femoral nerve stimulation with transcranial magnetic stimulation can provide greater insight into this (Taylor *et al.,* 2016). However, as discussed these methods cannot successfully be integrated with dynamic whole-body exercise as of yet and the invasive nature of these techniques makes them difficult in practice to implement on a larger scale, unlike the current protocol.

This study, and the previous work using isokinetic methods that attain $\dot{V}O_{2max}$, have only measured maximal voluntary power before and immediately after the exercise protocol. Consequently, although inferences can be made based on the available data (e.g. Figure 6; Ferguson *et al.,* 2016b), there is a lack of information on the development of fatigue during this exercise modality, without the need to dismount the ergometer. There is potential to use the short, maximal

isokinetic efforts during exercise to investigate this (Cannon *et al.,* 2011). If these could be implemented during exercise and not just at the LoT, this may improve our understanding of the fatigue kinetics during whole-body exercise and how this brings about task failure. Chapter 4 in this thesis was aimed at addressing these questions during high-intensity exercise.

3.4.7 Conclusions

At the limit of tolerance and $\dot{V}O_{2max}$ of ramp-incremental exercise with incrementation rates of 50 and 25 $W.min^{-1}$, no power reserve was present. However, when the incrementation rate was reduced to 10 $W.min^{-1}$, increasing exercise duration and reducing the power demand, at the LoT there was a significant reserve in skeletal muscle power generating capacity. Together, these findings suggest the origin of the fatigue that causes task failure at $\dot{V}O_{2max}$ in young-healthy participants is plastic, and can be altered by the exercise task, specifically it's duration and power requirement.

Chapter 4 Influence of fatigue dynamics on limitations to high-intensity exercise

4.1 Introduction

During whole-body maximal ramp-incremental exercise, fatigue (both central and peripheral) develops during the exercise until the power that can be produced voluntarily no longer exceeds the demands of the exercise task, thus resulting in task failure at $\dot{V}O_{2max}$ (Coelho *et al.,* 2015; Ferguson *et al.,* 2016b). During this "standard" ramp-incremental exercise protocol, the perceptual and physiological limits for exercise seem to be coupled such that they coincide at task failure. By altering the exercise task, Chapter 3 has shown that this coupling can be disrupted, leading to a dissociation of the perceived and the actual physiological capabilities at LoT. It was found that when the ramp rate was decreased, and as a consequence the peak power reduced and exercise duration increased, the LoT was attained with a significant skeletal muscle power reserve. This implies that different mechanisms may be ultimately causing task failure, evidencing the acute plasticity of fatigue, and suggesting there is an interaction present between the relative contributions to overall fatigue of central and peripheral mechanisms, and the task being performed. However, during ramp-incremental exercise it is difficult to quantify the effects of the exercise task on the rate of energy utilisation, as this will be continually changing throughout the exercise as the power demand and exercise intensity increases.

The tolerable duration (T_{lim}) of high-intensity exercise (above critical power; CP, the maximum power at which $\dot{V}O_2$ can attain a steady-state) has been well characterised by a hyperbola (Monod and Scherrer, 1965; Moritani *et al.,* 1981).

Within this power-T_{lim} (P-T_{lim}) relationship, time to task failure is determined by the curvature constant of the hyperbola W', which denotes a fixed volume of work that can be performed above CP, independent of the rate of utilisation (Monod and Scherrer, 1965). While still controversial, W' has been suggested to represent both a finite energy store and a fixed amount of fatigue metabolite build-up that can be tolerated, where the "depletion" of this W' coincides with the attainment of the limit of tolerance (LoT) (Monod & Scherrer, 1965; Hill, 1993; Gaesser & Poole, 1996). W' utilisation is considered to be mathematically linear (Fukuba *et al.,* 2003), or slightly curvilinear due to reconstitution during relaxation phases (the upstroke during cycling) (Skiba *et al.,* 2012, 2014; Broxterman *et al.,* 2016). Importantly, this means that throughout constant-power supra-CP exercise, at the same percent of exercise duration, W' utilisation is the same, independent of the power demand. Consequently, the P-T_{lim} relationship and the associated mathematical construct provides a robust framework to investigate the development of fatigue, allowing accurate alterations of the exercise task to be made (between ~2-30 min (Poole *et al.,* 1988; Hill, 1993; Fukuba and Whipp, 1999). Additionally, given the suggested origins for W', this model of exercise implies a more peripheral exercise limitation. Therefore, this potentially allows insight into the role that skeletal muscle perturbations and changes in efficiency, that manifest as the $\dot{V}O_2$ slow component ($\dot{V}O_{2SC}$) and have been linked to peripheral fatigue (Keir *et al.,* 2016), might play on exercise tolerance, influences that although present, can't accurately be assessed in ramp-incremental exercise where the $\dot{V}O_{2SC}$ magnitude is difficult to discern (Rossiter, 2011).

Using the P-T_{lim} relationship, de Souza *et al.* (2016) reported that after 70 % W' depletion at two different rates (3 and 10 min exercise duration), maximal voluntary isokinetic cycling power measured immediately at end-exercise was

not different. This suggests that fatigue had developed to the same level, independent of rate of W' utilisation. However, in this study participants were not required to complete the exercise to LoT, so fatigue during the final 30 % of W' utilisation was not assessed. This is important as these authors reported only a ~20 % drop in isokinetic power, significantly less than reported at LoT (i.e. 100 % W' depletion) of ramp-incremental exercise, in previous work using the same methodology (Coelho *et al.,* 2015; ~45 %, Ferguson *et al.,* 2016b; ~50 %, Chapter 3; ~50 %). Additionally, the fatigue measured by de Souza *et al.* (2016) is likely a result of predominantly peripheral mechanisms, occurring from exercise onset during high-intensity constant-power exercise, a result of the O_2 deficit and the consequent fatigue metabolite build up (Murgatroyd and Wylde, 2011), causing peripheral fatigue to be more prevalent during the early part of exercise (Froyd *et al.,* 2013). Therefore, the drop in power when central fatigue mechanisms are predominant, in the latter part of exercise near LoT (Decorte *et al.,* 2012; Gruet *et al.,* 2014) are potentially not measured by this study. Together this shows the importance of measuring fatigue development all the way to LoT, in order to determine what primary mechanisms ultimately cause task failure.

The dynamics of peripheral and central fatigue have also been shown to be influenced by the exercise task being performed. It has been found using the P-T_{lim} relationship that at the LoT of single leg constant-power isometric exercise, when the power output and therefore the W' utilisation rate and exercise duration are altered (~3-17 min), although peripheral fatigue developed to a similar level, the magnitude of central fatigue is increased after lower power, longer duration exercise (Burnley *et al.,* 2012). Similar responses have been found in whole-body constant-power exercise, where there is early onset of peripheral fatigue and delayed central fatigue development, associated with task failure (Decorte

et al., 2012). Again these kinetics seem to result in greater prevalence of peripheral fatigue at LoT of high-power exercise and conversely increased contributions of central fatigue at task failure of lower power, longer duration exercise (Thomas *et al.,* 2016).

However, by using external stimulation techniques to remove the volitional effort component, these whole-body studies are constrained by limitations such as the need to switch ergometers to measure fatigue, using different exercise modalities, but more importantly allowing for significant rapid neuromuscular recovery preceding fatigue assessment (Froyd *et al.,* 2013; Coelho *et al.,* 2015), limitations which can be overcome using instantaneous isokinetic measurements.

Therefore, the aim of this study were to use the P-T_{lim} relationship to determine the origin of the fatigue predominantly causing the LoT during high-intensity exercise by:

1) investigating how altering the power output and exercise duration affected the maximal voluntary isokinetic power capacity at LoT;

2) characterising the fall in maximal voluntary power and development of fatigue, during exercise in which the rate of W' depletion was changed.

We hypothesised that by reducing the power demands, and as a consequence the rate of W' utilisation, this would cause a reserve in maximal voluntary power capacity at LoT. Additionally, the fall in maximal power output during supra-CP was hypothesised to coincide with the proposed response of W' depletion, independent of the rate of utilisation.

4.2 Methods

4.2.1 Ethical Approval

All procedures in this study were approved by the Faculty of Biological Sciences ethics committee, University of Leeds. All volunteers provided written informed consent prior to involvement in the study and were free to withdraw at any point without explanation.

4.2.2 Participants

Nine habitually active and healthy male individuals volunteered to participate (age 25 ± 6 yr; height 179 ± 6 cm; weight 76 ± 7 kg). Prior to any exercise testing participants were screened using a health and activity questionnaire to ensure they were safe to partake in high-intensity exercise. All exclusion criteria for healthy individuals were the same as those outlined previously (section 2.1.1).

4.2.3 Exercise protocols

Participants were required to visit the laboratory on 7-8 occasions, with each visit at a similar time of day and separated by at least 24 hours. In the time leading up to the exercise test participants were asked to avoid strenuous exercise (24 hours), excessive alcohol (24 hours), caffeine (3 hours) and food consumption (3 hours).

4.2.3.1 Pre-exercise isokinetic power assessment

In the first phase of every exercise visit a series of 4 variable effort (~25, ~50, ~75 and 100 %) short (~6 s) isokinetic bouts of cycling were performed, with each bout preceded by 30 s unloaded cycling (20 W). This process was repeated a second time (i.e. 8 total efforts), with the intention to improve the characterisation of the unfatigued isokinetic power (P_{ISO})-EMG relationship.

4.2.3.2 Ramp-incremental test

The first test performed by each participant was a ramp-incremental test (RIT) to measure non-invasive lactate threshold, $\dot{V}O_{2peak}$ and peak power. The details of this protocol are outlined previously (section 2.5.5). Briefly, after ~2 min of seated rest and ~4 min of warm-up exercise at 20 W (50-60 rpm) power was increased at a rate of 25 $W.min^{-1}$, until the LoT was attained: defined as when cadence could no longer be maintained above 50 rpm, despite strong verbal encouragement. Once the LoT was attained, the isokinetic mode was instantaneously selected on the ergometer and participants performed a final ~6 s bout of maximal effort isokinetic cycling.

4.2.3.3 Characterisation of the power- tolerable duration relationship

To characterise the P-T_{lim} relationship for each participant, a series of 4 randomised constant-power exercise tests were performed to the LoT. The power required by these tests was selected based on the peak work rate from the initial RIT, and were designed to produce exercise durations spanning ~3-15 min (Poole *et al.,* 1988; Hill, 1993). Specific details of constant-power exercise protocols are described earlier (section 2.5.6). Briefly, following the same rest (~2 min) and warm up (~4 min at 20 W) phases, the required flywheel power for the specific test was imposed instantaneously (1000 $W.s^{-1}$) using the standard hyperbolic cycling mode, with participants instructed to continue for as long as they could, with LoT determined when 50 rpm could no longer be maintained, despite encouragement.

The P-T_{lim} relationship for each participant was then characterised by a hyperbolic function; where the asymptote and curvature constant provided estimates of CP and W', respectively. The standard error (SE) of each participant

was; CP ≤ 4 W (< 2 %) and W' ≤ 1 kJ (< 7 %). When four tests did not sufficiently characterise the P-T_{lim} relationship (1 participant only), a 5th test was performed at a different power to improve parameter estimates. A high level of confidence was paramount so that power determined for the final two visits were accurate, extrapolated from the P-T_{lim} relationship to induce tolerable durations of 6 min (WR6) and 12 min (WR12):

$$WR6 = (W'/360\ s) + CP$$

Equation 4.1

$$WR12 = (W'/720\ s) + CP$$

Equation 4.2

A ~6 s maximal voluntary effort isokinetic cycling bout was performed instantaneously at the LoT of the constant-power tests; with the protocols for this phase of the study outlined in Figure 4.1 A.

4.2.3.4 WR6 and WR12 to assess fatigue kinetics

Following the same warm-up procedures described above, WR6 and WR12, extrapolated from the P-T_{lim} relationship (Equation 4.1 and Equation 4.2) were performed to the LoT, with short (~6 s) maximal isokinetic efforts interleaved at 1/3rd and 2/3rd T_{lim} (i.e. at 2 and 4 min for WR6 and at 4 and 8 min for WR12; Figure 4.1 B). For the interleaved efforts, participants were instructed to reduce their cadence from self-selected (generally 80-90 rpm) to 70-75 rpm 10 s prior to the isokinetic phase (performed at 80 rpm). This instruction was always preceded by a verbal warning 30 s before the next maximal effort, with both these instructions designed to allow for a smoother transition between different cycling phases. In addition to this, participants were informed beforehand of how the protocol was going to be run and had by this point completed at least 5 exercise tests with isokinetic efforts, so were well familiarised with the protocol. Once each

maximal effort was completed, the ergometer was immediately switched back into hyperbolic mode with participants free to self-select their cadence again. After the second interleaved effort was completed, participants were instructed to continue exercising for as long as they could, until the LoT (cadence not maintained > 50 rpm) was reached, with no further cues as to how much time had elapsed. At LoT, as described previously (section 2.5.7), a final maximal isokinetic power measurement was performed, before a phase of active recovery.

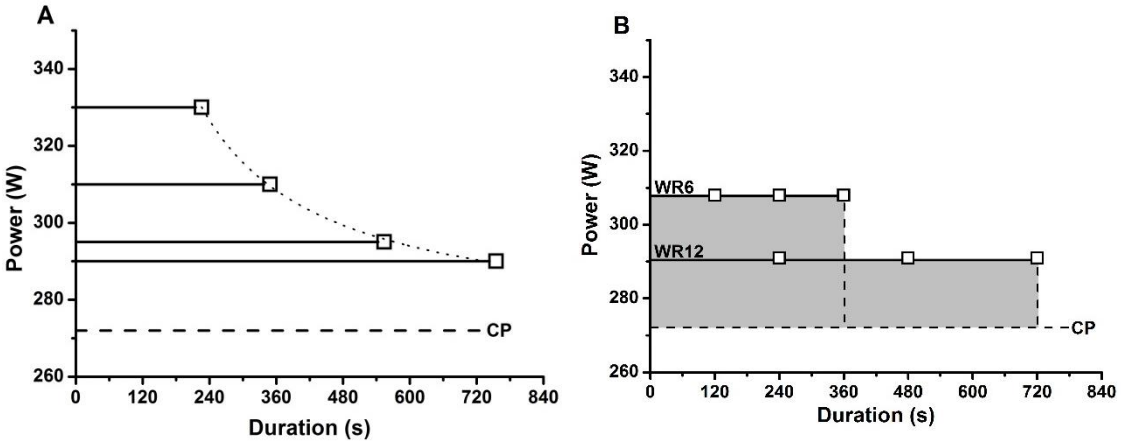

Figure 4.1: A schematic representation of the protocols performed in this study. A; characterisation of the power-tolerable duration relationship. The required power is shown as the solid lines, with the hyperbolic fit to the power-tolerable duration relationship (dotted line). Open squares denote a maximal isokinetic effort. B; the WR6 and WR12 protocols determined from the relationship in A. Power required is shown by the solid lines, with maximal isokinetic efforts (open squares) performed at 1/3rd and 2/3rd exercise duration, as well as the limit of tolerance. The grey shaded area denotes W', the same for both protocols. Critical power is also shown for reference on both figures.

