Central and Peripheral Fatigue in Response to the Acute and Chronic Metabolic Demand: Implications for Exercise Tolerance

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Abstract

Fatigue and the associated exercise limitation has wide reaching human performance and health implications, however the mechanisms are poorly understood. Muscle fatigue during exercise originates from a combination of peripheral and central processes, and their interaction, which are influenced by exercise-induced metabolic stress. Developments in non-invasive brain stimulation techniques such as transcranial magnetic stimulation (TMS), have allowed for measurement of central nervous system (CNS) processes alongside measurements of muscle performance and metabolic disturbances, but there are few studies to do so during locomotor exercise. Furthermore, although endurance training is a potent enhancer of exercise tolerance, the effect of training on fatigue mechanisms and the limiting factors to exercise tolerance is poorly understood. The aim of this thesis is to examine the central and peripheral contributions to fatigue in response to the exercise-induced metabolic demand before and after training in order to better understand the integrated physiology of fatigue as well as the mechanisms contributing to fatigue resistance and exercise tolerance.

Study 1 examined the within- and between-day reliability of a number of motor nerve stimulation and single- and paired-pulse TMS (ppTMS) techniques that examine aspects of neuromuscular and corticospinal function which have been implicated in fatigue. The results confirmed that measures of neuromuscular and corticospinal function demonstrate good reliability and provide the first evidence that ppTMS can be reliably measured in a functional locomotor muscle of the knee extensors, however the stimulation parameters should be considered in order to optimise reliability and minimise variability. Study 2 investigated the relationships between corticospinal and neuromuscular function with exercise capacity in order to better understand the peripheral and central factors underpinning exercise tolerance. This study revealed a number of neuromuscular and motor cortical properties related to submaximal

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and maximal exercise capacity which could be indicative of central fatigue resistance. Study 3 examined the central and peripheral contributions to fatigue resulting from non-exhaustive and exhaustive exercise of high and low metabolic stress in order to better understand the integration between peripheral and central mechanisms and how they contribute to exhaustion. This study revealed that high metabolic stress accelerates the development of peripheral and central fatigue, however central fatigue was similar at exhaustion, suggesting this is an important mechanism in exercise termination. Additionally, a number of disturbances in cortical cell function were identified in a manner dependent on the exercise-induced metabolic strain. Study 4 examined the effect of endurance training on high-intensity exercise tolerance and the associated central and peripheral fatigue mechanisms. High-intensity interval training, but not work-matched moderate-intensity continuous endurance training, increased tolerance of exercise that elicited the same metabolic demand as pre-training. Better exercise tolerance was accompanied by a greater tolerance of peripheral fatigue and ischaemic muscle pain, and attenuated central fatigue. These studies provide novel insight to the central and peripheral contributions to fatigue and exercise tolerance, and the associated adaptations to exercise training.

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Contents

Abbreviations

Δ	Delta
0.5RT	Half Relaxation Time
^{31}P-MRS	^{31}P-Magnetic Resonance Spectroscopy
ADP	Adenosine Diphosphate
aMT	Active Motor Threshold
AP	Action Potential
ATP	Adenosine Triphosphate
BF	Biceps Femoris
Ca^{2+}	Calcium
CBF	Cerebral Blood Flow
Cl^-	Chloride
cm	Centimetre
CMEP	Cervicomedullary Motor Evoked Potential
CNS	Central Nervous System
CO_2	Carbon Dioxide
CONT	Continuous Moderate-Intensity Endurance Training
CP	Critical Power
Cr	Creatine
CS	Conditioning Stimulus
cSP	Cortical Silent Period
CT	Time to Peak Torque
d	Days
DHPR	Dihydropyradine Receptors
EEG	Electroencephalography
EMG	Electromyography
ENS	Electrical Nerve Stimulation
ERT	Estimated Resting Twitch
ES	Effect Size
ETC	Electron Transport Chain
FAD^-	Flavin Adenine Dinucleotide
$FADH_2$	Reduced Flavin Adenine Dinucleotide
fMRI	Functional Magnetic Resonance Imagining
GABA	γ-Aminobutyric Acid
G-6-P	Glucose-6-phosphate
h	Hours
H^+	Hydrogen Ions
H_2O	Water
HIIT	High-Intensity Interval Training
HR	Heart Rate
HR_{max}	Maximal Heart Rate
Hz	Hertz
ICF	Intracortical Facilitation
ISI	Interstimulus Interval
ITT	Interpolated Twitch Technique
K^+	Potassium
kcal	Kilocalories
kg	Kilograms
kJ	Kilojoules
l	Litres

[La$^-$]	Blood Lactate Concentration
LDH	Lactate Dehydrogenase
LICI	Long-Interval Intracortical Inhibition
LT	Lactate Threshold
LTP	Lactate Turnpoint
m	Metres
MCT	Monocarboxylate Transporter
MEG	Magnetoencephalography
MEP	Motor Evoked Potential
MHC	Myosin Heavy Chain
min	Minutes
ml	Millilitre
MLSS	Maximal Lactate Steady State
mm	Millimetres
M_{max}	Maximal M-wave Amplitude at Rest
mmol	Millimole
MNS	Magnetic Nerve Stimulation
ms	Milliseconds
mV	Millivolt
MVC	Maximal Voluntary Isometric Contraction
$MVCM_{max}$	Maximal M-wave Amplitude during an MVC
N	Newtons
Na$^+$	Sodium
NAD$^+$	Nicotinamide Adenine Dinucleotide
NADH	Reduced Nicotinamide Adenine Dinucleotide
NIRS	Near-Infrared Spectroscopy
N·m	Newton Metres
NMDA	N-methyl-D-aspartate
O$_2$	Oxygen
P$_a$CO$_2$	Arterial CO$_2$ Tension
P$_a$O$_2$	Arterial O$_2$ Tension
PCr	Phosphocreatine
PDH	Pyruvate Dehydrogenase
PET	Positron Emission Topography
P$_i$	Inorganic Phosphate
PO	Power Output
ppTMS	Paired-pulse Transcranial Magnetic Stimulation
$Q_{tw,pot}$	Potentiated Quadriceps Twitch
$Q_{tw,unpot}$	Unpotentiated Quadriceps Twitch
RCP	Respiratory Compensation Point
RER	Respiratory Exchange Ratio
rms	Root Mean Squared
rmsM	Root Mean Square of Maximal M-wave
rMT	Resting Motor Threshold
RPE	Ratings of Perceived Exertion
rpm	Revolutions Per Minute
RT	Resting Twitch
RTD	Rate of Torque Development
RyR	Ryanodine Receptors
s	Seconds

SD	Standard Deviation
SE	Standard Error
SICI	Short-Interval Intracortical Inhibition
SIT	Superimposed Twitch
SO	Stimulator Output
spTMS	Single-pulse Transcranial Magnetic Stimulation
SR	Sarcoplasmic Reticulum
TES	Transcranial Electrical Stimulation
TMS	Transcranial Magnetic Stimulation
TS	Test Stimulus
TTE	Time to Exhaustion
μV	Microvolt
V	Volts
VA	Voluntary Activation
$\dot{V}CO_2$	Carbon Dioxide Production
$\dot{V}_E$	Minute Ventilation
$\dot{V}O_2$	Oxygen Uptake
$\dot{V}O_{2max}$	Maximal Oxygen Uptake
VL	Vastus Lateralis
VT	Ventilatory Threshold
W	Watts
$\dot{W}_{max}$	Peak Power Output
$\dot{W}\text{-}\dot{V}O_{2max}$	Power Output at Maximal Oxygen Uptake

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Publications and Presentations Arising From This Thesis

Papers

O'Leary, T. J., Morris, M. G., Collett, J. & Howells, K. (2015). Central and peripheral fatigue following non-exhaustive and exhaustive exercise of disparate metabolic demands. *Scan J Med Sci Sport (in press).* [Chapter 5]

O'Leary, T. J., Morris, M. G., Collett, J. & Howells, K. (2015). Reliability of single and paired-pulse transcranial magnetic stimulation in the vastus lateralis. *Muscle Nerve, 52,* 605-615. [Chapter 3]

Abstracts

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O'Leary, T. J., Morris, M. G. & Collett, J. (2014). High but not moderate intensity fatiguing cycling increases short-interval intracortical inhibition. *J Sports Sci, 32,* s5. [Chapter 5]

O'Leary, T. J., Morris, M. G. & Collett, J. (2014). The reliability of magnetic stimulation for measurement of neuromuscular function in the knee extensors. *J Sports Sci, 32,* s82. [Chapter 3]

Other Presentations

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Chapter 1 - Introduction

1.1 Summary

The following Chapter will provide an introduction to the background of fatigue and aims of this thesis. Section 1.3 will clarify current fatigue definitions and fatigue mechanisms are discussed in section 1.4.

1.2 Introduction

The exercise limitation imposed by, and mechanisms of, exercise–induced fatigue has been an area of interest across basic, clinical and applied science for its far reaching implications in human health and performance. Fatigue is a major limitation to exercise performance (Hargreaves, 2008, McKenna and Hargreaves, 2008) and a symptom of a number of clinical disorders that are also accompanied by poor exercise tolerance (Kent-Braun et al., 2012). Exercise tolerance is an important predictor of all-cause mortality (Myers et al., 2002) and a fundamental requirement for normal human function in order to be able to complete activities of daily living and sustain quality of life (McKenna and Hargreaves, 2008). Fatigue also profoundly affects healthy individuals by impairing performance in physical occupational duties and restricting exercise completed for recreation or health (McKenna and Hargreaves, 2008). The mechanisms of fatigue are poorly understood, and alongside advancing our fundamental understanding of basic human function and physiology (Hargreaves, 2008, Kent-Braun et al., 2012), a better understanding of fatigue is essential for improving physical work tolerance, overall human function and health, quality of life and longevity.

Global muscle fatigue is an exercise-induced reversible loss in the maximal force producing capacity of the muscle and occurs immediately from the onset of exercise until the task can no

longer be sustained (Gandevia, 2001). Fatigue can occur anywhere along the motor pathway from brain to muscle and has mechanisms characterised as either peripheral or central in origin; peripheral fatigue occurs within the muscle at sites distal to the neuromuscular junction whilst central fatigue reflects the inability of the central nervous system (CNS) to activate the muscle and occurs proximal to the neuromuscular junction (Gandevia, 2001). The contributions from peripheral and central mechanisms are task and intensity specific (Enoka and Duchateau, 2008, Taylor and Gandevia, 2008) but likely involve a number of physiological systems mediated through a complex integration of peripheral and central factors (Nybo and Secher, 2004, Meeusen et al., 2006, Hargreaves, 2008, McKenna and Hargreaves, 2008, Amann, 2011, Kent-Braun et al., 2012).

There is growing recognition of the importance of central mechanisms of fatigue in limiting exercise tolerance (Davis and Bailey, 1997, Nybo and Secher, 2004, Abbiss and Laursen, 2005, Hargreaves, 2008, Amann, 2011, Sidhu et al., 2013a), however these mechanisms are poorly understood. Recent developments in neuroimaging techniques (e.g. functional magnetic resonance imaging (fMRI)) allow assessment of CNS processes involved in fatigue. However, the use of these techniques is restricted due to imposed limitations to the experimental environments; participants are required to restrict motion which confines the exercise protocol, and the procedures are difficult and time-consuming to administer. In order to develop understanding of central fatigue, techniques that are non-invasive, do not restrict exercise and are easily and rapidly administered are required. As such a number of electrophysiological studies combining non-invasive techniques such as electromyography (EMG), motor nerve stimulation and brain stimulation techniques such as transcranial magnetic stimulation (TMS) have been employed to better understand fatigue (Ross et al., 2007, Sidhu et al., 2009b, Goodall et al., 2012).

TMS can provide definitive evidence of changes in the corticospinal pathways involved in fatigue following locomotor exercise (Sidhu et al., 2013a). The motor cortex is responsible for the generation of motor drive during exercise and measuring changes in motor cortex function with TMS is key to understanding central fatigue (Ament and Verkerke, 2009, Sidhu et al., 2013a). A number of single- and paired-pulse TMS techniques have been developed to help better understand these central changes. The muscle responses to single-pulse TMS (spTMS) allow assessment of corticospinal excitability and cortical voluntary activation (Goodall et al., 2014a) whereas paired-pulse TMS (ppTMS) allows the inhibitory and excitatory cortical cells that modulate motor cortex output to be studied (Kujirai et al., 1993). These techniques have identified that fatigue is accompanied by: i) a fall in motor cortex output, and ii) complex changes in corticospinal function (Gandevia, 2001, Sidhu et al., 2013a). Although TMS has shown to have important applications in fatigue research (Gruet et al., 2013, Sidhu et al., 2013a, Goodall et al., 2014a), many studies have used isolated models of muscle fatigue and the potential of these techniques to examine the mechanisms of endurance exercise fatigue is not fully realised (Sidhu et al., 2013a). ppTMS could be instrumental in understanding corticospinal function and central fatigue (Pereira and Keller, 2012) and a better understanding of cortical responses to locomotor exercise is key in understanding central fatigue (Sidhu et al., 2013a, Sidhu et al., 2013c):

'The responsiveness of the cortical cells might be an important constraint on how the CNS regulates locomotor exercise....it would lead to a better understanding of the sites and mechanism of changes in the CNS in response to a bout of whole-body exercise. It would also broadly contribute to the debate on what causes people to interrupt a whole-body dynamic activity....'   Sidhu et al. (2013a) p.444-445

It has been proposed that the mechanisms of central fatigue involve inhibitory group III/IV afferent feedback from the muscle in response to peripheral metabolic stress in order to preserve a metabolic reserve (Gandevia, 2001, Taylor and Gandevia, 2008, Amann, 2011, Sidhu et al., 2013a) and that trained individuals are better able to tolerate the physiological and sensory effects of these afferents and access this reserve (Nybo and Secher, 2004, Mauger, 2013, Jones et al., 2014, Zghal et al., 2015). The exercise-induced metabolic disturbance thus contributes to fatigue within the muscle and CNS, and studies exploring how metabolic and peripheral fatigue processes influence the cortical circuits and motor cortical output are required (Sidhu et al., 2013a). A better understanding of fatigue therefore requires an integration of neurophysiological, central motor drive, metabolic, and skeletal muscle measurements (Hargreaves, 2008, Kent-Braun et al., 2012, Sidhu et al., 2013a) alongside appropriate prescription of exercise intensity to control the metabolic strain (Burnley and Jones, 2008). However, how the exercise-induced metabolic stress contributes to peripheral and central fatigue processes is poorly understood. Whilst exercise training is a potent enhancer of fatigue resistance, the effect of training status on fatigue mechanisms is also not well understood. Although anecdotally trained individuals are considered to be able to 'push' themselves harder than untrained counterparts and better tolerate unpleasant afferent signals (i.e. metabolic strain/muscle pain/muscle fatigue), there is little evidence to support this notion. Whilst fatigue and pain are inextricably linked (Mauger, 2013), better understanding of the fatigue processes and/or the ability to tolerate sensory disturbance (i.e. muscle pain and muscle fatigue) in response to training could be important for better understanding exercise tolerance and adherence as an individual moves from the untrained to the trained state.

The aim of this thesis is to investigate mechanisms of fatigue following locomotor exercise of differing metabolic stress and following endurance training. The reliability of a range of

stimulation techniques that measure peripheral and central fatigue were investigated in Chapter 3 and how the function of these peripheral and central systems relates to exercise capacity (Chapter 4) and respond to exercise-induced metabolic stress before (Chapter 5) and after training (Chapter 6) were subsequently investigated.

1.3 Defining Fatigue

Fatigue is a term that has been used to describe a number of psychological, cognitive and physiological processes as well as functional outcomes (Zwarts et al., 2008). For example in disease, fatigue is a symptom distinct from the fatigue-induced by exercise, although the mechanisms may be related (Davis and Bailey, 1997). For the remainder of this thesis exercise-induced fatigue will be discussed. Exercise-induced fatigue is also a term that has been used interchangeably to describe separate phenomena, such as a conscious sensation or rise in perception of effort, decrement in muscle performance or an inability to continue sustained activity (Enoka and Duchateau, 2008). This latter definition that fatigue occurs at a set point i.e. task-failure implies that the 'point of fatigue' occurs suddenly and only begins at exhaustion. This definition is incompatible with the physiological mechanisms and perceived sensations of fatigue that occur from the onset of activity. Thus fatigue occurs regardless of whether the task can be sustained. For the purpose of this thesis muscle fatigue is defined as:

'any exercise-induced reversible loss in the ability to exert muscle force whether or not the task can be sustained.'   Gandevia (2001) p. 1732

Muscle fatigue is therefore an objective, quantifiable and developing phenomenon that can be measured at any time-point. In comparison, exhaustion is a distinct event and describes task-failure (Gandevia, 2001), with exercise tolerance the duration over which exercise can be

maintained before exhaustion. Fatigue also has a sensory component which must be considered due to its profound impact on exercise tolerance (Enoka and Stuart, 1992).

1.4 Mechanisms of Fatigue

Before fatigue during locomotor exercise can be understood, the underlying mechanisms must be first considered. Muscle fatigue occurs from the onset of exercise up until exhaustion (Gandevia, 2001). Despite extensive research, the mechanisms are poorly understood and widely debated. It is almost impossible to identify the exact cause of exhaustion (Barry and Enoka, 2007), therefore the study of fatigue should focus on any mechanism that contributes to performance impairment (Enoka and Stuart, 1992). Fatigue can occur anywhere along the motor pathway, from motor cortex output to muscle contractile performance (Figure 1.1), and has mechanisms characterised as either peripheral or central; peripheral fatigue occurs at sites distal to the neuromuscular junction (i.e. within the muscle) whilst central fatigue reflects the inability of the CNS to activate the muscle and occurs proximally to the neuromuscular junction (Gandevia, 2001). The whole motor pathway must be studied as it is likely that many systems fail when stressed as it would be wasteful to over-engineer any individual part of the chain (Jones, 2010). The contributions from peripheral and central mechanisms are task and intensity specific (Enoka and Duchateau, 2008, Taylor and Gandevia, 2008) but likely involve a complex interaction (Nybo and Secher, 2004, Meeusen et al., 2006, McKenna and Hargreaves, 2008, Hargreaves, 2008, Amann, 2011, Kent-Braun et al., 2012).

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Figure 1.1. Possible sites of fatigue (Boyas and Guevel, 2011).

1) activation of motor cortex; 2) spinal propagation from CNS to motoneuron; 3) activation of motor units and muscle; 4) neuromuscular propagation; 5) excitation-contraction coupling; 6) substrate availability; 7) state of intracellular medium; 8) performance of contractile apparatus; 9) blood flow.

1.4.1 Peripheral Fatigue

Peripheral fatigue refers to processes distal to the neuromuscular junction (4–9, Figure 1.1) that impair muscle contractile performance. The following section will provide a brief overview of potential mechanisms broadly categorised as processes relating to energy supply (metabolic) and contractile function (muscular).

1.4.1.1 Metabolic

Muscle Bioenergetics

Adenosine triphosphate (ATP) hydrolysis (Equation 1.1) provides the energy required by the working muscles. Intramuscular ATP stores are limited, ~25 mmol·kg^{-1}·dm^{-1} in the vastus lateralis (VL) (Bogdanis et al., 1995, Bogdanis et al., 1996), and so must be continually resynthesised from adenosine diphosphate (ADP) and inorganic phosphate (P_i). Impairment in the ability to supply ATP will compromise muscle performance. Despite high rates of hydrolysis, muscle ATP stores are well maintained during submaximal exercise (Sahlin et al., 1998) but may decrease ~30% during maximal exercise (Bogdanis et al., 1995, Bogdanis et al., 1996). Modest reductions in muscle ATP are therefore not a contributing factor to fatigue. The pathways through which ATP is resynthesised: i) ATP-phosphocreatine (ATP-PCr) system, ii) anaerobic glycolysis and, iii) oxidative metabolism, differ in terms of their rate of ATP resynthesis (power) and amount of ATP that can be resynthesised (capacity) each presenting individual limitations to exercise. The ATP-PCr system and anaerobic glycolysis take place in the cytosol of the cell and do not require oxygen (O_2) whereas oxidative metabolism takes place in the mitochondria and involves reactions requiring O_2.

$$ATP + H_2O \xrightarrow{\textit{ATPase}} ADP + P_i + H^+$$
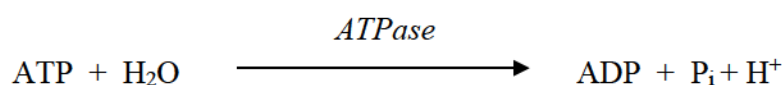
Equation 1.1. Hydrolysis of ATP by the enzyme ATPase.

The ATP-PCr system rapidly resynthesises ATP from the breakdown of PCr (Equation 1.2). The capacity of this system is sufficient for ~10 s maximal exercise and is limited by intramuscular PCr stores (70-80 $mmol \cdot kg \cdot dm^{-1}$) which can be near depletion following maximal cycling and align to the muscle power output profile (Bogdanis et al., 1995, Bogdanis et al., 1996). Muscle PCr stores therefore limit the capacity of this energy system and contribute to fatigue during very high-intensity exercise.

$$PCr \ + \ ADP \ + \ H^+ \quad \xrightarrow{\textit{Creatine Kinase}} \quad ATP \ + \ Cr$$
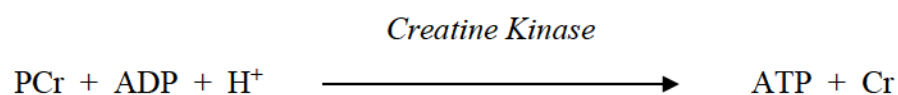
Equation 1.2. Resynthesis of ATP by the ATP-PCr system.

Albeit at a slower rate than the ATP-PCr system, anaerobic glycolysis generates ATP quickly by converting glucose or glycogen to pyruvate (Figure 1.2) and is an important source of ATP resynthesis during high-intensity exercise. The hydrogen ions (H^+) produced from ATP hydrolysis and glycolysis are received by nicotinamide adenine dinucleotide (NAD^+) in conversion to its reduced form (NADH). As the cell contains limited NAD^+, a mechanism for regeneration is required for glycolysis to continue. When ATP demand is matched by mitochondrial ATP resynthesis, NADH can transfer H^+ to the electron transport chain (ETC). However, when the glycolytic flux is high, pyruvate is generated faster than it can enter oxidative metabolism and the production of NADH exceeds the maximum rate at which NAD^+ can be regenerated. A key step in the maintenance of cell equilibrium is the conversion of pyruvate to lactate in a reaction which consumes a H^+ and regenerates NAD^+ to maintain the cell redox potential (Robergs et al., 2004). Muscle H^+ production that overcomes the capacity of mitochondrial uptake results in intracellular accumulation and a drop in muscle pH (Robergs

et al., 2004), inhibition of glycolysis (Sahlin et al., 1998) and potential implications in the manifestation of other peripheral and central fatigue processes.

Glucose ———————————— Glycogen

Glucose-6-phosphate

NAD^+

NADH

Pyruvate

Lactate Dehydrogenase

Pyruvate Dehydrogenase

NADH

NAD^+

NAD^+

NADH

Lactate

Acetyl-CoA + CO_2
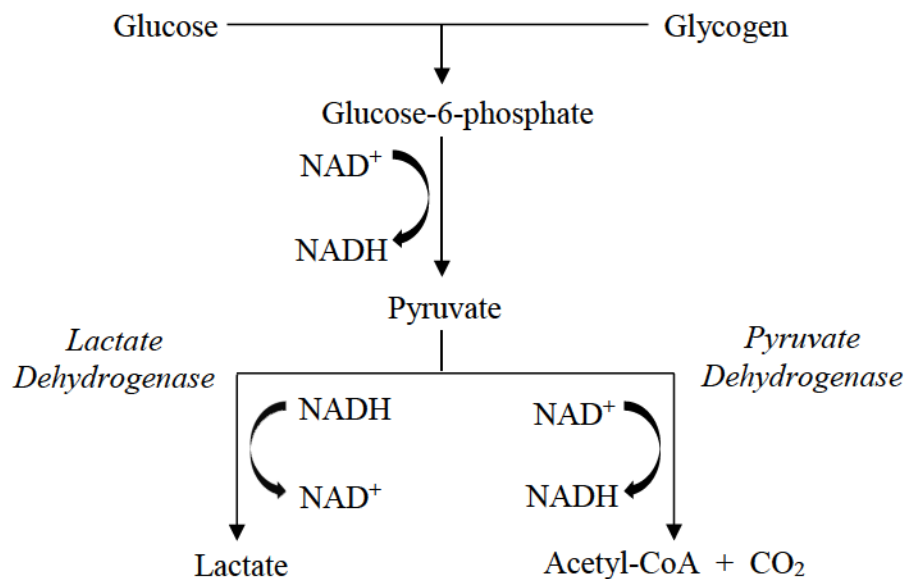
Figure 1.2. Key steps of glycolysis.

Although the power of oxidative metabolism is lower than anaerobic pathways, the capacity is far greater. Oxidative metabolism involves the breakdown of predominantly carbohydrate and fats through the Kreb's cycle and then ETC to be oxidised to carbon dioxide (CO_2) and water (H_2O). Pyruvate and fat are converted to acetyl-CoA which then enters the Kreb's cycle to be oxidised to CO_2, forming H^+ in the process. The H^+ are taken by NAD^+, forming NADH, and flavin adenine dinucleotide (FAD), forming $FADH_2$, which then enter the ETC. Once the H^+ enter the ETC, electrons are extracted which eventually pass to O_2 to form H_2O. The transfer of the electrons through the ETC causes H^+ to be pumped across the inner mitochondrial membrane resynthesising ATP in the process. The major determinant of muscle fatigability is the density of mitochondria and therefore potential for oxidative metabolism (Allen et al., 2008b). Endurance training is a rapid and potent improver of muscle aerobic enzyme level and activity as well as mitochondria content and function (Holloszy and Coyle, 1984, Bishop et al., 2014). Increased mitochondrial capacity and higher sensitivity to ADP results in less oxidative

demand per mitochondria, a tighter coupling between ATP demand and oxidative supply, less disturbance in cytosolic phosphorylation potential and a greater coupling between pyruvate oxidation and glycolytic flux (Holloszy and Coyle, 1984). Improvements in muscle oxidative capacity are considered key for increased fatigue resistance and exercise tolerance following endurance training (Holloszy and Coyle, 1984).

Substrate Availability

The capacity of oxidative metabolism is limited by supply of substrate. Muscle glycogen depletion has been associated with exhaustion during prolonged exercise (Bergstrom et al., 1967), however this relationship is poorly understood and likely not causal (Sahlin et al., 1998, Allen et al., 2008b). Muscle glycogen content at exhaustion is highly variable but never depleted (Abbiss and Laursen, 2005, Rauch et al., 2005), time-trial end-spurts are produced when glycogen is lowest (Rauch et al., 2005) and low glycogen does not impair Kreb's cycle activity (Baldwin et al., 2003). Low glycogen increases perception of effort (Noakes, 2004), structurally impairs contractile function (Ortenblad et al., 2013) and may disturb neurotransmission due to increases in fat oxidation (Meeusen et al., 2006) and ammonia production (Nybo et al., 2005) or reductions in cerebral fuel availability (Watson, 2008).

Oxygen Delivery

Maximal oxygen uptake ($\dot{V}O_{2max}$) reflects the capacity to take up, transport and utilise O_2 and sets the upper limit to the power of oxidative metabolism. The capacity of muscle O_2 use outweighs the O_2 delivering capacity of the cardiovascular system with a cardiac limitation to $\dot{V}O_{2max}$ (Bassett and Howley, 2000). Blunted O_2 delivery accelerates the rate of peripheral and central fatigue development during high-intensity exercise (Amann and Calbet, 2008). Insufficient O_2 supply to the muscle impairs mitochondrial aerobic metabolism and increases

the accumulation of metabolites associated with anaerobic metabolism (Amann and Calbet, 2008, Kent-Braun et al., 2012). Endurance training increases left ventricular size, myocardial contractility and end diastolic volume leading to a larger ejection fraction, stroke volume and maximal cardiac output; $\dot{V}O_{2max}$ is increased enhancing the ability to deliver O_2 from the atmosphere to the muscle mitochondria (Blomqvist and Saltin, 1983, Jones and Carter, 2000, Bassett and Howley, 2000), which is considered a major factor in the improved fatigue resistance during high-intensity exercise (Abbiss and Laursen, 2005).

1.4.1.2 Muscular

Mechanism of Contraction

Once excited by the initiation of an action potential (AP), fatigue could occur anywhere along the chain of events that lead to contraction (Figure 1.3). The AP is transmitted along the membrane of the muscle fibres and conducted down the transverse (T-) tubules. Voltage-sensor dihydropyridine receptors (DHPR) in the T-tubules change their 'charge movement' causing the opening of adjacent ryanodine receptors (RyR) in the sarcoplasmic reticulum (SR) and subsequent calcium (Ca^{2+}) release. The SR-Ca^{2+} release increases myoplasmic Ca^{2+} concentrations which bind with troponin C instigating movement of tropomyosin allowing actin and myosin cross-bridge formation. The myosin heads are first weakly and then strongly bound to the actin filaments which then undergo the power stroke. The cycle ends with relaxation once Ca^{2+} is removed from the myoplasm by SR-Ca^{2+} reuptake pumps.

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Figure 1.3. Sequence of the excitation-contraction coupling and possible sites and causes of muscle fatigue (Allen et al., 2008a, Allen et al., 2008b).

AP SL, sarcolemma action potential; AP TT, T-tubule action potential; CaP$_{i,ppt}$, calcium phosphate precipitation in the sarcoplasmic reticulum; $[Ca^{2+}]_{myo}$, myoplsmic calcium concentration; $[Ca^{2+}]_{SR}$, sarcoplasmic reticulum calcium concentration; $[K^+]_{ext}$, extracellular potassium concentration; $[Na^+]_{int}$, intracellular sodium concentration; $[P_i]_{myo}$, myoplsmic inorganic phosphate concentration; $[P_i]_{SR}$, sarcoplasmic reticulum inorganic phosphate concentration; SR Ca^{2+} RC / RyR1, sarcoplasmic-calcium release channels or ryanodine receptors; VS / DHPR, voltage-sensitive dihydropyradine receptors.

Sodium (Na$^+$) and Potassium (K$^+$)

In order to trigger adequate SR-Ca^{2+} release, the AP must be propagated through the T-tubular system which depends on sarcolemma membrane potentials mediated by sodium (Na$^+$) and potassium (K$^+$) exchange. Resting gradients are tightly regulated by the Na$^+$-K$^+$-pump with the depolarisation and repolarisation phases of each AP occurring through the passive influx of Na$^+$ followed by the efflux of K$^+$ via their respective channels. During exercise, each repeated AP causes small levels of K$^+$ to leave the cell (Allen et al., 2008b) and interstitial K$^+$ increases and intracellular K$^+$ decreases (McKenna et al., 2008). Extracellular Na$^+$ may also decrease but there are marked increases in intracellular Na$^+$ (McKenna et al., 2008). The extracellular increase in K$^+$ causes depolarisation of sarcolemma and t-tubule membranes and inactivates Na$^+$ channels, reducing excitability which could reduce force production (Allen et al., 2008a, McKenna et al., 2008). Reduced Na$^+$-K$^+$-ATPase activity may also contribute to reduced ability to maintain K$^+$ equilibrium (Leppik et al., 2004). However, the magnitude of membrane depolarisation is considerably less than would be expected considering the change in K$^+$ concentrations and any minor AP impairment is still sufficient to trigger adequate SR-Ca^{2+} release (Allen et al., 2008a, Allen et al., 2008b, McKenna et al., 2008). It is likely that Cl$^-$ movements and Na$^+$-K$^+$-pump activity assist in maintaining membrane potential and repolarisation, particularly in the T-tubules, avoiding K$^+$ accumulation (Allen et al., 2008a, McKenna et al., 2008). A reduction in neural stimulation rate required for tetani also serves to minimise the required number of APs and K$^+$ efflux (Allen et al., 2008a, Allen et al., 2008b). A reduction in muscle pH helps maintain excitability by reducing Cl$^-$ conductance in T-tubules

(Pedersen et al., 2004). Endurance training upregulates the activity and content of Na^+-K^+ pumps allowing better K^+ clearance and improved homeostatic control of muscle cell excitability (Nielsen et al., 2004).

Lactate, Hydrogen (H^+) and pH

The accumulation of lactic acid has historically been viewed as a major cause of muscle fatigue. Upon production, lactic acid dissociates into lactate and H^+. Maximal exercise can result in a ~30 fold increase in muscle lactate concentrations which is tightly coupled to decreases in muscle pH (Gaitanos et al., 1993, Bogdanis et al., 1995). An increase in lactate was therefore originally considered to cause muscle acidosis whereas lactate actually alkalises the muscle cell by receiving H^+ (Robergs et al., 2004). Although produced together, they are now known to have separate physiological effects. Lactate is a precursor to gluconeogenesis, metabolic cell signaller and fuel source during exercise being oxidised either within muscle, heart or brain (Brooks, 2009, van Hall, 2010). Thus lactate accumulation has no effect on muscle fatigue (Allen et al., 2008b). The acidosis is instead caused by H^+ accumulation when production exceeds the rate of mitochondrial uptake (Robergs et al., 2004). The H^+ production necessitates intracellular buffering (via proteins, small peptides, bicarbonate, free phosphate) and intracellular removal via a number of membrane transporters (Juel, 2008, Thomas et al., 2012). Monocarboxylate transporters (MCT1 and MCT4) mediate the majority of H^+ efflux from muscle in a bidirectional 1:1 ratio with lactate, with other proteins in the sarcolemmal membrane (Na^+/H^+ and Na^+/bicarbonate transporters) complementing the actions of the MCTs (Juel, 2008, Thomas et al., 2012).

Normal muscle pH is ~7.05 but can decrease by up to ~0.5 with extreme exercise (Bogdanis et al., 1995, Bogdanis et al., 1996, Kent-Braun, 1999). Data on animal muscle fibres show that

low pH impairs contractile force and velocity, however the effect is less pronounced when completed closer to physiological temperatures ($\geq 30°C$) (Pate et al., 1995, Westerblad et al., 1997). In human muscle there is a strong relationship between decline in muscle pH and force (Kent-Braun, 1999, Westerblad et al., 2002) or power output (Gaitanos et al., 1993). However, the recovery of muscle performance is quicker than that of muscle pH (Bogdanis et al., 1995) and pH and muscle force can be dissociated (Bangsbo et al., 1996). Muscle acidity has even been demonstrated to have a positive effect on muscle force by preserving membrane excitability (Pedersen et al., 2004). The effect of H^+ and the associated drop in muscle pH is therefore not considered the main cause of peripheral fatigue (Sahlin et al., 1998, Allen et al., 2008b) but may play a role in other sensory and central fatigue processes.

Whilst the effect of pH on fatigue is controversial, *in vitro* (intracellular) buffer capacity is correlated with 40 km cycling time-trial performance (Weston et al., 1997) whereas *in vivo* buffer capacity is correlated with fatigue resistance during sprint exercise (Bishop et al., 2004). High-intensity cycling time to exhaustion (TTE) also correlates with the ability to extrude H^+ from the muscle by numerous transporters (Messonnier et al., 2007). *In vitro* buffer capacity (Weston et al., 1997, Edge et al., 2006) and muscle content of MCT1, MCT4, Na^+/H^+ and Na^+/bicarbonate transporters (Juel, 2008, Thomas et al., 2012) increase in response to endurance training. These adaptations result in improved lactate kinetics (shuttling and clearance) (Brooks, 2009) and improved intracellular control of H^+ and pH (Juel, 2008, Thomas et al., 2012).

Calcium (Ca^{2+}) Release and Uptake and Inorganic Phosphate (P_i)
There are two important factors related to Ca^{2+} kinetics and muscle fatigue: whether there is sufficient SR-Ca^{2+} release and uptake to cause tetani and relaxation, and the sensitivity of the

contractile apparatus to Ca^{2+}. Impaired SR-Ca^{2+} release is considered a key component in muscle fatigue although there is no consensus on the cause (Allen et al., 2008a). High rates of ATP hydrolysis impair the activation of SR-Ca^{2+} channel opening directly through reductions in cellular ATP and increases in free magnesium, ADP and adenosine monophosphate (Allen et al., 2008a, Allen et al., 2008b). Degradation of intramuscular glycogen also impairs SR-Ca^{2+} release either through a metabolic or structural role (Ortenblad et al., 2013). A reduced fatigue index in response to sprint training is accompanied by an increase in SR-Ca^{2+} release due to increased SR volume (Ortenblad et al., 2000), whereas endurance training results in increased efficiency (slowing) of Ca^{2+} kinetics (Green et al., 2003).

A major cause of impaired SR-Ca^{2+} release is an increase in intramuscular P_i. Muscle P_i accumulation is considered to be a major contributor to fatigue with wide spread effects on contractile function (Westerblad et al., 2002, Allen and Trajanovska, 2012). Resting muscle P_i levels are ~3 $mmol \cdot kg \cdot dm^{-1}$ but can increase to ~20 $mmol \cdot kg \cdot dm^{-1}$ with maximal exercise (Bogdanis et al., 1995, Bogdanis et al., 1996). The main source of P_i is from the hydrolysis of ATP (Equation 1.1) which accumulates when P_i production exceeds mitochondrial P_i uptake (Robergs et al., 2004). In humans there are strong relationships between muscle P_i and decline in force (Allen and Trajanovska, 2012). P_i impairs SR-Ca^{2+} release through directly inhibiting the RyR and by reducing the available Ca^{2+} for release by Ca^{2+}-P_i precipitation in the SR; the SR is P_i permeable which allows equilibrium between myoplasmic and SR P_i, forming calcium phosphate in the process and reducing available Ca^{2+} (Westerblad et al., 2002, Allen et al., 2008a, Allen et al., 2008b). P_i also directly impairs cross-bridge peak force, inhibits high force cross-bridge formation and reduces myofibrillar Ca^{2+} sensitivity (Allen et al., 2008a, Fitts, 2008, Allen and Trajanovska, 2012).

1.4.2 Central Fatigue

Central fatigue is a progressive reduction in voluntary activation of muscle and occurs proximal to the neuromuscular junction, encompassing a number of spinal and supraspinal processes involved in the generation of muscle activation (1–3, Figure 1.1; Figure 1.4) (Gandevia, 2001). Central fatigue is now understood to contribute significantly to the decline in muscle performance (Gandevia, 2001, Taylor and Gandevia, 2008) and during a submaximal task such as locomotor exercise, exhaustion may not be caused by failure of the principle muscles involved (Enoka and Duchateau, 2008). Central fatigue has therefore been considered a key contributor to locomotor exercise tolerance (Davis and Bailey, 1997, Kayser, 2003, Abbiss and Laursen, 2005, Hargreaves, 2008, McKenna and Hargreaves, 2008, Amann, 2011, Decorte et al., 2012, Sidhu et al., 2013a).

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Figure 1.4. Chain of motor command during voluntary muscle activation. Fatigue can occur anywhere from the brain to muscle pathway (Gandevia, 2001).

Central motor drive during exercise is the sum of many CNS centres acting on the motor cortex (Ament and Verkerke, 2009). The mechanisms of central fatigue are poorly understood but can originate from impairments in the circuits that generate motor cortical output or circuits within the motor cortex (Gandevia et al., 1996, Sogaard et al., 2006, Smith et al., 2007, Sidhu et al., 2009b, Sidhu et al., 2012a, Sidhu et al., 2013c), alterations to spinal cord reflexes (Racinais et al., 2007, Klass et al., 2008) and intrinsic motoneuron properties (McNeil et al., 2011a, McNeil et al., 2011b, Williams et al., 2014). The sub-optimal output from the motor cortex as measured by TMS is termed supraspinal fatigue (Gandevia, 2001), part of which could be a result of inhibitory and excitatory process in the motor cortex that modulate cortical output (Taylor and

Gandevia, 2008, Ranieri and Di Lazzaro, 2012). The recent introduction of TMS in locomotor exercise studies has provided new insight into central fatigue (Ross et al., 2007, Sidhu et al., 2009b, Ross et al., 2010b, Goodall et al., 2012, Sidhu et al., 2012a, Sidhu et al., 2013c, Temesi et al., 2013, Thomas et al., 2015a) and findings from these studies are considered in sections 2.3.2 and 2.3.3. Other central factors such as arousal, lethargy, motivation, mood (Davis and Bailey, 1997), sensations of fatigue and pain (St Clair Gibson et al., 2003, Nybo and Secher, 2004, Ament and Verkerke, 2009, Mauger, 2013) and perception of effort (Marcora and Staiano, 2010), whilst distinct from direct measurement of voluntary activation of the muscle, must also be considered given their potential capacity to reduce the level of CNS drive (Barry and Enoka, 2007). Central fatigue has also been considered as a safety mechanism manifesting as a reduction in motor unit recruitment to preserve whole-body homeostasis and maintain a physiological reserve as described by the central governor model (St Clair Gibson and Noakes, 2004, Noakes et al., 2005). Locomotor exercise induces disturbances to CNS function that influence motor output and sensations of effort and fatigue; these processes are considered in sections 2.3.4 - 2.3.6.

Chapter 2 – Literature Review

2.1 Summary

The following review will provide a synopsis of the literature pertaining to mechanisms of exercise-induced fatigue as they relate to locomotor exercise. Fatigue measurement techniques are discussed in sections 2.2. The development of fatigue in response to locomotor exercise is considered in section 2.3. Finally, a critique of methods used to prescribe exercise intensity to investigate fatigue is provided in section 2.4.

2.2 Measurement of Fatigue

Numerous measurement techniques have been developed to assess the processes implicated in fatigue. These techniques are targeted at identifying processes at the level of the muscle or within the CNS (i.e. peripheral or central fatigue). Muscle fatigue is an exercise-induced impairment in force generating capacity and quantified by the reduction in force produced from a maximal voluntary contraction (MVC) (Gandevia, 2001). However, an impairment in MVC torque is a measure of global muscle fatigue as it depends on the contractile performance of the muscle as well as descending drive from the CNS (Gandevia, 2001) and therefore provides no information regarding site of impairment. The aim of the following section is to briefly consider the techniques that measure both peripheral and central fatigue.

2.2.1 Peripheral Fatigue

Human muscle can be studied through direct sampling from a biopsy or using non-invasive techniques such as near-infrared spectroscopy (NIRS), ^{31}P magnetic resonance spectroscopy (^{31}P-MRS) and EMG. The muscle biopsy technique involves the removal of a small sample of muscle for analysis whereas NIRS, ^{31}P-MRS and EMG assess muscle oxygenation (and blood flow), muscle metabolism and muscle electrical activity, respectively. The muscle biopsy technique allows single fibres to be examined, and therefore distinctions between fibre types, and a high level of experimental control so intramuscular changes can be studied in isolation. Biopsy techniques have consequently identified important muscle metabolic, ionic and contractile processes implicated in fatigue during locomotor exercise (Bergstrom et al., 1967, Coyle et al., 1988, Bogdanis et al., 1995, Leppik et al., 2004, Ortenblad et al., 2013). However, the biopsy technique is invasive, the volume of muscle sampled is small and assumes that the findings represent the entire muscle, and the analysis is *in vitro* absent of other key *in vivo* physiological processes.

Whilst techniques such as NIRS, ^{31}P-MRS and EMG also only measure a small sample of muscle, they help combat some of these issues by allowing non-invasive assessment of *in vivo* muscle processes during exercise. ^{31}P-MRS measures high-energy phosphates and pH by placing the muscle in a high magnetic field. Due to technical restraints induced by the equipment, only isolated muscle exercise can be employed, i.e. single leg knee extensions (Jones et al., 2008). NIRS involves passing near-infrared light through muscle in order to determine muscle oxygenation and blood flow. The muscle NIRS signal is therefore severely affected by the thickness of the subcutaneous layer below the optodes and is subject to other issues such as large movement artefacts. Signal quantification is also problematic due to an inability to measure absolute values and only relative changes can be quantified. As such ^{31}P-

MRS and muscle NIRS have restricted applications to locomotor exercise. Surface EMG allows assessment of muscle electrical activity and therefore an assessment of muscle fibre recruitment, although the signal can be affected by factors such as electrode placement, subcutaneous fat and muscle properties, position and length (Farina et al., 2004). The EMG electrodes do not impair ongoing activity, however, the voluntary EMG signal is generated from processes along the pathway from cortical firing to the muscle membrane and must be combined with other stimulation techniques that can help discern central and peripheral contributions (Allen et al., 2008b). Whilst data generated from these non-invasive techniques is useful in examining processes that contribute to the impairment in muscle performance, the data is associative and collected from a small sample of muscle. Therefore, these techniques do not provide direct evidence of impairment in muscle performance across an entire muscle group which means the relevance to fatigue (decline in contractile performance) is unclear. As such, muscle stimulation techniques that directly assess changes in muscle performance in the absence of CNS input are required and a number of motor nerve stimulation techniques (section 2.2.3) have been employed to assess locomotor exercise fatigue (section 2.3.1). Muscle stimulation techniques offer the advantage of directly quantifying peripheral fatigue, are non-invasive, quick and easy to administer, do not impede ongoing activity and allow simultaneous assessment of peripheral and central fatigue.

2.2.2 Central Fatigue

Measurement of central fatigue processes is difficult due to inaccessibility of the CNS. Theories about the role of the CNS in the development of fatigue, or as a limiting factor to exercise tolerance, have been based upon theoretical models derived from indirect observations of performance, pacing or perceptual responses to exercise (St Clair Gibson and Noakes, 2004, Noakes et al., 2005) or pharmacological attempts to manipulate performance (Meeusen et al.,

2006, Roelands and Meeusen, 2010). A major limitation of research to date is a lack of data demonstrating direct changes in CNS function.

A number of brain imaging techniques, such as electroencephalography (EEG), magnetoencephalography (MEG), positron emission topography (PET), NIRS and fMRI have been developed that allow non-invasive assessment of CNS processes during fatigue (Tanaka and Watanabe, 2012). These techniques vary in their spatial and temporal resolution and their use is restricted due to imposed limitations to the experimental environments; participants are required to restrict motion which confines the exercise protocol that can be employed, and the procedures are difficult and time-consuming to administer. Therefore, when using imaging techniques such as PET, MEG and fMRI, fatigue is induced by contraction of the limb or hand (Tanaka and Watanabe, 2012). Processes such as EEG and NIRS help to solve this problem by providing portable solutions, however the signal quality is impaired by movement and they require cumbersome equipment to be worn by the participant which interferes with the exercise. Therefore, in order to develop understanding of central fatigue, techniques that are non-invasive, do not restrict exercise and are easily and rapidly administered are required. As such a number of electrophysiological studies combining EMG, motor nerve stimulation and TMS have been employed to better understand central fatigue (sections 2.3.1 – 2.3.3.). TMS can provide definitive evidence of changes in the corticospinal pathways involved in fatigue following locomotor exercise (Sidhu et al., 2013a). These techniques were therefore employed to measure neuromuscular fatigue throughout this research.

2.2.3 Neuromuscular Fatigue

The gold standard measurement of fatigue is the intact, perfused muscle under central control (Allen et al., 2008b) and quantified by a reduction in MVC (Vollestad, 1997). Studying fatigue

in the intact organism with multiple complimentary techniques that examine the entirety of the integrated system responsible for generating muscle force is crucial to understanding human muscle fatigue (Kent-Braun et al., 2012). Therefore, it is important to measure impairments along the motor cortex to muscle pathway. As measurement techniques such as EMG only provide associative physiological data, other stimulation techniques are required to identify impairments in the chain of muscle activation (Vollestad, 1997). Non-invasive muscle and brain stimulation techniques have therefore been developed as tools for assessing neuromuscular function, offering key insights into fatigue.

2.2.3.1   Motor Nerve Stimulation

Supramaximal stimulation of the motor nerve using electrical nerve stimulation (ENS) or magnetic nerve stimulation (MNS) can be used to elicit a muscle response in order to examine neuromuscular function and determine central and peripheral components of fatigue (Millet et al., 2011). The resultant contractile torque and electrophysiological responses provide key insights into fatigue.

Evoked Twitch Parameters

Motor nerve stimulation applied at rest elicits a muscle twitch and allows assessment of muscle contractile performance. By recording the peak torque produced by the muscle twitch peripheral fatigue can be quantified (Amann, 2011, Millet et al., 2011). When elicited after an MVC, the twitch is potentiated which provides a more sensitive indicator of peripheral fatigue than the unpotentiated twitch (Kufel et al., 2002). A number of characteristics from the muscle twitch can be examined to quantify muscle rates of shortening (rate of torque development, RTD; contraction time, CT) and relaxation (rate of relaxation, RR; half relaxation time, 0.5RT). The reduction in RTD is indicative of an inhibition of the transition in cross-bridge attachment

from low to high force states whereas a prolonged CT reflects prolonged duration of the intracellular Ca^{2+} transients (Fitts, 2008, Kent-Braun et al., 2012). The slowing of contractile relaxation with fatigue represents a slowing of cross-bridge detachment rates and decrease in maximum rate of weak to strong cross-bridge binding (Jones et al., 2009) although impaired $SR-Ca^{2+}$ uptake could contribute (Allen et al., 2008a, Kent-Braun et al., 2012). Due to the distinct contractile properties of different fibre types, the contractile characteristics can also provide information regarding muscle fibre type (Hamada et al., 2000b). The evoked muscle twitch is accompanied by a compound muscle action potential (M-wave), recorded using EMG, and represents a measure of muscle membrane excitability (Lepers et al., 2002).

Peripheral Voluntary Activation

Voluntary activation (VA) of the muscle is defined as the ability to recruit the motor units voluntarily through descending neural drive from the motor cortex (Gandevia, 2001, Taylor, 2009). Merton (1954) was the first to apply ENS at the motor nerve to investigate whether during an MVC all the abductor pollicis muscle fibres could be voluntarily recruited using the interpolated twitch technique (ITT). An external stimulus applied to the motor nerve during an MVC elicits a superimposed twitch (SIT) by activating motor units not recruited or not recruited at maximum firing rates (Merton, 1954, Shield and Zhou, 2004). Based on the inverse linear relationship between the size of the SIT and the level of voluntary force, the size of the SIT on the MVC can be expressed relative to a resting twitch (RT) to determine the level of VA (Merton, 1954). The RT is elicited after the MVC so as to be as equally potentiated as the SIT (Shield and Zhou, 2004). The SIT is consequently inversely proportional to the level of VA, although this relationship may be curvilinear due to greater co-activation of synergists and antagonists at higher forces or their inadvertent stimulation, small increases in muscle length, antidromic collision or failure to detect small twitches with maximum efforts (Shield and Zhou,

2004, Taylor, 2009). In the literature a reduction in VA in response to exercise is widely used to determine the level of central fatigue, indicating fatigue at or above the neuromuscular junction (Gandevia, 2001). However, other stimulation techniques such as TMS are required to better understand processes above the neuromuscular junction.

