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Sex differences in the fatigability of locomotor muscles

Paul Ansdell

PhD

2020

Sex differences in the fatigability of locomotor muscles

A thesis submitted in partial fulfilment of the requirements of Northumbria University for the degree of Doctor of Philosophy

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ABSTRACT

Fatigability during exercise is determined by a myriad of factors including characteristics of the task being performed and the individual performer. When the latter is considered, the sex of the individual can influence the physiological responses, and therefore the underpinnings of fatigue during a wide range of tasks. Typically, fatigability of males and females has been compared following tasks normalised to maximum capacity (e.g. maximum voluntary contraction). Whilst these approaches have identified that females might experience less fatigue, they cannot provide further insight into the differences between the two sexes, as they do not account for potential differences in metabolic thresholds. The aim of this thesis was to therefore compare fatigability of males and females during exercise normalised to the intensity-duration relationship. It was hypothesised that due to anatomical and physiological differences between males and females, greater critical intensities would be observed in females, however, when exercise was subsequently normalised to these thresholds fatigability would be similar. In Study 1, the neuromuscular function and fatigability of females was compared across the eumenorrheic menstrual cycle. Alterations in nervous system function were observed in line with changes in neuro-excitatory and inhibitory hormone concentrations, meaning that in subsequent Chapters, hormonal status had to be controlled. Study 2 then demonstrated that assessments of neuromuscular function and fatigability were repeatable in a hormonally-constant population of monophasic oral contraceptive pill users. This finding indicated the suitability of this population for comparison with males in studies involving repeated visits. In Study 3, the intensity-duration relationship was compared between males and females for intermittent, isometric exercise, then fatigability and physiological responses were observed for exercise normalised to the critical intensity. Females demonstrated a greater relative critical intensity, however contrary to the original hypothesis, still demonstrated greater fatigue-resistance during

metabolically-matched intensities. Near-infrared spectroscopy and neurostimulation data showed lesser deoxygenation and contractile dysfunction, respectively, within female knee-extensors during these normalised exercise trials, implying that the locus of the sex difference resided in the musculature. Study 4 then developed a novel method for assessment of subcortical excitability of descending tracts for the knee-extensor muscles. This study confirmed that lumbar stimulation was capable of activating the corticospinal tract and evoking responses at rest and during contractions, for use in subsequent study. In a similar study design to Study 3, Study 5 compared the intensity-duration relationship during cycling exercise between males and females, and assessed physiological responses and fatigability during metabolically-matched severe and heavy intensity exercise. In contrast to the original hypothesis, critical power was not different between sexes, however during exercise at 110 and 90% of critical power, time to task failure was the same between sexes. Despite similar exercise time at metabolically-matched intensities, females again demonstrated lesser deoxygenation and contractile dysfunction of the knee-extensors following exercise. Collectively, the work in this thesis extends the understanding of the sex difference in fatigability during exercise, offering insight into the difference in metabolic and neuromuscular consequences of single-limb and locomotor exercise which can be used to explain previous observations. Furthermore, the data implies that for the same relative volume of exercise, female skeletal muscle experiences less disruption compared to males, which has consequences for acute and chronic exercise prescription in a range of populations.

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LIST OF SYMBOLS AND ABBREVIATTIONS

$5\text{-}HT_{1A}$	Serotonin
^{31}P-MRS	Phosphorous magnetic resonance spectroscopy
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
AMT	Active motor threshold
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
BF	Biceps femoris
Ca^{2+}	Calcium
CI	Confidence interval
Cl^-	Chloride
CMEP	Cervicomedullary evoked potential
CNS	Central nervous system
CP	Critical power
CS	Conditioning stimulus
CT	Critical torque
CV	Coefficient of variation
D-wave	Direct wave
E-C coupling	Excitation-contraction coupling
EMG	Electromyography
ERT	Estimated resting twitch
fMRI	Functional magnetic resonance imaging
GABA	Gamma-aminobutyric acid
H^+	Hydrogen
HHb	Deoxyhaemoglobin
HR	Heart rate
H-reflex	The Hoffman reflex
ICC	Intraclass correlation coefficient
ICF	Intracortical facilitation
ISI	Interstimulus interval
ITT	Interpolated twitch technique
I-wave	Indirect wave
K^+	Potassium
KE	Knee extensors
LEP	Lumbar evoked potential
LS	Electrical stimulation of the lumbar spinal segments
M1	Primary motor cortex
MAP	Mean arterial pressure
MCP	Menstrual cycle phase
M_{max}	Maximal compound action potential
MNS	Motor nerve stimulation
mOCP	Monophasic oral contraceptive

MVC	Maximum voluntary contraction
Na^+	Sodium
NIRS	Near infrared spectroscopy
O2Hb	Oxyhaemoglobin
PCr	Phosphocreatine
P_i	Inorganic phosphate
$\dot{Q}$	Cardiac output
$Q_{tw,pot}$	Potentiated quadriceps twitch force
RF	Rectus femoris
RMS	Root mean squared
rMT	Resting motor threshold
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RyR1	Ryanodine receptor 1
SD	Standard deviation
SICI	Short-interval intracortical inhibition
SIT	Superimposed twitch
SO	Stimulator output
SP	Silent period
SR	Sarcoplasmic reticulum
SV	Stroke volume
TE	Typical error
TMS	Transcranial magnetic stimulation
TOI	Tissue oxygenation index
TS	Test stimulus
TTF	Time to task failure
VA	Voluntary activation
VL	Vastus Lateralis
VA_{MNS}	Voluntary activation measured with motor nerve stimulation
$\dot{V}CO_2$	Rate of carbon dioxide production
$\dot{V}O2$	Rate of oxygen consumption
VA_{TMS}	Voluntary activation measured with transcranial magnetic stimulation
W'	Curvature constant of the intensity-duration relationship
η_p^2	Partial eta squared

PUBLICATIONS

Peer-reviewed publications arising from this thesis

Ansdell P, Thomas K, Hicks K, Hunter S, Howatson G, Goodall S. (2020). Physiological sex differences affect the integrative response to exercise: Acute and chronic implications. *Experimental Physiology.* In Press

Ansdell P, Škarabot J, Atkinson E, Corden S, Tygart A, Hicks K, Thomas K, Hunter S, Howatson G, Goodall S. (2020). Sex differences in fatigability following exercise normalised to the power-duration relationship. *Journal of Physiology*. In Press.

Ansdell P, Škarabot J, Brownstein C, Hicks K, Howatson G, Hunter S, Thomas K, Goodall S. (2019). Sex differences in fatigability and recovery relative to the intensity duration relationship. *Journal of Physiology*. *597*(23): 5577-5595.

Škarabot J*, **Ansdell P*,** Brownstein C, Thomas K, Howatson G, Goodall S, Durbaba R. (2018). Electrical stimulation of human corticospinal axons at the level of the lumbar spinal segments. *European Journal of Neuroscience. 49*(10): 1254-1267.

* joint first authorship

Ansdell P, Škarabot J, Brownstein C, Simões D, Hicks K, Thomas K, Howatson G, Goodall S. (2019). Menstrual cycle associated modulations in neuromuscular function and fatigability of the knee extensors in eumenorrheic females. *Journal of Applied Physiology. 126*(6), 1701-1712.

Conference communications arising from this thesis

Ansdell P, Škarabot J, Brownstein C, Thomas K, Howatson G, Goodall S, Durbaba R. Exploring the validity of lumbar stimulation for the assessment of lower limb motoneuron excitability in humans. Europhysiology; 21-23 September 2018; London, United Kingdom.

Ansdell P, Brownstein C, Škarabot J, Simões D, Hicks K, Thomas K, Howatson G, Hunter S, Goodall S. Alterations in neuromuscular function and fatigability of the knee-extensors across the menstrual cycle. European College of Sport Science Annual Congress; 4-7 July 2018; Dublin, Ireland.

Ansdell P, Brownstein C, Škarabot J, Thomas K, Howatson G, Hunter S, Goodall S. Reliability of knee-extensor neuromuscular function assessment and fatigue in a healthy female population. XXII Congress of the International Society for Electrophysiology and Kinesiology; 29 June-2 July 2018; Dublin, Ireland.

Ansdell P, Brownstein C, Škarabot J, Simões D, Hicks K, Thomas K, Howatson G, Hunter S, Goodall S. Alterations in neuromuscular function and fatigability of the knee-extensors across the menstrual cycle. UK Sensory Motor Conference; 21-23 June 2018; Leeds, United Kingdom.

DECLARATION

I declare that the work contained in this thesis has not been submitted for any other award and that it is all my own work. I also confirm that this work fully acknowledges opinions, ideas and contributions from the work of others.

Any ethical clearance for the research presented in this thesis has been approved. Approval has been sought and gained by the Faculty of Health and Life Sciences Ethics committee for each study.

Name: Mr Paul Ansdell

Signature: 

Date: 14th February 2020

CHAPTER 1 - INTRODUCTION

1.1 Introduction

Fatigue is a universal phenomenon, defined by the Oxford Dictionary as “extreme tiredness resulting from mental or physical exertion or illness”. Throughout history, the underpinning causes and influences of fatigue have been at the forefront of scientific endeavour, with many investigators attempting to create an objective model of fatigue that can accurately describe its influence in an exercise model. Angelo Mosso published *La Fatica* in 1891 and following multiple experiments, concluded that exhaustion is a form of poisoning by waste material and can include impairments to aspects of both the central nervous system and working musculature (Mosso, 1891). Mosso’s interpretation was ahead of his time, as almost a century later, Gandevia (2001) suggested that the exercise-induced decrease in maximal voluntary muscle force (termed ‘muscle fatigue’) arises from both muscular (peripheral) and neural (central) factors. However, Gandevia proposed that reductions in supraspinal drive act to protect the working muscle from further peripheral dysfunction, avoiding a ‘catastrophic state’ and compromising other homeostatic process required for survival of the organism. More recently, Enoka & Duchateau (2016) proposed a taxonomy for understanding exercise-induced fatigue derived and adjusted from a clinical perspective (Kluger *et al.*, 2013). Stating that fatigue, defined as a symptom, is a result of interactions between two components: performance fatigability – the decline in an objective measure of performance over a discrete period of time; and perceived fatigability – changes in the sensations that regulate the integrity of the performer. Whilst these models of fatigue slightly differ in understanding and terminology, a consistent aspect is that fatigue, or fatigability, and the contributing processes, are modulated by the specificity of task performed and the characteristics of the muscle(s) or organism performing it.

Within the field of exercise physiology, the sex of the individual performing a given exercise task has been shown to influence exercise tolerance and fatigability (Hunter, 2014). During

certain tasks, females are less fatigable than males, purportedly due to physiological and anatomical differences. At a muscular level, female and male tissues differ considerably, with a sexual dimorphism of gene expression (Welle *et al.*, 2008) leading to differences in phenotypic expression (e.g. muscle mass; Janssen *et al.*, 2000), substrate utilisation (Roepstorff *et al.*, 2002), and fibre type distribution (Staron *et al.*, 2000). Consequently, males are usually stronger and more powerful, however, for sustained or repeated contractions, females exhibit a greater tendency to be more fatigue-resistant than males (Hunter, 2016*a*). Importantly, this sex difference is muscle-specific, and the knee-extensors appear to be more fatigue-resistant in females. The sex difference in knee extensor fatigability poses a dilemma for researchers and practitioners, for instance, if training or rehabilitation programming is based upon research conducted in male populations, then such conclusions would not apply to female counterparts. The knee extensors have obvious involvement in locomotor exercise, therefore understanding how adjustments in the neuromuscular properties of locomotor musculature underpin fatigue in males and females, could lead to better outcomes in terms of athletic performance and rehabilitation.

Advances in technology and understanding have enabled physiologists to evaluate neuromuscular function in clinical (Hallett & Rothwell, 2011) and healthy (Goodall *et al.*, 2014*a*) populations, via the use of electrical and magnetic stimulation of muscle and/or nervous tissue. Using such techniques, sex differences in knee extensor properties have been shown. Specifically, lesser declines in contractile function have been shown in females compared to males following isometric exercise (Wüst *et al.*, 2008), cycling (Glace *et al.*, 2013), and ultra-endurance running (Temesi *et al.*, 2015). However, there is also literature to suggest that the central nervous system (CNS) plays a mediating role in the sex differences in fatigability. In an unfatigued state, maximal voluntary activation (VA) of skeletal muscle does not differ between sexes (Hunter *et al.*, 2006*a*; Keller *et al.*, 2011). However, during fatiguing isometric exercise of the knee extensors, males experience greater reductions in VA than

females (Russ & Kent-Braun, 2003; Martin & Rattey, 2007). Collectively, the aforementioned evidence suggests that any sex difference in knee extensor fatigability is likely a multi-factorial phenomenon involving several metabolic and physiological processes. However, the metabolic and physiological insight that previous investigations can offer is limited due to the fact that exercise intensities are either standardised to percentages of maximum capacity (e.g. % MVC), or self-paced in nature.

In order to provide clarification on the underlying mechanisms behind the sex difference in fatigability, this thesis will firstly examine the influence of the menstrual cycle on female neuromuscular function and fatigability, then profile the 'intensity-duration' relationship in both sexes. This paradigm of exercise tolerance and fatigability defines exercise intensity domains, and the aetiology of fatigue elicited in each domain (Burnley & Jones, 2018). It provides information such as the maximal sustainable intensity (often termed critical intensity) and the finite work capacity above critical intensity (W'; Jones *et al.*, 2010). Above the critical intensity, endurance performance can be predicted using the following equation:

$$T_{lim} = W' \div (I - CI)$$

In which T_{lim} is time to task failure, W' is the curvature constant parameter, I is the intensity of exercise and CI is critical intensity (expressed as power for cycling, velocity for running, or torque/force for isometric exercise). One advantage of modelling exercise tolerance using this framework is that the characteristics of fatigability can be compared between different populations during metabolically-matched exercise intensities.

The primary aim of this thesis was to investigate the underpinning mechanisms of the sex difference in exercise tolerance, relative to a metabolic threshold (the critical intensity) to ensure equivalence in the exercise task demand between males and females. Using non-invasive neurostimulation in conjunction with cardiorespiratory and metabolic assessment before, during, and following exercise, the present thesis provides a comprehensive overview of the sex differences in the physiological underpinnings of exercise tolerance. Chapter 2 reviews the literature pertaining to the study of fatigue, and the physiological differences between sexes that could contribute to a difference in exercise tolerance and fatigability. Before the two sexes can be compared, the influence of female sex hormones (the menstrual cycle) on neuromuscular function and fatigability is discerned (Chapter 4). Then, the repeatability of measures is quantified in Chapter 5, using a hormonally-constant female population. Consequently, the intensity-duration relationship is defined, using an isometric model, in both sexes (Chapter 6). Subsequently, a methods development section (Chapter 7) contains information about how non-invasive electrical stimulation of the spinal cord was adapted to facilitate lower limb motoneuron excitability quantification. The final experimental section (Chapter 8) details fatigability during cycling in both sexes using the power-duration relationship. Several additional techniques are employed to shed light on the mechanisms underpinning exercise tolerance in the intensity domains. Finally, the findings of the thesis are discussed collectively (Chapter 9), with practical implications and directions for future research closing this Chapter.

CHAPTER 2 - LITERATURE REVIEW

2.1. Introduction

This chapter will provide a comprehensive synopsis of the literature pertaining to the mechanisms of exercise-induced fatigue in healthy adults, and how these might differ between males and females. Section 2.2 and 2.3 will define fatigue and outline the mechanisms that underpin central and peripheral neuromuscular adjustments to exercise. How these mechanisms of fatigue interact within distinct exercise intensity domains will be covered in Section 2.4. Following this, Section 2.5 will cover differences between males and females within the motor pathway, whilst literature on the topic of sex differences in fatigability will be critically discussed in section 2.6. Finally, the aims and hypotheses of the investigations will be presented in section 2.7.

2.2. Defining fatigue

The study of fatigue has troubled exercise physiologists for centuries. Even today, an unequivocal definition and understanding of the topic does not exist (Marino *et al.*, 2011). The seminal work of Angelo Mosso published in his book *La Fatica* (Mosso, 1891) still underpins much of the present day's understanding. Mosso suggested that fatigue entails two phenomena: *"The first is the diminution of the muscular force. The second is fatigue as a sensation. That is to say, we have a physical fact which can be measured and compared, and a psychic fact which eludes measurement"* (p154, cited in Marino *et al.*, 2011). Typically, research concerned with fatigue in exercise physiology has focused on the measurable aspect of fatigue, applying definitions such as *"a failure to maintain the required or expected force"* (Edwards, 1981), *"a loss of maximal force generating capacity"* (Bigland-Ritchie *et al.*, 1986; Gandevia, 2001), and *"a reversible state of force depression"* (Fitts & Holloszy, 1978). While the experiments that test hypotheses based upon these definitions all contribute to our understanding of the objective aspect of fatigue, they do little to aid the understanding of how these 'force diminutions' contribute to the sensation of fatigue that Mosso eluded to. Only more

recently have exercise scientists considered the effect that the sensations can have on exercise tolerance. St. Clair Gibson *et al.* (2003, p167) suggested that fatigue is an emotion based upon *"a conscious awareness of changes in subconscious homeostatic control systems"*. The authors also suggest that the emotion can be modulated by factors such as motivation, anger and fear. Thus, the same group suggested that exercise tolerance was regulated by a complex, dynamic, non-linear system, later termed the 'central governor model' (St. Clair Gibson & Noakes, 2004; Noakes *et al.*, 2005). Whilst this model has been critiqued and reviewed (Weir *et al.*, 2006; Marcora, 2008), it has created more of an awareness that fatigue is not a simple, linear process involving only objective declines in motor performance; something which the original definition of Mosso eluded to.

A recent review proposed a new taxonomy for fatigue based upon the work of Mosso, but broadened to incorporate a more contemporary understanding (Enoka & Duchateau, 2016). The review stems from a similar classification proposed in clinical populations (Kluger *et al.*, 2013) but adjusted to relate to fatigue experienced by healthy performers of exercise. The authors suggest that fatigue consists of two main components: perceived fatigability and performance fatigability, with interactions between the two resulting in the disabling symptom of fatigue (Enoka & Duchateau, 2016).

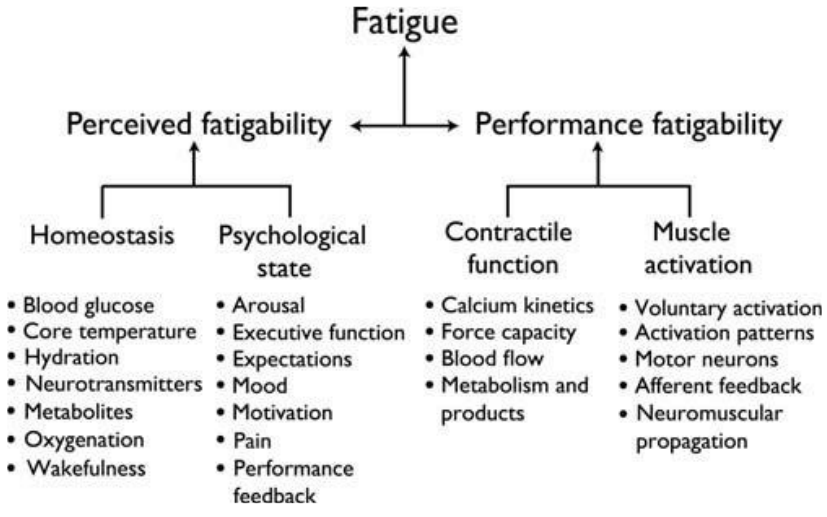

Figure 2-1: The proposed taxonomy from Enoka and Duchateau (2016)

Performance fatigability refers to the classically studied limiting factors of the neuromuscular system during exercise and is defined as *"the decline in an objective measure of performance over a discrete period"*. The mechanisms underpinning performance fatigability are dictated by the task demands, muscle group(s) involved, and characteristics of the performer(s) assessed (Hunter, 2018). Enoka & Duchateau (2016), and consequent authors using this definition (Hunter, 2018), suggest that performance fatigability is affected by contractile function of the muscles involved, as well as the level of activation of the muscles by the central nervous system, terms that have previously been classified as peripheral and central fatigue, respectively (Gandevia, 2001). Perceived fatigability refers to *"changes in the sensations that regulate the integrity of the performer"* and is modulated by homeostatic disturbances and the psychological state of the performer. The definition of perceived fatigability is similar to the description of fatigue by St. Clair Gibson *et al.* (2003), in that the sensation evoked from changes to homeostasis can be modulated by psychological aspects such as motivation or mood, etc. However, the model proposed by Enoka & Duchateau (2016) acknowledges that the two contributing factors (performance and perceived fatigability) are interdependent. One example the authors give is that the capacity of the central nervous system to voluntarily activate the muscle can be modulated by blood glucose (Nybo, 2003), core temperature (Nybo, 2008), arousal (Klass *et al.*, 2012) and mood (Steens *et al.*, 2012), all of which contribute to perceived fatigability, but have physiological consequences and affect performance fatigability as well.

This thesis will utilise the taxonomy proposed by Enoka & Duchateau (2016) to describe fatigue and the mechanisms that contribute to the symptom. However, the notion that the term 'fatigue' cannot be modified by an accompanying adjective (i.e. central fatigue) is not one this thesis will subscribe to. Modifying adjectives are commonly utilised in the literature investigating fatigue to refer to the locus of adjustment(s) within the motor pathway that limit force producing capacity. Performance fatigability during a given task is underpinned by

decreases in the performance of the neuromuscular system (neuromuscular fatigue), and as described by Enoka & Duchateau (2016), involves dysfunction within contractile apparatus (peripheral fatigue, Fitts, 2008) and/or muscle activation by the CNS (central fatigue, Gandevia, 2001). Therefore, for the purpose of clarity, this thesis will utilise modifying adjectives in front of the word fatigue when referring to specific mechanisms that contribute to the symptom of fatigue. The following section (2.3) will outline the contributing factors to fatigue from the beginning of the motor pathway to the resultant contraction of muscles.

2.3. Mechanisms of exercise-induced neuromuscular fatigue

Neuromuscular fatigue refers to suboptimal performance of the neuromuscular system, this manifests as a reduction in maximum force production (Gandevia, 2001). The production of voluntary force is result of multiple complex processes in the motor system (Figure 2-2). The process begins with the planning of movement in supra-cortical structures. Neural drive from the motor cortex travels via the descending tracts and innervates spinal motor neurons, which excite motor axons and lead to the recruitment of motor units. The result of this process is a muscle contraction, and force output. Adjustments in these processes as a result of activity can lead to a sub-optimal motor performance. Changes that occur proximal to the neuromuscular junction (above the red line in Figure 2-2) are termed central fatigue, whereas adjustments below the red line are termed peripheral fatigue.

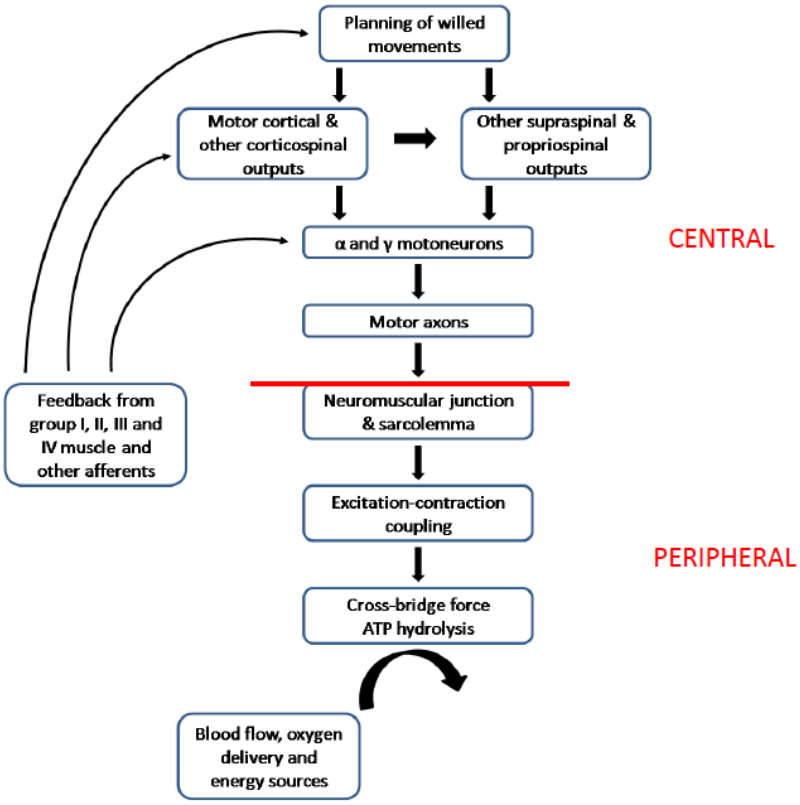

Figure 2-2: The processes involved in voluntary force production. Central mechanisms of fatigue are shown above the red line, whereas peripheral mechanisms are shown below the red line. Adapted from Gandevia (2001) in Goodall (2011).

2.3.1. Peripheral fatigue

Contraction of the muscles involves excitation-contraction coupling resulting in force production via cross bridge cycling (Dulhunty, 2006). Peripheral fatigue refers to an exercise-induced reduction in contractile function, this can be manifest as a reduction in maximal force output of muscle fibres, and can be caused by alterations in any process distal to the neuromuscular junction (Fitts, 2008). As seen in Figure 2-2, E-C coupling has multiple stages, and theoretically a change in any of the stages could lead to suboptimal performance (Sjøgaard, 1996). The following sub-sections will cover the quantification of these potential changes in the excitation-contraction coupling process that lead to peripheral fatigue.

2.3.1.1. Quantifying Peripheral Fatigue

Alterations in the excitation-contraction processes can be quantified using non-invasive neurostimulation, specifically, by stimulating the motor nerve of a muscle at rest (Millet et al. 2011). The amplitude of the mechanical response to nerve stimulation is typically used to assess E-C coupling, with decreases observed following both single-limb (Place *et al.*, 2007; Goodall *et al.*, 2010; Burnley *et al.*, 2012; Gruet *et al.*, 2014; Vernillo *et al.*, 2018) and whole-body (Thomas *et al.*, 2014*a*, 2016; Temesi *et al.*, 2017*b*) exercise commonly interpreted as an impairment distal to the neuromuscular junction.

Stimulation of the motor nerve can be performed in multiple ways; a single stimulus evokes an electromyographical response termed the M-wave, as well as a mechanical response or 'twitch'. This method can only be used for muscles innervated by a motor nerve without simultaneous co-activation of the antagonist muscle(s), for example the knee-extensors (femoral nerve) can be assessed, but not the plantar flexors (tibial nerve). The M-wave is commonly considered to reflect propagation of the action potential across the sarcolemma (Bigland-Ritchie & Woods, 1984; Place *et al.*, 2010), a key step in the excitation and recruitment of motor units. However, this interpretation has been questioned previously (Dimitrova & Dimitrov, 2002; Rodriguez-Falces & Place, 2018). One factor to consider when using single electrical stimuli is the balance between potentiation and fatigue (Millet & Lepers, 2004). The net mechanical output of the twitch, as well as the sensitivity to fatiguing tasks are influenced by the degree of potentiation (Place *et al.*, 2007). Three prior maximum voluntary contractions (MVCs) are thought to be required for maximising potentiation of the twitch (Kufel *et al.*, 2002), and thought to increase calcium ion (Ca^{2+}) sensitivity (Rassier & MacIntosh, 2000), and permit exercise-induced changes to be determined when twitch amplitudes are compared pre-post activity.

Other methods of motor nerve stimulation utilise the force-frequency relationship to infer different characteristics about E-C coupling, with trains of stimuli delivered at different frequencies permitting mechanistic insight into the aetiology of peripheral fatigue (Binder-Macleod & McDermond, 1992). *In vivo* studies investigating human contractile function typically employ a combination of high (80-100 Hz) and low frequency (10-20 Hz) stimulations in the form of tetanic stimuli (Duchateau & Hainaut, 1984) or doublet pulses (Place *et al.*, 2007; Verges *et al.*, 2009). Tetanic stimuli are infrequently used, however, due to its 'brutality' (Millet *et al.*, 2011) when applied to large muscle groups; indeed, a report of a dislocated knee-cap exists in the literature utilising this technique (Bigland-Ritchie *et al.*, 1978). Low frequency stimulation is performed below the fusion frequency, and is therefore purported to reflect either impaired intracellular Ca^{2+} release (Hill *et al.*, 2001; Westerblad *et al.*, 2017) or reduced Ca^{2+} sensitivity of myofibrils (Bruton *et al.*, 2008). High frequency stimulation is performed above the fusion frequency of muscle and is therefore not expected to be altered by Ca^{2+} properties, instead, extracellular potassium (K^{+}) accumulation is implicated in force loss (Jones, 1996). Single stimulations are used as a global measure of peripheral fatigue, and are sensitive to detecting relatively small exercise-induced changes in contractile function (Mador *et al.*, 2000); the limitation is, however, that mechanistic underpinnings of peripheral fatigue are difficult to discern, as the physiological alterations within exercising muscle often occur concurrently. The intricacies of the aforementioned ionic changes are discussed in further detail throughout the subsequent sections.