4.2.3.5 Validity of the interleaved isokinetic protocol

To confirm that interleaving maximal isokinetic efforts had no effect on subsequent responses, two participants completed a series of 6 additional exercise tests. These consisted of performing each phase of the WR6 and WR12 tests individually, on separate days, stopping at the point where an interleaved

effort would occur and performing a single maximal isokinetic effort. The power produced for the individual tests could then be compared to the powers produced during the interleaved phases of the WR6 and WR12 tests. Both participants showed no difference in power between the two groups of isokinetic efforts, above what would be expected from natural fluctuation. Most importantly, power was seemingly not different at the LoT of either test, showing no additional fatigue was present at the LoT as a consequence of the additional interleaved maximal efforts (Table 4.1).

Table 4.1: Mean power (W) of the two participants that performed each phase of the interleaved protocols (WR6 and WR12) independently in addition to the standard interleaved tests.

	WR6			WR12		
	2 min	**4 min**	**LoT**	**4 min**	**8 min**	**LoT**
Stopped	602 ± 86	549 ± 17	405 ± 30	666 ± 40	602 ± 49	439 ± 63
Interleaved	606 ± 34	510 ± 3	398 ± 47	631 ± 61	567 ± 11	465 ± 13

4.2.4 Measurements

4.2.4.1 Isokinetic power

The cycle ergometer (Lode Excalibur Sport PFM, Lode BV, Groningen, NL) allowed instantaneous switching from hyperbolic to isokinetic cycling modes. The isokinetic mode was used to measure velocity-independent power prior to, during, and immediately post exercise protocols. P_{ISO} was measured at the crank axis of each pedal every 2° of angular rotation, calculated as the product of force (Nm) and angular velocity (rad.s^{-1}) measured by strain gauges and light sensors sampling in series, respectively. Further details about the ergometer capabilities, calibration and isokinetic power measurements can be found in section 2.2.

4.2.4.2 Electromyography

As in the previous study, during each isokinetic bout of cycling surface electromyography (EMG) measurements were recorded at 1500 Hz to assess electrical activity within the predominant exercising muscles during cycling (Noraxon, Telemyo 2400T G2, Noraxon U.S.A Inc, Scottsdale, AZ, USA). Five muscles were assessed in the right leg only during exercise; *gastrocnemius lateralis, biceps femoris, vastus lateralis, rectus femoris and vastus medialis*.

Before the EMG electrodes were attached the skin was prepared to improve the signal by shaving excess hair, abrading dead skin cells and sterilising the desired site. More details on EMG measurements, including exact anatomical positions are detailed in section 2.4.

4.2.4.3 Pulmonary gas exchange

Respired gases were measured using a breath-by-breath pulmonary gas analysis system (Medgraphics D series, Medgraphics, Medical Graphics Corporation, St Paul, MN, USA) throughout each of the exercise visits, excluding the pre-exercise isokinetic cycling phase. Details of this gas analysis system can found in section 2.3. Briefly, participants breathed through a mouthpiece attached to a flow sensor while wearing a nose clip. Respired gases were then passed down an umbilical to the analysis system.

Prior to the participants arrival the system was calibrated according to manufacturer guidelines. The flow sensor was calibrated by passing 10 strokes of varying flow rates over the flow sensor using a 3 L syringe (Hans Rudolph, Kansas City, MO) to mimic flow at rest and maximal exercise. The O_2 and CO_2 sensors were then calibrated using gases of known concentrations (O_2 12 %, CO_2 5 %, Bal N_2 and O_2 21 %, CO_2 0 %, Bal N_2), independently verified before

and after the exercise test to monitor any drift in the signal. Finally, a 12 lead Mortara ECG system was attached to the participants to measure heart rate.

4.2.5 Data Analysis

4.2.5.1 Isokinetic power

Initially, left and right pedals were summed to give an overall P_{ISO} for each pedal stroke. The first 3 consecutive pedal strokes of each isokinetic phase that were correctly constrained at 80 rpm were then isolated, with these the data reported for each phase. Typically this was the 2nd, 3rd and 4th pedal strokes. No difference was found between the two pre-exercise 100 % isokinetic efforts for any of the protocols ($p > 0.05$) therefore, the higher of the two powers was used for further analysis. For more details on how the isokinetic power was determined and how other power measures were calculated such as; RI-Flywheel$_{peak}$, performance fatigue, power reserve, power accessed and the natural fluctuation in power, see section 2.6.2.

4.2.5.2 Electromyography

Raw EMG signals were rectified, band pass filtered (10-500 Hz) and smoothed via a root-mean-square (RMS) with a 100 ms moving window, without overlap. The 3 contractions that coincided with the 3 selected isokinetic pedal strokes were then isolated for each of the 5 muscle groups. Peak EMG activity for each muscle group was found and the 5 muscle groups summed for each pedal stroke. As with P_{ISO} a mean of the 3 pedal strokes was then calculated to provide an overall EMG activity to match the isokinetic data for the 3 pedal strokes selected. The P_{ISO}-EMG relationships were determined as described in section 2.6.4, with these relationships found to be linear in a fresh state (mean $r^2 = 0.93 \pm 0.05$), as previously shown (Coelho *et al.,* 2015). However, similar issues as those reported in Chapter 3 (section 3.3.3) were found at the LoT, therefore these data

are not reported and EMG amplitude is defined throughout this study as a percent of the EMG amplitude during the maximal baseline effort.

4.2.5.3 Gas exchange

Breath-by-breath gas exchange data were initially edited using 99 % prediction bands to remove coughs, sighs and swallows that were not part of the underlying physiological response (Lamarra *et al.,* 1987). $\dot{V}O_{2peak}$ and all other gas exchange values were found for the relevant tests, as previously described (section 2.6.1), with $\dot{V}O_{2max}$ confirmed by comparing $\dot{V}O_{2peak}$ values across the different tests. Finally, the magnitude of the $\dot{V}O_2$ slow component was determined as the difference between $\dot{V}O_2$ at 3 min exercise duration and $\dot{V}O_{2peak}$ at LoT.

4.2.6 Statistics

For the four constant-power exercise tests, comparisons between protocols were made using a one-way repeated measures ANOVA with Bonferroni post-hoc. The comparisons of the power data within an individual protocol were made using a one-way repeated measures ANOVA with Dunnett post-hoc. With WR6 and WR12, paired sample t-tests were used for all gas exchange data. For power and EMG data a two-way repeated measures ANOVA was used, comparing the effects of the protocol (WR6 or WR12) and of the time point during the exercise (1/3rd, 2/3rd, LoT). Values are reported as mean ± SD; significance level set at 0.05.

4.3 Results

4.3.1 Ramp-incremental exercise

Mean T_{lim} was 785 ± 88 s, attaining a $\dot{V}O_{2peak}$ of 4.39 ± 0.49 L.min^{-1} (57.4 ± 3.8 ml.min^{-1}kg^{-1}) and HR_{peak} of 193 ± 11 bpm at a RI-Flywheel$_{peak}$ power 347 ± 36 W. Mean lactate threshold was 2.05 ± 0.38 L·min^{-1}. LoT P_{ISO} of the RIT (376 ± 49 W) was not significantly different from RI-Flywheel$_{peak}$ task requirement ($p > 0.05$), consistent with the voluntary limits for power production being attained, no power reserve, and with previous results from ramp-incremental exercise in healthy participants (Coelho *et al.,* 2015; Ferguson *et al.,* 2016b; Chapter 3).

4.3.2 Constant-power exercise tests

The mean responses to the four constant-power tests are presented in Table 4.2; importantly $\dot{V}O_{2peak}$ was not different across the four protocols, despite the difference in power ($p > 0.05$). As in Chapter 3, increasing the required power and therefore decreasing the duration resulted in a significantly greater $\dot{V}CO_{2peak}$, and as a consequence a greater RER_{peak}. Conversely, by reducing the power and increasing duration, this caused an increased $\dot{V}O_{2SC}$ magnitude. Additionally, a small but significant difference in HR_{peak} was again found between the shortest and longest protocols.

The P-T_{lim} relationship was well characterised by a hyperbola in each participant, from which critical power (236 ± 34 W) and W' (19.6 ± 7 kJ) could be estimated. A high confidence in this characterisation was confirmed by low SE and CI_{95} of CP (1.2 ± 0.5 W and 10 ± 3.9 W) and SE of W' (0.8 ± 0.3 kJ).

Table 4.2: Mean pulmonary gas exchange responses to the 4 supra-CP constant-power exercise tests

	Supra-CP constant-power exercise tests →			
	1	**2**	**3**	**4**
Flywheel power (W)	325 ± 35[2,3,4]	302 ± 34[1,3,4]	282 ± 34[1,2,4]	266 ± 37[1,2,3]
Duration (s)	220 ± 34[3,4]	306 ± 48[3,4]	432 ± 66[1,2,4]	705 ± 114[1,2,3]
$\dot{V}O_{2peak}$ (L.min^{-1})	4.19 ± 0.68	4.22 ± 0.46	4.18 ± 0.43	4.19 ± 0.51
$\dot{V}O_{2SC}$ (L.min^{-1})	0.09 ± 0.07[2,3,4]	0.26 ± 0.07[1,3,4]	0.49 ± 0.17[1,2]	0.59 ± 0.13[1,2]
$\dot{V}CO_{2peak}$ (L·min^{-1})	5.10 ± 0.67[3,4]	4.81 ± 0.58[4]	4.57 ± 0.53[1]	4.30 ± 0.62[1,2]
RER_{peak}	1.24 ± 0.18[3,4]	1.14 ± 0.08	1.09 ± 0.09[1]	1.03 ± 0.07[1]
$\dot{V}_{Epeak}$ (L·min^{-1})	176 ± 13	177 ± 14	174 ± 15	170 ± 18
HR_{peak} (bpm)	185 ± 8[4]	185 ± 8[4]	188 ± 9	190 ± 8[1,2]

Values are mean ± SD. Arrow denotes increasing exercise duration. $\dot{V}O_{2SC}$, $\dot{V}O_2$ slow component. All variables compared with ANOVA and bonferroni post-hoc: [1] significantly different from test 1; [2] significantly different from test 2; [3] significantly different from test 3; [4]significant different from test 4.

All P_{ISO} measurements were constrained at the desired cadence, confirmed by *post-hoc* analysis, baseline P_{ISO}; 80.4 ± 0.2 (test 1), 80.4 ± 0.2 (test 2), 80.2 ± 0.2 (test 3), 80.2 ± 0.2 (test 4) and LoT P_{ISO}; 80.4 ± 0.1 (test 1), 80.5 ± 0.2 (test 2), 80.4 ± 0.3 (test 3), 80.5 ± 0.2 rpm (test 4). Baseline P_{ISO} was not different across the 4 protocols ($p > 0.05$, Table 4.3), with a mean of 836 ± 105 W. P_{HYP} LoT was not different from the task requirement in all tests ($p > 0.05$, Table 4.3). During the high-power, shorter duration constant-power exercise (test 1 and test

2) performance fatigue developed throughout the test to the extent that at LoT and $\dot{V}O_{2max}$, P_{ISO} was not significantly different from the required power ($p > 0.05$; Table 4.3; Figure 4.2). However, during the lower power, longer-duration tests (test 3 and test 4), although performance fatigue was present at LoT, P_{ISO} was greater than the task requirement ($p < 0.05$; Table 4.3; Figure 4.2), a power reserve was present. The fluctuation in crank power during the exercise was -55 to 55 W (± 19 %), and is displayed in Figure 4.2 to highlight the magnitude of the power reserve.

Table 4.3: Mean power responses to the 4 supra-CP constant-power exercise tests

	Supra-CP constant-power exercise tests →			
	1	**2**	**3**	**4**
Baseline P_{ISO} (W)	828 ± 96[1]	846 ± 110[1]	834 ± 110[1]	836 ± 106[1]
Flywheel power (W)	325 ± 35[b,c,d]	305 ± 34[a,c,d]	282 ± 34[a,b,d]	266 ± 37[a,b,c]
P_{HYP} LoT (W)	280 ± 37[c,d]	273 ± 31[d]	255 ± 27[a]	253 ± 36[a,b]
LoT P_{ISO} (W)	338 ± 53[d]	348 ± 57[d]	356 ± 85[1,d]	420 ± 89[1,a,b,c]
Performance Fatigue (W)	491 ± 80[d]	498 ± 108[d]	478 ± 94[d]	416 ± 215[a,b,c]

Values are mean ± SD. Arrow denotes increasing exercise duration. Comparisons within each protocol (excluding performance fatigue); ANOVA with Dunnett post-hoc, [1]significantly different from Flywheel power control level. Comparisons between protocols; all variables compared with ANOVA and bonferroni post-hoc: [a] significantly different from test 1; [b] significantly different from test 2; [c] significantly different from test 3; [d] significant different from test 4.