2.2.3.2 Transcranial Magnetic Stimulation

TMS is a non-invasive stimulation technique used to excite neural tissue such as the cerebral cortex (Kobayashi and Pascual-Leone, 2003). Since its development by Barker et al. (1985), TMS has been shown to be an important tool in understanding human CNS physiology (Hallett, 2000), central mechanisms of muscle fatigue in isolated contractions (Gandevia, 2001, Taylor and Gandevia, 2008) and more recently in locomotor endurance exercise (Sidhu et al., 2009b, Sidhu et al., 2013a). TMS is based on Faraday's principle of electromagnetic induction, where a rapidly changing magnetic field induces a flow of electric current in a nearby conductor, including human neural tissue. When applied to TMS, the discharge of a capacitor delivers a brief high-powered electric current through a coil of wire to produce a rapidly changing magnetic pulse which induces electric current of opposite direction in underlying neural tissue. The lines of magnetic flux occur perpendicular to the magnetic coil, which is placed tangentially to the scalp, with the electric current then produced perpendicular to the magnetic field and parallel to the magnetic coil (Figure 2.1, Hallett (2000)). The magnetic pulses reach the brain with little attenuation which results in an electrical current sufficient to excite the motor cortex (Figure 2.2) and the corticospinal neurons (Kobayashi and Pascual-Leone, 2003).
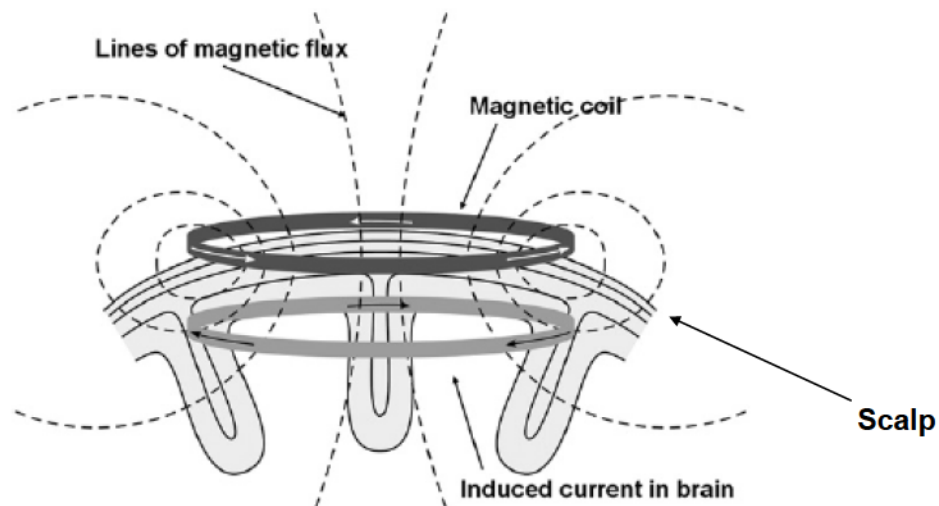
Figure 2.1. Direction of current flow in the magnetic coil and brain (Hallett, 2000) (reproduced with permission).

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Figure 2.2. Homunculus of the primary motor cortex by Penfield and Rasmussen (1950) cited in Goodall et al. (2014a).

The induced current activates superficial cortical layers beneath the scalp altering membrane potential causing an AP and muscle response. As the induced current within the cortex is horizontal, the horizontally lying cortical cells are preferentially activated rather than direct activation of vertical corticospinal neurons (Rothwell et al., 1991). TMS can activate the pyramidal cells *directly* (D-wave) via their axons, but predominantly activates the corticospinal neurons *indirectly* (I-wave) through their excitatory trans-synaptic inputs, with only a little (if any) involvement from a D-wave with non-preferential coil orientations or at high intensities of stimulation (Figure 2.3) (Di Lazzaro et al., 2004, Hallett, 2007, Groppa et al., 2012). Multiple I-waves at intervals of ~1.5 ms (I1, I2, I3, etc.) originate in the motor cortex which project onto the corticospinal neurons through monosynaptic excitatory cortico-cortical interneurons with the interval between I-waves due to activation of increasing long polysynaptic networks or recurrent synaptic networks (Di Lazzaro et al., 2004, Hallett, 2007,

Groppa et al., 2012). A number of TMS techniques have been developed to examine cortical activation of muscle (cortical VA) as well as excitability of corticospinal outputs to muscle.
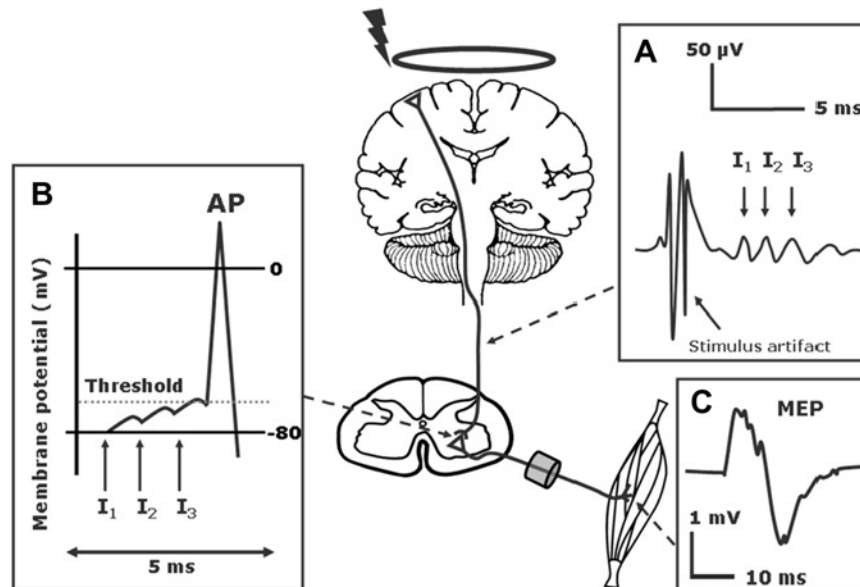
Figure 2.3. Mechanisms leading to the generation of the muscle response to TMS (Groppa et al., 2012) (reproduced with permission). A, I-waves from spinal epidural recordings; B, contribution of I-waves to depolarisation of the spinal motoneurons; C, muscle response recorded by EMG.

Cortical Voluntary Activation

Stimulation of the motor cortex during a sustained MVC evokes muscle twitches that grow in size with the development of fatigue, highlighting that motor cortex output becomes sub-optimal (Gandevia et al., 1996). From this work grew a technique that allows measurement of VA from the motor cortex; originally developed in the elbow flexors (Todd et al., 2003, Todd et al., 2004), this technique has now been validated in the knee extensors (Goodall et al., 2009, Sidhu et al., 2009a). Unlike motor nerve stimulation, the relationship between voluntary force and the SIT from TMS is more complicated. The reduced motoneuron and motor cortex excitability at rest and lower contraction intensities results in a reduced SIT compared with that

expected from extrapolation at higher intensities; it is therefore necessary to estimate the resting twitch (ERT) by linear extrapolation from the SIT at contraction intensities ≥50% MVC (Todd et al., 2003). Cortical VA allows a quantitative measure of output form motor cortex to muscle, and has been used to examine supraspinal fatigue following locomotor exercise (section 2.3.2).

Motor Evoked Potential

TMS evokes a short-latency contralateral muscle response that can be recorded using EMG termed a motor evoked potential (MEP). A number of characteristics can be derived from the MEP to reveal information about the corticospinal tract. The size of the MEP, usually expressed as peak-to-peak amplitude (Rossini et al., 2015), reflects the excitability of the corticospinal pathway (motor cortex, corticospinal tract, spinal cord, motoneurons as well as peripheral transmission to the muscle) (Gandevia, 2001, Kobayashi and Pascual-Leone, 2003). Therefore for in order to interpret MEP changes with fatigue, the MEP should be expressed relative to M_{max} to control for muscle excitability (Gruet et al., 2013).

Cortical Silent Period

When the motor cortex is stimulated during a muscle contraction, a ~200 ms period of near silence in the EMG succeeds the MEP, referred to as the cortical silent period (cSP). The cSP reflects an interruption of voluntary drive (Inghilleri et al., 1993). Whilst the first part of the cSP (≤100 ms) is thought to reflect spinal inhibition, the later part is a measure of γ-Aminobutyric acid type B (GABA$_B$) mediated cortical inhibition (Inghilleri et al., 1993, Chen et al., 1999, Werhahn et al., 1999). Administration of GABA reuptake inhibitors lengthens the cSP (Werhahn et al., 1999). The cSP is often used as a measure of intracortical inhibition in response to muscle fatigue (Taylor et al., 1996).

Principles of Paired-Pulse TMS

The final motor cortex output is the net result of the intracortical inhibitory and excitatory influences and their interaction (Figure 2.4) (Chen, 2004, Chen et al., 2008, Ni et al., 2011). These inhibitory and excitatory circuits can be studied using ppTMS (Valls-Sole et al., 1992, Kujirai et al., 1993) which may be instrumental in understanding CNS changes in motor output with fatigue (Pereira and Keller, 2012). ppTMS involves the discharge of two pulses through the same coil with the first and second pulses referred to as the conditioning stimulus (CS) and test stimulus (TS), respectively. The MEP generated by the CS and TS applied together is compared to the MEP when the TS is administered alone. When the ratio is <1.0 the CS has inhibited the TS and activated inhibitory circuits, whereas a ratio >1.0 indicates the TS is facilitated by the CS and facilitatory circuits have been activated. Whether the intracortical inhibitory or facilitatory circuits are recruited, depends on the intensity of the CS and TS and the interstimulus interval (ISI) (Valls-Sole et al., 1992, Kujirai et al., 1993). Two inhibitory (short-interval intracortial inhibition, SICI; long-interval intracortical inhibition, LICI), and one excitatory circuit (intracortical facilitation, ICF) are most commonly used.

Short-Interval Intracortical Inhibition

When a subthreshold CS is applied before a suprathreshold TS with an ISI of 1-6 ms, the CS inhibits the TS (Kujirai et al., 1993). As the CS does not inhibit a response to transcranial electrical stimulation (TES) or the H-reflex (Kujirai et al., 1993), or evoke descending activity in the spinal cord (Di Lazzaro et al., 1998), the inhibition occurs within the motor cortex. Administration of γ-Aminobutyric acid type A (GABA$_A$) receptor agonists increase SICI (Ilic et al., 2002, Di Lazzaro et al., 2005), therefore SICI reflects the excitability of GABA$_A$-

mediated intracortical inhibitory neurones (Chen et al., 2008). These synaptic mechanisms are relevant to the ISI of 2–5 ms as SICI at 1 ms appears to be mediated by axonal refractoriness (Fisher et al., 2002, Roshan et al., 2003). SICI is reduced with increasing muscle activation (Ridding et al., 1995, Zoghi et al., 2003, Ortu et al., 2008) in order to modulate excitatory corticospinal drive to the muscle (Floeter and Rothwell, 1999, Reynolds and Ashby, 1999).

Long-Interval Intracortical Inhibition

When both the CS and TS are supramaximal and separated by an ISI of 50–200 ms, the CS inhibits the TS and is referred to as LICI (Valls-Sole et al., 1992, Wassermann et al., 1996). Administration a $GABA_B$ receptor agonist, enhances LICI (McDonnell et al., 2006) and therefore it is likely that LICI reflects the activity of the $GABA_B$-mediated intracortical inhibitory neurons (Sanger et al., 2001, Chen, 2004). However, there is recent evidence that spinal mechanisms could be involved (McNeil et al., 2009). Whilst $GABA_A$ acts post-synaptically, $GABA_B$ acts both pre- and post-synaptically and can pre-synaptically inhibit $GABA_A$ at the cortical level (Figure 2.4) (Sanger et al., 2001, Ni et al., 2011).

Intracortical Facilitation

When a subthreshold CS is applied before a suprathreshold TS at ISI of 8-30 ms, the CS facilitates the TS (Kujirai et al., 1993, Ridding et al., 1995, Chen et al., 1998). ICF is reduced by N-methyl-D-aspartate (NMDA) receptor antagonists (Ziemann et al., 1998) and although far less studied than inhibitory measures, it is thought ICF reflects the activity of glutamate-mediated excitatory neurons at a cortical level (Chen, 2004, Chen et al., 2008). The threshold of the ICF neurons are higher than SICI and so often a higher CS may be used (Ziemann et al., 1996, Ni et al., 2007). ICF is reduced with muscle activation and although the mechanisms are

unknown it may be due to contamination of the ICF measurement with the reduction in SICI (Ridding et al., 1995, Ortu et al., 2008).
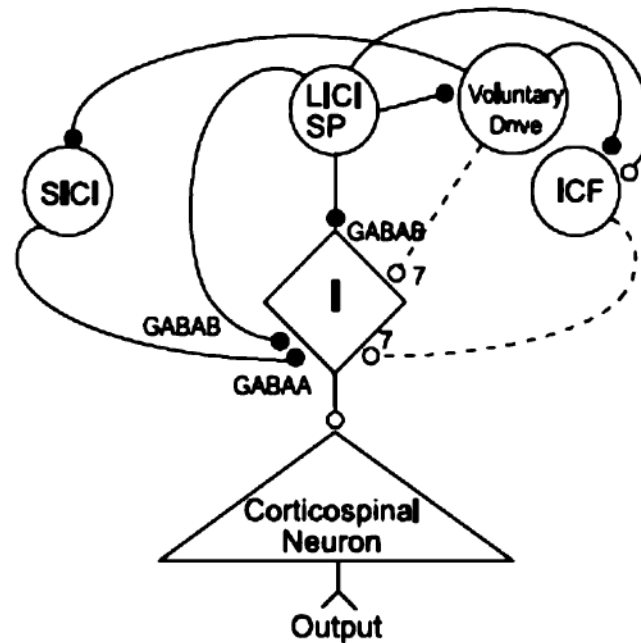
Figure 2.4. Interactions between SICI, LICI and ICF on motor cortex output (proposed by Sanger et al. (2001) and adapted by Ni et al. (2007)) (reproduced with permission).

I, I-waves generated by TMS; filled circles, inhibitory effects; empty circles, excitatory effects.

2.3   Fatigue Mechanisms Limiting Locomotor Exercise

Whilst *in vitro* studies have been invaluable in isolating cellular processes that impair contractile performance (Allen et al., 2008b, Kent-Braun et al., 2012), they pose obvious limitations by failing to view the whole organism, resulting in a fragmented view of fatigue. Investigations into isolated contractions, often in small muscles, have also proved useful in examining impairments to muscle performance (Taylor and Gandevia, 2008), however this mode of exercise has limited application to whole-body exercise where the intramuscular, metabolic, systemic, perceptual and CNS feedback mechanisms are different (Sidhu et al., 2013a). Whole-body endurance exercise investigations require the need for an integrative look

at multiple systems of fatigue. To gain insight into the mechanisms limiting locomotor exercise tolerance, neuromuscular and corticospinal function, and perception of effort have been studied.

2.3.1 Neuromuscular Fatigue

Locomotor exercise of various intensities and durations induces both peripheral and central fatigue as measured by assessment of neuromuscular function with motor nerve stimulation (Tables 2.1 and 2.2). The contribution of peripheral and central mechanisms is exercise mode specific. For example, running induces more central fatigue than cycling due to muscle damage (Millet and Lepers, 2004). However, less is known how intensity or duration of exercise plays a role in the development of fatigue; peripheral and central fatigue are evident after prolonged low-intensity ($\geq$60 min) (Lepers et al., 2002, Martin et al., 2010, Ross et al., 2010a, Jubeau et al., 2014) and shorter duration high-intensity (<60 min) (Lattier et al., 2004, Theurel and Lepers, 2008, Decorte et al., 2012, Goodall et al., 2015b) cycling and running.

A few studies have examined the evolution of fatigue throughout exercise, permitting insight into the development of fatigue as the exercise progresses towards high perceptual strain and exhaustion. Both prolonged (Lepers et al., 2002, Jubeau et al., 2014) and high-intensity intermittent cycling (Decorte et al., 2012) induce peripheral fatigue that contributes to the fatigue early in the exercise bout whereas central fatigue occurs towards the cessation of exercise or exhaustion. Lepers et al. (2002) reported a reduction in quadriceps twitch torque ($Q_{tw,pot}$) after 1 h of a 5 h cycle (55% $\dot{W}_{max}$) which stabilised over time whereas peripheral VA was only reduced at 5 h. Jubeau et al. (2014) reported similar findings with measurements every 80 min during 4 h cycling (45% $\dot{W}_{max}$). The 'late' development of central fatigue has also been demonstrated during prolonged running even in the absence of peripheral fatigue (20 km time-trial) (Ross et al., 2010a) or even with enhanced muscle contractility post-exercise (5 h at 55%

$\dot{V}_{max}$) (Place et al., 2004). Decorte et al. (2012) measured neuromuscular function between bouts of intense cycling completed until exhaustion. Peripheral fatigue was evident after 20% of the total exercise time with no further fatigue from 50% of the time completed. In contrast central fatigue was only evident at 80% of total exercise time and at exhaustion, leading to the suggestion that central processes play an important role in exhaustion. In support of this, when the exercise is maximal intensity, significant peripheral and central fatigue are evident after 2 of 12 maximal 30 m running sprints with minimal decline thereafter (Goodall et al., 2015b).

Similar to isolated contractions (Taylor and Gandevia, 2008), prolonged endurance exercise has been associated with greater central fatigue compared to higher intensity efforts which are more peripherally dominated (Millet and Lepers, 2004). Whilst central fatigue seems exercise duration dependent, and strong relationships have been demonstrated between loss in peripheral VA and MVC after prolonged exercise (>60 min), often with moderate or no peripheral fatigue (Millet et al., 2003, Place et al., 2004, Ross et al., 2010a), recent evidence indicates that the higher the intensity of contraction the greater the rate of peripheral and central fatigue development (Burnley et al., 2012). Therefore, it appears that both duration and intensity are important, however this has yet to be fully addressed during locomotor exercise.

Greater central and peripheral fatigue has been seen after variable intensity (lower intensity bouts interspersed with high-intensity bouts) compared with work-matched constant intensity cycling (Theurel and Lepers, 2008). The greater peripheral fatigue is likely be induced by the greater metabolic disturbance as post-exercise [La$^-$] have shown to correlate with peripheral impairment (Sidhu et al., 2009b, Thomas et al., 2015a) which may also cause the greater central fatigue (Theurel and Lepers, 2008) (section 2.3.6). In contrast, Thomas et al. (2015a) reported that peripheral fatigue was greater and central fatigue attenuated following a 4 km (~6 min)

versus a 20 km (~32 min) and 40 km (~66 min) cycling time-trial. These intensities were all still relatively high with the mean recorded intensities at 87-96% $\dot{V}O_{2max}$ producing mean blood lactates ($[La^-]$) of 5.1–9.6 $mmol \cdot l^{-1}$ and peak $[La^-]$ of 8.1–14.5 $mmol \cdot l^{-1}$, therefore it is difficult to make conclusions regarding metabolic strain and fatigue. The shorter time-trials may have induced a faster rate of central fatigue development but the reduced time-trial duration likely contributed to the difference between trials. Furthermore, time-trial data must be interpreted with the consideration that the intensity is variable and self-paced. Indeed, the range of protocols used make assessment of fatigue mechanisms difficult with open-loop (TTE), closed-loop (time trials and set durations) as well as self-paced, intermittent and continuous exercise trials employed. Therefore, it is currently difficult to fully appreciate the mechanisms underpinning fatigue and in particular the role of metabolic stress as to date no direct comparison has been made between the fatigue profiles of non-exhaustive and exhaustive exercise of differing metabolic strains.

Table 2.1. Studies examining peripheral and central fatigue using peripheral stimulation techniques to measure neuromuscular function following locomotor exercise.

Study	Participants	Task	MVC Change (%)	RT Change (%)	VA Change (%)
Cycling – Knee Extensors					
Lepers et al. (2000)	Trained male cyclists/triathletes (n = 8)	2 h at 65% $\dot{W}_{max}$	−13	−24	N/A
Lepers et al. (2001)	Trained male triathletes (n = 8)	30 min at 80% $\dot{W}_{max}$ FCC	−13	−16*	−16
		30 min at 80% $\dot{W}_{max}$ -20%FCC	−12	−17*	−13
		30 min at 80% $\dot{W}_{max}$ +20%FCC	−9	−19*	−15
Lepers et al. (2002)	Trained male cyclists/triathletes (n = 9)	5 h at 55% $\dot{W}_{max}$	−18	−16	−9
Lepers et al. (2008)	Trained male triathletes (n = 8)	30 min at 75% $\dot{W}_{max}$	−9	−11*	−5
		30 min variable intensity: 5 min at ±5, ±10 and ±15% of 75% $\dot{W}_{max}$ (mean 75% $\dot{W}_{max}$)	−13	−10*	−5
Theurel and Lepers (2008)	Trained male cyclists (n = 10)	33 min at 70% $\dot{W}_{max}$	−8*	−7*	−1*
		33 min at 50% $\dot{W}_{max}$ interspersed with periods at 100, 150 and 200% $\dot{W}_{max}$ (mean 70% $\dot{W}_{max}$)	−12*	−11*	–2*
Ross et al. (2010b)	Trained male cyclists (n = 8)	Simulated Tour de France (22d)			
		9d	−16	−22	−12*
		17d	−20	−21	−8*
Decorte et al. (2012)	Moderately-trained males (n = 13)	5 min at 80% $\dot{W}_{max}$ with 4 min rest repeated until exhaustion	−21	−45	−6

Running – Knee Extensors

Millet et al. (2002)	Trained male runners (n = 9)	65 km time-trial	−30	+19	−28
Millet et al. (2003)	Trained male runners (n = 12)	30 km time-trial	−24	−8	−8
Lattier et al. (2004)	Trained male runners (n = 8)	10 x 1 min at 120% $\dot{V}_{max}$ with 2 min rest	−7	−28	NS
Place et al. (2004)	Trained runners (n = 8) *sex not stated*	5 h at 55% $\dot{V}_{max}$	−28	+18	−16
Martin et al. (2010)	Trained male runners (n = 12)	24 h treadmill run	−41	−25	−33
Ross et al. (2010a)	Trained male runners/triathletes (n = 8)	20 km time-trial	−15	NS	−13

FCC, freely chosen cadence; N/A, data not reported or measured; NS, no significant change; RT, twitch are potentiated muscle twitch from single stimulus; $\dot{V}_{max}$, peak treadmill velocity during incremental exercise test; $\dot{W}_{max}$ peak power output during incremental cycle test; *percentage change estimated from data or figures provided. All data reported relative to pre-exercise. All trials completed in normal environmental conditions, without intervention unless stated. Trained refers to endurance trained.

2.3.2 Supraspinal Fatigue

Part of the central fatigue seen after locomotor exercise arises from supraspinal mechanisms. A limited number of studies have employed TMS to assess supraspinal fatigue and, similar to peripheral VA, deficits in cortical VA have been evidenced after high-intensity short-duration ($\leq$60 min) as well as prolonged exercise (>60 min) (Table 2.2). Sidhu et al. (2009b) demonstrated supraspinal fatigue after high-intensity intermittent cycling which persisted for 45 min post-exercise, demonstrating that locomotor exercise impairs the ability to generate motor cortical output to the knee extensors. The authors proposed that intramuscular or systemic fatigue signals act to limit cortical output upstream of the motor cortex. There is some support for this with evidence that the levels of supraspinal and peripheral fatigue are positively correlated (Temesi et al., 2014). Whilst this could be a mechanism contributing to the supraspinal fatigue seen after sprint exercise (Fernandez-del-Olmo et al., 2013, Goodall et al., 2015b) and high-intensity endurance (Sidhu et al., 2009b, Goodall et al., 2012, Thomas et al., 2015a), supraspinal fatigue is also evident during prolonged exercise where metabolic stress is minimal (Jubeau et al., 2014). Therefore, the mechanism may be distinct between these intensities; however, the role of muscle metabolism on supraspinal fatigue is yet to be investigated. Nevertheless, similar to the findings for peripheral VA, cortical VA develops at the end of prolonged exercise whereas peripheral fatigue develops early during exercise and stabilises (Jubeau et al., 2014), and is greater following shorter compared to longer cycling time-trials (Thomas et al., 2015a). Supraspinal fatigue can originate from mechanisms that reduce descending output from the motor cortex or reduce the efficacy of the output from motor cortex (i.e. changes in the properties of, or input to, corticospinal neurons) (Taylor et al., 2006). More recent studies using TMS have examined the responsiveness of the corticospinal pathway following locomotor exercise (Sidhu et al., 2012a, Sidhu et al., 2013a, Sidhu et al., 2013c) and these studies are considered in the following section.

Table 2.2. Studies examining supraspinal fatigue using TMS following locomotor exercise.

Study	Participants	Task	MVC Change (%)	RT Change (%)	ERT Change (%)	VA Change (%) Peripheral	Cortical
Cycling – Knee Extensors							
Sidhu et al. (2009b)	Moderately active males (n = 5) and females (n = 5)	5 x 5 min at 80% $\dot{W}_{max}$ with 1 min rest	−23	−40*	−50*	−5*	−11
Goodall et al. (2012)	Trained male cyclists (n = 9)	60%Δ to exhaustion - hypoxia *(3.6 min)* 60%Δ for 3.6 min 60%Δ to exhaustion *(8.1 min)*	−25 −7 −17	−30 −9 −19	−47 −20 −36	−7 −5 −6	−18 −5 −9
Klass et al. (2012)	Trained male cyclists/triathletes (n = 10)	55% $\dot{W}_{max}$ followed by time-trial *(equivalent to 30 min at 75% $\dot{W}_{max}$)*	−9*	−12	−30	NS	NS
Bowtell et al. (2013)	Male Taekwondo athletes (n = 9)	90 min at 80% HR_{max} - 40° *(Euhydrated)* 90 min at 80% HR_{max} - 40° *(Hypohydrated)*	−5 −15	NS NS	NS NS	NS NS	NS NS
Fernandez-del-Olmo et al. (2013)	Recreationally active males (n = 10)	Wingate 1 Wingate 2 *(separated by 35 min rest)*	−16 −23*	−36 −44*	N/A N/A	N/A N/A	−30 −35
Girard et al. (2013)	Recreationally active males (n = 12)	15 x 6 s sprints over 15 min	−11	−43	−40	NS	NS
Temesi et al. (2013)	Trained males (n = 12)	35 min at 65% $\dot{W}_{max}$ 5 min at 65% $\dot{W}_{max}$ with 5% $\dot{W}_{max}$ increases every 5 min until exhaustion	−11 −16	−23 −28	−32 −49	−3* −5*	NS NS

Study	Group	Task					
Goodall et al. (2014b)	Recreationally active males (n = 5) and females (n = 2)	50% $\dot{W}_{max}$ to exhaustion - acute hypoxia *(10.1 min)*	−14	−21	−31*	N/A	−11
		50% $\dot{W}_{max}$ for 10.1 min	NS	NS	NS	N/A	NS
		50% $\dot{W}_{max}$ for 10.1 min - chronic hypoxia	−8	−18	NS	N/A	NS
Jubeau et al. (2014)	Recreationally training males (n = 10)	4 h at 45% $\dot{W}_{max}$	−25	−28	−37	−14	−8
Thomas et al. (2015a)	Trained male cyclists (n = 13)	4 km time-trial	−18	−40	−41	−7	−6
		20 km time-trial	−15	−31	−32	−11	−12
		40 km time-trial	−16	−29	−36	−10	−10
Goodall et al. (2015a)	Trained male cyclists (n = 7)	60%Δ to exhaustion - heat *(11.4 min)*	−13	−16	−24	−9	−8
		60%Δ for 11.4 min	−9	−21	−9	−7	−3

Running – Knee Extensors

Study	Group	Task					
Goodall et al. (2015b)	Males engaged in team-sports (n = 12)	12 x 30 m sprints with 30 s recovery	−12	−23	−27*	−9	−9
Temesi et al. (2014)	Trained male (n = 14) and female (n = 11) runners	110km time-trial	−34	−11	N/A	−26	−16
Temesi et al. (2015)	Trained male (n = 10) and female (n = 10) runners	110 km time-trial					
		Males	−38	−14	N/A	−24	−14
		Females	−29	−5	N/A	−19	−12

Running – Ankle Dorsiflexors

Study	Group	Task					
Ross et al. (2007)	Trained male runners (n = 9)	42.2 km time-trial	−18	−35	N/A	N/A	−18

Δ, difference between gas exchange threshold and maximal oxygen uptake; ERT, estimated resting twitch; HR$_{max}$, maximal heart rate; N/A, data not reported or measured; NS, no significant change; RT, potentiated muscle twitch from single stimulus; $\dot{W}_{max}$ peak power output; *percentage change estimated from data provided. All data reported relative to pre-exercise. All trials completed in normal environmental conditions, without intervention unless stated. Trained refers to endurance trained.

2.3.3 Corticospinal Function

The motor pathway responsible for muscle activation (motor cortex and corticospinal tract, Figure 1.4) undergoes complex changes with fatigue and disturbances in the excitability of this pathway are an important contributor to fatigue (Gandevia, 2001). The motor cortex generates motor drive during exercise and testing changes in the function of the motor cortex with TMS is key to understanding central fatigue (Ament and Verkerke, 2009).

There is a pronounced depression in MEP amplitude in the resting muscle following maximal sprint rowing (Fulton et al., 2002) incremental exhaustive treadmill running (Verin et al., 2004) as well as prolonged time-trial running (Ross et al., 2007) and cycling (Ross et al., 2010b, Thomas et al., 2015a). Verin et al. (2004) reported that an incremental exhaustive treadmill test depressed MEP amplitude by 41% in the rectus femoris (RF) which took ~60 min to recover. Ross et al. (2007) reported a 57% reduction in MEP amplitude in the tibialis anterior ~20 min after a treadmill marathon. These authors also reported that 20 days (d) simulated Tour de France cycling induced a depression in VL MEP amplitude after days 8 (44%) and 16 (48%) as well as 2 d post-exercise (30%) (Ross et al., 2010b). The depression in corticospinal excitability is considered an important part of the central fatigue induced by locomotor exercise (Ross et al., 2007, Ross et al., 2010b). However, these studies failed to report MEP amplitude relative to M_{max} and so any reported reductions in muscle excitability (e.g. Ross et al. (2010b)) cannot be dissociated. Nevertheless, when normalised for M_{max}, a depression still presents which appears exercise duration and/or intensity dependent. Thomas et al. (2015a) reported reductions in MEPs after 20 and 40 km but not 4 km time-trial cycling which were also accompanied by greater supraspinal fatigue. The mechanisms of the MEP depression are unclear but have been dissociated from impairments in VA as shown by preventing the recovery of VA by holding the muscle ischemic (Gandevia et al., 1996, Taylor et al., 2000).

These studies also recorded MEPs in the resting muscle which may not represent the behaviour of the motor pathway during fatigue; when post-exercise MEPs are recorded during a contraction the depression is offset by voluntary effort (Gruet et al., 2013).

Post-exercise MEPs elicited in contracting muscle, the majority of which have been recorded during the measurement of cortical VA (50%, 75% and 100% of MVC), show a different pattern of change. High-intensity cycling has no effect on MEP size or cSP duration at any contraction strength in the RF (Sidhu et al., 2009b). This finding has been replicated in the knee extensors after time-trial cycling (Klass et al., 2012, Thomas et al., 2015a), repeated sprint cycling (Girard et al., 2013) and running (Goodall et al., 2015b), and exhaustive and non-exhaustive constant load cycling (Goodall et al., 2012, Goodall et al., 2015a). Increased MEP amplitudes during contractions at 50% and 75% MVC but not at MVC were reported after repeated 30 s sprint cycling (Fernandez-del-Olmo et al., 2013). This study however failed to account for the fatigue-induced reduction in MVC and so the post-exercise measures were at a higher relative force. Following more prolonged exercise (4 h cycling 45% $\dot{W}_{max}$), increases in knee extensor amplitudes have been reported during 50%, 75% and 100% MVC contractions with the cSP duration remaining unaltered (Jubeau et al., 2014). Similar MEP findings have been reported following a pre-loaded (35 min, 65% $\dot{W}_{max}$) incremental cycle to exhaustion (~20 min) (Temesi et al., 2013) and 110 km ultra-trail run (Temesi et al., 2014). Whilst Temesi et al. (2014) reported increases in cSP duration at 50% MVC, Temesi et al. (2013) reported a reduction in cSP duration after the pre-load and exhaustive cycle. Repeated sprint-cycling also reduced the increase in cSP duration during a 30 s sustained MVC (Girard et al., 2013). This discrepancy could be due to the exercise type, duration and intensity but suggests locomotor exercise may differently impact the cortical cells compared to isolated muscle fatigue where the cSP is prolonged (Taylor et al., 1996).

Using a different methodology, Sidhu et al. (2012a) applied TMS at the knee extensor activation phase during 30 min cycling at 75% $\dot{W}_{max}$ followed by a cycle to exhaustion at 105% $\dot{W}_{max}$. When expressed to M_{max} there was no difference in MEP or cervicomedullary motor evoked potential (CMEP) amplitude throughout, however when the MEPs and CMEPs were expressed to the rising background EMG there was a reduction in MEP amplitude with no change in CMEP amplitude over time. Therefore motor cortex excitability appears to decrease at exhaustion, further supporting different modulation of cortical excitability compared to isolated muscle fatigue where the MEP increases (Taylor et al., 1996). In a follow up study, Sidhu et al. (2013c) reported an increase in intracortical inhibition in the last 5 min compared to the first 5 min of 30 min sustained cycling at 75% $\dot{W}_{max}$, as measured with a subthreshold TMS technique. However, more established ppTMS measures of inhibition have yet to confirm these findings and the reliability of ppTMS has yet to be addressed in the knee extensors. To date only one study has employed ppTMS and these authors reported ICF was reduced by ~40% in the resting RF after an exhaustive incremental treadmill run (Verin et al., 2004). Therefore, the cortical response to locomotor exercise remains poorly understood.

2.3.4 Effects of Locomotor Exercise on CNS Function

Intense or prolonged locomotor exercise has the capacity to impact a number of CNS processes implicated in central fatigue and exhaustion. Alterations in neurotransmission (particularly the monoamines: serotonin, dopamine and noradrenaline) have been considered key processes in the development of central fatigue (Davis and Bailey, 1997, Meeusen et al., 2006). The most important is the altered synthesis of serotonin which has wide-spread effects on mood, arousal and lethargy and contributes to altered perception of effort, pain tolerance and the loss in central drive (Davis et al., 2000, Meeusen et al., 2006). Dopamine plays a role in motivation and reward (Watson, 2008) and a high dopamine/serotonin ratio favours enhanced central drive

(Davis and Bailey, 1997). An increase in tryptophan crossing the blood brain barrier increases serotonin synthesis (Meeusen et al., 2006). As tryptophan crosses the blood brain barrier in its free form (unbound to albumin), and competes with branch-chain amino acids to do so, exercise favours the entry of free-tryptophan into the brain (Figure 2.5). However, attempts to manipulate neurotransmission have been unconvincing (Meeusen et al., 2006, Watson, 2008, Roelands and Meeusen, 2010) although noradrenaline reuptake inhibition reduces cycling time-trial performance and induces greater supraspinal fatigue (Klass et al., 2012). Prolonged exercise also increases cerebral ammonia uptake which may contribute to central fatigue and elevated RPE through neurotransmitter disturbances (Nybo et al., 2005). As the brain has no effective urea cycle, ammonia combines with glutamate to form glutamine, disturbing both glutamate and GABA concentrations (Guezennec et al., 1998, Nybo and Secher, 2004).
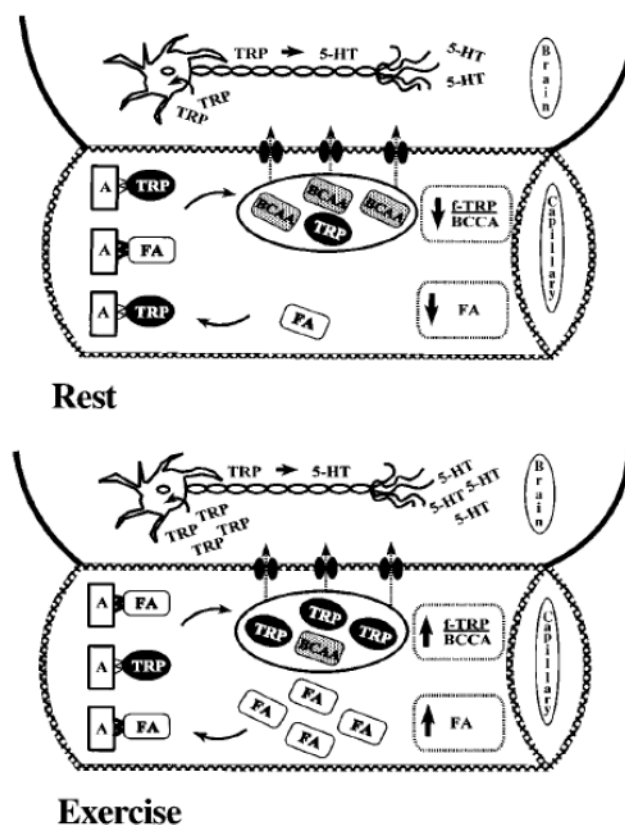
Figure 2.5. Serotonin and the central fatigue hypothesis (Davis et al., 2000) (reproduced with permission).

A, albumin; BCAA, branch-chain amino acid; FA, fatty acid; (f-)TRP, (free-) tryptophan; 5-HT, serotonin.

Impairments to cerebral O_2 delivery and fuel supply during exercise could also contribute to central fatigue by impairing oxidative energy supply required for continued motor activation of muscle (Nybo and Rasmussen, 2007, Secher et al., 2008). With increasing exercise intensity, the greater neuronal activity requires increased cerebral blood flow (CBF) for delivery of O_2 and substrate (Secher et al., 2008). The widespread increase in neuronal activity and demand for neurotransmitter synthesis also requires a supply of blood glucose or other fuel source (ketone bodies or lactate) as neuronal glycogen is limited (Nybo and Secher, 2004). CBF is driven by the arterial CO_2 tension (P_aCO_2) and therefore depends on the exercise-induced metabolic demand; hyperventilation during intense exercise lowers P_aCO_2 and results in constriction of cerebral arterioles reducing CBF close to resting values at maximal exercise (Nybo and Rasmussen, 2007, Secher et al., 2008). Cerebral oxygenation follows a similar pattern (Subudhi et al., 2009) and impairments to cerebral oxygenation appear to increase RPE and induce supraspinal fatigue (Rasmussen et al., 2010, Goodall et al., 2012). Endurance training may increase CBF and oxidative capacity in the motor cortex to support an increased metabolic demand (Adkins et al., 2006). Increases in brain mitochondrial content contribute to improved oxidative capacity which might be important in central fatigue resistance (Steiner et al., 2011). Fitter individuals have lower cerebral oxygenation at submaximal workloads and higher cerebral oxygenation at high exercise intensities (Rooks et al., 2010) and better maintenance of cerebral oxygenation may contribute to suprapinal fatigue resistance (Goodall et al., 2014b).

2.3.5 Interaction Between Peripheral and Central Fatigue

Skeletal muscle is richly innervated by group III (myelinated) and IV (unmyelinated) afferents sensitive to a number of chemical, thermal and mechanical stimuli which project via the lumbar dorsal horn of the spinal cord to various spinal and supraspinal sites (Amann et al., 2015).

During exercise the increased concentration of intramuscular metabolites (lactate, ATP, K^+, H^+, P_i) increases the discharge of these sensory neurons (Laurin et al., 2015). Numerous subtypes of the chemosensitive afferents exist and are classified as ergoreceptive, which respond to levels of metabolites seen during moderate- to high-intensity exercise and are involved in the general homeostasis of muscle and evoking the sensation of fatigue, or nociceptive, which respond to noxious levels of metabolites and generate sensations of pain (Pollak et al., 2014, Amann et al., 2015). The sensation of both fatigue and pain depend on the concentration and combination of metabolites (Pollak et al., 2014). Group III/IV afferent firing informs the CNS regarding the metabolic condition of the muscle (Amann, 2011). As well as adjusting the cardio-respiratory response to exercise, these afferents also play a key role in the regulation of motor command (Laurin et al., 2015). During high-intensity locomotor exercise the muscle metabolic disturbance increases group III/IV muscle afferent firing and inhibits neural drive to the muscle impairing exercise tolerance, possibly to restrict the exercise-induced homeostatic disturbance (Figure 2.6) (Amann, 2011, Amann et al., 2015). As such supraspinal fatigue protects further peripheral fatigue at the expensive of truly maximal performance (Gandevia, 2001).
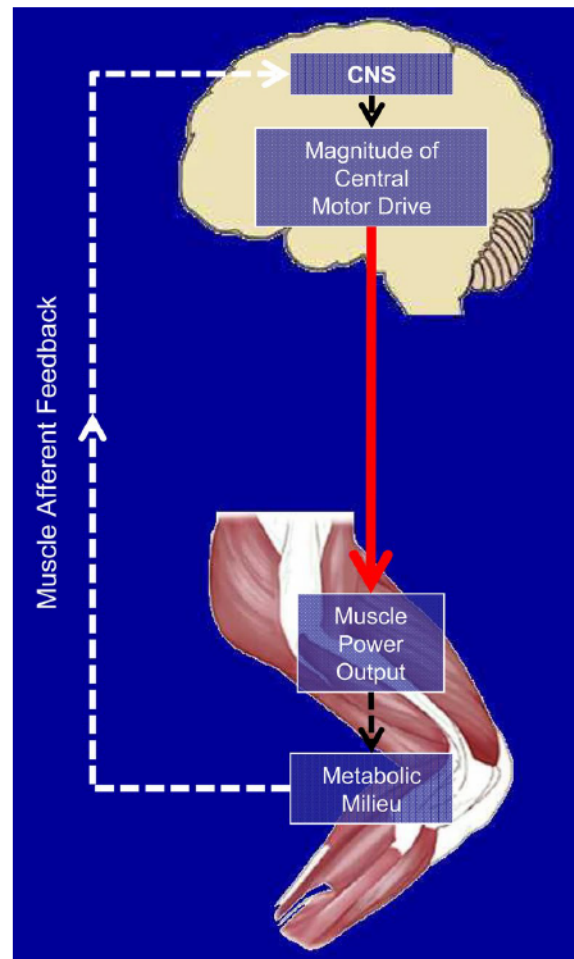
Figure 2.6. Inhibitory effect of muscle afferents on central motor drive during high-intensity exercise (Amann, 2011) (reproduced with permission). The magnitude of central drive is hypothesised to be inhibited in proportion to the disturbance to the muscle metabolic milieu in order to restrict peripheral locomotor fatigue development and the associated sensory feedback.

The most convincing evidence for this effect has come from Amann and colleagues (Amann et al., 2006a, Amann and Dempsey, 2008, Amann et al., 2009, Amann et al., 2011). By manipulating environmental O_2, a faster rate of peripheral fatigue development and poorer 5 km cycle time-trial performance were observed in hypoxia vs normoxia vs hyerpoxia (Amann et al., 2006a). At the end of exercise, the level of peripheral fatigue (~35% reduction in $Q_{tw,pot}$) was the same amongst the conditions, however during exercise the greater rate of peripheral fatigue development was accompanied by a reduction in neural drive (estimated from cycling

EMG). Similar findings have been reported after inducing pre-exercise fatigue by cycling at either 83% $\dot{W}_{max}$ to exhaustion, 67% $\dot{W}_{max}$ for the same duration or no cycling (reduction in $Q_{tw,pot}$ of −36, −20 or −0, respectively) (Amann and Dempsey, 2008). In this study 5 km time-trial performance was reduced by pre-existing fatigue with concomitant reductions in neural drive, however the level of post-exercise peripheral fatigue was not different (~35% reduction in $Q_{tw,pot}$). These authors proposed central motor drive is inhibited in response to afferent feedback to limit peripheral fatigue within a 'sensory tolerance limit' or 'critical threshold.' Once this limit is attained, the CNS mediates exhaustion during constant workload exercise, or a reduction in intensity if the exercise is self-paced (Amann, 2011).

To test this hypothesis further, these authors blocked spinal opioid receptor-sensitive lower limb muscle afferents by infusion of lumbar intrathecal fentanyl (Amann et al., 2009, Amann et al., 2011). Compared with administration of a placebo, a greater level of peripheral fatigue was tolerated by the participants during a 5 km time-trial (Amann et al., 2009) and a TTE test at 80% $\dot{W}_{max}$ (Amann et al., 2011). In the 5 km time-trial, neural drive was higher during the first 2.5 km and was completed with a significantly greater degree of peripheral fatigue compared to the placebo (−46% vs −33%). During the exercise tolerance test at 80% $\dot{W}_{max}$, there were similar increases in the level of peripheral fatigue (−44% vs −34%, Figure 2.7A) and central motor drive (Figure 2.7A), however TTE was reduced due to the blunted cardiopulmonary response to exercise. The authors suggest a naïve CNS releases the 'brake' on central motor drive and 'tolerated' a greater level of peripheral fatigue, but with the intact CNS the sensory sensations accompanying fatigue beyond these levels are intolerable (Amann et al., 2011).
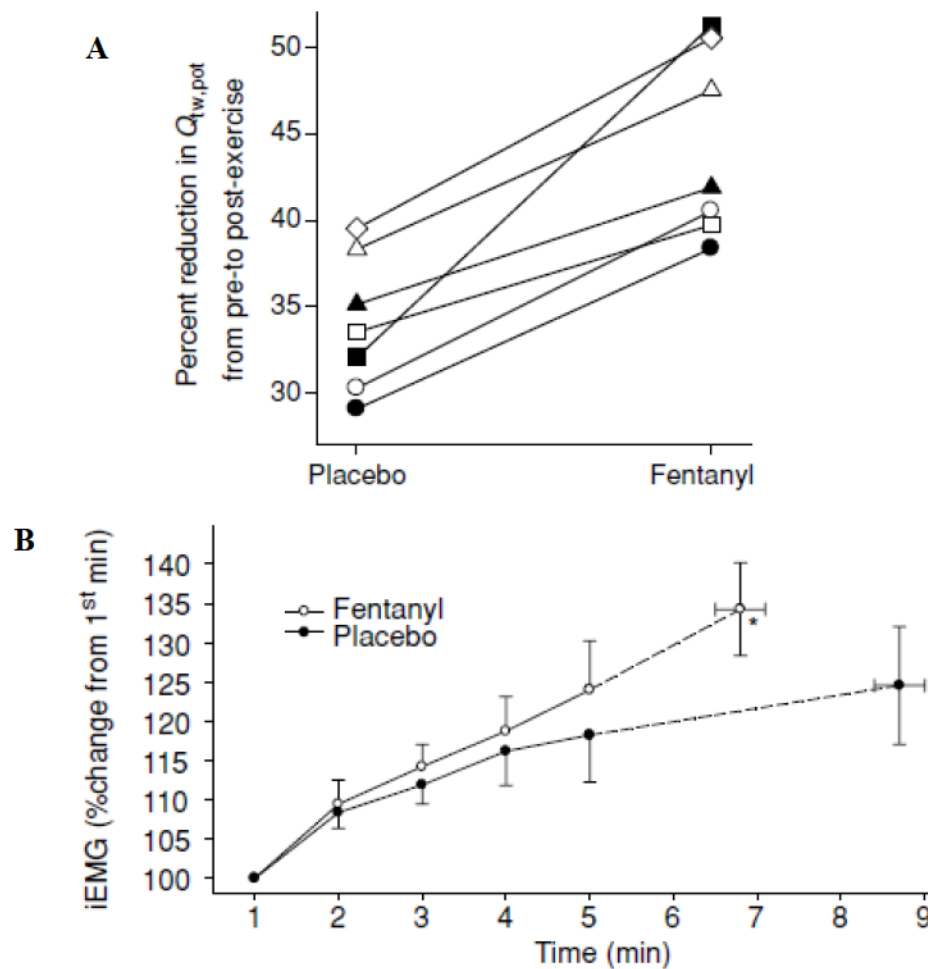
Figure 2.7. Level of peripheral fatigue determined by reduction in potentitated quadriceps twitch torque ($Q_{tw,pot}$) in response to supramaximal magnetic stimulation of the femoral nerve (A) and integrated EMG (iEMG) activity of the vastus lateralis in response to a time to exhaustion trial at 80% $\dot{W}_{max}$ (Amann et al., 2011) (reproduced with permission). Trials were either completed with administration of a placebo or fentanyl to block sensory feedback.

In order to overcome disturbances to the cardiopulmonary response to exercise with pharmacological blocking of afferents, a recent number of one-legged models of exercise have been employed. Compared to single-leg knee extensor exercise to exhaustion, there is a reduced endurance time, less neural drive, and peripheral fatigue is less tolerated when the other leg is exercised to exhaustion immediately before (Amann et al., 2013b). Additionally, compared to

two-leg knee extension exercise, single-leg knee extensor exercise (both performed to exhaustion at mode specific 85% $\dot{W}_{max}$) induces a greater level of peripheral fatigue and allows a greater level of neural drive during exercise (Rossman et al., 2014). Taken together, these studies suggest greater sensory afferent feedback reduces the level of neural drive and peripheral fatigue. Whilst these studies provide insight into the role of afferents on fatigue mechanisms and the subsequent exercise performance, they must be considered alongside their limitations. Pharmacological manipulation of afferents affects whole-body physiology, in particular the exercise pressor reflex, and single-leg exercise could also induce a number of systematic disturbances that could contribute to fatigue.

In a similar study design, Triscott et al. (2008) found that exhausting the dominant arm impaired exercise tolerance in the non-dominant arm in resistance-trained or sedentary individuals but failed to affect endurance time in endurance-trained individuals. The authors suggest this represents an adaptation to central fatigue specific to endurance training. Based on the data from Amann et al. (2013b), it may be that the endurance trained individuals have increased resistance to the inhibitory effects of group III/IV afferent firing. This is supported by a recent study in which untrained males completed an isometric knee extension at 15% MVC to exhaustion before and after 8 weeks isolated muscle low force endurance training (Zghal et al., 2015). Compared to a control group, the training group increased exercise tolerance (~3.5 fold) and had a greater level of peripheral fatigue at exhaustion compared to pre-training suggesting better CNS tolerance of peripheral fatigue, either through altered group III/IV afferent firing or better central tolerance of their inhibitory effects. The degree of supraspinal fatigue and magnitude of increase in the cSP were unaffected by training, however when the post-training contraction was completed again but terminated at the pre-training exhaustion time, the level of supraspinal fatigue and increase in the cSP was attenuated. The

reduced fatigability of the muscle fibres may have resulted in less afferent feedback preserving cortical function.

A number of proposed methods for group III/IV afferent impairment of exercise performance have been proposed. Group III/IV afferents have inhibitory effects at numerous parts of the motor pathway including the motoneurons, motor cortex and the circuits that generate motor cortex output (Figure 1.4) (Gandevia, 2001). Following a 2 min sustained MVC, maintenance of group III/IV afferent firing using inflation of a cuff to prevent metabolite clearance, maintains supraspinal fatigue of the exercised muscle (Gandevia et al., 1996) as well as other muscles in the same limb not involved in the task (Kennedy et al., 2013, Kennedy et al., 2014), but does not prevent recovery of the cortical cells (Taylor et al., 1996) or motoneurons (Taylor et al., 2000, Butler et al., 2003). Hypertonic saline infusion depresses cortical excitability at rest and during activation (Martin et al., 2008) and increases SICI and reduces ICF in a resting muscle (Schabrun and Hodges, 2012), however has little impairment on measures of VA (Khan et al., 2011). It may therefore be that a specific firing pattern of group III/IV afferents activated only by fatiguing exercise is required to induce VA impairments (Kennedy et al., 2013, Kennedy et al., 2014). Infusion of lumbar intrathecal fentanyl prevents the increase in the knee extensors cSP during isolated muscle fatigue (Hilty et al., 2011) and the MEP depression and supraspinal fatigue in the elbow flexors induced by exhaustive lower limb cycling (Sidhu et al., 2014). Group III/IV afferents may also act up-stream of the motor cortex to disturb the RPE and motor output relationship (Carson et al., 2002, Smith et al., 2007) and contribute to the exercise-induced sensations of muscle pain and fatigue (Mastaglia, 2012, Mauger, 2013, Pollak et al., 2014). Fatigue and pain have been inextricably linked, with muscle pain and pain tolerance considered important factors in both central fatigue development and the sensation

of fatigue with exercise terminated once muscle pain becomes intolerably exacerbated (Nybo and Secher, 2004, Mastaglia, 2012, Mauger, 2013).

It has been suggested that part of the adaptation to chronic exercise is the supraspinal processing of muscle pain and unpleasant afferent signals accompanying exercise (Nybo and Secher, 2004). Indeed, athletic success is characterised by an ability to tolerate pain (Mauger, 2013). Endurance training increases ischaemic muscle pain tolerance induced with a tourniquet test (Jones et al., 2014). However, the results from this study must be received with caution: the training and control groups were not randomised which resulted in the training group having a lower initial $\dot{V}O_{2max}$; exercise training was performed at 75% HR reserve, an intensity that produces profoundly different perceptual, physiological and metabolic responses amongst individuals (Mann et al., 2013); the control group performed no exercise and so any placebo or behavioural artefacts cannot be excluded, and; endurance performance was quantified by $\dot{V}O_{2max}$. Therefore, the effect of the training stimulus on pain tolerance, or relevance of an increase in pain tolerance to the training induced changes in peripheral and central fatigue, and exercise tolerance, are not well understood.