2.3.1.2. Sodium (Na+) and Potassium (K+) activity

The excitation-contraction coupling process begins with electrical excitement of the muscle fibre. This begins at the neuromuscular junction, in which the terminal branches of motor axons reach the motor unit. Action potentials are transmitted from axon to muscle via synapses, utilising the transmitter acetylcholine to rapidly propagate the action potential along the muscle fibre sarcolemma and throughout the t-tubule system (Pavelka & Roth, 2010). The action

potential travels throughout the t-tubules stimulating Ca^{2+} release, and consequent contraction (Westerblad *et al.*, 1990). Intra and extracellular maintenance of Na^+ and K^+ ions are crucial to maintaining resting membrane potential, and propagation of action potentials occurs via rapid efflux of Na^+, and K^+ to a lesser extent, followed by an influx of K^+ (Allen *et al.*, 2008*a*).

Repeated muscle activation causes net K^+ efflux (Hodgkin & Horowicz, 1959; Clausen & Nielsen, 2007), with resting extracellular concentration thought to be ~4 mM (Overgaard & Nielsen, 2001) and over ~9 mM following repeated activity (Sejersted & Sjøgaard, 2000). This extracellular efflux causes a change in the electrochemical gradient of the sarcolemma that can lead to failures in excitation (Sjøgaard, 2011), and consequently, reduced force production (Nielsen & de Paoli, 2007). Another consequence of repeated muscle activity is a progressive dysfunction of Na^+ channels, leading to reduced excitability due to inhibited Na^+ efflux upon innervation (Ruff *et al.*, 1988; Ruff, 1996; Filatov *et al.*, 2005). Combined, these mechanisms cause a failure of action potential propagation throughout the sarcolemma and t-tubule system, leading to reduced force production (Chua & Dulhunty, 1988). It should be noted however, that this is not a linear process with impaired force production as an inevitable consequence of activity. Na^+-K^+ pumps act to restore the electrochemical gradients of Na^+ and K^+ (Clausen & Nielsen, 2007). Nevertheless, excitability is ultimately determined by the net result of the aforementioned passive ion leaks and Na^+-K^+ pump activity, with passive leaks often playing a dominant role during repeated activity (Clausen, 2003).

2.3.1.3. Calcium (Ca2+) handling and Inorganic Phosphate (Pi) interactions

In human skeletal muscle fibres, Ca^{2+} is stored within the sarcoplasmic reticulum (SR), and released for use in cross bridge cycling (Huxley & Niedergerke, 1954) upon stimulation by an action potential. In 'fast-twitch' fibres, Ca^{2+} release per action potential is ~3 times greater than 'slow-twitch' fibres (Baylor & Hollingworth, 2003). As an action potential is detected by voltage-

sensitive molecules in the t-tubules (dihydropyridine receptors, Araya *et al.*, 2002), and ryanodine receptor-Ca^{2+} channels (RyR1) located in the SR are opened, causing an efflux of Ca^{2+}. This causes an elevation of myoplasmic Ca^{2+} concentration, leading to Ca^{2+} binding with Troponin C, and initiating muscle contraction via cross bridges. The reuptake of Ca^{2+} into the SR via the SR-Ca^{2+} pump then causes muscle relaxation (Allen *et al.*, 2008*b*).

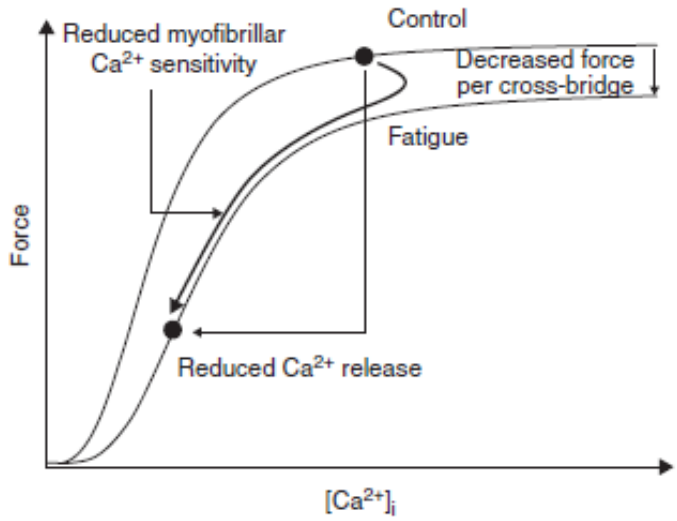

Figure 2-3: The relationship between force-Ca^{2+} release during fatigue. In a fatigued state, Ca^{2+} sensitivity is decreased, meaning less force is produced for the same magnitude of Ca^{2+} release. Ca^{2+} release from the sarcoplasmic reticulum is also impaired. Collectively these adjustments result in less force per cross bridge. Taken from Cheng et al (2017).

In a fatigued state, there is a reduced free Ca^{2+} concentration, leading to less Ca^{2+} binding with troponin C, reduced cross bridge formation, and attenuated force output (Allen *et al.*, 1995; Macintosh & Rassier, 2002; Cheng *et al.*, 2017). During repeated stimulation, Ca^{2+} stores within the SR are depleted, meaning that the amount released per action potential is diminished, reducing the force response (Posterino *et al.*, 2000). Muscle fibres *in vitro* have shown that a ~35% decrease in SR Ca^{2+} content resulted in a 46% decrease in tetanic force (Dutka *et al.*, 2005).

Increased ATP hydrolysis during high intensity activity results in a net decrease in ATP concentrations due to the inability of energy systems to synthesise ATP at an equal rate. This decrease in ATP limits the activity of RyR1 as adenosine monophosphate (AMP) and adenosine diphosphate (ADP, the resultants of ADP and ATP hydrolysis, respectively) are strong inhibitors (Dutka & Lamb, 2004). This inhibition of RyR1 channels leads to reduced Ca^{2+} release per action potential, and consequently, attenuated force production. Another way in which Ca^{2+} release is impaired is when Ca^{2+} within the SR forms a precipitate with a by-product of ATP hydrolysis - inorganic phosphate (Pi), to form calcium phosphate (B in Figure 2-, Ca^{2+}-Pi), reducing the amount of available Ca^{2+} for release (Allen *et al.*, 2008*a*). Inorganic phosphate may also impair force producing capacity through inhibition of the ryanodine receptor-Ca^{2+} channels (A in Figure 2-4), further reducing the amount of Ca^{2+} release per action potential (Allen *et al.*, 2008*b*) . Additionally, Pi has also been reported to alter cross-bridge function directly via the myosin-actin binding sites by reducing the myofibrillar sensitivity to Ca^{2+} (Fitts, 1994). Finally, reactive oxygen and nitrogen species (ROS/RNS) have been suggested to contribute to prolonged low-frequency force depression by RyR1 channel fragmentation (Place *et al.*, 2015), impairing Ca^{2+} release. It has also been suggested that the accumulation ROS cause reduced myofibrillar Ca^{2+} sensitivity, attenuating force production by impairing cross bridge cycling (Cheng *et al.*, 2016). These deleterious processes occur concurrently during high-intensity exercise, inducing reductions in the force production of the muscle (peripheral fatigue).

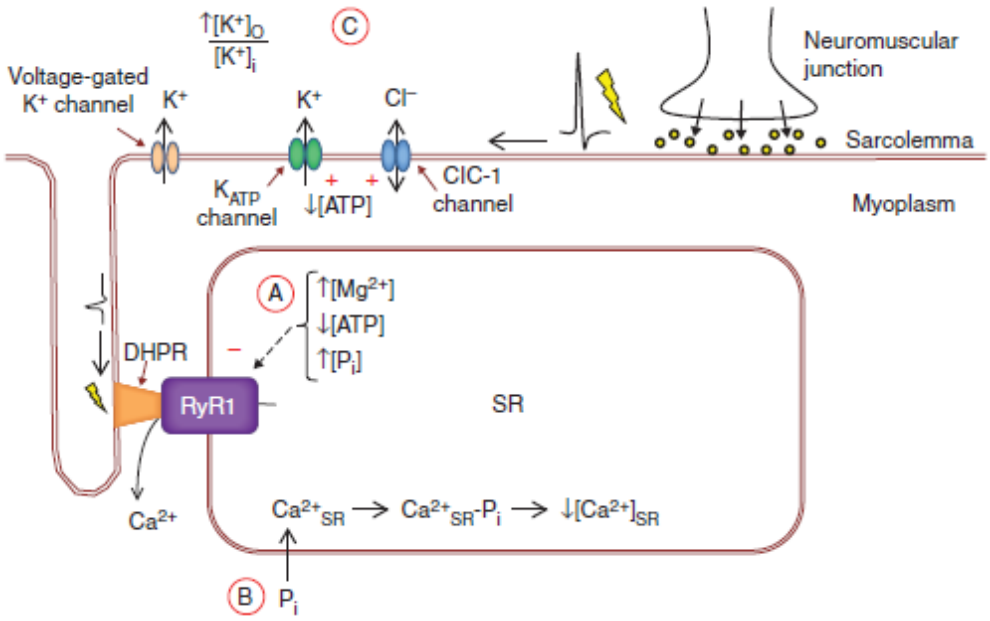

Figure 2-4: Cellular mechanisms of decreased sarcoplasmic reticulum Ca^{2+} release. (A) Inhibition of rynaodine receptor 1 (RyR1) channel opening. (B) Reduction in 'releasable' Ca^{2+} concentration ($[Ca^{2+}]_{SR}$) via Ca^{2+}-Pi precipitation. (C) Impaired action potential propagation via extracellular K^+ accumulation. Taken from Cheng et al (2017).

2.3.1.4. Acidosis

The notion that lactic acid and the associated acidity of the muscle is a cause of fatigue during exercise stems from studies published in the early 20th century. Such studies observed elevated lactic acid concentration in skeletal muscles at exhaustion, and that the reversal of this accumulation would restore muscle function (Fletcher & Hopkins, 1907; Hill *et al.*, 1924). During high intensity activity, the accumulation of H^+ causes intracellular pH to decrease by ~0.5 pH units, and correlational evidence would suggest that the temporal changes in pH mimic the reduction in force output of human muscle (Troup *et al.*, 1986; Spriet *et al.*, 1987; Cady *et al.*, 1989). As ATP is hydrolysed, H^+, that is utilised for oxidative phosphorylation in the mitochondria in the presence of oxygen, is not consumed during anaerobic processes, and can accumulate causing acidosis (Robergs *et al.*, 2004). Indeed, low pH has been shown to reduce peak power of skeletal muscle fibres *in vitro* by ~18% (Knuth *et al.*, 2006), and in combination with elevated Pi (a state mimicking exercise-induced fatigue) peak power was reduced by ~70% (Karatzaferi *et al.*, 2008). Mechanisms purported to explain this effect are

that H^+ compete with Ca^{2+} on Troponin C, lowering the sensitivity of contractile apparatus and reducing force output (Bolitho Donaldson *et al.*, 1978; Fabiato & Fabiato, 1978). Additionally, it has been suggested that low pH also inhibits activity of muscle fibre ATPases (Fitts, 2016), meaning that the ATP cost of a given force output is elevated. However, the deleterious effects of acidosis on skeletal muscle performance are equivocal, with debate on the topic still a contemporary issue (Fitts, 2016; Westerblad, 2016). Conflicting evidence has been shown regarding the effects of acidosis on Ca^{2+} sensitivity, with ergogenic effects of acidosis being reported (Westerblad & Allen, 1993; Pedersen *et al.*, 2004). The lack of association between H^+ and impaired muscle performance is reinforced by the observation that recovery of muscle force is far more rapid than the recovery of pH (Sahlin & Ren, 1989). Whilst the detrimental effect of acidosis to the excitation-contraction coupling process is disputed, it might also potentially contribute to neuromuscular fatigue via stimulation of group III/IV afferent neurons, which have implications in the development of fatigue and exercise tolerance (Amann, 2011).

2.3.2. Central Fatigue

Processes within the CNS can reduce the capacity of the organism to activate skeletal muscle, a phenomenon commonly termed central fatigue (Gandevia, 2001). As Gandevia (2001, p1726) suggested, *"if muscle is regarded as a motor, then the way it behaves depends not only on its intrinsic properties but also on the way that it is driven and the way feedback systems maintain its output"*. The aetiology of these CNS impairments is dependent on the task(s) performed, but can be defined as a reduction in the voluntary activation of the muscle.

2.3.2.1. Voluntary Activation

Voluntary activation (VA) is typically quantified using the interpolated twitch technique (ITT) developed by Merton (1954). This involves the delivery of a stimulation during a maximal voluntary contraction, and is commonly performed by stimulating the nerve that innervates a

target muscle group. If extra force is elicited by the stimulation (a superimposed twitch, SIT), VA is considered sub-maximal, and some motor units were not recruited or firing at a high enough rate to produce tetanic contraction (Taylor & Gandevia, 2008). To quantify VA, the SIT is normalised to the response to the same stimulus delivered in the potentiated, relaxed muscle (potentiated twitch, $Q_{tw,pot}$) using the formula shown in the equation below.

$$\mathbf{VA = (1 - (SIT / POT)) \times 100}$$

Whilst the ITT is subject to methodological (Folland & Williams, 2007) and conceptual (Contessa *et al.*, 2016) debate, it is generally accepted as the most useful tool for assessing VA (Taylor, 2009). Pre-exercise, in the resting knee-extensors, typical values range between 90-95% (Place *et al.*, 2007). The ITT is considered to be a more accurate index of VA compared to the central activation ratio due to the effect that synergist co-contraction has on the CAR value, but not the ITT (Stackhouse *et al.*, 2000). This leads to the CAR overestimating VA (Place *et al.*, 2007) and being less sensitive to exercise-induced change when compared to the ITT (Bilodeau, 2006). Exercise-induced central fatigue is typically characterised by a decrease in VA (Bigland-Ritchie *et al.*, 1978; Thomas *et al.*, 1989), implying further decrements in the ability of the central nervous system to recruit and maximally fire the available motor units (Gandevia *et al.*, 1995). One limitation of motor nerve-assessed ITT assessment is that it does not provide information about the locus or aetiology of central fatigue, therefore to discern information regarding the aetiology of central fatigue, further methods must be employed.

2.3.2.2. TMS-assessed Voluntary Activation

Advances in the use of TMS in assessing exercise-induced change within the nervous system has permitted the assessment of VA from a different perspective. Stimulation of the primary motor cortex produces a motor response in the contralateral limb, first demonstrated using non-invasive electrical stimulation through the scalp (Merton & Morton, 1980), and later using electromagnetic stimulation (Barker *et al.*, 1985). The latter method enabled investigation of the motor cortex without accompanying pain, and has drastically changed the state of human research, being widely adopted throughout neurophysiological investigations (Rothwell *et al.*, 1991).

The mechanism in which TMS activates the motor cortex is electromagnetic conduction. An electrical current is passed through wire coils placed over the target muscle representation on the motor cortex, causing a rapidly-changing magnetic field along the winding of the coils (Terao & Ugawa, 2002). This magnetic current reaches a depth of 2-4 cm depending on the formation of the coil (Rudiak & Marg, 1994; Deng *et al.*, 2013), with a trade-off between focality and depth apparent. As mentioned, the activation of cortical muscle representations elicits a motor response, or 'twitch', in the target muscle if a sufficient stimulus intensity is used, and when delivered during a maximum contraction a SIT is observed (Gandevia *et al.*, 1996).

A modified interpolated twitch technique is necessary for calculation of TMS-assessed VA (VA_{TMS}) as normalisation of the SIT to a resting twitch is not appropriate. As corticospinal and motoneuronal excitability is much lower at rest, TMS is unable to evoke an adequate twitch (Ugawa *et al.*, 1995*a*; Di Lazzaro *et al.*, 1998). This issue was resolved by Todd *et al.* (2003), who estimated the resting twitch via linear regression of SITs evoked at 50-100% of maximum, with the y-intercept of the regression used as the estimated resting twitch (ERT). First utilised in the elbow flexors (Todd *et al.*, 2003, 2004), the method was subsequently validated and

refined in other muscle groups such as the knee extensors (Goodall *et al.*, 2009; Sidhu *et al.*, 2009; Dekerle *et al.*, 2019)

As the SIT is evoked cortically in this context, it could imply submaximal output at the motor cortical level. Indeed, it has been suggested that an exercise-induced increase in SIT amplitude is caused by impairments within or upstream of the motor cortex (Gandevia *et al.*, 1996; Todd *et al.*, 2004). However, as more recently acknowledged, an increased SIT could be caused by submaximal output at the motor cortical or motoneuronal level (Todd *et al.*, 2016). Regardless, the use of VA_{TMS} in addition to the 'traditional' ITT permits further detail about the aetiology of central fatigue, by stimulating prior to the corticospinal-motoneuronal synapse rather than the projecting motor nerve. Specific detail about the methodology can be found in Chapter 3, section 3.3.2.

2.3.2.3. Corticospinal Excitability

If TMS is delivered at a sufficient intensity, the axons of pyramidal neurons and cortical interneurons are depolarised in the area beneath the stimulation (Salvador *et al.*, 2011). As a result, a single TMS pulse evokes a combination of both direct (D waves) and indirect (I waves) activation of pyramidal neurons (Di Lazzaro & Rothwell, 2014). This descending activation travels primarily via the corticospinal pathway, with implanted electrodes in the epidural space confirming this assumption (Di Lazzaro *et al.*, 2012; Di Lazzaro & Rothwell, 2014). Using non-invasive surface electromyography (EMG), the response to TMS (motor evoked potential, MEP) can be recorded on the targeted muscle(s). Whilst the MEP is not analogous to descending pathway activation via volitional motor command (Bestmann & Krakauer, 2015), it is commonly used as a quantitative marker for state, time, and task-dependent changes in the excitability of the corticospinal tract (Rothwell *et al.*, 1991; Kalmar, 2018*a*). The MEP is frequently normalised to the maximum compound muscle action potential (M_{max}), to account

for changes in peripheral neurotransmission, and is considered to reflect a net balance between faciliatory and inhibitory inputs at both cortical and spinal levels (Gruet *et al.*, 2013). The link between excitability and exercise relates to the notion that an alteration in excitability changes the amount of synaptic input (i.e. neural drive/motor command) required to maintain a given task (Weavil & Amann, 2018). If an increase in neural drive is insufficient to overcome decreased corticospinal excitability, the ability of the CNS to activate the muscle will decrease (Martin, 2006).

2.3.2.4. Spinal Excitability

Distinguishing the cortical and spinal contributions to central fatigue is challenging as the organisation of spinal interneuron networks is not fully understood in humans (Nielsen, 2004). Excitability of the spinal portion of the corticospinal tract can be isolated however, with direct subcortical stimulation, and can permit segmental assessment of the motor pathway when used concurrently with TMS (Taylor & Gandevia, 2004). This technique was initially performed at the cervicomedullary junction, with a cervicomedullary evoked potential (CMEP) evoked (Taylor *et al.*, 2002). Similar to a MEP, the size of a CMEP is dependent on the intensity of background contraction, an indication that it is evoked trans-synaptically (i.e. not direct activation of the motoneuron), via depolarization of the spinal tracts (Taylor *et al.*, 2002). It is likely not possible to solely activate the corticospinal tract with direct spinal stimulation (McNeil *et al.*, 2013), however evidence from studies investigating antidromic collisions from cortical and spinal stimulation suggest that CMEPs and similar stimulation at the thoracic spinal level (TMEPs) consist of a large corticospinal component (Taylor *et al.*, 2002; Martin *et al.*, 2008). Other methods exist to investigate the excitability of spinal structures, such as the H-reflex (Hoffman, 1918), F-wave (Eccles & Pritchard, 1937), and V-wave (Upton *et al.*, 1971), with advantages and disadvantages to each one (McNeil *et al.*, 2013). The responses to direct stimulation of the descending tract (i.e. CMEPs/TMEPs) are considered the most direct

assessment of spinal excitability, due to the fact that they are not affected by pre-synaptic inhibition (Jackson *et al.*, 2006).

2.3.2.5. Neural Inhibition

In addition to the excitability of neural structures, TMS can be used to quantify the effects of inhibitory influences within the nervous system. When single-pulse TMS is delivered during a contraction, both excitatory (MEP), and an inhibitory responses are evoked. The inhibitory response manifests as a period of silence in the EMG signal following the MEP (Fuhr *et al.*, 1991; Uozumi *et al.*, 1991; Wilson *et al.*, 1993). Historically, the silent period was thought to reflect intracortical mechanisms of inhibition (Schnitzler & Benecke, 1994), however an abundance of evidence exists suggesting there are both spinal and cortical components (Fuhr *et al.*, 1991; Ziemann *et al.*, 1993). This notion was based on depression of H-reflexes evoked within the silent period, which as mentioned, is affected by pre-synaptic factors; however a recent investigation utilising CMEPs demonstrated the silent period has a longer spinal component than previously thought (Yacyshyn *et al.*, 2016). A critical discussion of the underpinning mechanisms and confounding factors has been recently published (Škarabot *et al.*, 2019), however the authors concluded that methodological confounds involved in data collection, as well as the lack of evidence for purported physiological inference could limit the comparability between studies and usefulness of the measure.

Paired-pulse TMS can provide a reflection of the properties of the diverse populations of intracortical neurons that are implicated in the control of voluntary movement (Kujirai *et al.*, 1993). These intracortical neurons can be classified as either inhibitory of facilitatory based on their electrophysiological properties and how they affect post-synaptic neurons. Inhibitory neurons release neurotransmitters that increase the permeability of the post-synaptic membrane to K^+ and Cl^- ions, increasing the cell's resting membrane potential. This has the

effect of hyperpolarising the post-synaptic neuron, and making it less likely to reach the action potential threshold in response to an excitatory input (Rosenthal *et al.*, 1967). In the motor cortex, the most abundant inhibitory neurotransmitter is γ-aminobutyric acid (GABA; Jones, 1993), with $GABA_A$ and $GABA_B$ receptor sub-types being the most commonly observed in the mammalian central nervous system (Watanabe *et al.*, 2002). On the contrary, facilitatory intracortical neurons induce changes in post-synaptic membrane permeability so that Na^+ can diffuse into the neuron. This moves the resting membrane potential closer to the action potential threshold, and increases the chance of that neuron firing in response to excitatory input.

Using paired-pulse TMS, the effects of intracortical inhibitory neurons can be quantified when a sub-threshold conditioning stimulation is delivered 1-5 ms prior to a suprathreshold test stimulation, a phenomenon termed short-interval intracortical inhibition (SICI; Kujirai *et al.*, 1993). The neurotransmitter thought to mediate this inhibition is GABA, with the $GABA_A$ receptor responsible (Kujirai *et al.*, 1993). The result is a reduced MEP amplitude in response to the test stimulation, when the conditioning stimulus is delivered at appropriate timings. Similarly, the influence of facilitatory intracortical neurons can be assessed with a subthreshold stimulus 6-20 ms prior to a test stimulation, a technique termed intracortical facilitation (ICF; Ziemann *et al.*, 1996). The underpinning physiology of ICF is less clear, however, it has been suggested that the MEP facilitation could be mediated by glutamate-mediated N-methyl-D-aspartate excitatory interneurons (Liepert *et al.*, 1997; Nakamura *et al.*, 1997). Paired-pulse TMS has previously been used to quantify the changes in these neuronal populations following fatiguing isometric (Maruyama *et al.*, 2006; Hunter *et al.*, 2016; Goodall *et al.*, 2018) and locomotor exercise (Tergau *et al.*, 2000). Additionally, these techniques have recently been optimised in the knee-extensors, potentially facilitating research in a pertinent locomotor muscle (Brownstein *et al.*, 2018*b*).

2.3.2.6. Mechanisms of Central Fatigue

The aetiology of exercise-induced central fatigue can be multi-factorial, with alterations in processing from the supraspinal to the motoneuronal levels of the motor pathway. The aetiology of central fatigue is task-specific, and can manifest as several of the subsequently described phenomena (Todd *et al.*, 2016).

2.3.2.7. Neurobiological Mechanisms

Neurotransmitters modulate the communication between neurons and broadly speaking, can be inhibitory or excitatory in nature. *In vivo* studies in human populations are difficult to draw conclusions from due to the complexity of brain functioning, however, in 1987, Newsholme *et al* proposed a link between the neurotransmitter 5-hydroxitryptamine (serotonin) and central fatigue. It was suggested that exercise-induced increases in the concentration of this neurochemical led to negative effects on arousal, lethargy, sleepiness, and mood (Newsholme *et al.*, 1987). The experimental literature regarding the influence of serotonin and fatigue is mixed (see review Roelands & Meeusen, 2010), likely due to the complex interactions between different receptors and subsequent functions. Recent evidence does however suggest that serotonin might influence CNS adjustments at the spinal level, Kavanagh *et al* (2019) demonstrated that paroxetine administration (a selective serotonin reuptake inhibitor) improved VA in an unfatigued state. However, the drug also induced a greater degree of motoneuronal excitability depression and exercise-induced VA decrease during fatiguing exercise. The authors suggested the latter observation was due to increased extracellular concentrations of serotonin activating inhibitory 5-HT_{1A} receptors, resulting in reduced motoneuronal activity. Indeed, *in vitro* work performed in the spinal cord of the adult turtle would support this notion (Perrier & Cotel, 2008; Cotel *et al.*, 2013). The available evidence suggests that rather than the original notions that serotonin augmented central fatigue at

supraspinal sites (Newsholme *et al.*, 1987; Meeusen *et al.*, 2006), this neurotransmitter plays a key role in the regulation of spinal motoneuron excitability during exercise.

Other neurobiological mechanisms of central fatigue that have been investigated include alterations in the concentrations of catecholamines such as noradrenaline and dopamine (Roelands & Meeusen, 2010). Ingestion of a noradrenaline reuptake inhibitor has been demonstrated to worsen endurance performance (Piacentini *et al.*, 2002) and in terms of neurophysiological responses to exercise, the pharmacological manipulation induces a greater decrease in VA following prolonged cycling exercise (Klass *et al.*, 2012, 2016). Conversely, dopamine manipulation did not alter endurance performance in normal ambient temperatures, only elevated (30°C; Roelands *et al.*, 2008). Furthermore, Swart *et al.* (2009) demonstrated that dopamine reuptake inhibitors enabled participants to work at higher work rates, and experience greater cardiorespiratory stress for longer, with identical perceived 'exercise stress' compared to a placebo trial. Despite this, Klass *et al.* (2012) demonstrated no effect on cycling time trial performance or indices of corticospinal excitability and VA. In summary, it appears that changes in neurochemistry are implicated in the regulation of central fatigue during exercise, with serotonin likely regulating properties of spinal motoneurons. However, the supraspinal effects of changing neurochemical concentrations are challenging to discern in humans due to the complex interactions between neurotransmitter systems (Meeusen & Roelands, 2018).

2.3.2.8. Neural Drive and Excitability

During sustained maximal contractions, the amplitude of voluntary EMG recorded at the muscle is at its greatest, however, progressively decreases as the task progresses due to a reduction in the responsiveness of motoneurons (see Section 2.3.2.9), decreased excitatory afferent input from Ia neurons, and decreased descending drive due to supraspinal fatigue

(Todd *et al.*, 2005; Taylor & Gandevia, 2008). Indeed, multiple studies have demonstrated changes in the excitability of cortical and motoneuronal neurons during two-minute MVCs (Taylor *et al.*, 1996; Kennedy *et al.*, 2016), which have been thought to account for ~25% of the force decreased (Gandevia *et al.*, 1996). However, during submaximal sustained contractions, EMG amplitude increases as descending drive increases (Hunter & Enoka, 2001; Søgaard *et al.*, 2006; Klass *et al.*, 2008). This is thought to be a compensatory mechanism for contractile dysfunction in the working muscles, and is commonly observed alongside an initial increase in corticospinal excitability (Lévénez *et al.*, 2007; Klass *et al.*, 2008). This initial increase in corticospinal excitability with fatiguing exercise appears to be transient, as studies investigating the pre-post exercise response commonly demonstrate reduced or unchanged corticospinal excitability at task-failure (Sidhu *et al.*, 2009, 2017; Klass *et al.*, 2012; Girard *et al.*, 2013; Goodall *et al.*, 2014*b*; Jubeau *et al.*, 2014; Thomas *et al.*, 2016). Although several studies report an increase (Fernandez-del-Olmo *et al.*, 2013; Temesi *et al.*, 2013, 2014*b*; Jubeau *et al.*, 2017) or a decrease (Sidhu *et al.*, 2014; Thomas *et al.*, 2014*a*; Pearcey *et al.*, 2015). The task-specificity of the exercise-induced change in MEP amplitude likely explain the discrepancy between results, as well as differences in the methodology of testing (Weavil & Amann, 2018). Despite the discrepancies in MEP changes following exercise, the literature investigating CMEP changes is more conclusive. Studies in both upper (Pearcey *et al.*, 2015) and lower limbs (Weavil *et al.*, 2016; Sidhu *et al.*, 2017; Finn *et al.*, 2018) have demonstrated that even in the presence of an unchanged MEP, CMEPs or TMEPs decrease with exercise-induced fatigue.

In short, exercise-induced changes in the excitability of the corticospinal tract appear to be dependent on the exercise task performed. Changes during single-limb exercise being almost completely related to sensory feedback from the exercising muscle or activity-dependent changes in the motoneurons (see Sections 2.3.2.9 and 2.3.2.10), whereas, in whole-body exercise, the influence of autonomic activity and sensory feedback from cardiovascular

systems has been shown to modulate the corticospinal pathway (Buharin *et al.*, 2013). Therefore, the generalisability of single-limb findings to whole-body exercise is limited.