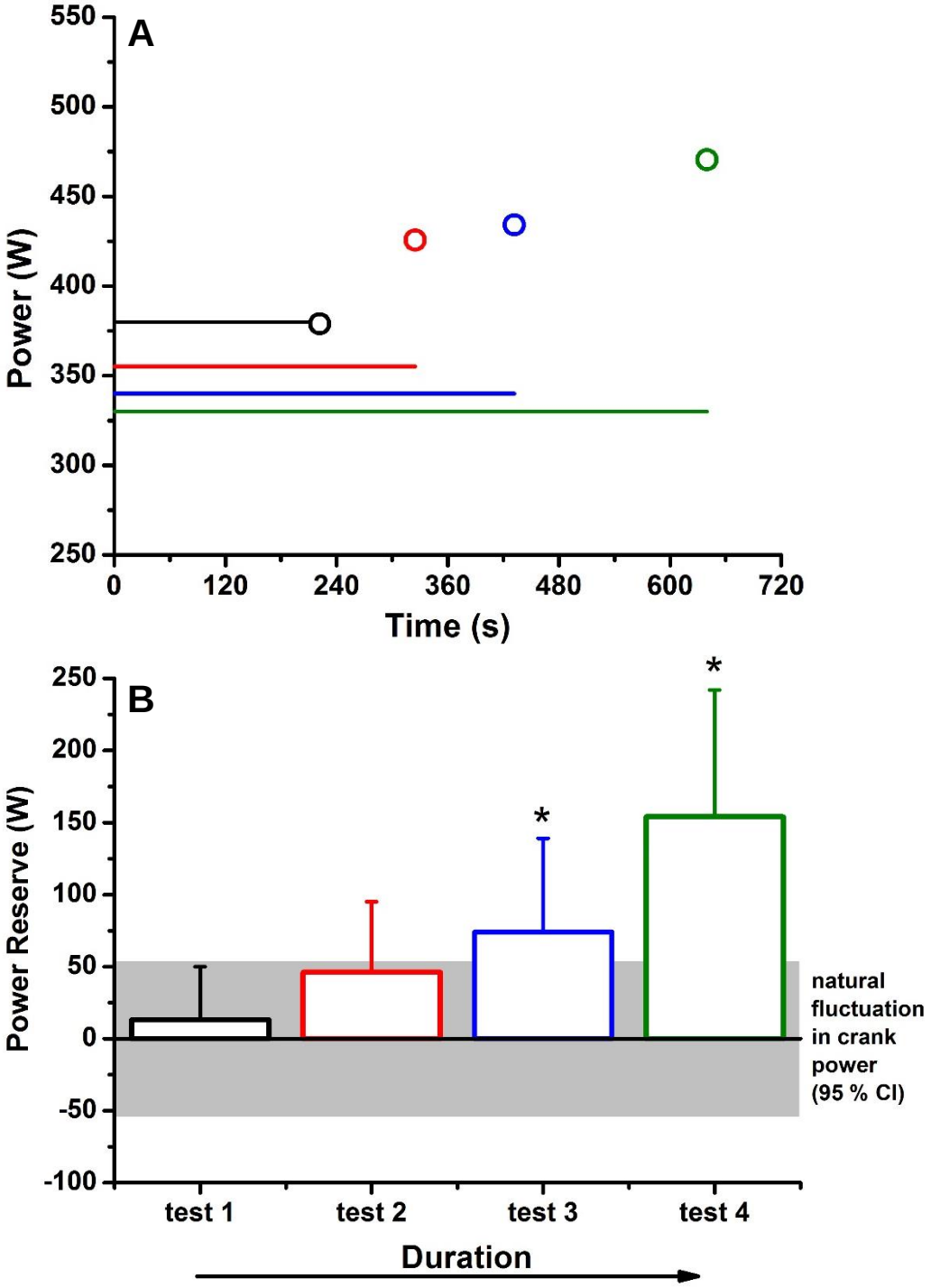

Figure 4.2: A; representative participants power responses to the 4 constant-power exercise tests. The power required (solid line) and the maximal isokinetic power at the limit of tolerance for each protocol is shown in the corresponding colour (open circle). B; group mean (± SD) power reserve for the 4 constant-power protocols. Also shown is the natural fluctuation for context (grey box). *isokinetic power at LoT significantly greater than the required power.

Maximal EMG decreased at the LoT of constant-power exercise, with no difference in the magnitude of decline between the protocols; test 1; 72 ± 14, test 2; 67 ± 16, test 3; 69 ± 17, test 4; 74 ± 13 $\%_{max}$ ($p > 0.05$).

4.3.3 WR6 and WR12

WR6 and WR12 derived from the P-T_{lim} relationship were 291 ± 38 and 264 ± 35 W, respectively. The validity of these powers was confirmed with a T_{lim} of 359 ± 44 and 738 ± 116 s for WR6 and WR12, respectively with no difference in $\dot{V}O_{2peak}$ between the two protocols ($p > 0.05$). The greater $\dot{V}CO_{2peak}$ in WR6 was not significant, although this difference resulted in a significantly greater RERpeak in WR6. As before, reducing the power required and thus extending the duration caused a greater $\dot{V}O_{2SC}$ in WR12. However, unlike in the previous constant-power tests, this also caused a small yet significantly reduction in $\dot{V}_{Epeak}$ for WR12. These responses to WR6 and WR12 are displayed in Table 4.4.

Table 4.4: Pulmonary gas exchange responses to work rate 6 and work rate 12 protocols

	WR6	**WR12**
Flywheel power (W)	291 ± 38	264 ± 35*
Duration (s)	359 ± 44	738 ± 116*
$\dot{V}O_{2peak}$ ($L.min^{-1}$)	4.27 ± 0.50	4.34 ± 0.51
$\dot{V}O_{2SC}$ ($L.min^{-1}$)	0.30 ± 0.16	0.62 ± 0.22*
$\dot{V}CO_{2peak}$ ($L{\cdot}min^{-1}$)	4.67 ± 0.53	4.32 ± 0.42
RER_{peak}	1.10 ± 0.10	0.99 ± 0.08*
$\dot{V}_{Epeak}$ ($L{\cdot}min^{-1}$)	176 ± 18	171 ± 18*
HR_{peak} (bpm)	188 ± 8	189 ± 11

Values are mean ± SD. Values are compared with a paired samples T-test. *significantly greater than WR6.

All isokinetic phases were constrained at the desired cadence in both protocols; WR6; 80.3 ± 0.1 (baseline), 80.3 ± 0.2 (1/3rd), 80.3 ± 0.2 (2/3rd), 80.4 ± 0.2 rpm (LoT) and WR12; 80.3 ± 0.1 (baseline), 80.3 ± 0.3 (1/3rd), 80.3 ± 0.2 (2/3rd), 80.4 ± 0.3 rpm (LoT). Baseline P_{ISO} was not different between the 2 protocols ($p > 0.05$). During the exercise, there was no main effect of the protocol on the P_{ISO} at each time point ($p > 0.05$), thus performance fatigue had developed to a similar extent at each time point in WR6 and WR12. Performance fatigue during WR6 was progressive, inducing a fall in P_{ISO} that was significant between all time points as the test progressed ($p < 0.05$; Figure 4.3 A). However, during WR12 the fall in P_{ISO} was only significant between baseline to 1/3rd and between 1/3rd to 2/3rd exercise duration ($p < 0.05$), with no additional fatigue developing in the final 1/3rd of exercise of WR12 ($p > 0.05$; Figure 4.3 B).

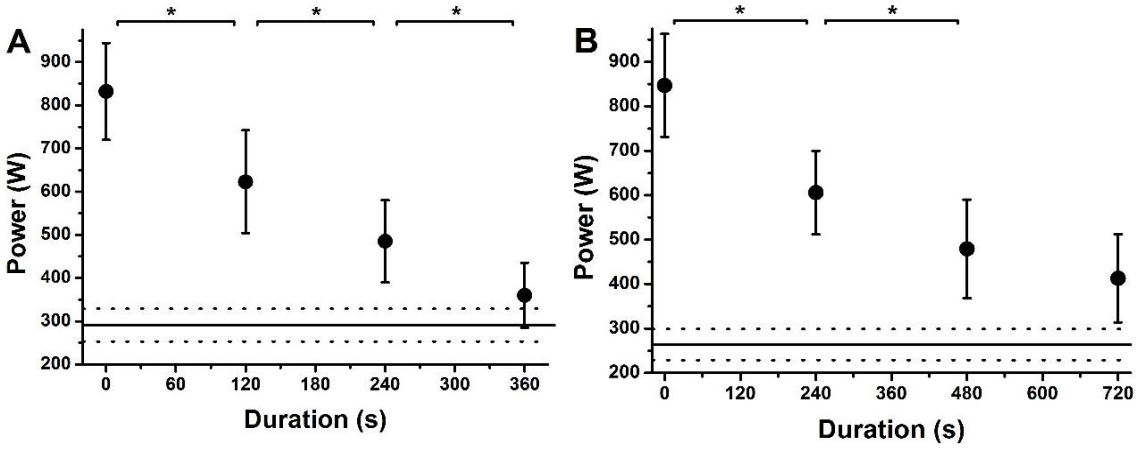

Figure 4.3: Mean (± SD) isokinetic power during WR6 (A) and WR12 (B) protocols. Shown is the baseline power (at time 0 s) followed by the 3 isokinetic power measurements at 1/3rd, 2/3rd exercise duration and at LoT. Also displayed is the mean (solid line) and SD (dotted line) required power of the group for each protocol. *denotes significantly less than the previous power measurement within the same protocol.

EMG amplitude fell throughout both WR6 and WR12, with the same magnitude of decline in WR6 and WR12 ($p > 0.05$; Table 4.5). While there was a progressive fall in EMG throughout both protocols ($p < 0.05$), displaying the increasing

activation fatigue as the exercise progressed, *post-hoc* analysis revealed no significant differences between the individual time points for either protocol ($p > 0.05$; Table 4.5).

Table 4.5: Fall in EMG during WR6 and WR12 protocols. Displayed is the EMG ($\%_{max}$) at the 3 measured time points during the exercise.

	EMG ($\%_{max}$)		
	1/3rd	**2/3rd**	**LoT**
WR6	79 ± 13	72 ± 14	66 ± 11
WR12	75 ± 15	75 ± 13	68 ± 14

Values reported as mean ± SD.

When all six protocols completed by each participant were collated for each of the 9 participants, a moderate but significant positive correlation was observed between the $\dot{V}O_{2sc}$ and the magnitude of the power reserve at LoT (Figure 4.4).

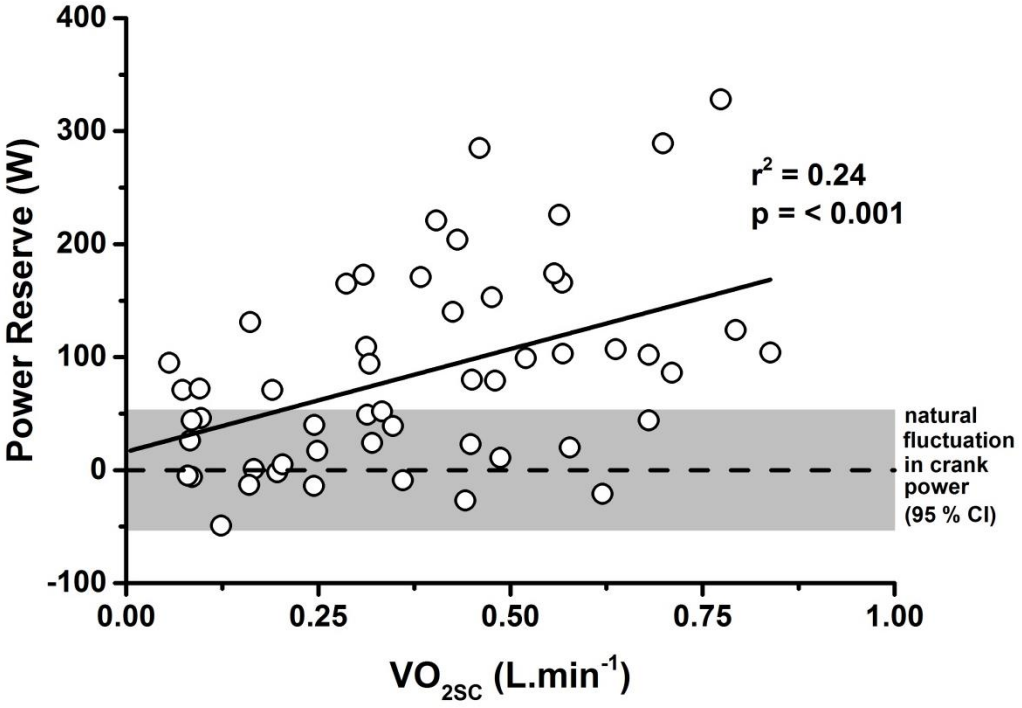

Figure 4.4: A correlation between the magnitude of the $\dot{V}O_2$ slow component ($\dot{V}O_{2SC}$) and the power reserve (W) for all 6 constant-power exercise tests performed by each participant. The natural fluctuation in power is also shown to provide context (grey shaded area).

4.4 Discussion

The first aim of this study was to use the P-T_{lim} relationship as a framework to investigate how the dynamics of the exercise task (power output) interacted with the accumulation of fatigue to determine the point and origin of task failure during whole-body supra-CP cycling in which $\dot{V}O_{2max}$ was attained. It was found that by reducing the power required and therefore reducing the rate of W' depletion and increasing the exercise duration, this caused a reduction in performance fatigue during the exercise. As a result the power generating capacity of the skeletal muscles at the LoT was progressively greater than power required by the task. The second aim was to characterise the development of fatigue during supra-CP exercise by measuring the fall in maximal voluntary isokinetic power during the exercise. There was a progressive fall in voluntary power output throughout the exercise that seemingly mirrored the proposed kinetics of W' depletion, with these dynamics altered depending on the task being performed. Together, these findings suggest that the physiological and perceptual limitations to exercise can be dissociated, leading to task failure before the skeletal muscle limits for exercise have been attained.

4.4.1 Power reserve at $\dot{V}O_{2max}$

The P-T_{lim} relationship provided a robust construct within which to prescribe the power required during exercise in this study to achieve a given T_{lim}. The P-T_{lim} relationship also enabled a fixed volume of supra-CP work to be accumulated in each protocol, represented by W'. In the current study, reducing the rate of W' depletion by reducing the power demand caused a reduction in performance fatigue, which in turn resulted in an increasing power reserve, alongside an increasing $\dot{V}O_{2SC}$ magnitude at LoT. Previous work has suggested that

peripheral fatigue develops up to a critical threshold and no further (Amann and Dempsey, 2008; Burnley *et al.,* 2012), although this remains debated (Froyd *et al.,* 2016; Thomas *et al.,* 2016). If this is the case, peripheral fatigue would be expected to be the same at LoT independent of exercise task. Therefore, it is possible that increased central fatigue and perception of effort during the lower power tasks caused participants to reach task failure with a reserve in voluntary skeletal muscle power capacity. These data would suggest that changing the power output alters the contributions of peripheral and central fatigue, possibly affecting the primary fatigue mechanisms causing the attainment of LoT.