2.3.6 Perception of Effort

The perception of effort, as measured by the ratings of perceived exertion (RPE) (Borg, 1982), has been widely used to examine the degree of physical effort during a task and is an important measure for examining central fatigue during submaximal efforts (Taylor and Gandevia, 2008). An increase in the sense of effort is usually the first sign of fatigue and distinctive of impaired motor performance (Enoka and Stuart, 1992). The sense of effort appears to arise from the corollary discharges from corticofugal motor commands projecting to the somatosensory cortex (Enoka and Stuart, 1992) and has been described as the sensation of the central command

generated in the motor cortex (Kayser, 2003). Whilst perception of effort is therefore distinct from other unpleasant sensations during exercise, such as muscle pain and metabolic stress, it is likely an interaction between sense of effort and physiological feedback that contribute to the sensory response to exercise (Smirmaul, 2012). The effect of such sensory disturbances on exercise tolerance is poorly understood (Smirmaul, 2012), partly due to the difficulty in measuring and distinguishing between these sensations. Investigations that can examine sensory afferent tolerance and provide links with exercise tolerance are therefore required.

Perception of effort, as well as other sensory signals such as muscle pain, are considered key factors contributing to the cessation of exercise (Kayser, 2003, Smirmaul, 2012). RPE demonstrates linear relationships with time up until exhaustion during constant workload cycling (Presland et al., 2005, Marcora and Staiano, 2010). This relationship is irrespective of exercise intensity (Pires et al., 2011) and holds true in response to interventions that reduce TTE such as mental fatigue (Marcora et al., 2009), prior muscle fatigue (Marcora et al., 2008), heat (Crewe et al., 2008) and reductions in muscle glycogen (Noakes, 2004). Recent arguments therefore suggest RPE is the limiting factor to exercise tolerance (Marcora and Staiano, 2010). Altered RPE with fatigue may be related to the fall in motor output (i.e. mismatch between motor cortex output and RPE possibly due to neurophysiological alterations up-stream of the motor cortex) (Sogaard et al., 2006, Smith et al., 2007) or disturbances in neurotransmission (Meeusen et al., 2006) and cerebral metabolism and oxygenation (Rasmussen et al., 2010). The transfer of effort to motor output and muscle work depends on the input-output characteristics of the corticospinal tract, i.e. excitability of the motor pathway and a reduction in excitability may necessitate increased effort to generate the required muscle work (Kalmar and Cafarelli, 2006). Therefore RPE increases during exercise due to the required increase in motor command to overcome reductions in cortical, motoneuron and muscles responsiveness (Marcora, 2008).

However, it is likely an interaction between sense of effort and unpleasant sensory feedback signals contribute to exhaustion (Smirmaul, 2012).

2.4 Methods of Determining Intensity for Exercise Prescription

The exercise-induced disturbances in muscle and CNS function that contribute to fatigue, sensory disturbance and exhaustion are heavily influenced by the metabolic demand of the exercise. The complexity of fatigue can therefore be reduced through appropriate definition of exercise intensity (Burnley and Jones, 2008). Investigations into mechanisms of fatigue typically prescribe exercise intensity as a proportion of $\dot{V}O_{2max}$, $\dot{W}_{max}$ or HR_{max}. These markers have a variable relationship with metabolic markers (i.e. lactate threshold, LT and ventilatory threshold, VT) and so making prescription based on these alone results in differing levels of physiological, metabolic and perceptual stress (Whipp et al., 2005, Scharhag-Rosenberger et al., 2010, Mann et al., 2013). Scharhag-Rosenberger et al. (2010) reported that when cycling for 60 min at 65% $\dot{V}O_{2max}$, 22% of participants were exercising above the VT which significantly impaired exercise tolerance (10% exhausted before 60 min) (Figure 2.8). In a repeated bout at 75% $\dot{V}O_{2max}$, 78% of participants were exercising above the VT, 15% above maximal lactate steady-state (MLSS) and 81% experienced exhaustion. Similarly, Coyle et al. (1988) examined TTE at 88% $\dot{V}O_{2max}$ in cyclists with a similar $\dot{V}O_{2max}$ (4.6–5.0 l·min^{-1}) but half had a *low* LT (~66% $\dot{V}O_{2max}$) and half a *high* LT (~82% $\dot{V}O_{2max}$). TTE was ~29 min ([La$^-$], ~15 mmol·l^{-1}) in the *low* group and ~61 min ([La$^-$], ~7 mmol·l^{-1}) in the *high* group with 92% of the variance explained by the LT.
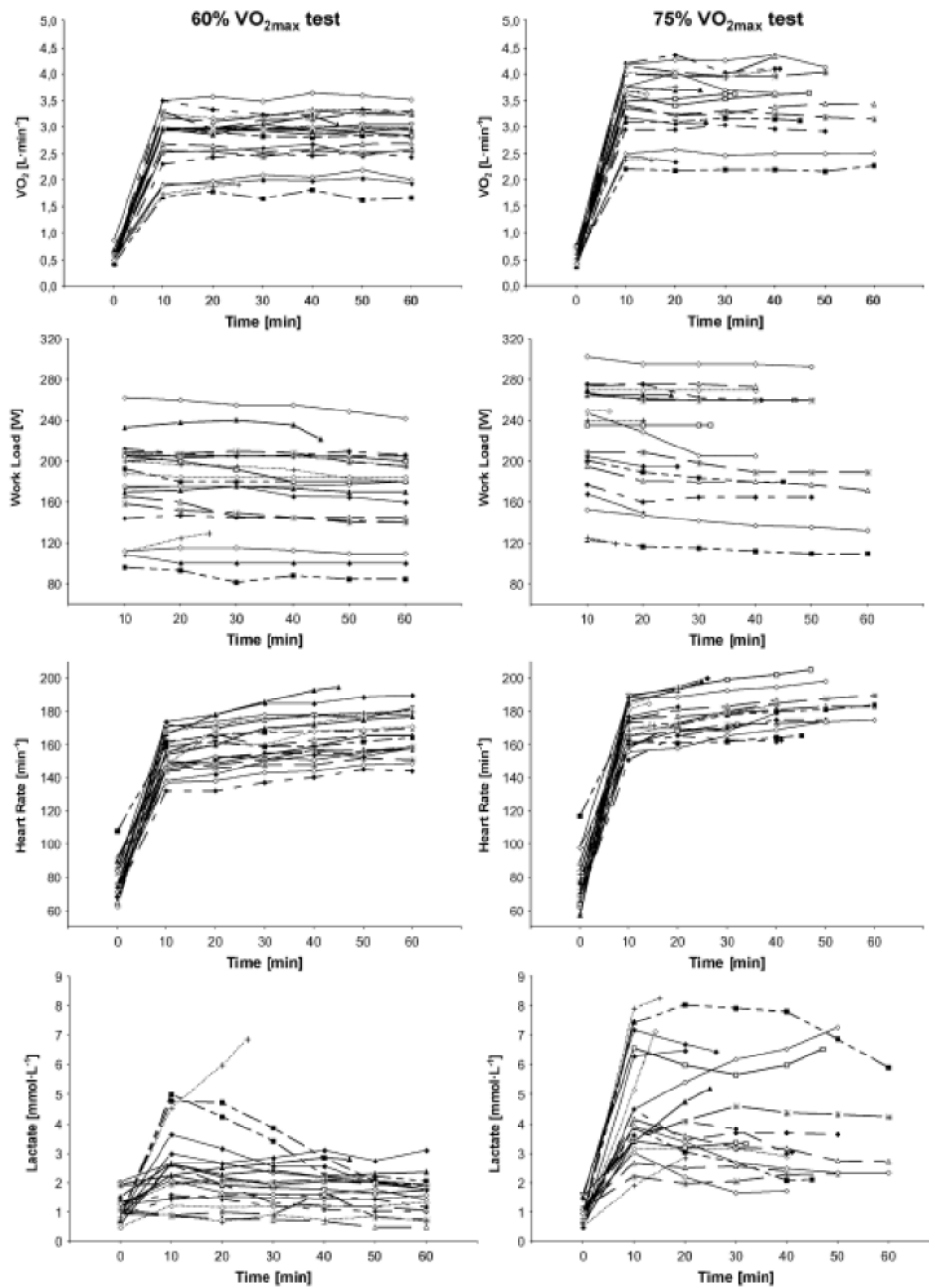
Figure 2.8. Inter-individual responses to exercise prescribed relative to maximal oxygen uptake (%$\dot{V}O_{2max}$) (Scharhag-Rosenberger et al., 2010) (reproduced with permission).

Assigning work rate as %$\dot{V}O_{2max}$ is therefore flawed as the metabolic stress and the fatigue mechanisms experienced by participants are different (Burnley and Jones, 2008). Normalisation of the blood acid-base profile, gas exchange and muscle metabolism is vital to

understand the integration between metabolism and neurophysiology and provide insight into the nature of fatigue (Burnley and Jones, 2008). In order for common physiological and metabolic stress profiles to be elicited, muscle metabolite and pulmonary gas exchange responses must be accounted for (Whipp et al., 2005) and as such fatigue research would benefit from controlled prescription of exercise intensity relative to metabolic markers such as the LT alongside $\dot{V}O_{2max}$ (Burnley and Jones, 2008). This is also key for delivering a homogenous training stimuli (Mann et al., 2013). As such three domains of exercise intensity have been described (Whipp et al., 2005): the moderate-intensity domain, at intensities below the LT; the heavy intensity domain, between the LT and MLSS or critical power (CP), and; the severe intensity domain, between MLSS or CP and $\dot{V}O_{2max}$. The intensity domains can be characterised by the $\dot{V}O_2$ and [La$^-$] response (Figure 2.9) (Carter et al., 2000, Pringle and Jones, 2002). The moderate-intensity domain is characterised by a steady-state $\dot{V}O_2$ achieved within 2-3 mins with [La$^-$] remaining at resting levels. The heavy intensity domain is characterised by a $\dot{V}O_2$ slow component and increases in [La$^-$] above resting levels that both eventually reach a delayed steady-state. The severe domain is characterised by the failure to achieve a steady-state; the $\dot{V}O_2$ slow component continues until $\dot{V}O_{2max}$ or exhaustion and [La$^-$] increases throughout exercise. There is also a progressive depletion of PCr with rapid accumulation of metabolites (Pi) and drop in pH compared to below CP (Jones et al., 2008).
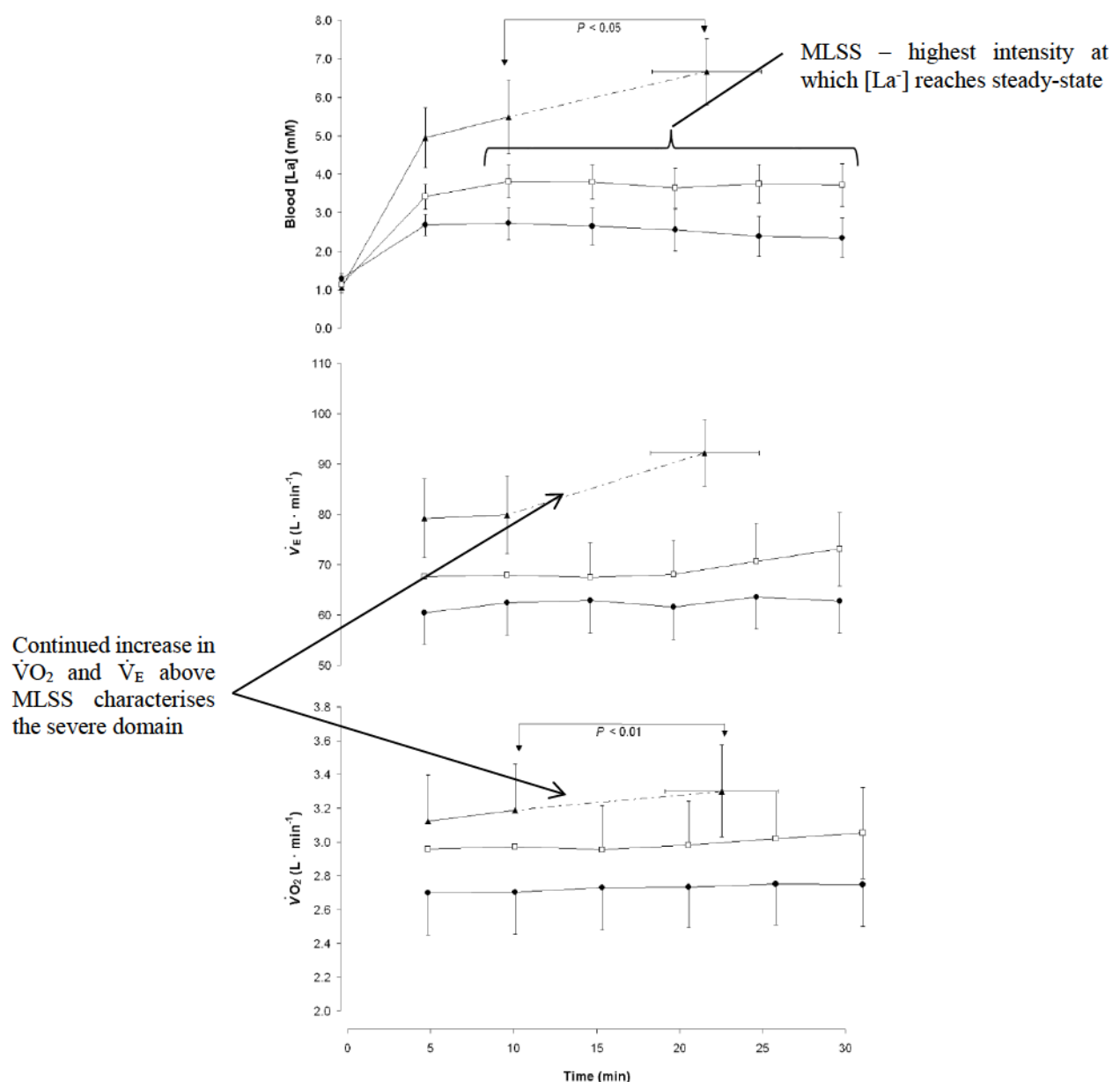
Figure 2.9. Determination of the maximal lactate steady-state during cycling and accompanying pulmonary gas measurements (Pringle and Jones, 2002) (reproduced with permission). Three exercise trials were completed at increasingly higher power outputs between LT and 50%Δ.

2.5 Investigations, Aims and Hypotheses

Locomotor exercise induces a number of physiological and metabolic disturbances that contribute to fatigue, sensory disturbance and exhaustion. Fatigue is therefore an integrative

physiological process rather than a failure of a system. At the heart of this appears the role of metabolic disturbance; however, the role of metabolic strain on fatigue mechanisms has yet to be investigated using controlled exercise intensity prescription. Whilst endurance training is a potent enhancer of exercise tolerance and anecdotally increases the tolerance to fatigue and the associated sensory disturbances, it is unknown how the peripheral and central fatigue mechanisms adapt to a period of endurance training. The aim of the following studies was to investigate mechanisms of fatigue in response to the exercise-induced metabolic demand before and after endurance training.

Study 1 – Assessment of Corticospinal and Neuromuscular Function in the Knee Extensors: A Reliability Study

Background: Fatigue and training adaptation originate from changes in muscle and CNS function, however the reliability of techniques to measure neuromuscular and corticospinal function are poorly established the knee extensors.

Aims: To examine the within- and between-day reliability and variability of single and ppTMS in the VL muscle and established measures of neuromuscular function used to examine peripheral and central fatigue.

Hypotheses: Single and ppTMS measures of corticospinal function in the VL muscle will demonstrate similar repeatability to that reported for other muscles. Measures of neuromuscular function will demonstrate similar repeatability to those previously reported.

Study 2 – Corticospinal and Neuromuscular Function and their Relationship with Exercise Capacity

Background: Multiple sites within the pathway from motor cortex to muscle contribute to human performance and fatigue resistance, however the relationship between corticospinal and neuromuscular function to exercise capacity is poorly understood.

Aims: To examine the relationship between measures of corticospinal and neuromuscular function and endurance exercise capacity.

Hypotheses: Those with a higher exercise capacity will demonstrate a greater ability to activate the motor cortex (cortical VA), higher corticospinal excitability (MEP amplitudes) and lower intracortical inhibition (SICI, cSP and LICI).

Study 3 – Central and Peripheral Fatigue Following Non-Exhaustive and Exhaustive Exercise of Disparate Metabolic Demands

Background: Both central and peripheral fatigue are influenced by the metabolic demand of the exercise, however fatigue processes after differing levels of metabolic stress using controlled prescription of exercise intensity has yet to be investigated.

Aims: To investigate central and peripheral fatigue resulting from non-exhaustive and exhaustive exercise of disparate metabolic demands. Secondary aims were to investigate the reliability of measures of central and peripheral fatigue.

Hypotheses: Compared with moderate-intensity steady-state exercise, the greater metabolic disturbance induced by severe-intensity exercise will result in a greater level of peripheral and central fatigue in response to non-exhaustive exercise, but there will be no difference between intensities at exhaustion. Severe-intensity exercise will result in disturbances in the response of the inhibitory cortical cells (SICI and cSP).

Study 4 – The Effect of Endurance Training on Central and Peripheral Contributions to Fatigue: Implications for Exercise Tolerance

Background: Endurance training is a potent enhancer of exercise tolerance, however it is unknown whether training affects the fatigue mechanisms limiting exercise tolerance. Whilst anecdotally trained individuals are better able to tolerate muscle fatigue and muscle pain however there is little evidence to support this.

Aims: To examine the effect of high-intensity interval training on high-intensity exercise tolerance and the accompanying central and peripheral fatigue mechanisms compared to a control group completing an equal volume of training at a moderate-intensity.

Hypotheses: Markers of aerobic fitness will increase similarly in both groups, however a greater enhancement of exercise tolerance willd be seen following high-intensity interval training, which will be accompanied by greater tolerance of ischaemic muscle pain and peripheral fatigue with unchanged central fatigue.

Chapter 3 - General Methods

3.1 Summary

This Chapter highlights the common methods used throughout this thesis. The specifics and applications are presented within respective Chapters. Measurement of exercise capacity and exercise tolerance, as well as their reliability, are covered in section 3.8. As reviewed in Chapter 2, a number of protocols have been developed to measure corticospinal and neuromuscular function in order to understand the mechanisms of fatigue induced by locomotor exercise; the repeatability of these techniques is examined in section 3.9.

3.2 Pre-Trial Preparations

All procedures received approval from the Oxford Brookes University Research Ethics Committee (Appendix A) and were completed according to the latest Declaration of Helsinki. All experimental trials were completed within the Oxford Brookes' British Association of Sport and Exercise Science accredited Human Performance Laboratories.

3.3 Participants

All participants were recreationally active and free of illness, neuromuscular, neurological or cardiovascular disorder, non-smokers and not taking any drug known to affect the CNS. All experimental sessions were performed at a time of day that was kept constant for each participant. Participants arrived at the laboratory post-prandial (2–3 h) and after abstaining from caffeine (24 h), alcohol (48 h) and exhaustive exercise (48 h). The purpose, procedures and risks of each study were fully explained to each participant (Appendix B). A physical activity readiness questionnaire was completed to determine suitability to exercise (Appendix

C) followed by an adapted TMS suitability questionnaire (Appendix D) (Rossi et al., 2009, Rossi et al., 2011). Participants then provided written informed consent (Appendix E).

3.4 Exercise Responses

Cycle Ergometry

All exercise trials were completed on an electromagnetically braked cycle ergometer (Excalibur Sport, Lode, Netherlands). During the first visit, the seat and handlebar height were customised for each participant to ensure a comfortable fit and the bike characteristics were recorded and replicated. All tests were completed with the bike in hyperbolic mode and so cadence remained independent of power output.

Cardiopulmonary

Expired gases were sampled breath-by-breath using an online gas analyser (Metalyser 3B, Cortex, Germany) for the determination of oxygen uptake ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), minute ventilation ($\dot{V}_E$) and respiratory exchange ratio (RER). Expired gases passed through a low resistance volume transducer (Triple V, Cortex, Germany) and were sampled using a gas sample line (Cortex, Germany) connected to a face mask (Oro-Nasal 7450 V2 Mask, Hans Rudolph, USA). Before each exercise test, the analyser was calibrated according to manufacturer guidelines; the sample line was calibrated using ambient air and gases of a known concentration (15% O_2/5% CO_2), and flow volume was calibrated using a 3 L syringe (5530, Hans Rudolph, USA). Data were recorded (Metasoft, Cortex, Germany) and exported in 1 s epochs for subsequent analysis. Heart rate (HR) was measured continuously using an online telemetry system (T31, Polar, Finland) interfaced with the online gas analyser.

Blood Lactate

Blood lactate concentrations ([La$^-$]) were determined from a fingertip capillary whole-blood sample using a portable analyser (Lactate Pro, Arkray, Japan). The fingertip area was sterilised with an alcohol wipe and then punctured using a lancet. The first drop of blood was removed and the second drop was extracted for analysis. Finger-tip capillary samples accurately reflect venous and arterial [La$^-$] (Williams et al., 1992).

Ratings of Perceived Exertion

RPE was recorded using the Borg 15-point category scale (6–20) (Borg, 1982). The 6–20 scale is a valid indicator of the degree of physical effort during exercise and has demonstrated linear relationships with physical (power output or speed) and metabolic measures of exercise intensity where RPE is given in response to fixed work rates (Faulkner and Eston, 2007, Scherr et al., 2013). This relationship has also been demonstrated in 'active production' tasks where RPE is used to regulate intensity (Eston and Williams, 1988) demonstrating this tool is effective in guiding training intensity. Each participant was briefed regarding the use of RPE according to the guidelines outlined by Borg (1998).

3.5 Muscle Torque and Electromyography

Muscle Torque

Isometric torque (N·m) produced from the right knee extensors during voluntary and stimulated contractions was measured on a semi-recumbent fully adjustable strength chair (Figure 3.1). The chair had an adjustable moment lever, footplate and back support. The lever was attached to four strain gauges which measured rotational torque at the knee. The knee was flexed at 90° and the foot placed behind a padded resistance lever. The lever length was adjusted so the resistance pad was ~2 cm above the malleolus and the lever's centre of rotation was aligned to

the centre of the knee joint. The upper body was secured using straps across the shoulders and waist in order to isolate the knee extensors. This setup elicits the greatest activation of the knee extensors (Kubo et al., 2004) and is the method in which measurements of cortical VA have been validated (Goodall et al., 2009, Sidhu et al., 2009a). The chair characteristics were optimised for each participant during the first visit and then recorded and replicated in subsequent visits. An oscilloscope displaying torque on a computer screen was placed at eye level in front of the chair to assist in providing maximal effort during MVCs as well as providing the target force for submaximal contractions. The output from the torque transducer was amplified ($\times$1000, NL107, Neurolog Systems, Digitimer, UK), digitised (1000 Hz, Micro 1401 mk-II, CED, UK) and recorded on a PC for subsequent analysis (Spike 2 v.5.2, CED, UK). The chair was calibrated by suspending loads of a known mass across physiological ranges with the V-N·m linear relationship ($r^2 > 0.99$) providing the regression analysis for converting V to N·m.
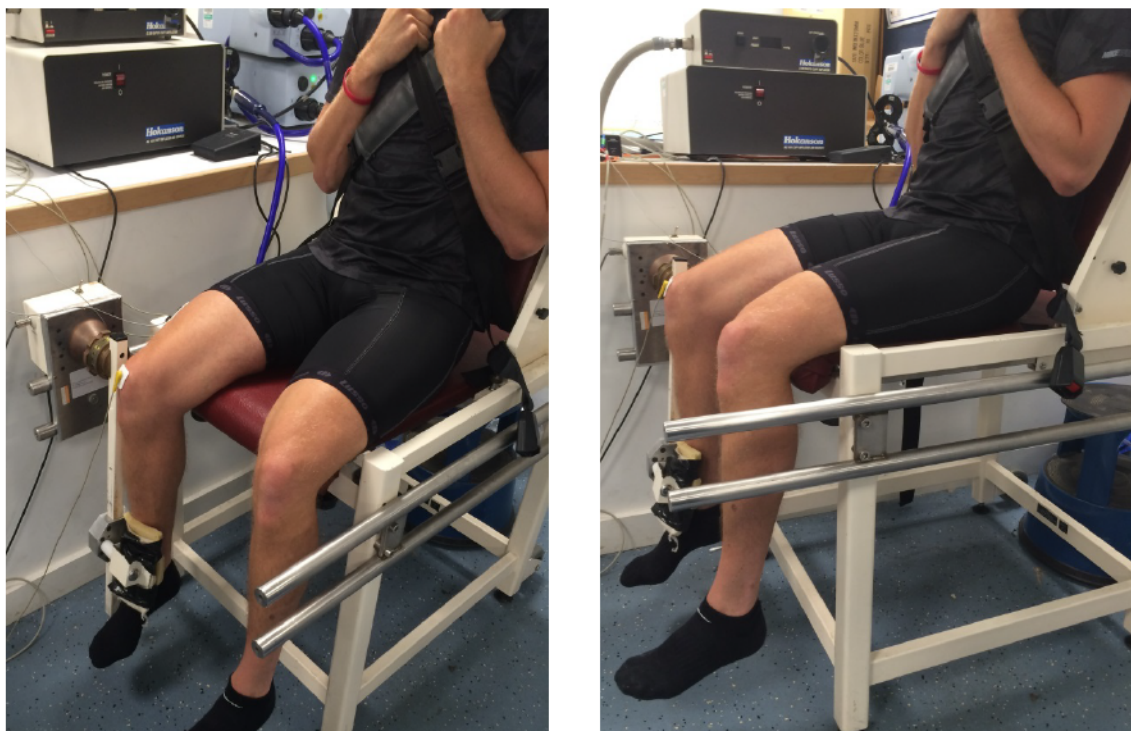
Figure 3.1. Strength chair used for recording muscle torque.

Electromyography

EMG was recorded using self-adhesive surface EMG bipolar Ag-AgCl electrodes (10 mm diameter, 2 cm inter-electrode distance). All electrode placements were made according to the guidelines set by the SENIAM research project (Hermens et al., 2000, SENIAM, 2014). Electrodes were placed on the belly of the right VL, at $^2/_3$ of the line from the anterior spina iliac superior to the lateral side of the patella, and the right biceps femoris (BF), at 50% of the mid line between the ischial tuberosity and lateral epicondyle of the tibia. A ground electrode was placed on the patella. Each area was shaved and cleaned using alcohol wipes and an abrasive gel. During within-day trials, the electrode remained in place throughout the day and during between-day trials, the electrode position was photographed to allow for accurate relocation. The VL was targeted for recordings, as in cycling research this muscle has been frequently studied in fatigue work (Amann et al., 2011, Goodall et al., 2012, Thomas et al., 2015a) and has shown to be directly driven by the motor cortex (Sidhu et al., 2012b, Sidhu et al., 2013b). The BF was monitored for antagonist co-activation during measures of cortical VA (Sidhu et al., 2009a). The EMG signal was amplified (×1000, NL844, Neurolog, Digitimer, UK), band-pass filtered with a 50 Hz notch (10 - 2000 Hz, NL135, Neurolog, Digitimer, UK), digitised (2000 Hz, Micro 1401 mk-II, CED, UK) and collected on PC for subsequent analysis (Spike 2 v.5.2, CED, UK).

3.6 Motor Nerve Stimulation

Knee extensor function was assessed with supramaximal magnetic stimulation of the femoral nerve (Polkey et al., 1996, Goodall et al., 2009, Verges et al., 2009, Tomazin et al., 2010, Decorte et al., 2012, Bachasson et al., 2013). Two monophasic MagStim 200 magnetic stimulators (BiStim², The MagStim Company Ltd, UK) were discharged through a 55 mm double branding iron coil (maximum output 2.2T, 1 ms pulse duration) (Figure 3.2). The

branding iron coil is designed for stimulation of deep lying nerves and higher powered than coils used for cortical stimulation. Smaller branding iron coils produce greater activation than other coils (i.e. 70 mm flat coils) due to more concentrated delivery of the magnetic field (Tomazin et al., 2010). Two magnetic stimulators allow summation of the power from each Magstim 200^2 in order to give a higher intensity pulse to ensure supramaximal stimulation (Hamnegard et al., 2004). MNS is less painful than ENS (Han et al., 2006) allowing supramaximal stimulation which can be problematic with ENS due to tolerance of the required intensity (Millet et al., 2011). MNS has been validated against ENS, producing comparable electrophysiological and contractile responses in the rested and fatigued knee extensors without producing antagonist co-activation (Verges et al., 2009).
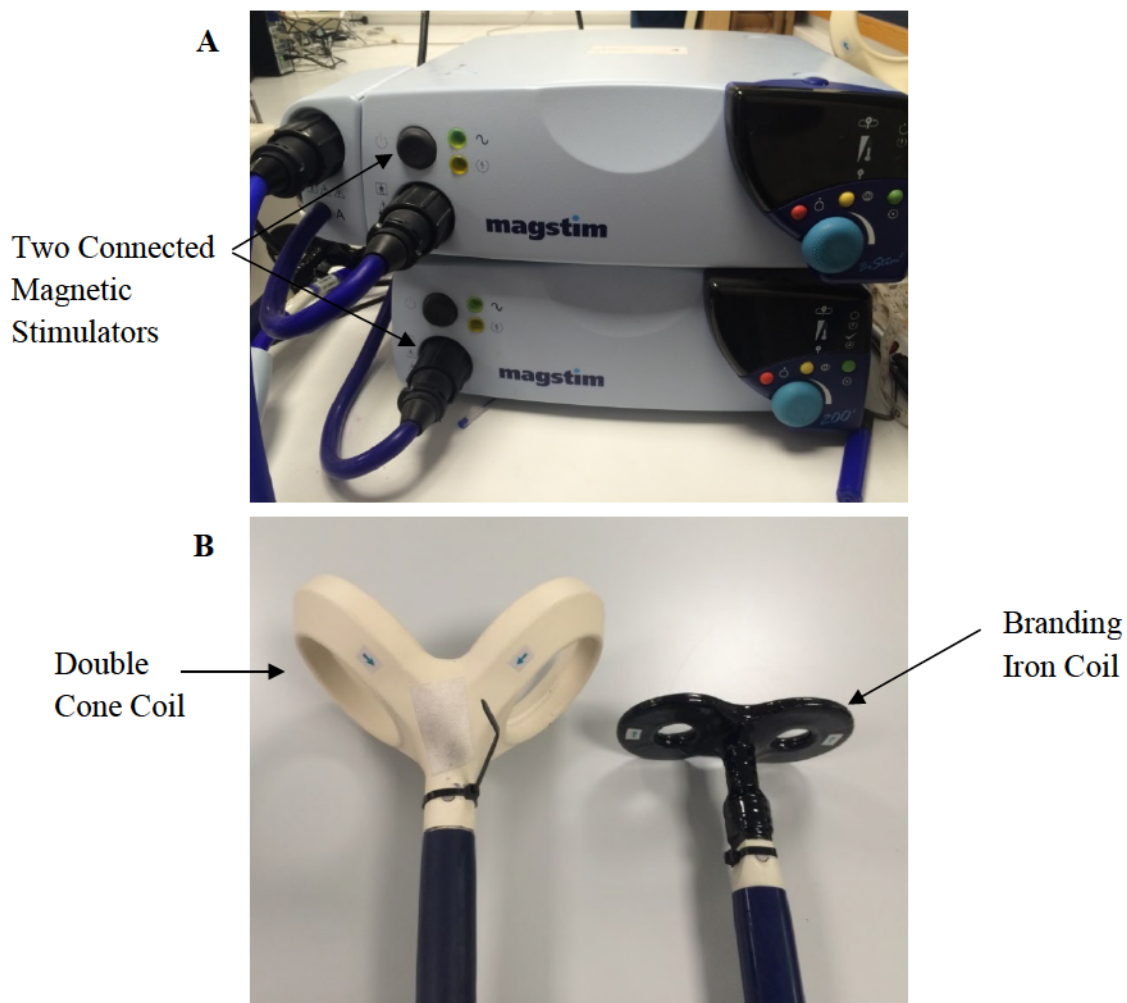
Figure 3.2. Magnetic stimulators (A) and stimulating coils (B) used in these experiments.

The femoral triangle was palpated to locate the femoral nerve and the coil was initially placed on the motor point high in the femoral triangle lateral to the femoral artery (Figure 3.3) (Polkey et al., 1996). The site evoking the largest unpotentiated quadriceps twitch ($Q_{tw,unpot}$) and EMG response (M-wave) at rest was determined with the coil pressed firmly in the femoral groove. The area was marked on the skin using indelible ink for repeat application. In order to ensure stimulation was supramaximal, a stimulus-response curve was constructed for each participant during each visit. Two muscle responses were recorded every 20 s at each of the stimulator outputs (SO) of 50%, 60%, 70%, 80%, 90% 95% and 100% of maximum (Figure 3.4). A plateau in $Q_{tw,unpot}$ and M-wave amplitude ensures maximal depolarisation of the femoral nerve (Polkey et al., 1996, Amann et al., 2006a, Romer et al., 2006a, Goodall et al., 2009, Tomazin et al., 2010, Decorte et al., 2012). All subsequent stimulations were at 100% SO. The repeatability of femoral MNS measurement protocols of peripheral and central fatigue are discussed in section 3.9.

Figure 3.3. Magnetic stimulation of the femoral nerve.
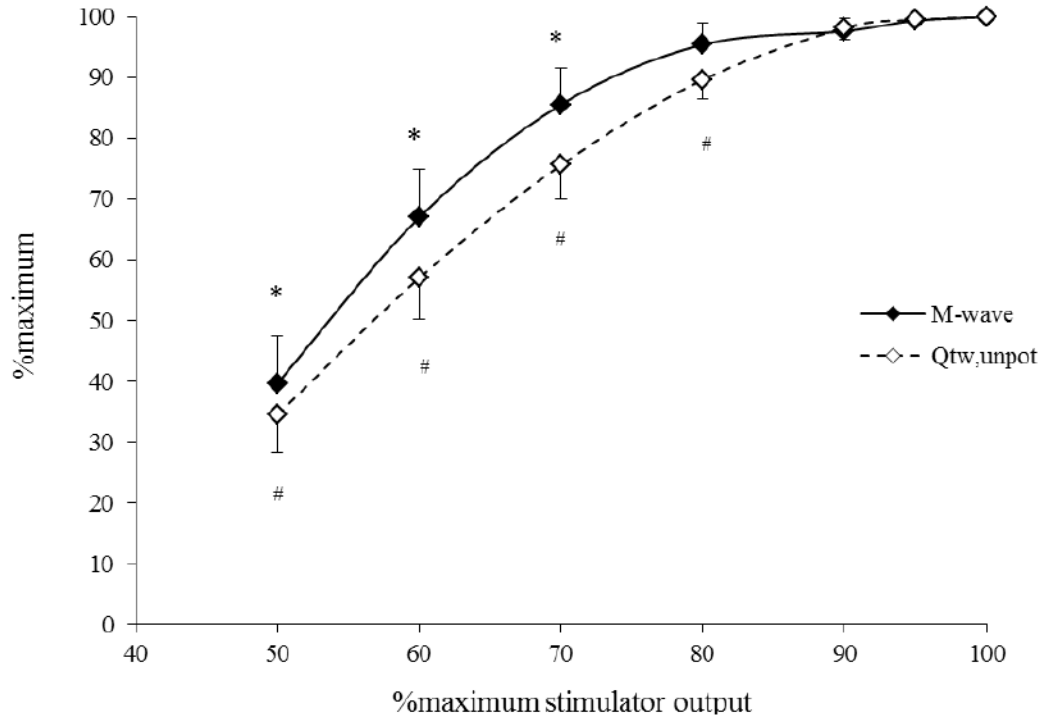
Figure 3.4. M-wave and $Q_{tw,unpot}$ responses to magnetic stimulation of the femoral nerve from the stimulus-response curve.

*$P < 0.05$ vs 100% for M-wave. #$P < 0.05$ vs 100% for $Q_{tw,unpot}$.

3.7 Transcranial Magnetic Stimulation

All TMS procedures were performed over the left motor cortex in order to cause activation in the contralateral right VL. Two connecting monophasic Magstim 200 magnetic stimulators (BiStim^2, The MagStim Company, UK) were discharged through a 110 mm double cone coil (maximum output 1.4T, 1 ms pulse duration) (Figure 3.2). The cortical representation of the lower limbs lie ~3–4 cm deep at the interhemispheric fissure (Terao and Ugawa, 2002) of which the double cone coil is designed for activating (Rossi et al., 2009). The junction of the coil was held tangentially to the sagittal plane above the vertex and orientated so as to induce an intracranial posterior to anterior current. This orientation activates a significant proportion of knee extensors motor units (Goodall et al., 2009, Sidhu et al., 2009a) and effectively recruits the knee extensor intracortical circuitry (Sidhu et al., 2013b). The mid-point of the lines

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between the nasion and inion and both preauricular points were measured and marked on the scalp; the intersection of these two points was taken as the vertex. The search for the optimal location for the VL began with the coil placed 1 cm to the lateral of the vertex over the left hemisphere with the SO set at 60% of maximum. The optimal location or 'hotspot' for activating the VL lies ~1–2 cm left of the vertex (Goodall et al., 2009, Sidhu et al., 2013b). Single TMS pulses were administered during brief (~3–5 s) 10% MVC contractions. The coil was moved in 1 cm steps laterally and medially, and anteriorly and posteriorly, until the site that elicited the largest VL MEP was identified. This site was marked by drawing around the coil on the scalp using permanent marker to ensure accurate relocation within-session and defined as the 'hotspot.' This location was held consistent within each trial. For between-day retesting, the optimal location was reassessed.

The resting motor threshold (rMT) was determined by reducing the SO until the lowest intensity that elicited a minimum MEP of 50 µV in 5 out of 10 trials was identified with the muscle relaxed (Rossini et al., 2015). Active motor threshold (aMT) was determined during an isometric contraction of 10% MVC and was taken as the SO required to elicit a minimum response of 200 µV in 5 out of 10 trials (Ortu et al., 2008, Groppa et al., 2012) or an MEP response distinguishable from back ground activity (Sidhu et al., 2013b). The target level of torque was displayed on the screen and each participant was instructed to maintain the 10% MVC as closely as possible. Each contraction was held in 30 s blocks separated by 30 s rest until the aMT was determined. Both the rMT and aMT were determined to the nearest 1%.

3.8 Assessment of Exercise Capacity and Exercise Tolerance: A Reliability Study

3.8.1 Introduction

Accurate performance testing is a key aspect of exercise physiology. It is therefore imperative to understand the repeatability of tests that examine exercise capacity and exercise tolerance. Cycling tests of various intensities and durations have been employed to induce and investigate fatigue (sections 2.3.1 – 2.3.3). Cycling avoids the confound of muscle damage when examining post-exercise muscle performance compared to running (Millet and Lepers, 2004). Cycle to exhaustion tests are important determinants of exercise performance (Coyle et al., 1988), sensitive to changes in endurance induced by training (Burgomaster et al., 2005), as well as important methods to examine exercise tolerance via inducing significant peripheral and central fatigue (Tables 2.1 and 2.2). Compared with time-trials or fixed duration exercise tests, TTE tests allow the mechanisms associated with task failure (exhaustion) to be studied. The constant workload also allows a controlled level of metabolic strain to be elicited in order to examine the mechanisms of fatigue. The aim of this section is determine the reliability of tests of exercise capacity (LT, lactate turnpoint, LTP and $\dot{V}O_{2max}$) and exercise tolerance (TTE).

3.8.2 Methods

3.8.2.1 Participants

Ten recreationally active males (mean ± SD, age 27 ± 5 years, height 1.82 ± 0.05 m, mass 82.7 ± 9.8 kg) volunteered to participate in the first part of this study. A further ten recreationally active males (age 27 ± 5 years, height 1.75 ± 0.10 m, mass 80.3 ± 10.3 kg) volunteered to participate in the second part of this study.

3.8.2.2 Experimental Procedures

In the first part of this study, ten participants completed a series of exercise capacity tests on two separate occasions; a submaximal test for the determination of the LT and LTP, followed 20 min later by a maximal test for the determination of $\dot{V}O_{2max}$ and $\dot{W}_{max}$. In the second part of the study, a different ten participants completed three visits, the first of which involved completing a LT and $\dot{V}O_{2max}$ test. The remaining two visits each involved the completion of a TTE trial at an intensity equal to halfway between the LT and $\dot{V}O_{2max}$ (50%Δ). All visits were completed at the same time of day and separated by $\geq$5 d.

3.8.2.3 Exercise Tests

Lactate Threshold and Lactate Turnpoint

The LT and LTP were determined from a submaximal incremental step test. Each participant completed a series of 4 min stages beginning at 50 W with the intensity increased 20-25 W every 4 min (Carter et al., 2000). A finger-tip [La⁻] sample taken at the end of each stage and steady-state $\dot{V}O_2$ and HR were taken as the average of the last 60 s of each stage. The test was terminated once a [La⁻] of $\geq$4 mmol·l⁻¹ was recorded. The LT was defined as the first sudden and sustained increase in [La⁻] above resting levels (Carter et al., 2000). The LTP was defined as the second sudden and sustained increase in [La⁻] between LT and $\dot{V}O_{2max}$ (Smith and Jones, 2001). The LT and LTP were identified by two independent reviewers from graphs of the power output - [La⁻] relationship (Figure 3.5). Although complex, the mechanisms determining the LT relate to the oxidative capacity of the muscle (Joyner and Coyle, 2008). The LT is a key determinant of exercise tolerance (Coyle et al., 1988), sensitive to endurance training (Jones and Carter, 2000) and an important marker in the prescription of training intensity (Faude et al., 2009).
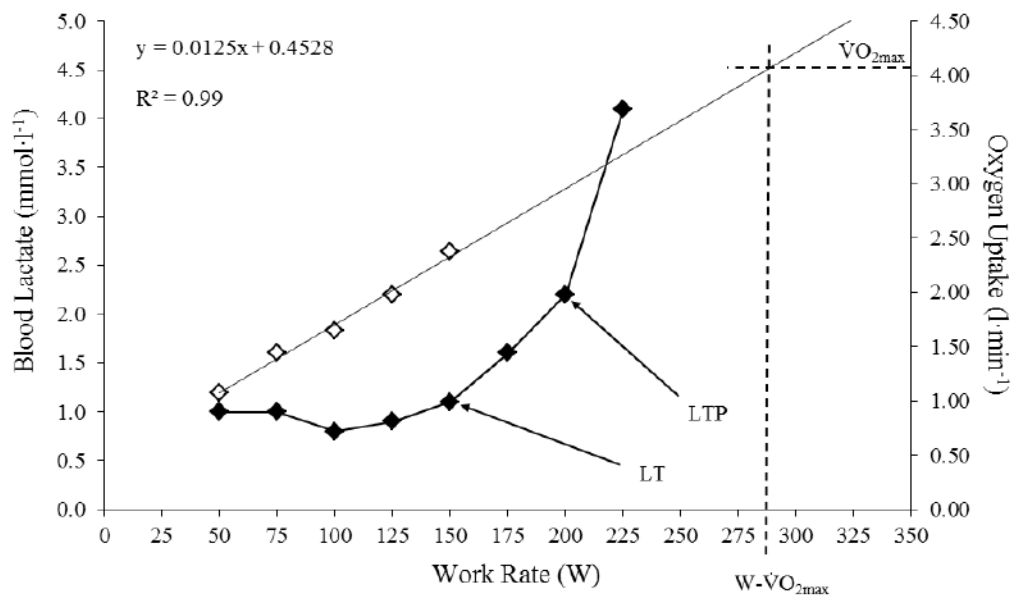
Figure 3.5. Relationship between power output and capillary lactate (black diamonds) and oxygen uptake (empty diamonds) during the lactate threshold test from a representative participant. Extrapolation of oxygen uptake to maximal oxygen uptake can be seen.

LT, lactate threshold; LTP, lactate turnpoint; $\dot{V}O_{2max}$, maximal oxygen uptake; $\dot{W}$-$\dot{V}O_{2max}$, power output at maximal oxygen uptake

Maximal Oxygen Uptake

$\dot{V}O_{2max}$ was determined from an incremental ramp protocol (Figure 3.6). The test began with one min at 100 W before a ramp increase of 25 $W \cdot min^{-1}$ until exhaustion, defined as an inability to maintain a cadence above 60 rpm. Expired gases and HR were collected continuously throughout the test and RPE and [La⁻] were determined at the test termination. The attainment of $\dot{V}O_{2max}$ was confirmed according to the following criteria: end [La⁻] ≥ 8 $mmol \cdot l^{-1}$, peak RER ≥ 1.15, end RPE ≥ 19, peak HR $\geq 90\%$ age-predicted maximum and volitional exhaustion (Midgley et al., 2007). Due to critique of these methods as individual criteria, it was ensured that all were satisfied (Midgley et al., 2007). Maximal heart rate (HR_{max}) was taken as the peak HR recorded. $\dot{V}O_{2max}$ was determined as the highest 30 s average recorded $\dot{V}O_2$ before volitional exhaustion (Morris et al., 2012) and $\dot{W}_{max}$ was defined as the corresponding average power output of the last 30 s.

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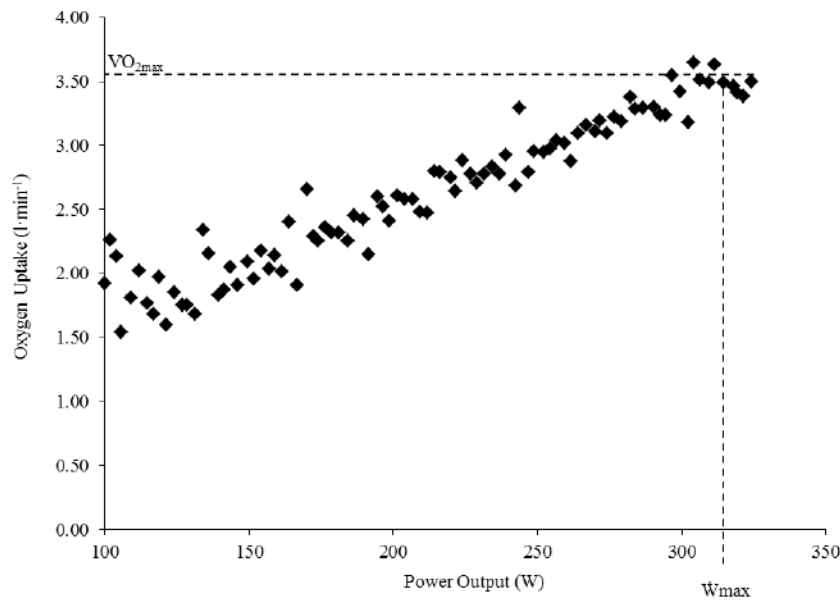
Figure 3.6. Oxygen uptake recorded during the ramp test from a representative participant.

Prescription of Exercise Intensity

Methods of exercise intensity prescription have been discussed in section 2.4. Based on those conclusions, the %Δ concept was used to prescribe exercise intensity where Δ represents the difference between the LT and $\dot{V}O_{2max}$ (Carter et al., 2000). This ensures comparable physiological, metabolic and perceptual responses between individuals (Carter et al., 2000, Mann et al., 2013). $\dot{V}O_2$ was plotted against power output recorded from the LT test and extrapolated to $\dot{V}O_{2max}$ in order to determine the power output at $\dot{V}O_{2max}$ (W-$\dot{V}O_{2max}$) (Figure 3.5). Only sub-LT power outputs were included due to the $\dot{V}O_2$ slow component at supra-LT intensities. The equation derived from the linear power output - $\dot{V}O_2$ relationship was then used to prescribe subsequent exercise intensity. Moderate intensity exercise was set as the power output required for 90% of the LT (Chapter 5). This was to ensure a metabolic steady-state was achieved (no $\dot{V}O_2$ slow component or [La$^-$] increase). In contrast, high-intensity exercise was prescribed at 50%Δ (half way between LT and $\dot{V}O_{2max}$): 50%Δ = LT + (0.5 x ($\dot{V}O_{2max}$ - LT)) (Chapters 5 and 6). This was to induce significant metabolic and physiological stress ($\dot{V}O_2$ slow component and [La$^-$] accumulation) and no steady-state (Carter et al., 2000).

The prescription of 50%Δ is the most common form of exercise prescription to elicit a 'heavy' exercise demand and significant $\dot{V}O_2$ slow component (Burnley and Jones, 2007) and so allows comparison with previous work examining exercise tolerance. This intensity is also above MLSS and CP (Pringle and Jones, 2002). CP during cycling is reported to occur between ~40–46%Δ (Poole et al., 1988, Pringle and Jones, 2002, Coats et al., 2003, Burnley et al., 2006, Vanhatalo et al., 2007) however it could occur as high as ~50%Δ (Vanhatalo et al., 2011), whereas MLSS is reported to occur at ~35%Δ (Pringle and Jones, 2002). Direct determination of MLSS or CP requires multiple exercise tests over separate days. As the MLSS and CP are considered to occur at an equivalent exercise intensity (Burnley and Jones, 2007, Keir et al., 2015), [La⁻] measurements allow approximation of the intensity domains, with the LTP providing an accurate estimate of MLSS (Faude et al., 2009).

Cycle to Exhaustion

Each cycle to exhaustion test was preceded by a 5 min warm-up and completed at a self-selected cadence (≥60 rpm). The test was terminated when the participant could no longer maintain a cadence above 60 rpm or they fell below this cadence three times. Participants were given no physiological feedback and were unaware of expired time. Strong verbal encouragement was given to encourage each participant to continue for as long as possible.

3.8.2.4   Statistical Analysis

Statistical analyses were completed in SPSS for Windows v21 (SPSS Inc., USA). Differences between trials were tested using paired-sampled t-tests. Reliability was tested with a two-way mixed-effect models intraclass correlation coefficient ($ICC_{3,1}$) with 95% confidence intervals (95%CI) for absolute agreement and mean bias (±95%CI). Variability was assessed with the coefficient of variation (CV): SD / mean x 100.

3.8.3 Results

Exercise Capacity

There was no difference between trials 1 and 2 for measures of LT (124 ± 39 vs 125 ± 37 W; 53 ± 10 vs $54 \pm 9\%$ $\dot{V}O_{2max}$), LTP (182 ± 41 vs 175 ± 44W; 69 ± 10 vs $69 \pm 8\%$ $\dot{V}O_{2max}$), $\dot{V}O_{2max}$ (3.60 ± 0.68 vs 3.61 ± 0.63 l·min^{-1}), $\dot{W}$-$\dot{V}O_{2max}$ (300 ± 56 vs 302 ± 53 W) and $\dot{W}_{max}$ (324 ± 43 vs 324 ± 48 W), respectively (all $P > 0.05$). All markers of exercise capacity demonstrated good repeatability with minor variability (ICC ≥ 0.73, CV $\leq 5.7\%$) (Table 3.1).

Exercise Tolerance

The 50%Δ elicited an intensity of ~80% $\dot{V}O_{2max}$ which was above the LTP in all participants (Table 3.2). There was no difference between trials 1 and 2 for time to exhaustion (19.9 ± 7.9 vs 21.7 ± 8.1 min) which also demonstrated good reliability and minor variability (Table 3.1).

Table 3.1. Reliability and variability of exercise capacity and exercise tolerance.