2.3.2.9. Motoneuronal Properties and Fatigue

The decrease in spinal excitability with exercise-induced fatigue is one line of evidence that motoneuronal properties are modulated by exercise. Other factors include activity-dependent changes with constant input, which manifest as a decrease in firing rate (Kernell & Monster, 1982; Miles *et al.*, 2005; Brownstone, 2006). Furthermore, through motor unit recordings during submaximal contractions, it is evident that the number of active motor units increases, implying that to maintain the same output (force), the motoneuron requires greater synaptic input (Johnson & Heckman, 2014). Whilst increases in descending drive can compensate for some of the impairments in motoneuron excitability (Finn *et al.*, 2018), it would appear that the intrinsic properties of low-threshold motoneurons are more negatively affected by exercise-induced fatigue (McNeil *et al.*, 2011; Finn *et al.*, 2018). This led to the conclusion that the intrinsic properties of low-threshold motoneurons are more negatively impaired as they are recruited prior to high-threshold counterparts and are more active during fatiguing contractions (Carpentier *et al.*, 2001). Therefore, if repetitively active motoneurons are less excitable than less active or inactive neurons, it implies that it is the repeated activity inducing the change, as altered descending or afferent input would modulate motoneurons regardless of threshold (Todd *et al.*, 2016).

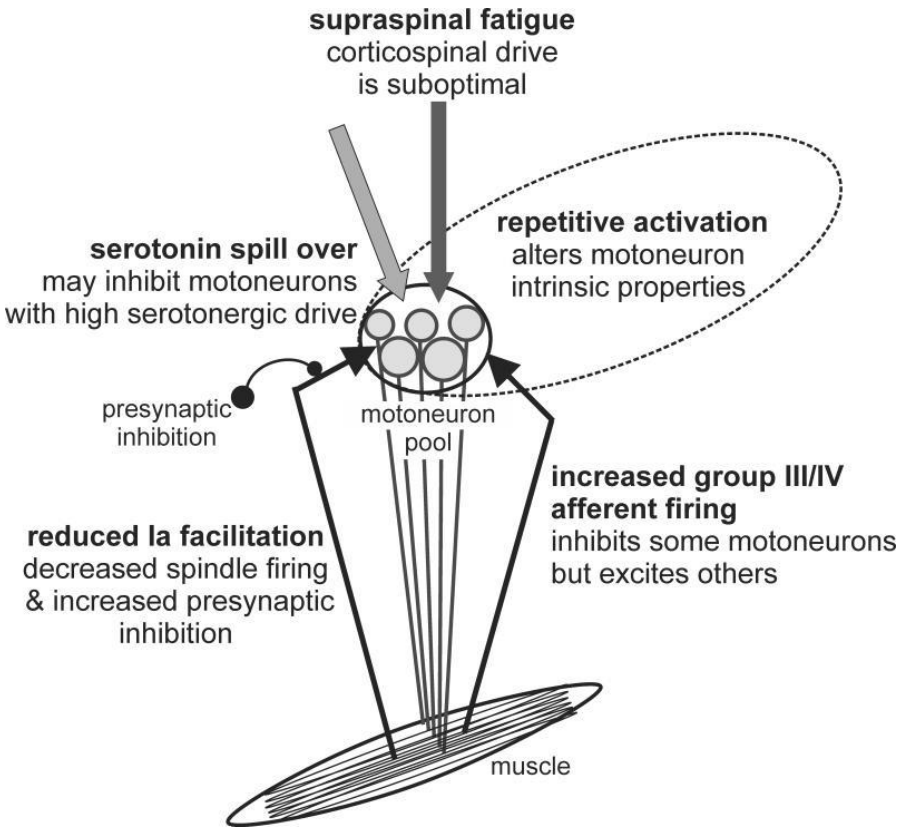

Figure 2-5: Contributing factors to the reduction in motoneuron firing rate during fatiguing exercise. Taken from Taylor et al*. (2016).*

The change in excitability of a motoneuron likely reflects a combination of changes in the intrinsic properties of the motoneuron, as well as the modulation of synaptic input to the motoneuron (see Figure 2-5). The firing properties of a motoneuron depends on the integration of excitatory input (i.e. descending drive and Ia afferents) as well as inhibitory influences (group Ib, III, IV afferents and Renshaw cell input; Enoka 2012). Whilst the role of golgi tendon organ (Ib) feedback and Renshaw cells are reduced with fatigue (Gandevia, 2001), group III/IV afferent input is increased (Amann & Light, 2015). The aetiology of the inhibitory effects of group III/IV afferents is multifactorial and widespread throughout the nervous system, as discussed in the subsequent section.

2.3.2.10. Group III/IV Afferent Feedback

Group III/IV afferent neurons are activated in response to mechanical and metabolic stimuli (Kaufman *et al.*, 1987; Pollak *et al.*, 2014) and the role of this afferent feedback is purported to play a large role in the development of central fatigue (Gandevia *et al.*, 1996; Gandevia, 2001; Kennedy *et al.*, 2014). Studies employing transient pharmacological blockades of these neurons provide evidence that the activation of these neurons increases intracortical inhibition (Hilty *et al.*, 2011; Sidhu *et al.*, 2018), and reduces cortical and spinal excitability (Sidhu *et al.*, 2017). These factors impair the synaptic input to the motoneuron as well as directly affecting motoneuron transmission of motor commands, and amalgamate in an impaired ability to activate the muscle (Sidhu *et al.*, 2017). Indeed group III/IV neurons have been demonstrated to project to spinal and supraspinal sites within the motor pathway (Light *et al.*, 2008; Amann & Light, 2015), and while the firing properties of these neuronal populations can only be directly assessed using invasive techniques in humans, this link between peripheral and central fatigue is thought to be vital in the determination of exercise tolerance (Amann, 2011). Gandevia (2001) first proposed that afferent feedback contributed to a sensory limit to exercise, at which point a fatiguing task became 'sufficiently unattractive' to continue. Subsequently, (Amann & Dempsey, 2008) proposed that afferent feedback was a mechanism in which the central nervous system limited the exercise performer to a 'critical threshold' of peripheral disturbance, beyond which the extremely large sensory input would not be tolerated. This theory was recently expanded upon, with the suggestion that afferent feedback from other physiological systems (e.g. respiratory, cardiac systems) as well as corollary discharge from the motor cortex is integrated into a feed-forward mechanism that would influence the exercise performer (Hureau *et al.*, 2018). These neurons are also vital for the regulation of autonomic control during exercise, reflexively increasing cardiac output, mean arterial pressure, and ventilation (Amann, 2011; Amann *et al.*, 2011), enabling the exerciser to maintain adequate haemodynamic function to perform the given task. Thus, group III/IV feedback cannot be classified as solely negative to the exercise performer, and the intensity

of exercise likely mediates the net balance of positive to negative consequences of sensory feedback. One counter-argument to this model suggests that perception of effort is the predominant limiting factor to high-intensity exercise performance, and that the neurophysiological origins of this sensation are not related to group III/IV feedback (Marcora, 2010). Instead, corollary discharge from motor to sensory areas of the brain is responsible, and as greater central motor drive is required to maintain the required exercise task, an equal increase in corollary discharge occurs. In constant-load exercise, this increases until maximum effort is attained, whereas during self-paced exercise, power output can be reduced, to allow full completion of a given time/distance. Critical discussion of the two conflicting theories has been previously offered (Perrey *et al.,* 2010), with researchers highlighting a multitude of opinions and alternate explanations.

2.4. Fatigue throughout the Exercise Intensity Domains

Exercise intensity can be categorised into four distinct ‘domains’, and the physiological responses to each domain elicit different profiles of neuromuscular fatigue (Burnley & Jones, 2018). These differing aetiologies of fatigue all eventually elicit task failure during constant-load exercise via different means, and these can broadly be predicted by the intensity-duration relationship. During ramp exercise, the boundaries between intensity domains can begin to be established using physiological and performance markers to demarcate the four domains. Exercise tolerance in the severe intensity domain in particular becomes predictable if profiled correctly (see Section 2.4.3.1).

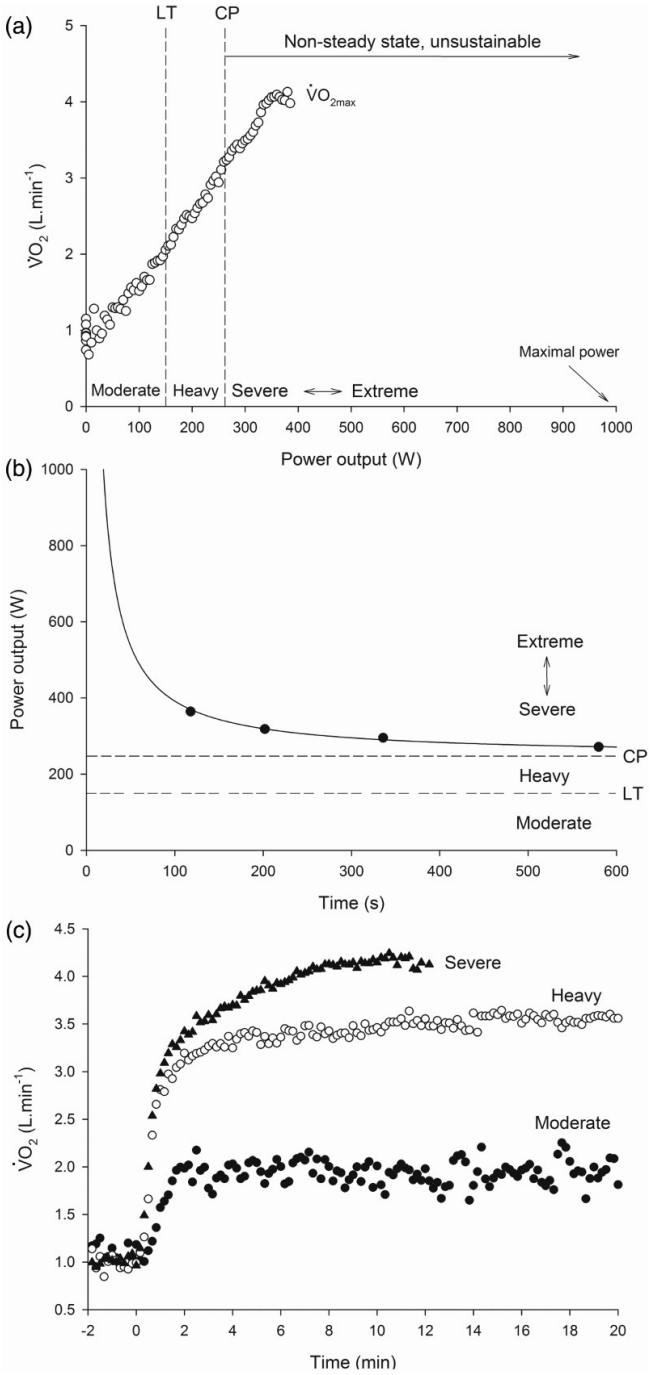

Figure 2-6: Visualisations of the exercise intensity domains. A: the oxygen uptake response to incremental exercise. B: the powe-duration relationship during cycling exercise. C: the $\dot{V}O_2$ response to constant load cycling in each intensity domain. Taken from Burnley and Jones (2018).

2.4.1. Moderate Intensity Domain

The lactate threshold is the initial physiological intensity border, beneath which, exercise is termed ‘moderate’ intensity. During constant-load trials in the moderate domain, oxygen

consumption ($\dot{V}O_2$) rises to match the demands of work, then remains constant without further increase (see Figure 2-6, Burnley & Jones, 2018). Exercise in this domain is typically observed during ultra-endurance events, as this intensity can be sustained for hours without any progressive perturbation within the exercising musculature (Black *et al.*, 2016). Rather, depletion of muscle glycogen, and subsequent impairment of calcium release, is a contributing factor to peripheral fatigue (Chin & Allen, 1997; Ørtenblad *et al.*, 2013). The predominant cause of neuromuscular fatigue in this domain however, is central fatigue; following both moderate intensity running (Martin *et al.*, 2010) and cycling (Lepers *et al.*, 2002), decreases in VA have been observed. The cause of this central fatigue is likely to be activity-dependent changes in motoneuron properties (Section 2.3.2.9), as group III/IV afferent feedback is minimal due to negligible metabolite accumulation (Black *et al.*, 2016). As acknowledged by Burnley & Jones (2018), moderate intensity exercise is commonly terminated prior to task failure, therefore it is unclear whether neuromuscular fatigue limits performance of these tasks.

2.4.2. Heavy Intensity Domain

Above the lactate threshold, exercise intensity is deemed 'heavy' and the time to task failure becomes shorter, typically ~40 minutes to 3 hours (Coyle *et al.*, 1986). During heavy intensity exercise, $\dot{V}O_2$ initially rises to match the energy demands of the task, however, a subsequent smaller rise is then observed, termed the $\dot{V}O_2$ slow component (Figure 2-6, Burnley & Jones, 2018). This slow component eventually reaches a steady-state in the heavy intensity domain, and is presumed to be a result of additional type II muscle fibre recruitment, possibly as a compensatory response to contractile dysfunction, thus increasing the muscle $\dot{V}O_2$ as a result (Poole *et al.*, 1991; Whipp, 1994). Indeed, more recent evidence would suggest that exercise in the heavy intensity domain results in metabolic perturbation within the exercising muscle, however this reaches a steady-state and is therefore unlikely a limiting factor to heavy intensity exercise (Jones *et al.*, 2008; Vanhatalo *et al.*, 2010; Black *et al.*, 2016).

When neuromuscular responses are compared pre-post exercise in this domain, both central and peripheral fatigue are observed (Thomas *et al.*, 2016). It is suggested that peripheral fatigue develops in the early phases of heavy intensity exercise because of the aforementioned metabolic perturbation (Burnley & Jones, 2018). Later in heavy intensity exercise, this peripheral fatigue is potentially exacerbated due to glycogen depletion, with 33-49% decreases observed following 30 minutes of cycling at 85% of $\dot{V}O_{2max}$ (Ahlquist *et al.*, 1992). This glycogen depletion could impair excitation-contraction coupling (Chin & Allen, 1997; Ørtenblad *et al.*, 2013), leading to a reduction in the evoked amplitude of resting muscle twitches (Thomas *et al.*, 2016).

Central fatigue during heavy intensity exercise is unlikely a result of exaggerated group III/IV feedback, as the metabolic disturbance in the exercising muscle reaches a steady state (Jones *et al.*, 2008; Vanhatalo *et al.*, 2016), therefore is unlikely to increase the activity of metabo-sensitive afferents. More likely, the extended duration of exercise leads to activity-dependent changes in the properties of motoneurons (see Section 2.3.2.9), which leads to a progressive inability to activate the exercising muscle. As a result, increased central motor command is required to maintain the task (Burnley & Jones, 2018). The combination of peripheral disturbances and reduced CNS efficacy likely both contribute to the attainment of task failure or termination in the heavy intensity domain, with no single mechanism responsible.

2.4.3. Severe Intensity Domain

2.4.3.1. Modelling Severe Intensity Exercise Tolerance

Above a certain intensity, the metabolic response to exercise becomes unsustainable; this threshold is the lower boundary of the severe intensity domain and can be discerned by a number of techniques. For example, during a ramp test the lactate turnpoint, onset of blood

lactate accumulation, and respiratory compensation point are all considered to be indicators of unsustainable metabolism (Faude *et al.*, 2009; Jones *et al.*, 2019*a*, 2019*b*). The limitations of these variables are that they represent distinct physiological processes, and might occur at contrasting metabolic rates (Jones *et al.*, 2019*b*). Indeed, controversy exists over the 'gold-standard' for determining the lower boundary of the severe intensity domain, with two commonly used methods purported to be the most valid (Jones *et al.*, 2019*a*). The maximum lactate steady state (Beneke & Von Duvillard, 1996; Billat *et al.*, 2003; Faude *et al.*, 2009) is determined by performing multiple (usually 4-5) 30 minute exercise bouts, with the work rate at which lactate remains constant (< 1mmol.L^{-1} change in the final 20 minutes) deemed the MLSS. Alternatively 'critical power' (CP) can be calculated from the hyperbolic relationship between exercise intensity (e.g. speed or power) and the time to task failure (Hill, 1925; Monod & Scherrer, 1965; Hill & Smith, 1999; Hill *et al.*, 2002; Jones *et al.*, 2010). A comparison and critique of both methods has recently been published (Jones *et al.*, 2019*a*), however the authors argue that the evidence demonstrating that CP represents the metabolic boundary between sustainable (heavy) and non-sustainable (severe) exercise (Figure 2-7) suggests it should be considered the 'gold standard' demarcation.

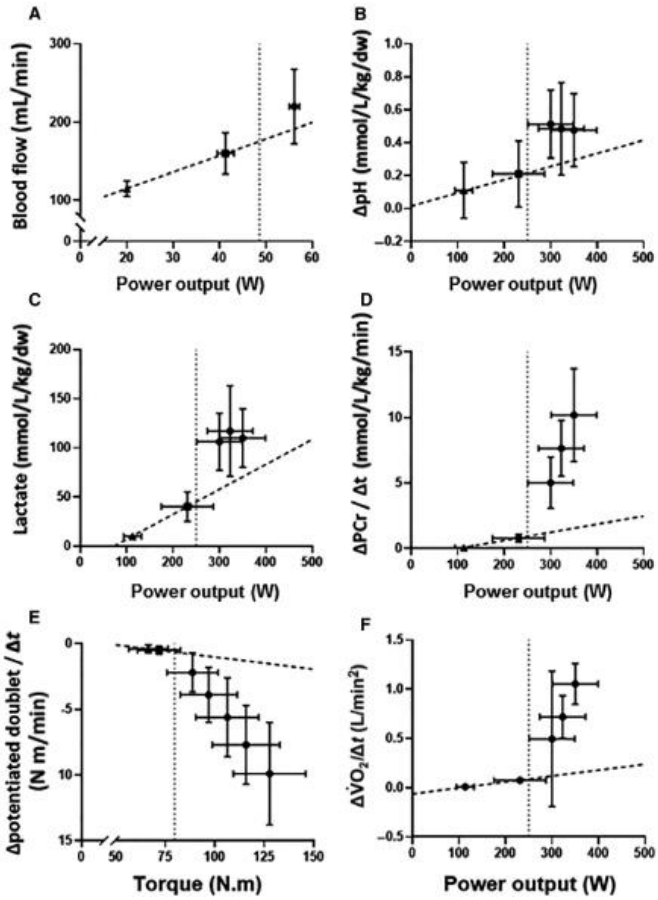

Figure 2-7: Mean and SD muscle blood flow (panel A; Copp et al., 2010), muscle metabolic perturbation (pH, panel B; lactate, panel C; Blacket al. 2017), and the rates of change in muscle [PCr] (panel D; Black et al. 2017), neuromuscular excitability (panel E; Burnley et al. 2012), and pulmonary V̇O2(panel F; Black et al. 2017) following moderate-intensity (triangles), heavy-intensity (squares), and severe-intensity (circles)exercise. The dotted vertical line indicates CP, and a line of best fit has been drawn for all trials performed below CP (i.e., moderate- and heavy-intensity exercise; dashed line). Taken from Jones et al (2019)

Critical power is considered to be the greatest exercise intensity that results in 'wholly oxidative' energy provision (Poole *et al.,* 2016). Therefore energy provision during exercise above CP must come from anaerobic sources, which results in an intramuscular disturbance (Jones *et al.*, 2010). As mentioned, the non-sustainable nature of exercise tolerance above CP results in a hyperbolic relationship between exercise intensity and duration. Typically, this relationship is profiled using multiple constant-load trials to task failure, with the most accurate estimations discerned from trials between 2-20 minutes (Bishop *et al.*, 1998; Jones *et al.*, 2010; Mattioni Maturana *et al.*, 2018). The asymptote of the hyperbolic relationship is used as

CP, and the curvature constant (W') the maximum work capacity above CP. As described in Chapter 1, W' can be used to predict time to task failure using the following equation:

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

In which T_{lim} is time to task failure, W' is the curvature constant parameter, P is the intensity of exercise (power) and CP is critical intensity (expressed as power for cycling). Other statistical methods of discerning these parameters can be used. For instance plotting work (in Joules) versus time to task failure creates a linear relationship, with the intercept of the linear regression taken as CP, and the slope as W' (Moritani *et al.*, 1981). Similarly, the inverse linear model can be used when exercise intensity (power) is plotted against the inverse (1/time) of the time to task failure, and a linear relationship is observed, again, with the intercept of the linear regression taken as CP, and the slope as W' (Whipp *et al.*, 1982). A common approach is to model the power-duration relationship in multiple ways, and use the model with the lowest error to determine subsequent exercise intensities (Mitchell *et al.*, 2018*a*).

2.4.3.2. Fatigability in the Severe Intensity Domain

Below critical power (i.e. the heavy intensity domain), metabolic responses reach a steady state, as described above, however above CP, the muscle metabolic milieu increases progressively, until task failure is reached (Vanhatalo *et al.*, 2010). This physiological response to unsustainable exercise is reflected in the $\dot{V}O_2$ response as well, as the $\dot{V}O_2$ slow component rises to $\dot{V}O_{2max}$, at which point task failure is imminent (Figure 2-6; Poole *et al.*, 1988; Burnley & Jones, 2007; Murgatroyd *et al.*, 2011). An increase in exercise intensity above CP increases the rate of muscle metabolic perturbation and the $\dot{V}O_2$ slow component increases (Vanhatalo *et al.*, 2010), however the end-exercise metabolic milieu and consequent contractile

dysfunction is remarkably consistent, regardless of work rate (Jones *et al.*, 2008; Vanhatalo *et al.*, 2010, 2016; Black *et al.*, 2016; Schäfer *et al.*, 2019). This relationship is also observed during single-limb exercise as well as cycling, when the rate of fatigue above critical torque is augmented (Burnley *et al.*, 2012).

It is thought that the attainment of $\dot{V}O_{2max}$ and the maximum intramuscular disturbance are tightly linked with W' (Murgatroyd *et al.*, 2011; Vanhatalo *et al.*, 2011). W' was originally considered to be a finite 'anaerobic work capacity' (Monod & Scherrer, 1965; Moritani *et al.*, 1981), however interventions such as hyperoxic breathing (Vanhatalo *et al.*, 2010), and endurance exercise training (Gaesser & Wilson, 1988; Vanhatalo *et al.*, 2008) elicit divergent effects on CP and W'. Therefore, the parameters cannot be considered solely aerobic or anaerobic, and are likely a result of an integrated physiological system that acts to determine exercise tolerance in the severe intensity domain. Recent work has suggested that W' during single-limb exercise is closely related to the depletion of intramuscular energy stores and accumulation of metabolites (Broxterman *et al.*, 2015*b*), however it is well established that the determinants of exercise tolerance differ between single-limb and whole-body exercise (Hureau *et al.*, 2018; Thomas *et al.*, 2018). Indeed Poole *et al* (2016) suggested that in whole-body exercise, W' is likely influenced by cardiopulmonary limiting factors to exercise in addition to muscle dysfunction.

The mechanisms underpinning peripheral fatigue in the severe intensity domain are primarily related to the uncontrollable muscle metabolic perturbation (Jones *et al.*, 2008; Vanhatalo *et al.*, 2010, 2016; Black *et al.*, 2016). Which leads to a necessary recruitment of additional motor units as the contractile function of previously recruited units decreases (Burnley *et al.*, 2012). The severe intensity domain is also where the role of group III/IV afferent neurons is implicated in the development of central fatigue (Section 2.3.2.10), as metabo-sensitive receptors are

activated by the presence of metabolic stimuli (e.g. Pi, H^+, and K^+). The effects of III/IV afferent feedback include a decrease in the excitability of various levels of the central nervous system (see Section 2.3.2.10), which, when combined with activity-dependent impairments in motoneuron properties (Section 2.3.2.9) amalgamates in a reduced capacity to voluntarily activate the exercising muscle (Sidhu *et al.*, 2017).

The ultimate limiting mechanism to severe intensity exercise is debated (Amann, 2011; Hureau *et al.*, 2018; Thomas *et al.*, 2018), with researchers suggesting that the modest degree of central fatigue experienced post-exercise likely does not limit exercise tolerance (Thomas *et al.*, 2014*a*, 2016). The consistent degree of post-exercise peripheral fatigue following trials above CP fits with the hyperbolic nature of the power-duration relationship, and consistent depletion of W' at task failure (Poole *et al.*, 2016*a*; Burnley & Jones, 2018), suggesting that contractile dysfunction of the exercising muscle limits performance. Whilst this might hold true for single-limb exercise (i.e. Burnley *et al.*, 2012), whole body exercise tolerance is likely a result of the integration of cardiovascular, respiratory, and muscular responses to exercise. Indeed, as recently acknowledged by Poole *et al* (2016), a physiological equivalent of W' has not be demonstrated, and is likely task- and population-specific.

2.5. Sex Differences in Neuromuscular Function

Considering sex as a biological variable has been promoted as an area of importance for biomedical research, highlighted by a series of review articles (Cahill, 2006; Beery & Zucker, 2011; McCarthy *et al.*, 2012; Shansky & Woolley, 2016). These sex differences also extend to exercise performance; all exercise requires coupling of the nervous system with skeletal muscle(s), therefore sex differences at any stage of the motor pathway have the potential to influence exercise performance. Understanding how sex influences the neuromuscular system in health and disease will enable practitioners to optimise the prescription of exercise

in athletic and clinical populations. This section aims to detail the current scientific knowledge of sex differences in neuromuscular function, whilst outlining areas for future research.

2.5.1. A brief history of the scientific study of sex and performance

The anatomical and physiological differences between males and females have been thought to determine the absolute limits to human performance for centuries. The scientific understanding of this topic has developed considerably over the past 150 years, mainly due to advances in technology. However, in the 19th century, medical literature consisted mainly of hypotheses and opinion. For instance, Dr Edward Clark published '*Sex in Education; or, A Fair Chance For Girls*' in 1873, which suggested that if women pushed themselves to compete with males, they would experience nervous collapse and sterility. However, similar to other essays of that era, the conclusions were not based on empirical data. The first to comprehensively study female performance was Dr Mary Putnam-Jacobi, who studied a range of parameters including muscle strength, pulse pressures and daily physical activity levels. The data showed that menstruation did not hinder female performance, and her essay "The Question of Rest for Women during Menstruation" was awarded Harvard University's esteemed Boylston Prize in 1876. Since the pioneering work of Dr Putnam-Jacobi, technological advances have permitted further insight into the how the sex of an individual might influence physiological responses to exercise.

2.5.2. Sex differences throughout the motor pathway

The chain of events that results in muscle contraction outlined in Section 2.3 is susceptible to influences that can augment or impair the process. While aspects of the motor pathway can be trained and augmented, the characteristics of the exercise performer ultimately define the maximal neuromuscular output in a given scenario; it is for this reason why certain individuals are capable of athletic feats that others are not. Indeed, the sex of the exerciser influences

characteristics of the motor pathway and leads to both subtle and clear differences in performance of a wide range of tasks between males and females.

2.5.2.1. Pre-motor processes

Beginning at the top of the motor pathway, sex differences in brain structure and function are prevalent, including greater connectivity between the sensory and motor cortices in males (Ritchie *et al.*, 2018). Techniques such as functional and structural magnetic resonance imaging, positron emission tomography, and single photon emission computed tomography have identified sex differences in cerebral blood flow and neurochemistry (Cosgrove *et al.*, 2007), indicating that males and females have distinct cerebral features that could contribute to sex differences in a wide range of both healthy and pathological states. However, how these differences relate to motor function is unclear, and whether these sex differences are physiological or related to social factors remain undetermined. Further evidence supports the notion that a sex difference in cortical and sub-cortical activation occurs during both simple and complex motor tasks (Lissek *et al.*, 2007). Whilst sensorimotor activation, assessed by functional MRI (fMRI), has been implicated in the production of force (Cramer *et al.*, 2002), and exercise-induced fatigue (Liu *et al.*, 2003), the causal role of interaction between brain areas on neuromuscular function during exercise is still unclear (Robertson & Marino, 2016; Meeusen *et al.*, 2016). As suggested by Hunter (2014), the present challenge is to determine the functional significance of sex differences in brain activity during motor output.

2.5.2.2. Intracortical and corticospinal neurons

Understanding whether neurotransmitter systems might differ between sexes can aid in optimising a wide range of physiological processes, such as adaptation to strength training (Weier *et al.*, 2012), and exercise-induced fatigue (Sidhu *et al.*, 2017; Goodall *et al.*, 2018), both of which have been suggested to be influenced by sex (Crewther *et al.*, 2006; Hunter,

2009). To date, only one investigation has addressed the question of sex differences in TMS-assessed intracortical neurotransmission directly, reporting no sex difference in the resting first dorsal interosseous muscle (Cahn *et al.*, 2003). However, as acknowledged by Kalmar (2018), excitability of intracortical and corticospinal structures is state, time, and task dependent, therefore resting data should not be extrapolated to an active state. On this note, cortical representations of different muscle groups also exhibit different input-output properties in response to single and paired-pulse TMS (Chen *et al.*, 1998). Thus, a comprehensive investigation into the dynamics of intracortical properties in different muscle groups, during different tasks is required before conclusions can be made about sex differences in neurotransmission in motor regions of the brain.