This effect of exercise task is supported by previous research that suggests peripheral fatigue begins to develop almost immediately at exercise onset, a result of the O_2 deficit and the compensatory substrate level phosphorylation, causing fatigue metabolite build up (Allen *et al.,* 2008). Central fatigue however, is slower to develop, possibly in response to the peripheral fatigue stimulus (Amann and Dempsey, 2008). As a consequence of these differing kinetic responses, central fatigue has been found to be more prevalent in lower power, longer duration exercise (Froyd *et al.,* 2016; Thomas *et al.,* 2016), a notion seemingly supported by the current data.

Accordingly, Burnley *et al.* (2012) performed a study similar to and of particular relevance to the current work, whereby the P-T_{lim} relationship was used to vary the exercise duration, and the effects of this on neuromuscular fatigue during single-leg isometric contractions were assessed. When fatigue was measured at LoT, it was found that peripheral fatigue was not different independent of the power output and exercise duration. Conversely, the magnitude of central fatigue was found to increase as the power output demand and thus rate of W' utilisation,

decreased. Despite this increased central fatigue, when maximal voluntary power was assessed at LoT no reserve in power generating capacity was observed in any of the exercise tasks. However, the single-leg isometric exercise modality utilised in their study means there would have been little cardiorespiratory demand present, in contrast to the data presented here performed in whole-body exercise to $\dot{V}O_{2max}$. This increased cardiorespiratory demand and the associated afferent feedback stimuli, such as that from the work of breathing, discussed in detail in the previous chapter has the potential to magnify central fatigue (Sidhu *et al.,* 2013) and increase the perception of effort (Hureau *et al.,* 2016), to a greater extent than reported by Burnley *et al.* (2012). Therefore, this increased overall afferent stimulus driving increased central fatigue may be amplified here in whole-body exercise, resulting in the LoT occurring with a skeletal muscle reserve in voluntary power generating capacity.

It is important to note that these alterations as a consequence of the exercise task are not only duration specific. This is evident when these data are compared with Chapter 3, where the RI25 was an exercise task of 732 ± 93 s that resulted in no power reserve at LoT. This exercise duration was longer than the constant-power protocols presented here for which a power reserve was present at LoT (test 3; 432 ± 66 s and test 4; 705 ± 114 s). Therefore, it is not solely changes in the exercise duration that seem to alter the mechanisms of fatigue, but how the power demand of the task interacts with dynamics of fatigue that determines both the exercise duration and the mechanisms that eventually cause the LoT.

4.4.2 The rate of W' utilisation and magnitude of $\dot{V}O_{2SC}$

It has been suggested that from the onset of supra-CP exercise, a fatigue cascade is initiated as a result of increased reliance on anaerobic energy

systems and the resultant energy depletion and fatigue metabolite build-up (i.e. W' utilisation), the effects of which result in skeletal muscle fatigue (Murgatroyd and Wylde, 2011). This causes a reduction in the ability of the recruited muscle to produce the power required; therefore, in the presence of fatigue, for exercise to continue there is an additional O_2 cost to maintain power and additional muscle fibres must be recruited, which together manifest as the $\dot{V}O_{2SC}$ (Pringle *et al.,* 2003; Krustrup *et al.,* 2004; Cannon *et al.,* 2011). Consequently, the $\dot{V}O_{2SC}$ is now widely considered to originate, at least 80 % from within the exercising muscles (Poole *et al.,* 1991; Rossiter *et al.,* 2002). In the current work, the rate of utilisation of W' was slowed by reducing the power demand. The exercise tasks with a lower power demand and thus slower W' utilisation rate resulted in an increased magnitude of the $\dot{V}O_{2SC}$, a relationship that has been characterised by previous work (Murgatroyd *et al.,* 2011) and suggests a greater inefficiency is present within the exercising muscles when W' utilisation rate is slowed. Furthermore, increasing $\dot{V}O_{2SC}$ magnitude has been found to be associated with both increased peripheral fatigue during high-intensity exercise (r^2= 0.69 $p < 0.05$; Keir *et al.,* 2016), and peak isokinetic power at end-exercise (Cannon *et al.,* 2011). These findings, in addition to the significant correlation found between the $\dot{V}O_{2SC}$ and the power reserve at LoT in the current work (Figure 4.4), suggest a significant role of the changes in W' utilisation rate and consequent increased $\dot{V}O_{2SC}$ in contributing to the increasing power reserve at LoT of high-intensity constant-power exercise. There is potential that the increased intramuscular inefficiency ($\dot{V}O_{2SC}$) may cause a disproportionate increase in afferent feedback from the skeletal muscle to the CNS, as in order to meet this increased $\dot{V}O_2$ requirement there will be an increased $\dot{V}_E$ and HR response. The increased afferent feedback associated with these mechanisms may contribute further to

an increased central drive and the development of additional central fatigue, when W' utilisation rate is slowed.

4.4.3 Kinetics of fatigue during supra-CP exercise

In the second part of this study, the dynamics of fatigue development during supra-CP exercise were investigated using maximal isokinetic efforts during two different exercise protocols. In both of the exercise tasks (designed to be 6 min (WR6) and 12 min (WR12) in duration), there was significant fatigue between all maximal isokinetic efforts up to 2/3rd of exercise duration, with no difference in the magnitude of this fatigue between the different protocols. Therefore, it seems that the different rates of W' utilisation had no effect on overall fatigue up to ~67 % W' depletion, corroborating previous research using the same methods to assess fatigue after 70 % W' depletion at two different rates (exercise durations of 3 and 10 min; de Souza *et al.,* 2017). While there was a significant fall in maximal voluntary power output in the final 1/3rd of WR6 exercise, there was no significant decline in the last 1/3rd of the WR12 test. Overall, the kinetics of the fall in power seem largely linear throughout WR6 and the first 2/3rd of WR12 exercise. However, due to the small fatigue development in the final 1/3rd of WR12, the overall profile for this protocol seems slightly more curvilinear.

This overall profile of the fall in power, perhaps unsurprisingly, seems to mirror the proposed (mathematically derived) dynamics of W' depletion during supra-CP exercise, which has been modelled as primarily linear (Fukuba *et al.,* 2003) or slightly curvilinear, due to W' reconstitution during relaxation phases (the upstroke during cycling) (Skiba *et al.,* 2012, 2014; Broxterman *et al.,* 2016). This coupled kinetic response between W' and overall fatigue seems to add to the evidence that suggests a direct link between W' depletion rate and skeletal

muscle fatigue (Murgatroyd *et al.,* 2011; Cannon *et al.,* 2011), potentially influencing the power reserve at LoT.

The small amount of fatigue that developed in the final 1/3rd of WR12 suggests a dissociation between the physiological and perceptual limits near the end of this exercise protocol. During the final part of the exercise the skeletal muscle inefficiency is greatest (Grassi *et al.,* 2015), evidenced by a larger $\dot{V}O_{SC}$ in the WR12 protocol (WR6; 0.30 ± 0.16 vs. WR12; 0.62 ± 0.22 l.min^{-1}). It is potentially here that the disproportionate increase in afferent feedback (from skeletal muscle and respiratory demands) occurs, with the ensuing increased central fatigue and overall perception of effort causing the LoT to be attained with only a small fall in maximal voluntary power output in these final stages of exercise.

4.4.4 Activation fatigue

As in the previous chapter, EMG data were collected during each of the isokinetic phases of these exercise protocols, to provide an indication of the development of fatigue mechanisms linked to activation of the working muscles. EMG amplitude was reduced at the LoT in all of the constant-power protocols. In addition, when assessed during the interleaved efforts (WR6 and WR12) an overall time effect was found, as the amplitude of EMG decreased during the exercise. Together these findings indicate the importance of central fatigue mechanisms at LoT, and suggest that significant contributions are present throughout exercise. However, no difference in the fall in EMG was observed between any of the six protocols at LoT. This lack of difference implies that there was in fact no difference in the development of the mechanisms associated with central fatigue between these different protocols. However, this more likely represents a lack of sensitivity with this surface EMG methodology, in that while

it can provide information about muscle activation and the motor unit action potentials (Farina *et al.,* 2014), it doesn't provide a direct measurement of neural drive to the muscle (Enoka and Duchateau, 2015), an issue compounded by fatigue (Dideriksen *et al.,* 2011) and even more so by whole-body dynamic contractions where there are changes in muscle length (Farina, 2006), all of which are present here. Moreover, without the presence of stimulation techniques (e.g. TMS), the current work cannot determine the specific origins of this reduced EMG amplitude; therefore, whether the fatigue induced fall in EMG is supraspinal or closer to the muscle level, remains unknown. Finally, the initially proposed method of separating fatigue contributions from activation and muscle origins using the EMG-P_{ISO} relationship, as used previously (Coelho *et al.,* 2015), was not implemented in the current work due to the presence of a power reserve and its effects on this relationship (discussed in section 3.3.3).

4.4.5 Limitations

In previous work, maximal isokinetic power measurements were isolated to before and immediately post-exercise (Cannon *et al.,* 2011; Coelho *et al.,* 2015; Ferguson *et al.,* 2016b; de Souza *et al.,* 2017). The current study has built on this by performing interleaved isokinetic measurements to further our understanding of the kinetics of fatigue during exercise. However, findings in this study are still based on measurements made at 3 discrete time points, something which could be improved by including additional measurements. For example, in future work extra measurements may determine at what point between 67 % W' utilisation and LoT the dissociation of WR6 and WR12 fatigue kinetics occurs. Although the two extra measurements did not increase the overall fatigue development here, adding extra maximal effort measurements during exercise would need to be validated, to ensure this is not an issue.

By necessity the exercise durations assessed in this study were kept within the construct of the P-T_{lim} relationship (~2-30 min (Poole *et al.,* 1988; Hill, 1993; Fukuba and Whipp, 1999) and are all performed to the LoT. Consequently, the findings presented here may not translate to either very short or longer exercise protocols, in addition to protocols not performed to LoT such as time trials. Previous research does suggest that the kinetics of central and peripheral fatigue are consistent during much longer exercise and during time trials (Lepers *et al.,* 2002; Thomas *et al.,* 2015). However, confounding factors that may contribute to the development of fatigue during these much longer protocols cannot be discounted, such as; substrate provision, thermoregulation and hydration status (Fukuba and Whipp, 1999).

Finally, as discussed in detail previously (section 3.4.6) the lack of external stimulation for neuromuscular fatigue assessment means these protocols rely on maximal effort of participants throughout. This maximal effort is evidenced in the current work by $\dot{V}O_{2max}$ and HR_{max} that are not different across protocols, in addition to the P_{HYP} data that is not different from the task requirement, in the final few pedal strokes before LoT is attained. However, the simplicity provided by the current protocol and the ability to measure power and fatigue without delay, during whole-body dynamic cycling (the same modality as the fatiguing exercise) provides an important and more ecological alternative to external stimulation techniques. Nevertheless, future work that enables externally stimulated power measurements that mirror the neural recruitment patterns during exercise to be performed immediately post whole-body dynamic exercise remains a key goal for this research field (e.g. Black *et al.,* 2016; Temesi *et al.,* 2017), with the aim to determine what is ultimately limiting exercise. If this can be determined on an individual and task basis, then interventions to target these

mechanisms with the aim of improving exercise tolerance can be designed and implemented (specific interventions are explored in Chapter 6).

4.4.6 Conclusions

Using the P-T_{lim} relationship to provide a robust framework for control of supra-CP work done, it has been demonstrated that reducing the power demand and consequently reducing W' utilisation rate and increasing exercise duration caused a power reserve at the LoT of whole-body supra-CP constant-power cycling. This reserve suggests that during lower power exercise the perceived and actual fatigue stimulus are dissociated, with this possibly linked to the increased skeletal muscle inefficiency manifest as the $\dot{V}O_{2SC}$.

Additionally, we have shown that the kinetics of the fall in maximal power during supra-CP exercise primarily follow the proposed kinetics of W' utilisation, although this may have the potential to be altered depending on exercise task.

Chapter 5 Limitations to exercise in chronic heart failure

5.1 Introduction

Chronic heart failure (CHF) is a condition that is initiated by an event or disease within the myocardium (e.g. myocardial infarction and myocarditis) resulting in cardiac dysfunction. This cardiac dysfunction most commonly causes a reduced left ventricular ejection fraction (LVEF), stroke volume and heart rate response, ultimately reducing cardiac output (Poole *et al.,* 2011), the consequences of which can affect multiple physiological systems. While LVEF can be preserved (termed HFpEF), generally associated with a diastolic dysfunction (Sharma and Kass, 2014), all patients in the current work had reduced LVEF, therefore the effects of the separate HFpEF phenotype were not investigated here. A cardinal symptom of CHF, independent of phenotype, is a reduced exercise tolerance, which limits physical activity, reduces quality of life, and ultimately predicts mortality in CHF (Keteyian *et al.,* 2016). Traditionally, the reduced cardiac function was thought to be the primary cause for the reduced exercise tolerance in CHF; where the reduced cardiac output limits O_2 delivery to the working muscles. However, while this may play a role either directly or indirectly, it is becoming increasingly evident that the degree of cardiac function (as measured by LVEF) is not closely related to either exercise tolerance (defined by $\dot{V}O_{2max}$) or symptoms in CHF (Davies *et al.,* 1992; Carell *et al.,* 1994; Witte *et al.,* 2004). Accordingly, researchers have focused their efforts on the consequences of CHF within the periphery, considering the influence of vascular and skeletal muscle dysfunctions (e.g. Esposito *et al.,* 2011; Bowen *et al.,* 2012b; Middlekauf *et al.,* 2013).

While the specific underlying mechanisms reducing exercise tolerance in CHF remain elusive, it is well characterised that the two primary symptoms during exercise are sensations of dyspnoea and fatigue. Interestingly, in a cohort of CHF patients neither of these symptoms were found to be more prevalent than the other when assessed at $\dot{V}O_{2max}$ (Witte and Clark, 2008). While this is not a uniform finding (Chase *et al.,* 2008), it is clear that the CHF population report either dyspnoea or fatigue as their primary reason for exercise intolerance, with the relative contributions of each symptom seemingly different on an individual patient basis.