	Mean bias (95%CI) Visit 1 − Visit 2	ICC$_{(3,1)}$ (95% CI)	CV (%)	P
Exercise Capacity				
LT (W)	1 (−8 − 6)	0.97 (0.90 − 0.99)	2.9	0.76
LT (%$\dot{V}O_{2max}$)	1.5 (−4.4 − 1.3)	0.93 (0.76 − 0.98)	5.7	0.26
LTP (W)	9 (−2 − 19)	0.96 (0.82 − 0.99)	3.2	0.11
LTP (%$\dot{V}O_{2max}$)	0 (−5 − 5)	0.73 (0.20 − 0.93)	5.1	0.99
$\dot{V}O_{2max}$ (l·min^{-1})	0.00 (−0.09 − 0.09)	0.98 (0.90 − 0.99)	2.2	0.94
$\dot{W}$- $\dot{V}O_{2max}$ (W)	−3 (−18 − 13)	0.93 (0.74 − 0.98)	3.9	0.71
$\dot{W}_{max}$ (W)	2 (−8 − 11)	0.95 (0.82 − 0.99)	2.3	0.69
Exercise Tolerance				
50%Δ TTE (min)	−1.8 (−4.3 − 0.8)	0.90 (0.65 − 0.97)	11.8	0.15

LT, lactate threshold; LTP, lactate turnpoint; $\dot{V}O_{2max}$, maximal oxygen uptake; $\dot{W}$-$\dot{V}O_{2max}$, power output at maximal oxygen uptake; $\dot{W}_{max}$, peak power output; TTE, time to exhaustion.

Table 3.2. Comparison of the power output at the LTP and 50%Δ.

Participant	LTP		50%Δ	
	(W)	(%$\dot{V}O_{2max}$)	(W)	(%$\dot{V}O_{2max}$)
1	210	73	233	81
2	190	66	236	82
3	170	60	219	76
4	130	52	206	78
5	170	70	204	79
6	130	60	182	80
7	150	76	178	87
8	210	62	273	76
9	150	55	217	73
10	190	75	199	79
Mean	**170**	**65**	**215***	**79***
SD	**28**	**8**	**27**	**4**

*$P < 0.01$ vs LTP

3.8.4 Discussion

Measures of submaximal and maximal exercise capacity were reliable and demonstrated minor variability. TTE was highly repeatable but demonstrated greater variability. Whilst the physiological determinants of submaximal and maximal exercise capacity are defined, the fatigue mechanisms underpinning exhaustion are poorly understood and likely multifactorial, which may contribute to greater variability. As 50%Δ elicited an intensity above LTP and the TTE was considerably shorter than those reported at CP (Brickley et al., 2002) and MLSS (Baron et al., 2008), this appears a suitable method to elicit severe intensity exercise. This intensity also corresponded to ~80% $\dot{V}O_{2max}$, similar to exercise tolerance tests employed previously (Burgomaster et al., 2005). These performance measures therefore appear suitable to examine exercise capacity and exercise tolerance.

3.9 Assessment of Corticospinal and Neuromuscular Function in the Knee Extensors: A Reliability Study

3.9.1 Introduction

Both spTMS and ppTMS offer a non-invasive and painless method for assessing human CNS physiology (Hallett, 2000) and have been shown to be important tools for understanding central mechanisms of fatigue (Gandevia, 2001, Gruet et al., 2013, Sidhu et al., 2013a). spTMS MEP amplitudes demonstrate significant pulse-to-pulse variability due to constant fluctuations in corticospinal excitability (Kiers et al., 1993, Ellaway et al., 1998). This variability is reduced by taking measurements in an active muscle compared to the relaxed condition (Darling et al., 2006), increasing the stimulation intensity (Pitcher et al., 2003, Darling et al., 2006) or by increasing the number of pulses (Cuypers et al., 2014, Lewis et al., 2014). Maintenance of a low level of background force with a visual display can help control for corticospinal excitability as well as any attentional and somatosensory affects (Darling et al., 2006). Furthermore, obtaining measurements in relaxed muscle compared to active muscle may not be indicative of motor cortical function during fatigue (Gruet et al., 2013). Nevertheless, MEP amplitudes have shown to be reliable in the relaxed and active muscles of the upper (Carroll et al., 2001, Kamen, 2004, Ngomo et al., 2012) and lower limbs (van Hedel et al., 2007, Cacchio et al., 2011, Tallent et al., 2012, Lewis et al., 2014).

Despite its increasing popularity, surprisingly few studies have examined the reliability of ppTMS. High variability has been reported between individuals (Boroojerdi et al., 2000, Wassermann, 2002, Orth et al., 2003, Du et al., 2014) as well as between- and within-days (Boroojerdi et al., 2000, Orth et al., 2003). Some studies have reported that SICI but not ICF could be reliably measured (Maeda et al., 2002, Fleming et al., 2012) whereas others have found good reliability for both measures (Du et al., 2014) as well as LICI (Farzan et al., 2010)

in the relaxed muscles of the hand. Comparisons between studies are difficult due to a number of methodological issues such as the muscle tested and its activity, the stimulation protocol (i.e. intensity and number of pulses, ISI or type of stimulating coil) and data analysis technique. For example, very few studies have examined reliability using accepted reliability analyses (ICC) and none have used an active muscle contraction.

There is a body of work examining cortical function in the small muscle groups of the upper limbs, however more recently TMS has been shown to be a valuable tool to examine the larger muscle groups of the knee extensors to investigate mechanisms of locomotion (Sidhu et al., 2012b, Sidhu et al., 2013b), central fatigue resulting from endurance exercise (Verin et al., 2004, Ross et al., 2010b, Gruet et al., 2013, Sidhu et al., 2013a, Sidhu et al., 2013c) as well as revealing neural adaptations to exercise training (Goodwill et al., 2012, Weier et al., 2012). Unlike studies in small isolated muscles, locomotor exercise studies require investigations involving the larger muscle groups of the knee extensors. Many studies have examined TMS responses at multiple time-points either within a day (Verin et al., 2004, Sidhu et al., 2013b) (i.e. in response to fatigue) or over multiple visits (Goodwill et al., 2012, Weier et al., 2012) (i.e. in response to training). In order to functionally quantify neural responses to acute or chronic interventions, such as fatigue and exercise training, it is important to understand the sensitivity of the TMS measure to detect change both within- and between-days. A reliable technique to examine neural function in this muscle group may help in understanding mechanisms of fatigue that affect the knee extensors restricting locomotor exercise.

Despite the increased utility of TMS as a research tool only a few studies have investigated the reliability of MEP amplitudes in the knee extensors (Sidhu et al., 2009a, Wheaton et al., 2009, Luc et al., 2014). Of these studies only one has examined the reliability of MEP amplitudes in

the VL (Wheaton et al., 2009). These authors reported good reliability in the active VL in chronic hemiparetic stroke survivors in the non-paretic side only. Although ppTMS has been previously measured in the knee extensors (Chen et al., 1998, Verin et al., 2004, Goodwill et al., 2012, Weier et al., 2012, Sidhu et al., 2013b, Stevens-Lapsley et al., 2013), the reliability of this technique has yet to be described.

TMS has also been employed to assess cortical VA of the knee extensors (Sidhu et al., 2009a, Goodall et al., 2009). This technique has contributed to the understanding of supraspinal fatigue following locomotor exercise (section 2.3.2), as well as training adaptation (Zghal et al., 2014). Compared with motor nerve stimulation derived measures of VA (peripheral VA), which cannot discriminate between spinal and supraspinal mechanisms, cortical VA allows assessment of neural drive from the motor cortex (Todd et al., 2003). Motor nerve stimulation however allows assessment of muscle function alongside peripheral VA. MNS offers an attractive alternative to ENS due to pain free application (Han et al., 2006) and has been validated against ENS, producing comparable electrophysiological, contractile and peripheral VA responses in the rested and fatigued state (Newman et al., 2003, Verges et al., 2009), whilst also giving reliable measures of central and peripheral fatigue following exhaustive exercise (Bachasson et al., 2013). Although a few studies have reported good test-retest reliability of cortical VA (Sidhu et al., 2009a, Goodall et al., 2009) and MNS measures of neuromuscular function (Bachasson et al., 2013, Bachasson et al., 2014), a comprehensive longer-term analysis (>21 d) of the reliability of these techniques has yet to be conducted. The aim of the present study was to comprehensively investigate the reliability of TMS in a large locomotor muscle. MEP amplitude, cSP duration, SICI, LICI and ICF in the active VL were studied to determine within- and between-day reliability. Secondary aims were to examine the reliability of cortical VA and MNS measures of neuromuscular function.

3.9.2 Methods

3.9.2.1   Participants

Sixteen healthy males (mean ± SD, age 26 ± 5 years, height 1.80 ± 0.08 m, mass 80.7 ± 10.0 kg) volunteered to participate in the study.

3.9.2.2   Neuromuscular and Corticospinal Function

Maximal Voluntary Isometric Torque

Maximal voluntary isometric contractions (MVC) of the knee extensors were performed according to the guidelines of Gandevia (2001): i) MVCs were preceded by clear instruction and practice; ii) feedback was displayed on an oscilloscope placed directly in front of the chair; iii) verbal encouragement was delivered by the same investigator; iv) participants were allowed to discard any contractions judged not to be maximal, and; v) 'real time' visual feedback was disguised so post-exercise decrements could not be calibrated against pre-exercise. Isometric contractions of the knee extensors produce greater peripheral VA (Babault et al., 2001) and are more sensitive to detecting central fatigue than concentric contractions (Babault et al., 2006). A post-exercise reduction in MVC is considered a marker of global fatigue (Gandevia, 2001). Once secured into the chair, participants were instructed to hold their arms across the chest and to concentrate on delivering the highest level of torque possible against the lever plate as displayed on an oscilloscope on the computer screen. The root mean square (rms) amplitude of the EMG signal accompanying each MVC was calculated to determine the power of the voluntary EMG (De Luca, 1997) which represents the net activity of the motor unit pool (recruitment and discharge rates) (Farina et al., 2004). The rms amplitude was analysed during a 1 s window at peak torque and expressed relative to the M_{max} rms (rms/rmsM) to account for changes in muscle excitability (Lepers et al., 2002).

Peripheral Voluntary Activation

Peripheral VA was measured using the ITT (Merton, 1954). A single stimulation was applied during the MVC at peak torque to elicit a SIT; a further stimulation was given at rest ~3 s after the MVC in order to produce a potentiated quadriceps twitch ($Q_{tw,pot}$) which was also used for the determination of peripheral fatigue (Figure 3.7A). The size of the SIT and $Q_{tw,pot}$ were determined by subtracting twitch onset torque from peak twitch torque (Figure 3.7B) (Sidhu et al., 2009a). MNS produces reliable estimates of peripheral VA in the knee extensors (Amann et al., 2006b, Romer et al., 2006a, Romer et al., 2006b, Amann and Dempsey, 2008, O'Brien et al., 2008, Goodall et al., 2009), similar to those produced with ENS (Newman et al., 2003, O'Brien et al., 2008), and has been used extensively to examine central fatigue following cycling (Amann et al., 2006b, Romer et al., 2006a, Amann et al., 2007, Amann and Dempsey, 2008, Amann et al., 2009, Amann et al., 2011, Decorte et al., 2012, Bowtell et al., 2013, Rossman et al., 2014).
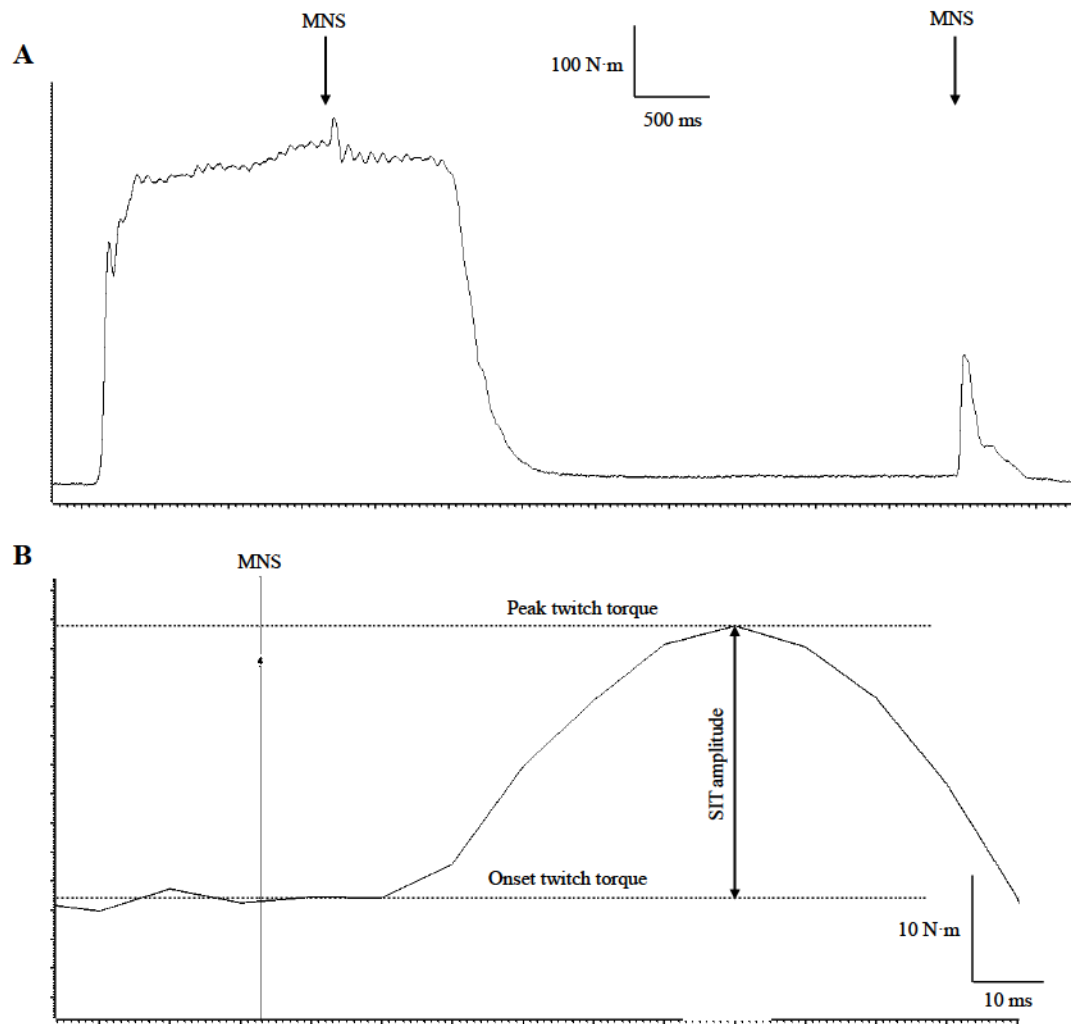
Figure 3.7. A, raw torque trace for calculation of peripheral voluntary activation. B, calculation of the superimposed twitch torque during the MVC.

MNS, magnetic nerve stimulation; SIT, superimposed twitch.

Cortical Voluntary Activation

Cortical VA was determined using the methods originally described in the elbow flexors (Todd et al., 2003, Todd et al., 2004) and more recently validated in the knee extensors (Goodall et al., 2009, Sidhu et al., 2009a). Single TMS pulses at 130% rMT were applied at the optimal location for evoking responses in the VL during a set of contractions at 50%, 75% and 100% of MVC (Figure 3.8A) (Goodall et al., 2009). Participants had the target torque displayed on the screen and were instructed to reach and maintain this level of torque. The peak-to-peak

amplitude of the VL and BF MEPs were expressed relative to the peak-to-peak amplitude of the VL M_{max} recorded during the MVC ($MVCM_{max}$) (Sidhu et al., 2009a). This was to ensure a large VL MEP ($\geq 50\%$ $MVCM_{max}$) and small antagonist BF MEP ($\leq 10\%$ $MVCM_{max}$) were evoked, confirming a large number of motoneurons were recruited with minimal antagonist co-activation (Goodall et al., 2009, Sidhu et al., 2009a). The SIT response to each TMS pulse was calculated by subtracting twitch onset torque from peak twitch torque (Sidhu et al., 2009a). The resting twitch was estimated (ERT) from linear regression between the voluntary torque and the SIT torque (Figure 3.8B) and used for calculation of cortical VA (Todd et al., 2003, Todd et al., 2004). The linearity of this relationship was examined to ensure accurate determination of the ERT (Goodall et al., 2009, Sidhu et al., 2009a).
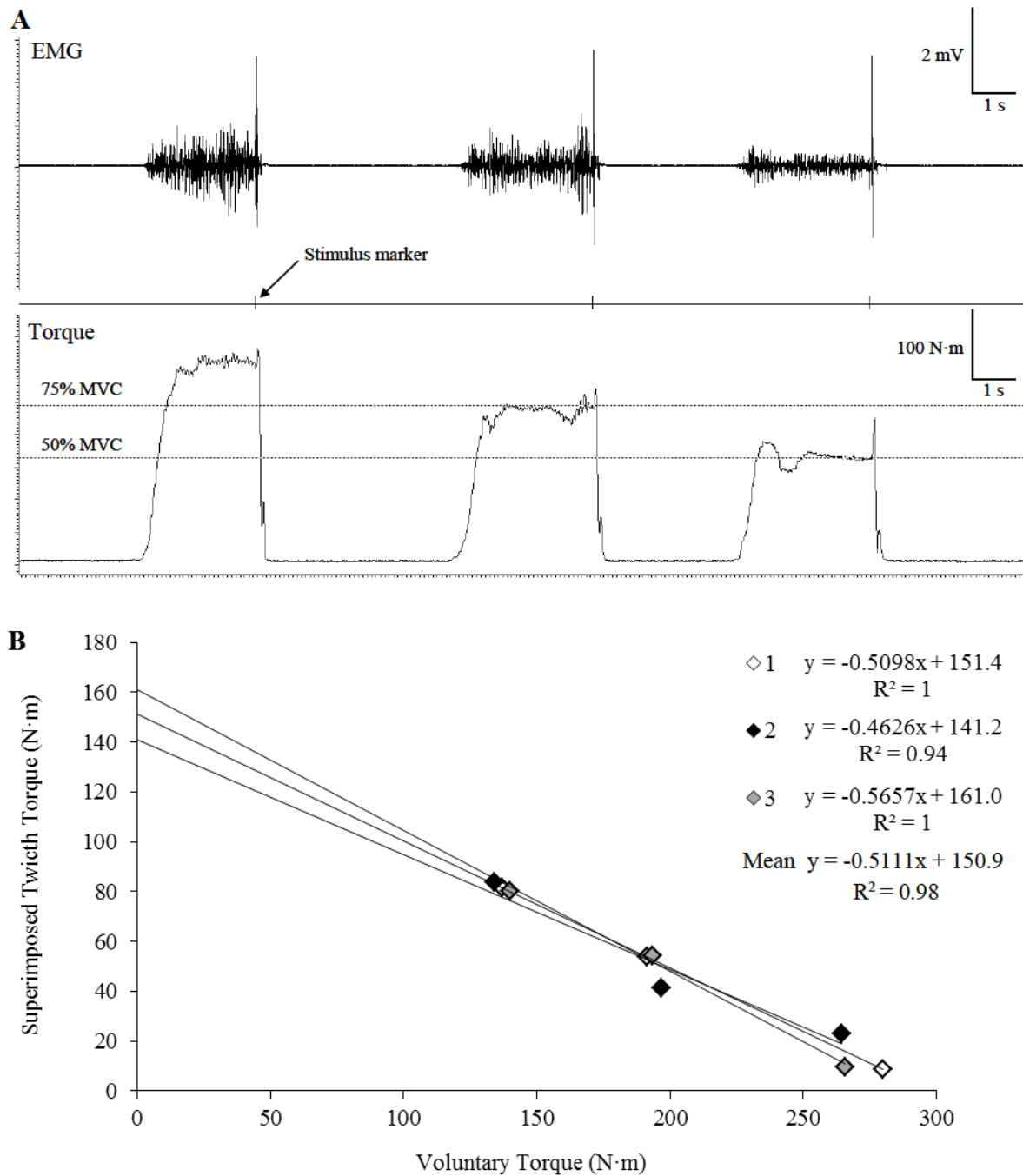
Figure 3.8. Calculation of cortical voluntary activation from a representative participant. A, raw EMG and torque trace for one set of contractions (50%, 75% and 100% MVC) with target torque highlighted. B, estimation of the resting twitch based on linear regression between voluntary and superimposed torque.

Contractile Function

Knee extensor contractile function was examined from the $Q_{tw,pot}$ succeeding each MVC (Figure 3.9A). The prior MVC potentiates the muscle twitch by increasing the sensitivity of actin-myosin to Ca^{2+} from myosin light chain phosphorylation (Palmer and Moore, 1989). The $Q_{tw,pot}$ is less variable and a more sensitive indicator of peripheral fatigue than the $Q_{tw,unpot}$ (Kufel et al., 2002) and due to possible potentiating effects of exercise (Place et al., 2004) is preferred for peripheral fatigue assessment. Femoral MNS produces reliable measurements of knee extensor fatigue (Bachasson et al., 2013), similar to the fatigue-induced impairments in contractile and electrophysiological measured by ENS (Verges et al., 2009), and has been used extensively to quantify peripheral fatigue following cycling (Amann et al., 2006a, Romer et al., 2006a, Amann et al., 2007, Amann and Dempsey, 2008, Amann et al., 2009, Amann et al., 2011, Decorte et al., 2012, Bowtell et al., 2013, Rossman et al., 2014). The following characteristics were calculated from each $Q_{tw,pot}$: peak torque; contraction time (CT), defined as the time from twitch onset to peak torque; rate of torque development (RTD), defined as peak torque / CT, and; half relaxation time (0.5RT), defined as the time between peak torque and return to half that value (Figure 3.9B) (Lepers et al., 2000, Lepers et al., 2002, Morris et al., 2012). The M_{max} accompanying each $Q_{tw,pot}$ was analysed for peak-to-peak amplitude (Figure 3.9C).
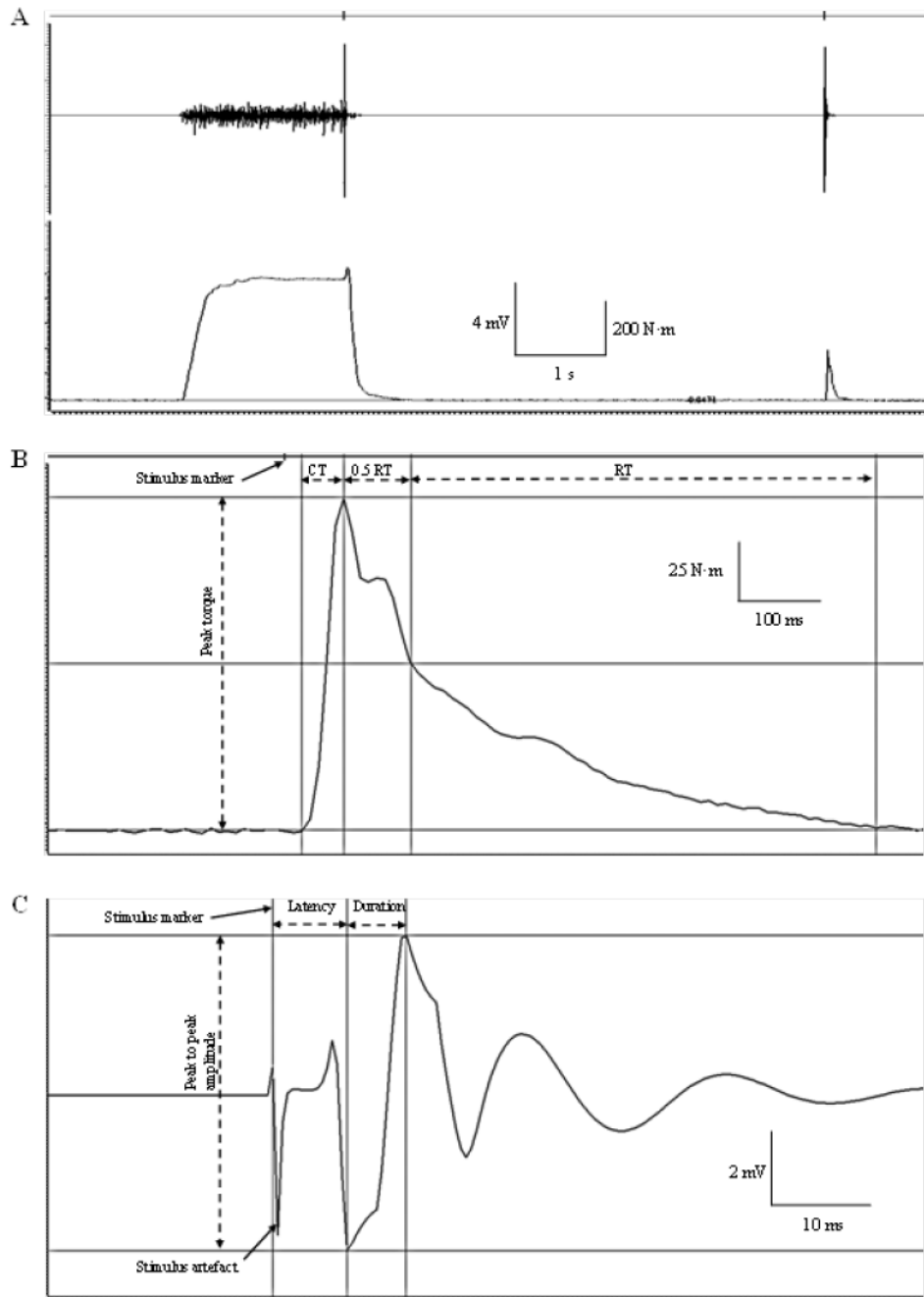
Figure 3.9. Raw EMG and torque traces from the measurement of muscle contractility from a representative participant. A, motor nerve stimulation following a maximal voluntary contraction. B, muscle contractile characteristics derived from the potentiated quadriceps muscle twitch. C, M-wave characteristics accompanying the potentiated muscle twitch.

CT, time to peak torque; 0.5RT, half relaxation time; RT, relaxation time.

Corticospinal Function

MEP responses to spTMS and ppTMS were recorded from EMG electrodes placed on the VL during a light background contraction at 10% MVC, calculated from the MVC recorded at the same time-point to account for any fatigue-induced changes. Participants had torque displayed on a visual oscilloscope on a computer screen at eye level and told to focus on maintaining the 10% MVC as closely as possible. Measurements in the active muscle are more indicative of motor function during fatigue (Gruet et al., 2013), increase the efficacy of the measure (Groppa et al., 2012), reduce MEP variability (Darling et al., 2006) and temporal dispersion (Groppa et al., 2012), and increase test-retest reliability (Tallent et al., 2012). Maintenance of a light contraction with visual display helps stabilise corticospinal excitability and control attentional and somatosensory affects (Darling et al., 2006). It can also be difficult to elicit resting MEPs in lower limb muscles in some participants (Chen et al., 1998). A 10% MVC is similar to the torque produced during high-intensity cycling (Lollgen et al., 1980), has previously been used in spTMS and ppTMS investigations in the knee extensors (Goodwill et al., 2012, Weier et al., 2012) and is likely to not induce any fatigue. This intensity of contraction has shown to be sufficient to markedly reduce MEP variability (Darling et al., 2006) but light enough to measure SICI as higher contraction intensities suppress SICI (Ortu et al., 2008). Contractions below 10% MVC may produce fluctuating recruitment patterns whereas this intensity produces consistent recruitment of the VL (Kouzaki et al., 2002).

To examine corticospinal excitability, MEPs and cSP durations were recorded from single TMS pulses at an intensity of 120% rMT (Tallent et al., 2012, Goodall et al., 2014a). The intensity was anchored to the rMT rather than aMT, as MEP variability reduces at this intensity in active and relaxed muscle (Pitcher et al., 2003, Darling et al., 2006) and produces stable measures of the cSP (Saisanen et al., 2008). From each recorded MEP the peak-to-peak

amplitude was determined (Figure 3.10) (Rossini et al., 2015). TMS can cause motor units to fire more than once which affects the MEP area and makes the area a more variable measure (Magistris et al., 1998). Nevertheless peak-to-peak amplitude and area are extremely well correlated (Lewis et al., 2014). All MEP amplitudes were expressed relative to resting M_{max} recorded at the same time-point to account for alterations in muscle membrane excitability (Gruet et al., 2013); M_{max} does not differ between rest and 10% MVC (Behm et al., 1996). The cSP duration was measured as the time between stimulus and return of background EMG (Figure 3.10) (Taylor et al., 1996, Sidhu et al., 2009b, McNeil et al., 2011a). Visual inspection provides less variable cSP estimates than mathematical modelling (Damron et al., 2008). Whilst a number of methods of determining cSP duration have been tested (Damron et al., 2008, Saisanen et al., 2008), inclusion of the MEP duration allows better standardisation of the cSP onset (Saisanen et al., 2008), with stimulus onset to return of EMG providing the least variable measure (Damron et al., 2008).
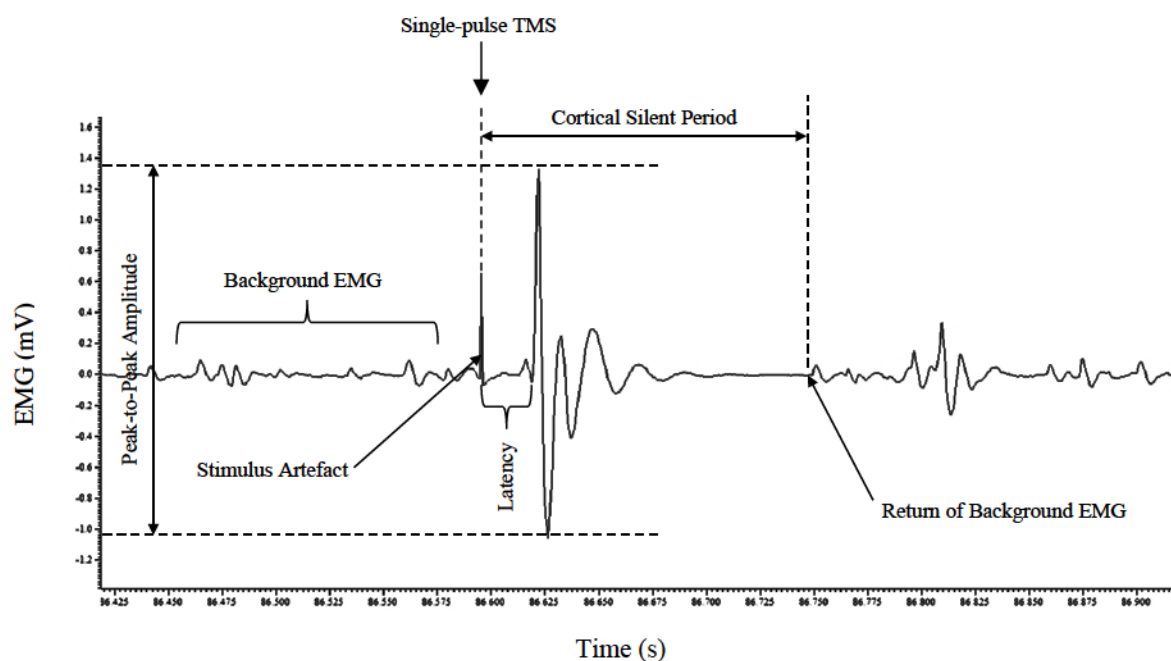
Figure 3.10. Raw motor evoked potential trace elicited by single pulse TMS at 120% of resting motor threshold during a 10% of maximal voluntary contraction in the vastus lateralis.

100

SICI, ICF and LICI were measured using the conditioning-test ppTMS paradigm (Valls-Sole et al., 1992, Kujirai et al., 1993). The first and second pulse acted as the CS and TS, respectively. The MEP amplitude evoked by the CS and TS administered together was compared to the amplitude of the MEP evoked by the TS alone (Figure 3.11). SICI and ICF were measured using a subthreshold CS – suprathreshold TS paradigm (Kujirai et al., 1993). It is unclear at which CS intensity SICI and ICF are most effectively recruited in the active muscle so the CS of 70%, 80% and 90% aMT for SICI (Ridding et al., 1995, Chen et al., 1998, Zoghi et al., 2003, Ortu et al., 2008, Sidhu et al., 2013b) and 90%, 100% and 110% of aMT for ICF (Ziemann et al., 1996) were initially explored. The threshold for the ICF circuits is higher than SICI and so necessitates a higher CS (Ziemann et al., 1996, Ortu et al., 2008).

For SICI the ISI was held at 3 ms. Whilst peak SICI can occur between an ISI of 2-3 ms, a number of investigations have reported an ISI of 3 ms produced peak SICI (Ridding et al., 1995, Zoghi et al., 2003, Benwell et al., 2006). The inhibition at an ISI of 1 ms is due to axonal refractoriness (Fisher et al., 2002, Roshan et al., 2003). While 2 ms has also been previously used (Chen et al., 1998, Sanger et al., 2001, Sidhu et al., 2013b), the use of 3 ms prevents inadvertent stimulation of neurons mediating short-interval intracortical facilitation (Ortu et al., 2008) and pharmacological studies show $GABA_A$-mediated inhibition is stronger at 3 ms than 2 ms (Di Lazzaro et al., 2005, McDonnell et al., 2006). During muscle activation a ISI $\geq$3 ms is recommended to isolate GABAergic activity as a 2 ms ISI is likely to cause a collision between CS and TS or occlusion of the CS by the TS (Hanajima et al., 2003). The 3 ms ISI is also sensitive to interventions such as muscle activation (Zoghi et al., 2003), muscle fatigue (Benwell et al., 2006) and exercise training (Weier et al., 2012). For ICF the ISI was set at 15 ms as this has shown to produce consistent ICF (Ridding et al., 1995, Kujirai et al., 1993) and the most reliable measure of ICF (Du et al., 2014). This ISI is also sensitive to muscle fatigue

(Tergau et al., 2000, Verin et al., 2004). The use of a longer ISI also allows for a greater decay in SICI to better recruit the ICF circuits (Ziemann et al., 1996) although ICF appears consistent using 8–15 ms (Ridding et al., 1995, Ziemann et al., 1996, Stokic et al., 1997, Chen et al., 1998).

For both SICI and ICF, the TS intensity was held constant at 120% rMT, as this intensity produces the greatest degree of SICI in the active muscle (Garry and Thomson, 2009). This intensity of TS has also been used to successfully identify ICF impairments following treadmill running (Verin et al., 2004). Pilot work found this elicited an MEP of ~2 mV in amplitude similar to that used in the only previous study of ppTMS in the active VL (Sidhu et al., 2013b). However, estimates of SICI are unaffected by alterations in TS intensities that elicit muscle response ≥ 1 mV (Sanger et al., 2001, Roshan et al., 2003, Chen, 2004, Benwell et al., 2006) or TS intensities from 110–130% rMT (Garry and Thomson, 2009, Opie and Semmler, 2014), although it has been recommended that measures of SICI should be standardised to the TS intensity (Zoghi et al., 2003, Garry and Thomson, 2009). LICI was measured using a suprathreshold CS and TS (Valls-Sole et al., 1992, Wassermann et al., 1996). The intensity of both the CS and TS was set at 120% rMT as this produces the greatest degree of LICI during a 10% MVC (McNeil et al., 2011c). The ISI was set at 100 ms as this also gives the greatest degree of LICI (Benwell et al., 2007), reflects cortical inhibition as spinal motoneuron excitability is recovered (Nakamura et al., 1997), has shown to be GABA$_B$-mediated (McDonnell et al., 2006) and has been used to successfully examine muscle fatigue (Benwell et al., 2007, McNeil et al., 2009, McNeil et al., 2011a).
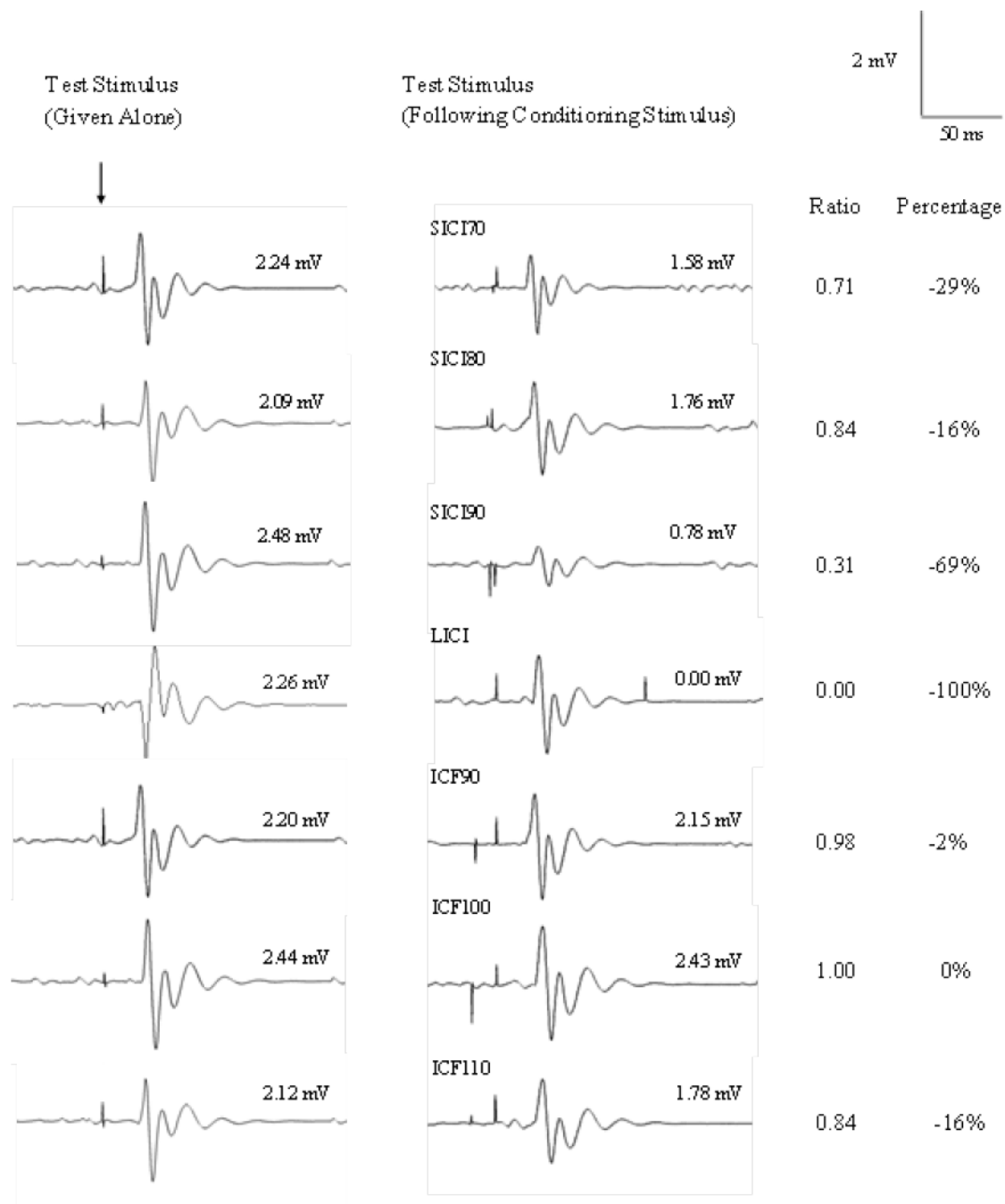
Figure 3.11. Raw traces of single (left traces) and paired-pulse TMS (right traces) from a representative participant. The left traces shows the motor evoked potential peak-to-peak amplitude with the test simulus given alone. The right traces show the motor evoked potetnial peak-to-peak amplitude when the same test stimulus intensity was preceded by a conditioning stimulus. The right column demonstrates the ratio of the paired-pulse to single-pulse TMS response for quantification of inhibtion or facilitation.

3.9.2.3   Experimental Procedures

Within-Day Reliability

Within-day reliability and variability were examined in 10 participants. Each participant arrived at the laboratory at 9 am. At the beginning of each visit a series of submaximal and maximal contractions were performed in order to familiarise participants. The familiarisation was completed with a minimum of three MVCs until a plateau in torque, and followed by the determination of the rMT and aMT. Three MVCs were then performed with MNS applied at peak torque and ~3 s post MVC. These muscle responses to MNS were used to determine peripheral VA, $Q_{tw,pot}$, M_{max} and $MVCM_{max}$. Each MVC was held for 3 s and accompanied by strong verbal encouragement with 15 s separating each effort. The peak of the three MVCs was taken as peak torque and used for setting the remaining submaximal contraction intensities at that time-point. A set of three contractions at 100%, 75% and 50% MVC were then completed with single TMS pulses at 130% rMT administered during each contraction for the determination of cortical VA. Each contraction was separated by 3 s and each set was repeated three times separated by 15 s (Goodall et al., 2012, Thomas et al., 2015). A series of contractions were then performed at 10% MVC, during which MEP amplitude, cSP duration, SICI (SICI70, SICI80, SICI90), LICI, and ICF (ICF90, ICF100, ICF110) were measured. Ten pulses were administered for each parameter as per guidelines (Rossini et al., 2015). TMS pulses were applied in blocks of 10 with each pulse separated by ~3-5 s in random intervals to avoid anticipation. The 10% MVC contraction was maintained for a set of 10 TMS pulses (~40 s) with the order of the pulses assigned randomly. Each block of TMS pulses was separated by 30 s rest. The testing protocol was repeated three times throughout a single day at 4 h intervals (0 h, 4 h and 8 h) to assess within-day reliability. During the testing participants refrained from exercise, caffeine and alcohol.

Between-Day Reliability

Between-day reliability was tested in 16 participants, including the 10 from the within-day experiment. Each participant visited the laboratory five times over a 10 week period with each trial separated by a minimum of 5 d. Each trial was repeated at the same time of day with the protocol identical to the within-day study.

3.9.2.4   Data Analysis

The aMT and rMT were expressed as % maximum SO. MVC torque was identified as the highest instantaneous torque achieved. The $Q_{tw,pot}$ torques were averaged across the three MNS given after each MVC. Peripheral VA was calculated with the ITT using the formula: $1 - SIT / Q_{tw,pot} \times 100$ and averaged across the three MVCs. On the rare occasion MNS was not applied during peak torque or if MVC torque was declining, a correction factor was applied: $1 - (SIT \times (Torque\ at\ SIT\ onset / MVC)) / Q_{tw,pot} \times 100$ (Strojnik and Komi, 1998). For the determination of cortical VA, the resting twitch was estimated (ERT) from the y-intercept of the linear relationship (mean within-day $r^2 = 0.93 \pm 0.06$, mean between-days $r^2 = 0.93 \pm 0.04$) between voluntary torque and SIT torque across the contraction intensities of 50%, 75% and 100% MVC. Cortical VA was calculated using the formula: $1 - SIT / ERT \times 100$ (Goodall et al., 2009, Sidhu et al., 2009a). Cortical VA and ERT amplitude were taken as the mean of the three sets of contractions. For all MNS and TMS pulses, the peak-to-peak amplitude of the EMG responses were calculated offline. The M-wave response accompanying each MNS was averaged across the three trials at each time-point. spTMS MEP amplitudes were taken from the average of the ten pulses and normalised relative to M_{max}. MEP amplitudes during 50%, 75% and MVCs (MEP_{50}, MEP_{75} and MEP_{MVC}, respectively) were taken as the average of the three contractions at each time-point and expressed relative to $MVCM_{max}$. The cSP duration following each MEP was determined by manually placing a cursor at the stimulus onset and

return of the continuous EMG and the mean of the ten pulses was taken. For the determination of SICI, LICI and ICF, the peak to peak amplitude was averaged across the ten pulses at each CS and expressed as a ratio relative to the TS given alone.

3.9.2.5   Statistical Analysis

All data are presented as mean ± SE in the figures and mean ± SD in the tables and text. Statistical analyses were completed in SPSS for Windows v21 (SPSS Inc., USA). Normal distribution was tested with the Shapiro-Wilk test. Separate one-way repeated-measures ANOVAs for each variable with time as the repeated-measures factor were performed for the within- (0 h, 4 h and 8 h) and between day (visits 1 − 5) trials. At the 0 h time-point only, additional one-way repeated measures ANOVAs for SICI and ICF with CS as the repeated measures factor were conducted to examine whether there was significant inhibition or facilitation of the TS (i.e. different from 1.0) (Sidhu et al., 2013b) for each CS. A paired-samples t-test was conducted to examine whether LICI inducted significant inhibition. If a one-way ANOVA revealed a significant effect of time, post-hoc pairwise comparison was performed with a Bonferroni correction for multiple comparisons. Within- and between-day reliability were examined with an $ICC_{3,1}$ (95% CI) for absolute agreement: ≥0.6 was accepted as reliable with 0.6-0.8 considered as moderate reliability and ≥0.8 as good reliability (Du et al., 2014). CV was determined for each variable: SD / mean x 100. Each participant had the within-participant CV calculated across the three time-points, for the within-day trial, and across the five visits, for the between-day trial. As such each participant had one CV value for the within-day trial and one value for the between-day trial with the mean of all participants presented. Significance was accepted at $P \leq 0.05$.

3.9.3 Results

The reliability and variability of all measures can be seen in Table 3.3.

Table 3.3. Within- (n = 10) and between-day (n = 16) reliability and variability of all measures.

	ICC$_{3,1}$ (95% CI)		CV (%)		P	
	Within	*Between*	*Within*	*Between*	*Within*	*Between*
Corticospinal						
rMT	0.99 (0.98 – 1.00)	0.91 (0.83 – 0.96)	1.9	5.1	0.653	0.450
aMT	0.98 (0.93 – 0.99)	0.92 (0.84 – 0.97)	2.3	4.8	0.809	0.497
MEP	0.85 (0.64 – 0.96)	0.82 (0.68 – 0.92)	9.5	20.7	0.676	0.771
MEP$_{50}$	0.97 (0.90 - 0.99)	0.66 (0.46 – 0.84)	9.1	17.0	0.770	0.117
MEP$_{75}$	0.95 (0.86 - 0.99)	0.50 (0.26 – 0.74)	12.3	19.8	0.058	0.756
MEP$_{MVC}$	0.88 (0.69 - 0.97)	0.36 (0.12 – 0.66)	12.5	22.8	0.712	0.394
cSP	0.97 (0.92 - 0.99)	0.83 (0.70 – 0.93)	3.6	8.4	0.818	0.274
SICI70	0.88 (0.69 – 0.96)	0.67 (0.46 - 0.85)	9.6	21.2	0.739	0.827
SICI80	0.84 (0.61 – 0.95)	0.68 (0.47 – 0.85)	14.3	23.9	0.723	0.686
SICI90	0.92 (0.79 – 0.98)	0.84 (0.71 – 0.94)	8.8	23.6	0.532	0.167
LICI	0.96 (0.88 – 0.99)	0.47 (0.25 – 0.72)	11.7	72.1	0.189	0.246
ICF90	0.83 (0.60 – 0.95)	0.61 (0.38 – 0.82)	11.8	16.4	0.640	0.894
ICF100	0.73 (0.42 – 0.92)	0.56 (0.34 – 0.78)	12.9	18.0	0.482	0.128
ICF110	0.73 (0.41 – 0.92)	0.51 (0.28 – 0.74)	12.5	18.2	0.890	0.887
Neuromuscular						
MVC	0.82 (0.58 – 0.95)	0.70 (0.51 – 0.87)	3.7	7.3	0.549	0.325
Q$_{tw,pot}$	0.91 (0.76 – 0.97)	0.84 (0.65 – 0.93)	3.6	7.2	0.139	0.476
M$_{max}$	0.97 (0.90 – 0.99)	0.62 (0.30 - 0.90)	4.9	11.5	0.723	0.777
Peripheral VA	0.92 (0.79 – 0.98)	0.80 (0.65 – 0.91)	1.0	2.2	0.884	0.731
ERT	0.92 (0.78 – 0.98)	0.90 (0.81 – 0.96)	8.3	13.6	0.022	0.959
Cortical VA	0.94 (0.84 – 0.99)	0.85 (0.76 – 0.94)	1.5	3.2	0.953	0.519

rMT, resting motor threshold; aMT, active motor threshold; MEP, motor evoked potential; MEP$_{50}$, MEP during 50%MVC; MEP$_{75}$, MEP during 75%MVC; MEP$_{MVC}$, MEP during MVC; cSP, cortical silent period; SICI, short-interval intracortical inhibition; LICI, long-interval intracortical inhibition; ICF, intracortical facilitation; MVC, maximal voluntary contraction; Q$_{tw,pot}$, potentiated quadriceps twitch; M$_{max}$, maximal M-wave amplitude; VA, voluntary activation; ERT, estimated resting twitch.

3.9.3.1 Neuromuscular Function

There were no differences for MVC, $Q_{tw,pot}$, M_{max}, peripheral VA or cortical VA within- or between-days ($P > 0.05$) (Table 3.4). There was a significant effect of time on measures of within-day ERT ($P < 0.05$). Post-hoc analysis revealed that the ERT tended to be greater at 4 h than 0 h ($P = 0.063$). There was no difference in ERT between-days ($P > 0.05$). All measures of neuromuscular function demonstrated good within-day reliability (ICCs ≥ 0.82) and minimal variability (CV $\leq 8.3\%$). Between-day MVC and M_{max} were moderately reproducible (ICC ≥ 0.62) with all other measures of neuromuscular function demonstrating good between-day reliability (ICCs ≥ 0.80). All neuromuscular function measurements had minimal between-day variability (CV $\leq 13.6\%$). Additionally, there were no differences in MEP amplitudes during the measurement of cortical VA (MEP_{50}, MEP_{75}, or MEP_{MVC}) within- or between-days ($P > 0.05$).

Table 3.4. Within- (n = 10) and between-day (n = 16) measures of neuromuscular function. Data are mean ± SD.

	Within-Day			Between-Day				
	0 h	*4 h*	*8 h*	*Visit 1*	*Visit 2*	*Visit 3*	*Visit 4*	*Visit 5*
MVC	523	516	512	484	478	475	480	497
(N·m)	± 41	± 35	± 35	± 68	± 50	± 73	± 92	± 82
$Q_{tw,pot}$	164	156	160	164	162	157	159	160
(N·m)	± 29	± 23	± 26	± 31	± 28	± 25	± 32	± 29
M_{max}	5.13	5.12	5.05	6.74	6.80	6.75	7.06	7.09
(mV)	± 1.70	± 1.47	± 1.54	± 0.75	± 0.85	± 1.24	± 1.10	± 1.28
Peripheral VA	96.0	95.7	95.8	93.6	94.1	93.4	94.3	94.2
(%)	± 4.4	± 4.6	± 3.6	± 5.0	± 5.7	± 6.7	± 5.5	± 7.2
ERT	95	104	98	103	102	102	103	100
(N·m)	± 30	± 34	± 34	± 48	± 39	± 36	± 37	± 39
Cortical	96.5	96.5	96.6	95.4	96.0	96.2	96.0	95.8
VA (%)	± 2.3	± 2.9	± 2.4	± 4.3	± 4.0	± 3.6	± 3.9	± 3.6
$MEP_{50\%}$	59.2	60.3	58.5	62.5	55.4	54.6	59.0	57.6
	± 29.5	± 29.1	± 27.7	± 18.8	± 21.4	± 21.2	± 17.7	± 16.3
$MEP_{75\%}$	57.1	61.3	55.3	59.0	53.2	55.2	58.0	54.1
	± 27.2	± 27.2	± 27.7	± 16.5	± 17.1	± 22.1	± 19.5	± 14.5
MEP_{MVC}	49.1	48.7	51.1	51.3	46.3	46.3	48.9	48.7
	± 23.3	± 21.6	± 28.7	± 16.5	± 20.9	± 16.2	± 17.6	± 15.3

MVC, Maximal voluntary contraction; $Q_{tw,pot}$, potentiated quadriceps twitch; M_{max}, Maximal M-wave amplitude; VA, voluntary activation; ERT, estimated resting twitch; $MEP_{50\%}$, Motor evoked potential during 50%MVC; $MEP_{75\%}$, Motor evoked potential during 75%MVC; MEP_{MVC}, Motor evoked potential during MVC.

3.9.3.2   Corticospinal Function

Motor Thresholds

There were no differences for rMT or aMT within- or between-days ($P > 0.05$) (Table 3.5). Both within- and between-day rMT and aMT showed good reliability (ICC ≥ 0.91) and minor variability (CV ≤ 5.1%).

Table 3.5. Within- (n = 10) and between-day (n = 16) motor thresholds and cortical silent period durations. Data are mean ± SD.