At rest, the properties of the corticospinal tract in response to single-pulse TMS are known to be similar between males and females (Pitcher *et al.*, 2003; Rozand *et al.*, 2019). Similarly, both sexes experience changes in corticospinal excitability following fatiguing exercise (Hunter *et al.*, 2006*a*; Senefeld *et al.*, 2018), suggesting that the responsiveness of this descending tract is not responsible for any sex differences in aspects of neuromuscular performance. However, to fully discern the aetiology of change in corticospinal properties, direct subcortical stimulation must be delivered in addition to TMS (Petersen *et al.*, 2002; Martin *et al.*, 2008). To date, no studies exist comparing the contribution of cortical and subcortical structures to indices of neuromuscular performance in fatigued or non-fatigued states.

2.5.2.3. Motor Unit Properties

A variety of techniques exist to assess motor unit discharge properties, however, there is a lack of literature directly comparing sex differences. Higher discharge rates have been observed in males compared to females at low (15%) contraction intensities (Harwood *et al.*, 2014), possibly due to the fact that males have a lower proportion of low-threshold motor units

(Kukulka & Clamann, 1981). These factors, amongst others, are suggested to contribute to a sex difference in force steadiness (Jakobi *et al.*, 2018), that appears to be exacerbated by exercise-induced fatigue (Ansdell *et al.*, 2018*b*). Although Jakobi *et al.* (2018) concluded that despite a clear sex difference in steadiness, the underlying mechanisms are unclear. A recent investigation using high-density EMG (Pereira *et al.*, 2019) demonstrated no differences in discharge rate, but rather showed that females experienced greater oscillations in the common synaptic input to motor units. This greater coefficient of variation (CV) in the common synaptic input has been associated with the CV of force during sustained submaximal contractions (Farina & Negro, 2015). The present challenge is to assess how the interaction between sex, motor unit firing properties, and fatigue interact to affect functional tasks, which as suggested by Jakobi *et al.* (2018) is a challenging task.

2.5.2.4. Skeletal Muscle Physiology

The morphological differences in skeletal muscle are likely the area of the motor pathway that differ the most between sexes when exercise-induced fatigue is of interest (Hunter, 2016*a*). A simple observation is that males possess a greater quantity of skeletal muscle, which leads to greater maximum strength (Miller *et al.*, 1993; Lindle *et al.*, 1997; Ivey *et al.*, 2000; Welle *et al.*, 2008). This sex difference in maximum strength varies between muscles (Miller *et al.*, 1993; Senefeld *et al.*, 2013), however, the VA of skeletal muscle does not differ between males and females (Hunter *et al.*, 2006*a*; Yoon *et al.*, 2007*b*), indicating the properties of muscle are the reason for the strength difference. This strength difference can have implications for sex differences during certain fatiguing exercises, as detailed in Section 2.6.1.

In terms of muscle metabolism during exercise, females oxidise more fat, but less carbohydrate and amino acids compared to males (Tarnopolsky, 2008). This is likely due to the fact that males have greater glycolytic capacity (Esbjörnsson *et al.*, 1993), whereas

females have greater whole-muscle oxidative capacity (Russ *et al.*, 2005). All of which, are likely a result of a difference in the proportional area of type I muscle fibres, which has been shown to be consistently greater in females, particularly in the knee extensors (see Figure 2-8, Simoneau & Bouchard, 1989; Staron *et al.*, 2000; Roepstorff *et al.*, 2006; Welle *et al.*, 2008). These differences result in observations such as females exhibiting a smaller decrease in ATP concentrations and increases in products of ATP breakdown (Esbjörnsson-Liljedahl *et al.*, 1999; Esbjornsson-Liljedahl *et al.*, 2002), leading to the suggestion that differences in skeletal muscle metabolism contribute to the sex difference in fatigability (Hunter, 2014).

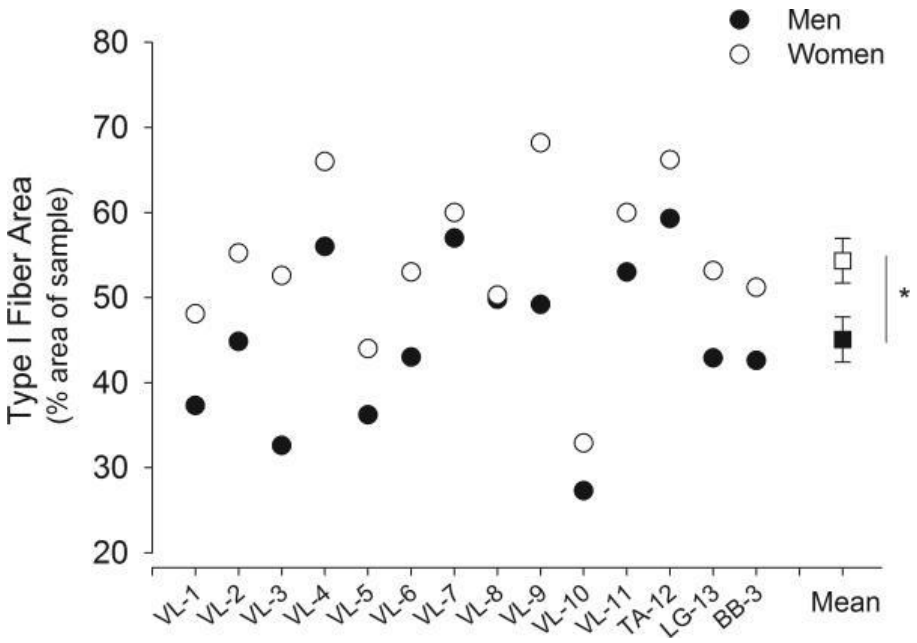

Figure 2-8: Type I fibre area (% proportional area of the sample) of skeletal muscle in men and women across 13 studies. The mean (± SEM) proportional area of type I fibers of all the muscles from the 13 studies is plotted on the right side of the figure. Women had greater type I fiber area (%) than men for the 13 studies when pooled (P<0.05). Taken from Hunter et al. (2016).

This fibre type difference between sexes contributes to more than just metabolism during exercise and has implications for the contractile properties of the skeletal muscle(s). Male skeletal muscle exhibits faster relaxation rates than female muscle (Hunter *et al.*, 2006*a*), which agrees with the notion that type I fibres demonstrate slower Ca^{2+} kinetics, lower power

generation, slower shortening and relaxation velocities than type II fibres (Schiaffino & Reggiani, 2011). Indeed, evidence from biopsies has shown females have a 24% lower rate of maximal sarcoplasmic reticulum Ca^{2+}ATPase activity than males (Harmer *et al.*, 2014). Slower Ca^{2+} ATPase activity and Ca^{2+} reuptake into the sarcoplasmic reticulum is linked with slower rates of relaxation (Gollnick *et al.*, 1991), with a positive relationship observed between Ca^{2+} ATPase and type II fibre proportional area (Madsen *et al.*, 1994). This slower activity does not mean a 'less active' female muscle, as this parameter is not altered in response to resistance training (Hunter *et al.*, 1999), or immobilisation (Thom *et al.*, 2001). However, as suggested in Section 2.3.1.3, dysfunction in calcium-related processes can cause peripheral fatigue, which has lead Hunter (2014) to suggest that females possess slower, yet more fatigue-resistant skeletal muscle properties than males.

Another result of greater type I fibre proportional area is the haemodynamic and perfusion properties of the muscle, which has implications for oxygen delivery and the accumulation of metabolites during exercise. Vasodilatory responses of the feed arteries to exercising skeletal muscles appear to be greater in females, for instance, the femoral artery exhibits greater vascular conductance and blood flow during incremental exercise, as a result of greater increases in artery diameter compared to males (Parker *et al.*, 2007). This sex difference could promote greater muscle perfusion during exercise; however, this would also depend on the capillarisation of muscle. Evidence from muscle biopsies also demonstrates a higher density of capillaries per unit of skeletal muscle in females compared to males in the *vastus lateralis* (Roepstorff *et al.*, 2006). Collectively, the greater delivery of oxygenated blood and muscle capillarity implies that muscle perfusion during exercise would be greater in females compared to males. Indeed, a recent study employing near-infrared spectroscopy (NIRS) demonstrated that forearm muscle oxygenation decreases to a greater degree during handgrip exercise in males (Mantooth *et al.*, 2018). However, this study employed sustained contractions, of which pressure-related occlusion might influence the sex difference (see Section 2.6.2). More recent

evidence demonstrated a lesser deoxygenation in females during high-intensity intermittent contractions (resistance training) compared to males (Marshall *et al.*, 2019). Therefore, it remains to be seen whether a similar phenomenon occurs during endurance exercise, and whether a potential sex difference in oxygenation affects fatigability.

2.5.2.5. The Influence of the Menstrual Cycle on Neuromuscular Function

Sex differences cannot be discussed without acknowledging the potent effects of sex hormones on neural function. Throughout the female lifespan, women experience changes in sex hormone concentrations from menarche to menopause (Brown & Thomas, 2011). These sex hormones can modulate activity within the nervous system, which has led researchers to refer to them as 'neurosteroids' (Paul & Purdy, 1992). The effects of these hormones can be simplified into net excitatory or inhibitory, with the amalgamation of all hormonal concentrations at a given time point creating a 'dynamic ecosystem' within the nervous system (Tenan, 2016). Typically, oestrogens have excitatory effects, as they attenuate the release of GABA at inhibitory synapses (Schultz *et al.*, 2009), leading to decreased firing thresholds and increased discharge frequency of cerebral neurons (Smith *et al.*, 1989; Wong & Moss, 1992). On the contrary, progesterone and its metabolites have a net inhibitory effect on the nervous system, as the activity and effects of GABA are potentiated, leading to decreased neuronal discharge rate (Smith *et al.*, 1989) and in an animal model, increased inhibition of pyramidal neurons (Hsu & Smith, 2003). Collectively, this *in-vitro* evidence suggests human neuronal function could be affected; however, *in-vivo* studies are relatively sparse in comparison.

Research employing TMS to investigate the neurosteroidal effects of these hormones often use the menstrual cycle to study changes, due to the predictable cyclical changes in oestrogen and progesterone. Smith *et al.* (1999) first demonstrated the inhibitory effects of these hormones by assessing SICI in the resting hand muscles during the follicular (low oestrogen

and low progesterone) and luteal (high oestrogen and high progesterone) phases. The authors reported greater inhibition in the luteal phase, which supported the notion that progesterone potentiated GABAergic processes (Smith *et al.*, 1989; Hsu & Smith, 2003). The authors then built on their findings by demonstrating that in the late follicular phase (high oestrogen, low progesterone) oestrogen reduced intracortical inhibition and increased facilitation (Smith *et al.*, 2002). These changes have since been suggested to cause changes in motor unit firing properties across the menstrual cycle (Tenan *et al.*, 2013); specifically, a lower initial firing rate at recruitment was observed in the early follicular phase, when hormone concentrations are lowest. Similarly, inhibition in other locations of the nervous system has been shown to be modulated by hormones. Using TMS, Hausmann *et al.* (2006) demonstrated that ipsilateral silent period, an index of transcallosal inhibition (Ferbert *et al.*, 1992), was reduced in the late follicular phase. Furthermore, lower in the motor pathway, pre-synaptic inhibition of sensory Ia axons is reduced concurrently with elevated oestrogen concentrations (Hoffman *et al.*, 2018*b*). This theme of inhibitory pathways being modulated by sex hormones is evidently common within the nervous system.

The aforementioned neuronal changes throughout the menstrual cycle have been purported to elicit functional consequences too, for instance a body of literature suggests that females can produce greater maximal force in the late follicular phase, when intracortical inhibition is reduced (Sarwar *et al.*, 1996; Birch & Reilly, 2002; Tenan *et al.*, 2016*a*). Despite this, there are a number of studies showing no difference in strength across the menstrual cycle (Dibrezzo *et al.*, 1991; Janse De Jonge *et al.*, 2001; Elliott *et al.*, 2003; Kubo *et al.*, 2009). During submaximal contractions, force steadiness is decreased in the mid luteal phase (Tenan *et al.*, 2016*a*), with the authors suggesting this could potentially be due to reduced motor unit synchronisation, however, this mechanistic index of neuromuscular function is yet to be examined across the menstrual cycle. Discrepancies between studies could be caused by factors such as the identification and confirmation of menstrual cycle phases (e.g. counting

days since menstruation vs. obtaining serum hormone concentrations); time of day (Birch & Reilly, 2002); and variability in the cycle phases durations (Fehring *et al.*, 2006). However, efforts are now being made to reduce ambiguity in study designs involving females (Elliott-Sale *et al.*, 2013; Sims & Heather, 2018), which, if followed could aid with reducing heterogeneity in study designs and methodologies for menstrual cycle research.

2.6. Sex Differences in Fatigability: Current Evidence & Purported Mechanisms

First noted by Petrofsky & Lind (1975) during handgrip exercise, females frequently outlast males during open-ended exercise tasks, and the current body of evidence shows a sex difference in fatigability occurs during a variety of exercise tasks and muscle groups. However, it is not a universal phenomenon, and the aetiology of the sex difference is not consistent across tasks (Hunter, 2014). It is well established that the mechanisms of neuromuscular fatigue are specific to the task being performed (Enoka & Stuart, 1992), and as suggested by Hunter (2009), this specificity can be different between males and females. The following are examples of how the mechanisms of fatigue can differ between sexes across various exercise tasks.

2.6.1. Sustained Isometric Contractions

Sustained isometric contractions are a commonly used task to assess performance fatigability, with neuromuscular responses compared pre-post a contraction held for a given time period, or through the use of open-ended tasks with the time to task failure used as the variable of interest. When sex comparisons are performed using these tasks, females typically outperform males at submaximal intensities (Hunter & Enoka, 2001; Clark *et al.*, 2005; Hunter *et al.*, 2006*b*). Interestingly, whilst a sex difference is still apparent during maximal contractions (Russ & Kent-Braun, 2003; Hunter *et al.*, 2006*a*), the magnitude becomes lesser as

contraction intensity increases (see Figure 2-9 from Hunter, 2014). This relationship helps to provide insight into the aetiology of the sex difference in fatigability during sustained contractions, as noted by Hunter, (2009). When contraction intensities are matched for relative intensity, this is typically done using a percentage of maximum voluntary contraction (% MVC, e.g. Hunter & Enoka, 2001; Hunter *et al.*, 2006*b*). This means that for the same % MVC, males will typically produce greater absolute forces than females (Yoon *et al.*, 2007*b*), which results in greater intramuscular pressures and occlusion of blood flow into the contracting muscle(s) (Hicks *et al.*, 2001). This negative correlation between maximum strength and time to task failure was observed by Hunter & Enoka (2001), who suggested that a lesser reduction in muscle blood flow and perfusion permitted a greater time to task failure in females compared to males. Indeed, Hunter (2009) later reviewed the literature on sex differences in fatigability during sustained contractions, and demonstrated a negative linear relationship (r^2 = 0.42) between contraction intensity and the percentage difference between sexes. Further experimental evidence for this notion includes matching sexes by maximum strength (Hunter *et al.*, 2004*a*), or artificial blood flow occlusion (Clark *et al.*, 2005), both of which result in no sex difference in fatigability.

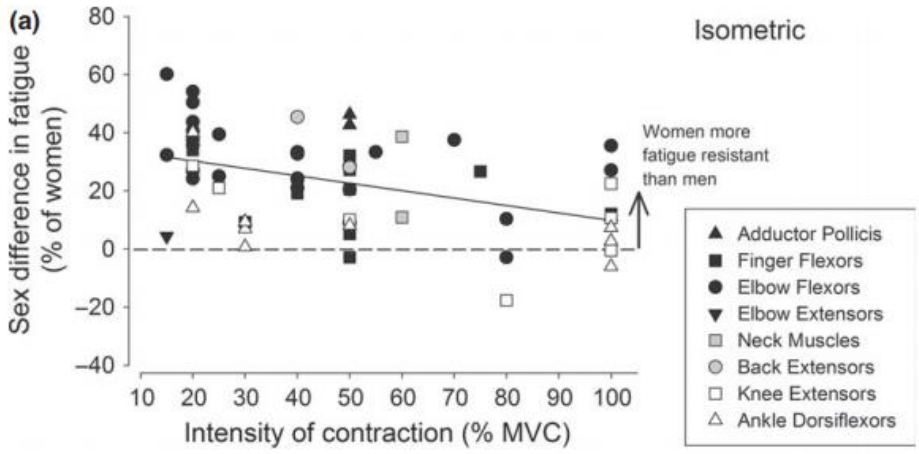

Figure 2-9: Sex differences in fatigability during isometric contractions. The solid line demonstrates the relationship between intensity of contraction and the magnitude of the sex difference. Taken from Hunter (2014).

2.6.2. Intermittent Isometric Contractions

When research concerning the sex difference in fatigability employs intermittent isometric contractions, the sex difference is still evident (Hunter, 2009). A fundamental difference between sustained and intermittent contractions is that muscle blood flow is not occluded during the latter, provided that an adequate duty cycle is used (Hunter *et al.*, 2009). Indeed, when sexes are matched for maximum strength, the sex difference is maintained (Hunter *et al.*, 2004*b*; Ansdell *et al.*, 2018*b*), implying that a factor other than strength and or occlusion is influencing fatigability. As suggested by (Hunter, 2009), the sex difference during this contraction modality is likely meditated by the differences in the composition of skeletal muscle (e.g. Section 2.5.2.4). For instance, Wüst *et al.* (2008) demonstrated that female muscle has slower relaxation rates following electrical stimulation, an indirect suggestion of a greater proportion of type I fibres. This was then suggested by the authors to be related to the 26% lesser decline in force following an electrical stimulation fatiguing task in females. Similarly, Hunter *et al.* (2004*b*) demonstrated the same finding when correlating TMS-evoked twitch properties with fatigability following an intermittent isometric voluntary fatiguing task. The sex difference observed during this contraction modality also appears to be affected by the intensity of the task. As shown by Hunter (2009), a greater difference is shown at lower contraction intensities (≤ 50% MVC) compared to higher intensity tasks, this does however remain an under-researched area of the sex difference in fatigability. Given the functional relevance of intermittent contractions to everyday tasks (Hunter, 2016*a*), discerning the underpinnings of an intensity-dependent sex difference could inform aspects of exercise prescription.

2.6.3. Whole Body Exercise

The majority of the literature pertaining to sex differences in fatigability utilises single-limb contraction modalities to explore the underpinning mechanisms, which might not necessarily

translate to whole-body exercise. The available data suggest that females exhibit greater fatigue-resistance following multiple-sprint cycling (Esbjörnsson *et al.*, 1993; Esbjornsson-Liljedahl *et al.*, 2002; Billaut & Bishop, 2012) and running (Laurent *et al.*, 2010). Furthermore, the ability to reconstitute power in the 20 minutes between 30 s Wingate tests was shown to be superior for females (Esbjornsson-Liljedahl *et al.*, 2002), with authors reporting a lesser decrease in muscle glycogen content and blood lactate production in females. Despite these apparent metabolic differences, when changes in multiple sprint studies are expressed as a function of strength, or maximum power output, the sex difference is negated (Esbjornsson-Liljedahl *et al.*, 2002; Billaut & Smith, 2009; Billaut & Bishop, 2012). As a result, a consequence is that whilst males are able to exert greater absolute force, females are able to recover from repeated exertions at a faster rate (Laurent *et al.*, 2010).

In terms of sex differences in fatigability following continuous whole-body exercise, the literature is relatively sparse in comparison to literature pertaining to single-limb protocols (Hunter, 2016*a*). Following long-duration exercise, evidence suggests that females experience less neuromuscular fatigue, as shown by a lesser decrease in MVC following two hours of running at ventilatory threshold (Glace *et al.*, 1998). Similarly, the same group then demonstrated that a long duration cycling protocol (two hours at ventilatory threshold, interspersed with one minute sprints, followed by a three km time trial) elicited peripheral fatigue in males only, with no observable decrease in evoked force in females (Glace *et al.*, 2013). Furthermore, Temesi *et al.* (2014) showed that following a 110 km ultra-endurance trail race, males experienced greater MVC declines in the knee-extensors, and greater peripheral fatigue in the plantar flexor muscles. This data fits with the notion that the sex difference in competitive race performance is lowest at ultra-endurance distances, and a greater fatigue-resistance in females could lead to females outperforming males at distances above 66 km (Bam *et al.*, 1997). Temesi *et al.* (2014) also suggested that the sex difference in plantar flexor evoked force decrease could be due to a sex difference in muscle stiffness and reduced

exercise-induced muscle damage, which could provide a plausible explanation given the demands of trail running.

Interestingly, data is equivocal regarding shorter-duration events. Recent evidence showed no sex difference in indices of central and peripheral fatigue following a half marathon (21 km, ~110 minutes, Boccia *et al.*, 2018). Whereas O'Leary *et al.* (2018) demonstrated a greater decrease in MVC for males following a 9.7 km loaded-march (~87 minutes) in military recruits. One potential reason for the discrepancies in the aforementioned literature is that the sex difference in fatigability following whole-body exercise is typically assessed following self-paced tasks. Whilst these tasks provide greater ecological validity in the assessment of exercise-induced fatigue, self-paced tasks often involve fluctuating exercise intensities (i.e. pacing) in an effort to conserve energetic resources for crucial moments of a race (Tucker & Noakes, 2009). The consequence of pacing strategies means that often, exercise is performed in multiple intensity domains (Hettinga *et al.*, 2006; Thomas *et al.*, 2012), with different fatigue-characteristics observed in each domain (Burnley & Jones, 2018). As recently demonstrated by Azevedo *et al.* (2019), different phases of an 'inverted U' race strategy have different effects on neuromuscular fatigue. Specifically, the fast-start and end-spurt can occur in higher intensity domains than the even-paced middle portion, with decrements in neuromuscular function observed at the elevated work rates. Conceivably, these effects could influence the degree of end-exercise neuromuscular function in studies utilising self-paced exercise tasks (Glace *et al.*, 2013; Temesi *et al.*, 2015; Boccia *et al.*, 2018; O'Leary *et al.*, 2018).

2.6.4. The Possibility of Sex Differences within Intensity Domains

The use of self-paced exercise tasks could hinder the mechanistic insight that sex comparisons of fatigability can offer, and as highlighted by (Hunter, 2016*a*), more studies are needed to bridge the gap between single-limb and whole-body exercise when considering sex

differences. Furthermore, it has been suggested that accounting for exercise intensity domains is vital when modelling fatigability (Burnley & Jones, 2018), therefore a comprehensive sex comparison of fatigability at metabolically-matched exercise intensities could permit the most mechanistic insight to be gained about the sex difference in fatigability.

As outlined in Section 2.4.2, peripheral fatigue in the heavy intensity domain is related to depletion of muscle glycogen stores, as well as progressive impairment in excitation-contraction coupling. Thus, the augmented rate of fat oxidation (Tarnopolsky, 2008), and slower calcium handling kinetics (Harmer *et al.*, 2014) in females could contribute to reduced peripheral fatigue in this intensity domain. Furthermore, if CP (the boundary between heavy and severe domains) is related to the exercise performer's ability to deliver and utilise oxygen to working muscle (Section 2.4.3), a sex difference could also be expected. This rationale is based on recent evidence suggesting that CP is positively correlated with the proportional area of type I muscle fibres and muscle capillarisation (Vanhatalo *et al.*, 2016; Mitchell *et al.*, 2018*a*). As outlined in Section 2.5.2.4, female knee-extensors are consistently observed to have a greater percentage area of type I fibres compared to males, therefore it could be possible that when CP is expressed relatively (e.g. as a % of maximum ramp test power for cycling, or MVC for isometric exercise) females demonstrate greater values.

Exercise in the severe intensity domain is terminated once $\dot{V}O_{2max}$ is reached, and W' is fully utilised (Section 2.4.3). The contributing processes to task-failure depend on the modality of exercise (e.g. single-limb versus whole-body, Hureau *et al.*, 2018; Thomas *et al.*, 2018), however a critical accumulation of metabolites and depletion of substrates is assumed to play a key role in task failure in this domain (Section 2.4.3.1). It could be hypothesised that if females are able to maintain perfusion of the muscle, this could augment the delivery of oxygenated blood and the clearance of metabolites to attenuate the aforementioned

accumulation and depletion processes. During whole-body exercise, the limitations of other physiological systems (e.g. cardiovascular, ventilatory, etc) are considered to contribute to the attainment of task failure. Evidence suggests females experience a slower rate of neuromuscular fatigue in respiratory muscles such as the diaphragm (Guenette *et al.*, 2010; Welch *et al.*, 2018), which could reduce the degree of afferent feedback from this physiological system, slowing the attainment of the sensory threshold and task failure (Hureau *et al.*, 2018; Thomas *et al.*, 2018).

Despite these potential contributors to superior fatigue resistance in females for whole-body exercise, it remains to be seen whether these factors are offset by sex differences in physiology that might impair female performance in comparison to males. For instance, equivalently-trained females have a ~10% lower $\dot{V}O_{2max}$, relative to body mass (Joyner, 2017), likely as a result of differential body composition between sexes. Furthermore, during exercise females experience a greater oxygen cost of breathing (Guenette *et al.*, 2007; Dominelli *et al.*, 2015), have smaller lung volumes (Schwartz *et al.*, 1988), and lower haemoglobin concentrations (Cureton *et al.*, 1986) compared to males. These factors contribute to females being prone to exercise-induced arterial hypoxemia (Harms *et al.*, 1998). This is thought not to affect sub-maximal exercise performance as muscle oxygen extraction can be increased as a compensatory mechanism, however, at maximal exercise intensities (i.e. the severe intensity domain), no room exists for oxygen extraction increases, leading to reduced arterio-venous oxygen difference, and impaired performance. Indeed, recent evidence suggests that these physiological sex differences could mask any potential sex differences in fatigability of locomotor muscles during whole-body exercise (Dominelli *et al.*, 2017). Therefore, it would be remiss to suggest that all physiological sex differences would contribute to improved fatigue-resistance, however, different modalities of exercise (i.e. single-limb versus whole-body) might be differentially influenced by sex. Therefore, a comprehensive sex comparison of fatigue-resistance should take into account different forms of exercise and consider the limiting factors

to each when forming mechanistic conclusions about the underpinnings of any sex difference(s).

2.7. Investigations, Aims, and Hypotheses

As this literature review has outlined, neuromuscular fatigue is an unavoidable consequence of exercise, and is specific to the task being performed, as well as the exerciser performing the task. Biological sex has been shown to influence physiology, which in turn affects the underpinnings of neuromuscular fatigue. What is unknown, however, is how sex differences in physiology contribute to sex differences in fatigue throughout distinct exercise intensity domains, when the metabolic demands of the task are matched between males and females. However, before this can be performed, the influence of the menstrual cycle on locomotor muscle function and fatigability must be discerned. Following this, endogenous hormones can potentially be controlled for, and the sex comparison of fatigability through exercise intensity domains in single-limb and whole-body exercise can be conducted.

Chapter 4 – Study 1

Title: The effect of the eumenorrheic menstrual cycle on neuromuscular function and fatigability.

Aim: To investigate the influence that changing endogenous hormone concentrations have on neuromuscular parameters at baseline, and following an open-ended, intermittent, isometric fatiguing task.

Hypothesis: The menstrual cycle would induce changes in maximum strength, as well as excitability and inhibition within the nervous system. It was also hypothesised that fatigue-resistance would be improved in the luteal phase of the menstrual cycle.

Chapter 5 – Study 2

Title: Test-retest reliability of neuromuscular function and fatigability in hormonally-constant females.

Aim: To investigate the repeatability of indices of neuromuscular function and fatigability used in Chapters 4, 5, and 7 in a population of monophasic oral contraceptive users.

Hypothesis: Neuromuscular function and fatigability would not differ between two testing sessions on separate days, and reliability would be similar to previously reported data in males.

Chapter 6 – Study 3

Title: A sex comparison of fatigability and recovery following exercise normalised to the intensity-duration relationship

Aim: To compare the intermittent isometric intensity-duration relationship between sexes, and assess fatigability and recovery following exercise 10% above and below the critical intensity.

Hypothesis: The critical intensity would be greater in females when normalised to MVC. However, when exercise intensity was matched relative to the critical intensity, no sex difference would be observed. It was also hypothesised that females would recover at a faster rate than males.

Chapter 7 – Study 4

Title: Methods development: assessing the validity of lumbar electrical stimulation for the assessment of subcortical contributions to corticospinal excitability.

Aim: To investigate the interaction between transcranial magnetic stimulation and lumbar electrical stimulation at rest and during voluntary muscle contraction.

Hypothesis: Facilitation and inhibition of the electromyographic response would be observed when the two stimuli were delivered at appropriate interstimulus intervals. The two stimuli would exhibit similar relationships between voluntary contraction intensity and response size.

Chapter 8 – Study 5

Title: A sex comparison of fatigability following exercise normalised to the power-duration relationship.

Aim: To compare the power-duration relationship in both sexes, and assess fatigability during exercise 10% above and below critical power.

Hypothesis: Females would demonstrate a greater critical power when expressed as a percentage of peak ramp test power. However, when exercise intensity was matched relative to the critical power, no sex difference would be observed.

CHAPTER 3 - GENERAL METHODS

3.1. Introduction

The general methods used within the studies included in this thesis are outlined in this chapter. Each of these methods were conducted within the British Association of Sport and Exercise Science (BASES) accredited exercise physiology laboratories at Northumbria University. Specific details pertaining to their application are found in the respective experimental chapters.

3.2. Pre-test procedures

3.2.1. Ethical approval

Prior to data collection, institutional ethical approval was obtained from the Department of Sport, Exercise, and Rehabilitation ethics committee at Northumbria University. All studies were completed according to the World Medical Association's Declaration of Helsinki.