However, the primary symptom reported seems to have no influence on objective measures of exercise tolerance (e.g. $\dot{V}O_{2peak}$ and minimum $\dot{V}_E/\dot{V}CO_2$) (Clark *et al.,* 1995; Russell *et al.,* 1998; Witte and Clark, 2008). Therefore, the ability to determine an individual's primary exercise limitation has been restricted to self-report measures such as rating of perceived exertion (Borg, 1978). The accuracy of these subjective measures may be limited; participant bias, interpretation by experimenter or participant, the exercise modality and the specific exercise protocol performed can all influence the reported reason for task failure (Chase *et al.,* 2008; Dube *et al.,* 2016). Therefore, an objective measure of the primary exercise limitation may provide a more robust indication of how the exercise intolerance of an individual patient should be treated, targeting either the skeletal muscle or symptoms of dyspnoea.

Recently, Cannon *et al.* (2016) used short bouts of isokinetic cycling in chronic obstructive pulmonary disease (COPD) patients, to objectively assess whether the sensations of dyspnoea and/or the presence of fatigue were enough to reduce the maximal voluntary locomotor power output, causing task failure.

While COPD patients were more fatigable than healthy controls, and significant locomotor fatigue was present, this was insufficient to limit the continuation of exercise in COPD, with patients able to produce a power ~2.6 times that required, when measured immediately (< 1 s) post the limit of tolerance (LoT). Thus, the authors concluded that patients were ultimately limited by dyspnoea arising as a consequence of their COPD, and not an inability of the exercising muscles to produce the required power (i.e. fatigue) (Cannon *et al.,* 2016), as is the case when this protocol performed by healthy individuals (Coelho *et al.,* 2015).

Thus, the aim of this study chapter was to investigate exercise intolerance in CHF by determining whether or not CHF patients are ultimately limited by the ability of the exercising muscles to generate power at $\dot{V}O_{2max}$. It was hypothesised that the power generating capacity of the working muscles at task failure would be variable among CHF patients, whereby some patients are limited by the working muscles, and others are ultimately limited by more central symptoms, such as dyspnoea.

5.2 Methods

5.2.1 Ethical Approval

This study and all the procedures involved were approved by the Leeds Teaching Hospital NHS Trusts Research & Innovation Ethics Committee. Written informed consent was obtained from all participants prior to their involvement in the study, with participants free to withdraw from the study at any point without explanation.

5.2.2 Participants

Thirteen male CHF participants volunteered to partake in the study (NYHA I-III; Webber class A-C). Participant characteristics were, (mean ± SD): Age 67 ± 10 years; Height 170 ± 10 cm; Weight 91 ± 23 kg; LVEF 26 ± 8 %. All participants were suffering from symptomatic but stable CHF (7 dilated cardiomyopathy and 6 ischaemic heart disease) and were on optimal therapy at the time of testing. More details regarding the recruitment process and specific exclusion/inclusion criteria can be found in section 2.1.2. Additionally, a flow chart illustrating the recruitment during this study can be found in Figure 5.1.

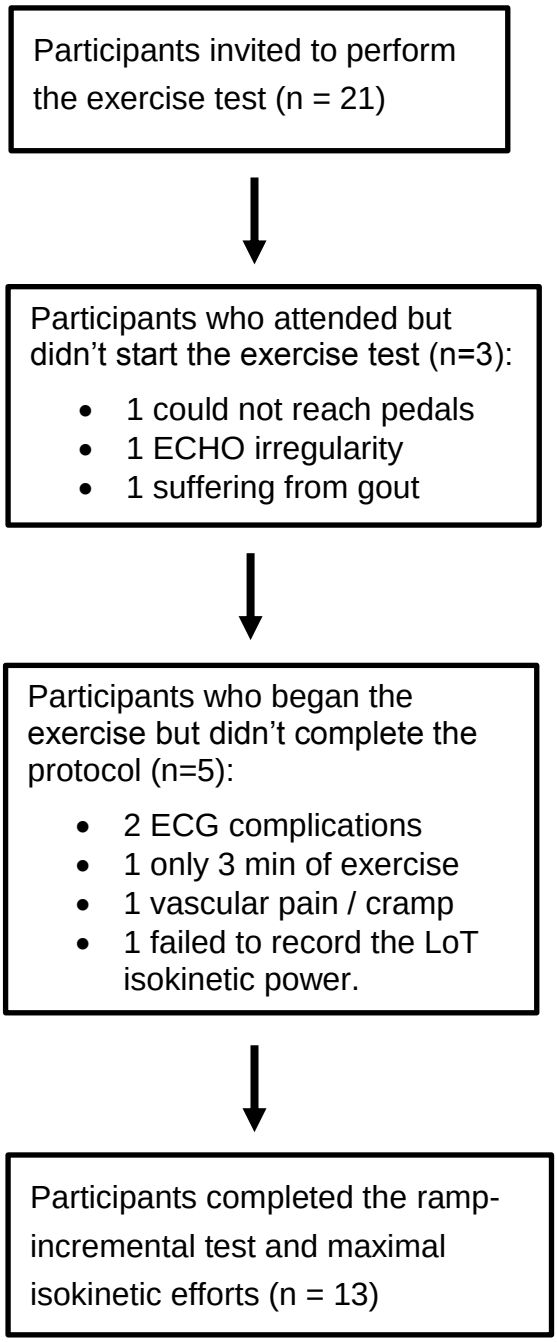

Figure 5.1: Flow diagram of participant recruitment

5.2.3 Exercise Protocols

All exercise tests in the study were performed on an electromagnetically braked, computer-controlled upright cycle ergometer (Excalibur Sport PFM, Lode BV, Groningen, NL). Each participant completed a single visit to the Cardiac Research Facility at Leeds General Infirmary, consisting of two maximal isokinetic efforts, followed by a ramp-incremental test and final bout of maximal effort isokinetic cycling, details of which can be found below.

First, participants were required to perform two short (~6 s) bouts of isokinetic cycling at 100 % maximal voluntary effort. The isokinetic velocity in this chapter was constrained at 70 rpm (rather than 80 rpm), as this was deemed a more appropriate cycling cadence for CHF participants. Each maximal effort was preceded by a period of cycling at 10 W, with the duration of this determined by the participant (usually ~1-2 min).

Participants were then given as much time as required to recover (without dismounting the ergometer), before the second phase of the test commenced. Details of the ramp-incremental exercise test can be found in section 2.5.5. Briefly, participants sat on the ergometer at rest for ~ 2 min, followed by ~ 4 min of cycling at a low power (10 W) for warm-up. The power was then increased as a smooth function of time, at a rate designed to induce the LoT after 8-12 min of exercise (Buchfuhrer *et al.,* 1983). This incrementation rate varied from 5-15 $W.min^{-1}$, selected based on knowledge of the participants health status and/or previous exercise test performance. The test was continued until the LoT was reached, defined as when participants could no longer maintain cadence above 50 rpm, despite strong verbal encouragement. Upon reaching LoT, the mode on the ergometer was immediately switched to isokinetic, causing flywheel

resistance to fall to zero, and participants were instructed to increase cadence to 70 rpm (~1-2 s) and perform a final short (~6 s) maximal voluntary effort bout of isokinetic cycling. The exercise test was completed with 6 min of monitored active recovery (10 W) and a further 6 min of monitored rest after dismounting the ergometer.

5.2.4 Measurements

The isokinetic mode on the ergometer was used to measure maximal voluntary isokinetic power before and instantaneously at the LoT of the ramp-incremental test, calculated as the product of force and angular velocity. For more details on the cycle ergometer and isokinetic power measurements, see section 2.2.

Respired gases were measured during the ramp-incremental phase of the exercise test only, using methods described previously (section 2.3). Briefly, participants breathed into a facemask designed to cover the mouth and nose, with gases sampled and then analysed by the breath-by-breath analysis system (MedGraphics D-Series, Medgraphics, Medical Graphics Corporation, St Paul, MN, USA). A facemask was chosen in this study as opposed to a mouthpiece for participant comfort, with the best fit (to avoid any leaks that may occur) ensured by using differing size masks. Prior to the exercise test both the flow sensor and the O_2 and CO_2 gas analysers were calibrated using standard procedures. Additional details regarding the gas analysis system and the calibration procedures can be found in section 2.3. A 12 lead ECG was attached to the participant and was continuously monitored by the cardiac physiologist who was present for all testing. If any abnormality in the ECG trace occurred at any point during the exercise, the test was immediately halted at the discretion of the cardiac physiologist. Details of instances of this can be found in Figure 5.1.

5.2.5 Data Analysis

For each bout of isokinetic cycling, the first 3 pedal strokes constrained at 70 rpm were isolated. The left and right power of these were then summed and the mean of these 3 pedal strokes reported. Additionally, the natural fluctuations in hyperbolic cycling power during the ramp-incremental test were calculated for each participant, as described previously (section 2.6.2.2). Participants in this study were less accustomed to exercise testing and the specific cycling modality so were prone to much larger natural fluctuations in power, therefore more conservative 99 % prediction bands were used to characterise the natural fluctuation. Details of all power calculations, including the power reserve, performance fatigue and power accessed can be found in section 2.6.2.

Breath-by-breath data were initially edited to remove erroneous breaths, those outside of 99 % prediction band of the local mean. Data were then analysed to determine a range of peak gas exchange variables (e.g. $\dot{V}O_{2peak}$). Additionally, the lowest 8 breath average of $\dot{V}_E/\dot{V}CO_2$ ratio was found for each participant, as this has been shown to be a stable and reproducible measure of ventilatory efficiency, in addition to being a strong prognostic marker in CHF (Sun *et al.,* 2002; Myers *et al.,* 2009). For more details on the editing procedures and analysis of pulmonary gas exchange data see section 2.6.1.

5.2.6 Statistics

One way repeated measures ANOVA with Dunnet post-hoc (power required at LoT as control level) was used to compare all power measurements. Two sample T tests were used when comparing the two different CHF groups. Significance level was set at 0.05, values reported as mean ± SD.

5.3 Results

The 2 baseline maximal isokinetic efforts for the group were 358 ± 170 W and 375 ± 182 W, with good reproducibility shown between efforts; CoV 7.2 %. As a result, a mean of these two efforts was taken for each individual to represent the isokinetic power (P_{ISO}) at baseline.

The physiological responses to the ramp-incremental test for each individual participant can be found in Table 5.1. As previously reported, there was no correlation between cardiac function and exercise tolerance (r^2 = -0.12, p > 0.05; Figure 5.2).

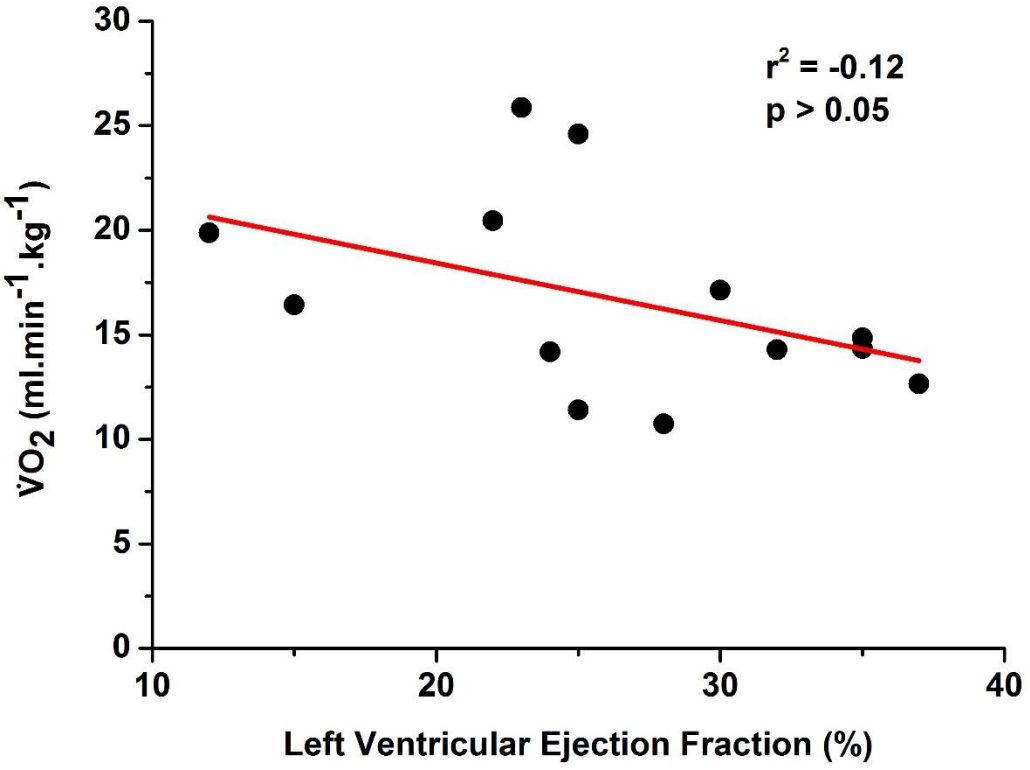

Figure 5.2: Correlation between $\dot{V}O_2$ and left ventricular ejection fraction for all 13 participants.

Table 5.1: Responses of each participant to the ramp-incremental exercise

Participant	Ramp Rate ($W.min^{-1}$)	Ramp Duration (s)	WR_{peak} (W)	$\dot{V}O_{2peak}$ ($l.min^{-1}$)	$\dot{V}O_{2peak}$ ($ml.min^{-1}.kg^{-1}$)	HR_{peak} (bpm)	HR_{rise} (bpm)	Min $\dot{V}_E/\dot{V}CO_2$	RER_{peak}
1	5	550	56	1.09	14.34	106	37	34.3	1.02
2	5	579	58	0.98	14.18	103	25	47.2	1.26
3	7	652	86	1.27	14.29	111	32	38.8	1.14
4	10	760	136	1.79	20.46	136	68	33.8	1.19
5	7	733	95	1.55	19.87	91	40	27.5	1.26
6	7	710	92	1.71	11.40	126	31	30.3	0.89
7	10	502	93	1.20	12.66	119	39	28.0	1.24
8	15	590	154	1.82	24.59	98	32	31.4	1.11
9	10	709	126	1.68	17.14	145	80	2.4	1.10
10	15	411	112	1.56	14.86	132	48	27.8	1.04
11	10	345	65	0.75	10.74	92	38	41.4	1.05
12	15	699	185	1.94	25.87	129	66	34.2	1.03
13	12	629	136	1.84	16.43	137	46	29.4	1.09
Mean		**605**	**107**	**1.48**	**16.70**	**117**	**45**	**33.3**	**1.11**
SD		**127**	**39**	**0.38**	**4.76**	**18**	**17**	**6.0**	**0.11**

Power accessed during the ramp-incremental test was 29 ± 11 % baseline P_{ISO}, with performance fatigue at LoT 139 ± 78 W (Figure 5.3). Immediately at task failure, group mean LoT P_{ISO} was significantly greater than the task requirement (107 ± 39 vs. 223 ± 142 W; $p < 0.05$), displaying a power reserve 108 ± 130 % task requirement (Figure 5.3).