	Within-Day			Between-Day				
	0 h	*4 h*	*8 h*	*Visit 1*	*Visit 2*	*Visit 3*	*Visit 4*	*Visit5*
rMT	55 ± 12	55 ± 11	56 ± 11	57 ± 12	57 ± 11	56 ± 12	56 ± 12	55 ± 11
aMT	40 ± 7	40 ± 6	40 ± 6	40 ± 8	39 ± 7	41 ± 8	41 ± 8	39 ± 8
cSP (ms)	176 ± 39	174 ± 40	176 ± 40	188 ± 46	184 ± 40	190 ± 44	190 ± 43	199 ± 62

aMT, Active Motor Threshold; cSP, Cortical Silent Period; rMT, Resting Motor Threshold.

Single-pulse TMS

There was no difference in MEP amplitude within- or between-days (P > 0.05) (Figure 3.12). MEPs showed good reliability within- and between-days (ICC ≥ 0.82, CV ≤ 20.7%). There was no difference in cSP duration within- or between-days (P > 0.05) (Table 3.5). cSP showed good reliability within- and between-days (ICC ≥ 0.83, CV ≤ 8.4%).
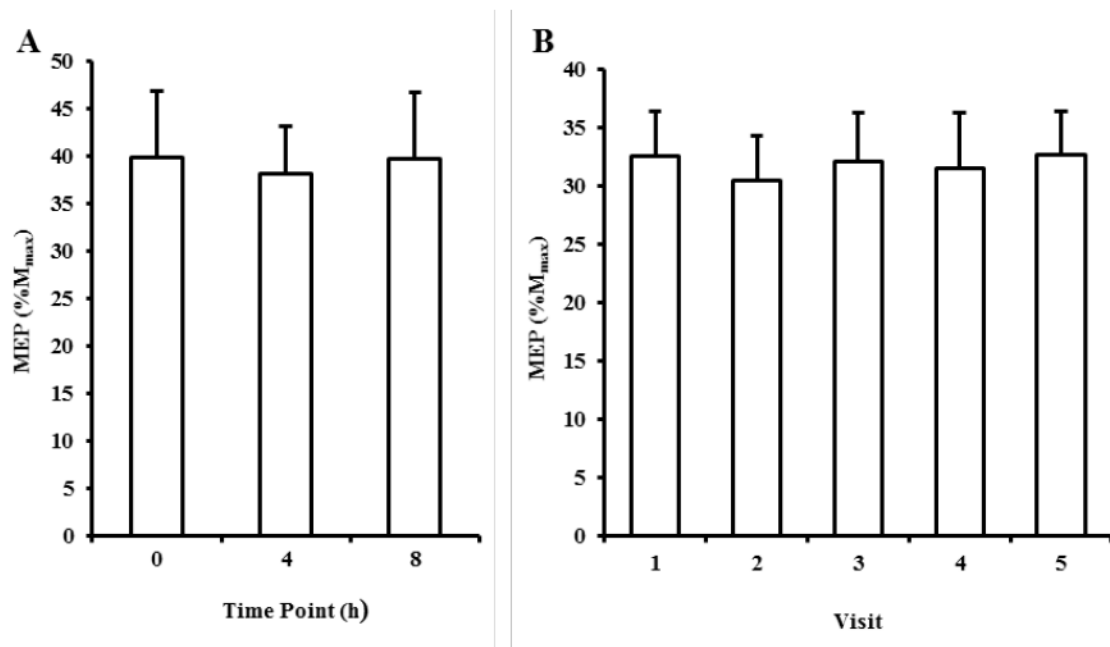
Figure 3.12. Within- and between-day peak to peak MEP amplitude (%M_{max}). A: Within-day MEP amplitude across the three time-points (0 h, 4 h and 8 h, n = 10). B: Between-day MEP amplitude across the five visits (n = 16). Data are mean ± SE.

Paired-pulse TMS

Raw EMG traces from a representative participant can be seen in Figure 3.11. There were no differences for within-day measurements of SICI, LICI or ICF (P > 0.05) (Figures 3.13A and 3.14A). All within-day measures of SICI and LICI showed good reliability (ICC ≥ 0.84) and moderate variability (CV ≤ 14.3%). SICI90 demonstrated the highest reliability and lowest variability amongst the SICI measurements. Measurements of ICF showed similar variability (CV ≤ 12.9%), however only ICF90 demonstrated good reliability (ICC = 0.83) with ICF100 and ICF110 showing moderate reliability (ICC = 0.73). There was a significant effect of CS on induction of SICI with only SICI90 inducing inhibition of the TS (P < 0.05). There was no effect of CS on induction of ICF with ICF110 actually inducing inhibition of the TS (P < 0.05) with a similar trend for ICF 100 (P = 0.057). LICI induced significant inhibition of the TS (P < 0.001). There were no differences for between-day measurements of SICI, LICI or ICF (P > 0.05) (Figures 3.13B and 3.14B). Between-day measures of SICI showed moderate to good reliability (ICC = 0.67 - 0.84) and moderate variability (CV ≤ 23.9%) with only SICI90 demonstrating good reliability. Measurements of LICI showed poor reliability and substantial variability (ICC = 0.47, CV = 72.1%). Only ICF90 showed moderate reliability (ICC = 0.61, CV = 16.4%) compared with ICF100 and ICF110 which both showed poor reliability (ICC = 0.51 - 0.56, CV ≤ 18.2%).
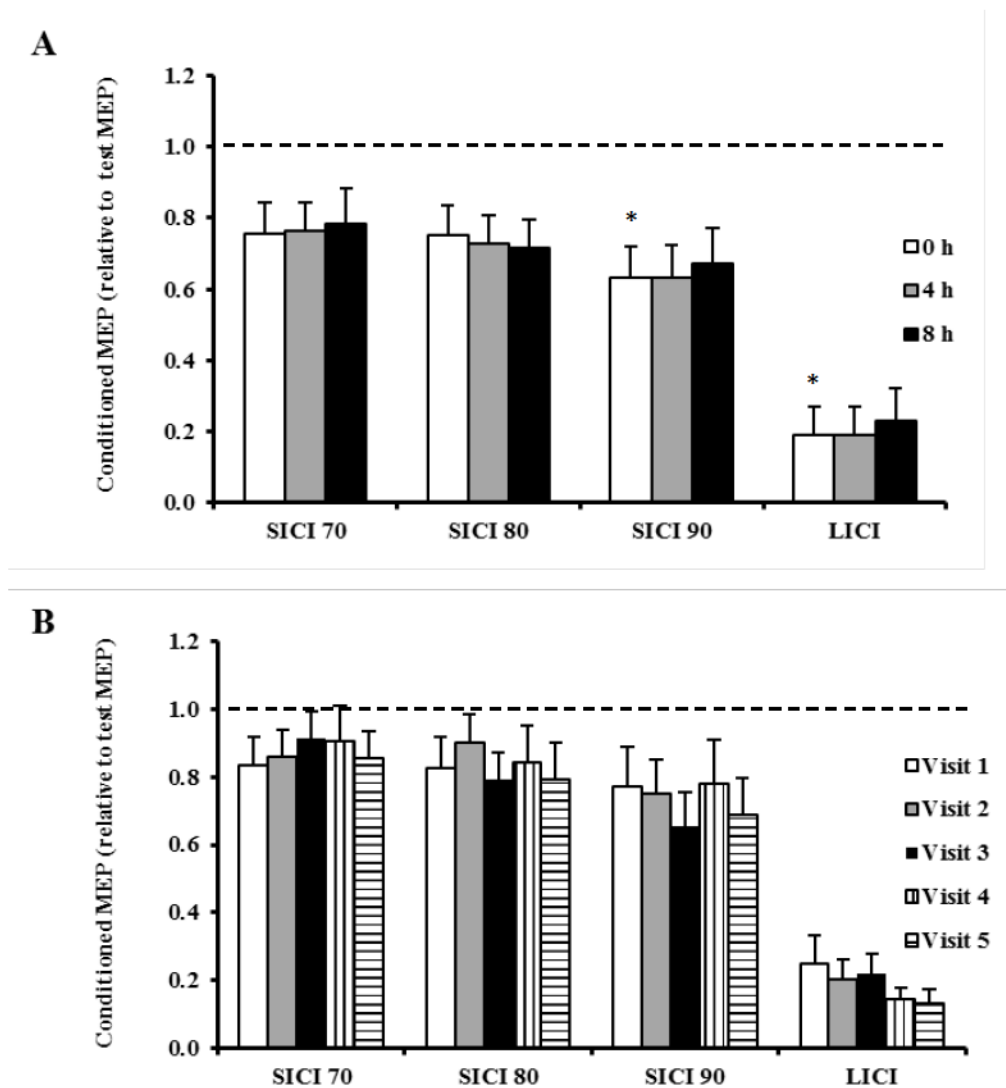
Figure 3.13. Within- and between-day SICI and LICI (ratio compared to TS alone). A: Within-day SICI and LICI across the three time-points (0 h, 4 h and 8 h, n = 10). B: Between-day SICI and LICI across the five visits (n = 16). Dashed line represents the test stimulus given alone. Data are mean ± SE.

*P<0.05 vs TS
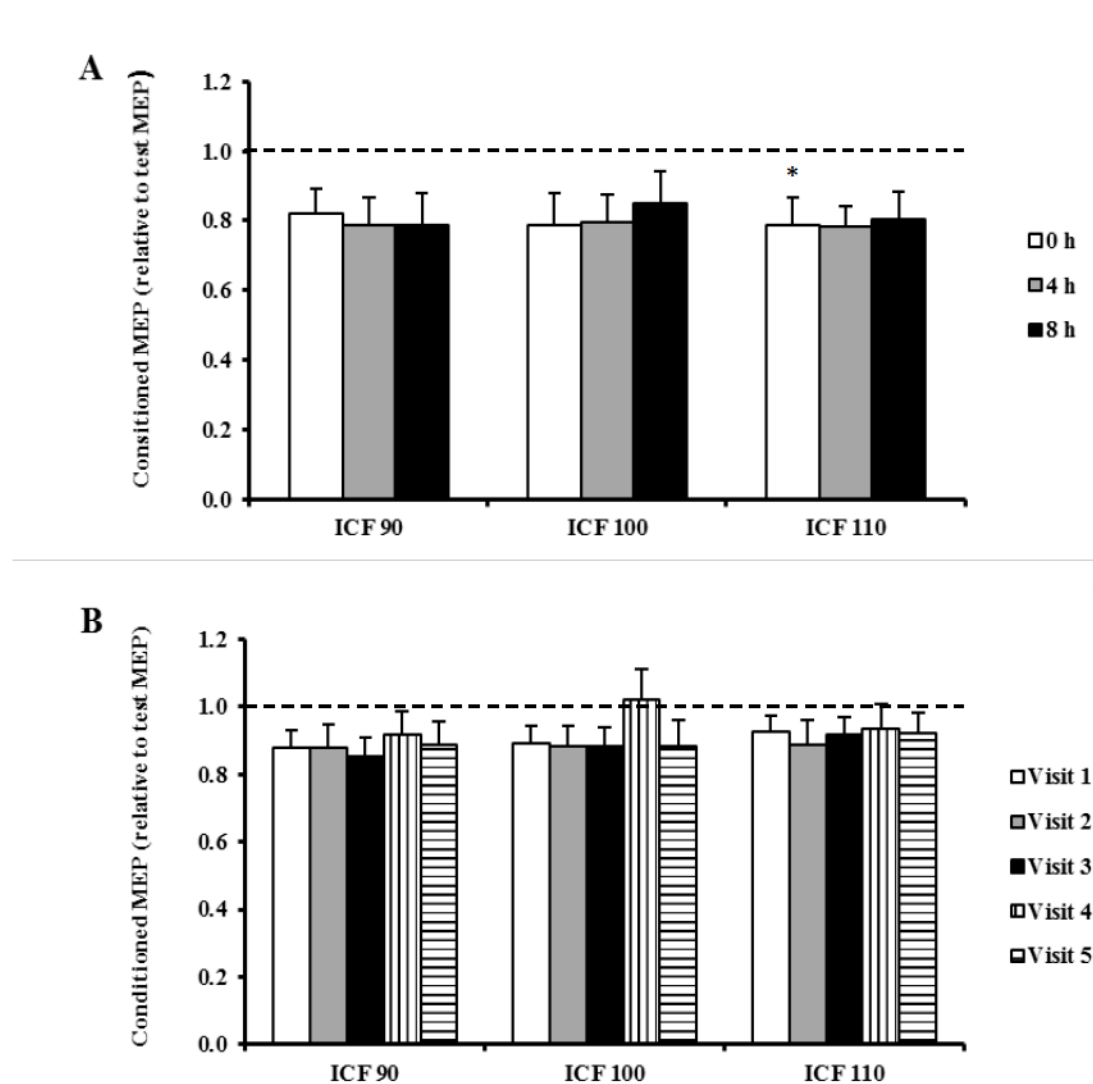
Figure 3.14. Within- and between-day ICF (ratio compared to TS alone). A: Within-day ICF across the three time-points (0 h, 4 h and 8 h) (n = 10). B: Between-day ICF across the five visits (n = 16). Dashed line represents the test stimulus given alone. Data are mean ± SE. *P<0.05 vs TS

3.9.4 Discussion

This study provides the first demonstration that MEPs, cSP duration, SICI, LICI and ICF can be reliably measured in the VL both within- and between-days but supports the use of a CS of 90% aMT for both SICI and ICF to increase measurement reliability. However, ICF did not elicit a facilitated response and the variability in LICI should be considered with long term

study designs. Although smaller variability was reported for all spTMS and ppTMS measures within-day, the greater between-day variability should be noted when conducting studies that require repeated measurements over a longer period of time. This is an important advance as a tool to be used in research and demonstrates that reliable estimates of MEP amplitude, SICI, LICI and ICF could be obtained during a short testing session (10 pulses per condition). Previous ppTMS reliability work has been conducted in the muscles of the upper extremities (Maeda et al., 2002, Wassermann, 2002, Orth et al., 2003, Fleming et al., 2012, Du et al., 2014). While these studies are useful regarding the reliability of ppTMS, it was previously unknown whether cortical excitability in the knee extensor representation of the motor cortex (Figure 2.2) could be reliably measured. Given the role of the knee extensors in locomotor activities, reliably measuring cortical function in this muscle group is an important consideration in fatigue research. The assessment of within- and between-day reliability allow for conducting effective research implementing acute (i.e. fatigue) or chronic interventions (i.e. training).

TMS is an inherently variable technique due to numerous physiological and methodological factors. Physiological variability can arise through intrinsic, constant fluctuations in corticospinal excitability (Kiers et al., 1993, Ellaway et al., 1998). Using a light isometric contraction likely reduced some of this variability by controlling for corticospinal excitability and attentional and somatosensory influences (Darling et al., 2006). A number of methodological factors also contribute to the witnessed variability such as accurate relocation of the coil and EMG electrodes, and accurate determination of motor thresholds. However, the level of stimulation required to excite the muscle demonstrated high reliability within- and between-days so can be ruled out as a source of significant variability. This is in agreement with previous work demonstrating between-day stability in rMT (Malcolm et al., 2006,

Wheaton et al., 2009, Cacchio et al., 2009, Cacchio et al., 2011, Tallent et al., 2012) and aMT (Ngomo et al., 2012, Lewis et al., 2014).

Single-pulse TMS

As demonstrated in previous work in other active muscles, MEP amplitudes (Carroll et al., 2001, Kamen, 2004, Cacchio et al., 2011, Tallent et al., 2012) and cSP duration (Fritz et al., 1997, Tallent et al., 2012) could be reliably measured between-days; we also demonstrated MEP amplitudes and cSP duration were also highly reproducible within-day. spTMS allows for corticospinal excitability measurements through assessment of the MEP amplitude (Kobayashi and Pascual-Leone, 2003). During a contraction, the MEP is followed by a period of near silence in the EMG signal termed the cSP. Whilst the initial component of the cSP (≤100 ms) is thought to reflect spinal inhibition, the later part is a measure of $GABA_B$-mediated cortical inhibition (Inghilleri et al., 1993, Chen et al., 1999, Werhahn et al., 1999). To our knowledge this is the first study to investigate the cSP reliability in the knee extensors. The MEP amplitude and cSP have been investigated in the knee extensors in a number of studies investigating cortical function in CNS disease (Tremblay and Tremblay, 2002), ageing (Stevens-Lapsley et al., 2013), muscle fatigue (Ross et al., 2010b, Hilty et al., 2011) and response to exercise training (Latella et al., 2012); our findings support this work.

Wheaton et al. (2009) reported similar reproducibility of MEP amplitudes (ICC = 0.87) in the non-paretic active VL in stroke survivors. Despite similar reproducibility for within- and between-day MEPs, we found the between-day variability was twice that of the within-day variability and should be taken into account when conducting longitudinal studies. Administering TMS during active contractions can increase potential methodological error as the level of force produced across days must be matched. MEP amplitudes are increased with

increasing muscle activity (Darling et al., 2006, Tallent et al., 2012) and so the ability to repeat MVCs can therefore influence estimates of corticospinal excitability. Unsurprisingly, MVCs were more reliable within-day compared to between-day measurements which may have increased the reported between-day MEP variability. Despite this, MVCs were still repeatable between-days with minor variability. MEP amplitudes are also unlikely to vary substantially with small changes in force output (Darling et al., 2006). By expressing MEP amplitude relative to M_{max} any effects of muscle or peripheral nerve transmission were also controlled for. This also allows the reliability of corticospinal excitability and peripheral excitability to be examined in isolation. A more likely major source of this greater variability is likely to stem from fluctuations in corticospinal excitability combined with a greater number of potential methodological sources of error including accurate coil relocation at the site of stimulation between-days as well as EMG electrode replacement. Indeed, due to the period between measurement times (~2 weeks) it was necessary to re-establish the optimal site of stimulation or 'hot spot' on the scalp. This however is common practice in longer term trials, and clinical investigations may not have the luxury of repeat within-day measures and so is important to consider as part of the witnessed reproducibility of the measures.

Paired-pulse TMS

SICI and LICI reflect the activity of $GABA_A$ and $GABA_B$-mediated intracortical inhibitory neurons, respectively (Hanajima et al., 1998, Ilic et al., 2002, Chen, 2004, McDonnell et al., 2006), whereas ICF reflects the activity of glutamate-mediated NMDA excitatory neurons (Chen, 2004, Chen et al., 2008). ppTMS has been used in the knee extensors to examine cortical function with aging (McGinley et al., 2010, Stevens-Lapsley et al., 2013), mechanisms of muscle fatigue (Verin et al., 2004), adaptations to exercise training (Goodwill et al., 2012, Weier et al., 2012) and cortical contributions to locomotion (Sidhu et al., 2013b). The data

presented in these studies are supported by the findings in the present study. ppTMS has also been used in a number of other muscle groups to examine cortical function in CNS disease (Curra et al., 2002, Kobayashi and Pascual-Leone, 2003, Chen et al., 2008), some of which are classified as movement disorders, and we propose that the technique can be reliably applied to a more functional locomotor muscle.

Previous investigations in resting muscles of the upper limbs have found SICI (Fleming et al., 2012, Du et al., 2014) and LICI (Farzan et al., 2010) had good repeatability whilst contrasting reports have found ICF with either good (Du et al., 2014) or poor reliability (Fleming et al., 2012). As estimates of SICI, LICI and ICF depend on the intensity of both the CS and TS (Chen et al., 1998), the increased variability in these measures between-days may be due to the increased variability of the responses to the TS given alone. The increased variability in ICF using a CS at or above the motor threshold found in this study seems to be a consistent finding (Orth et al., 2003). Although previous work reports a greater level of ICF with increasing the CS above 90% of motor threshold (Kujirai et al., 1993) it is likely that a suprathreshold stimulus invokes a descending volley and affects the excitability of the spinal cord and motoneurons (Ziemann et al., 1996) possibly inducing greater variability in the measure. However, we also report that there was no induction of ICF with the 90% aMT CS which is in agreement with previous work in other active muscles (Ridding et al., 1995, Ortu et al., 2008), the mechanism of which remains unknown. Indeed, the higher CS intensities actually inhibited the TS response. Therefore, it must be concluded that with the methodology employed here, ICF could not be measured in the active VL. From the protocol employed here it is unclear why LICI would be ~7 times more variable between-days. In some participants we found complete inhibition of the TS by the CS during some visits but not during others which contribute to large estimates of variability. The exact mechanisms of LICI appear complex with a spinal

component to the inhibition recently demonstrated (McNeil et al., 2009). Hence the large variability may be due to the number of physiological fluctuations that contribute to the conditioned response.

Generally, SICI was more reliable than either LICI or ICF. SICI has consistently reported to be more reliable than ICF (Maeda et al., 2002, Fleming et al., 2012, Du et al., 2014), but with a greater between session variability (Boroojerdi et al., 2000, Wassermann, 2002, Orth et al., 2003, Fleming et al., 2012). This is in agreement with this work, also demonstrating that SICI was more variable than ICF but only for between-day measures. Other studies have reported substantially larger variance in SICI and ICF compared to the present study (Orth et al., 2003) potentially due to methodological differences such as the stimulation parameters employed and muscle activity. We propose that the use of an active muscle rather than the resting state may reduce the variability due to the stabilisation of corticospinal excitability, attentional and somatosensory influences. During an active contraction the level of SICI is reduced compared to rest due to a reduced excitability of intracortical inhibitory circuits projecting to the muscle or concurrent increase in excitability of facilitatory circuits (Ridding et al., 1995, Zoghi et al., 2003, Ortu et al., 2008). As SICI is expressed as a ratio of the CS to TS, less SICI will result in a larger ratio and consequently could result in the smaller variances when expressed relative to the mean value. However, SICI in the resting muscle has reported to be more reliable than SICI in the active muscle (Ngomo et al., 2012). Unusually, these authors reported no difference in SICI in the resting compared to the active condition possibly due to the light contraction intensity used (7.5% MVC) as well as stimulating at 120% aMT in the active and 120% rMT in the resting condition, whereas the use of 120% rMT seems to result in the greatest level of SICI regardless of excitability state (Garry and Thomson, 2009). Furthermore, TS elicited at lower intensities lower than 120% rMT results in greater variability in single-pulse MEP

amplitudes (Pitcher et al., 2003, Darling et al., 2006) and may result in greater variability in SICI.

To date only two studies have measured SICI in the active knee extensors (Sidhu et al., 2013b, Thomas et al., 2015b). Whilst Thomas et al. (2015b) found a CS of 70% aMT could elicit SICI in the RF, Sidhu et al. (2013b) reported a CS of 90% aMT resulted in the greatest degree of SICI in static activation of the VL. This is in agreement with our data as well as contractions of the upper limb (Ridding et al., 1995, Zoghi et al., 2003). Although the exact mechanism is unclear, the increased reliability of a higher CS in our study may be due to a larger number of inhibitory interneurons activated by the higher stimulus intensity (Ridding et al., 1995, Sidhu et al., 2013b) producing a more consistent response whereas lower CS (i.e. 70% or 80% aMT) produce no significant inhibition of the TS response (Sidhu et al., 2013b). Other authors have however reported that SICI is reduced with increasing CS intensity during muscle contraction in upper limb muscles, perhaps due to an increased excitability and consequent recruitment of facilitatory circuits by the higher intensity CS (Ortu et al., 2008). Thus comparisons between muscles and activity states must be made with caution.

Neuromuscular Function

TMS measures of cortical VA and ERT were reliable within- and between-day in agreement with previous work (Goodall et al., 2009, Sidhu et al., 2009a). Whilst it is unclear why measures of ERT tended to be greater at 4 h vs 0 h, systematic biases within-day have previously been reported (Goodall et al., 2009) and should be considered in interpretation of this measure. Whilst the accompanying MEPs were highly repeatable within-day, the between-day MEPs were not repeatable during contractions $\geq$75% MVC. This is in contrast to previous studies demonstrating between-day stability across all contraction intensities (Sidhu et al., 2009a).

However, higher intensity shortening contractions (80% MVC) have previously shown to produce poorer reliability than those at lower intensities ($\leq$50% MVC) (Tallent et al., 2012). This is possibly due to greater synchronisation of motor unit recruitment, which results in an increased likelihood of the TMS pulse discharging during the neuron refractory period, desynchronization of the AP at the muscle membrane and the intermittent arrival of the AP at the muscle through phase-out cancellation, which can all affect the MEP shape (Rosler, 2001, Darling et al., 2006, Tallent et al., 2012).

In agreement with previous work, MNS provided reliable measures of peripheral VA and muscle contractility (Bachasson et al., 2013, Bachasson et al., 2014) allowing for accurate determination of central and peripheral fatigue. We also demonstrate stability of these measures over a longer time period and so these techniques have potential for tracking training adaptation. Previous work has reported low within- and between-day variability for measures of the $Q_{tw,pot}$ (CV $\leq$8.5%), M_{max} (CV $\leq$4.9%) and peripheral VA (CV $\leq$4.7%) (Polkey et al., 1996, Amann et al., 2006a, Amann et al., 2006b, Romer et al., 2006a, Romer et al., 2006b, Amann et al., 2007, Amann and Dempsey, 2008, Bachasson et al., 2013, Bachasson et al., 2014). MNS also achieved supramaximal stimulation of the femoral nerve in agreement with previous work (Polkey et al., 1996, Amann et al., 2006a, Romer et al., 2006a, Verges et al., 2009, Bachasson et al., 2013, Bachasson et al., 2014).

Limitations

Although the aim of the present study was to examine the reliability of a short testing protocol, limitations of this approach include the failure to assess a number of other potential ppTMS parameters. For example, altering the CS affects the reliability of the technique but it is not clear from this investigation whether other ISI or TS intensities will produce differing results.

Furthermore, although measurements were made in a tonically active muscle in order to examine the motor pathway during muscular work, it is likely that the reliability of the technique will differ with the muscle relaxed. These findings should therefore be interpreted with caution when examining other ppTMS protocols or other muscles of differing activity states. The use of MNS can pose methodological limitations due to issues ensuring supramaximal stimulation in some individuals. However, this appears to be mainly a problem in overweight individuals (Tomazin et al., 2011). Nevertheless, a number of precautions were taken to ensure maximal depolarisation of the femoral nerve. Firstly, a 55 mm branding iron coil was used; smaller branding iron coils produce greater activation than 70 mm flat coils due to more concentrated delivery of the magnetic field (Tomazin et al., 2010). The coil was also discharged by two stimulators which increase the output (Hamnegard et al., 2004).

Conclusion

The results from this Chapter show that within-day measures of both spTMS and ppTMS demonstrate good reliability and minor variability. The between-day measures showed less reliability and greater variability but are still supported as reliable. The stimulation parameters should be considered when using ppTMS and these data suggest a CS of 90% aMT as the most reliable for measuring SICI and ICF. However, it was not possible to produce a facilitated response with the ICF ppTMS paradigm, confirming results from studies in other muscles that ICF cannot be elicited during a contraction. These results also support the use of MNS and TMS measures of neuromuscular function in acute and chronic studies. This study provides first evidence spTMS and ppTMS can be used as a reliable tool in motor cortex representations of the active VL and supports use in future investigations. The next Chapter will investigate how these markers of neuromuscular and corticospinal function relate to exercise capacity in order to better understand the central and peripheral factors underpinning exercise tolerance.

Chapter 4 – Corticospinal and Neuromuscular Function and their Relationship to Exercise Capacity

4.1 Summary

Chapter 3 demonstrated the reliability of measures of exercise capacity and corticospinal and neuromuscular function in the knee extensors. Based on these methods, the following Chapter outlines how corticospinal and neuromuscular function relates to aerobic exercise capacity in order to better understand the peripheral and central factors contributing to locomotor exercise tolerance. The results from this Chapter demonstrate that a number of measures of cortical and neuromuscular function were associated with a number of markers of exercise capacity.

4.2 Introduction

It is now appreciated that not only do sites within the muscle, but also within the CNS, contribute to exercise tolerance (Hargreaves, 2008, McKenna and Hargreaves, 2008, Sidhu et al., 2013a). Numerous sites within the motor cortex to muscle pathway contribute to exercise-induced fatigue (Gandevia, 2001, Sidhu et al., 2013a) and the adaptive response to training (Adkins et al., 2006), and so to understand limits to exercise capacity, an integration of neurophysiological, central motor drive and skeletal muscle measurements are required (Hargreaves, 2008, Sidhu et al., 2013a). The responses to stimulation of the motor nerve and motor cortex (TMS) can reveal information about muscle contractile and neurophysiological function. spTMS allows quantification of the ability to generate motor cortical drive (Goodall et al., 2009) and excitability of the corticospinal pathway and ppTMS allows the intracortical inhibitory and excitatory interneurons that modulate motor cortex output to be studied (Kujirai

et al., 1993). The motor cortex and spinal cord undergo adaptations in response to training (Adkins et al., 2006) and adaptations in these structures involved in motor performance may contribute to enhanced exercise endurance (Tergau et al., 2000, Triscott et al., 2008, Vila-Cha et al., 2012b, Goodall et al., 2014b). However, neuromuscular and corticospinal function correlates to locomotor exercise capacity are not well understood.

Neuromuscular function differs between endurance-trained, power-trained and sedentary individuals (Hamada et al., 2000a, Maffiuletti et al., 2001, Lattier et al., 2003, Garrandes et al., 2007), responds distinctively to endurance and strength training (Vila-Cha et al., 2010, Vila-Cha et al., 2012a, Vila-Cha et al., 2012b) and contributes to the increased resistance to fatigue during isolated contractions in endurance-trained individuals (Paasuke et al., 1999, Garrandes et al., 2007, Vila-Cha et al., 2010, Vila-Cha et al., 2012a, Vila-Cha et al., 2012b). Neuromuscular fatigue resistance is also an important contributor to locomotor exercise capacity (Morris et al., 2008, Morris et al., 2010) and impaired neuromuscular function affects exercise tolerance (Decorte et al., 2012). Locomotor exercise tolerance is also compromised by an inability to generate motor cortical output (Sidhu et al., 2009b) and disturbances in the responsiveness of the corticospinal pathway and motor cortical cell function (Verin et al., 2004, Sidhu et al., 2012a, Sidhu et al., 2013a), however the corticospinal factors contributing to locomotor exercise capacity are poorly understood. To date only one study has investigated corticospinal function and exercise capacity and these authors reported positive associations between intracortical excitability and the volume of work that could be performed during exhaustive pull-ups suggesting a cortical resistance to fatigue (Tergau et al., 2000). The relationship between corticospinal function and locomotor exercise capacity is yet to be investigated.

Taken together these observations suggest that impairments in neuromuscular and corticospinal function contribute to locomotor exercise-induced fatigue, and enhanced fatigue resistance is accompanied by alterations in neuromuscular and corticospinal function. This study set out to investigate corticospinal and neuromuscular function correlates with established markers of locomotor exercise capacity in order to better understand the CNS factors contributing to exercise capacity. It was hypothesised that those with a higher exercise capacity will demonstrate a greater ability to activate the motor cortex (cortical VA), higher corticospinal excitability (MEP amplitudes) and lower intracortical inhibition (SICI, cSP and LICI).

4.3 Methods

4.3.1 Participants

Twenty six healthy males (mean ± SD, age 24 ± 4 years, height 1.81 ± 0.07 m, mass 80.8 ± 10.2 kg) volunteered to participate in the study. Participants were recreationally active in a range of resistance and endurance activities but not engaged in regular structured training. This allowed assessment across a continuum of individuals rather than discrete groups.

4.3.2 Experimental Procedures

Each participant completed two trials separated by 2-14 d. The first trial involved familiarisation to the neuromuscular and corticospinal function tests. The second trial involved assessment of neuromuscular and corticospinal function followed by completion of completion of a submaximal and maximal exercise test to determine exercise capacity.

4.3.3 Muscle Torque and Electromyography

Isometric torque and EMG were recorded according to the procedures previously described (section 3.5).

4.3.4 Motor Nerve Stimulation

MNS was applied according to procedures previously described (section 3.6). The stimulus-response curve revealed an increment in $Q_{tw,unpot}$ and M-wave amplitude from 90 to 95% SO of 0.00% (P = 0.77) and 0.27% (P = 0.41), respectively; the increment in $Q_{tw,unpot}$ and M-wave amplitude from 95% to 100% SO was 0.00% (P = 0.79) and 0.34% (P = 0.28), respectively. A plateau in torque and M-wave amplitude was confirmed in all participants.

4.3.5 Transcranial Magnetic Stimulation

TMS was applied according to procedures previously described (section 3.7).

4.3.6 Neuromuscular and Corticospinal Function

In order to examine neuromuscular and corticospinal function, MNS and TMS evoked torque and EMG responses were determined. MVC, peripheral VA, cortical VA muscle contractile characteristics ($Q_{tw,pot}$ torque, CT and RTD), and corticospinal excitability (MEP amplitude, SICI, LICI and cSP duration) were assessed as described previously (section 3.9.2.2 - 3.9.2.3). Due to the findings reported in Chapter 3 (O'Leary et al., 2015b), ICF was not measured as it cannot be elicited in the active knee extensors, and SICI was measured with a CS of 90% aMT as this proved most repeatable (section 3.9). During the measurement of cortical VA, the TMS stimulus elicited a large MEP in the VL (peak-to-peak amplitude of $60.5 \pm 23.3\%$, $55.0 \pm 20.5\%$ and $50.2 \pm 19.1\%$ MVCM$_{max}$ during 50%, 75% and 100% MVC, respectively) and small MEP in the BF ($6.5 \pm 3.9\%$ of the VL MVCM$_{max}$ during a 50% MVC).

4.3.7 Exercise Capacity

All exercise tests were completed on an electromagnetically braked cycle ergometer and expired gases, HR, RPE and [La⁻] were measured as described previously (section 3.4).

Exercise capacity was determined with submaximal and maximal cycling tests, to determine the LT, $\dot{V}O_{2max}$ and $\dot{W}_{max}$ as described previously (section 3.8.2.3).

4.3.8 Data Analysis

MVC, peripheral VA, cortical VA, $Q_{tw,pot}$ torque and corticospinal excitability (MEP amplitude, SICI, LICI and cSP duration) were analysed as described previously (section 3.9.2.4). Each $Q_{tw,pot}$ was analysed for CT and RTD as previously described (3.9.2.2). MVC and $Q_{tw,pot}$ were expressed relative to body mass ($N \cdot m \cdot kg^{-1}$).

4.3.9 Statistical Analysis

Data are presented as mean ± SD. Statistical analyses were completed in SPSS (v.21, SPSS Inc., USA). Normal distribution was tested with the Shapiro-Wilk test. Pearson's correlation coefficients were used to examine associations between exercise capacity (LT (%$\dot{V}O_{2max}$ and $W \cdot kg^{-1}$), $\dot{V}O_{2max}$ ($ml \cdot kg^{-1} \cdot min^{-1}$) and $\dot{W}_{max}$ ($W \cdot kg^{-1}$)) and measures of neuromuscular (MVC torque, $Q_{tw,pot}$ torque, CT and RTD) and corticospinal function (MEP amplitude, SICI and cSP duration). Non-parametric data (peripheral VA, cortical VA and LICI) were examined with Spearman's rank. Stepwise multiple linear regression was then used to better understand how markers of the transition to anaerobic capacity (LT), aerobic capacity ($\dot{V}O_{2max}$), neuromuscular function (MVC torque, peripheral VA, $Q_{tw,pot}$ torque, CT and RTD) and corticospinal function (cortical VA, MEP amplitude, SICI, LICI and cSP duration) integrate to predict performance during the incremental exercise test ($\dot{W}_{max}$). Non-parametric data were log transformed. Paired-samples t-tests were used to compare the conditioned MEP (SICI and LICI) and unconditioned MEP. Significance was accepted as $P \leq 0.05$.

4.4 Results

All measures of neuromuscular and corticospinal function can be seen in Table 4.1.

Table 4.1. Measures of neuromuscular and corticospinal function.

	Mean ± SD
MVC $(N \cdot m \cdot kg^{-1})$	5.97 ± 1.13
Voluntary Activation	
Peripheral (%)	94.0 ± 4.8
Cortical (%)	94.7 ± 6.0
$Q_{tw,pot}$	
Peak torque $(N \cdot m \cdot kg^{-1})$	2.00 ± 0.54
CT (ms)	44 ± 5
RTD $(N \cdot m \cdot kg^{-1} \cdot s^{-1})$	47.3 ± 16.8
M_{max} (mV)	6.65 ± 1.09
Corticospinal Excitability	
MEP $(\%M_{max})$	34.8 ± 20.5
cSP (ms)	184 ± 42
SICI	0.81 ± 0.46
LICI	0.31 ± 0.35

MVC, maximal voluntary contraction; $Q_{tw,pot}$, potentiated quadriceps twitch; CT, time to peak torque; RTD, rate of torque development; M_{max}, maximal M-wave at rest; MEP, motor evoked potential; cSP, cortical silent period; SICI, short-interval intracortical inhibition; LICI, long-interval intracortical inhibition.

Neuromuscular Function and Exercise Capacity

VA measured with both MNS and TMS were high with good agreement between measures (rho = 0.75, P < 0.001). All markers of exercise capacity can be seen in Table 4.2. The LT ($\%\dot{V}O_{2max}$) was associated with slower CT (r = 0.72, P < 0.001, Figure 4.1) and RTD (r = -0.33, P < 0.05) but was unrelated to any other marker of neuromuscular function (P > 0.05).

Similar relationships were seen for the power output at LT ($W \cdot kg^{-1}$) (CT, $r = 0.61$, $P < 0.001$; RTD, $r = -0.44$, $P < 0.05$). $\dot{V}O_{2max}$ and $\dot{W}_{max}$ did not relate to any marker of neuromuscular function ($P > 0.05$).

Table 4.2. Physiological measures of exercise capacity.

	Mean ± SD
Lactate Threshold	
Watts	132 ± 42
W·kg^{-1}	1.62 ± 0.42
%$\dot{V}O_{2max}$	55.3 ± 9.6
$\dot{V}O_{2max}$	
l·min^{-1}	3.54 ± 0.62
ml·kg^{-1}·min^{-1}	44.0 ± 5.4
$\dot{W}_{max}$	
Watts	333 ± 50
W·kg^{-1}	4.14 ± 0.44

LT, lactate threshold; $W \cdot kg^{-1}$, Watts per kilogram of body mass; $\dot{V}O_{2max}$, maximal oxygen uptake; $ml \cdot kg^{-1} \cdot min^{-1}$, millilitres per kilogram of body mass per minute; $\dot{W}_{max}$, peak power output.
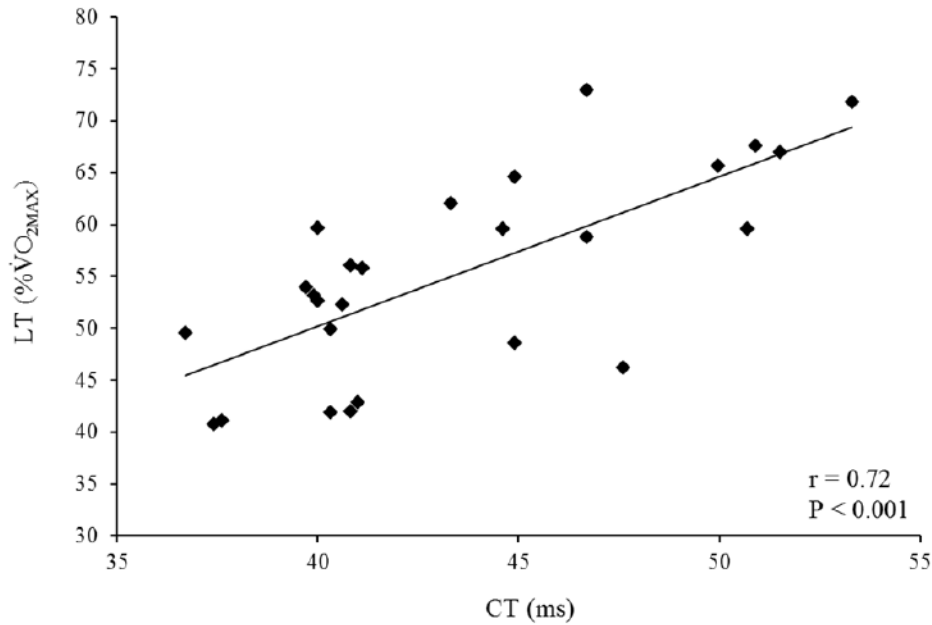
Figure 4.1. Relationship between muscle function (CT) and the LT.

Corticospinal Excitability and Exercise Capacity

The rMT and aMT occurred at $55 \pm 12\%$ and $37 \pm 8\%$ of maximum SO, respectively. The CS inhibited the TS during measures of SICI ($P < 0.05$) and LICI ($P < 0.001$). Those with a higher LT had less SICI ($r = 0.54$, $P < 0.01$, Figure 4.2A) but the LT did not relate to any other measure of corticospinal excitability ($P > 0.05$). A higher $\dot{V}O_{2max}$ was associated with more LICI (rho = -0.48, $P < 0.01$) but no other measure of corticospinal excitability ($P > 0.05$). Those who achieved a higher $\dot{W}_{max}$ had more LICI (rho = -0.40, $P < 0.05$, Figure 4C) and a longer cSP ($r = 0.37$, $P < 0.05$, Figure 4D) but $\dot{W}_{max}$ was not related to SICI (Figure 4.2C) or MEP amplitude.

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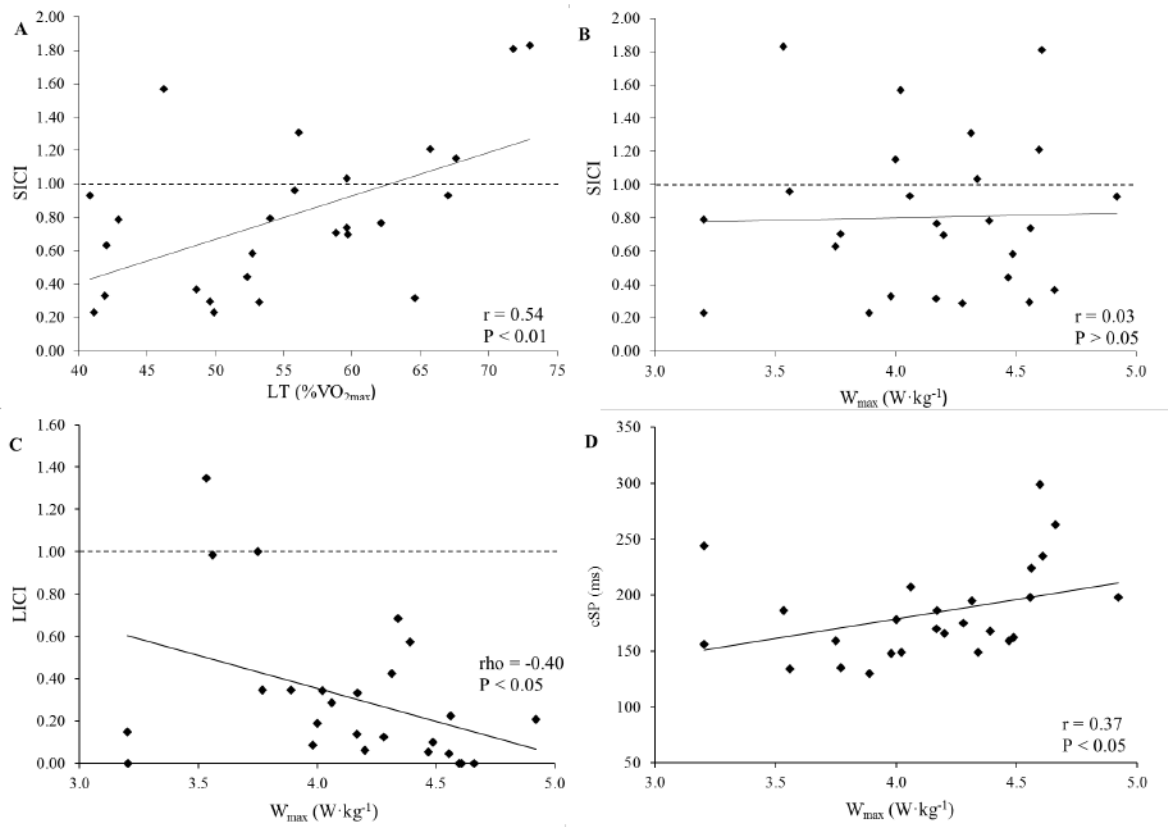
Figure 4.2. Relationships between markers of exercise capacity and intracortical excitability. A, SICI and LT; B, SICI and $\dot{W}_{max}$; C, LICI and $\dot{W}_{max}$ D, cSP and $\dot{W}_{max}$. Dashed line represents the test stimulus given alone.

Regression Analysis

Multiple linear regressions could be used to successfully predict $\dot{W}_{max}$ (P < 0.001). The model explained 57% of the variance in $\dot{W}_{max}$. $\dot{V}O_{2max}$ was the dominant predictor of $\dot{W}_{max}$ explaining 46% of the variance (both P < 0.001), with cortical VA explaining the remaining 11% (P = 0.022). The regression equation was: $\dot{W}_{max}$ (W·kg^{-1}) = (0.052 $\dot{V}O_{2max}$ (ml·kg^{-1}·min^{-1})) + (0.025 cortical VA (%)) − 0.533

4.5 Discussion

The aim of the present study was to investigate corticospinal and neuromuscular correlates of exercise capacity in order to better understand the CNS mechanisms underpinning human performance. Motor function is a key contributor to human performance, however this is the first study to report the associations between corticospinal physiology and locomotor exercise capacity. The main novel findings are that exercise capacity was associated with markers of inhibition within the motor cortex projecting to the exercising muscle and the ability to generate motor cortical output (cortical VA). More specifically, cortical VA was a predictor of maximal exercise performance during a standardised incremental ramp test. The motor cortex contributes to driving the VL during cycling (Sidhu et al., 2012b), and the intracortical inhibitory circuits are important in modulating corticospinal output and have been implicated in locomotor exercise fatigue. Therefore, a lower level of inhibition which results in greater motor cortical output in response to excitatory drive could be a central mechanistic link with exercise capacity. This data provides new insights to how CNS function may contribute to locomotor exercise capacity.

Neuromuscular Function and Exercise Capacity

Those with a slower CT, and to a lesser extent RTD, had a higher LT. Time to peak torque provides an indirect measure of whole muscle fibre type distribution (i.e. slower CT represents higher proportion of type I fibres) (Hamada et al., 2000b) and those with a higher proportion of type I muscle fibres have a higher LT due to the greater oxidative capacity of these fibres (Coyle et al., 1988). Although muscle biopsies allow direct determination of muscle fibre types, they allow only a small sample of one muscle to be measured whereas muscle contractile characteristics allow direct measurement of muscle performance across a whole muscle group (Morris et al., 2010). Here we present new data on how a reliable magnetic stimulation

technique can be used to assess muscle function as it relates to submaximal exercise capacity. To our knowledge, only one other investigation has examined relationships between neuromuscular function and aerobic exercise capacity. Morris et al. (2008) previously reported that individuals with less neuromuscular fatigue during a 3 min electrical stimulation protocol had a higher LT, however electrical stimulation techniques can be uncomfortable and may be unsuitable for some populations. Additionally, multiple linear regression analysis revealed that $\dot{W}_{max}$ could be predicted from $\dot{V}O_{2max}$ and cortical VA; therefore, the hypothesis can be accepted. $\dot{W}_{max}$ is an important marker of endurance capacity and cross-sectional data has revealed endurance-trained individuals also have increased peripheral VA and MVC of the knee extensors compared with sedentary individuals (Lattier et al., 2003). Conversely, 8 weeks endurance running training failed to improve MVC or cortical VA of the knee extensors (Zghal et al., 2014). Nevertheless, impairments in MVC, and peripheral and cortical VA, contribute to fatigue following cycling (Sidhu et al., 2009b, Decorte et al., 2012), and this data suggests cortical VA being a predictor of maximal exercise capacity.

Corticospinal Excitability and Exercise Capacity

Submaximal but not maximal exercise capacity was associated with reduced SICI and therefore this hypothesis can be accepted. The LT is a key marker of muscle oxidative capacity (Joyner and Coyle, 2008) and exercise tolerance (Coyle et al., 1988) whereas SICI reflects the activity of $GABA_A$-mediated intracortical inhibitory neurons (Chen, 2004, Chen et al., 2008). The SICI circuits inhibit corticospinal output (Chen, 2004, Chen et al., 2008, Petersen et al., 2010), focus corticospinal excitatory drive to the muscle (Floeter and Rothwell, 1999, Petersen et al., 2010, Weier et al., 2012, Sidhu et al., 2013b) and are modulated in response to fatigue (Benwell et al., 2006) and training (Weier et al., 2012). Corticospinal output plays an important role in locomotor exercise fatigue (Sidhu et al., 2013a) and increases in intracortical inhibition as

measured using subthreshold TMS (Sidhu et al., 2013c) are implicated in fatigue induced by high-intensity cycling. Therefore, a lower level of SICI could lead to better exercise tolerance allowing greater cortical output, however SICI was unrelated to maximal exercise capacity and therefore appears an unlikely mechanism. SICI is reduced by strength training (Weier et al., 2012) and although not engaged in structured training, our participants were recreationally active in a range of endurance and strength based activities. The LT is highly adaptive (Joyner and Coyle, 2008) and therefore contractile changes, as well as improvements in neuromuscular control induced by resistance exercise, could improve exercise economy (Jones and Carter, 2000). However, alongside reductions in SICI, resistance training of the knee extensors increases MEP amplitudes (Weier et al., 2012) and reduces the cSP (Latella et al., 2012) which are findings hard to align to this study and further work is required to better understand this relationship.

$\dot{V}O_{2max}$ was associated with more LICI whereas a higher $\dot{W}_{max}$ was associated with both higher LICI and a longer cSP duration and therefore the hypothesis must be rejected. As a measure of muscular performance, $\dot{W}_{max}$ is more likely to be influenced by factors such as central motor drive compared to $\dot{V}O_{2max}$ which is limited by cardiovascular function. However, it should be noted that LICI demonstrates more variability compared to other corticospinal measures as demonstrated in Chapter 3. $GABA_B$-mediated intracortical inhibition is thought to mediate both the cSP (Werhahn et al., 1999, Inghilleri et al., 1993) and LICI (Chen, 2004, Chen et al., 2008). A reduction in $GABA_B$ inhibition pre-synaptically increases $GABA_A$ inhibition of the corticospinal neuron (Chen, 2004). Both increases in LICI and the cSP are considered to play important roles in neural mechanisms of isolated muscle fatigue (Benwell et al., 2007, McNeil et al., 2009). The responses to locomotor exercise are less well understood, however reductions in cSP duration in response to exhaustive high-intensity cycling have been recently reported

(Temesi et al., 2013) suggesting different modulation of the cSP in response to isolated muscle and locomotor exercise. This may explain why we report a larger cSP with enhanced exercise capacity whereas resistance training shortens the cSP (Latella et al., 2012). In an animal model, administration of a $GABA_B$ agonist delays exhaustion during a treadmill run (Abdelmalki et al., 1997) and our data would support this as a mechanism contributing to maximal exercise tolerance. Individuals engaged in endurance activities do not differ in cSP duration compared to sedentary individuals (Cirillo et al., 2009), however this study investigated a hand muscle not engaged in the task. A more recent study failed to find any changes in cSP durations in response to 8 weeks running training (Zghal et al., 2014), however these responses were only elicited during an MVC and compared to running, cycling is likely to produce more localised neural activation patterns. The neural responses to endurance training are poorly understood and this study opens up new areas for investigation. Future work should examine the effect of endurance training on corticospinal function which could have important implications for improving motor function as well as developing fatigue resistance in a range of populations.

Limitations

Some of the correlations reported here vary in strength despite statistical significance; this is an expected finding as ppTMS displays variability and the factors determining exercise capacity are multi-factorial. This study also failed to record physical activity history which may help better understand the nature of these relationships. The participants in this study were also homogenous in terms of exercise capacity whereas a more heterogeneous group would increase the inter-individual variability and might help to better reveal some of the relationships between neural function and exercise capacity.

Conclusion

These results show that those with a higher LT had slower contractile times suggesting muscle contractile characteristics play an important role in submaximal exercise capacity. Better exercise capacity was also accompanied by differences in the circuits that modulate motor cortical output and greater cortical VA which could be important factors in central fatigue resistance. This data may be useful for future investigations targeted at developing, and better understanding, interventions that improve neural function and could have important implications for understanding decrements in physical performance induced by the neural alterations seen in neurological fatigue, muscle disuse, aging and motor disorders.