3.2.2. Health and safety

All experimentation was carried out with in line with Northumbria University standard operating procedures. All researchers involved had received departmental ethical and health and safety training. Risk assessments were completed and approved for the use of all laboratories, exercise modalities, and techniques involved ensuring safety of the participants and researchers. The control measures outlined by the risk assessments were enforced during all aspects of testing. During each data collection session, a minimum of two researchers were always present, and it was explained to female participants that the option of a female chaperone was available if they wished. If participants exhibited signs of disproportionate discomfort, or symptoms such as chest pain, nausea, vomiting or syncope, experimental sessions were terminated prematurely. However, this did not occur in the studies contained in this thesis.

3.2.3. Confidentiality and Data Protection

This research was conducted in accordance with the Data Protection Act 1998, and the General Data Protection Regulations 2018. The privacy, rights and dignity of participants was maintained throughout all aspects of testing. Confidentiality was ensured by assigning an alpha-numerical code (i.e. P01) to each participant; all subsequent data pertaining to the participant were anonymised and stored using this code. Data stored on institutional computers were secured using password access, and subsequently exported to an external hard disk and a cloud-based storage service, both requiring passwords to access. Any subsequent use of the data (e.g. publication) was, and will, remain anonymous. Hand-written data from participant screening questionnaires and informed consent forms were locked in a cabinet in the lead investigators office to prevent unauthorised access, these data will be stored for ten years, then securely disposed of as confidential waste. The participants were informed of these procedures in the information sheets and informed consent forms. If a participant completed the screening questionnaire (see Appendix 3) and/or did not meet the inclusion and exclusion criteria for the study, the records were securely disposed of immediately.

3.2.4. Recruitment of Participants

Participants were recruited from the student population at Northumbria University, as well as the general population (Chapters 4-7) via an introductory email or poster. Recruitment for Chapter 8 involved emailing and using social media to contact members of local cycling/triathlon clubs, as well as the student-athlete population of Northumbria University. All means of advertisement provided a brief outline of the study requirements and the contact details of the lead investigator, with no obligation to respond. Potential participants were asked to express their interest in the study and/or request further information by emailing the lead investigator. Subsequently, the respective information sheet (see Appendix 1 for an example)

was sent in a preliminary invitational email, free from coercive or suggestive language. If participants indicated they wished to volunteer for the study, a familiarisation visit was arranged.

3.2.5. Informed Consent

Participants were given opportunities via email and in person to discuss the purpose and nature of the research, as well as risks and the commitment of taking part prior to any experimental sessions. Participants were also provided with the departmental ethics coordinator, who was not involved with the study, should they wish to discuss any queries with a scientist independent of the research team. Participants were informed that taking part in the study was completely voluntary, and that they were free to withdraw at any time. After this was clarified, the written informed consent form was signed (see Appendix 2), and the familiarisation visit was scheduled.

3.2.6. Familiarisation

To ensure participants were fully accustomed to the experimental techniques and procedures, comprehensive familiarisation visits were performed prior to experimental sessions. Care was taken to ensure that participants understood and could perform the neuromuscular protocols as well as the exercise task(s) of the study. Data from these visits was not used for subsequent analysis.

3.2.7. Experimental Controls

Prior to any experimental visits in the laboratory, participants were instructed to refrain from strenuous physical activity for 48 hours, and refrain from consuming alcohol and caffeine for 24 hours prior. The importance of refraining from alcohol and caffeine was stressed due to the

modulating effects on the motor pathway that the two substances cause (Gandevia & Taylor, 2006; Kalmar & Cafarelli, 2006; Ziemann, Lönnecker, & Paulus, 1995). Experimental trials were conducted at the same time of day (± 1 hour) to account for diurnal effects on corticospinal excitability and maximal force production (Tamm *et al.*, 2009), as well as endurance performance (Bessot *et al.*, 2006). Trials were separated by a minimum of 48 hours in order to ensure full recovery from neuromuscular fatigue between sessions (Carroll *et al.*, 2016). During experimental sessions participants received standardised encouragement from researchers to avoid influencing motivation (Andreacci *et al.*, 2002).

3.3. Apparatus and procedures

3.3.1. Anthropometry

The date of birth of each participant was recorded and converted into age (years). Stature was measured to the nearest mm using a wall-mounted stadiometer (Seca, Bonn, Germany) using the stretch-stature method (Marfell-Jones *et al.*, 2012). Participants stood upright with their heels, buttocks, and upper back in contact with the stadiometer and instructed to fully inhale and briefly hold the breath while the experimenter adjusted the headboard to make full contact with the vertex of the head. Body mass was calculated to the nearest 0.1 kg using a precision balance scale (Seca 200, Vogel and Halke, Germany) with participants wearing minimal or lightweight clothing and no footwear.

3.3.2. Assessment of Neuromuscular Function

3.3.2.1. Data Acquisition

Neuromuscular data were captured using a data acquisition system (Power1401, Cambridge Electronic Design, Cambridge, UK). Force and electromyography (EMG) data were pre-amplified (1902 amplifier, Cambridge Electronic Design, Cambridge, UK). Data were recorded

online via Spike2 software (v8, Cambridge Electronic Design, Cambridge, UK), and saved on a password secured computer.

3.3.2.2. Isometric force measurement

All experimental Chapters involved measuring isometric knee extensor force (N) production during both voluntary and evoked contractions using a calibrated load cell (MuscleLab force sensor 300, Ergotest technology, Norway). The load cell was fixed to a custom-built chair and connected to a non-compliant strap around the participants right leg, 2 cm superior to the ankle malleoli. The height of the load cell was adjusted so that force was applied in a direct line away from the cell. The load cell was calibrated by suspending objects of known masses (kg), and using regression analysis to convert raw analogue signals (mV) to force (N). This procedure was performed prior to each round of data collection. During knee extensor force recordings participants were sat upright with hips and knees at 90˚ flexion, hands were placed on handles attached to the chair at waist height.

3.3.2.3. Surface Electromyography

Surface electromyography was recorded from the right knee extensors (Chapters 4-8) and accessory respiratory muscles (Chapter 8 only). Activity of the vastus lateralis (VL), rectus femoris (RF), and lateral head of the biceps femoris (BF) muscles were recorded during isometric and cycling trials to quantify locomotor muscle activation. These muscles were chosen as previous work from our laboratory has demonstrated the optimal transcranial magnetic stimulation variables for the assessment of corticospinal and intracortical properties in the RF (Brownstein *et al.*, 2018*b*). Whilst the VL was chosen as it is considered a 'power generator' during cycling exercise (Ryan & Gregor, 1992). The BF was selected to quantify antagonist co-activation in response to TMS during neuromuscular function assessments.

To lower impedance and ensure good contact between skin and electrodes, skin was prepared by shaving and cleansing the area with an alcohol wipe, before leaving skin to dry (Basmajian & DeLuca, 1985). In chapters 4-7, single use Ag/AgCl electrodes (Kendall H87PG/F; Covidien, Mansfield, MA, USA) were used in a bipolar arrangement, whereas, in Chapter 8, wireless surface EMG electrodes (Trigno Sensors, Delsys, Natick, MA, USA) were used as movement during cycling and transitions from ergometer to isometric chair were not feasible with wired electrodes. For the locomotor muscles, SENIAM guidelines were used to determine muscle belly location (Hermens *et al.*, 2000), then electrode position was adjusted during the initial stages of testing sessions to a position eliciting the largest amplitude maximum compound action potential (M wave) at a submaximal intensity stimulation. Electrode placement was marked with indelible ink to ensure consistent placement between trials on separate days. For the wired electrodes (Chapters 4-7) the raw EMG signal was amplified (×1000), bandpass filtered (20-2000 Hz), digitised (5 kHz), and analysed offline. The wireless electrodes (Chapter 8) were bandpass filtered (20-450 Hz), digitised (2 kHz), and analysed offline.

3.3.2.4. Electrical Stimulation of the Femoral Nerve

In all experimental Chapters, electrical stimuli (0.2 ms duration) were applied to the right femoral nerve using a constant-current stimulator (DS7AH, Digitimer Ltd, Welwyn Garden City, UK) via adhesive surface electrodes (CF3200, Nidd Valley Medical Ltd, Harrogate, UK). The cathode was placed high in the femoral triangle, in the position that elicited the greatest the greatest twitch amplitude (Q_{tw}) and M-wave in the RF at rest. The anode was placed halfway between the greater trochanter and iliac crest. Single electrical stimuli were delivered for the assessment of contractile function and VA (see below), as the number of stimuli delivered in a train has not been reported to affect the interpolated twitch technique (Bampouras *et al.*, 2006).

Before the baseline neuromuscular assessment in each visit to the laboratory, stimulations were delivered at an intensity of 20 mA and increased by 20 mA intervals until a plateau in evoked Q_{tw} and M wave were observed. To ensure maximal depolarisation of the femoral nerve, despite activity-dependent changes in axonal excitability (Burke, 2002), the plateau intensity was increased by 30%; the mean ± standard deviation for current used are reported in individual Chapters. Stimulation electrodes remained attached and in the same position throughout testing visits, and were marked with indelible ink to replicate position between visits on separate days.

3.3.2.5. Electrical Stimulation of the Spinal Cord

Single electrical stimuli (1 ms duration) were delivered with a constant current stimulator (DS7AH, Digitimer Ltd, Welwyn Garden City, UK) via self-adhesive electrodes (Nidd Valley Medical Ltd, Harrogate, UK). The cathode (5 × 9 cm) was centred over the first lumbar spinous process, with the long axis of the electrode aligned to the centre of the vertebral column. The anode (3.2 cm diameter) was placed in the midline of the vertebral column, 5 cm above the upper edge of the cathode (Ugawa *et al.*, 1995*b*), corresponding to the level of the eighth thoracic spinal process (T_8). Specific rationale for using this stimulation setup is contained in Chapter 7, and the mean ± standard deviation current used are contained in individual Chapters.

3.3.2.6. Transcranial Magnetic Stimulation

Magnetic stimuli were delivered to the motor cortex via a concave double cone coil (diameter: 110 mm; maximum output 1.4 Tesla) powered by two linked monopulse magnetic stimulators (Magstim 200^2 and BiStim, The Magstim Company Ltd, Whitland, UK). A double cone coil (Type 9902, The Magstim Company Ltd, Whitland, UK) was chosen instead of a figure of eight coil, as the lower limb cortical representation lies close to the midline of the brain, within the

depths of the inter-hemispheric fissures (Ugawa *et al.*, 1994). The angulation of the double cone coil permits deeper penetration of the induced electrical field into cortical tissue (Deng *et al.*, 2013) thus, delivering the most appropriate form of magnetic stimuli for activation of the lower limb area of the motor cortex. The coil was held over the left motor cortex to stimulate with a postero-anterior intracranial flow. Transcranial magnetic stimulation was used for the determination of VA_{TMS}, measures of corticospinal excitability, and short-interval intracortical inhibition (SICI) in the RF.

A standardised procedure was employed prior to each use of TMS to determine optimal coil position. First, the vertex of the cranium was located by measuring the midlines between the tragions (a point in the notch above the tragus of the ear), and the inion (prominent projection of the occipital bone) to the nasion (a projection superior to the bridge of the nose). These distances were measured using an anatomical tape measure, and the midlines were marked with indelible ink. Following this, stimulations were delivered during a 10% contraction to find the optimal coil position, which was defined as that which elicited the largest motor evoked potential (MEP) in the agonist muscle (RF), with a concurrent small MEP in the antagonist (BF). These stimulations were delivered at 50% of maximum stimulator output (MSO), or if MEPs could not be found at this intensity this was increased as necessary. Optimal coil position was measured relative to the vertex (i.e. 1-2 cm lateral and 1-2 cm posterior), and marked on the scalp with indelible ink to ensure consistent positioning throughout testing sessions.

In Chapters 4-6 and 8, active motor threshold (aMT) was used to normalise stimulus intensities for the assessment of corticospinal excitability and SICI pre and post exercise, and was defined as the stimulus intensity required to obtain peak-to-peak MEP amplitudes ≥ 0.2 mV in three out of five stimulations (Brownstein *et al.*, 2018*b*). The use of an active motor threshold,

and delivery of subsequent stimuli during contraction was chosen rather than performing these tasks in relaxed muscle as it was specific to the motor tasks performed during exercise (Chapters 4-6), and similar to the state of the motor pathway during exercise (Kalmar, 2018). To ascertain aMT, stimuli were delivered while participants performed contractions at 10% of maximum voluntary contraction (MVC). These stimuli were first delivered at 50% MSO, and increased or decreased by 5% where necessary, then refined by 1% changes in stimulator output. Mean ± standard deviation for stimulator intensities are contained within individual chapters.

Short-interval intracortical inhibition was used to quantify $GABA_A$ related inhibition within the motor cortex (Kujirai *et al.*, 1993). The stimulation variables for conditioned MEPs were those determined by Brownstein *et al.* (2018), and were as follows: 2 ms interstimulus interval, conditioning pulse intensity 70% aMT, test pulse intensity 120% aMT, contraction intensity 10% MVC.

Stimulation intensity for VA_{TMS} was determined using a step-wise procedure. Participants were instructed to maintain a 50% contraction for ~5-6 s whilst single pulse TMS was delivered twice (~2 s between stimulations). Participants were given 30 s rest between contractions. The size of the superimposed twitches (SITs) was measured and averaged, and the stimulator intensity was set at 50% and increased in 5% intervals until SIT amplitude either plateaued or decreased. For the knee extensors, as stimulator intensity is increased the agonist MEP increases until a plateau is reached and amplitude does not change further, meanwhile the agonist MEP increases linearly with no plateau (Temesi *et al.*, 2014, see Figure 3-1). Therefore, the largest SIT in this procedure should correspond to the largest agonist MEP, with a concurrently small antagonist MEP, in the example Figure 3-1, this would correspond

to 60% MSO. Mean ± standard deviations for VA_{TMS} stimulation intensities are provided within individual chapters.

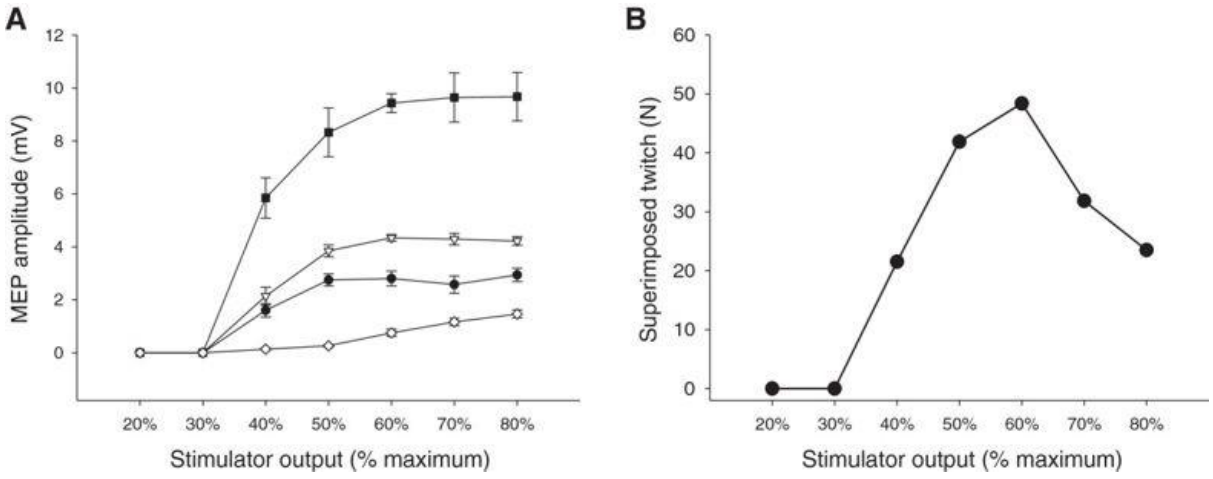

Figure 3-10: A representative stimulus-response curve during a 50% MVC. Peak to peak MEP amplitudes for vastus lateralis (●), rectus femoris (▽), vastus medialis (■) and biceps femoris (◊) are provided in Panel A. The corresponding means of four SITs are displayed in Panel B. Taken from Temesi et al (2014).

3.4. Neuromuscular Function Assessments

3.4.1. Assessment Protocol

The pre and post exercise neuromuscular assessment protocols used in the experimental Chapters of this thesis remained consistent throughout. In Chapter 8, there was a longer delay (30 s) between task termination and the start of the assessments due to the need to transfer participants from the cycle ergometer to the isometric force chair. Figure 3-2 shows a schematic of the order of the assessment. These began with five MVCs, the first two were to maximise potentiation of quadriceps twitches ($Q_{tw.pot}$) from the final three MVCs. Potentiated twitched were chosen as they are known to be more sensitive to fatigue-induced changes in contractile function (Kufel *et al.*, 2002). Electrical stimulation was delivered at the plateau of peak force and 2 s following MVCs to quantify VA_{MNS}, and contractile function of the quadriceps ($Q_{tw.pot}$). Following these, the maximum of the five MVCs (peak instantaneous force), was used to set guidelines for the assessment of VA_{TMS}. Five guidelines were used between 50-100% MVC, and the contractions were performed in two sets (Dekerle *et al.*,

2018). The TMS stimuli were delivered on the plateau of force at the target guideline; care was taken to avoid stimulating as force was increasing or decreasing. Following the quantification of VA_{TMS}, corticospinal excitability and SICI were assessed. Single- and paired-pulse TMS were delivered alternately in two sets of 10 stimulations; the aforementioned variables are discussed in further detail below.

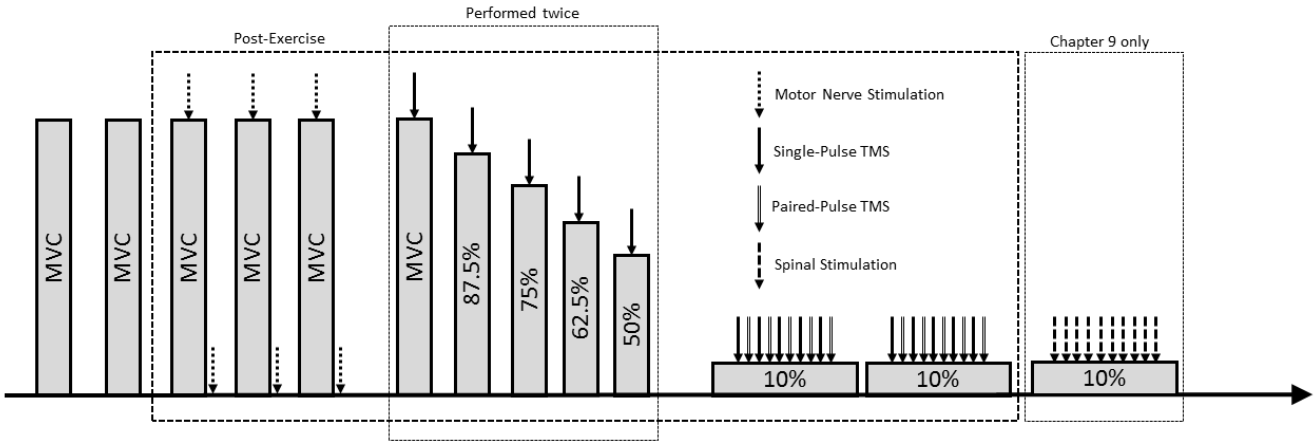

Figure 3-2: Schematic of the neuromuscular function assessments performed in Chapters 4-6 and 8.

3.4.2. Voluntary Activation

The twitch interpolation technique was used to quantify VA, which can be defined as the level of voluntary drive to a muscle, resulting in force generation (Gandevia, 2001). First described by Merton (1954), VA relies on the assumption that recruitment of motor neurons by voluntary descending drive is sub-maximal (Taylor, 2009), potentially due to excitatory/inhibitory influences from other areas of the central nervous system (Rekling *et al.*, 2000). The presence of a SIT during an MVC indicates that the electrical stimulation of the motor nerve, and subsequent action potential arriving at the muscle fibres, was able to recruit motor units that were not firing at maximal rates, resulting in extra force. This SIT is then compared to the $Q_{tw.pot}$ which is delivered shortly (2-3 s) following the MVC. The relationship between voluntary force and SIT is inversely linear, therefore, the SIT during MVC to be interpolated against

$Q_{tw.pot}$, to account for between individual or conditions (e.g. fatigue) differences, using the following equation:

$$\mathbf{VA = (1 - SIT / Q_{tw.pot}) \times 100}$$

When TMS is used for the assessment of VA, the resting twitch must be estimated, due to the inability of a TMS pulse to recruit the entire motoneuron pool at rest (Todd *et al.*, 2003). This estimated resting twitch (ERT), is a result of regression of SITs and voluntary force between 50-100% MVC (see figure 2-3), these high forces are required as corticospinal excitability is relatively similar, and the SIT – force relationship is linear above 50% MVC in the knee extensors (Goodall *et al.*, 2009; Sidhu *et al.*, 2009). The *y*-intercept of this regression is then used as the estimation for the TMS-evoked twitch at rest, and entered into the aforementioned equation.

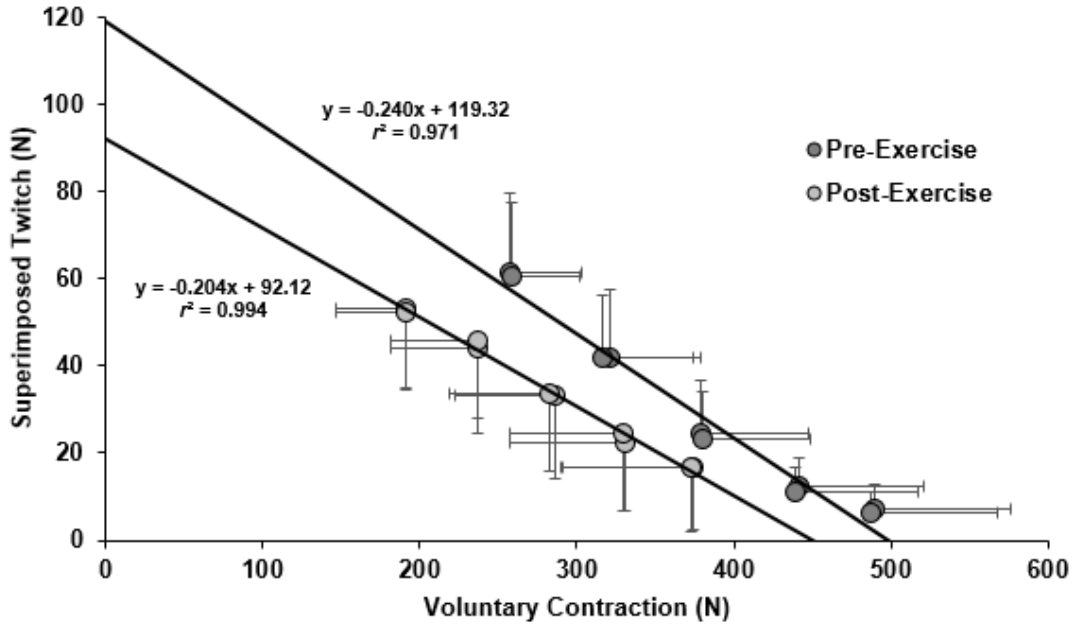

Figure 3-3: The linear relationship between SIT and voluntary force pre and post-exercise in Chapter 5.

The conceptual benefit of quantifying VA using TMS is that it provides more detail about the site within the motor pathway that causes an increase or decrease in the value compared to VA_{MNS} (Nuzzo *et al.*, 2019). Theoretically, if VA_{TMS} changes in response to an intervention, voluntary drive from the motor cortex is better or less able to recruit the motoneuron pool (Todd *et al.*, 2003). Technical challenges exist with VA_{TMS} (Todd *et al.*, 2016), however, methodological choices can be made to minimise the influence of factors such as antagonist activation (see *Transcranial Magnetic Stimulation* paragraph above), resulting in a more valid quantification of VA_{TMS}. Throughout this thesis, attempts have been made to maximise validity of VA_{TMS} by addressing the technical challenges outlined by Todd *et al.* (2016). Details are provided in Table 1.

Table 3-1: Technical challenges of assessing VATMS from Todd et al. *(2016). The consequences and compromises that were addressed in the present thesis are presented.*

Technical Challenge	**Result if unaddressed**	**Methodological adjustment**
TMS activates antagonist muscle(s).	Smaller SIT due to inadvertent antagonist torque generation. Occurs particularly at high forces (> 75% MVC), resulting in an overestimation of ERT.	TMS intensity chosen to elicit the greatest SIT. Corresponding to greatest agonist MEP and relatively small antagonist MEP. See *Transcranial Magnetic Stimulation.*
TMS pulse might not activate enough of the motoneuron pool.	Extra motor unit activation is submaximal, resulting in smaller SITs, and therefore an underestimation of ERT.	Agonist activation (MEP) is required to be >40% Mmax during a 50% contraction.
The regression for estimation of ERT might not be linear.	Invalid estimation of the y-intercept, resulting in under/overestimation of ERT.	If the SIT-force regression was not linear ($P > 0.05$), outliers from the 10 regression points were identified and removed.
Intra- and inter-individual variability	Larger measurement error associated with ERT might limit the observation of meaningful changes.	A 10-point regression is used, which has been shown to have superior reliability values to 3- and 5-point alternatives.

Whilst debate exists as to whether 'full activation' can be accurately quantified using the interpolated twitch technique (de Haan *et al.*, 2009), it is generally established that when methodological constraints are controlled for (i.e. stimulation intensity and location, joint angle, and dynamometer sensitivity), VA provides a tool for discerning changes in the nervous system's ability to drive muscle (Nuzzo *et al.*, 2019).

3.4.3. Contractile Function

The $Q_{tw.pot}$ elicited by electrical stimulation of the femoral nerve was used to assess contractile function of the knee-extensors pre- and post-exercise. The twitches were potentiated by preceding MVCs, which elicited the post-activation potentiation phenomenon (Hodgson *et al.*, 2006). This is thought to occur via the phosphorylation of myosin regulatory chains, increasing the sensitivity of the actomyosin complex to Ca^{2+}, causing the myosin cross bridges to move from weak to strong binding states (Allen *et al.*, 2008*a*). Thus, increasing the size of the evoked twitch; the sensitivity of which to a fatiguing task is greater than an unpotentiated equivalent (Kufel *et al.*, 2002). The peak-to-peak amplitude of the evoked twitches was used as an index of contractile function.

3.4.4. Evoked Electromyographical Responses

Electrical and magnetic evoked potentials were recorded using EMG and the peak-to-peak amplitude was analysed offline. Responses to supramaximal electrical stimulation of the femoral nerve (M_{max}) were used to assess maximum muscle membrane excitability. When the motor cortex was stimulated magnetically (Chapters 4-8), the resultant MEP was used to quantify corticospinal excitability, and when the spinal cord was stimulated electrically (Chapter 8), the LEP was used to assess spinal excitability. The MEP and LEP were expressed as percentages of M_{max} to account for any potential day-to-day variation in electrode placement (Lefebvre *et al.*, 2004). The peak-to-peak amplitude and area of evoked

responses were calculated, with area defined as the integral of the reflected value of the entire response. Further detail of EMG analyses can be found within the relevant chapters.

Following an MEP, a period of silence is observed in the ongoing EMG, termed the TMS silent period (SP). The duration of the SP is measured in milliseconds (ms) and is believed to reflect a combination of both intracortical and spinal mechanisms of inhibition (Škarabot *et al.*, 2019). As outlined by Škarabot *et al.* (2019), several methodological factors can confound the SP, with a moderate contraction intensity (40-60% MVC) and moderate stimulus intensity (between aMT and ~80% MSO) recommended when delivering TMS. Therefore, studies within the present thesis assessed SP during 50% contractions, using the TMS intensity chosen for VA_{TMS} assessment (typically between 50-70% MSO).

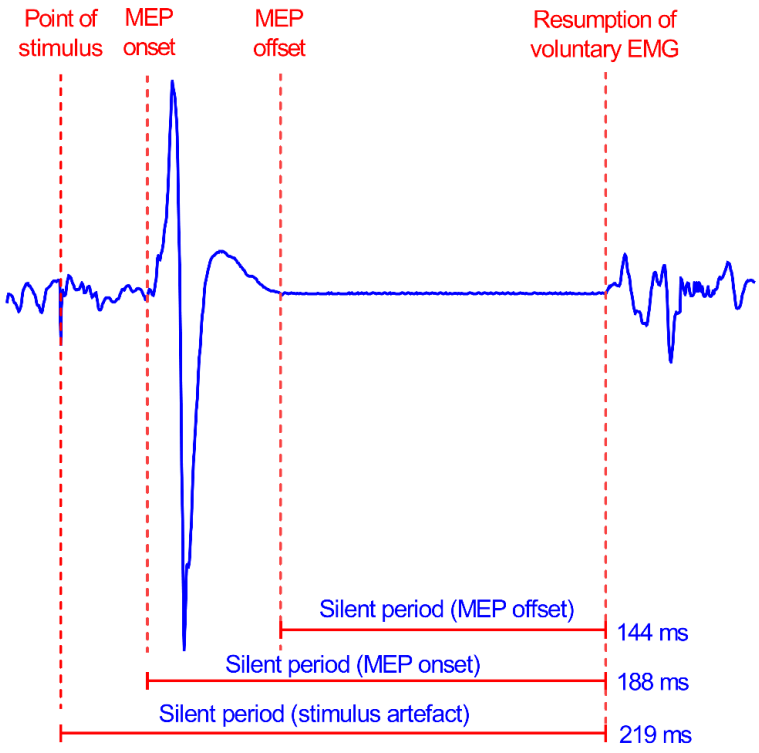

Figure 3-4: The TMS silent period, with options for SP onset and offset marked. Taken from Skarabot et al. (2019).