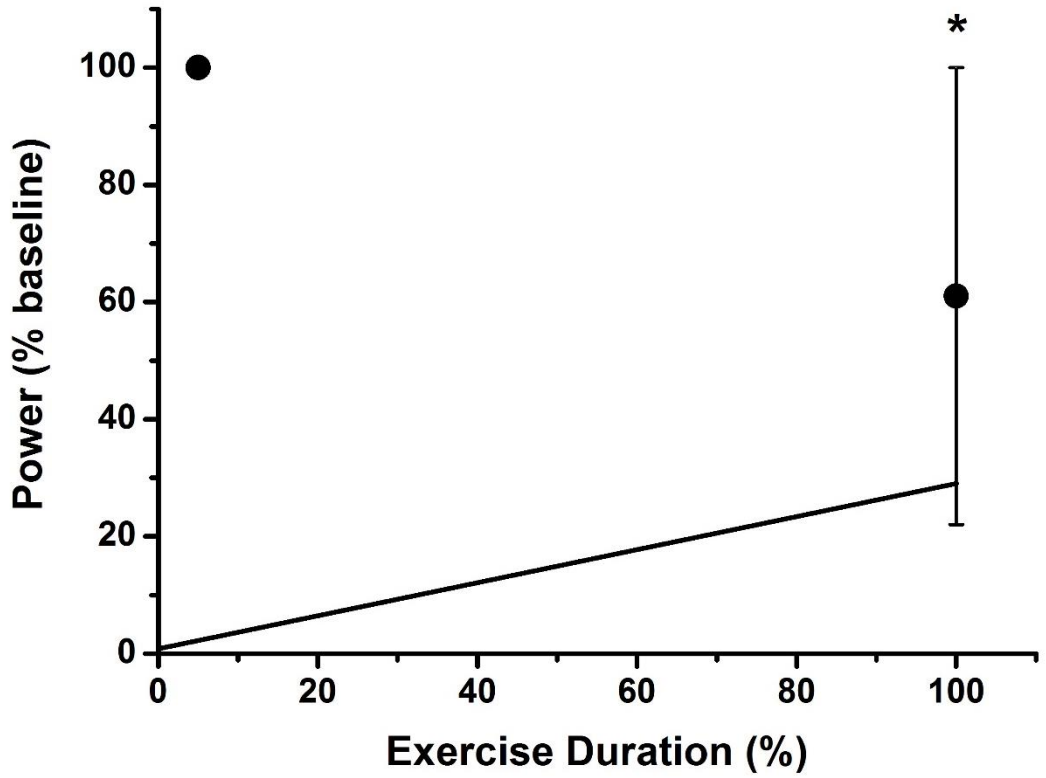

Figure 5.3: Group mean (± SD) ramp-incremental test power responses, displayed as a % of baseline isokinetic power. Displayed is the group mean ramp-incremental required power normalised to % exercise duration (solid line), and the isokinetic power produced immediately at the limit of tolerance. *significantly greater than power demand at task failure.

However, there was high variation between the individual participants isokinetic power at LoT and thus, the magnitude of power reserve at LoT (Figure 5.4). Consequently, participants were separated into two groups depending on if they displayed a significant power reserve at LoT or not (i.e. did LoT P_{ISO} exceed the natural fluctuation in power, characterised in this group as 55 % task requirement). In 6 participants there was no power reserve at LoT (92 ± 29 vs.

120 ± 36 W; 32 %; $p > 0.05$; Figure 5.4; Figure 5.5 A), whereas in 7 participants LoT P_{ISO} was greater than the task requirement and in excess of the natural power fluctuation (120 ± 43 vs. 310 ± 140 W; 173 %; $p < 0.05$; Figure 5.4; Figure 5.5 B).

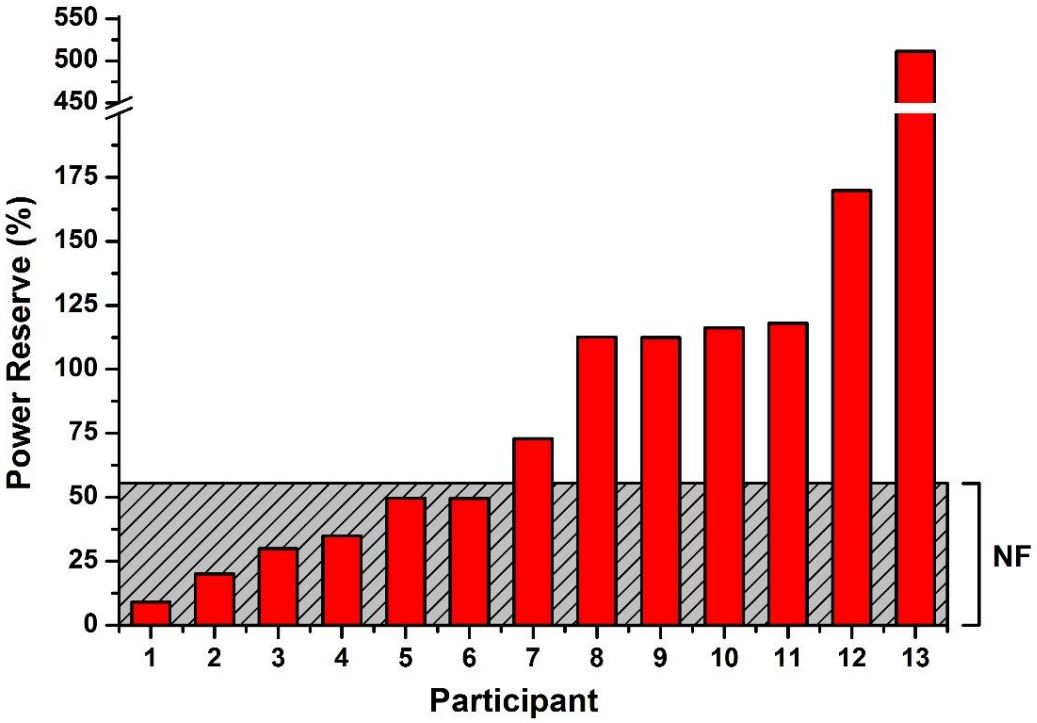

Figure 5.4: The power reserve (%) of each participant, shown to highlight the variation in participant responses. Power reserve = the difference between peak work rate during the ramp-incremental test and the isokinetic power produced immediately post the limit of tolerance. Also shown if the mean natural fluctuation (NF) in power of this group of participants (55 %), displayed to highlight the 2 different responses observed; either below (n = 6) or above (n = 7) the NF, determining the absence or presence of a power reserve, respectively.

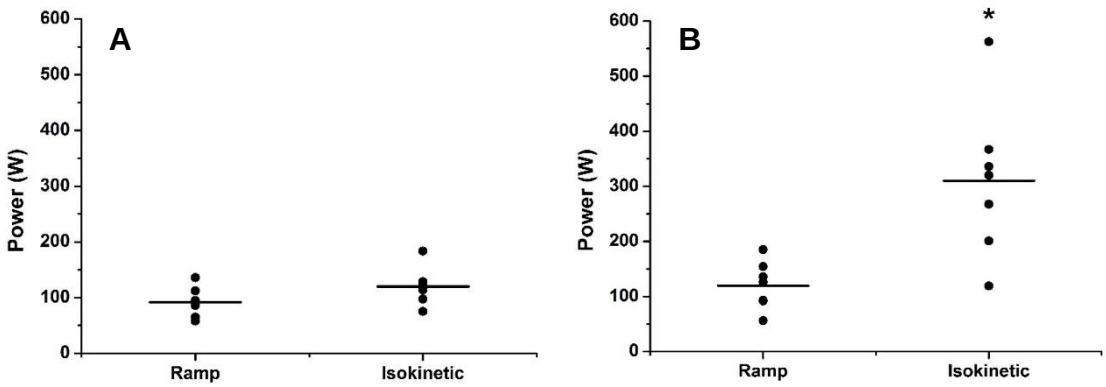

Figure 5.5: The power required at the limit of tolerance (Ramp) and isokinetic power produced immediately post-task failure (Isokinetic) of the 2 groups. No power reserve (n = 6; A) or a significant power reserve (n = 7; B). The mean of each phase is also shown (solid lines). * Isokinetic significantly greater than Ramp power.

A clinical overview of the 2 distinct groups is provided in Table 5.2. No difference was found between the 2 groups when comparing demographics, exercise data, blood markers or medication ($p > 0.05$).

Table 5.2: Clinical overview of the two CHF groups with or without a significant power reserve

	No Power Reserve (n = 6)	Power Reserve (n = 7)
Age (yr)	70 ± 9	65 ± 11
Height (m)	1.67 ± 0.1	1.75 ± 0.1
Weight (Kg)	79 ± 19	88 ± 14
BMI	28.1 ± 4.3	28.7 ± 3.7
Ejection Fraction (%)	25 ± 8	27 ± 8
CRT Device (n)	6 (100 %)	6 (86 %)
Atrial Fibrillation (n)	2 (33 %)	3 (43 %)
Diabetes (n)	3 (50 %)	2 (29 %)
Hypotension (n)	2 (33 %)	2 (29 %)
$\dot{V}O_{2peak}$ ($ml.min^{-1}.kg^{-1}$)	15.73 ± 3.73	17.49 ± 5.66
Min $\dot{V}_E/ \dot{V}CO_2$	36.08 ± 7.84	30.85 ± 2.60
Peak heart rate (bpm)	111 ± 20	123 ± 17
Haemoglobin (g/L)	132 ± 16	148 ± 14
Sodium (mmol/L)	139 ± 5	139 ± 4
Potassium (mmol/L)	4.7 ± 0.5	4.5 ± 0.5
Estimated GFR	71 ± 17	68 ± 11
ACE inhibitor (n)	4 (67 %)	6 (86 %)
Beta blocker (n)	6 (100 %)	7 (100 %)
Loop Diuretic (n)	3 (50 %)	3 (43 %)

Values reported as either Mean ± SD or n (%). All continuous variables compared with a two samples T test. GFR = glomerular filtration rate. ACE inhibitor = angiotensin-converting enzyme inhibitor.

5.4 Discussion

The aim of this study was to characterise whether at $\dot{V}O_{2max}$, CHF patients attained task failure due to a limited power generating capacity of the working muscles. When maximal evocable power was measured immediately at LoT of the ramp-incremental exercise test, P_{ISO} of the group as a whole was significantly greater than the task requirement. However, when considered in the context of the natural fluctuation in power, two distinctly different responses were observed, with some individuals able to produce a power significantly greater than the task (i.e. power reserve), whereas in others the maximal evocable power measured was not different (i.e. no power reserve). This suggests that while some participants were limited by the exercising muscles, others were in fact limited more by other more central symptoms that have the potential to increase the discomfort of exercise, such as the sensation of dyspnoea present in CHF.

5.4.1 Participants with no reserve in maximal evocable power

The absence of a reserve in the capacity to generate power at intolerance was observed in 6 participants (92 ± 29 vs. 120 ± 36 W $p > 0.05$). In this instance, the maximal evocable power produced suggests that the LoT was reached because the exercising muscle can no longer meet the power requirements of the task. Thus, peripheral mechanisms are proposed to be the primary cause of exercise intolerance in these individuals, factors that will be exacerbated by the alterations within the vasculature and skeletal muscle, consequent of CHF. Muscle atrophy and myopathy have been consistently observed in CHF (Mancini *et al.,* 1992). Alterations in the fibre composition are present, with an increased proportion of glycolytic, easily fatigable type II muscle fibres in CHF (Mancini *et al.,* 1989; Sullivan *et al.,* 1990; Middlekauff, 2010). These structural changes in skeletal

muscle are accompanied by a reduced mitochondrial volume, density and enzyme function (Sullivan *et al.,* 1990; Drexler *et al.,* 1992), which together with the structural changes, cause an impaired overall oxidative capacity of the skeletal muscles. A direct influence of CHF on the oxidative capacity, rather than the effects of muscle inactivity is evidenced by a reduced oxidative capacity in the wrist flexors, a muscle group not involved with locomotion (Vescovo *et al.,* 1996; Southern *et al.,* 2015). These skeletal muscle alterations will contrive to cause a reduced skeletal muscle efficiency and greater reliance on anaerobic energy systems, resulting in increased muscle fatigue and al reduced power generating capacity of the working muscles.

In addition to reduced muscle capacity, CHF causes vascular dysfunction that reduces blood flow to the exercising muscles, exacerbating fatigue further. Increased sympathetic vasoconstrictor tone, the result of enhanced peripheral chemoreceptor sensitivity in addition to hyperactive hormonal systems such as renin-angiotensin, cause a poor vasodilation response in CHF, initially as a compensatory mechanism to maintain cardiac output, becoming problematic when chronic, primarily due to a link to hypertension (LeJemtel *et al.,* 1986; Fisher *et al.,* 2009; Triposkiadis *et al.,* 2009). Finally, there is a potential role of reduced capillarity reducing blood flow to muscles and reducing exercise tolerance (Duscha *et al.,* 1999), although this remains debated (Esposito *et al.,* 2010). A combination of these peripheral muscle and vascular dysfunctions, causing increased skeletal muscle fatigue is suggested to be contributing to a greater extent to exercise intolerance in the group with no reserve in maximal evocable power generating capacity at LoT.

5.4.2 Participants with a reserve in maximal evocable power

A reserve in the ability of the skeletal muscle to produce power above that required by the task at LoT was observed in 7 participants; 120 ± 43 vs. 310 ± 140 W ($p < 0.05$). This magnitude of reserve in evocable power (173 %) was importantly above the natural fluctuation in power observed in this group (55 % required power), showing this reserve was physiological in origin and not the result of the fluctuation in power that occurs during exercise.