Chapter 5 – Central and Peripheral Fatigue Following Non-Exhaustive and Exhaustive Exercise of Disparate Metabolic Demands

5.1 Summary

This Chapter will build on the findings from Chapter 4. Whilst corticospinal and neuromuscular function relates to exercise capacity, this Chapter explores how they are impacted by non-exhaustive and exhaustive exercise of disparate metabolic demands. The results from this Chapter demonstrate high metabolic stress accelerates the rate of central and peripheral fatigue development, with a relationship between peripheral and supraspinal fatigue supporting peripheral inhibition of central motor drive. Central fatigue was similar at exhaustion suggesting an important role in task failure. Additionally, the metabolic stress of the exercise differentially effects the cortical cells with severe-intensity exercise disturbing intracortical inhibition.

5.2 Introduction

Metabolic responses to exercise are dependent on exercise intensity and duration (Carter et al., 2000, Pringle and Jones, 2002) and have important implications for the exercising human by contributing to fatigue processes at the level of the muscle (Allen et al., 2008b, Morris et al., 2012) and within the CNS (Theurel and Lepers, 2008, Amann, 2011, Sidhu et al., 2013a) affecting exercise tolerance. However, the mechanisms underpinning exercise-induced fatigue are poorly understood. Investigating fatigue induced by both non-exhaustive and exhaustive exercise (i.e. exercise which induces task failure) of different intensities with controlled metabolic stress may help understanding of the factors limiting exercise tolerance. Adequately

controlling the metabolic strain induced by exercise and studying the subsequent sites of fatigue has important implications for informing how individuals tolerate exercise in order to develop strategies to enhance exercise performance and adherence. The focus of the following study is to examine central and peripheral mechanisms of fatigue in response to non-exhaustive and exhaustive exercise of disparate metabolic demands.

Muscle fatigue is characterised by a loss in MVC and occurs due to processes distal to the neuromuscular junction (peripheral fatigue) as well as within the CNS (central fatigue), whereas exhaustion refers to task failure (Gandevia, 2001). Locomotor exercise induces progressive muscle fatigue, with mechanisms both peripheral and central in origin, up until exercise cessation or exhaustion (Lepers et al., 2002, Decorte et al., 2012). Peripheral fatigue can occur due to impairments in contractile function from substrate degradation or metabolite accumulation (Allen et al., 2008b). Activation from the CNS may be disturbed by altered neurotransmission associated with increased lipolysis (Meeusen et al., 2006) or inhibited by metabolic afferent feedback (Taylor and Gandevia, 2008, Amann, 2011). Therefore the central mechanisms of fatigue, considered important for exercise tolerance (Amann, 2011, Decorte et al., 2012), appear to be influenced by the muscle metabolic response and accordingly the exercise intensity and duration.

Central and peripheral fatigue are evident following prolonged low-intensity exercise (i.e. cycling at 55% $\dot{W}_{max}$, 5 h) (Lepers et al., 2002) and high-intensity short duration exercise (80% $\dot{W}_{max}$, <30 min) (Decorte et al., 2012). Central and peripheral fatigue can be measured by stimulation of the femoral nerve using the ITT. A supraspinal component to central fatigue has been demonstrated following prolonged low-intensity exercise (45% $\dot{W}_{max}$, 4 h) (Jubeau et al., 2014) and high-intensity exercise (~80% $\dot{W}_{max}$) (Sidhu et al., 2009b, Goodall et al., 2012).

Supraspinal fatigue can be examined by TMS of the motor cortex allowing quantification of untapped motor cortical output (Goodall et al., 2009). Only a few studies have compared fatigue after different exercise demands. Compared to constant power output cycling (50-70% $\dot{W}_{max}$, ~30 min), work and duration matched high-intensity intermittent cycling results in greater peripheral (Morris et al., 2012) and central fatigue (Theurel and Lepers, 2008). In contrast, Thomas et al. (2015a) reported peripheral fatigue was greater and central and supraspinal fatigue attenuated following 4 km (~6 min) compared to 20 km and 40 km time-trial cycling (>30 min). Therefore, the role of intensity, but also duration, and their relation to mechanisms of fatigue remains unclear. The exercise protocols employed (constant vs intermittent and different distance time-trials) make direct interpretation difficult and an intensity comparison between constant work rate exercise matched for workload and/or at exhaustion is not available. A comparison between non-exhaustive and exhaustive exercise will help allude to factors that contribute to task failure. Furthermore, few studies have prescribed intensity anchored to metabolic markers in order to elicit controlled levels of metabolic stress, preferring percentages of $\dot{V}O_{2max}$ or $\dot{W}_{max}$ which can lead to profoundly different inter-individual metabolic and physiological responses (Mann et al., 2013).

As well as identifying supraspinal fatigue, TMS of the motor cortex can also reveal changes in the excitability of the cortical output neurons which may be important in understanding central fatigue and CNS regulation of locomotor exercise (Sidhu et al., 2013a). As the response of these cortical cells to exercise are likely to be affected by the homeostatic disturbances induced by the exercise and subsequent afferent feedback signals to the CNS (Sidhu et al., 2013a), adequately manipulating the exercise metabolic demand is key. A single TMS pulse elicits a muscle response termed a MEP, followed by a period of silence in the EMG signal when elicited during a contraction, termed the cSP. Whilst the MEP amplitude reflects the excitability

of the corticospinal pathway, the cSP represents a measure of $GABA_B$-mediated intracortical inhibition (Rossini et al., 2015, Chen et al., 2008). ppTMS allows the assessment of the excitability of the intracortical $GABA_A$-mediated inhibitory interneurons (SICI) that modulate motor cortex output to be studied (Kujirai et al., 1993, Chen, 2004, Chen et al., 2008). Increases in intracortical inhibition (Sidhu et al., 2013c) have been implicated in locomotor exercise fatigue, however the response of the cortical cells to locomotor exercise fatigue is poorly understood (Sidhu et al., 2013a) and established ppTMS techniques have yet to be employed to better understand central fatigue following cycling. Examining the response of these cortical cells to both exhaustive and non-exhaustive exercise of different metabolic demands can provide key insights to how factors such as feedback from fatigued muscle, physiological disturbances and perception of effort contributes to alterations in central drive (Sidhu et al., 2013a). The aim of the present study was to examine central and peripheral fatigue resulting from *non-exhaustive* and *exhaustive* cycling of differing intensities with disparate, controlled metabolic demands. It was hypothesised that the greater metabolic disturbance induced by severe-intensity exercise, compared with moderate-intensity steady-state exercise, would result in a greater level of peripheral and central fatigue in response to non-exhaustive exercise but that there will be no difference between intensities in the level of peripheral or central fatigue at exhaustion. It was also hypothesised that severe-intensity exercise would result in disturbances in the response of the inhibitory cortical cells (SICI and cSP).

5.3 Methods

5.3.1 Participants

Sixteen males (mean ± SD, age 25 ± 5 years, height 1.80 ± 0.08 m, mass 80.5 ± 9.9 kg, $\dot{V}O_{2max}$ 43.5 ± 5.7 ml·kg^{-1}·min^{-1}) volunteered to participate in the study. All participants were recreationally active but not taking part in structured endurance training.

5.3.2 Experimental Procedures

Each participant completed five trials, each separated by 5-14 d (Figure 5.1A). The first trial (Visit 1, Figure 5.1A) involved familiarisation to the neuromuscular and corticospinal testing procedures before completion of submaximal and maximal cycle tests to determine the LT and $\dot{V}O_{2max}$, respectively. Each of the remaining visits (Visits 2–5, Figure 5.1A) involved three cycling bouts with neuromuscular and corticospinal function tested after each bout to examine peripheral and central fatigue, as well as intracortical inhibition (SICI and cSP) (Figure 5.1B). The exercise was completed at either 50%Δ or 90% of the LT (90%LT) so as to elicit disparate metabolic responses (Carter et al., 2000). The first two cycling bouts were for fixed durations so as not to induce task failure (non-exhaustive exercise) and matched for workload between intensities (equivalent to 10 min at 50%Δ), whereas the third bout was a TTE trial and so was completed until task failure (exhaustive exercise). 50%Δ was chosen to target an intensity above MLSS (severe-intensity, SI) so a metabolic steady-state could not be achieved, whereas 90%LT was chosen to ensure a moderate-intensity (MI) (Carter et al., 2000, Pringle and Jones, 2002). Each participant completed two trials (one at each intensity) during which SICI was measured (SICI and SI, SICI and MI). The remaining two repeat trials (one at each intensity) were completed to examine the repeatability of the fatigue measures. The order of intensity (either SI or MI) was randomised and counterbalanced in a cross-over design.

Figure 5.1. Overview of study design (A) and neuromuscular and corticospinal testing protocol (B). *SICI (once at each intensity) was measured with ppTMS.

5.3.3 Muscle Torque and Electromyography

Isometric torque and EMG were recorded according to the procedures previously described (section 3.5).

5.3.4 Motor Nerve Stimulation

MNS was applied according to procedures previously described (section 3.6). During the first visit, the increment in $Q_{tw,unpot}$ and M-wave amplitude from 90 to 95% SO was 0.59% (P = 0.35) and 0.89% (P = 0.43), respectively; the increment in $Q_{tw,unpot}$ and M-wave amplitude from 95% to 100% SO was 0.08% (P = 0.88) and 0.32% (P = 0.46), respectively. Supramaximality of the stimulation was also confirmed in all subsequent visits.

5.3.5 Transcranial Magnetic Stimulation

TMS was applied according to procedures previously described (section 3.7).

5.3.6 Neuromuscular and Corticospinal Function

In order to examine the effect of exercise on peripheral and central fatigue, pre- to post-exercise changes in voluntary, MNS and TMS evoked torque and EMG responses were determined (Figure 5.1B). MVC, peripheral VA, $Q_{tw,pot}$, M_{max} and $MVCM_{max}$, cortical VA, MEP amplitude, cSP duration and SICI were determined as described previously (sections 3.9.2.2 - 3.9.2.3). The measurement of cortical VA elicited a large MEP in the VL during a 50% MVC (mean amplitude of $61.3 \pm 21.4\%$ $MVCM_{max}$ and 58.1% $MVCM_{max}$ for SI and MI, respectively) and small MEP in the BF (mean amplitude of $7.5 \pm 4.3\%$ and 6.5 ± 3.9 of VL $MVCM_{max}$ for SI and MI, respectively). During two trials (one at each exercise intensity) SICI was measured with a subthreshold CS of 90% aMT; ten single-pulse and ten paired-pulses were given at each time-point in a random order.

5.3.7 Locomotor Exercise

All exercise tests were completed on an electromagnetically braked cycle ergometer and expired gases, HR, RPE and [La$^-$] were measured as described previously (section 3.4).

Visit 1 - Familiarisation Session and Exercise Tests

Upon familiarisation to the neuromuscular and corticospinal testing, participants completed a submaximal and maximal exercise test for the determination of the LT and $\dot{V}O_{2max}$ as described previously (3.4.2.4). The LT occurred at 138 ± 46 W ($55 \pm 10\%$ $\dot{V}O_{2max}$). $\dot{V}O_{2max}$ was 3.50 ± 0.61 l·min^{-1} and occurred at a power output of 297 ± 52 W. $\dot{W}_{max}$ was 337 ± 37 W.

Visits 2 and 3 - Constant Workload Trials

During each trial, three bouts of cycling were completed at either a MI or SI each separated by 20 min rest. The work rates equated to 124 ± 41 W ($51 \pm 9\%$ $\dot{V}O_{2max}$) for the MI and 218 ± 41 W ($78 \pm 6\%$ $\dot{V}O_{2max}$) for the SI. The first two bouts were of a fixed duration (non-exhaustive), matched for work completed (kJ) between intensities, followed by a cycle to exhaustion (Figure 5.1A). During these visits, SICI was measured at each intensity with the order of exercise intensity assigned in a random, counterbalanced order.

Non-Exhaustive Cycling

A 5 min warm-up below LT preceded the first exercise bout. The first two bouts were 10 min duration in the SI and 18.63 ± 3.96 min in the MI, manipulated so as to match the work completed in each SI bout (130.8 ± 24.3 kJ). The neuromuscular and corticospinal testing protocol was repeated pre-exercise (pre) and ≤ 2 min after each exercise bout (post 1 and post 2). Expired gases and HR were recorded continuously throughout and RPE was noted every minute. [La$^-$] was measured before and after each exercise bout.

Exhaustive Cycling

The cycle to exhaustion was terminated once a cadence ≥ 60 rpm could no longer be maintained. Participants were blinded to elapsed time and verbally encouraged to continue as long as possible. The neuromuscular and corticospinal testing protocol was repeated ≤ 2 min post-exhaustion (post TTE). During the SI trial, expired gases and HR were recorded throughout and RPE was taken every ~2 min. During the MI trial, expired gases, HR and RPE were recorded for the first 10 min. [La$^-$] was measured pre-exercise and every 5 min in both trials for the first 10 min. All variables were measured at exhaustion.

Visits 4 and 5 – Repeated Constant Workload Trials

All participants repeated the constant workload trials to determine the repeatability of TTE and the fatigue measures (change (%) from pre- to post-exercise at exhaustion).

5.3.8 Data Analysis

All data analyses were completed blinded to exercise intensity. MVC, $Q_{tw,pot}$, peripheral and cortical VA, ERT, and corticospinal excitability (MEP amplitude, SICI and cSP duration) were analysed as described previously (section 3.9.2.4). Each $Q_{tw,pot}$ was analysed for CT, RTD and 0.5RT as previously described (3.9.2.2). Regression analysis confirmed the linearity of the SIT and voluntary torque relationship for the determination of the ERT ($r^2 = 0.93 \pm 0.05$, mean of all contractions across all trials and time-points). Data for cortical VA and ERT were excluded from one participant due to inability to obtain twitches at $\geq 75\%$ MVC.

5.3.9 Statistical Analysis

Data are presented as mean $\pm$ SD in the tables and text and mean $\pm$ SE in the figures. All within exercise and fatigue data were analysed, and are presented, from the first trial completed at each intensity; fatigue data from the second trials was used to examine repeatability. Statistical analyses were completed in SPSS (v.21, SPSS Inc., USA). Normal distribution was tested with the Shapiro-Wilk test. Within-exercise measurements ($\dot{V}O_2$, HR, RER, [La$^-$], RPE) were analysed to detect differences between intensities with two-way (intensity $\times$ time) repeated-measures ANOVAs for the non-exhaustive exercise, and paired-samples t-tests at exhaustion. Additional one-way repeated-measures ANOVAs were performed to detect within trial changes in [La$^-$] and RER. Pre-exercise measurements of neuromuscular and corticospinal function were compared between trials using paired-samples t-tests. The same variables were examined with one-way repeated-measures ANOVAs (for non-exhaustive exercise) and

paired-samples t-tests (for exhaustive exercise) for each intensity to determine changes from pre-exercise, and two-way repeated-measures ANOVAs (intensity × time) to detect differences between trials for non-exhaustive and exhaustive exercise. SICI data were analysed relative to pre-exercise (Verin et al., 2004, Williams et al., 2014) to minimise effects of normal large inter-individual and day-to-day variability (section 3.9). If an ANOVA revealed a significant effect, contrasts and pairwise comparisons were performed using a Bonferroni correction. Effect sizes (ES) were calculated with Cohen's D as small ($0.20 - 0.50$), medium ($0.50 - 0.80$) and large (> 0.80). Repeatability of fatigue measures were determined using an $ICC_{3,1}$ and mean bias. To test associations between the level of peripheral fatigue with supraspinal fatigue (Amann, 2011, Sidhu et al., 2013a) and cortical cell function (Sidhu et al., 2013a), Pearson's correlation coefficients were used to examine associations between changes in $Q_{tw,pot}$ with changes in cortical VA, cSP and SICI at exhaustion. Significance was accepted as $P \leq 0.05$.

5.4   Results

Cardio-Respiratory and Perceptual Responses

Mean exhaustion time was 17.9 ± 7.6 min in the SI trial and 117.6 ± 47.2 min in the MI trial. During non-exhaustive cycling, the SI trial induced greater metabolic, physiological and perceptual demands as evidenced by greater increases in $\dot{V}O_2$, HR, [La⁻] and RPE than the MI trial at all time-points, whilst RER was greater in the SI vs MI trial from 2 min onwards (all $P < 0.001$) (Figure 5.2). Each SI cycling bout increased [La⁻] compared to the corresponding pre-exercise value ($P < 0.001$) whereas [La⁻] remained unchanged following each MI bout ($P > 0.05$). $\dot{V}O_2$ ($92 \pm 10\%$ vs $51 \pm 9\%$ $\dot{V}O_{2max}$), HR ($93 \pm 4\%$ vs $72 \pm 8\%$ HR_{max}), [La⁻] and RER were all greater at exhaustion in the SI versus the MI trial, respectively (all $P < 0.001$), however there was no difference in RPE ($P > 0.05$) (Figure 5.3). During the exhaustive MI cycle there was gradual decline in RER from 40 min onwards compared to 10 min ($P < 0.01$).
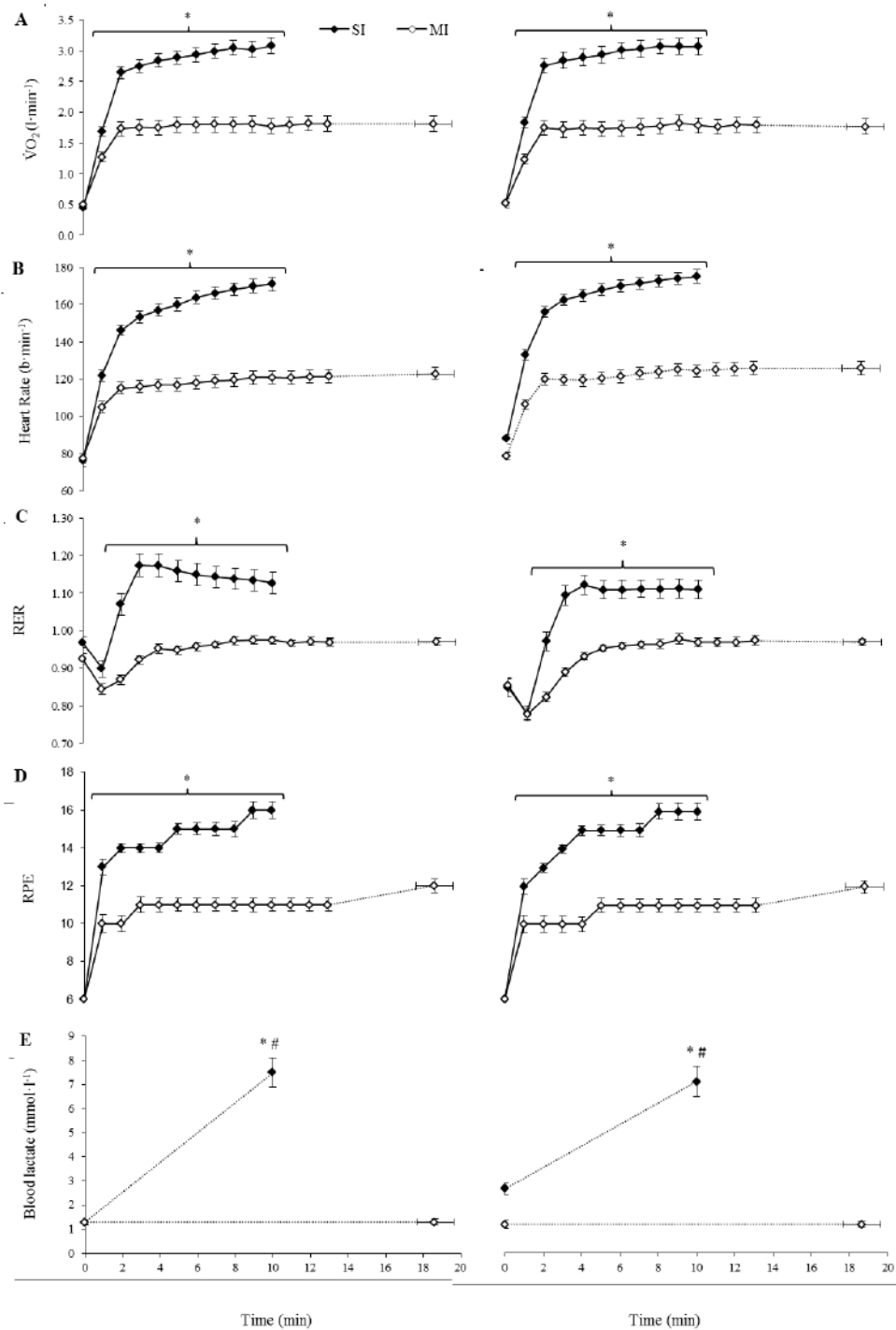
Figure 5.2. Physiological responses to non-exhaustive exercise bouts 1 (left) and 2 (right). A, oxygen uptake; B, heart rate; C, respiratory exchange ratio; D, ratings of perceived exertion; E, blood lactate. Data are for 10 min in the SI trial (filled diamonds) and the shortest exercise duration completed by all participants in the MI trial (hollow diamonds) extrapolated to mean end time. Data are mean ± SE.

*$P < 0.001$ vs MI at same time-point; # $P < 0.001$ vs pre-exercise.

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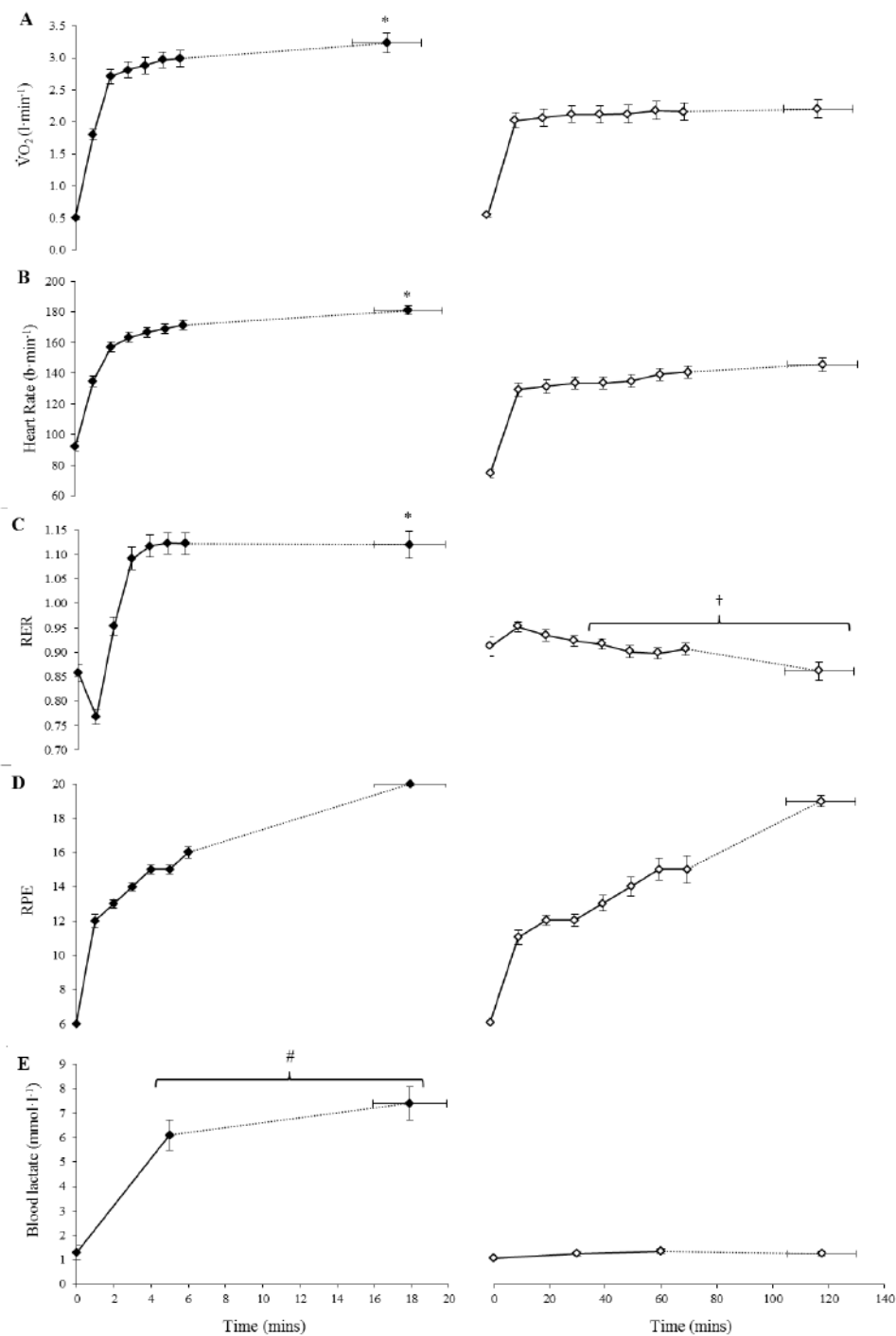
Figure 5.3. Physiological responses during exhaustive exercise (SI trial are left figures and filled diamonds; MI trial are right figures hollow diamonds). A, oxygen uptake; B, heart rate; C, respiratory exchange ratio; D, ratings of perceived exertion; E blood lactate. Data are plotted for the shortest time completed by all participants extrapolated to group mean exercise exhaustion time. Data are mean ± SE.

*$P \leq 0.001$ for HI vs MI at exhaustion; #$P \leq 0.001$ vs pre-exercise; †$P \leq 0.01$ vs 10 min within MI.

Non-Exhaustive Cycling

Global and Peripheral Fatigue

There were no differences in pre-exercise MVC, $Q_{tw,pot}$ or ERT between the SI and MI trials, respectively (all $P \geq 0.42$). MVC torque was reduced below pre-exercise in the SI trial (post 1: $-9 \pm 8\%$ and post 2: $-12 \pm 8\%$, $P < 0.01$) with a tendency for a reduction in the MI trial at post 1 ($-3 \pm 5\%$, $P = 0.058$) which reached significance at post 2 ($-6 \pm 7\%$, $P < 0.05$) (Figure 5.4A). The MVC tended to be impaired more for SI than MI cycling at post 1 ($P = 0.055$, ES $= 0.90$) and was significantly more impaired at post 2 ($P < 0.05$, ES $= 0.80$). Similar to MVC, the SI trial induced a loss in $Q_{tw,pot}$ from pre-exercise (post 1: $-18 \pm 10\%$ and post 2: $-24 \pm 9\%$, $P < 0.001$) with a trend for a reduction in the MI trial at post 1 ($-6 \pm 9\%$, $P = 0.061$) which was significant at post 2 ($-10 \pm 7\%$, $P \leq 0.001$) (Figure 5.4B). The $Q_{tw,pot}$ was impaired more in the SI trial at both time-points ($P \leq 0.001$, ES ≥ 1.26). Reductions in ERT were also evident following SI cycling (post 1: $-24 \pm 23\%$ and post 2: $-37 \pm 23\%$, $P \leq 0.01$) but not after MI cycling (post 1: $5 \pm 24\%$ and post 2: $1 \pm 19\%$, $P > 0.05$) with a significant difference between trials at both time-points ($P \leq 0.001$, ES ≥ 0.81) (Figure 5.4C). All contractile and EMG data are in Table 5.1. RTD was impaired from pre-exercise at both time-points in the SI trial ($P < 0.01$) but unchanged in the MI trial ($P > 0.05$) with significant differences between intensities at both time-points (both $P < 0.01$). 0.5RT was reduced similarly after both bouts irrespective of intensity. CT, $MVCM_{max}$, M_{max} and rms/rmsM were unaffected by exercise ($P > 0.05$).

Central Fatigue

There was no difference in pre-exercise rMT ($55 \pm 11\%$ vs $56 \pm 12\%$), aMT ($40 \pm 7\%$ vs $38 \pm 8\%$), peripheral VA ($95.2 \pm 5.2\%$ vs $94.7 \pm 5.8\%$), cortical VA ($96.6 \pm 3.4\%$ vs $96.1 \pm 3.5\%$), MEP amplitude, cSP duration or SICI (0.86 ± 0.73 vs 0.81 ± 0.53) between the SI and MI, respectively (all $P \geq 0.17$). Peripheral VA remained unchanged following MI cycling (post 1:

−2 ± 5% and post 2: −3 ± 5%, P > 0.05) whilst the SI trial impaired peripheral VA (post 1: −4 ± 6% and post 2: −6 ± 7%, P < 0.05) (Figure 5.4D). The loss in peripheral VA was greater following SI cycling compared to MI cycling post bout 2 (P < 0.05, ES = 0.49). Cortical VA was maintained during the MI (post 1: −1 ± 3%, and post 2: −2 ± 2%, P > 0.05) but there was a progressive loss in the SI trial (post 1: −4 ± 4%, P < 0.05 and post 2: −5 ± 4%, P ≤ 0.001) (Figure 5.4E) with a significant difference between intensities at both time-points (P < 0.01, ES ≥ 0.85). MEP amplitude (Table 5.1) and SICI (Figure 5.5) were unaffected by exercise (P > 0.05). SI cycling reduced the cSP duration from pre-exercise (post 1: −7 ± 9%, P < 0.05 and post 2: −12 ± 9%, P ≤ 0.001) but the cSP remained unchanged after the MI bouts (P > 0.05) with a significant difference between trials at both time-points (P ≤ 0.01, ES ≥ 0.59) (Table 5.1).

Exhaustive Cycling

Figure 5.6 shows an example torque trace for measurement of cortical VA (Figure 5.6A), MVC torque, peripheral VA and $Q_{tw,pot}$ torque (Figure 5.6B) and an example EMG trace for measurement of MEP amplitude, cSP duration and SICI (Figure 5.6C) at pre-exercise and post-exhaustion for both intensities of exercise.

Global and Peripheral Fatigue

SI and MI cycling both reduced MVC torque (−19 ± 15% vs −17 ± 15%, respectively, P ≤ 0.001) and $Q_{tw,pot}$ (−31 ± 10% vs MI: −17 ± 12%, respectively, P < 0.001) below pre-exercise. The MVC loss was not different between trials (P > 0.05, ES = 0.13) however there was a trend for greater impairments in $Q_{tw,pot}$ after SI cycling (P = 0.051, ES = 1.23). The ERT was reduced after SI cycling (−41 ± 19%, P < 0.001) and MI cycling (−19 ± 27%, P < 0.05) with the impairment greater after SI cycling (P < 0.01, ES = 0.94). Exhaustion impaired muscle

contractility (RTD, 0.5RT) and the rms/rmsM ratio similarly irrespective of intensity (Table 5.1). CT, M_{max} and $MVCM_{max}$ were unaffected by exercise in either trial ($P > 0.05$).

Central Fatigue

Both the SI and MI trials reduced peripheral VA ($-7 \pm 8\%$, $P < 0.001$ vs $-7 \pm 10\%$, $P \leq 0.01$, respectively) and cortical VA ($-7 \pm 9\%$, $P < 0.01$ vs $-7 \pm 6\%$, $P < 0.001$, respectively) below pre-exercise with no differences between intensities ($P > 0.05$, ES = 0.00). There were significant correlations between cortical VA and $Q_{tw,pot}$ reductions after SI cycling ($r = 0.52$, $P < 0.05$) but not MI cycling ($r = 0.18$, $P = 0.26$). Figure 5.4E and 5.4F illustrates the relationships between the level of peripheral and supraspinal fatigue (individual and grouped mean data, respectively) after each exercise bout suggesting the two components of fatigue develop together. MEP amplitude was unaffected by exhaustion at either intensity ($P > 0.05$). cSP duration was reduced below pre-exercise in the SI ($-14 \pm 10\%$, $P < 0.001$) but not MI trial ($P > 0.05$) with these different between intensities ($P < 0.001$, ES = 1.07). There was no relationship between cSP reduction and the level of peripheral fatigue ($r = 0.23$, $P > 0.05$). SICI was increased from pre-exercise in the SI as evidenced by a reduction in the SICI ratio ($-24 \pm 22\%$, $P < 0.001$), denoting an increased inhibition of the test stimulus by the conditioning stimulus, with the increase in SICI correlated with impartments in $Q_{tw,pot}$ ($r = 0.49$, $P < 0.05$). SICI remained unchanged in the MI trial ($P > 0.05$) with a significant difference between intensities ($P < 0.05$, ES = 0.66).
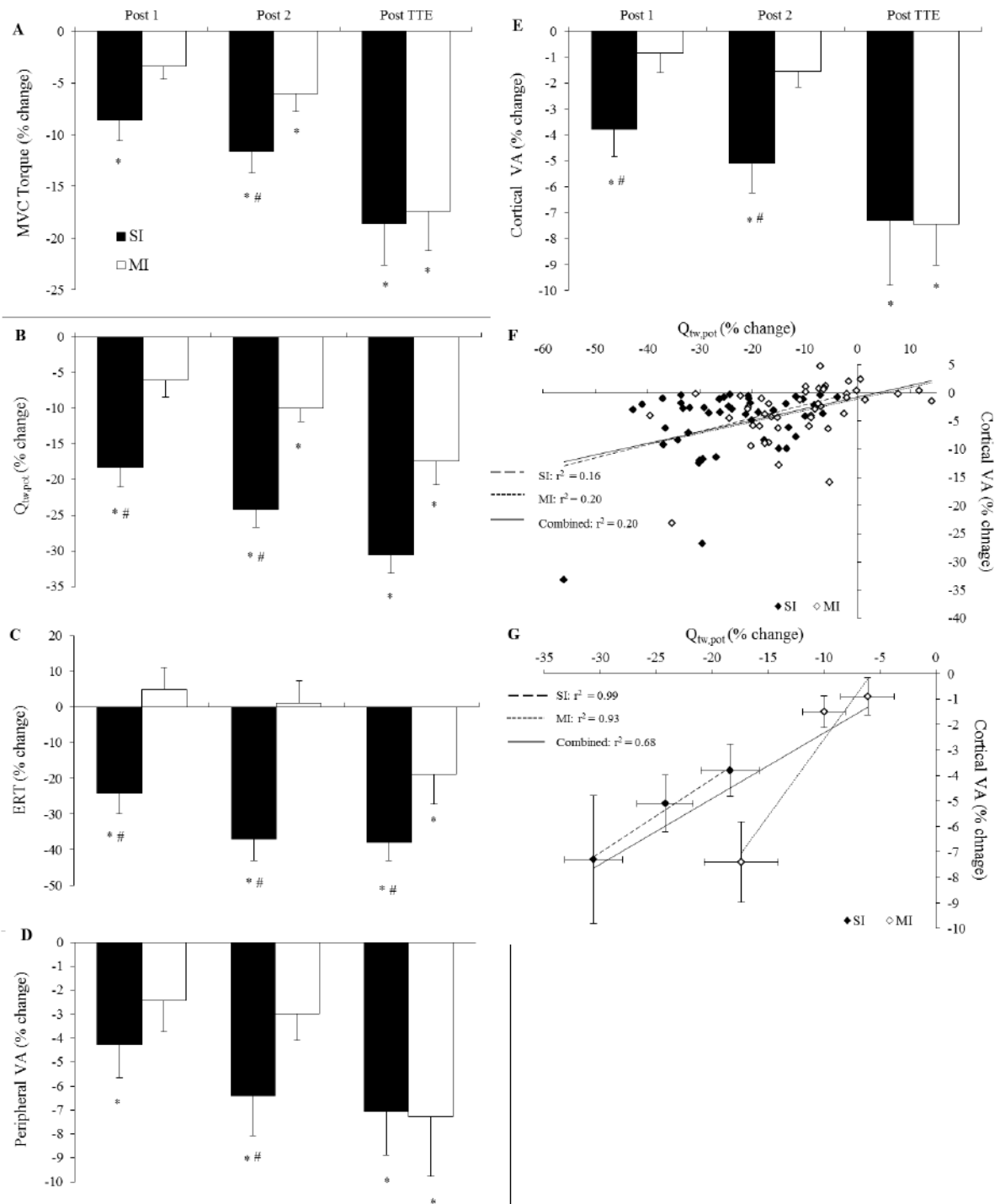
Figure 5.4. Peripheral and central fatigue throughout the SI (filled bars) and MI (hollow bars) trials. A, maximal voluntary torque; B, potentiated quadriceps twitch torque; C, estimated resting twitch; D, peripheral voluntary activation; E, cortical voluntary activation; F, individual data showing relationship between change in $Q_{tw,pot}$ and cortical VA across all post-exercise time-points; G, group data showing relationship between change in $Q_{tw,pot}$ and cortical VA

across all post-exercise time-points. Data expressed as change (%) from pre-exercise (mean ±

SE).

*P ≤ 0.05 vs pre-exercise; #P ≤ 0.05 vs MI at same time-point.
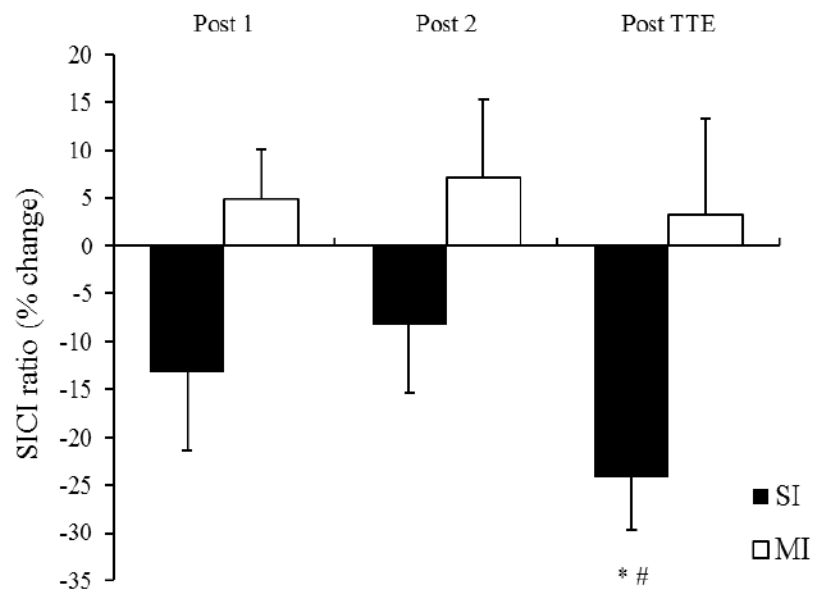
Figure 5.5. Short-interval intracortical inhibition throughout the SI (filled bars) and MI (hollow

bars). Data expressed as change (%) from pre-exercise (mean ± SE).

*P < 0.05 vs pre-exercise; #P < 0.05 difference between intensities at same time-point.

Table 5.1. Electromyographical and contractile characteristics throughout the SI and MI. Data are mean ± SD.

	Severe Intensity				Moderate Intensity			
	Pre	Post 1	Post 2	Post TTE	Pre	Post 1	Post 2	Post TTE
MVC								
N·m	482 ± 60	439 ± 57*	425 ± 54*#	388 ± 64*	480 ± 71	463 ± 70	450 ± 66*	392 ± 90*
MVCM$_{max}$ (mV)	5.69 ± 2.27	5.87 ± 2.01	6.07 ± 1.81	5.84 ± 1.98	5.47 ± 1.93	5.74 ± 2.01	5.77 ± 2.07	5.90 ± 2.06
rms/ rmsM	0.24 ± 0.07	0.22 ± 0.06	0.21 ± 0.07	0.21 ± 0.06	0.24 ± 0.07	0.22 ± 0.05	0.21 ± 0.06	0.19 ± 0.07*
ERT								
N·m	106 ± 49	76 ± 35*#	65 ± 36*#	62 ± 35*#	102 ± 49	104 ± 59	99 ± 48	84 ± 48*
Q$_{tw,pot}$								
N·m	156 ± 22	127 ± 24*#	117 ± 15*#	108 ± 21*	158 ± 31	148 ± 30	142 ± 28*	127 ± 27*
M$_{max}$ (mV)	6.37 ± 1.70	6.51 ± 1.33	6.58 ± 1.06	6.17 ± 1.42	6.45 ± 1.30	6.32 ± 1.57	6.26 ± 1.60	6.43 ± 1.60
CT (ms)	44.1 ± 5.9	47.2 ± 5.5#	47.4 ± 6.2#	47.4 ± 5.8	45.1 ± 6.8	43.9 ± 6.8	44.4 ± 6.0	44.7 ± 5.3
RTD (Nm·ms)	3.31 ± 0.80	2.65 ± 0.65*#	2.33 ± 0.56*#	2.32 ± 0.57*	3.49 ± 1.10	3.37 ± 0.93	3.33 ± 1.10	2.95 ± 0.77*
0.5 RT (ms)	103.8 ± 23.5#	65.8 ± 33.1*	64.4 ± 38.9*	47.4 ± 25.8*	93.5 ± 19.8	60.8 ± 18.4*	54.3 ± 19.6*	54.7 ± 21.9*
MEP								
amp (%M$_{max}$)	26.2 ± 15.8	26.9 ± 13.3	27.1 ± 15.3	26.9 ± 15.7	29.3 ± 14.4	30.1 ± 16.3	29.5 ± 14.3	27.4 ± 14.7
cSP (ms)	193 ± 43	180 ± 51*#	171 ± 48*#	166 ± 50*#	194 ± 57	197 ± 60	194 ± 64	196 ± 68

MVC, maximal voluntary contraction; MVCM$_{max}$ maximal M-wave during MVC; rms/rmsM, root mean squared relative to root mean squared of MVCM$_{max}$; ERT, estimated resting twitch; Q$_{tw,pot}$ potentiated quadriceps twitch; M$_{max}$, maximal M-wave at rest; CT, time to peak torque; RTD, rate of torque development; 0.5RT, half relaxation time; MEP, motor evoked potential; cSP, cortical silent period.

*P ≤ 0.05 vs pre-exercise; #P ≤ 0.05 vs MI at same time-point.
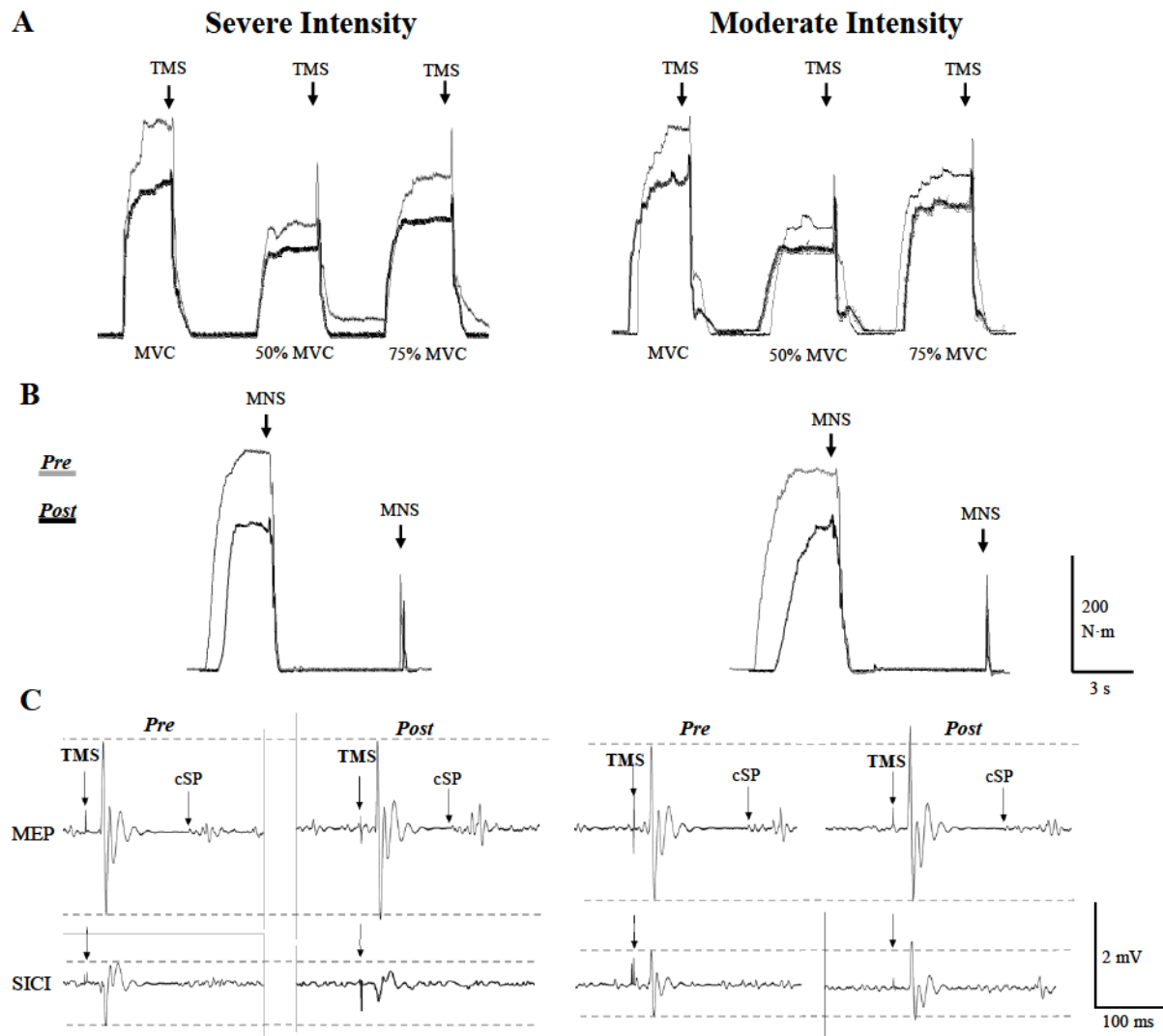
Figure 5.6. Raw torque (A and B) and EMG traces (C) for a representative participant pre-exercise and post-exhaustion for each intensity (left figures are SI; right figures are MI). A: Torque responses to TMS during contractions at 100%, 75% and 50% MVC for the measurement of cortical VA. B: Torque responses to MNS during MVC and at rest for measurement of MVC torque, peripheral VA and $Q_{tw,pot}$. Pre-exercise data are represented by grey traces and post-exercise data are represented by black traces. C: EMG responses to spTMS (MEP with cSP duration) and ppTMS (SICI). Horizontal dashed lines represent pre-exercise peak-to-peak amplitude.

Reliability

Relative change (%) in pre- to post-exhaustion values for fatigue measures (MVC, $Q_{tw,pot}$ and ERT, peripheral VA and cortical VA) demonstrated moderate to good repeatability for the SI trial (ICC = 0.62 − 0.88) with no systematic bias (all P > 0.5, mean bias −2.1 − 0.9%). The reduction in the cSP demonstrated no systematic bias (P = 0.46, mean bias −2.1%) however was less repeatable (ICC = 0.51). The MI trial demonstrated no systematic bias (all P > 0.6, mean bias −2.9 − 0.5%) but the fatigue measures were less repeatable (ICC = 0.30 − 0.70).

5.5 Discussion

The aim of the present study was to examine peripheral and central contributions to fatigue following *non-exhaustive* and *exhaustive* exercise of different metabolic demands. This study found greater peripheral and central fatigue following non-exhaustive severe intensity compared to moderate intensity exercise, but that exhaustion occurs with similar levels of central fatigue. For the first time ppTMS has been employed to investigate supraspinal processes following cycling and this study demonstrated distinct impairments in intracortical excitability in response to exercise intensity; an increase in SICI and shortening of the cSP was evident after SI exercise. Although there was a tendency for a greater level of peripheral fatigue after exhaustive SI exercise, in contrast to the hypothesis peripheral fatigue would be equal at exhaustion which must now be rejected, the remaining hypotheses can be accepted. These findings contribute to better understanding of the multi-faceted, integrated mechanisms of fatigue and to the growing interest on supraspinal mechanisms of exercise tolerance.

Global and Peripheral Fatigue

Non-exhaustive SI exercise induced greater global and peripheral fatigue (MVC and $Q_{tw,pot}$ impairments, respectively). Although a small reduction in $Q_{tw,pot}$ was found after the second MI

exercise bout, the ERT was unchanged. It is unclear why this discrepancy would arise, however measures of ERT were twice as variable (CV ~14%) as measures of $Q_{tw,pot}$ (CV ~8%), as confirmed by others (Sidhu et al., 2009a), and may therefore be less sensitive to detecting small levels of peripheral fatigue in response to submaximal exercise (<LT). The level of peripheral fatigue after MI post 2 (−10%) exceeded the CV for the $Q_{tw,pot}$ but not for the ERT, which suggests that measures of $Q_{tw,pot}$ were sensitive enough to detect this level of fatigue whereas measure of ERT were not. In contrast, there were similar patterns of change in the $Q_{tw,pot}$ and ERT following exhaustive exercise when levels of peripheral fatigue were above the ERT CV. Whilst a reduction in the ERT is indicative of peripheral fatigue (Goodall et al., 2009), compared with the $Q_{tw,pot}$, measurement of ERT also involves mechanisms at cortical and spinal sites and there are differences in the motor units recruited by MNS and TMS (Todd et al., 2003, Sidhu et al., 2009a). Therefore, the ERT and $Q_{tw,pot}$ appear to reflect different underlying processes which may contribute to these results. Nevertheless, other work has reported that the ERT remains unchanged following prolonged exercise (Bowtell et al., 2013), and the ERT and $Q_{tw,pot}$ do not follow the same pattern in response to cycling in the heat versus an ambient condition (Goodall et al., 2015a). This study reported a ~3-fold greater reduction in the ERT after cycling in the heat versus an ambient condition, with no difference in $Q_{tw,pot}$ impairment between conditions.

High-intensity intermittent cycling has previously resulted in greater impairments in MVC and contractile function in comparison to lower intensity work-matched trials (Theurel and Lepers, 2008, Morris et al., 2012). Similarly, higher intensity time-trials resulted in greater peripheral fatigue with no difference in MVC (Thomas et al., 2015a). The SI trial [La⁻] data suggest an accumulation of intramuscular metabolites (such as P_i and H^+), which impair muscle contractile function (Allen et al., 2008b). There were similar reductions in MVC between intensities at

exhaustion, comparable in magnitude to those reported previously across a range of cycling protocols (Lepers et al., 2002, Decorte et al., 2012, Goodall et al., 2012, Jubeau et al., 2014, Thomas et al., 2015a), however there was a tendency for greater peripheral fatigue following SI exhaustive cycling. The high repeatability of $Q_{tw,pot}$ following SI (ICC = 0.74) but not MI (ICC = 0.30) exhaustive cycling support the notion of a critical fatigue threshold whereby high-intensity exercise tolerance is disturbed by the attainment of a critical level of peripheral fatigue (Amann, 2011). The more variable end $Q_{tw,pot}$ values following MI exercise suggest exercise tolerance was not impaired by peripheral fatigue which is unsurprising as the MI exercise likely failed to disturb muscle metabolic homeostasis. The reduction in RER (from 0.95 after 10 min to 0.86 at exhaustion) during this trial supports the limited muscle glycogen as a contributing factor to exhaustion.

Central Fatigue

Cortical VA was reduced after all SI bouts, but only at exhaustion in the MI trial where the fall in cortical VA was similar to the SI trial. These reductions are similar to those reported previously following high-intensity (Goodall et al., 2012) and prolonged cycling (Jubeau et al., 2014). A decline in cortical VA demonstrates that TMS could produce extra motor cortical output and therefore an impaired ability of the motor cortex to drive the motoneurons maximally (Todd et al., 2003, Taylor and Gandevia, 2008). This component of fatigue is defined as supraspinal fatigue (Gandevia, 2001, Taylor and Gandevia, 2008). Peripheral VA followed a similar pattern with reductions similar to those reported previously (Lepers et al., 2002, Sidhu et al., 2009b, Goodall et al., 2012). Theurel and Lepers (2008) also reported greater peripheral VA loss after 33 min work-matched high-intensity intermittent compared to constant load cycling (70% $\dot{W}_{max}$). These authors suggest this was partly due to the greater metabolite accumulation caused by high-intensity exercise activating inhibitory group III/IV muscle

afferents. However, prescription of exercise relative to $\dot{W}_{max}$ can lead to variable individual metabolic responses depending on proximity to metabolic thresholds thus affecting fatigue processes. Nevertheless, our data support a metabolic involvement in the development of central fatigue when controlled for metabolic thresholds. The significant relationship between post-exhaustion supraspinal and peripheral fatigue in the SI, but not MI trial, support a metabolic mechanism distinct to high-intensity exercise. This is a mechanism through which CNS motor output is hypothesised to be inhibited in response to developing peripheral muscle fatigue and disturbed metabolic homeostasis (Amann, 2011) i.e. motor output is inversely related to the level of peripheral fatigue. Although the correlations between peripheral and supraspinal fatigue (Figure 5.4F) support such an interaction, from the methodology employed here it is not possible to determine if this relationship is causal. Conversely, supraspinal fatigue has shown to be exacerbated when cycling in the heat whilst peripheral fatigue is unchanged (Goodall et al., 2015a), and augmented following longer duration time-trials whilst peripheral fatigue is attenuated (Thomas et al., 2015a), demonstrating the extremely task specific nature of fatigue.