When determining SP onset, three options exist: stimulus artefact, MEP onset, and MEP offset, with potential confounds existing for all three. As the present studies did not include neurologically diseased, or elderly participants, MEP latency was not expected to change. Additionally, evoked potential duration can be increased in a fatigued state (Gandevia *et al.*, 2013), which could confound SP duration if MEP offset was used for SP onset. Therefore, in the present thesis, SP duration was calculated from the stimulus artefact to avoid this confound. The offset of the SP can be determined visually (Todd *et al.*, 2005; Sidhu *et al.*, 2009), whilst other researchers have used mathematical modelling (King *et al.*, 2006). An alternative method is to use a minimum value (i.e. ± 2 standard deviations of the pre-stimulus EMG; Goodall *et al.,* 2010) as the offset point, which also avoids the contamination of SP with reflexive low-level 'bursts' of EMG activity (Butler *et al.*, 2012). Thus, the present thesis utilised this method of SP offset determination.

3.5. Metabolic variables

3.5.1. Near Infrared Spectroscopy

The oxygenation status of biological tissues was quantified throughout this thesis using Near Infrared Spectroscopy (NIRS; INVOS 5100C, Somanetics, Troy, MI, USA). In Chapters 6 and 8, the right VL was monitored. Two wavelengths (765 and 855 nm) were used, with the intensity of transmitted light continuously monitored at 1 Hz. Based on the absorption and scattering coefficients of light at each wavelength, determined by Beer-Lambert law, concentrations were estimated for oxy (HbO2), deoxy (HHb), and total haemoglobin (THb). Tissue oxygenation (TOI) index was also calculated using the following equation:

$$\textbf{TOI} = \frac{[\boldsymbol{HbO2}]}{[\boldsymbol{HbO2}]+[\boldsymbol{HHb}]\ \boldsymbol{x}\ \mathbf{100}}$$

For the VL, optodes were placed over the muscle belly 20 cm superior from the fibular head (Keane *et al.*, 2018). Data was recorded and stored offline for further analysis, specific details of the analyses performed can be found within the respective chapters.

3.5.2. Cardiovascular Measurements

In Chapters 6 and 8 cardiovascular variables were monitored during exercise via a finger plethysmography (Finometer MIDI, TNO Biomedical Instrumentation, Netherlands). This non-invasive technique uses a finger-clamp method, whereby the diameter of the finger cuff was maintained at a constant, while changes in intra-arterial pressure occurred during the cardiac cycle. Changes in the diameter of the cuff are measured by an infrared photoplethysmograph (wavelength: 950 nm) and sensor within the inflated finger cuff. The cuff then applies the required counter (extramural) pressure, to avoid changes in volume (Bogert & Van Lieshout, 2005). When extramural and intra-arterial pressures are matched (termed set-point) at the onset of recording, changes in the latter can be measured. The in-built calibration program (Physiocal, Finometer Medical Systems, Netherlands), acts as a calibration tool during recording to ensure the set point remains constant (Imholz *et al.,* 1991; Imholz, *et al.,* 2006). Systolic, diastolic, and mean arterial pressures (MAP) were obtained from the pressure changes within the cardiac cycle. Left ventricular stroke volume (SV) was estimated from the integral of the arterial pressure-flow wave, with the aortic cross-section adjusted for age, gender, height, and body mass of the participant (Langewouters *et al.*, 1984). Instantaneous heart rate (HR) measurements were then multiplied by SV to obtain cardiac output ($\dot{Q}$).

The reliability of this technique for estimating cardiovascular variables during exercise has been established (Waldron *et al.*, 2017*a*), with low measurement errors reported for estimates of blood pressure (1.6-3.2%), heart rate (3.4-3.9%), and cardiac output (3.8-4.8%), however,

stroke volume exhibited larger error at rest (7.4%). The validity of the technique has also been established during exercise by comparing non-invasive estimates to aortic arch catheterisation (Eckert & Horstkotte, 2002).

CHAPTER 4 - THE EFFECT OF THE EUMENORRHEIC MENSTRUAL CYCLE ON NEUROMUSCULAR FUNCTION AND FATIGABILITY

4.1. Introduction

Prior to the comparison of neuromuscular function and fatigability between males and females, the effect of cyclical sex hormone changes on these parameters must be discerned. The eumenorrheic menstrual cycle presents cyclical changes in concentrations of multiple sex hormones, including oestrogen and progesterone (Sherman & Korenman, 1975). These sex hormones can act as neurosteroids and affect CNS function due to their ability to cross the blood-brain barrier (Stoffel-Wagner, 2001). *In vitro* models have shown direct evidence for the effect of sex hormones on neuronal function. For instance, estradiol (an estrogenic steroid) binds to estrogen receptor α (ER-α) sites on GABA-ergic neurons, causing an attenuation in GABA synthesis and release, resulting in a net excitatory effect (Wallis & Luttge, 1980; Schultz *et al.*, 2009). Additionally, estrogen has been shown to decrease firing thresholds and increase discharge frequency of cerebral neurons (Smith, 1989*a*; Wong & Moss, 1992). On the contrary, progesterone has a net inhibitory effect on the nervous system, as the activity and effects of GABA are potentiated, leading to decreased neuronal discharge rate (Smith, 1989*b*) and increased inhibition of pyramidal neurons in rats (Hsu & Smith, 2003). These alterations have also been established *in vivo*, with TMS studies showing increased intracortical excitability and reduced intra-cortical inhibition mid-cycle, when estradiol concentration is high and progesterone low (Smith *et al.*, 1999, 2002), reflecting the alterations seen in the aforementioned *in vitro* studies.

Previous research shows clear changes in human CNS function, however, they were conducted in the resting upper limbs, specifically in hand muscles associated with fine motor tasks. It is established that properties of intracortical and corticospinal circuits vary between upper and lower limb projections (Brouwer & Ashby, 1992; Chen *et al.*, 1998), and the neural control of large locomotor muscle groups, such as the KE, are implicated in athletic performance, everyday tasks, and rehabilitation from injury. To date, there is relatively little

research investigating menstrual cycle induced changes in nervous system function with regard to the KE. Some evidence suggests that factors such as motor unit firing rates are augmented when oestrogen concentrations are elevated (Tenan *et al.*, 2013), with the authors proposing that increased excitability of corticospinal neurons and decreased intracortical inhibition could be an explanatory factor (Smith *et al.*, 1999, 2002).

Literature concerning the functional consequences of altered CNS function across the menstrual cycle is equivocal. Previous studies investigating motor functions such as MVC are unclear. Several studies have shown that females can produce 8-23% more force with the KEs mid-cycle compared to early follicular and luteal phases (Sarwar *et al.*, 1996; Birch & Reilly, 2002; Tenan *et al.*, 2016*b*). However, multiple studies report no difference in maximal strength of the KEs across the menstrual cycle (Dibrezzo *et al.*, 1991; Janse De Jonge *et al.*, 2001; Elliott *et al.*, 2003; Kubo *et al.*, 2009). Furthermore, two studies have shown that VA of the knee extensors is unaltered by the menstrual cycle (Janse De Jonge *et al.*, 2001; Kubo *et al.*, 2009), further contradicting the studies reporting strength changes. Other aspects of motor performance, such as performance fatigability (Hunter, 2018), have also been studied throughout the menstrual cycle with inconclusive results. Sarwar *et al.* (1996) showed that the knee extensors of eumenorrheic females were less fatigable in the luteal phase, however, this is a finding that has not been consistently observed (Dibrezzo *et al.*, 1991; Janse De Jonge *et al.*, 2001), potentially due to heterogeneity in contraction type, and the use of open-ended versus fixed duration tasks.

The effects of hormonal fluctuations on neuromuscular function and fatigability still remain unclear. Conflicting literature exists for the majority of neuromuscular variables in the knee extensors, despite a rationale for change. One potential issue is that information about potential mechanistic responses (i.e. CNS excitability and inhibition) is typically derived from

the resting upper limb. Therefore, the present study aimed to investigate changes in TMS and MNS-evoked variables as well as neuromuscular function and fatigability across the eumenorrheic menstrual cycle at hormonally-distinct time points in the KE. The findings of this Chapter will discern whether endogenous hormone concentrations need to be controlled for in subsequent work when females are compared to males.

4.2. Methods

4.2.1. Participants

Fifteen eumenorrheic females (EFs; age: 25 ± 4 years; statue: 169 ± 6 cm; mass: 68.3 ± 7.8 kg; mean cycle duration: 29 ± 3 days, range: 24-34 days) provided written informed consent to volunteer for the study. Participants reported having regular menstrual cycles (at least 4 cycles of 25-35 days duration in the previous 6 months) without using any hormonal contraceptives for 6 months.

4.2.2. Experimental Design

Eumenorrheic females visited the laboratory four times, completing a familiarisation session prior to three experimental visits. Participants completed experimental visits on days 2 (early follicular phase), 14 (late follicular phase), and 21 (luteal phase) of the menstrual cycle. Testing days were counted from the onset of menstruation and fasted blood samples were taken between the hours of 06:00 – 09:00 on testing days to analyse serum estradiol and progesterone concentration and confirm menstrual cycle phase. The order of visits was pseudorandomized and counterbalanced to minimize order effects, with 5 participants beginning on each testing day. All testing visits occurred within the same menstrual cycle (order: d2, d14, d21), or two consecutive cycles (order d14, d21, d2 or d21, d2, d14). This experimental design was based on previous recommendations (Sims & Heather, 2018; Janse

de Jonge *et al.*, 2019). Experimental visits consisted of a baseline neuromuscular function, performance of intermittent, isometric contractions at 60% MVC until task failure, followed immediately by a post-task neuromuscular function assessment.

4.2.3. Experimental Procedures

Upon arriving between the hours of 06:00 – 09:00, fasted blood samples were taken following 10 minutes of seated rest. Participants were then instructed to consume a typical breakfast and return to the laboratory at their designated testing time. The breakfast and time of testing were replicated (± 1 hour) for each experimental visit to control for diurnal variations in corticospinal excitability and maximal force production (Tamm *et al.*, 2009). Experimental sessions began with participants completing a standardised voluntary isometric contraction warm up (two contractions at 25, 50, and 75% perceived maximal effort) followed by a baseline neuromuscular assessment (as described in Chapter 3). The fatiguing task involved sets of intermittent (3 s contraction, 2 s rest), isometric contractions to task failure. Contractions were paced with an audible metronome to ensure the duty cycle was maintained. One set was defined as 11 submaximal contractions followed by a MVC with MNS during and after, lasting one minute. Task failure was defined as an inability to reach the target force three times. Rating of perceived exertion (RPE; Borg, 1982) was recorded following each MVC during the fatiguing task. Real-time visual force feedback using guidelines set as percentages of maximum force was provided to participants on a computer screen to help maintain a constant force level. The post-task neuromuscular assessment began immediately following task failure.

4.2.4. Transcranial Magnetic Stimulation

Active motor threshold was not different on testing visits (43 ± 9, 42 ± 8, and 43 ± 9%, P = 0.874). Similarly, the mean stimulator intensity was not different between trials (63 ± 10, 63 ±

11, and 63 ± 12%, *P* = 0.984). The stimulator output activated a large proportion of the KE motoneuron pool at baseline in each experimental visit, with no difference between trials (61 ± 17; 57 ± 17; 55 ± 14% M_{max}; *P* = 0.788). The TMS pulse also concurrently elicited a small MEP in the antagonist muscle group (0.53 ± 0.39, 0.71 ± 0.42 and 0.60 ± 0.34 mV, *P* = 0.211).

4.2.5. Motor Nerve Stimulation

The optimum stimulus intensity for MNS was not different between visits (233 ± 72, 244 ± 68, and 262 ± 67 mA, *P* = 0.125).

4.2.6. Blood Sampling and Hormone Analysis

Venous blood sampling was performed on the morning of each testing session. A 10 mL blood sample was drawn from an antecubital vein into a silica coated tube by a trained phlebotomist, then left upright for 15 minutes to coagulate before centrifuging. Samples were centrifuged at 2,500 rpm for 10 minutes at room temperature (Allegra-X22R, Beckman Coulter, USA). Using a 500-1000 *μ*l pipette, the supernatant serum was separated into three aliquots (~1000 *μ*l each) and stored at −80°C until oestradiol and progesterone analysis were performed. Total concentrations of 17-β oestradiol and progesterone were measured in duplicate using hormone-specific enzyme-linked immunoassay kits (Cayman Chemical, Ann Arbor, MI). All samples were analysed using the ELISA technique with absorbance detection (wavelength 405 nm). The minimal oestradiol and progesterone detection was 15 pg/ml and 7.5 pg/mL, respectively. To calculate 17-β oestradiol and progesterone levels, a standard curve was plotted using eight standards against their absorbance. Using the mean absorbance from the duplicate of each sample, the concentration of the sample was interpolated directly from the standard curve. The coefficients of variation (CV) for the ELISA kits, as provided by the manufacturer, were 8-12% for 17-β oestradiol, and 5-8% for progesterone. In one instance, the CV of a duplicate sample exceeded the manufacturer's CV due to an excessively high

(non-physiological) reading in one well. Therefore, the lower of the two was used for data analysis. Participants' hormonal profiles were deemed 'acceptable' when a peak in progesterone concentration was observed during the luteal phase (D21) and an increase in 17-β oestradiol was observed from D2 to D14. If neither peak was observed, then participants were deemed anovulatory and excluded from further analyses. This occurred in two of the 15 participants.

4.2.7. Data Analysis

The regression between TMS-evoked SIT amplitudes and voluntary force for estimation of resting twitch was not significantly linear ($p > 0.05$, $r^2 < 0.85$) in four out of 60 cases. As a result, outliers were identified (as described in Chapter 3) and six out of 870 (0.7%) SITs were excluded. This meant that there were 86 ten-point regressions, three nine point regressions, and one eight point regression used to estimate resting twitches. The mean ± SD r^2 values were 0.92 ± 0.05 pre exercise, and 0.89 ± 0.07 post-exercise.

4.2.8. Statistical Analysis

Data are presented as mean ± SD within the text and figures. Normal Gaussian distribution of data was confirmed using the Kolmogorov–Smirnov test. If a violation was detected, the data were logarithmically transformed. The alpha for all statistical tests was set at $p \leq 0.05$. One-way repeated measures ANOVAs were performed for all pre-exercise dependent variables to assess MCP changes in neuromuscular function and hormone concentrations. Sphericity was assessed using Mauchly's test and if necessary, controlled using the Greenhouse-Geisser correction. Two-way repeated measures ANOVAs were run using pre and post exercise variables to obtain both fatigue, and menstrual cycle phase × fatigue interaction effects. To explore potential differences in the fatigue profiles of neuromuscular and perceptual variables, two-way repeated measures ANOVAs were run including data points from baseline, 25, 50,

75, and 100% of TTF. Significant main and interaction effects were explored using Bonferroni-corrected tests.

4.3. Results

4.3.1. Hormonal Concentrations

Thirteen out of 15 participants presented a regular hormonal profile (see Table 4). Two participants had no increase in progesterone on D21; given the hypothesis that changing hormone concentrations would modulate neuromuscular function, and these participants did not exhibit any change in hormone concentrations, they were excluded from further statistical analyses. The repeated measures ANOVAs showed an effect of MCP on 17-β oestradiol ($F_{1.4,19.5}$ = 3.55, p = 0.040, η_p^2 = 0.18) and progesterone concentration ($F_{=1.0,14.1}$ = 8.35, p = 0.012, η_p^2 = 0.37). Post hoc tests revealed that 17-β oestradiol concentrations were greater on D14 compared to D2 (p = 0.033), and greater on D21 than D2 (p = 0.029). Progesterone was greater on D21 than D2 and D14 (p = 0.011, and 0.012, respectively).

*Table 4-1: Group average concentrations for 17-β oestradiol and progesterone across the three tested phases of the menstrual cycle. * = greater than d2, # = greater than d14 (all P < 0.05).*

	Day 2	**Day 14**	**Day 21**
17-β Oestradiol (pg·ml^{-1})	248 ± 129	328 ± 160*	341 ± 186*
Progesterone (ng·ml^{-1})	1.27 ± 0.50	1.38 ± 0.69	4.41 ± 4.60*#
E:P ratio	0.20 ± 0.13	0.28 ± 0.18	0.12 ± 0.10*#

4.3.2. Baseline Neuromuscular Function

MVC force was unaffected by MCP (Figure 4-1A, $F_{1.4,16.8}$= 0.15, p = 0.790, η_p^2 = 0.01). Potentiated twitch force was also unchanged (Figure 4-1B, $F_{2,24}$ = 0.25, p = 0.782, η_p^2 = 0.02);

however, the SIT elicited by MNS was affected by MCP ($F_{2,28}$ = 3.69, p = 0.040, η_p^2 = 0.24), with greater SITs on D14 compared to D2 (mean difference: 2 N, p = 0.031). The reduced SIT on D14 meant that VA_{MNS} was affected by MCP (Figure 4-1C, $F_{2,28}$ = 9.23, p = 0.001, η_p^2 = 0.44) with post hoc tests showing greater VA_{MNS} on D14 compared to D2 (mean difference: 1.9%, p = 0.007), however, there was no difference between D14 and D21 (mean difference: 1.0%, p = 0.059). VA_{TMS} was also affected by MCP (Figure 4-1D, $F_{2,28}$ = 5.89, p = 0.008, η_p^2 = 0.33) with greater values on D14 compared to D21 (mean difference: 3.0%, p = 0.016), however, D14 and D2 were not different (mean difference: 2.5%, p = 0.080). Despite the change in VA_{TMS}, neither of its constituent parts were altered by MCP: ERT ($F_{1.3,15.3}$ = 0.25, p = 0.784, η_p^2 = 0.02) and SIT elicited by TMS ($F_{1.3,15.3}$ = 2.17, p = 0.136, η_p^2 = 0.15).

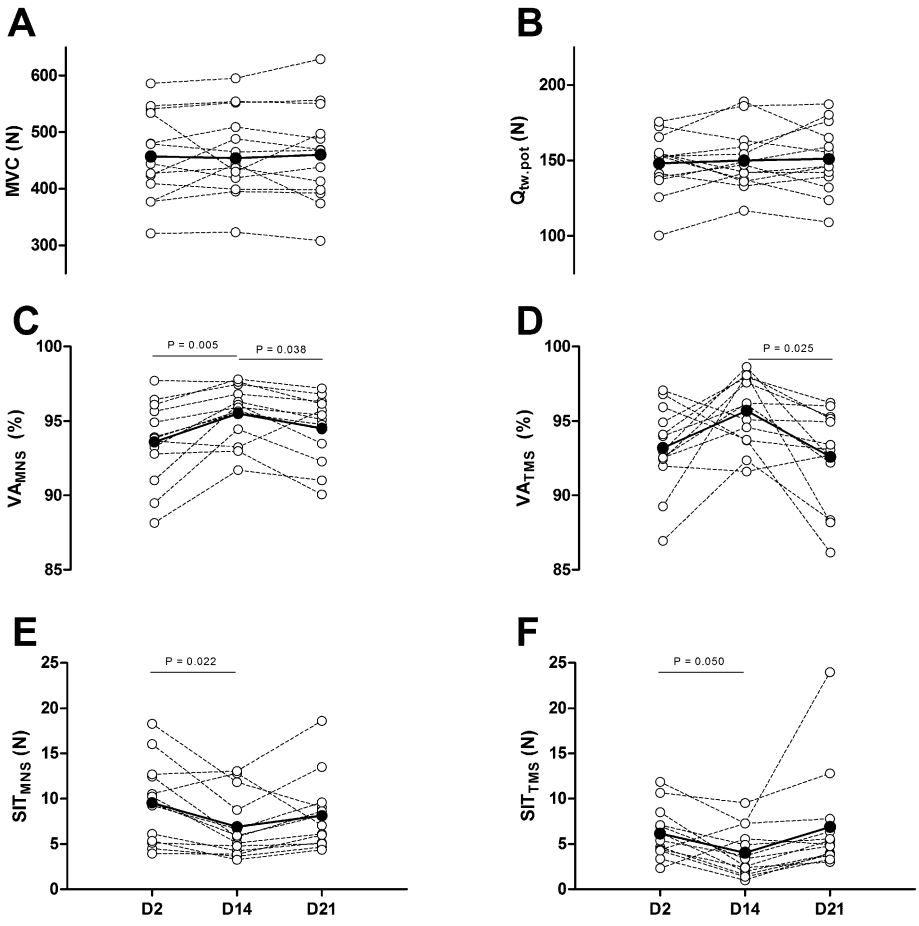

Figure 4-11: Baseline neuromuscular measures across the three time points. Panel A: maximum voluntary contraction; Panel B: potentiated twitch force; Panel C: voluntary activation assessed with motor nerve stimulation; Panel D: voluntary activation assessed; Panel E: superimposed twitch evoked by motor nerve stimulation; Panel F: superimposed twitch evoked by transcranial magnetic stimulation. Individual data are shown with mean data overlaid as the filled symbols and connecting line.

As shown in Table 3, M_{max} was unaffected by MCP ($F_{2,28} = 0.24$, $p = 0.786$, $\eta_p^2 = 0.02$), nor was normalized MEP amplitude (Figure 4-2A, $F_{2,28} = 2.24$, $p = 0.129$, $\eta_p^2 = 0.16$). However, SICI was affected (Table 5 and Figure 4-2B, $F_{1.4, 16.8} = 13.52$, $p < 0.001$, $\eta_p^2 = 0.53$) with post hoc tests showing greater inhibition on D21 compared to D2 (mean difference: −10%, $p = 0.048$) and D14 (mean difference: −14%, $p = 0.001$). The pre-stimulus normalised rmsEMG activity was not different between MCPs (D2: 1.16 ± 0.43, D14: 1.07 ± 0.53, D21: 1.20 ± 0.64% M_{max}, $F_{2,28} = 0.31$, $p = 0.736$, $\eta_p^2 = 0.025$) and neither was the SP (Figure 4-2C, Table 5, $F_{2,28} = 0.53$, $p = 0.594$, $\eta_p^2 = 0.04$).

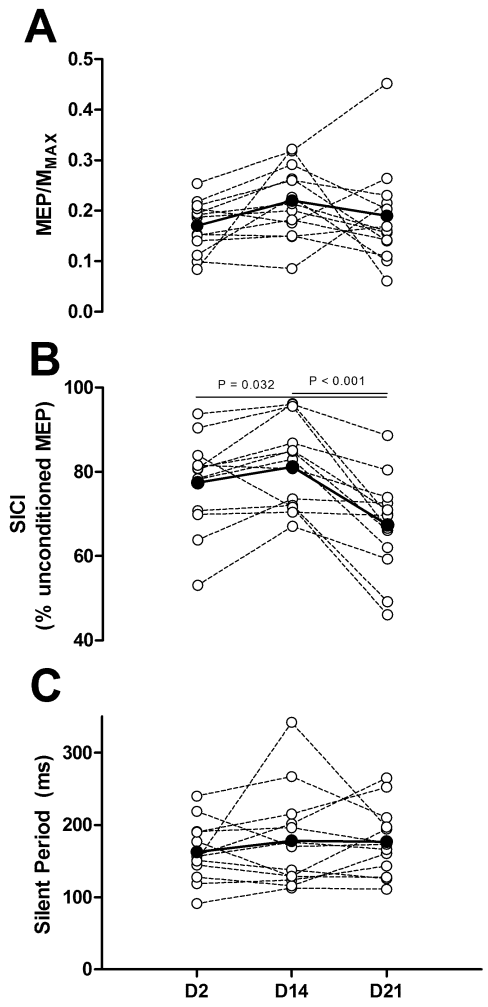

Figure 4-2: Transcranial magnetic stimulation evoked responses across the three testing time points. Panel A: corticospinal excitability; Panel B: short interval cortical inhibition; Panel C: TMS evoked silent period. Individual data are shown with mean data overlaid as the filled symbols and connecting line.

4.2.4. Fatigability

Time to task failure during the intermittent, isometric, fatiguing task was significantly affected by MCP (see Figure 4-3, $F_{1.4, 14.8} = 6.89$, $p = 0.030$, $\eta_p^2 = 0.32$), with post hoc tests showing greater TTF on D21 compared to D2 (mean difference: 187 s, $p = 0.025$). However, there was no difference between D21 and D14 (mean difference: 135 s, $p = 0.103$), or D2 and D14 ($p = 0.594$). The two-way ANOVA (MCP × time) time effect showed that MVC decreased pre-post exercise ($F_{1,11} = 80.056$, $p < 0.001$, $\eta_p^2 = 0.88$), as did $Q_{tw.pot}$ ($F_{1,11} = 123.53$, $p < 0.001$, $\eta_p^2 = 0.92$), VA_{MNS} ($F_{1,11} = 15.219$, $p = 0.002$, $\eta_p^2 = 0.58$), and VA_{TMS} ($F_{1,11} = 13.99$, $p = 0.003$, $\eta_p^2 = 0.56$). SP also increased pre-post exercise ($F_{1,11} = 9.68$, $p = 0.010$, $\eta_p^2 = 0.468$). The MCP × time interaction effects for the aforementioned variables that changed pre-post exercise indicated no difference between MCPs (all $p \geq 0.128$). The only exception to this was VA_{TMS} ($F_{2,22} = 3.48$, $p = 0.049$, $\eta_p^2 = 0.24$), however, post hoc tests revealed that the differences were only apparent pre-exercise (as indicated above), and not post-exercise ($p \geq 0.670$). Despite no time effect ($p = 0.578$), SICI displayed a MCP × time interaction effect ($F_{1.4, 15.0} = 5.26$, $p = 0.028$, $\eta_p^2 = 0.32$), however, the only differences were evident pre-exercise (as indicated above), with no post-exercise difference ($p \geq 0.247$).

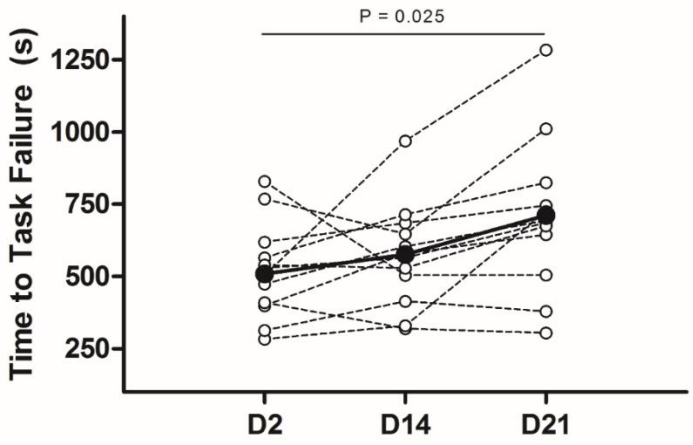

Figure 4-3: Time to task failure during the submaximal intermittent isometric fatiguing task at the three testing time points. Individual data are shown with mean data overlaid as the filled symbols and connecting line.

All variables measured during the fatiguing tasks (see Figure 4-4) demonstrated time effects ($p \leq 0.024$), however, only some (MVC, $Q_{tw.pot}$, VA_{MNS}) demonstrated an absence of MCP × time interaction effects ($p \geq 0.205$). MVC (Figure 4-4A) decreased progressively from baseline to 75% TTF (all intervals $p \leq 0.001$), however, between 75 and 100% TTF no further decrease was observed ($p = 0.776$). A similar pattern was observed with $Q_{tw.pot}$ (Figure 4-4B), with decreases exhibited until 50% TTF (both intervals $p \leq 0.009$), however, between 50-100% TTF $Q_{tw.pot}$ did not further decrease ($p \geq 0.593$). VA_{MNS} (Figure 4-4C) demonstrated the inverse time course, with no change from 0 to 50% TTF ($p \geq 0.345$), then a progressive decrease from 50 to 100% TTF ($p \leq 0.034$). rmsEMG (Figure 4-4D) and RPE (Figure 4-4E) exhibited phase × time interaction effects ($p \leq 0.032$). RPE increased progressively throughout all trials ($p \leq 0.008$), however, at 25% TTF RPE was greater on D21 compared to D14 (+2, $p = 0.006$), at 50% TTF D21 was greater than D2 (+2, $p < 0.001$), and at 75% TTF D21 was greater than D2 (+1, $p = 0.005$). The only significant increase in rmsEMG was between 25 and 50% TTF ($p = 0.003$), and despite the phase × time interaction effect, no post hoc differences between phases were apparent ($p \geq 0.205$).