In these participants, although performance fatigue developed during the ramp-incremental test, this was not sufficient to limit continuation of exercise, as the LoT was attained when still able to voluntarily evoke a power greater than that required by the task. Consequently, as the skeletal muscle can still produce the power required, this suggests that the physiological limits were not reached at LoT, and other factors are contributing to the attainment of task failure. As well as fatigue, the other primary symptom associated with reduced exercise tolerance in CHF is an increased sensation of dyspnoea. Increased sensations of dyspnoea have been linked to central fatigue, whereby the increased respiratory demand causes increased afferent feedback to the CNS, amplifying the overall perception of effort (Hureau *et al.,* 2016). Evidence for this is provided by COPD patients, where the primary exercise limitation is an increased sensation of breathlessness (Cannon *et al.,* 2016). Spinal injections of fentanyl, used to suppress these dyspnoeic sensations and reduce afferent feedback, caused both a reduced perception of effort and an increased exercise tolerance (Gagnon *et al.,* 2012). Furthermore, when using the same isokinetic cycling protocol as this study, it was found that COPD patients attain the LoT with a significant reserve in maximal evocable power (~260 %), suggesting the LoT in

COPD was consequent to dyspnoea induced by the participants COPD, and not muscle fatigue (Cannon *et al.,* 2016). Together, while it has not been directly measured here, these data provide additional indications that the primary limitation in the “power reserve group” in the current study may reside in the sensations of dyspnoea.

In CHF, the mechanisms that cause an increased dyspnoeic sensation are complex and not fully understood. The first potential origins are an impaired pulmonary diffusing capacity, causing increased deadspace during exercise and a ventilation-perfusion mismatch (Lewis *et al.,* 1996). In addition, there is reduced lung compliance, consequent of pulmonary oedema, that can cause increased loading of respiratory muscles (Gehlbach and Geppert, 2004); reducing this oedema has been shown to improve symptoms and exercise tolerance in CHF (Agostoni *et al.,* 1995). Finally, there is a potential link between the peripheral maladaptation’s and sensations of dyspnoea. Increased activation of ergoreceptors in the skeletal muscle, reacting to mechanical and metabolic alterations during exercise, are postulated to increase afferent feedback, inducing increased ventilation and sympathetic activation (Piepoli *et al.,* 1996; Ponikowski *et al.,* 2001). Similarly to the increased feedback from sensations of dyspnoea, increasing the afferent feedback from skeletal muscles increases the overall perception of effort, potentially causing participants to stop exercising with a skeletal muscle power reserve. This feedback from skeletal muscle will also be blocked in the presence of fentanyl, probably contributing to the reduced effort perception and improved exercise tolerance in COPD patients (Gagnon *et al.,* 2012).

5.4.3 Exercise tolerance and primary symptoms

The group as a whole was found able to access only 29 % of maximal power generating capacity, compared with 42 – 45 % in healthy individuals (Coelho *et al.,* 2015; Cannon *et al.,* 2016; Ferguson *et al.,* 2016b; Chapter 3 and 4). While higher than power accessed in COPD (~20 %; Cannon *et al.,* 2016), this displays the severe level of exercise intolerance present. However, as previously reported (Witte *et al.,* 2004) a poor relationship between the level of exercise intolerance ($\dot{V}O_{2peak}$) and cardiac function (assessed by LVEF) was found in the current participants, further suggesting the exercise limitation was not a consequence of reduced cardiac function.

While the study was limited in sample size, it was found that a reserve in power generating capacity was present and absent in 7 and 6 participants, respectively. These proportions seem to corroborate with the proportions of patients for which fatigue and dyspnoea are reported as the primary reason for exercise intolerance (Witte and Clark, 2008). While other studies have found a greater prevalence of either fatigue (Hamilton *et al.,* 1996) or dyspnoea (Clark *et al.,* 1995), it is widely accepted that both mechanisms contribute significantly to exercise intolerance, with the magnitude of contribution likely different on an individual participant basis.

Traditionally, information regarding symptoms is acquired by asking the participant during and after the exercise for a rating of exertion in relation to these two primary exercise symptoms, and of which they felt was limiting their continuation of the exercise (e.g. RPE). While the simplicity of asking participants how their symptoms are developing and how they feel the exercise is limiting them should not be disregarded, these subjective assessments are open to

misinterpretation and may be misunderstood by some participants (Chase *et al.,* 2008), which may lead to the incorrect exercise limitation being determined.

Consequently, the importance of objective measures of exercise tolerance (e.g. $\dot{V}O_{2peak}$), implemented alongside subjective symptom assessments is now widely accepted (Myers *et al.,* 2015). However, $\dot{V}O_{2peak}$, considered the best predictor of mortality in CHF (Keteyian *et al.,* 2016) was not different between the two groups in this study; 15.73 ± 3.73 vs. 17.49 ± 5.66 $ml.min^{-1}.kg^{-1}$ ($p > 0.05$). Additionally, while it may be expected that participants suggested to have greater sensations of dyspnoea may also present with a reduced ventilatory efficiency (represented by $\dot{V}_E/\dot{V}CO_2$ ratio), there was again no difference found between the two participant groups. This lack of correlative evidence is supported by previous research, where irrespective of the limiting symptom reported, $\dot{V}O_{2peak}$ and $\dot{V}_E/\dot{V}CO_2$ were not different (Clark *et al.,* 1995; Russell *et al.,* 1998). These findings together display how it is potentially not an increased ventilatory response that caused LoT in some individuals, rather it is an increased perception of the sensations of dyspnoea. Accordingly, it is believed that in the group with a power reserve, the perceptual and physiological limits of exercise are dissociated, in contrast to the group with no power reserve, where these are closely matched. There is potential that the objective, maximal isokinetic efforts may be used in conjunction with current measures, to understand where the limitation to exercise is coming from, on an individual basis. Finally, it is of note that all other clinical variables showed no link to whether a power reserve was present at LoT (Table 5.2). This is supported by previous literature finding no relationship between the aetiology of CHF, ejection fraction and the primary symptoms reported (Witte *et al.,* 2004; Arena *et al.,* 2005).

5.4.4 Limitations and future directions

By necessity, the exercise in this study was performed on a cycle ergometer to enable velocity independent measure of power, not possible using treadmill exercise. However, the exercise modality has potential to influence the limiting symptoms in CHF, with cycling exercise reported to result in fatigue as the primary reason for intolerance, more frequently than when exercise is performed on a treadmill (Witte and Clark, 2005). This increased perception of dyspnoea in treadmill exercise is probably due to the increased volume of muscle recruited for this weight baring exercise. However, due to the required isokinetic component of the power assessment in the current protocol, and the need to measure this immediately at LoT (negating the option of switching ergometers), the exercise is limited to cycling at this time. Other isokinetic modalities that could be used such as knee-extension would, due to the small cardiorespiratory demand in this exercise, also be dominated by the sensations of leg fatigue (Sidhu *et al.,* 2013). Consequently, the influence of the exercise modality on the power generating capacity of skeletal muscle at LoT remains unclear.

CHF is a complex condition that is often associated with comorbidities which may have had an effect on the data reported. While participants diagnosed with COPD were excluded from this study, the coexistence of any limitations within the lungs and airways, that would increase an individual's sensations of dyspnoea during exercise, cannot be overlooked (Dube *et al.,* 2016). Spirometry assessments, especially effective when resting measures are supplemented with assessments performed during exercise, would be beneficial in negating this as a factor. Furthermore, the presence of diabetes mellitus (DM) is a common comorbidity, with 29 % of CHF participants reported to be suffering from DM (van

Deursen *et al.,* 2014). The presence of DM, and the associated mitochondrial dysfunction, may ultimately increase skeletal muscle fatigue in these participants (Kelley *et al.,* 2002; Lowell and Shulman, 2005). Although 5 of our 13 participants were diagnosed with DM, as with all other variables there was no link between this and the power produced at LoT, with 2 of these participants in the reserve group and 3 in the no reserve group. However, as with respiratory dysfunctions, the ultimate influence of DM cannot be discounted, with a much larger cohort of participants required to investigate this. While the different implications of comorbidities cannot be isolated in this study, it is the overall symptoms ultimately causing the LoT that are of interest here, and it is those which will be assessed by the exercise protocol used.

The current protocol, as in the rest of this thesis, does not utilise external stimulation techniques and therefore, is reliant on participants performing a maximal voluntary effort throughout. This may be considered of even greater relevance in a patient population not accustomed to maximal exercise. However, the utility and reliability of maximal exercise tests have been shown in many clinical populations, including CHF (Myers *et al.,* 2015; Keteyian *et al.,* 2016). In addition, the repeatability of the maximal efforts in this study (CoV 7.2 %) and the fact that the protocol was well tolerated in both the CHF patients in this study and COPD patients previously (Cannon *et al.,* 2016) allows good confidence in the measures collected. For additional discussion regarding the voluntary effort component of the protocols used, see section 3.4.6.

While participants were split in this study depending on whether or not the power produced at LoT exceeded the natural fluctuations in power, there were some individuals within this fluctuation who could be considered to have a power

reserve. The variation in responses is clear from Figure 5.4 and suggests that it is not one symptom causing LoT in participants, but rather differing contributions of fatigue and sensations more proximal to this, on an individual basis. The cut-off in this study was purposely conservative, due to the large fluctuations in power, small sample size and need to be confident in any participant displaying a power reserve. Even by using these conservative cut-offs, we have still been able to demonstrate some individuals for which the exercise was not primarily limited by the working muscles ability to generate power.

While the utility of this power reserve at LoT remains unknown, and whether the maximal power produced could be maintained much longer than the 6 second effort is unlikely, the data presented here provides the important distinction of whether task failure occurred when the skeletal muscle can no longer produce the power required by the task or not. One potential way for investigating this further is the use of specific interventions, aimed at reducing symptoms in CHF, on the maximal power generating capacity at LoT and moreover, how any changes in maximal power translate to improvements in overall exercise tolerance. Whether interventions such as spinal injections of fentanyl, that have been shown to reduce dyspnoea in COPD (Gagnon *et al.,* 2012), allows participants for whom symptoms proximal to the exercising muscle are the primary limitation, to access some of their reserve in power generating capacity and thus, improve exercise tolerance, remains unknown. Further discussion of potential interventions that could be implemented in future work can be found in Chapter 6.

5.4.5 Conclusions

The current study displays the variation in primary symptoms limiting exercise in CHF. We have found that at the LoT in our small patient population, around half were characterised by an inability to produce the power required by the task, while the other half could produce a power significantly greater than required, suggesting their primary limitation to continuation of exercise originates proximal to the exercising muscles, such as sensations of dyspnoea. If information can be gained about the primary symptom limiting exercise, on an individual basis, this may provide opportunities for individualised treatment that may ultimately lead to greater improvements in exercise tolerance and quality of life in CHF.

Chapter 6 General Discussion

The overall aim of this thesis was to investigate the origin of exercise intolerance and how this is altered depending on the task being performed and the presence of chronic heart failure (CHF). Importantly, the focus was on what limits the continuation of whole-body dynamic exercise in which $\dot{V}O_{2max}$ is attained, as opposed to exercise in smaller muscle groups (e.g. knee extension) or exercise of a lower intensity. The primary method used throughout the work presented was short bouts of maximal isokinetic cycling before and immediately at LoT, employed to determine if task failure occurs when the skeletal muscle can no longer produce the power required by the task. The findings of these studies help further our understanding of the mechanisms that ultimately cause intolerance in whole-body exercise, so that hopefully future studies can be implemented that target these, and as a consequence improve exercise tolerance.

6.1 Summary of key findings

In the first study, both the power required at LoT and the duration of exercise was altered by changing the incrementation rate of a standard ramp-incremental exercise test. Maximal voluntary power output before and immediately at task failure of each exercise test was assessed using short bouts of isokinetic cycling. At LoT of the standard duration (10-12 min) 25 $W.min^{-1}$ test, maximal isokinetic power (P_{ISO}) was not different from the power required at task failure, corroborating previous research (Coelho *et al.,* 2015; Ferguson *et al.,* 2016b). Similarly, when the incrementation rate was increased to 50 $W.min^{-1}$, no reserve in power generating capacity was found. Together this suggests that in both of these protocols, an inability of the skeletal muscles to produce the power

required was ultimately limiting the continuation of exercise. Moreover, it seems as if in these protocols the physiological and perceptual limits remain coupled at task failure. However, when the incrementation rate was reduced to 10 $W.min^{-1}$ and as a consequence power required at LoT reduced and the exercise duration increased, the LoT P_{ISO} was significantly greater (43 ± 22 %) than the task requirement. This reserve in power generating capacity suggests task failure occurred when the skeletal muscle was still capable of producing the power required and therefore, the primary limitation to continuation of exercise originates elsewhere. Contrary to RI25 and RI50 it seems as if the physiological and perceptual limits during RI10 are dissociated, with LoT occurring due to increased contributions of central fatigue mechanisms, possibly as a consequence of increased afferent feedback to the CNS from the exercising muscles and/or the work of breathing. Together the findings from the first experimental chapter suggest that the dynamics of the exercise task have implications for the contributions of central and peripheral mechanisms ultimately causing the LoT, exemplifying the acute plasticity of fatigue in the presence of different exercise tasks.

Findings from the first study informed the development of the second experimental chapter presented here. The first study was designed to build upon recent research that has utilised this isokinetic methodology in ramp-incremental exercise, by altering the ramp-incrementation rate only. However, ramp-incremental exercise itself has some potential limitations. Primarily, there isn't control over the rate of energy utilisation, and therefore the development of fatigue is complicated by the fact the test spans the entire intensity domain construct. Additionally, the influence of muscle inefficiency, shown to be linked to peripheral fatigue (Keir *et al.,* 2016) and manifest as the $\dot{V}O_2$ slow component

($\dot{V}O_2sc$), is difficult to determine in ramp-incremental exercise as the magnitude of the $\dot{V}O_2sc$ while present, is somewhat obscured (Rossiter, 2011). The power-tolerable duration (P-T_{lim}) relationship of supra-CP exercise is a robust, well characterised mathematical construct that describes how there is a fixed amount of work that can be done above CP (defined by the constant W'), independent of changes in supra-CP power output (Poole *et al.,* 1988), addressing the issues with incremental exercise. This second study utilised the P-T_{lim} construct, and the same isokinetic cycling methodology to further investigate how changing the exercise task altered the maximal voluntary power capacity at LoT. Similarly to chapter 3, reducing the power output and as a consequence reducing the rate of W' utilisation, and increasing the exercise duration, caused an increasingly greater reserve in LoT P_{ISO}. Tightly controlling the work done during exercise, this study further strengthens the impact of the exercise task on the mechanisms ultimately limiting exercise. It seems that when the power demand is lower, the energy utilisation rate reduced and thus the exercise duration increased, there is an increasing contribution of central fatigue mechanisms to overall fatigue, potentially as a consequence of increased afferent feedback from both ventilation and the working muscles (Amann *et al.,* 2009; Hureau *et al.,* 2016), causing this reserve in power at LoT.