Cortical output was preserved following MI exercise until exhaustion suggesting a duration component to this fatigue. The reduction in RER during this trial suggests an increase in lipolysis to meet the energy requirements which is thought to indirectly contribute to central fatigue (Meeusen et al., 2006). Although not measured in the present study, the attainment of a higher, or critical core temperature, has also been linked to the development of supraspinal fatigue (Goodall et al., 2015a). The greater level of supraspinal fatigue following each non-exhaustive SI vs MI bout could therefore be due to a greater rate of bodily heat accumulation, with the equal level of supraspinal fatigue at exhaustion due to the attainment of a critical core temperature of which the MI trial took longer to attain. Central and supraspinal fatigue has

previously shown to be greater after longer (20 and 40 km, >30 min) compared to shorter duration time-trials (4 km, ~6 min) (Thomas et al., 2015a). Thomas et al. (2015a) reported no further central or supraspinal fatigue by increasing time-trial duration from ~30 min to >60 min, a finding similar to this study. This component of fatigue may therefore not be exacerbated by increasing exercise time beyond a certain duration, of which both our trials may have exceeded. Nevertheless, available data suggests peripheral mechanisms contribute to the initial (non-exhaustive) fatigue whereas central impairments contribute to the fatigue at exhaustion (Decorte et al., 2012). The greater supraspinal fatigue seen after SI bouts 1 and 2 may therefore represent the proximity to exhaustion as 10 min SI cycling was close to the maximal tolerable time (~18 min). Therefore central fatigue appears an important mechanism in task failure during constant workload cycling (Decorte et al., 2012) and our findings suggest this may be further localised to supraspinal mechanisms.

In order to better understand supraspinal processes involved in fatigue, we employed ppTMS to examine SICI. Whilst MEP amplitudes were unaffected by exercise, in agreement with previous work (Sidhu et al., 2009b, Goodall et al., 2015a), exhaustive SI cycling reduced the cSP duration and increased SICI. A shortened cSP duration denotes a decrease in $GABA_B$-mediated intracortical inhibition whereas an increase in SICI denotes an increase in $GABA_A$-mediated intracortical inhibition (Chen, 2004, Chen et al., 2008, Rossini et al., 2015). A reduction in $GABA_B$ inhibition pre-synaptically increases $GABA_A$ inhibition of the corticospinal neuron (Chen, 2004). These results are contrasting to those reported during isolated muscle fatigue where there are increases in cSP duration with reductions in SICI (Gandevia, 2001, Taylor and Gandevia, 2008, Williams et al., 2014). Compared with isolated muscle fatigue, whole-body exercise results in greater disturbances in a number of systemic and local factors that could differentially influence homeostasis and affect the cortical cells

(Sidhu et al., 2013a). However, comparisons between studies should be made with caution due to differences in the muscle tested, level of muscle activity and time of measurement relative to fatigue.

Other authors have also reported disturbances in cortical inhibitory processes following high-intensity cycling. Temesi et al. (2013) found that incremental exhaustive cycling shortens the cSP whereas Sidhu et al. (2013c) used a subthreshold TMS technique to show 30 min cycling at 75% $\dot{W}_{max}$ increased intracortical inhibition. Sidhu et al. (2013c) proposed group III/IV muscle afferents may have acted at the level of the motor cortex to inhibit the motor cortex to muscle pathway. This is supported by our finding that disturbances in inhibitory function were induced by SI, but not MI exercise. There is also evidence that the increased cSP in response to isolated muscle fatigue can be prevented by blocking the central projection of group III/IV muscle afferents (Hilty et al., 2011). The intracortical inhibitory circuits are important in modulating the final cortical output (Chen, 2004, Chen et al., 2008). Therefore, the motor cortex is less responsive to voluntary descending excitatory input, which could be an important mechanism of central fatigue. As MEPs and therefore corticospinal excitability was unchanged, it is unclear whether there were compensatory effects for decreased supraspinal excitability at the level of the spinal cord and future methodologies may benefit from the use of techniques that examine mechanisms at a spinal level. It should be noted that whilst the EMG responses to TMS provide measures of excitability along the corticospinal pathway (MEP) and within the motor cortex (cSP and SICI), these are distinct from measures of supraspinal fatigue and have been dissociated under certain circumstances (Taylor and Gandevia, 2008). Whilst this study provides new insight into the response of the cortical inhibitory responses to endurance cycling, further work is required regarding the functional impact and significance of fatigue-

induced changes in various parts of the motor pathway and the effects these have on fatigue and exercise tolerance.

Limitations

Firstly, measurements were made post-exercise and may not translate to mechanisms during exercise. The neuromuscular and cortical testing procedure itself could have also induced a small amount of fatigue, or present a different interaction with each exercise intensity, thereby masking or exacerbating fatigue. To address this, future study designs could include a control condition. These limitations are nonetheless common amongst locomotor exercise and fatigue studies which assume that differences between the fatigue measurement protocols form pre- to post-exercise are distinct mechanisms induced by the exercise. Other studies employing similar fatigue measurement protocols have shown to be effective in detecting differences in mechanisms of fatigue following different environmental conditions (Goodall et al., 2012, Goodall et al., 2015a) as well as following different distance time-trials (Thomas et al., 2015a). The fatigue measurement protocol was identical between trials and time-points and the observation that there was significant fatigue induced within a trial (pre- to post-exercise) as well as differences between intensities suggest that the protocol was adequate in detecting differences in central and peripheral fatigue between exercise of disparate metabolic demands. However, the sample size may have been insufficient to detect subtle differences between trials (i.e. peripheral fatigue at exhaustion).

TMS responses were also recorded in the right VL and so findings may not translate to the whole knee extensor muscle group. It should be noted that ppTMS measures demonstrate significant inter-individual variability such that some individuals fail to show inhibition with measures of SICI. This is however an accepted finding with an active muscle contraction,

which suppresses SICI (Ridding et al., 1995, Ortu et al., 2008), and the level of SICI we report here are similar to those previously reported (Ridding et al., 1995, Ortu et al., 2008, Sidhu et al., 2013b). Indeed SICI is a complicated measure and more likely reflects a balance between inhibition and facilitation rather than inhibition alone (Chen et al., 2008, Rossini et al., 2015). In humans it is not possible to study the inhibitory and excitatory systems in isolation with the final cortical output the result of many interacting systems; therefore when there is no apparent inhibition it cannot be assumed that the circuits are not recruited (Chen, 2004). The results presented here should therefore be interpreted as disturbances in intracortical excitability either through a reduction in excitation as well as an increase in inhibition and inferences to specific neurotransmitters and receptors made with caution (Chen, 2004). Future investigations may benefit from individualising the ppTMS protocol to ensure optimal stimulation parameters to elicit SICI. Nevertheless, the methodology was consistent across exercise conditions suggesting adequacy in detecting differences between intensities.

The SI exercise was also prescribed according to the %Δ method rather than measuring MLSS directly. Measurement of MLSS requires multiple visits to the laboratory and was not feasible in this study, however the [La⁻] data support the setting of the exercise above this intensity. Although the order of trials was randomised and counterbalanced, the multiple visits required in the present study would undoubtedly elicit potential learning effects which could affect the inter-individual response to each condition. The participants were also untrained and it is unclear whether the results translate to trained individuals who are highly motivated and used to tolerance of fatigue and high-intensity and/or prolonged exercise; this area is poorly understood and the effect of training on fatigue mechanisms is an area worth pursuing; the next Chapter will investigate some of these issues.

Conclusions

Better understanding of fatigue mechanisms has far reaching human health and performance implications, however a comparison between non-exhaustive and exhaustive exercise with controlled levels of metabolic disturbance had yet to be investigated. Compared to MI exercise, SI exercise accelerates the development of peripheral and central fatigue. The level of central fatigue was similar at exhaustion suggesting a role in task failure. The function of the motor cortical cells was also disturbed by SI exercise. This study contributes further to the understanding of the role of the CNS in limiting exercise tolerance. The next Chapter will examine the role of training on the fatigue mechanisms described in this Chapter.

Chapter 6 – The Effect of Endurance Training on Central and Peripheral Contributions to Fatigue: Implications for Exercise Tolerance

6.1 Summary

Chapter 5 demonstrated exercise of high metabolic stress accelerates the rate of central fatigue development, potentially as a result of inhibition of motor drive in response to developing peripheral fatigue, and that central fatigue appears to play an important role in exhaustion. Whilst endurance training is a potent enhancer of exercise tolerance, the effect of training on mechanisms of fatigue has yet to be investigated. This Chapter investigated the effect of high-intensity interval training (HIIT) on high-intensity exercise tolerance and the associated fatigue mechanisms in comparison to a control group performing an equal volume of work at a moderate-intensity (CONT). The findings demonstrate that the HIIT induced increases in exercise tolerance is accompanied by greater tolerance of peripheral fatigue and ischaemic muscle pain with attenuated central fatigue.

6.2 Introduction

In Chapter 5, it was demonstrated that the exercise-induced metabolic stress is an important contributor to central and peripheral fatigue development with a potential interaction (O'Leary et al., 2015a). Whilst central and peripheral fatigue are task and intensity specific (Enoka and Duchateau, 2008, Taylor and Gandevia, 2008), the effect of training has yet to be addressed. Endurance training induces adaptations in cardiovascular and skeletal muscle function that contribute to fatigue resistance and enhanced exercise tolerance (Blomqvist and Saltin, 1983, Holloszy and Coyle, 1984, Jones and Carter, 2000), however it is unknown how fatigue

mechanisms adapt and whether the same limiting mechanisms contribute to exhaustion following training.

HIIT is a potent enhancer of exercise tolerance through increasing muscle oxidative capacity (Burgomaster et al., 2005, Daussin et al., 2008) and muscle buffer capacity (Weston et al., 1997, Edge et al., 2006). These adaptations appear specific to a higher metabolic demand of training compared to lower intensity training (Edge et al., 2006, Daussin et al., 2008). Improved skeletal muscle function leads to an attenuated development of peripheral fatigue and better exercise performance (Jacobs et al., 2013). In addition to peripheral adaptations, endurance training may induce central adaptations that enhance the ability to maintain the motor drive necessary to continue exercise (Nybo and Secher, 2004), however the CNS adaptations to endurance exercise have received limited attention.

An important aspect of the central component of fatigue is the CNS intolerance to the increased sensory feedback signals such as those induced by muscle pain, muscle fatigue and metabolic stress which all likely contribute to exhaustion (Smirmaul, 2012). This has led to the promotion of high-intensity training in order to accustom individuals with the induced discomfort (Westerblad et al., 2002). High-intensity exercise tolerance is hypothesised to be limited by increased group III/IV muscle afferent firing which inhibits central motor drive and restricts the development of peripheral fatigue and sensory feedback to within a critical threshold (Amann, 2011). The high-repeatability of peripheral fatigue after SI exercise, and linear relationship with supraspinal fatigue shown in Chapter 5, supports this. Exercise is therefore terminated with a physiological reserve and it has been suggested that training may enhance the ability to access this security reserve (Millet, 2011). Although anecdotally trained individuals are considered to be able to 'push themselves harder' than their untrained

counterparts, perhaps through better tolerance of unpleasant afferent signals (i.e. muscle pain and fatigue) (Nybo and Secher, 2004, Mauger, 2013), there is little evidence to support this notion. An increased tolerance to muscle pain (Jones et al., 2014) and peripheral fatigue (Zghal et al., 2015) suggests endurance training increases the resistance to the inhibitory effects of group III/IV afferent firing and the sensory tolerance limit. As well as evoking sensory disturbances (Pollak et al., 2014), group III/IV afferent firing also facilitates supraspinal fatigue (Taylor and Gandevia, 2008, Amann et al., 2015), depresses corticospinal excitability (Martin et al., 2008, Sidhu et al., 2014) and increases intracortical inhibition (Hilty et al., 2011, Sidhu et al., 2013c). Therefore an increase in peripheral fatigue at exhaustion suggests an ability to override central fatigue mechanisms in response to inhibitory afferent firing in order to extrude greater performance from the muscle (Zghal et al., 2015). However, the effect of endurance training on the tolerance to peripheral fatigue, the sensory tolerance of afferent firing, mechanisms of central fatigue and their relationships to improvement in high-intensity exercise tolerance remains unclear.

Experimental approaches to address this problem include examining ischaemic pain tolerance (Jones et al., 2014) and the level of peripheral fatigue that is tolerated following exhaustive exercise (Zghal et al., 2015); although both increase following a period of training, these studies are marked by a number of limitations. Jones et al. (2014) did not randomise the training and control groups; the training was completed at 75% HR reserve, an intensity that produces profoundly different perceptual, physiological and metabolic responses amongst individuals (Scharhag-Rosenberger et al., 2010); endurance performance was quantified by $\dot{V}O_{2max}$ rather than exercise tolerance, and; the control group performed no exercise and so behavioural and placebo aspects associated with training cannot be excluded. Equally, the study by Zghal et al. (2015) also employed a non-exercising control group. These authors also employed an

isometric contraction at 15% MVC to examine exercise tolerance and fatigue and it is unclear if the effects are transferable to a more functional mode of locomotor exercise where the muscular and systemic feedback signals are different.

HIIT is more effective at improving high-intensity exercise tolerance than CONT (Daussin et al., 2008, Seiler et al., 2013) but when work-matched produce similar improvements in exercise capacity (LT and $\dot{V}O_{2max}$) (Edge et al., 2006). Accordingly, the aim of this study was to determine the effect of HIIT on high-intensity exercise tolerance, and the associated mechanisms of peripheral and central fatigue, and ischaemic muscle pain tolerance compared to a control group performing the same volume of training at a moderate-intensity. It was hypothesised that although markers of aerobic fitness would increase similarly in both groups, a greater enhancement of exercise tolerance would be seen in the HIIT which would be accompanied by greater tolerance of ischaemic muscle pain and peripheral fatigue with unchanged central fatigue.

6.3 Methods

6.3.1 Participants

Twenty healthy adults (four females) volunteered to participate in the study (Table 6.1). All participants were recruited from the Oxford Brookes University staff and student population between September 2014 and June 2015. Participants were included if they were recreationally active but not engaged in structured endurance training. Participants were randomly assigned to either a HIIT (n = 10) or CONT (n = 10) group (Figure 6.1). Based on previous study sample sizes and reported effect sizes of > 0.6 for training-induced improvements in high-intensity exercise tolerance and ischaemic pain tolerance, and effect sizes of > 0.8 calculated from the differences between SI and MI exercise of equal bouts for peripheral and supraspinal fatigue

in Chapter 5, it was likely that 12 - 16 participants would be required to detect differences between training groups on the selected primary outcome variables with a statistical power of 80% and alpha of 0.05.

Table 6.1. Participant characteristics for the HIIT (n = 10) and CONT (n = 10) groups. Data are mean ± SD.

	HIIT		CONT	
	Pre-training	*Post-training*	*Pre-training*	*Post-training*
M/F	8/2	-	8/2	-
Age (y)	27 ± 6	-	27 ± 4	-
Height (m)	1.75 ± 0.10	-	1.76 ± 0.11	-
Mass (kg)	79.0 ± 13.2	79.2 ± 12.7	75.4 ± 12.9	75.4 ± 12.8
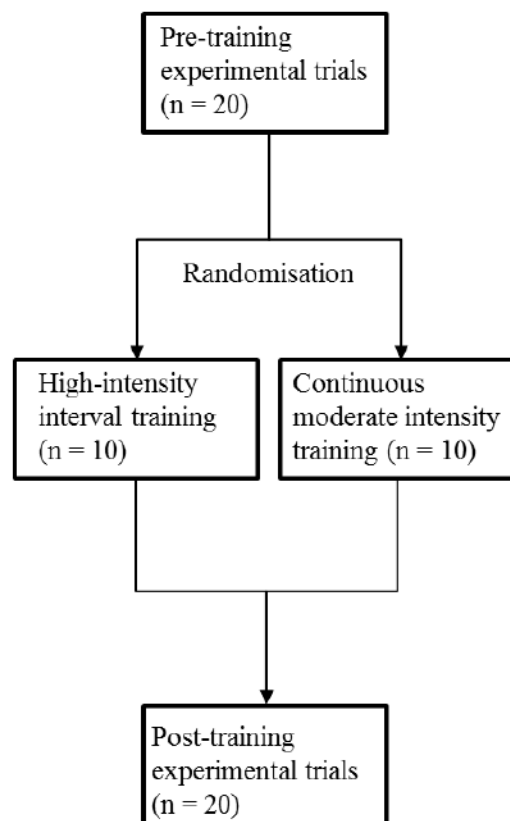
Figure 6.1. Overview of randomisation procedures.

6.3.2 Experimental Procedures

Each participant completed three experimental trials, each separated by 48–72 h before and after 6 weeks of either HIIT or CONT (Figure 6.2). During the first visit, participants completed an ischaemic muscle pain tolerance test before undertaking a submaximal and maximal exercise test for the determination of the LT, LTP and $\dot{V}O_{2max}$. During the second visit, participants were familiarised with the neuromuscular and corticospinal function assessment procedures before completing a cycle to exhaustion test at an intensity corresponding to 50%Δ which served as a familiarisation trial. During the third visit participants repeated the cycle to exhaustion test with corticospinal and neuromuscular function tested pre- and post-exercise to examine exercise tolerance and peripheral and central fatigue. After the pre-training trials, participants were randomly assigned to either HIIT or CONT, matched for $\dot{V}O_{2max}$, in a parallel trial design. The randomisation was performed by an investigator not involved in the experimental data collection using a stratified block randomisation. The training was initiated 2–5 d after the final experimental trial. The three experimental trials were repeated post-training (within 4 d of completion of the training), however the familiarisation cycle to exhaustion was replaced by a cycle to exhaustion at the pre-training 50%Δ (same absolute intensity, ABS) whilst the second cycle to exhaustion was completed at the post-training 'new' 50%Δ (same relative intensity, REL). The primary outcome measures of interest were training-induced increases in TTE and pain tolerance, and exercise-induced levels of central (peripheral and cortical VA) and peripheral fatigue ($Q_{tw,pot}$). Secondary outcome measures were training-induced increases in aerobic fitness (LT, LTP, $\dot{V}O_{2max}$), within-exercise measure of physiological and perceptual strain (HR, $\dot{V}O_2$, RER, $[La^-]$, RPE) and exercise-induced impairments in contractile characteristics (CT, RTD), electromyographical characteristics (rms/rmsM, M_{max}, $MVCM_{max}$) and corticospinal excitability (MEP amplitude, cSP duration and SICI).

Figure 6.2. Overview of the study design (A) and neuromuscular and corticospinal testing protocol (B).

*SICI parameters were employed during the measurement of paired-pulse TMS. CONT, continuous endurance training; HIIT, High-intensity interval training; ABS, same absolute intensity as pre-training; REL, same relative intensity as pre-training.

6.3.3 Muscle Torque and Electromyography

Isometric torque and EMG were recorded according to the procedures previously described (section 3.5).

6.3.4 Motor Nerve Stimulation

MNS was applied according to procedures previously described (section 3.6). During the pre-training TTE trial, the stimulus-response curve revealed an increment in $Q_{tw,unpot}$ and M-wave amplitude from 90 to 95% SO of 1.0% (P = 0.29) and 0.3% (P = 0.48), respectively; the increment in $Q_{tw,unpot}$ and M-wave amplitude from 95% to 100% SO was −0.1% (P = 0.88) and −0.2% (P = 0.71), respectively. During the post-training ABS trial, the stimulus-response curve revealed an increment in $Q_{tw,unpot}$ and M-wave amplitude from 90 to 95% SO of 0.6% (P = 0.71) and 1.4% (P = 0.30), respectively; the increment in $Q_{tw,unpot}$ and M-wave amplitude from 95% to 100% SO was −0.6% (P = 0.66) and −0.2% (P = 0.81), respectively. Thus a plateau in torque and M-wave amplitude was confirmed in all participants.

6.3.5 Transcranial Magnetic Stimulation

TMS was applied according to procedures previously described (section 3.7).

6.3.6 Neuromuscular and Corticospinal Function

In order to examine peripheral, central and supraspinal fatigue, the pre- to post-exercise changes in MNS and TMS evoked torque and EMG responses were determined according to the procedures in Chapter 5 (section 5.3.6 - 5.3.7, Figure 5.1B) and described previously (section 3.9.2.2 - 3.9.2.3). MVC, peripheral VA, $Q_{tw,pot}$, M_{max}, MVCM$_{max}$, cortical VA, MEP amplitude, cSP duration and SICI were recorded pre- and post-exercise. Only SICI was recorded as high-intensity exercise affects the inhibitory circuits (Chapter 5). The measurement

of cortical VA elicited a large MEP in the VL during a 50% MVC (mean pre-training amplitude of $68.9 \pm 37.5\%$ MVCM$_{max}$; mean post-training amplitude of $60.2 \pm 25.4\%$ MVCM$_{max}$) and small MEP in the BF (mean pre-training amplitude of $7.3 \pm 3.3\%$ of VL MVCM$_{max}$; mean post-training amplitude of $8.0 \pm 3.5\%$ of VL MVCM$_{max}$), data grouped for both training groups and all time-points.

6.3.7 Exercise Tests

All exercise tests were completed on an electromagnetically braked cycle ergometer and expired gases, HR, RPE and [La$^-$] were measured as described previously (section 3.4).

Exercise Capacity

Exercise capacity was determined with submaximal and maximal cycling tests, to determine the LT, LTP, $\dot{V}O_{2max}$, W-$\dot{V}O_{2max}$ and W$_{max}$ as described previously (section 3.8.2.3).

Cycle to Exhaustion

Exercise tolerance was determined by a cycle to exhaustion test completed at 50%Δ with neuromuscular and corticospinal function tested pre- and post-exercise as described previously (sections 3.8.2.3 and 5.3.7). This intensity limits the tolerable duration of this workload, and induces significant central and peripheral fatigue and perceptual strain (Chapter 5). This intensity of exercise also induces repeatable levels of peripheral and central fatigue (Chapter 5) and is similar to previous training studies (Burgomaster et al., 2005). The CV calculated from the familiarisation and pre-training experimental trial was 12.0%. During the post-training trials, the 50%Δ was re-calculated from the post-training submaximal and maximal exercise test results to account for training-induced changes in fitness and so as to elicit comparable metabolic, physiological and perceptual strain to pre-training. Therefore, two TTE trials were

completed post-training; one at the pre-training 50%Δ power output (ABS) and one at the post-training 50%Δ power output (REL). The neuromuscular and corticospinal testing procedure was completed pre- and post-exercise to examine peripheral, central and supraspinal fatigue as performed in Chapter 5 (section 5.3.6 - 5.3.7).

Pain Tolerance

To examine the effect of training on ischaemic muscle pain tolerance, a modified tourniquet test was employed (Jones et al., 2014). Participants performed repeated isometric hand-grip exercise (MLT004/ST, ADInstruments, UK). The force was digitised (1000Hz, PowerLab 26T, ADInstruments, UK) and displayed on a computer screen (LabChart 7, ADInstruments, UK). Following 3 MVCs, a cuff was placed around the upper arm (SC10D, Hokanson, USA) which was then raised above the level of the heart for 60 s. The cuff was then inflated to 200 mmHg (E20, Hokanson, USA) before the arm returned to horizontal. Complete occlusion of blood flow was confirmed by Doppler ultrasound (UltraTec PD1v, Ultrasound Technologies, UK). Repeated contractions were performed under ischaemic conditions at 30% MVC for 4 s separated by 4 s rest to the limit of tolerance. Each contraction was prompted by an auditory stimulus and monitored by visual feedback. Pain tolerance was the total time that the contractions could be sustained before voluntary termination of the test. Pain was rated using a 0 (no pain) to 10 (worst possible pain) scale every 30s. This technique has shown to be reliable and sensitive to training induced increases in pain tolerance (Jones et al., 2014).

6.3.8 Training

Training was initiated within 2–5 d of the pre-training experimental trials. All training was supervised and completed on a cycle ergometer (either Excalibur Sport or Corival, Lode, Netherlands). Participants were randomly assigned to either completing a HIIT or CONT

protocol and instructed to maintain normal activity throughout the training. Both training groups completed 18 sessions over a 6-week period ($3 \cdot wk^{-1}$) with each session separated by at least 24 h. The endurance training intensities were prescribed relative to metabolic thresholds in order to minimise inter-individual responses to the training stimuli and elicit disparate metabolic and perceptual demands between groups (Mann et al., 2013). An adapted HIIT protocol was used (Weston et al., 1997) which involved 6 repeats of 5 min cycling at 50%Δ, each separated by 1 min rest, which was progressed to 8 repeats during weeks 4–6. The CONT protocol involved completing the same volume of work that would be completed in the HIIT protocol at an intensity equivalent to 90%LT. Training near the LT provides a high quality aerobic training stimulus without compromising duration and prevents the non-linear increase in metabolic, respiratory and perceptual stress seen with exercise above the LT (Jones and Carter, 2000). Intensity was re-assessed every 2 weeks (session 7 and 13) and increased if necessary to ensure HR and RPE were consistent throughout the training.

6.3.9 Data Analysis

All data analysis was completed blinded to training group. MVC, peripheral VA, $Q_{tw,pot}$, cortical VA, ERT, and corticospinal excitability (MEP amplitude, SICI and cSP duration) were analysed as described previously (section 3.9.2.4). Each $Q_{tw,pot}$ was analysed for contractile characteristics: CT and RTD as previously described (3.9.2.2). Regression analysis confirmed the linearity of the SIT and voluntary torque relationship for the determination of the ERT for pre-training ($r^2 = 0.93 \pm 0.05$, mean across all groups and time-points) and post-training ($r^2 = 0.93 \pm 0.06$, mean across all groups, trials and time-points).

6.3.10 Statistical Analysis

Data are mean $\pm$ SD in the tables and text and mean $\pm$ SE in the figures. Statistical analyses were completed in SPSS (v.22, SPSS Inc., USA). Normal distribution was tested with the Shapiro-Wilk test. ES were calculated with the partial eta squared (η_p^2) as small ($0.01 - 0.06$), medium ($0.06 - 0.14$) and large (> 0.14). Significance was accepted as $P \leq 0.05$.

Training Intensity and Volume

Training volume was compared between groups using an independent-samples t-test. To investigate whether progression of training was equal between groups, power output, HR, RPE and work completed was tested with a 2×2 (group [HIIT and CONT] $\times$ time [first session and last session]) mixed-design ANOVA.

Exercise Capacity, Exercise Tolerance and Pain Tolerance

Pre-training measures of exercise capacity (LT, LTP, $\dot{V}O_{2max}$, $\dot{W}_{max}$ and W-$\dot{V}O_{2max}$), exercise tolerance (TTE) and pain tolerance were compared between groups using independent-samples t-tests. Paired-samples t-tests were used to examine the effect of each training protocol on each variable. A 2×2 (group [HIIT and CONT] $\times$ time [pre-training and post-training]) mixed-design ANOVA was used to compare the effect of training group on each parameter. To examine the effect of each training protocol on within-exercise measurements during the exercise tolerance trials (HR, $\dot{V}O_2$, RER, [La$^-$], RPE), a series of 2×3 (trial [pre-training and ABS or REL] $\times$ time [rest, 10 min, exhaustion]) repeated-measures ANOVAs were performed. To compare the effect of training protocols on within-exercise measurements, $2 \times 3 \times 2$ (group [HIIT and CONT] $\times$ trial [pre-training and ABS or REL] $\times$ time [rest, 10 min, exhaustion]) mixed-design ANOVAs were used. If the ANOVA revealed a significant effect, post hoc contrasts were used. Pearson's correlation coefficients were used to examine any associations

between training induced improvement in exercise capacity and pain tolerance with exercise tolerance.

Peripheral and Central Fatigue

Paired-sampled t-tests were used for each trial to assess the impact of exercise on the induction of global fatigue (MVC), peripheral fatigue ($Q_{tw,pot}$, CT, RTD, $MVCM_{max}$, M_{max}) and central fatigue (peripheral VA, cortical VA, MEP, cSP, SICI, rms/rmsM) from pre- to post-exercise. To examine the effect of each training protocol on the development of fatigue, the same variables were examined with a series of 2×2 (trial [pre-training and ABS or REL] $\times$ time [pre-exercise and post-exercise]) ANOVAs. To compare each training protocol, the pre- to post-exercise change scores (%) were compared between trials using 2×2 (group [HIIT and CONT] $\times$ trial [pre-training and ABS or REL]) mixed-design ANOVAs.

6.4 Results

Training Intensity and Volume

All training data can be seen in Table 6.2. Power output was significantly higher during HIIT compared with CONT ($P < 0.001$) and during the first session compared with the last session for both groups ($P < 0.001$), however there was no difference between groups for power output progression ($P > 0.05$). HIIT induced greater physiological and perceptual strain compared with CONT as evidenced by the higher HR and RPE ($P < 0.001$). HR and RPE did not differ between the first and last sessions for either group ($P > 0.05$) demonstrating the training load was adequately increased in both groups to ensure a similar strain throughout. Work completed was similar between the groups during the first and last sessions ($P > 0.05$). As such the total training volume completed was similar between groups ($P > 0.05$).

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Table 6.2. Characteristics of the HIIT and CONT protocols. Data are mean ± SD.

	HIIT	CONT
First Session		
Power Output (W)	202 ± 47[#]	103 ± 40
% pre-training $\dot{V}O_{2max}$	80 ± 6[#]	50 ± 6
Protocol	6 x 5 min with 1 min rest	58.8 ± 9.8 min continuous
Total Work (kJ)	363 ± 84	346 ± 102
Mean HR (b·min^{-1})	164 ± 7[#]	125 ± 12
Mean RPE (6-20)	16 ± 1[#]	12 ± 1
Last Session		
Power Output (W)	228 ± 45[#*]	129 ± 42*
Protocol	8 x 5 min with 1 min rest	77.7 ± 13.7 min continuous
Total Work (kJ)	545 ± 103*	562 ± 148*
Mean HR (b·min^{-1})	164 ± 9[#]	128 ± 11
Mean RPE (6-20)	16 ± 2[#]	12 ± 2
No. of Sessions	18 ± 1	18 ± 1
Total Volume (kJ)	7918 ± 1541	8105 ± 2036

*P < 0.05 vs pre-training; [#]P < 0.05 vs CONT.

Exercise Capacity

All exercise capacity data can be seen in Table 6.3. There was no difference between groups for pre-training measures of LT (W or %$\dot{V}O_{2max}$), LTP (W or %$\dot{V}O_{2max}$), $\dot{V}O_{2max}$ (l·min^{-1} or ml·kg^{-1}·min^{-1}) or $\dot{W}_{max}$ (P > 0.05) demonstrating the groups were matched for aerobic fitness. Consequently, there was no difference between groups for pre-training 50%Δ intensity (W or %$\dot{V}O_{2max}$) (P < 0.05). Training increased the power at LT and LTP after HIIT (24 ± 20% and 19 ± 12%, respectively, P < 0.001) and CONT (23 ± 10% and 14 ± 10%, respectively, P < 0.001) with no difference between groups (P > 0.05, η_p^2 = 0.02 - 0.09). Similarly, training increased the $\dot{V}O_2$ (%$\dot{V}O_{2max}$) at LT and LTP after HIIT (6 ± 14% and 6 ± 8%, respectively, P

< 0.05) and CONT ($8 \pm 13\%$ and $4 \pm 7\%$, respectively, P < 0.05), with no difference between groups (P > 0.05, $\eta_p^2 = 0.00 - 0.02$). $\dot{V}O_{2max}$ ($l \cdot min^{-1}$) and the power at $\dot{V}O_{2max}$ ($\dot{W}\text{-}\dot{V}O_{2max}$) increased after HIIT ($8 \pm 7\%$ and $7 \pm 6\%$, respectively, P < 0.001) and CONT ($9 \pm 9\%$ and $7 \pm 8\%$, respectively, P < 0.001) with no difference between groups (P > 0.05, $\eta_p^2 = 0.00 - 0.04$). $\dot{W}_{max}$ increased after HIIT ($11 \pm 5\%$, P < 0.001) and CONT ($7 \pm 7\%$, P < 0.001) with a trend towards a greater improvement following HIIT (P = 0.08, $\eta_p^2 = 0.16$). The power at 50%Δ increased after both HIIT ($11 \pm 5\%$, P < 0.001) and CONT ($6 \pm 10\%$, P < 0.001) with no difference between groups (P > 0.05, $\eta_p^2 = 0.12$), however training did not alter the $\dot{V}O_2$ (%$\dot{V}O_{2max}$) at 50%Δ.

Table 6.3. Exercise capacity before and after 6 weeks HIIT or CONT. Data are mean $\pm$ SD.

	HIIT		CONT	
	Pre-training	*Post-training*	*Pre-training*	*Post-training*
LT				
W	112 ± 36	138 ± 43*	106 ± 37	128 ± 39*
%$\dot{V}O_{2max}$	52 ± 8	56 ± 10*	52 ± 9	55 ± 8*
LTP				
W	158 ± 38	186 ± 35*	158 ± 38	174 ± 40*
%$\dot{V}O_{2max}$	68 ± 8	72 ± 8*	68 ± 7	70 ± 8*
$\dot{V}O_{2max}$				
$l \cdot min^{-1}$	3.52 ± 0.71	3.80 ± 0.75*	3.33 ± 0.92	3.62 ± 0.99*
$ml \cdot kg^{-1} \cdot min^{-1}$	44.5 ± 5.4	47.9 ± 6.0*	43.5 ± 5.9	47.4 ± 8.0*
$\dot{W}\text{-}\dot{V}O_{2max}$	289 ± 62	308 ± 62*	265 ± 68	282 ± 70*
$\dot{W}_{max}$	310 ± 65	342 ± 66*	289 ± 75	307 ± 72*
50%Δ				
W	202 ± 47	223 ± 50*	189 ± 52	201 ± 51*
%$\dot{V}O_{2max}$	80 ± 6	80 ± 5	79 ± 5	78 ± 6

LT, lactate threshold; LTP, lactate turnpoint; $\dot{V}O_{2max}$, maximal oxygen uptake; $\dot{W}_{max}$, peak power output, 50%Δ, exercise intensity halfway between lactate threshold and $\dot{V}O_{2max}$.

*P < 0.05 vs pre-training; #P < 0.05 vs CONT.

Exercise Tolerance

Time to Exhaustion

There was no difference between groups for pre-training TTE ($P > 0.05$). Training increased TTE during the ABS trial compared with pre-training after HIIT ($148 \pm 74\%$, $P < 0.001$) and CONT ($38 \pm 52\%$, $P < 0.05$) with the increase greater after HIIT ($P < 0.001$, $\eta_p^2 = 0.52$) (Figure 6.3). Training also increased TTE during the REL trial compared to pre-training after HIIT ($43 \pm 56\%$, $P < 0.05$) but not after CONT ($P > 0.05$) with a difference between groups ($P < 0.05$, $\eta_p^2 = 0.27$). There was a significant relationship between the increase in $\dot{W}_{max}$ and increase in TTE during the ABS trial ($r = 0.50$, $P < 0.05$) but there were no other relationships between improvements in any marker of exercise capacity with exercise tolerance ($r \leq 0.35$, $P > 0.05$).
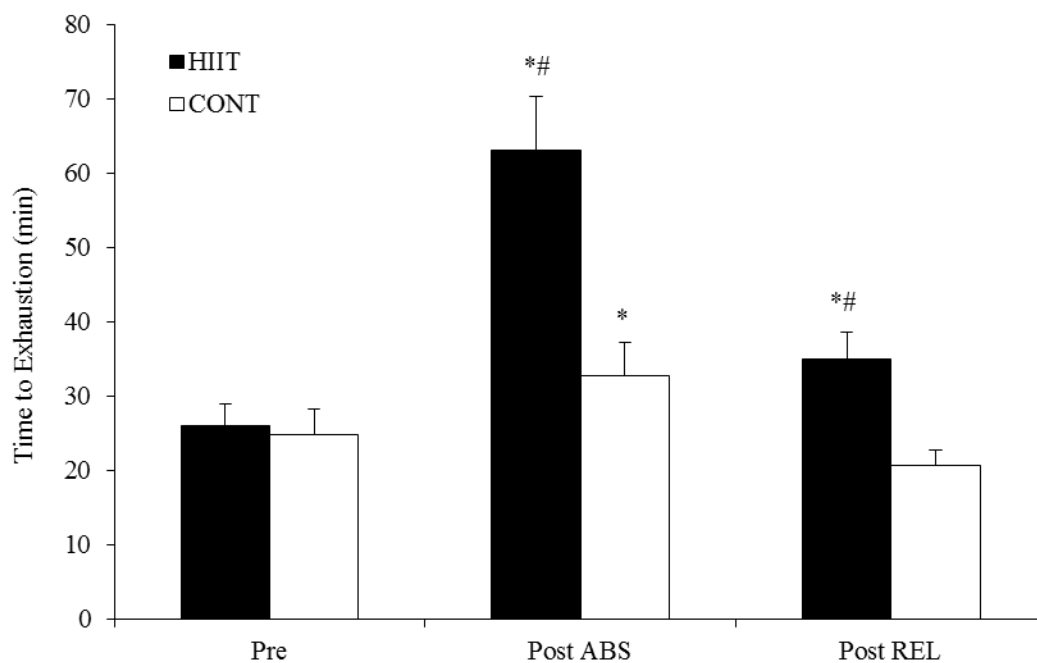
Figure 6.3. Exercise tolerance during the pre-training (Pre), post-training absolute (Post ABS) and post-training relative trials (Post REL) for the HIIT and CONT groups. Data are mean ± SE.

Cardio-Respiratory and Perceptual Responses

The physiological and perceptual responses to the exercise tolerance trials can be seen in Table 6.4. HIIT significantly reduced HR, RER and RPE after 10 min during the ABS trial compared with pre-training ($P < 0.05$), however there were no differences between trials during the final minute ($P > 0.05$). [La⁻] was also reduced after 10 min and at exhaustion following HIIT ($P < 0.001$) but HIIT did not change $\dot{V}O_2$ ($P > 0.05$). During the REL trial HR, RPE, RER or [La⁻] were unchanged at any time-point compared with pre-training following HIIT ($P > 0.05$), however $\dot{V}O_2$ was significantly greater after 10 min and at exhaustion ($P < 0.05$). CONT significantly reduced HR and RPE ($P < 0.05$) after 10 min during the ABS trial compared with pre-training, however there were no differences between trials during the final min ($P > 0.05$). [La⁻] was reduced after 10 min and at exhaustion following CONT ($P < 0.05$) but CONT did not change $\dot{V}O_2$ or RER ($P > 0.05$). During the REL trial HR, RPE, RER, [La⁻] or $\dot{V}O_2$ were unchanged at any time-point compared with pre-training following CONT ($P > 0.05$). There were no differences between groups during any trial for HR, RPE, RER or [La⁻] ($P > 0.05$, η_p^2 = 0.04 - 0.23), however compared with pre-training HIIT resulted in a greater increase in $\dot{V}O_2$ during the final min of the REL trial compared with CONT ($P < 0.05$, $\eta_p^2 = 0.23$).

Table 6.4. Physiological and perceptual responses during the exercise tolerance tests before and after 6 weeks of HIIT or CONT. Data are mean ± SD.

	Pre-Training			Post-Training Absolute			Post-Training Relative		
	Rest	*10 min*	*Last min*	*Rest*	*10 min*	*Last min*	*Rest*	*10 min*	*Last min*
HR (b·min⁻¹)									
HIIT	74	164	171	74	157	170	72	163	172
	± 13	± 7	± 12	± 12	± 11*	± 10	± 17	± 10	± 11
CONT	76	168	174	76	164	173	77	170	174
	± 12	± 14	± 12	± 9	± 14*	± 11	± 10	± 11	± 10
$\dot{V}O_2$ (l·min⁻¹)									
HIIT	0.44	2.96	3.14	0.46	2.85	3.07	0.44	3.20	3.40
	± 0.08	± 0.73	± 0.71	± 0.15	± 0.61	± 0.65	± 0.07	± 0.71*	± 0.78*#
CONT	0.40	2.75	3.00	0.40	2.65	2.93	0.41	2.81	3.09
	± 0.07	± 0.71	± 0.66	± 0.16	± 0.80	± 0.78	± 0.16	± 0.76	± 0.72
RER									
HIIT	0.95	1.13	1.13	0.97	1.06	1.11	0.98	1.12	1.14
	± 0.04	± 0.07	± 0.09	± 0.09	± 0.06*	± 0.08	± 0.11	± 0.08	± 0.09
CONT	0.96	1.14	1.13	0.97	1.12	1.13	0.95	1.15	1.15
	± 0.11	± 0.09	± 0.09	± 0.05	± 0.06	± 0.09	± 0.07	± 0.10	± 0.10
[La⁻] (mmol·l⁻¹)									
HIIT	1.4	6.8	8.6	1.3	4.1	5.8	1.4	6.2	8.8
	± 0.5	± 1.9	± 3.3	± 0.4	± 1.3*	± 2.4*	± 0.3	± 2.0	± 2.8
CONT	1.3	6.8	7.6	1.2	5.3	5.8	1.3	6.2	7.2
	± 0.3	± 2.1	± 2.8	± 0.4	± 1.8*	± 2.8*	± 0.4	± 1.1	± 1.0
RPE (6-20)									
HIIT	6 ± 0	16 ± 2	20 ± 1	6 ± 0	13 ± 2*	20 ± 1	6 ± 0	15 ± 2	20 ± 1
CONT	6 ± 0	16 ± 2	20 ± 1	6 ± 0	15 ± 3*	20 ± 0	6 ± 0	17 ± 2	20 ± 0

HR, heart rate; $\dot{V}O_2$, oxygen uptake; RER, respiratory exchange ratio; [La-], capillary lactate concentrations; RPE, ratings of perceived exertion.

*P < 0.05 vs pre-training; #P < 0.05 vs CONT.

Global and Peripheral Fatigue

The pre-training, ABS and REL trials induced MVC losses in the HIIT (−20 ± 11%, −16 ± 10% and −19 ± 9%, respectively, P < 0.01) and CONT groups (−22 ± 12%, −18 ± 10% and −16 ± 8%, respectively, P < 0.01) (Figure 6.4A). Neither HIIT nor CONT had any effect on MVC impairment (P > 0.05). The pre-training, ABS and REL trials also induced reductions in

$Q_{tw,pot}$ in the HIIT ($-34 \pm 17\%$, $-32 \pm 13\%$ and $-43 \pm 13\%$, respectively, $P < 0.001$) and CONT groups ($-38 \pm 21\%$, $-31 \pm 14\%$ and $-32 \pm 14\%$, respectively, $P < 0.001$) (Figure 6.4B). HIIT increased the exercise-induced reduction in $Q_{tw,pot}$ compared with pre-training at the REL intensity ($P < 0.05$), which was significantly different from the CONT group ($P < 0.01$, $\eta_p^2 = 0.35$), however HIIT had no effect on the $Q_{tw,pot}$ loss after the ABS trial ($P > 0.05$). CONT had no effect on the $Q_{tw,pot}$ reduction for any trial ($P > 0.05$). The muscle contractile and electrophysiological characteristics can be seen in Table 6.5. All trials impaired RTD in the HIIT ($P < 0.001$) and CONT groups ($P < 0.001$). At the REL intensity, HIIT tended to increase the impairment in RTD compared with pre-training ($P = 0.08$), which was significantly different from the CONT group ($P < 0.01$, $\eta_p^2 = 0.26$), however HIIT had no effect on the RTD loss after the ABS trial ($P > 0.05$). CONT had no effect on the exercise-induced RTD loss for any trial ($P > 0.05$). A significant slowing of CT was induced by the pre-training and REL trials for the HIIT group ($P < 0.01$) and after all trials in the CONT group ($P < 0.05$). HIIT attenuated the impairment in CT after the ABS trial compared with pre-training ($P < 0.05$), however there were no difference between groups ($P > 0.05$, $\eta_p^2 = 0.07$). ERT was reduced after all trials for both groups ($P < 0.01$), with no difference between trials or groups ($P > 0.05$). $MVCM_{max}$ and M_{max} were unaffected by exercise ($P > 0.05$).

Central Fatigue

The motor thresholds were similar across the pre-training, ABS and REL trials for the HIIT (rMT: $51 \pm 10\%$, $51 \pm 10\%$ and $53 \pm 10\%$, respectively, $P > 0.05$; aMT: $34 \pm 6\%$, $35 \pm 6\%$ and $35 \pm 6\%$, respectively, $P > 0.05$) and CONT groups (rMT: $47 \pm 9\%$, $46 \pm 8\%$, and $46 \pm 9\%$, respectively, $P > 0.05$; aMT: $32 \pm 5\%$, $32 \pm 5\%$ and $33 \pm 5\%$, respectively, $P > 0.05$). Pre-exercise peripheral VA was similar across the pre-training, ABS and REL trials for the HIIT ($97.6 \pm 1.2\%$, $98.0 \pm 1.1\%$ and $97.9 \pm 1.4\%$, respectively, $P > 0.05$) and CONT groups ($94.9 \pm$

6.4%, 95.9 ± 6.5% and 95.9 ± 6.5%, respectively, P > 0.05). The pre-training, ABS and REL trials induced peripheral VA impairments in the HIIT (−7 ± 7%, −3 ± 2% and −3 ± 3%, respectively, P < 0.05) and CONT groups (−5 ± 5%, −5 ± 5% and −6 ± 6%, respectively, P < 0.05) (Figure 6.4C). At the REL intensity, HIIT attenuated the peripheral VA loss compared with pre-training (P < 0.05), which was significantly different from CONT (P < 0.05, η_p^2 = 0.25), with a similar trend at the ABS intensity (P = 0.08), which was not different to CONT (P < 0.05, η_p^2 = 0.08). CONT had no effect on peripheral VA impairment for any trial (P > 0.05). Pre-exercise cortical VA was similar across the pre-training, ABS and REL trials for the HIIT (96.5 ± 3.7%, 96.9 ± 3.0% and 96.4 ± 2.9%, respectively, P > 0.05) and CONT groups (94.6 ± 5.0%, 94.4 ± 4.7% and 93.7 ± 5.7%, respectively, P > 0.05). The pre-training, ABS and REL trials induced cortical VA impairments in the HIIT (−8 ± 11%, −3 ± 2% and −3 ± 3%, respectively, P < 0.05) and CONT groups (−7 ± 8%, −7 ± 8% and −7 ± 7%, respectively, P < 0.01) (Figure 6.4D). HIIT tended to attenuate the cortical VA loss compared with pre-training at the REL (P = 0.09) and ABS intensity (P = 0.10), however there was no difference between groups (P < 0.05, η_p^2 = 0.04 - 0.10). The impairment in cortical VA was unaffected by CONT (P > 0.05). There was a trend for SICI to be increased during the pre-training exercise tolerance trial for HIIT (12 ± 17%, P = 0.08) and CONT (17 ± 23%, P = 0.09), which was significant when the two groups were pooled together (P < 0.05). SICI was unchanged by exercise after the ABS or REL trials for either group (P > 0.05). There was no effect of training on SICI for either group (P > 0.05) and no difference between groups (P > 0.05, η_p^2 = 0.02 - 0.08). The pre-training, ABS and REL trials all shortened the cSP in the HIIT (−14 ± 9%, −18 ± 11% and −13 ± 11%, respectively, P < 0.01) and CONT groups (−16 ± 14%, −13 ± 14% and −14 ± 6%, respectively, P < 0.05). There was no difference between trials for either group or any differences between groups (P > 0.05, η_p^2 = 0.00 - 0.05). MEP and rms/rmsM were unaffected by exercise in any trial for either group (P > 0.05).
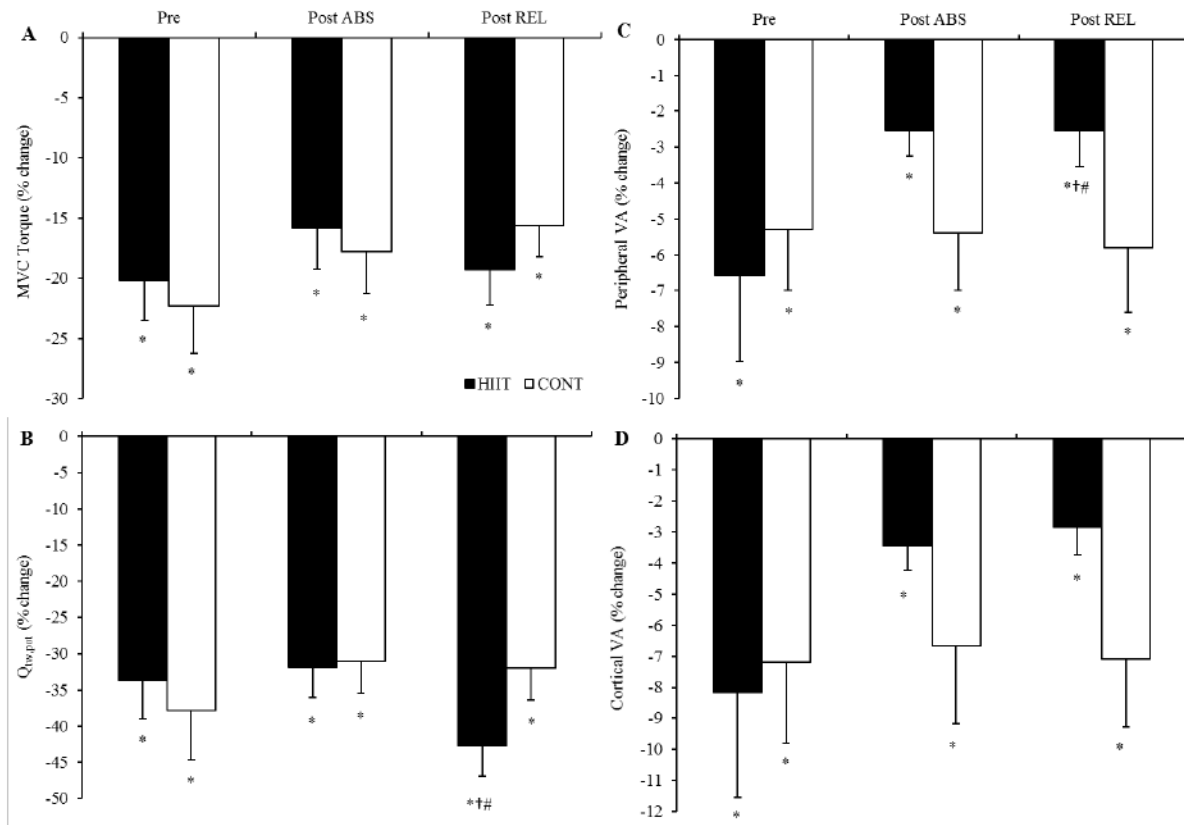
Figure 6.4. Peripheral and central fatigue in response to the pre-training (Pre), post-training absolute (Post ABS) and post-training REL (Post REL) trials before and after HIIT (filled bars) or CONT (hollow bars). A, maximal voluntary torque; B, potentiated quadriceps twitch torque; C, peripheral voluntary activation; D, cortical voluntary activation. Data expressed as change (%) from pre-exercise (mean ± SE).

*$P < 0.05$ vs pre-exercise; †$P < 0.05$ vs pre-training; #$P < 0.05$ vs CONT.