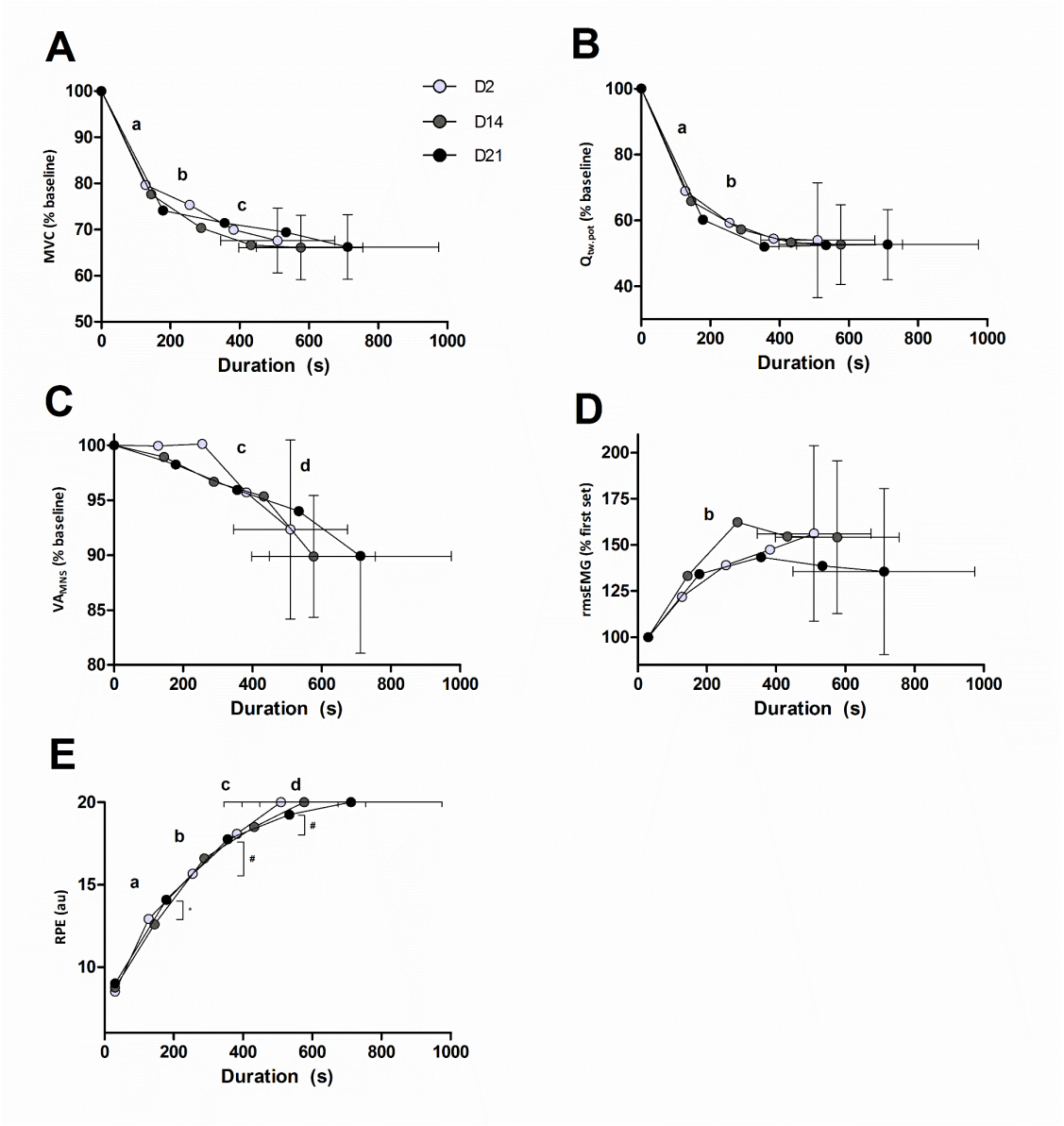

*Figure 4-4: Neuromuscular variables assessed at 25, 50, 75 and 100% TTF throughout the fatiguing tasks in each MCP. Panel A: maximum voluntary contraction; B: potentiated quadriceps twitch; C: voluntary activation assessed with motor nerve stimulation; D: root-mean- squared EMG; E: rating of perceived exertion. Data are means with the standard deviation shown in panels A-D for the final point. Data are displayed as % baseline, although statistical analyses were performed on absolute data. Statistical differences (P < 0.05) are depicted by a: significantly difference between baseline-25% TTF; b: significant difference between 25-50% TTF; c: significant difference between 50-75% TTF; d: significant difference between 75-100% TTF; *: significant difference between D21-D14; #: significant difference between D21-D2.*

*Table 4-2: Variables assessed throughout the pre- and post-exercise testing battery across the menstrual cycle. P values from the baseline ANOVA (1 × 3 repeated measures), and the pre-post exercise ANOVA (2 × 3 repeated measures) are reported. When a significant effect of exercise was found, the Δ in a variable from pre-post exercise was reported. * =greater than day 2, # = greater than day 14, † = greater than day 21.*

	Day 2			Day 14			Day 21			MCP effect	Pre-post exercise	MCP × Exercise
	Pre	**Post**	Δ	**Pre**	**Post**	Δ	**Pre**	**Post**	Δ	**1×3 ANOVA**	**2×3 ANOVA**	**2×3 ANOVA**
MVC (N)	457 ± 79	344 ± 59	−25%	454 ± 78	337 ± 67	−26%	460 ± 87	355 ± 93	−24%	0.790	**<0.001**	0.236
SIT_{MNS} (N)	9 ± 5#	11 ± 7	-	7 ± 4	10 ± 8	-	8 ± 4	11 ± 6	-	**0.040**	0.069	0.452
$Q_{tw.pot}$ (N)	148 ± 20	95 ± 24	−36%	150 ± 20	91 ± 22	−39%	151 ± 23	94 ± 26	−38%	0.782	**<0.001**	0.634
VA_{MNS} (%)	93.4 ± 2.8	88.4 ± 8.8	−6%	95.3 ± 1.9*	87.9 ± 6.9	−8%	94.3 ± 2.2	88.0 ± 5.6	−7%	**0.001**	**0.002**	0.303
SIT_{TMS} (N)	6 ± 2	10 ± 8	67%	5 ± 3	9 ± 7	80%	7 ± 6	10 ± 9	43%	0.136	**0.028**	0.292
ERT (N)	94 ± 43	74 ± 37	−35%	96 ± 39	64 ± 60	−40%	93 ± 43	70 ± 36	−35%	0.784	**0.001**	0.128
VA_{TMS} (%)	93.2 ± 2.8	87.5 ± 8.5	−6%	95.7 ± 2.4†	84.3 ± 9.4	−12%	92.6 ± 3.2	86.7 ± 9.7	−7%	**0.008**	**0.003**	**0.049**
MEP/M_{max} (%)	17 ± 5	17 ± 9	-	22 ± 7	18 ± 9	-	18 ± 10	15 ± 8	-	0.129	0.278	0.485
SICI (%)	77 ± 11*	84 ± 14	-	82 ± 10*	74 ± 22	-	67 ± 12	75 ± 19	-	**0.001**	0.578	**0.028**
SP (ms)	160 ± 42	176 ± 49	12%	173 ± 70	176 ± 54	2%	174 ± 48	176 ± 49	3%	0.594	**0.010**	0.360
M_{max} (mV)	4.05 ± 2.19	3.93 ± 2.27	-	4.39 ± 1.88	4.06 ± 2.12	-	4.30 ± 2.50	3.80 ± 2.28	-	0.786	0.087	0.436
TTF (s)	519 ± 164			571 ± 179			706 ± 262			**0.030**	-	-

MVC: maximum voluntary contraction, SIT_{MNS}: superimposed twitch elicited by motor nerve stimulation; $Q_{tw.pot}$: potentiated quadriceps twitch; VA_{MNS}: voluntary activation assessed with motor nerve stimulation; SIT_{TMS}: superimposed twitch elicited by transcranial magnetic stimulation; ERT: estimated resting twitch; VA_{TMS}: voluntary activation assessed with TMS; MEP/M_{max}: corticospinal excitability; SICI: short-interval cortical inhibition; SP: TMS evoked silent period; M_{max}: maximum compound muscle action potential; TTF: time to task failure.

4.4. Discussion

The present investigation aimed to assess the influence of modulations in female sex hormones across the eumenorrheic menstrual cycle on neuromuscular function and fatigability. The data showed that whilst one index of neuromuscular function (MVC) did not change, females experienced modulations in CNS control of muscle contraction. Specifically, VA was greatest on D14, concurrent with an increase in the concentration of oestrogen. Additionally, parallel to an increase in progesterone, SICI was greatest on D21. Time to task failure during the open-ended intermittent, isometric protocol was greatest on D21 of the cycle. Collectively, the present data suggest that neuromuscular function and fatigability are modulated by the eumenorrheic menstrual cycle.

4.4.1 Maximum strength and voluntary activation across the menstrual cycle

There was no effect of MCP on MVC force. As mentioned, previous data regarding maximum voluntary strength across the menstrual cycle is equivocal. In agreement with the present study, multiple studies have shown no effect (Janse De Jonge *et al.*, 2001; Elliott *et al.*, 2003; Kubo *et al.*, 2009), however, several studies have shown that strength peaks mid-cycle (Sarwar *et al.*, 1996; Birch & Reilly, 2002; Tenan *et al.*, 2016*b*). Previously, discrepancies such as the time of day (Birch & Reilly, 2002), or variability in menstrual cycle duration, as well as the chosen days of the menstrual cycle for testing (Fehring *et al.*, 2006) have been used as explanatory reasons for this discrepancy. The present study controlled both factors by testing at the same time of day and confirming participants were in the correct phase by serum hormone analysis, yet no effect of MCP was observed.

Interestingly, participants demonstrated changes in VA (assessed by both MNS and TMS) despite no change in MVC. VA_{MNS} peaked on D14 and VA_{TMS} was greater on D14 compared

to D21 (see Figure 4-1). As $Q_{tw.pot}$ and ERT were not affected by MCP, these changes in VA were mediated by a decreased SIT amplitude on D14 in response to both motor nerve and motor cortical stimulation. This could indicate that there was a decrease in the capacity of the CNS to elicit extra force in response to stimulation. The TMS and MNS evoked SITs represent the extra force from motor units that the CNS is not able to voluntarily recruit or discharge at a sufficient rate (Todd *et al.*, 2003). As acknowledged by Todd *et al.* (2003), a change in SIT force could be caused by changes in the CNS altering activation of the motoneuron, therefore changes within the motor cortex could provide an explanation for the change in VA. Previous studies that have shown VA_{MNS} not to change have used the central activation ratio equation (Janse De Jonge *et al.*, 2001; Kubo *et al.*, 2009), which is less sensitive to change than the ITT (Place *et al.*, 2007). It is therefore likely that the magnitude of menstrual cycle effect on VA_{MNS} and VA_{TMS} is marginally greater than the random error associated with the techniques used to assess it, thus based on current evidence, the true effect is unclear. Also of note is the MCP × time interaction effect for VA_{TMS}, which would indicate that the magnitude of change from pre-post exercise was different between MCPs. However, this appears to be driven by the increased VA_{TMS} pre-exercise on D14, as there were no differences in post-exercise values. Therefore, it is unlikely that participants experienced a greater degree of CNS adjustment following exercise during the late-follicular phase (D14).

4.4.2. Corticospinal and intracortical function across the menstrual cycle

As previously mentioned, the increase in both measurements of VA on D14 (VA_{MNS} and VA_{TMS}) could represent changes in supraspinal properties altering synaptic drive to the motoneuron pool across the menstrual cycle (Rekling *et al.*, 2000). To investigate the state of the corticospinal tract and motor cortex, the present study employed single- and paired-pulse TMS. No menstrual cycle effect was observed on corticospinal excitability, however, intracortical inhibition was increased on D21. Single pulse MEPs in the resting FDI muscle have previously been shown to be unaffected by oestrogen concentrations (day 1 vs. day 14

of the menstrual cycle, Inghilleri *et al.*, 2004), and the present study extends this conclusion to the active knee extensors, whilst demonstrating that the increase of progesterone concentrations on D21 is not concurrent with changes in corticospinal excitability. When considering paired-pulse responses, however, the increase in progesterone concentrations were concomitant with a ~14% increase in SICI, which when considered with previous evidence (Smith *et al.*, 1999, 2002) was likely through potentiation of $GABA_A$ inhibition. Indeed, GABA agonist pharmacological interventions (e.g. baclofen and gabapentin) have shown similar changes (Ziemann *et al.*, 1996*a*). Interestingly, SICI followed a similar pattern to the E:P ratio (see Table 4), with the only significant change demonstrated on D21 concurrent to a decrease in the E:P ratio. Furthermore, the MCP × time interaction effect for SICI would suggest that intracortical inhibition is differentially modulated by exercise throughout the menstrual cycle. Whilst this is a concept that has been postulated before (El-Sayes *et al.*, 2019), and the present data appears to show this phenomenon, the interaction should be treated with caution as the post exercise reliability of SICI is unknown, therefore further investigation is required to test this hypothesis.

The TMS SP, thought to partly reflect $GABA_B$ inhibitory mechanisms (Chen *et al.*, 1999), was not affected by MCP, supporting previous data recorded in the FDI muscle (Hausmann *et al.*, 2006). However, the conclusion that the menstrual cycle affects only $GABA_A$ neurotransmission cannot be made with the current data, as the SP has a large spinal contribution (Yacyshyn *et al.*, 2016; Škarabot *et al.*, 2019). Additionally, glutamatergic ICF was not measured in the present study, but has previously been shown to be affected by the menstrual cycle, with augmented ICF demonstrated mid-cycle (Smith *et al.*, 1999, 2002). Whilst the causal link between intracortical function and voluntary activation is under researched, it is possible that the adjustments of intracortical circuitry altered the capacity of TMS and MNS to evoke a SIT. For instance, if intracortical excitability was greatest on D14, there may have been a ‘ceiling effect’, meaning the stimulations were not able to induce

additional excitation in the motor cortex, thus, innervating fewer additive motor units during MVCs, and evoking a smaller SIT, and the contrary occurring on D21, when inhibition was greatest. The modulation of neurotransmitters has previously shown to affect VA, with pharmacological increases in noradrenaline (Klass *et al.*, 2016) and serotonin (Kavanagh *et al.*, 2019) resulting in a ~1-2% increase in VA. Indeed the effects of serotonin has been shown to be augmented by oestrogen (Bethea *et al.*, 2002), and inhibited by progesterone (Henderson & Bethea, 2008). Therefore, it is possible that the modulation of inhibitory and facilitatory intracortical circuitry across the menstrual cycle might collectively contribute to the changes in VA_{MNS} and VA_{TMS}.

4.4.3. Fatigability across the menstrual cycle

Fatigability, as measured by the TTF of the open-ended fatiguing protocol, was lowest on D21 (i.e. greatest TTF), thus supporting the findings of Sarwar *et al.* (1996), who showed that fatigue index was lowest in the luteal phase during a three-minute intermittent involuntary contraction protocol. The present data, however, contradicts Janse De Jonge *et al.* (2001), who showed no effect of MCP during voluntary or electrically evoked fatiguing protocols performed with the knee extensors. The differences between tasks could explain these discrepancies. The voluntary task used by Janse De Jonge *et al.* (2001) involved both dynamic knee extension and flexion, rather than a single muscle group. This anisometric, multi-muscle group exercise likely elicits a different pattern of sensory afferent feedback (Gandevia, 2001), and was not open-ended like the present study, which could explain the discrepancies in fatigability. The same reasons might also apply to why the findings of Dibrezzo *et al.* (1991) are inconsistent with the present study, who similarly demonstrated no menstrual cycle effect on fatigue during a set amount of dynamic contractions. Thus, the task employed in the present study likely permitted a greater degree of fatigue to develop, allowing the aforementioned MCP differences to be discerned.

As widely acknowledged, fatigability has both physiological and perceptual components that interact to determine exercise tolerance (Enoka & Duchateau, 2016; Hureau *et al.*, 2018; Thomas *et al.*, 2018). The fatiguing task in the present study involved high intensity (60% MVC) intermittent, isometric contractions, which were assumed to be far greater than the critical torque (~30% MVC, Burnley, 2009; Burnley *et al.*, 2012), and limited by decrements in neuromuscular adjustments (Amann, 2011; Burnley *et al.*, 2012). With no MCP x time interaction effects displayed for neuromuscular variables (MVC, Qtw.pot, and VA), the degree of pre-post exercise adjustment was not different between menstrual cycle phases. Accordingly, one hypothesis for why TTF was longer on D21 could be the influence of neurotransmitter systems on perceptions of fatigue. The present study measured GABAergic inhibition and demonstrated a large increase in SICI on D21 (Figure 4-2B), and it has previously been shown that GABA can have anti-nociceptive properties (Enna & McCarson, 2006) acting as an analgesic (Jasmin *et al.*, 2003). Indeed, it has recently been postulated that "luteal analgesia" occurs in eumenorrheic females when progesterone is elevated, where the affective response to nociceptive pain is reduced due to alterations in functional connectivity in the emotional regulation network (Vincent *et al.*, 2018). Thus, it could be possible that the analgesic effects of enhanced GABAergic neurotransmission permitted participants to continue exercising for a longer period due to a lower perception of pain. However, more evidence is needed to explore the effects of GABAergic inhibition on exercise-induced fatigue.

4.5. Conclusion

The present investigation demonstrated that when eumenorrheic females were tested at three distinct phases of the menstrual cycle the changing hormonal environment coincided with large changes in CNS function, which affected aspects of motor performance of a large locomotor muscle group. Specifically, oestrogen had neuro-excitatory effects that were

associated with an increase in VA on D14, whereas progesterone's neuro-inhibitory effects was concurrent with an increased intracortical inhibition and decreased VA. Additionally, fatigability was modulated by MCP, with the greatest TTF seen on D21, concurrent with an increase in progesterone. Thus, the menstrual cycle elicits changes in neuromuscular function and fatigability in locomotor muscle of eumenorrheic females, a finding that has implications for exercise prescription in female exercisers. Additionally, this evidence highlights a need to control for female endogenous hormone concentrations in future comparisons between males and females, for instance, the subsequent Chapters of this thesis.

CHAPTER 5 – REPEATABILITY OF NEUROMUSCULAR FUNCTION AND FATIGABILITY IN A HORMONALLY-STABLE FEMALE POPULATION

5.1. Introduction

As demonstrated in Chapter 4, neuromuscular function and fatigability of the knee-extensors in eumenorrheic females can change substantially across the menstrual cycle. With the increasing number of studies investigating sex differences in neuromuscular function and fatigability (Temesi *et al.*, 2015; Casamento-Moran *et al.*, 2017; Ansdell *et al.*, 2018*b*; Senefeld *et al.*, 2018), it is clear that in order for the true difference between males and females to be elucidated, sex hormones must be controlled for within and between female subjects. One opportunity to do so in healthy, young females is to test those using monophasic oral contraceptives (mOCP). Whilst it must be acknowledged that the dosage of exogenous sex hormones might differ between brands (Elliott-Sale *et al.*, 2013), the mOCP provides a consistent hormonal dosage throughout the period of consumption to maintain a nadir in circulating endogenous hormone concentrations and preclude ovulation (Frye, 2012). This was evidenced previously, where sex hormone concentrations did not vary during two time points during consumption of the mOCP (Elliott *et al.*, 2005).

One caveat to this suggestion of testing mOCP users is that, despite multiple studies assessing changes in neuromuscular function, the measurement error and repeatability of commonly used neurostimulation variables is yet to be elucidated in females. Without such information, many aspects of research can be impeded. For instance, sample size calculations require estimations of measurement error when determining the minimum number of participants required for a study design; furthermore, meaningful changes of a variable in response to an intervention also cannot be inferred (Hopkins, Schabort, & Hawley, 2001; Hopkins, 2000). This latter point is of particular importance for the field of TMS research, which is notorious for variability in evoked responses (Kiers *et al.*, 1993). Indeed, as recently highlighted by Furlan & Sterr (2018), including indices of measurement error in neurostimulation research is necessary to better elucidate the true effect(s) of an intervention.

While these measures have been reported in healthy populations, only one of these studies has involved females, and reported the reliability of a combined male and female cohort (Temesi *et al.*, 2017*a*). Additionally, the authors did not control for menstrual cycle phase or oral contraceptive usage when testing females on repeated visits 2-14 days apart. As emphasised by Sims & Heather (2018), the combination of sexes into one experimental group is inappropriate, and controlling for changes in the hormonal milieu is necessary for human physiological studies. Sex differences in various aspects of neuromuscular function (Lissek *et al.*, 2007; Tenan *et al.*, 2016*c*; Hoffman *et al.*, 2018*a*; Peng *et al.*, 2018) mean that it is incorrect to assume that previously published reliability data collected in males will also apply to females.

The concept of reliability testing to determine measurement error in exercise science relies upon the ability to limit confounding variables. In a male population this is relatively simple to do, with factors such as prior exercise, nutrition, stimulants and alcohol intake all usually controlled for between visits. However, the menstrual cycle and its associated effects on neuromuscular function and fatigability (see Chapter 4), means that unless sex hormones are the same during each testing session, there is a confounding factor potentially influencing the repeatability of measures. As suggested by Sims and Heather (2018), mOCP users can be used to examine outcome measures without the influence of fluctuations in endogenous hormones. Therefore, to understand the reliability of neuromuscular function tests before and after a fatiguing task in females, independent of fluctuations in the hormone levels typically observed during a eumenorrheic menstrual cycle, the present study aimed to assess test-retest reliability in a population of healthy young females taking a monophasic combined oral contraceptive pill. It was hypothesised that by controlling for neuroactive hormone concentrations, there would be no differences in neuromuscular function between visits, and reliability indices would be comparable to previously published data on male participants.

5.2. Methods

5.2.1. Participants

A total of fifteen healthy young females (mean ± SD age: 23 ± 2 years, stature: 169.7 ± 6.2 cm, body mass: 70.6 ± 8.5 kg) volunteered to take part in the study. Participants reported taking an mOCP for a minimum of six months in the prescribed manner (i.e. a 7 day break every 21 days). A full list of the mOCPs taken by the participants is provided below.

Table 5-1: Monophasic combined oral contraceptive pills taken by the participants.

OCP brand	No. Participants	Synthetic Estrogen	Dosage (μg)	Synthetic Progestin	Dosage (μg)
Rigevidon®	6	Ethinylestradiol	30	Levonorgestrel	150
Cilest®	3	Ethinylestradiol	35	Norgestimate	250
Yasmin®	2	Ethinylestradiol	30	Drospirenone	300
Gedarel®	1	Ethinylestradiol	20	Desogestrel	150
Gedarel®	1	Ethinylestradiol	30	Desogestrel	150
Microgynon®	1	Ethinylestradiol	30	Levonorgestrel	150
Levest®	1	Ethinylestradiol	30	Levonorgestrel	150

5.2.3. Procedures

Participants visited the laboratory three times, completing a familiarisation and two experimental visits. Experimental visits were completed during the final 14 days of the pill cycle, with a minimum of 48 h between visits to allow recovery (Carroll *et al.*, 2016). The time of testing was replicated (±1 hour) for both experimental visits to control for diurnal variations in corticospinal excitability and maximal force production (Tamm *et al.*, 2009). Additionally, participants consumed their mOCP a constant time prior to experimental sessions in order to standardise circulating exogenous oestrogen concentrations between visits. The procedures performed in the familiarisation trial replicated those of the experimental visits.

5.2.4. Experimental Visits

Experimental sessions began with participants completing a standardised voluntary isometric contraction warm up (2 contractions at 25, 50, and 75% perceived maximal effort) followed by a baseline neuromuscular assessment (as described in Chapter 3). The fatiguing task involved sets of intermittent isometric contractions (3 s contraction, 2 s rest at 60% MVC) to task failure. Contractions were paced with an audible metronome to ensure the duty cycle was maintained. One set was defined as 11 submaximal contractions followed by a 3 s MVC with MNS delivered at the peak force and 2 s post, lasting one minute. Task failure (TTF) was defined as an inability to reach the target force three times at any stage of the protocol. Rating of perceived exertion was recorded using a 6-20 scale following each MVC throughout the fatiguing task. Real-time visual force feedback using target forces set as percentages of maximum force was provided to participants on a computer screen to aid a constant force level. The post-task neuromuscular assessment began immediately following task failure.

5.2.5. Transcranial Magnetic Stimulation

Active motor threshold was not different between experimental visits (40 ± 6 vs. 40 ± 7%, p = 0.746). Similarly, the stimulation intensity used for the assessment of VA_{TMS} was not different between visits (67 ± 10 vs. 66 ± 10%, p = 0.737), and activated a large proportion of the agonist motoneuron pool (69 ± 35 vs. 68 ± 36% M_{max} amplitude, p = 0.916). The TMS pulse also concurrently elicited a small MEP in the antagonist muscle group (0.60 ± 0.37 vs. 0.72 ± 0.53 mV, p = 0.106).

5.2.6. Motor Nerve Stimulation

The optimum stimulus intensity for motor nerve stimulation was not different between visits (230 ± 61 vs. 241 ± 65 mA, p = 0.271).

5.2.7. Data Analysis

The regression between TMS-evoked SIT amplitudes and voluntary force for estimation of resting twitch was not significantly linear ($p > 0.05$, $r^2 < 0.85$) in four out of 60 cases. As a result, outliers were identified (as described in Chapter 3) and four out of 300 (1.3%) SITs were excluded. This led to four regressions containing nine data points (one pre-exercise, three post-exercise), rather than ten. The mean ± SD r^2 values were 0.94 ± 0.04 pre- exercise vs. 0.94 ± 0.04 post exercise.

5.2.8. Statistical Analysis

Data are presented as mean ± SD within the text and figures. Normal Gaussian distribution of data was confirmed using the Kolmogorov–Smirnov test. If a violation was detected, the data was logarithmically transformed. The alpha for all statistical tests was set at $p \leq 0.05$. For Study A, between session and pre-post exercise differences were explored using two-way (2 × 2) repeated measures ANOVAs, if assumptions of sphericity were violated, then the Greenhouse-Geisser correction was applied. If significant main or interaction effects were detected, Bonferroni-corrected post hoc tests were performed.

For between session test-retest reliability multiple indices were calculated (paired samples t-tests, typical error, intraclass correlation coefficient, Atkinson & Nevill, 1998; Hopkins, 2000) between the two time points. Typical error (TE) was calculated as the standard deviation of the mean differences divided by the square root of 2. Typical error was expressed as absolute raw values and as a percentage of the mean (coefficient of variation, CV). Intraclass correlation coefficients ($ICC_{3,1}$) were calculated according to Bland & Altman (1986). ICC values were defined as follows: <0.5 = poor, 0.5-0.75 = moderate, 0.75-0.9 = good, >0.9 = excellent (Koo & Li, 2016). Due to the ceiling effect (i.e. all values grouped close to 100%) associated with VA_{MNS} and VA_{TMS}, the ICCs were not calculated (Todd *et al.*, 2004; Clark *et al.*, 2007).

5.3. Results

5.3.1. Exercise performance and pre-post exercise changes

The TTF was not different between experimental trial 1 and 2 (560 ± 275 s and 603 ± 357 s respectively, p = 0.314). When assessing exercise-induced changes in neuromuscular function, the two-way ANOVAs detected no between-trial differences in the change values (trial × time interactions: $p \geq 0.331$), therefore to assess the pre-post exercise change, data from both visits was pooled. The MVC decreased pre-post exercise (time effect: 507 ± 95 vs. 379 ± 85 N; $F_{1,14}$ = 136.66, $p < 0.001$, η^2 = 0.907). Similarly, indices of contractile function ($Q_{tw.pot}$ and ERT) decreased pre-post trial ($Q_{tw.pot}$: 169 ± 24 vs. 109 ± 21 N; $F_{1,14}$ = 92.61, $p < 0.001$, η^2 = 0.869; ERT: 120 ± 36 vs. 93 ± 28 N; $F_{1,14}$ = 19.07, p = 0.001, η^2 = 0.557). Indices of voluntary activation also decreased pre-post trial: VA_{MNS} (93.6 ± 3.2 vs. 85.1 ± 6.8%; $F_{1,14}$ = 36.60, $p < 0.001$, η^2 = 0.723) and VA_{TMS} (94.6 ± 3.1 vs. 83.1 ± 10.6%; $F_{1,14}$ = 20.82, $p < 0.001$, η^2 = 0.598). Corticospinal excitability (MEP/M_{max}) was not different pre-post exercise (p = 0.057). There were no changes in SICI pre-post exercise (p = 0.667), whereas SP duration lengthened post-exercise (189 ± 46 vs. 202 ± 50 ms, $F_{1,14}$ = 5.49, p = 0.034, η^2 = 0.282). Lastly, M_{max} was not different pre-post exercise (p = 0.362).

Table 5-2: Reliability data for mechanical variables pre- and post-exercise. Pre-post change (Δ) is presented when a significant (P < 0.05) change was observed.