Additionally, this second study was used to investigate how the kinetics of fatigue during two further power outputs, by interleaving an extra two isokinetic efforts within these protocols. It was found that during the first 2/3^{rds} of exercise, independent of rate of W' utilisation, the reduction in P_{ISO} fell as a generally linear function. However, during the final 3rd of exercise, while significant fatigue continued to develop with the higher power demand, when W' utilisation was slowed, the LoT was attained without further significant reductions in P_{ISO}.

Therefore, the kinetics of the fall in maximal power, were similar to the suggested kinetics of W'. However, during the final stages of exercise a dissociation between the physiological and perceptual limits can occur, resulting in task failure being attained with a significant skeletal muscle power reserve.

In the final study, the same isokinetic methodology was implemented during ramp-incremental exercise in a group of CHF patients. Importantly, the protocol was well tolerated and provided reproducible measurements, indicating the potential for wider applications. It was found that at LoT, while some individuals produced a P_{ISO} not different from the task requirement, others were able to produce a significantly greater $P_{ISO.}$ This differing in response between individuals seems to suggest that the mechanism ultimately limiting exercise in CHF is patient specific, with some individuals limited by the skeletal muscles ability to generate power, whereas others are limited by other more proximal mechanisms, such as sensations of dyspnoea.

6.2 Methodological considerations

The isokinetic cycling method has clear benefits over traditional neuromuscular assessment; namely the ability to perform whole-body dynamic exercise during the fatigue assessment and to perform these measurements immediately at LoT without switching ergometers, avoiding the consequent delay that can allow for recovery (Froyd *et al.,* 2013; Coelho *et al.,* 2015). However, there are limitations associated with this protocol, discussed both here and throughout this thesis.

The overarching limitation is the reliance on volitional effort throughout all phases of the task, particularly the maximal LoT isokinetic efforts. While we believe this adds to the simplicity and utility of the protocol, clearly a lack of effort from the participant during the fatiguing task, followed by a maximal effort during the post-

exercise measurements has the potential to induce a false positive response in which a power reserve is observed, leading to misinterpretation of the data. The contrasting benefits of external stimulation (removing volitional effort) and isokinetic cycling fatigue assessments, raises the possibility of using these methods in conjunction. Moving forward, while no ergometer that allows external stimulation with force measurements during whole-body dynamic exercise currently exists, a protocol that involves performing both an isokinetic effort at LoT and then switching ergometers to perform traditional neuromuscular assessments may provide additionally information about fatigue not currently available, such as the relationship between single-joint and whole-body power output. Additionally, the correlation between these two separate fatigue measurements taken only minutes apart, would be of particular interest, potentially providing additional validation of the whole-body isokinetic cycling methodology.

While the protocol developed by Sidhu *et al.* (2012) has the potential for instantaneous stimulation at LoT, crucially force measurements are not possible at this time. An additional confounding factor is that the external stimulation can only assess fatigue in one muscle group, the knee-extensors. This is in contrast to the whole-body exercise task, during which significant muscle groups besides the knee-extensors will be contributing to the power being produced (Ericson *et al.,* 1986). To perform true external stimulation during dynamic exercise, the exercising muscle groups would need to be stimulated in series, contracting and relaxing at the correct time and for the correct duration to induce natural (yet maximal) locomotion. This technique has been used with spinal cord injury patients at sub-maximal stimulation intensities to induce a cycling motion (e.g. Fornusek and Davis, 2008), however clearly the safety concerns and potential

for injury with maximal stimulation in healthy individuals means this kind of protocol is currently unachievable.

Another benefit of the methods used here is the non-invasive approach to fatigue characterisation. However, this in itself has potential limitations, with more direct invasive approaches available that may have provided additional data. In the current research, while the potential importance of the work of breathing is discussed with regards to fatigue, the work of breathing was estimated from pulmonary gas exchange variables. More direct measurements of respiratory muscle fatigue using oesophageal and gastric pressures can be made, and are seemingly well tolerated, even in clinical populations (e.g. Vogiatzis *et al.,* 2011). Alternatively, and less invasively, in-exercise flow-volume loops can provide information about changes in breathing mechanics and the overall work of breathing (e.g. Guenette *et al.,* 2007). The data that could be gained about the work of breathing, and how this changes during different tasks and individuals from either of these two methodologies, could provide a better indication of the importance of respiratory fatigue to exercise intolerance. Interventions to alter this work of breathing, which may provide additional insights to the importance of this mechanism are discussed below (section 6.3).

Similarly, non-invasive surface EMG was used during the studies presented here. While the use of surface EMG remains controversial, with authors disagreeing about its level of utility and specificity, especially during dynamic contractions (Farina, 2008; von Tscharner and Nigg, 2008), it is still considered a useful tool to measure levels of muscle activation and alterations that may occur as a consequence of fatigue (Enoka and Duchateau, 2011). While some specific surface EMG limitations such as the effects of daily variations in skin

temperature, conductance, hydration and preparation were accounted for by normalising the EMG signal (discussed in section 2.6.3), perhaps the principal limitation to the current work and the understanding of fatigue, is the peripheral nature of surface EMG. Reductions in the amplitude of EMG during dynamic exercise gives no insight as to where, proximal to the neuromuscular junction, this fatigue has originated (Coelho *et al.,* 2015). When measured during isometric contractions, and coupled with external stimulation of the motor cortex, insights can be drawn as to the spinal or supraspinal nature of fatigue, although these methodologies come with their own limitations (discussed throughout, e.g. section 1.6.2). Finally, fine wire EMG is a more direct alternative to surface EMG, where electrodes are attached within the muscle of interest, rather than relying on signals from the skins surface where cross-talk from the surrounding muscles may or may not influence signals (Solomonow *et al.,* 1994). However, the benefits of fine wire have only really been reported in deeper muscles, where surface signals are harder to isolate. As long as the anatomical positions were carefully adhered to and care was taken in the placement of electrodes, the signals reported from surface EMG are general reported to be similar to the invasive fine wire methodology (Jacobson *et al.,* 1995). Therefore, this invasive method may not have actually provided us with any greater accuracy over the surface EMG signals.

While all of these methods would provide additional valuable data that may enhance further our insights to fatigue, for the reasons discussed at length, it was not possible to include these measurements at this time, keeping the current protocol simple and easy to implement.

6.3 Implications and future directions

The work in this thesis has demonstrated the acute plasticity of fatigue, in that changing the exercise task seems to alter the primary mechanism causing task failure. Reducing the power demand resulted in an increased exercise duration and ultimately, the LoT being attained with a reserve in maximal isokinetic power. The presence of a power reserve is similar to the response observed in COPD patients (Cannon *et al.,* 2016), suggesting that in these exercise protocols the origins of the limitation to exercise has been pushed from the skeletal muscle (during standard duration exercise), to mechanisms that originate more centrally. Additionally, this shows the potentially important role of work of breathing and respiratory fatigue to increasing the overall perception of the exercise, contributing to the dissociation between the physiological and perceptual limits in these specific exercise tasks, similar to COPD.

This has implications in that, by choosing a certain power output for exercise, this may be predetermining an individuals performance during the task depending on their primary exercise limitation. For example, in a COPD population where the primary limitation is dyspnoea (Cannon *et al.,* 2016), higher power and shorter duration exercise (such as a fast ramp-incrementation rate) may give the best chance for optimal performance, as the limitation should be pushed more to the periphery, away from the respiratory system.

Additionally, there is potential to use this interaction between the individual and the exercise task to help our understanding of different fatigue mechanisms. Artificially altering the development of different fatigue mechanisms and measuring the effect of this on the maximal power capacity at LoT could play a significant role in improving understanding, by manipulating fatigue symptoms in

an attempt to access the skeletal muscle power reserve. Accordingly, interventions designed to influence the development of fatigue during exercise should be a primary goal of future studies using this isokinetic methodology. Studies aimed at enabling participants to “access” any power reserve that is present and continue exercise, increasing the contributions of peripheral fatigue, until the skeletal muscle can no longer produce the power required, would be of significant interest. While the greatest benefits for this may be in centrally limited participants, initially healthy participants performing lower power long duration exercise, that display a similar response as the more centrally limited patients, would provide a suitable participant group.

There are a number of interventions that have been shown to reduce one of the primary central mechanisms, sensations of dyspnoea, that may be appropriate. Breathing supplementary oxygen or a mixture of oxygen and helium (Heliox) during maximal exercise has been shown to reduce the overall work of breathing and improve exercise performance in athletes (Wilkie *et al.,* 2015), although this is not conclusive (Ansley *et al.,* 2007). In COPD it has been shown that breathing this less dense gas has a beneficial impact, reducing the work of breathing, sensations of dyspnoea and improving exercise performance both acutely and with training (Dean *et al.,* 1992; Emtner *et al.,* 2003). However, the benefits are not limited to the respiratory system, the increased presence of oxygen has also been shown to increase oxygen delivery to the working muscles (Vogiatzis *et al.,* 2011; Louvaris *et al.,* 2012). Consequently, as there will also be improvements in muscle efficiency and reduced muscle fatigue, any improvements in performance cannot be isolated to changes in dyspnoea, potentially confounding this specific intervention.

Another intervention used in the literature is the pharmacological blocking of afferent feedback by fentanyl (e.g. Gagnon *et al.,* 2012). This provides a potentially more specific intervention, without the potential to also effect muscle efficiency during exercise. By blocking afferent feedback, originating from both working muscles and respiratory demand, it is suggested that this attenuates the increased central drive that ultimately causes an increased central fatigue (Amann and Dempsey, 2008). By administering this afferent blockade, it has been shown that the sensations of dyspnoea are reduced, the perception of effort is reduced and exercise performance is increased (Gagnon *et al.,* 2012). Additionally, the reduced feedback and central fatigue allows peripheral fatigue to increase to a greater extent compared with a placebo (Amann *et al.,* 2009). Whether reduced feedback, increased peripheral fatigue, and the continuation of exercise also results in the access to at least some of the power reserve at LoT, is something warranting further investigation.

Rather than reducing central fatigue by attenuating afferent feedback during exercise for which a reserve exists, another option is to attempt to increase the sensations of fatigue to induce a power reserve in protocols for which this is normally not evident (i.e. standard ramp-incremental exercise). Either increasing the resistance of breathing (e.g. Harms *et al.,* 2000) or simply increasing breathing frequency above the “usual” rate response for an individual should increase the work of breathing and subsequently, the afferent stimulus. While this would require compliant participants, with significant training in regulating these unnatural breathing patterns, monitoring of key variables such $\dot{V}_E$ and $PETCO_2$ could be used to confirm and increased dyspnoea. Whether increasing dyspnoeic sensations causes central fatigue to develop to the extent that it

becomes task limiting, and participants reach LoT with a power reserve would add to the body of evidence further.

Finally, it was found in chapter 4 that the skeletal muscle inefficiency manifest as the $\dot{V}O_{2SC}$ may be linked to the task dependant mechanisms ultimately causing LoT. Therefore, interventions aimed at reducing the $\dot{V}O_{2SC}$ in exercise where this is most prominent may be beneficial. While exercise training (Casaburi *et al.,* 1987; Carter *et al.,* 2000) and respiratory muscle training (Bailey *et al.,* 2010) have both been shown to chronically reduce the magnitude of $\dot{V}O_{2SC}$, acute interventions are the primary focus here. Priming exercise, the performance of a pre-exercise “warm-up” bout of at least heavy intensity exercise has been shown to reduce the $\dot{V}O_{2SC}$, a consequence of improved blood flow at exercise onset, delaying the build-up of muscle fatigue (Burnley *et al.,* 2000; Rossiter *et al.,* 2001; Bailey *et al.,* 2009). However, the performance benefits of a reduced $\dot{V}O_{2SC}$ remain contentious with some reporting improved tolerance after priming exercise (Burnley *et al.,* 2005) and others a detrimental effect (Ferguson *et al.,* 2007). This fact alongside the benefits to peripheral mechanisms that may be gained, confound to an extent this intervention for investigating the influence of reducing $\dot{V}O_{2SC}$ on solely central fatigue and the maximal voluntary power at task failure.

While acute interventions are the logical next step for this research in order to try and understand the primary limitations to exercise, it is hoped that in future this could lead to improved, individualised treatment for exercise intolerance in patient groups. Using CHF as an example; if a participant were found to be limited primarily by muscle fatigue, this could be directly targeted using for example; high-intensity interval exercise that has been shown to improve skeletal

muscle oxidative capacity (Burgomaster *et al.,* 2008), and found to be well-tolerated in CHF (Wisloff *et al.,* 2007). Other interventions such as eccentric exercise (e.g. LaStayo *et al.,* 2000) and blood flow restriction (e.g. Patterson and Ferguson, 2011) may also be beneficial, as these limit the need for high-intensity exercise, while still inducing beneficial physiological adaptations such as improved muscle strength and efficiency. Conversely, and perhaps more complex, if mechanisms proximal to the exercising muscles were identified to be limiting, while improvements to skeletal muscle function may be beneficial by reducing the afferent feedback stimulus, interventions that have been shown to reduce sensations such as exercise induced dyspnoea may be more valuable e.g. respiratory muscle training (Mancini *et al.,* 1995; Cahalin and Arena, 2015). Alternatively as discussed, the acute use of fentanyl has successfully reduced dyspnoea and as a consequence improved exercise tolerance in COPD participants (Gagnon *et al.,* 2012). Therefore, investigations into an equivalent yet more chronic intervention, may be another avenue for treatment of more centrally limited participants. Clearly for most participants both these symptoms are acting in combination, to varying degrees, and so this may require a multifaceted treatment programme. However, the ability to determine and then target independent symptoms allows movement away from a “one fits all” approach and towards more individualised medicine, that has the potential to markedly improve exercise tolerance and thus, the quality of life in patient populations.

6.4 Concluding Remarks

The studies presented here have shown how the origin of the fatigue ultimately causing exercise intolerance in whole-body dynamic exercise is plastic and can be altered depending on the task being performed and health status. It is hoped that these studies will add to previous work and help to improve the understanding of what is ultimately causing exercise intolerance. In turn, by improving this understanding, this can inform and help develop better targeted strategies aimed at improving exercise tolerance and as a consequence, quality of life and extending the lifespan.

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