Table 6.5. Contractile and electromyographical data in response to the pre-training, REL and ABS trials before and after 6 weeks of HIIT or CONT. Data are mean ± SD.

	Pre-Training			Post-Training Absolute			Post-Training Relative		
	Pre-	Post-	Change (%)	Pre-	Post-	Change (%)	Pre-	Post-	Change (%)
MVC (N·m)									
HIIT	460 ± 120	365 ± 103*	−20	453 ± 110	377 ± 90*	−16	472 ± 102	380 ± 90*	−19
CONT	388 ± 120	300 ± 100*	−22	391 ± 123	316 ± 93*	−18	393 ± 119	329 ± 97*	−16
MVCM$_{max}$ (mV)									
HIIT	6.38 ± 0.70	6.01 ± 1.27	−6	5.63 ± 1.33	5.58 ± 1.66	0	5.90 ± 1.48	6.12 ± 1.19	+8
CONT	5.66 ± 1.12	5.51 ± 1.11	+1	5.85 ± 1.64	6.04 ± 1.52	+5	5.52 ± 1.25	5.35 ± 1.68	0
rms/rmsM									
HIIT	0.26 ± 0.05	0.24 ± 0.06	−10	0.28 ± 0.14	0.24 ± 0.12	−12	0.28 ± 0.14	0.24 ± 0.10	−16
CONT	0.27 ± 0.11	0.23 ± 0.08	−12	0.22 ± 0.10	0.19 ± 0.08	−13	0.23 ± 0.10	0.20 ± 0.09	−14
Q$_{tw,pot}$ (N·m)									
HIIT	126 ± 37	83 ± 29*	−34	132 ± 29	91 ± 32*	−32	141 ± 29	80 ± 30*†	−43[#]
CONT	111 ± 34	70 ± 36*	−38	119 ± 26	82 ± 26*	−31	117 ± 29	80 ± 30*	−32
M$_{max}$ (mV)									
HIIT	6.57 ± 1.33	6.56 ± 1.74	−1	6.79 ± 1.40	6.41 ± 1.29	0	6.91 ± 1.15	6.96 ± 1.28	+1
CONT	6.20 ± 1.09	5.79 ± 1.13	−6	6.59 ± 0.83	6.53 ± 1.15	0	5.84 ± 1.24	5.81 ± 1.50	0
CT (ms)									
HIIT	48 ± 4	54 ± 3*	+13	50 ± 5	52 ± 3†	+6	49 ± 4	55 ± 3*	+11
CONT	47 ± 6	52 ± 3*	+11	48 ± 4	53 ± 4*	+11	50 ± 4	53 ± 3*	+7
RTD (N·m·ms)									
HIIT	2.75 ± 0.75	1.59 ± 0.53*	−41	2.67 ± 0.51	1.77 ± 0.61*	−34	2.98 ± 0.74	1.49 ± 0.51*	−49[#]
CONT	2.42 ± 0.96	1.35 ± 0.69*	−43	2.47 ± 0.46	1.60 ± 0.61*	−36	2.30 ± 0.54	1.52 ± 0.54*	−34
ERT (N·m)									
HIIT	97 ± 40	63 ± 38*	−35	91.3 ± 46.9	70 ± 47*	−27	96 ± 43	62 ± 41*	−37
CONT	81 ± 37	49 ± 22	−39	88 ± 34	61 ± 23	−29	84 ± 29	56 ± 24	−32

MEP (%M$_{max}$)

HIIT	31.2 ± 16.9	30.9 ± 10.7	+12	24.7 ± 12.9	21.8 ± 13.8	−5	24.6 ± 11.5	27.5 ± 13.9	+10
CONT	30.0 ± 14.3	23.9 ± 5.8	−3	28.9 ± 15.3	26.6 ± 9.7	−2	27.8 ± 17.2	29.0 ± 13.7	+10

cSP (ms)

HIIT	195 ± 33	170 ± 44*	−14	178 ± 24	147 ± 27*	−18	183 ± 34	160 ± 33*	−13
CONT	184 ± 42	153 ± 33*	−16	194 ± 31	169 ± 42*	−13	193 ± 54	167 ± 47*	−14

SICI

HIIT	0.67 ± 0.29	0.58 ± 0.23	−12	0.63 ± 0.26	0.58 ± 0.16	+10	0.74 ± 0.48	0.61 ± 0.21	−8
CONT	0.64 ± 0.23	0.53 ± 0.26	−17	0.55 ± 0.24	0.57 ± 0.30	0	0.57 ± 0.24	0.53 ± 0.26	−9

MVC, maximal voluntary contraction; MVCM$_{max}$, Maximal M-wave during MVC; rms/rmsM, root mean squared relative to root mean squared of MVCM$_{max}$; Q$_{tw,pot}$, potentiated quadriceps twitch; M$_{max}$, resting maximal M-wave; CT, time to peak torque; RTD, rate of torque development; MEP, motor evoked potential during 10% MVC; amp, amplitude; cSP, cortical silent period; SICI, short-interval intracortical inhibition.

*$P < 0.05$ vs pre-exercise; †$P < 0.05$ vs pre-training; #$P < 0.05$ vs CONT.

Pain Tolerance

There was no difference in pre-training pain tolerance between groups ($P > 0.05$). Isometric hand grip MVC was unaffected by HIIT (pre-training $467 ± 123$ N vs post-training $467 ± 122$, $P > 0.05$) or CONT (pre-training $446 ± 118$ N vs post-training $431 ± 109$, $P > 0.05$). Training increased pain tolerance following HIIT (41%, $P < 0.001$) but not CONT ($P > 0.05$) with a significant difference between groups ($P < 0.001$, $\eta_p^2 = 0.46$) (Figure 6.5). Pain perception after 2 min of contractions was unchanged after HIIT (pre-training $6 ± 1$ vs post-training $6 ± 1$, $P > 0.05$) or CONT (pre-training $6 ± 2$ vs post-training $6 ± 2$, $P > 0.05$). Similarly, pain perception at the limit of tolerance was unaltered by HIIT (pre-training $10 ± 0$ vs post-training $10 ± 0$, $P > 0.05$) or CONT (pre-training $10 ± 0$ vs post-training $10 ± 0$, $P > 0.05$). After HIIT there was a trend for a relationship between increase in pain tolerance and increase in REL TTE ($r = 0.50$, $P = 0.07$) but not ABS TTE ($r = 0.10$, $P = 0.39$).
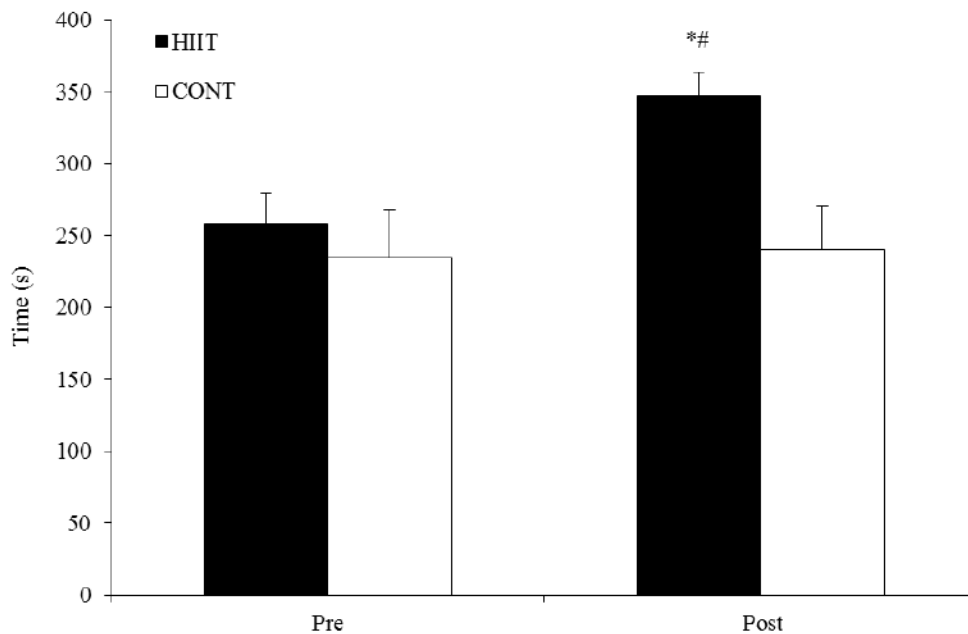
Figure 6.5. Pain tolerance time pre-training and post-training in response to HIIT and CONT. *P < 0.05 vs pre-training; #P < 0.05 vs CONT.

6.5 Discussion

The aim of the present study was to examine the effect of endurance training on mechanisms of fatigue induced by high-intensity exhaustive exercise. The main findings are that despite similar improvements in aerobic fitness, HIIT resulted in markedly greater improvements in exercise tolerance when completed at the same power output as pre-training (ABS) compared to CONT; when the post-training exercise tolerance test was completed at the same relative intensity as pre-training (REL) to account for changes in aerobic fitness, only HIIT improved exercise tolerance. Therefore, this component of the hypothesis can be accepted. This increase in REL exercise tolerance after HIIT was accompanied by a greater tolerance of both peripheral fatigue and ischaemic muscle pain with attenuated central fatigue. Whilst it was hypothesised that HIIT would result in increases in the tolerance of peripheral fatigue and ischemic muscle pain, and therefore these hypotheses can be accepted, it was also hypothesised central fatigue would be unhanged and therefore this hypothesis must be rejected. Although endurance training is a well-established potent enhancer of exercise tolerance, it was previously unknown

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whether this was through alterations to the fatigue mechanisms that limit exercise tolerance. Consequently, this study provides new insight into the mechanisms contributing to better tolerance of high-intensity exercise and contributes to the debate on central and peripheral limitations to exercise tolerance.

Exercise Tolerance

When completed at the same power output as pre-training (ABS), TTE was enhanced in both training groups, however the increase was greater following HIIT (147%) vs CONT (38%). This is in agreement with previous work showing HIIT as a better enhancer of high-intensity exercise tolerance than CONT (Daussin et al., 2008, Seiler et al., 2013). There were no differences between groups for improvements in metabolic thresholds (LT and LTP) or aerobic capacity ($\dot{V}O_{2max}$), suggesting these factors cannot explain the TTE differences. Other studies have also reported work-matched HIIT and CONT training induce similar physiological and metabolic adaptations (Poole and Gaesser, 1985, Edge et al., 2006). However, HIIT results in greater improvements in mitochondrial function, $\dot{V}O_2$ kinetics, maximal cardiac output (Daussin et al., 2008) and muscle buffer capacity (Edge et al., 2006) than work-matched CONT, which likely contribute to the better tolerance at the ABS intensity.

When the exercise tolerance trial was repeated at a power output that was increased to account for training-induced increases in aerobic fitness (REL), only HIIT resulted in improvements in TTE (43%). Most studies examining exercise tolerance employ workloads prescribed according to pre-training fitness where improvements in TTE can be explained by changes in $\dot{V}O_{2max}$ (Daussin et al., 2008) or metabolic thresholds (Seiler et al., 2013). Therefore, the post-training test is at a lower %$\dot{V}O_{2max}$ and/or at a different proximity to the LT and LTP (i.e. lower relative intensity) and it was previously unknown whether training allows the tolerance of the

same metabolic strain. The %Δ method allows improvements in both LT and $\dot{V}O_{2max}$ to be accounted for and 50%Δ was recalculated post-training to elicit a similar metabolic strain to pre-training. The metabolic stress has important implications for the development of peripheral and central fatigue, and exhaustion as previously demonstrated in Chapter 5. The similar HR, RER, [La-] and RPE responses compared with pre-training demonstrate a similar metabolic, perceptual and cardiovascular stress was induced and therefore improvements in these parameters cannot explain the enhanced TTE.

There is little data employing this methodology, however Demarle et al. (2001) reported a decrease in TTE at relative intensities following HIIT, which is in direct contrast to this study and likely due to differences between methodologies. The present study investigated an untrained group performing exercise tolerance tests at 50% of the difference between the LT and $\dot{V}O_{2max}$ compared with an endurance-trained group performing exercise tolerance tests at 50% of the difference between the LTP and $\dot{V}O_{2max}$ in the study by Demarle et al. (2001). The data here demonstrate an increase in $\dot{V}O_{2max}$ and thus the 50%Δ was at a similar %$\dot{V}O_{2max}$ pre- and post-training. Demarle et al. (2001) reported no increase in $\dot{V}O_{2max}$, likely due to the better pre-training aerobic fitness of their participants, however the post-training relative exercise intensity increased and was therefore closer to $\dot{V}O_{2max}$ than the pre-training trial, which may explain why the post-training exercise tolerance appears impaired. The endurance-trained participants in their study may also already be accustomed to high-intensity exercise and significant levels of muscle fatigue whereas are participants were not. Whether trained individuals can better tolerate the same physiological strain was therefore contentious (Millet, 2011). This study provides first evidence of this effect and demonstrates an enhanced TTE during exercise despite no attenuation of physiological stress suggesting that tolerance of the same physiological strain was improved. Although [La-] was unchanged, a greater $\dot{V}O_2$ at

exhaustion after HIIT vs CONT demonstrates a greater $\dot{V}O_2$ slow component during exercise and suggests better tolerance of physiological stress. In order to better understand the mechanisms that allow better REL tolerance, peripheral and central fatigue were examined.

Peripheral and Central Fatigue

The exercise tolerance trials all induced significant peripheral fatigue, as measured by the $Q_{tw,pot}$ reduction from pre- to post-exercise. Despite both HIIT and CONT reducing the metabolic strain induced by the ABS trial, peripheral fatigue was unchanged. Compared with pre-training, HIIT increased the level of peripheral fatigue at the end of the REL trial. This finding is strengthened by the demonstration of the repeatability of peripheral fatigue after cycling to exhaustion at 50%∆ in Chapter 5. This finding is in agreement with work during isolated knee extensions (Zghal et al., 2015), however those authors employed a no-exercise control group and a TTE task using an isometric contraction at 15% MVC. Therefore it was unclear whether the placebo or behavioural effects of training contributed to their findings, and whether the effects were transferable to a more functional mode of locomotor endurance exercise where the CNS afferent feedback signals are different (Sidhu et al., 2013a). The data here therefore supports that the inhibitory afferent feedback signals associated with high levels of peripheral fatigue developed during high-intensity locomotor exercise are better tolerated following HIIT. The increase in peripheral fatigue tolerance can therefore be attributed to HIIT and not increases in aerobic fitness or the behavioural or placebo aspects of training.

High-intensity exercise tolerance is hypothesised to be limited by the attainment of a critical peripheral fatigue threshold, beyond which the sensory sensations are intolerable; afferent feedback inhibits central motor drive once this level of fatigue has been attained in order to prevent excessive peripheral fatigue (Amann, 2011). Therefore a physiological reserve has

been proposed and it was suggested that training may enhance the ability to access this security reserve (Millet, 2011). This study supports exercise being terminated with a muscular reserve and demonstrates that HIIT allows greater access to this reserve. An increase in the level of peripheral fatigue after endurance training has been interpreted as an increased tolerance of muscle fatigue by the CNS due to altered and/or greater tolerance of group III/IV afferent firing (Zghal et al., 2015). Interestingly, the gain in peripheral fatigue after HIIT in this study is remarkably similar to that induced by group III/IV afferent blockade (Amann et al., 2011). This suggests an ability to override central fatigue mechanisms in response to inhibitory afferent feedback in order to extrude greater performance from the muscle or an increased 'sensory tolerance limit;' the pain tolerance and central fatigue data supports this.

Central and supraspinal fatigue was also induced by all trials as measured by reductions in VA. HIIT attenuated central fatigue (peripheral VA) following the REL trial and supraspinal fatigue (cortical VA) also tended to be attenuated, although it should be noted only the attenuation of peripheral VA was different between groups. Although there were small to moderate effect sizes for a training-induced attenuation of supraspinal fatigue after the REL trial (D = 0.53) compared with CONT ($\eta_p^2 = 0.10$), a lack of a significant effect of training group is likely due to the small sample size in the present study. Further analysis revealed that a total sample size of 32 would be required to reveal a significant effect of HIIT on attenuating supraspinal fatigue following high-intensity exhaustive exercise compared with a CONT group, a sample size not achievable for the present study but which informs future study design. Zghal et al. (2015) reported training attenuated central and supraspinal fatigue when an equal duration of exercise was performed compared with pre-training, however there were no differences at exhaustion. This study however demonstrates that HIIT develops central fatigue resistance during exhaustive high-intensity locomotor exercise. Other locomotor studies have demonstrated

central (Amann et al., 2013a) and supraspinal fatigue resistance (Goodall et al., 2014b) during hypoxic cycling following adaptation to hypoxia. A number of CNS adaptations in response to endurance training could contribute to central fatigue resistance such as improved handling of serotonin (Jakeman et al., 1994) and dopamine (Foley and Fleshner, 2008), attenuated cerebral ammonia uptake (Nybo et al., 2005), improved cerebral oxygenation (Rooks et al., 2010, Goodall et al., 2014b), enhanced spinal reflex excitability (Perot et al., 1991, Vila-Cha et al., 2012b), altered motor unit discharge properties (Vila-Cha et al., 2010), increased intracortical excitability (Tergau et al., 2000) or resistance to depressions in corticospinal excitability (Fulton et al., 2002, Triscott et al., 2008). Training had no effect on the cSP and SICI response to exercise, suggesting resistance to disturbances in cortical function are not a mechanism. Training may also result in better tolerance of high core temperature (Nybo, 2008), and resistance to the inhibitory effects of group III/IV afferent firing in inducing fatigue (Zghal et al., 2015) and the associated sensory disturbance (Jones et al., 2014).

The increased peripheral fatigue tolerance and attenuated central fatigue after HIIT, likely occurred due to training whilst under conditions of high metabolic and perceptual stress (and peripheral and central fatigue - Chapter 5). By prescribing training intensity with the %Δ method, homogenous metabolic and perceptual responses could be elicited within the groups as well as disparate responses between groups (HIIT above LTP/CONT below LT). HIIT therefore likely induced greater discharge of the sensory group III/IV afferents (Amann et al., 2015, Laurin et al., 2015) and evoked sensations of muscle pain and fatigue (Pollak et al., 2014) compared with CONT. The increase in peripheral fatigue with attenuated central fatigue demonstrates a disturbance in the peripheral and central fatigue relationship (Chapter 5) and supports the theory HIIT reduces the inhibitory effects of peripheral fatigue on central motor drive. Therefore the CNS may release the 'brake' on central motor drive to tolerate more

peripheral fatigue (Amann et al., 2011), which is supported by the peripheral and central fatigue data in this study. Triscott et al. (2008) reported that compared to resistance-trained or sedentary individuals, endurance-trained individuals had increased exercise tolerance in the non-dominant arm immediately after exhausting the dominant arm. Exhausting one limb before examining contralateral limb performance increases the inhibitory effect of group III/IV afferents on exercise tolerance (Amann et al., 2013b). Triscott et al. (2008) suggest endurance training develops central fatigue resistance, and other evidence supports this is due to increased resistance to group III/IV afferent firing. Therefore, it is likely that training under conditions of severe peripheral fatigue increases resistance to inhibitory group III/IV afferent feedback which contribute to reduced central fatigue an increased ability to tolerate peripheral fatigue allowing better REL exercise tolerance.

Pain Tolerance

HIIT, but not CONT, increased pain tolerance as measured by the tolerance to ischaemic muscle pain in an untrained limb. This is the first study to demonstrate training intensity dependent adaptations in the ability to tolerate pain. The ischaemic pain tolerance test stimulates chemosensitive group III/IV muscle afferents in a similar way to the metabolic disturbance induced by exercise (Jones et al., 2014). As the arm was untrained and the occlusion of blood flow excludes any central circulatory influence, the nociceptor stimulus is the same pre- and post-training. Other studies have reported that pain sensitivity is unchanged following training and therefore the effects are likely through better central tolerance of nociception (Jones et al., 2014); the unchanged reported pain sensation after 2 min supports this. This data helps to confirm previous suggestions that trained individuals have altered supraspinal processing of muscle pain signals (Nybo and Secher, 2004), however, for the first

time the data here demonstrate the effects are only apparent if the training is metabolically and perceptually straining.

Whilst six weeks of endurance cycling training has previously shown to increase ischaemic muscle pain tolerance (Jones et al., 2014), this study was marked by a number of limitations the present study attempted to address. Firstly, our training groups were randomised and matched for fitness. Secondly, the control group performed an equal volume of training which allows any behavioural or placebo aspects of training to be excluded. This resulted in similar improvements in aerobic fitness between groups which can also be dismissed as a contributor to the increased pain tolerance. The training intensity was also prescribed relative to metabolic thresholds so as to homogenise the perceptual stress within the training groups, as well as to induce disparate sensory strain between the groups. The training intensity of 75% HR reserve employed by Jones et al. (2014) would be below the LT in some participants and above the LTP in others in the present study. Therefore it was unclear whether the sensory disturbance induced by training is an important factor despite suggestions repeated exposure to noxious exercise stimuli were the mechanism contributing to increased pain tolerance (Jones et al., 2014). As the perceptual strain was markedly greater during the HIIT compared with CONT, the increase in muscle pain tolerance is likely due to training under conditions of unpleasant sensory stimuli, induced by controlled prescription of exercise intensity. Finally, in order to provide links between pain tolerance and exercise tolerance, participants completed a TTE trial. Whilst acetaminophen ingestion increases cycling TTE at 70% $\dot{V}O_{2max}$ in the heat (Mauger et al., 2014), there was little data to support enhanced pain tolerance as a contributor to the enhanced endurance following training. A trend for a moderate association between pain tolerance and TTE during the REL ($r = 0.50$) but not ABS trial, suggests increased REL TTE

is in part due to an increased ability to tolerate the sensory disturbances associated with the metabolic strain and increased level of peripheral fatigue.

Limitations

Although the tourniquet test is thought to stimulate sensory afferents in a similar way to exercising muscle (Jones et al., 2014), this assumption has been questioned (Amann et al., 2015) and numerous subtypes of group III/IV afferents have been identified which contribute independently to sensations of muscle pain and fatigue (Pollak et al., 2014). There are also a number of parameters relevant to exercise tolerance not measured in the present study, which could be used to better understand mechanisms underpinning improvements in exercise tolerance (e.g. MLSS or CP), however the LTP gives a good indication of MLSS (Faude et al., 2009) and both the pre-training and REL trials were above the LTP in all participants. The small number of participants in this study also likely restrict the conclusions of the findings as it is likely a number of potentially important findings were undetected. However, the number of participants per group were similar to those previously investigating fatigue mechanisms (Zghal et al., 2015), pain tolerance (Jones et al., 2014) and exercise tolerance (Seiler et al., 2013) after training, and this data is useful to inform study size estimates in future research.

Conclusion

Whilst there is an inextricable link between fatigue, pain and endurance performance (Mauger, 2013), there is little data to support a link between these components of human performance. This study demonstrates HIIT, but not CONT, allows better tolerance of exercise that elicits the same metabolic demand as pre-training, possibly through better tolerance of peripheral fatigue and muscle pain, and attenuated central fatigue. Although anecdotally trained individuals are considered to be able to 'push themselves harder' than their untrained

counterparts, there was little evidence to support this. Group III/IV afferents have been proposed as an important contributor to fatigue and exhaustion and the results from this study suggest that HIIT increases resistance to the inhibitory fatigue-inducing and sensory effects of group III/IV afferents; the effect is likely through repeated exposure to high metabolic stress and the accompanying noxious exercise stimulus and high levels of peripheral and central fatigue.

Chapter 7 - General Discussions and Conclusions

7.1 Summary

The aim of this thesis was to investigate the mechanisms of fatigue following locomotor exercise in order to better understand the processes limiting exercise tolerance; a number of different approaches were undertaken employing a range of acute and chronic exercise protocols. Chapter 3 examined the repeatability of measures of neuromuscular and corticospinal function in the knee extensors. Chapter 4 investigated relationships between neuromuscular and corticospinal function with exercise capacity. Chapter 5 employed both non-exhaustive and exhaustive exercise of disparate metabolic demands to examine the aetiology of fatigue. Chapter 6 examined the effect of endurance training on fatigue mechanisms following high-intensity exercise. This Chapter will provide an overview of the main findings of this thesis.

7.2 Main Findings

Motor Nerve and Cortical Stimulation as Tools to Examine Fatigue

Chapter 3 demonstrated the repeatability of MNS and TMS. Accurate measurement of neuromuscular and corticospinal function in the knee extensors is vital to better understanding locomotor exercise fatigue and training adaptation. Whilst both MNS and TMS have been used extensively to examine central and peripheral fatigue following locomotor exercise, only a few studies have investigated the repeatability of these techniques. Amongst the TMS measures, Chapter 3 provides first evidence of the repeatability of measures of cortical excitability in the knee extensors as measured by ppTMS. For both SICI and ICF, a 90% aMT CS was the most repeatable measure. However, ICF could not be elicited in the VL in agreement with previous research in other active muscle groups (Ridding et al., 1995, Ortu et al., 2008) and therefore

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this method was not taken forward into further investigations in this thesis. Although it should be noted that ICF has recently been demonstrated for the first time in the active RF (Thomas et al., 2015b). ppTMS allows a deeper understanding of supraspinal processes implicated in fatigue alongside other established MNS and TMS measures of peripheral (muscle contractility), central (peripheral VA) and supraspinal fatigue (cortical VA). Recent investigations have effectively used TMS in revealing mechanisms of fatigue following locomotor exercise (sections 2.3.2 – 2.3.3), however the potential of this technique has yet to be realised with a better understanding of the cortical responses to locomotor exercise considered key in understanding central fatigue and exercise tolerance (Sidhu et al., 2013a) with the use of ppTMS potentially instrumental (Pereira and Keller, 2012). Furthermore, TMS is emerging as an important tool to examine CNS adaptations to lower limb resistance training (Goodwill et al., 2012, Weier et al., 2012) and endurance training (Zghal et al., 2014, Zghal et al., 2015). Therefore, there was a need to examine the within- and between-day reliability of these techniques in a locomotor muscle. The results from this Chapter allowed this technique to be optimised and taken forward into investigations of fatigue.

Corticospinal and Neuromuscular Contributions to Exercise Capacity

Chapter 4 is the first study of the relationships between corticospinal and neuromuscular function with exercise capacity. Other cross-sectional studies have examined neuromuscular function differences between groups of individuals based on training status (i.e. endurance-versus resistance-trained or sedentary) (Maffiuletti et al., 2001, Lattier et al., 2003, Garrandes et al., 2007) but none have used TMS. Rather than analyse differences between individuals based on training history, we employed laboratory based measures of exercise capacity in order to provide links with well-established measures of functional capacity. This study revealed that the LT was associated with slower muscle contractile characteristics, likely representing a more

type I fibre dominant muscle (Hamada et al., 2000b), with greater oxidative capacity (Coyle et al., 1988) and fatigue resistance (Morris et al., 2008). A higher LT was also associated with less SICI which could occur as a result of adaptation to endurance or resistance training. $\dot{W}_{max}$ was associated with more LICI and a longer cSP. Further analysis using multiple linear regression revealed that exercise capacity during a standardised incremental exercise test ($\dot{W}_{max}$), could be explained by a combination of $\dot{V}O_{2max}$ and cortical VA. The better exercise capacity and accompanying alterations in the circuits that modulate motor cortical output could be important in central fatigue resistance; less inhibition may result in greater output in response to volitional drive. Whilst these adaptations may be induced by training, this remains to be confirmed. This Chapter provides first evidence of links between corticospinal function and locomotor exercise capacity.

Central and Peripheral Fatigue in Response to the Exercise-Induced Metabolic Demand

Chapter 5 employed exercise of disparate metabolic demands to better understand the mechanisms of fatigue during locomotor exercise. Although metabolic disturbances induced by exercise are thought to contribute to peripheral and central fatigue, there is yet to be an investigation comparing fatigue after exercise of disparate, controlled levels of metabolic stress. Previous investigations have employed work-matched intermittent vs constant power output cycling (Theurel and Lepers, 2008) or different distance time-trials (Thomas et al., 2015a) and have reported contrasting findings as to the development of central fatigue. Therefore, the role of intensity, but also duration of the exercise task, and their relation to mechanisms of fatigue was unclear. This study found that SI exercise accelerated the rate of both peripheral and central fatigue development compared with work-matched MI exercise, supporting the role of metabolic disturbance in central as well as peripheral fatigue

development. The greater metabolite accumulation caused by SI exercise likely activated inhibitory group III/IV muscle afferents (Theurel and Lepers, 2008).

Central and Peripheral Fatigue in Limiting Exercise Tolerance

Chapter 5 also employed non-exhaustive and exhaustive exercise of disparate demands in order to better understand the key factors contributing to exhaustion. At exhaustion there was no difference in central or supraspinal fatigue between intensities suggesting impairment in central motor drive role might play and important mechanistic role in exhaustion. The greater repeatability of peripheral fatigue after SI exercise compared with MI suggests a more important role in limiting exercise tolerance. Significant associations between peripheral and supraspinal fatigue at exhaustion after SI, but not after MI exercise, supporting the interaction of peripheral and supraspinal fatigue (Amann, 2011). It is therefore likely that although supraspinal fatigue appears an important mechanism contributing to exhaustion, the mechanisms causing the fatigue are different depending on intensity.

Cortical Contributions to Fatigue

In order to better understand the supraspinal processes involved in fatigue, a range of TMS techniques were employed to measure corticospinal excitability (MEP amplitudes) and intracortical inhibitory processes (cSP duration and SICI). Corticospinal excitability was unaffected by exercise. SI exercise reduced the cSP duration and increased SICI whereas in contrast MI had no effect on cortical inhibition. Taken together this suggests SI exercise decreases $GABA_B$-mediated intracortical inhibition and increases $GABA_A$-mediated intracortical inhibition. As such the cortical cells appear to respond differently to isolated muscle fatigue vs locomotor endurance exercise as well as to locomotor exercise of disparate metabolic demands. It was originally proposed that the systemic and local disturbances that

challenge homeostasis could affect the cortical cells (Sidhu et al., 2013a) and these data supports this. Sidhu et al. (2013c) proposed exercise-induced activation of group III/IV muscle afferents may act at the level of the motor cortex to inhibit the motor cortex to muscle pathway. This is supported by the findings that disturbances in inhibitory function were induced by SI, but not MI exercise, and the relationship between peripheral fatigue and increase in SICI. Other studies have shown the experimental induction of muscle pain suppresses cortical excitability (Martin et al., 2008) and increases SICI (Schabrun and Hodges, 2012) whereas blocking the central projections of muscle afferents prevents the fatigue induced increase in the cSP duration during isolated muscle contractions (Hilty et al., 2011). Taken together these studies suggest that type III/IV muscle afferents act at or above a cortical level to increase intracortical inhibition. The intracortical inhibitory circuits are important in modulating the final cortical output in response to excitatory input and this could represent an important mechanism involved in central fatigue.

Endurance Training Increases the Tolerance of the Exercise-Induced Metabolic Stress

Chapter 6 demonstrated that despite similar improvements in aerobic fitness, HIIT resulted in improvements in TTE at a workload eliciting the same metabolic strain as pre-training (REL) compared to CONT. Whether trained individuals can better tolerate the same physiological strain was previously questioned (Millet, 2011). The power output was increased to account for training-induced adaptations in fitness and there were no differences between groups for improvements in aerobic capacity, suggesting these factors cannot explain the TTE differences. Therefore, an aspect of training specific to HIIT induced this adaptation, independent of training alone. As the HIIT took place at the same intensity of the exercise tolerance trials, it is therefore likely that customisation to this level of metabolic strain allows better tolerance which

is supported by the greater $\dot{V}O_2$ at exhaustion after HIIT. In order to better understand the mechanisms, peripheral and central fatigue, and pain tolerance were examined.

Endurance Training Augments Peripheral Fatigue and Attenuates Central Fatigue

As well as increasing exercise tolerance during the REL trial, HIIT also increased the level of peripheral fatigue and attenuated central fatigue, suggesting a training-induced increased in the tolerance of peripheral fatigue by the CNS. This finding is strengthened by the demonstration of the repeatability of fatigue measures after cycling to exhaustion at 50%Δ in Chapter 5. By examining fatigue processes before and after an intervention, the significance of the mechanisms that confer exhaustion can be better understood (Enoka and Duchateau, 2008), however there are very few studies to adopt this approach. This data suggests that HIIT increases the sensory tolerance limit or critical fatigue threshold proposed by Amann (2011) and allows greater access to the physiological security reserve proposed by Millet (2011). The increase in peripheral fatigue and attenuated central fatigue is likely the result of increased CNS tolerance of and/or altered group III/IV afferent activity (Amann et al., 2011, Zghal et al., 2015). This suggests an ability to override central fatigue mechanisms in response to inhibitory afferent feedback in order to extrude greater performance from the muscle. Therefore the CNS may release the 'brake' on central motor drive to tolerate more peripheral fatigue (Amann et al., 2011). The resistance to central fatigue with concomitant increase in peripheral fatigue also demonstrates a disturbance in the peripheral and central fatigue relationship (Chapter 5) and supports the theory that HIIT reduces the inhibitory effects of peripheral fatigue on central motor drive. As the effect was only seen for HIIT and not CONT, it is likely that training under conditions of high peripheral and central fatigue, such as that induced by SI exercise (Chapter 5), might underlie this effect. Therefore the metabolic disturbance induced by HIIT likely induced greater discharge of the sensory group III/IV afferents (Amann et al., 2015, Laurin et

al., 2015) and sensations of muscle pain and fatigue (Pollak et al., 2014) whereas CONT failed to markedly disturb metabolic homeostasis and only induced small levels of peripheral fatigue (Chapter 5). As the sensations accompanying fatigue beyond the 'critical fatigue' threshold are considered to be intolerable (Amann et al., 2011) the effect of training on tolerance to unpleasant sensory afferent feedback (i.e. muscle pain) was investigated.

Endurance Training Increases the Tolerance of Ischaemic Muscle Pain

Whilst there is an inextricable link between pain, fatigue and endurance performance (Mauger, 2013), there was little data to support a link between these components of human performance. HIIT but not CONT increased pain tolerance as measured by the tolerance to ischaemic muscle pain induced by a tourniquet test, providing new evidence regarding training intensity dependent adaptations in the ability to tolerate pain. The adaptation likely occurred through better central tolerance of nociception as the reported pain sensitivity was unchanged. This data helps to confirm previous suggestions that trained individuals have better central tolerance of muscle pain signals (Nybo and Secher, 2004), however for the first time the data here demonstrate the effects are only apparent if the training is perceptually straining. As the group III/IV afferent response to the two training intensities was likely to be different and the perceptual strain was markedly greater during the HIIT, the increase in muscle pain tolerance induced by HIIT is likely due to training under conditions of unpleasant sensory stimuli. There was a moderate association between pain tolerance and TTE during the REL trial, suggesting the increased TTE in the REL trial is in part due to an increased ability to tolerate the sensory disturbances associated with the metabolic strain; this is supported by the peripheral fatigue data. Taken together this data suggests repeated exposure to high noxious exercise stimuli (metabolic strain and muscle fatigue) increases the tolerance of muscle pain and muscle fatigue which contributes to the increased exercise tolerance when the same metabolic strain is elicited.

7.3 Limitations

Alongside the specific limitations reported in the individual chapters, a number of general limitations throughout this thesis should be highlighted and considered. Firstly, the Nyquist theorem regarding data sampling was not adhered to when recording the EMG data. More specifically, the EMG signal was band pass filtered at 10 - 2000 Hz and sampled at 2000 Hz, whereas the Nyquist theorem states the sampling rate should be twice the highest frequency cut-off (Merletti, 1999). However, high frequency cut-offs at 500 Hz are recommended as sufficient for muscle analysis (De Luca, 1997) and the frequency of the VL EMG signal recorded during MVCs from the sixteen participants in Chapter 5 found a median frequency of 74.7 ± 17.0 Hz (range 42.6 - 102.9 Hz) and mean frequency of 84.4 ± 14.9 Hz (range 62.1 – 108.0 Hz). Therefore, the signals were sampled at a sufficient rate to capture all EMG data.

In Chapter 3, we could not induce facilitation of the test response with the ICF protocol. This is in agreement with previous studies in other active muscle groups, the mechanisms of which are unknown (Ridding et al., 1995, Ortu et al., 2008). To the authors knowledge, only one study appears to have demonstrated a facilitated response in the measurement of ICF in the active muscle (Thomas et al., 2015b). It is not clear whether in the present study employing a TS relative to rMT, which elicits a higher MEP than a TS relative to aMT, induced a ceiling effect with the MEP and therefore ICF could not be induced (Chen, 2004). Although this measurement was not taken any further in the studies that comprise this thesis, ICF may provide important insight to the cortical process involved in fatigue. Further work is required to develop ppTMS protocols that induce a facilitated response in the active muscle and also understand the underlying mechanisms of ICF.

7.4 Future Directions

Despite widespread interest, the mechanism of fatigue are poorly understood and fatigue remains a major issue in clinical and athletic populations. The data from this thesis provides new insight into the nature of fatigue and exhaustion during locomotor exercise and some of these findings warrant further research. In Chapter 3, the reported reliability for ppTMS suggests that manipulating the CS affects repeatability. It would benefit future investigations to better understand the relationship between TMS parameters and repeatability (i.e. ISI and TS). This may also help understanding the effect of the TMS parameters on inter-individual responses. TMS responses demonstrate significant inter-individual variability and it is assumed that administering TMS according to pre-determined motor thresholds standardises the response. Whilst the data in Chapter 4 suggests that some of this variability can be explained by differences in aerobic fitness, future investigations may benefit from individualising the ppTMS protocol to ensure optimal stimulation parameters for each individual. This approach may also help in developing methods that allow measurement of ICF in the active muscle. From the data demonstrating relationships between corticospinal function and exercise capacity reported in Chapter 4, it was suggested that cortical plasticity may occur in response to endurance training. The CNS adaptations to endurance training are poorly understood and future work should aim to better understand these adaptations which could have key implications for better understanding central fatigue resistance.

Part of the insight provided by this thesis is characterising the response of the cortical inhibitory responses to exhaustive cycling. However, further work is required regarding the functional impact of fatigue-induced changes in various parts of the motor pathway and the effects these have on exercise tolerance. Amongst locomotor exercise fatigue studies, non-invasive stimulation techniques offer an important advantage of measuring both central and peripheral

fatigue rapidly without disturbing the exercise. Compared with MNS, TMS has been a more recent important addition to better understand fatigue processes and provide direct evidence of CNS modulations. However, an important limitation with TMS is that the technique only allows probing of the excitability of the motor pathways. Undoubtedly fatigue induces widespread changes in the brain that may be responsible for modulating central motor drive and processing sensory information such as pain and effort. At present it is difficult to study these processes during locomotor exercise and better utilisation of more comprehensive imaging techniques that allow measurement of other CNS process such as cerebral metabolism, oxygenation, blood flow, neurotransmission and brain activity (not limited to but including EEG, fMRI, NIRS, PET, MEG) may help to better understand CNS physiology and fatigue.

The data presented here may be useful for future fatigue research that should identify and examine acute interventions that have the capacity to enhance exercise tolerance and/or alleviate the sensations of fatigue. For example, administration of a $GABA_B$ agonist delays exhaustion during a treadmill run in an animal model (Abdelmalki et al., 1997), which is supported by the findings in Chapters 4 and 5, but has yet to be explored in humans. Other innovative techniques that have the capacity to affect CNS function, such as transcranial direct current stimulation could also prove useful by providing targeted ways to manipulate motor cortex function. Such an approach could help the understanding of the physiology of fatigue, as well as providing strategies to alleviate fatigue, which could have important applications for clinical and athletic populations as well as increasing exercise adherence.

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Appendices

Appendix A – Example Ethical Approval

OXFORD BROOKES UNIVERSITY

Dr Martyn Morris
Director of Studies
Department of Sport and Health Sciences
Faculty of Health and Life Sciences
Oxford Brookes University
Gipsy Lane
Headington

27 November 2014

Dear Dr Morris

UREC Registration No: 140867
The effect of endurance training intensity on peripheral and central mechanisms of fatigue: implications for exercise tolerance

Thank you for the email of 13 November 2014 outlining your response to the points raised in my previous letter about the PhD study of your research student Thomas O'Leary, and attaching the revised documents. I am pleased to inform you that, on this basis, I have given Chair's Approval for the study to begin.

The UREC approval period for this study is two years from the date of this letter, so 27 November 2016. If you need the approval to be extended please do contact me nearer the time of expiry.

Should the recruitment, methodology or data storage change from your original plans, or should any study participants experience adverse physical, psychological, social, legal or economic effects from the research, please inform me with full details as soon as possible.

Yours sincerely

Hazel Abbott
Chair of the University Research Ethics Committee

cc   Johnny Collett, Supervisory Team
     Thomas O'Leary, Research Student
     Dido Green, Research Ethics Officer
     Jill Organ, Research Degrees Team
     Louise Wood, UREC Administrator

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Appendix B – Example Participant Information Sheet

Study title

The effect of endurance training intensity on peripheral and central mechanisms of fatigue.

Name, position and contact address of Researcher:

Tom O'Leary (tom.oleary-2012@brookes.ac.uk / 01865483272), PhD Student,

Movement Science Group, Room T426, Department of Sport and Health Sciences.

You are being invited to take part in this study. Before you decide whether or not to take part, it is important for you to understand why the research is being done and what it involves. Please take time to read the following information carefully.

What is the purpose of the study?

High-intensity exercise tolerance is limited by a number of peripheral and central fatigue mechanisms. Peripheral fatigue is the result of mechanisms within the muscle whilst central fatigue occurs within the central nervous system (CNS). The CNS mechanisms are unknown but likely involve the ability to tolerate muscle pain as well as disturbances in the brain to muscle pathway. Transcranial magnetic stimulation (TMS) is a technique where the brain can be stimulated in order to investigate CNS fatigue mechanisms. The aim of this study is to identify the peripheral and central fatigue adaptations induced by training.

Why have I been invited to participate?

You have been invited to participate as you have shown an interest in the study. Your eligibility will be determined before commencing the study. This study is recruiting healthy physically active individuals aged 18-39 years, but not engaged in endurance training.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part, you will be given this information sheet to keep and be asked to sign a consent form. You are free to withdraw at any time and without giving a reason.

What will happen to me if I take part?

Before taking part you will be emailed physical activity readiness and TMS suitability questionnaires to determine your eligibility. You will be then required to visit the Oxford Brookes Human Performance Laboratory over the course of 8 weeks.

Week 1 – Pre-training Testing: Three visits (each ~2 hours)

Before each visit you will abstain from caffeine for 12 hours, alcohol for 24 hours and avoid exhaustive exercise for 48 hours.

Visit 1: This visit will involve familiarisation to the muscle and brain stimulation procedures, some fitness tests and a pain tolerance test. Muscle stimulation and TMS will be applied at the femoral nerve and top of the head, respectively, in order to cause a contraction in the quadriceps. TMS produces induces a small electrical current in the brain which causes muscle activation. Electromyography will be applied at the quadriceps in order to record muscular electrical activity. Both muscle stimulation and TMS are safe and painless methods used to assess muscle and CNS function. You will be required to perform a series of maximal contractions with either stimulation to measure strength. The fitness test will be an incremental exercise test on a cycle ergometer until exhaustion to determine aerobic fitness, during which, heart rate and oxygen consumption using a face mask will be measured as well as blood lactate from fingertip blood samples. The pain tolerance test involves completing light hand-grip contractions for as long as possible with a blood pressure cuff wrapped around the arm. The cuff stops blood flow to the muscle which induces muscle pain. The pain is very brief and is alleviated immediately upon release of the cuff.

Visit 2: This will involve a repeat of the muscle stimulation and TMS before and after a short exercise tolerance test (A) (~25 min) during which you will be required to cycle at an intensity set according to your fitness for as long as possible.

Visit 3: This will involve a repeat of the tests completed during visit 2.

Weeks 2 – 7 – Training: Three visits each week (~1.5 hours).

Following the preliminary testing you will be randomly assigned to a training group. The training will involve visiting the lab for three 1.5-hour training session each week. The training will either be high- or moderate-intensity cycling, prescribed according to your fitness. The high-intensity training involves cycling in 5 min blocks with 1 min rest between repeated 6-8 times for each session. The moderate intensity will involve cycling for ~60-90 min at a moderate intensity. Throughout the training your heart rate will be monitored.

Week 8 – *Post-training Testing*: Three visits (each ~2-3 hours)

The post-training testing will be identical to pre-training; however, the exercise tolerance tests will be completed at intensities relative to your post-training fitness (B) in addition to the one set according to your pre-training fitness (A).

A participant undergoing TMS in the Oxford Brookes Human Performance Laboratory

Study Protocol

Week 1 – Pre-training testing	TMS, fitness and pain tolerance tests (~2 hours)	TMS and Exercise tolerance test **A** (~2 hours)	TMS and Exercise tolerance test **A** (~2 hours)

Week 2 – 7 Training Interventions (50% of participants in each group)	High-Intensity Training Group (6 weeks) – • 3 weekly sessions • ~1 hour per session • Each session involves 6-8 blocks of cycling (one block is 5 min with 1 min rest)	Moderate-Intensity Training Group (6 weeks) – • 3 weekly sessions • ~1.5 hour per session • Each session involves continuous cycling

Week 8 – Post-training testing	TMS, fitness and pain tolerance tests (~2 hours)	TMS and Exercise tolerance test **A** (~3 hours)	TMS and Exercise tolerance test **B** (~3 hours)

What are the possible benefits of taking part?

You will be compensated for your time with a £15 Amazon voucher on completion of the study. You will also receive detailed information about your fitness and 6 weeks of individual

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training. By participating you will also be contributing to the understanding of fatigue during exercise with clinical and athletic implications.

Are there any risks in taking part?

Maximal exercise can induce feelings of discomfort and breathlessness. Water and a fan will be provided if you feel thirsty or hot. Adverse reactions to exercise are rare and you should be accustomed to exercise in order to be eligible. Although TMS is painless and safe, and used regularly in the department, there are a number of rare low risk side effects including transient headache/discomfort at the site of stimulation for susceptible individuals. The pain tolerance test will induce feelings of local muscle discomfort which are alleviated immediately on release of the blood pressure cuff. All procedures are according to well-established safe and published methods. Only small drops of blood from a finger-tip will be collected and these samples will be taken by trained personnel; the number of samples taken will depend on each individual but will not exceed 8 in any visit. There will be trained first aiders present at all testing and training sessions.

Exclusion criteria

Before taking part you will be asked to complete a physical activity readiness questionnaire and TMS suitability questionnaire detailing exclusion criteria. Unfortunately, you cannot take part you meet any of the following criteria:

- Take part in regular endurance training (>30 min, >2 times per week).
- Suffered from recent illness or injury.
- Have any of the following i) epilepsy or susceptibility to fainting, ii) cardiovascular problems including heart and chest pain, hypertension or varicose veins, iii) diabetes, iv) metal implant in the head, or v) head trauma.

Will what I say and do in this study be kept confidential?

Due to the small sample size, complete anonymity cannot be guaranteed during your participation. However, all information and data collected during the study will be kept strictly confidential. Upon enrolling in the study, participants will be assigned a numbered code. All data will then be stored under this code to protect confidentiality. Only researchers will have access to this information as well as the de-identified data; data confidentiality will be upheld according to current UK law. Hard copies of data sets will be kept in locked filing cabinets and electronic data will be restricted by password. All published data will be anonymous. Following

completion of the study, the data generated will be kept securely in paper or electronic format for ten years and retained in accordance with the University's policy on Academic Integrity.

What should I do if I want to take part?

If after reading the participant sheet you decide you would like to take part please contact Tom O'Leary (tom.oleary-2012@brookes.ac.uk / 01865483272).

What will happen to the results of the research study?

The results from the study will form part of a PhD thesis and will be presented at academic conferences and published in peer-reviewed sources. You should feel free to request a copy of the study findings.

Who is organising and funding the research?

This research is being conducted as part of an Oxford Brookes University funded PhD in the Department of Sport & Health Sciences.

Who has reviewed the study?

This research has been approved by the University Research Ethics Committee (Registration No. 140867), Oxford Brookes University.

Contact for Further Information

Tom O'Leary (tom.oleary-2012@brookes.ac.uk)

If you have any concerns about the way in which the study has been conducted, you can contact the Chair of the University Research Ethics Committee on ethics@brookes.ac.uk

Thank you for taking time to read the participant information sheet.

Appendix C – Physical Activity Readiness Questionnaire

Please read the following carefully and answer as accurately as possible by ticking the appropriate box for each question.

		Yes	No
1.	Has a doctor ever said you have heart trouble?	☐	☐
2.	Do you ever suffer frequently from chest pains?	☐	☐
3.	Do you often feel faint or have spells of dizziness?	☐	☐
4.	Has a doctor ever said you have epilepsy?	☐	☐
5.	Has a doctor ever said you have high blood pressure?	☐	☐
6.	Has a doctor ever said you have diabetes?	☐	☐
7.	Has a doctor ever said you have asthma?	☐	☐
8.	Do you have a bone, joint or muscular problem which may be aggravated by exercise?	☐	☐
9.	Do you have any form of injury?	☐	☐
10.	Are you currently taking any prescription medications?	☐	☐
11.	Do you suffer from any form of cardiovascular disease (including varicose veins)?	☐	☐
12.	Have you suffered from a viral illness in the last two weeks?	☐	☐

	Yes	No
Have you eaten anything within the *last hour*?		
Have you consumed alcohol within the *last 24 hours*?		
Have you performed exhaustive exercise within the *last 48 hours*?		

If you have answered <u>YES</u> to any of the above questions, or know of any possible reason (physical or psychological) that might affect the safety or accuracy of the tests - please inform a member of staff.

Anything else you feel that we should know about:

Appendix D – TMS Suitability Questionnaire

Please read the following carefully and answer as accurately as possible by ticking the appropriate box for each question.

	Yes	No
1. Do you have epilepsy or have you ever had a convulsion or seizure?	☐	☐
2. Have you ever had a fainting spell (syncope)? (If yes, please describe in which occasions).	☐	☐
3. Have you ever had severe (i.e. followed by loss of consciousness) head trauma?	☐	☐
4. Do you have any hearing problems or ringing in your ears?	☐	☐
5. Are you pregnant or is there any chance you might be?	☐	☐
6. Do you have metal in the brain/skull (except titanium)? (e.g. splinters, fragments, chips, etc.)	☐	☐
7. Do you have cochlear implants?	☐	☐
8. Do you have an implanted neurostimulator? (e.g. DBS, epidural/subdural, VNS)	☐	☐
9. Do you have a cardiac pacemaker or intracardiac lines or metal in your body?	☐	☐
10. Do you have a medication infusion device?	☐	☐
11. Are you currently taking any medications? (Please list)	☐	☐
12. Have you ever had a surgical procedure to your spinal cord?	☐	☐
13. Do you have spinal or ventricular derivations?	☐	☐
14. Have you ever undergone TMS in the past?	☐	☐
15. Have you ever undergone MRI in the past?	☐	☐

	Yes	No
Have you eaten anything within the *last hour*?		
Have you consumed caffeine within the *last 12 hours?*		
Have you consumed alcohol within the *last 24 hours*?		
Have you performed exhaustive exercise within the *last 48 hours*?		

If you have answered <u>YES</u> to any of the above questions, or know of any possible reason (physical or psychological) that might affect the safety or accuracy of the tests - please inform a member of staff.

CONSENT FORM

Project: The effect of endurance training intensity on peripheral and central mechanisms of fatigue.

Name, position and contact address of Researcher:
Tom O'Leary (tom.oleary-2012@brookes.ac.uk / 01865483272), PhD Student
Movement Science Group, Room T426, Department of Sport and Health Sciences

Please initial box

I confirm that I have read and understand the information sheet for the above study and have had the opportunity to ask questions.

☐

I understand that my participation is voluntary and that I am free to withdraw at any time, without giving reason.

☐

I agree that my data, gathered anonymously in this study, may be stored in a specialist data centre and may be used for future research (optional).

☐

I agree to take part in the above study.

☐

_____   _____   _____
Name of Participant   Date   Signature

_____   _____   _____
Name of Researcher   Date   Signature