Measure		Visit 1	Visit 2	*P*	Bias	TE	CV (%)	ICC (95% CI)
MVC	Pre	502 ± 90	511 ± 103	0.314	−9	23	4.5	0.96 (0.89 - 0.98)
(N)	Post	375 ± 93	383 ± 81	0.356	−8	24	6.2	0.94 (0.82 - 0.98)
	Δ	−128 ± 43	−128 ± 51	0.972	0	30	23.9	0.62 (0.18 - 0.85)
$Q_{tw.pot}$	Pre	168 ± 27	170 ± 22	0.589	−2	9	5.0	0.90 (0.72 - 0.96)
(N)	Post	110 ± 22	110 ± 21	0.942	0	11	10.4	0.75 (0.40 - 0.91)
	Δ	−58 ± 29	−61 ± 21	0.643	−2	12	19.7	0.81 (0.53 - 0.93)
ERT	Pre	121 ± 38	118 ± 34	0.689	3	15	12.5	0.85 (0.62 - 0.95)
(N)	Post	94 ± 30	91 ± 27	0.605	3	14	14.9	0.79 (0.48 - 0.92)
	Δ	−27 ± 25	−27 ± 28	0.943	0	17	62.7	0.63 (0.20 - 0.86)
VA_{TMS}	Pre	94.3 ± 3.3	94.8 ± 2.9	0.679	−0.5	2.8	3.0	-
(%)	Post	82.3 ± 11.1	83.9 ± 10.4	0.406	−1.6	5.1	6.1	-
	Δ	−12.0 ± 11.2	-10.9 ± 9.6	0.573	1.1	5.2	45.4	0.78 (0.46 - 0.92)
VA_{MNS}	Pre	93.6 ± 3.0	93.7 ± 3.2	0.834	−0.1	1.6	1.7	-
(%)	Post	84.5 ± 7.2	85.8 ± 6.7	0.942	−1.3	3.6	4.2	-
	Δ	−9.1 ± 6.0	−7.9 ± 6.1	0.390	1.2	3.7	43.2	0.66 (0.24 - 0.87)
TTF (s)		560 ± 275	603 ± 357	0.338	−43	117	20.0	0.88 (0.69 - 0.96)

MVC: Maximum voluntary contraction; $Q_{tw.pot}$: Potentiated quadriceps twitch; ERT: Estimated resting twitch; VA_{TMS}: Voluntary activation assessed with TMS; VA_{MNS}: Voluntary activation assessed with MNS; TTF: Time to task failure

5.3.2. Reliability of NMF measures

Pre-exercise data from mechanical variables (Table 5-2) showed good (ERT, and TTF) and excellent (MVC, and $Q_{tw,pot}$) reliability. The TE and CV were also low for the majority of variables (CV ≤ 12.5%), except TTF (CV = 20.0%). Post-exercise reliability (Table 2) was slightly weaker, but was still interpreted as predominantly good ($Q_{tw,pot}$, ERT) or excellent (MVC). These values were all larger post-exercise; however, remained low (CV ≤ 14.9%). The relative reliability (ICCs) of the pre-post change was either moderate (MVC, ERT, VA_{MNS}) or good ($Q_{tw,pot}$), however there was a high degree of random error (CV range: 19.7 – 62.7%).

Table 5-3: Reliability values for electromyographical data pre- and post-exercise. Pre-post change (Δ) is presented when a significant (P < 0.05) change was observed.

Measure		Visit 1	Visit 2	*P*	Bias	TE	CV (%)	ICC (95% CI)
VA MEPs	Pre	68.9 ± 33.7	66.0 ± 24.6	0.566	2.88	13.4	19.9	0.82 (0.54 - 0.94)
(%M_{MAX})	Post	68.3 ± 33.9	59.6 ± 26.5	0.079	8.72	12.6	19.7	0.85 (0.61 - 0.95)
	Δ	0.6 ± 30.1	6.5 ± 12.9	0.329	-5.9	15.8	447.4	0.59 (0.13 - 0.84)
MEP10%	Pre	22.4 ± 12.0	19.8 ± 10.5	0.291	2.6	6.40	30.1	0.71 (0.34 - 0.89)
(%M_{MAX})	Post	17.8 ± 9.0	16.9 ± 10.1	0.677	0.9	5.4	31.0	0.72 (0.34 - 0.90)
	Δ	-	-	-	-	-	-	-
SICI	Pre	78.7 ± 15.0	82.9 ± 13.5	0.148	-4.2	7.40	9.2	0.75 (0.42 - 0.91)
(%)	Post	75.3 ± 13.3	84.3 ± 13.3	**0.031**	-9.1	10.4	13.0	0.42 (0.00 - 0.75)
	Δ	-	-	-	-	-	-	-
M_{MAX}	Pre	2.96 ± 1.13	3.08 ± 1.28	0.507	-0.12	0.48	15.9	0.86 (0.64 - 0.92)
(mV)	Post	2.74 ± 1.01	2.88 ± 1.03	0.466	-0.14	0.50	17.8	0.79 (0.47 - 0.92)
	Δ	-	-	-	-	-	-	-
SP	Pre	187 ± 45	190 ± 50	0.791	-3	31	16.4	0.60 (0.24 - 0.82)
(ms)	Post	201 ± 56	202 ± 46	0.947	-8	37	19.2	0.63 (0.19 - 0.86)
	Δ	14 ± 29	12 ± 23	0.815	2	19.8	155.1	0.44 (0.00 - 0.77)

MEP: Motor evoked potential, MVC: maximum voluntary contraction, SICI: Short interval cortical inhibition, M_{MAX}: maximum compound action potential

Surface EMG variables (Table 3) showed moderate (MEP10%, SP) or good (VA MEPs, SICI, M_{MAX}) reliability pre-exercise, however, displayed larger CVs than mechanical variables (range: 9 – 30%). Post-exercise reliability was similar to pre- for most variables, with ICCs either moderate (MEP10%, SP) or good (VA MEPs, M_{MAX}), and comparable CVs (range 13 – 31%). However, the post-exercise reliability of SICI was poor (ICC = 0.42), this was further supported by a significant bias between visits 1 and 2 (−9%, p = 0.031). When the pre-post change was significant for a variable, i.e. SP, the relative reliability of change value was deemed poor (ICC = 0.44), with a high degree of random error (CV: 155%).

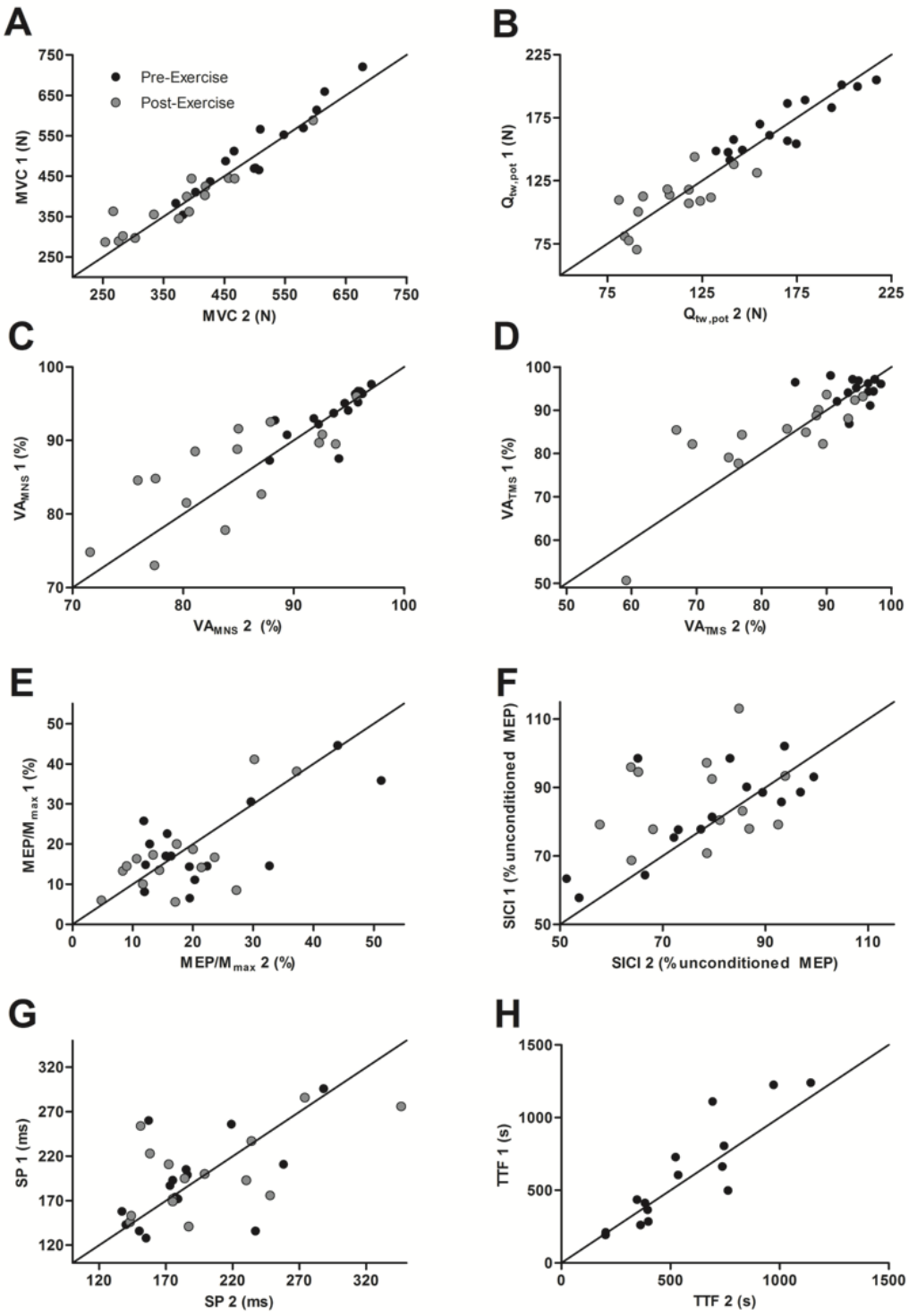

Figure 5-12: Agreement of values from visit 1 and 2 including the line of equality for NMF variables. Panel A: MVC, Panel B: $Q_{tw,pot}$, Panel C: VA_{MNS}, Panel D: VA_{TMS}. Panel E: MEP/M_{max} during a 10% MVC contraction, Panel F: SICI, Panel G: SP, Panel H: TTF. Black circles indicate pre-exercise values, whereas grey circles indicate post-exercise values, apart from panel H, which only contains TTF data.

5.4. Discussion

The present study assessed the test-retest reliability of neuromuscular function assessments before and after a fatiguing task in females taking a monophasic oral contraceptive pill. Previous studies have quantified the test-retest reliability of these measures in male or mixed sex populations; however, it was unclear whether commonly used KE neuromuscular function assessment techniques were reproducible in female populations due to the lack of control for factors such as neuroactive sex hormones that change across the menstrual cycle. The results demonstrate that elements of neuromuscular function can be reliably measured in females when hormones are controlled by taking the mOCP and provide a meaningful tool to assess changes in future experiments investigating neuromuscular function and/or fatigability, whilst contextualising previously reported changes in female populations.

Neuromuscular function was assessed using voluntary contractions and stimulation of nervous tissue. When force-derived variables were considered (Table 5-2), pre-exercise reliability was good to excellent, and similar to previously reported data in other populations. For instance, MVC, VA_{MNS}, VA_{TMS}, ERT, and $Q_{tw.pot}$ had CVs ranging from 2 – 13%, and ICCs ranging from 0.85 – 0.96. The only previous data in females (Hunter *et al.*, 2000) showed MVCs had ICCs of 0.91 and 0.95 in young and older females, respectively. In male populations (Place *et al.*, 2007; Thomas *et al.*, 2014*b*, 2016; Goodall *et al.*, 2017; Ansdell *et al.*, 2018*a*), pre-exercise CVs for the aforementioned force-derived variables ranged from 2 – 14%, and ICCs ranging from 0.70 – 0.96. When the same force-derived measures were performed immediately following the fatiguing task, the reliability was moderate to excellent for all variables (Table 5-2). However, all variables demonstrated poorer reliability than pre-exercise, for instance, the post-exercise CV range for force-derived variables was 4 – 15% and ICCs 0.75 – 0.94. This exercise-induced decrease in reliability is also common in male populations. For instance, Goodall *et al.* (2017) reported CVs of 10 – 18% for MVC, VA_{MNS}, and $Q_{tw.pot}$ at

several stages throughout 120 minutes of simulated soccer activity, whilst Place *et al.* (2007) reported increased variability of VA_{MNS} (CV: 3 to 6%) following a 2-minute MVC. In the present study, when a significant pre-post exercise change occurred, the reliability of the difference (Δ) was measured. All force-derived variables decreased from pre-post exercise, and whilst the relative reliability of these Δ was moderate to good (ICC range: 0.62 – 0.81), the random error was large (CV range: 20 – 63%). To date, no data exists on the reliability of Δ for common neuromuscular function variables. The present data showed that when neuroactive sex hormones are controlled, force-derived variables are a reliable method of quantifying neuromuscular function (MVC), central nervous system (VA_{MNS} and VA_{TMS}) and contractile function ($Q_{tw.pot}$, ERT) in females both pre- and post-fatigue, and the relative reliability (ICC values) of the Δ is also acceptable (Koo and Li, 2016).

Electromyographic variables evoked with TMS of the motor cortex or electrical stimulation of the motor neuron can be used to understand the integrity of different segments of the corticomotor pathway. The present study evoked EMG variables with TMS (MEPs, SICI) and MNS ($M_{max;}$ Table 5-3; pre-exercise, these variables showed moderate to good ICC values (range: 0.60 – 0.86), but large CVs (range: 9.2 – 30.1%). The maximal muscle response to MNS (M_{MAX}), was reliable in a non-fatigued state (CV: 16%, ICC: 0.86) and is comparable to previous data in male populations (CV range: 25 – 29% ICC range: 0.70 – 0.86; Thomas *et al*., 2016, 2014). M_{max} did not change with exercise, and retained good reliability (CV: 18%, ICC: 0.79) post-exercise.

The EMG responses to TMS however, are typically variable due to the fluctuations in the corticospinal and motoneuronal excitability (Kiers *et al.*, 1993; Ellaway *et al.*, 1998). The present study showed the reliability of TMS-assessed corticospinal (MEP ICC: 0.71, CV: 30%) and intracortical (SICI ICC: 0.75, CV: 9%) properties of the knee-extensors are similar to male

and mixed sex populations (ICC range: 0.74 – 0.80, CV range: 11 – 21%; Brownstein et al., 2018; O'Leary, Morris, Collett, & Howells, 2015; Temesi, Ly, & Millet, 2017). Experimental data exists in females for these measures (Smith *et al.*, 1999, 2002), however, these studies were conducted in small muscles of the hand, rather than large locomotor muscles such as the knee-extensors. Additionally, these previous data assessing the influence of changing hormonal state on measures such as SICI, have shown significant effects of sex hormone concentrations. Whereas the present study, that controlled for hormone concentrations, saw no significant difference between trials. Reliability data regarding paired pulse TMS in the knee-extensors is available in male (O'Leary *et al.*, 2015; Brownstein *et al.*, 2018*b*) and mixed-sex groups (Temesi *et al.*, 2017*a*), but to our knowledge, not in a solely female population. The present data established that test-retest reliability is good (CV: 9%, ICC: 0.75) when attempting to induce SICI in the knee-extensors of healthy females pre-exercise. However, whilst the mean SICI did not change following exercise, post-exercise reliability was poor (CV: 13%, ICC: 0.42), with a significant bias (−9%) between trials. These findings question the appropriateness of assessing SICI in the knee-extensors in a fatigued state (Goodall *et al.*, 2018), and similar investigations should test this in male populations.

Another feature of the MEP is the SP, which might reflect a combination of spinal and intracortical $GABA_B$ inhibition (Inghilleri *et al.*, 1993; Chen *et al.*, 1999). The present study measured SP during a 50% MVC contraction, using the stimulus intensity selected for the VA_{TMS} protocol. As mentioned in Chapter 3, these parameters were selected because it was previously shown that higher contraction intensities (>40% MVC) and stimulus intensities elicit a more stable SP duration (Säisänen *et al.*, 2008). In consideration of this, the present study showed that SP was moderately reliable pre- and post-exercise (ICCs: 0.60 and 0.63, respectively) in healthy females; whereas, the increase in SP (~13 ms) from pre-post exercise was not reliable (CV: 155%, ICC: 0.44). Collectively, it would appear that the measurements of neural inhibition used here (SICI and SP) were extremely variable when measured in

fatigued knee-extensors. However, the use of single- and paired-pulse TMS to ascertain cortical and corticospinal excitability in hormonally-constant females taking the mOCP appears to be valid and reliable in the non-fatigued muscle, eliminating previously seen changes due to fluctuating neuroactive hormones.

The primary measure of performance fatigability used in the present study was the TTF for an intermittent, isometric endurance task performed at 60% MVC. The present data showed a CV of 20%, which might be considered large, however, this is consistent with previously published TTF data (~10-30%, Amann, Hopkins, & Marcora, 2008). Additionally, Amann *et al.* (2008) investigated the differences between open-ended TTF tests compared to fixed-distance time trial (TT) performance and demonstrated that despite greater measurement error, TTFs had similar sensitivity to change as TTs, mainly due to the larger magnitude of effect physiological changes have on TTF duration. Therefore, the present data suggests that the TTF test used in the present study was reliable, when sex hormones are controlled and can be used to assess changes in performance of the knee-extensor muscles in female populations. Whilst variability in the TTF during the open-ended fatiguing task could explain the poorer reliability seen in the post-exercise NMF measures, the intensity (60% MVC) was selected so that it was far greater than a critical intensity (~30% MVC, Burnley, 2009; Burnley, Vanhatalo, & Jones, 2012). In theory, and as was demonstrated by the reliable pre-post Δ values (Table 5-2), task failure during constant-load exercise above critical intensity occurs once an individual reaches their respective task-specific maximum tolerable neuromuscular impairment, theoretically giving a similar degree of post-exercise neuromuscular adjustments.

Several studies have utilised MNS and TMS when assessing the NMF of females. Despite the lack of reliability data to assess whether meaningful changes occurred, previous authors have made conclusions about changes in NMF. Temesi *et al.* (2015) compared fatigability of males

and females following an ultra-trail marathon, reporting decreases of 29%, 5%, and 19% for MVC, $Q_{tw,pot}$ and VA_{MNS}, respectively, in females. The changes in MVC and VA_{MNS} were greater than the CVs (MVC: 5%, VA_{MNS}: 2%) in the present study, however, $Q_{tw.pot}$ was not (5%). Thus, the decrease in $Q_{tw.pot}$ in females in must be treated with caution. Data from our own laboratory (Ansdell *et al.*, 2018*b*), showed that females exhibit 15 and 24% decreases in MVC following intermittent isometric fatiguing tasks similar to the present study. As these decreases are greater than the present CVs, greater confidence can be placed in the results. More recently, Senefeld *et al*. (2018) demonstrated a 56 and 23% decrease in MVC, and ~70% and ~20% decrease in $Q_{tw.pot}$ following isometric and dynamic exercise, respectively. These reductions are large and meaningful, as they were greater than the CV demonstrated in our data. Finally, the present study demonstrated decreases in neuromuscular parameters (MVC, $Q_{tw.pot}$, VA_{MNS}, VA_{TMS}, and ERT) that were greater than the CVs for the associated measure (Table 5-2). Therefore, it can be concluded that measures of neuromuscular function are sensitive to detecting changes induced by exercise-related fatigue in female participants.

The data from the present chapter can also be used to contextualise the findings from Chapter 4. For instance, the eumenorrheic menstrual cycle associated changes in VA_{MNS} of 1.9% between D2 and D14, which is larger than the TE of 1.6% reported here. Similarly, in Chapter 4 VA_{TMS} decreased by 3.1% between D14 and D21, which is also larger than the respective TE of 2.8%. Even when more variable measures are considered (i.e. SICI, TE: 7%), the significant changes induced by the menstrual cycle were still larger than measurement error. For instance, D2 and D14 vs. D21 demonstrated 10 and 15% differences, respectively. Likewise, the menstrual cycle associated change in TTF between D2 vs. D21 was 36%, which was larger than the measurement error of 20% reported in this Chapter. Thus, using data from the hormonally-constant female population in the present Chapter, the hormone-induced changes in neuromuscular function exhibited across the menstrual cycle cannot be due to measurement error.

5.5. Conclusion

The present study determined reliability of neuromuscular function measures female population, by controlling for neuroactive sex hormone concentrations using the mOCP. The data showed that the measures investigated were reliable pre- and post-exercise and sensitive to exercise-induced change. The force derived variables such as the MVC, $Q_{tw.pot}$, ERT and VA were more reliable than EMG derived variables including MEPs, SICI, SP, and M_{max}. The data are comparable to previously published data in healthy male and mixed sex populations, due to the fact that hormone concentrations were controlled. The findings from the present study will be used to inform the forthcoming Chapters in this Thesis and contextualise previous findings involving healthy females taking the mOCP.

CHAPTER 6 – A SEX COMPARISON OF FATIGABILITY AND RECOVERY FOLLOWING EXERCISE NORMALISED TO THE INTENSITY-DURATION RELATIONSHIP

6.1. Introduction

As described in Chapter 2, insight into the metabolic demands of a fatiguing task and the mechanisms responsible for the attainment of task failure can be gained by determining the intensity-duration relationship. This relationship has been frequently reported during dynamic tasks (e.g. cycling and knee extension, Jones *et al.*, 2008; Vanhatalo *et al.*, 2010), and a similar relationship exists for intermittent, isometric tasks (Burnley, 2009; Burnley *et al.*, 2012). However, this has been described primarily in young males (Burnley, 2009; Burnley *et al.*, 2012). One study included both sexes, but did not conduct a sex comparison of the intensity-duration relationship (Pethick *et al.*, 2016). It is unknown whether the critical intensity differs between males and females for tasks where the sex difference in fatigability is commonly reported (e.g. intermittent isometric contractions, Hunter *et al.*, 2004; Ansdell *et al.*, 2018) and such an understanding could provide a physiological mechanism to explain previous findings.

A range of reported physiological differences between males and females would suggest the critical intensity could differ between sexes for intermittent isometric tasks. Females are reported to be less fatigable than males across a range of exercise tasks and muscle groups, for contractions performed at the same intensity relative to maximal strength (Hunter, 2009, 2016*a*). The sex difference in fatigability is dependent upon the intensity and contraction modality of the task (Yoon *et al.*, 2007*b*; Russ *et al.*, 2008; Hunter, 2016*b*, 2016*a*). During intermittent isometric contractions, females demonstrate greater fatigue-resistance compared to males, even when strength matched. The magnitude of the sex difference in fatigability might also be magnified at lower contraction intensities (Hunter *et al.*, 2004*b*; Ansdell *et al.*, 2018*b*), but it remains unclear whether the relationship between contraction intensity and task duration (time to task failure, i.e. fatigability) differs between males and females, and whether the underlying neural and contractile mechanisms of fatigue differ. A crucial determinant of the intensity-duration relationship is oxygen delivery to the skeletal muscle, with positive

correlations between critical intensity and the fraction of inspired oxygen (F_IO_2; Vanhatalo *et al.*, 2010; Dekerle *et al.*, 2012). Critical power (during cycling exercise), for example, is positively correlated with type I fibre proportion and muscle capillarity of knee extensor muscles (Vanhatalo *et al.*, 2016; Mitchell *et al.*, 2018*a*). Typically, females have a greater proportion of type I muscle fibres (Simoneau & Bouchard, 1989; Staron *et al.*, 2000; Roepstorff *et al.*, 2006), which are less fatigable than type 2 fibres (Schiaffino & Reggiani, 2011). Females also exhibit greater capillarisation per unit of VL muscle (Roepstorff *et al.*, 2006), and an augmented vasodilatory response of the femoral artery during exercise (Parker *et al.*, 2007). Furthermore, females exhibit greater skeletal muscle oxygenation and less deoxygenation during upper and lower limb exercise than males when assessed using NIRS (Mantooth *et al.*, 2018; Marshall *et al.*, 2019). Whether these physiological sex differences could influence the critical intensity of the intensity–duration relationship for intermittent isometric contraction tasks is unknown.

Finally, recovery of exercise is also influenced by the aforementioned properties of skeletal muscle and could therefore differ between males and females; however, the extent of possible sex differences and the involved mechanisms of neuromuscular recovery are not understood. Limited evidence exists examining the sex difference of recovery for short durations after exercise (10-20 minutes), showing that force producing capacity of female knee extensors recovers more rapidly than males (Senefeld *et al.*, 2018). Greater capillary density of the exercising muscle(s) can increase the rate of recovery from fatigue (Tesch & Wright, 1983; Casey *et al.*, 1996), possibly due to an increased rate of metabolite clearance and ATP/phosphocreatine re-synthesis post-exercise (Casey *et al.*, 1996; McDonough *et al.*, 2004), or a reversal in disruptions to calcium handling (Fitts & Balog, 1996). The latter has been shown to differ between sexes during exercise (Harmer *et al.*, 2014). However, there is a paucity of data relating to sex differences in recovery, and of the neural and contractile mechanisms involved following fatiguing exercise.

The present Chapter had three primary aims: 1) to compare the relative force (% MVC) at which critical intensity is achieved within the intensity-duration relationship for single-limb intermittent, isometric tasks in males and females; 2) determine the mechanisms that contribute to fatigability during intermittent isometric tasks at intensities of torque above and below the critical intensity in males and females; and 3) compare the rate of recovery following fatiguing exercise and the underpinning neuromuscular mechanisms. It was hypothesised that: 1) due to greater oxygen availability within the muscle, females would demonstrate a higher critical intensity than men when expressed relative to MVC. 2) There would be no sex difference in TTF when the tasks were compared at the same metabolic intensity of contraction, relative to critical intensity. 3) Recovery from fatiguing exercise would be more rapid in females than males due to the properties of contractile elements of the muscle.

6.2. Methods

6.2.1. Participants

Using the effect size for the sex difference in exercise tolerance at 50% MVC from Ansdell *et al.* (2018), a power calculation (alpha = 0.05, power 0.80) determined that a sample size of 16 participants was required. Therefore, to maximise statistical power, ten males (mean ± SD age: 26 ± 5 years, stature: 178 ± 8 cm, mass: 83.4 ± 14.4 kg) and ten females (age: 24 ± 2 years, stature: 168 ± 9 cm, mass 68.5 ± 7.7 kg) were recruited to take part in the study. The females that volunteered were all using monophasic oral contraceptive pills (>6 months), and were tested in the 21-day consumption period of the pill cycle in order to negate the effects of endogenous hormones on neuromuscular function and fatigability (see Chapter 4).

6.2.2. Experimental Design

All participants visited the laboratory seven times, completing a familiarisation visit, four constant intensity trials to estimate critical intensity, then trials at 10% above and below critical torque (see *Experimental Protocol*). Testing took place over a three to five week period, with a minimum of 48 h between visits to permit full recovery of fatigue (Carroll *et al.*, 2016). The time of day for each testing session was replicated (± 1 h) to account for diurnal variations in maximal force generating capacity and corticospinal excitability (Tamm *et al.*, 2009).

6.2.3. Experimental Protocol

Visit 1: Familiarisation. Participants were sat in the isometric dynamometer with hip and knee angles at 90˚. This set up was replicated for all visits. Electrical nerve stimulation threshold was determined, followed by TMS hotspot, aMT and VA stimulator intensity determination (described below). Following this, a baseline neuromuscular function assessment was performed. After five minutes of passive rest, participants performed the fatiguing task at 60%

MVC. An MVC and electrical stimulation was performed, each minute throughout the fatiguing task. Immediately following the fatiguing task, participants performed a 'post-exercise' neuromuscular assessment.

6.2.4. Visits 2-5: Critical Intensity Estimation Trials.

To establish critical intensity, participants performed four trials to task failure. These involved intermittent isometric knee-extensor contractions at submaximal intensities between 40-80% MVC. The first trial was set at 60% MVC, based on the pre-exercise MVC in the first trial. The following three estimation trials were set at intensities that elicit task failure between 2 and 15 minutes in a randomised order (Burnley, 2009; Burnley *et al.*, 2012). Participants were instructed to match a target force displayed using a visual guideline on a computer screen ~1 m in front of them, and were blinded to the time elapsed in each trial. The contraction regime for all trials involved 3 s contractions interspersed with 2 s rest, with an MVC and electrical stimulation performed at the end of each minute. This contraction duty cycle has previously displayed sex differences independent of strength, and therefore occlusion differences between males and females (Ansdell et al., 2017; Hunter et al., 2006). Task failure was deemed as a failure to meet the target force three consecutive times despite strong verbal encouragement. Participants were informed each time they failed to reach the target force. Before the submaximal task, participants performed five 3 s MVCs separated by 30 s, with electrical stimulation during and 2 s after the final three contractions. Immediately following task failure this was repeated with three MVCs and superimposed electrical stimulations.

6.2.5. Visits 6 and 7: Critical Intensity Trials.

The supra (+10%) and sub (–10%) critical intensity trials began with electrical nerve stimulation and TMS thresholds being determined. Baseline NIRS values were recorded once participants were sat in the dynamometer, in the same position as the fatiguing task. NIRS

data was captured for the entirety of the trials, and was used to measure changes in muscle oxygenation during the fatiguing task. Cardiac output ($\dot{Q}$) , heart rate (HR), and mean arterial pressure (MAP) were also measured throughout the trial via a fingertip arterial pressure cuff (Finometer Midi, Finapres Medical System, Arnhem, The Netherlands). Participants completed a standardised isometric warm up (Gruet et al., 2014), before a baseline assessment of neuromuscular function. After five minutes of passive rest participants completed an intermittent isometric fatiguing task to failure at an intensity relative to their critical intensity (+10 or −10%). An MVC with electrical stimulation during and ~2 s following was performed and delivered at the end of each minute of the task to assess neuromuscular function (see below). The −10% trial was terminated after 45 minutes, as this intensity contraction could theoretically be maintained indefinitely without task failure (Burnley et al., 2012). Therefore, male and female fatigability was compared after an identical 'dose' of exercise. The intensity for the first critical intensity trial was randomised and counterbalanced. Upon task failure or termination, a post-test neuromuscular function assessment (see below) was immediately performed, then repeated at 15, 30 and 45 minutes post-exercise.

6.2.6. Intensity-Duration Relationship

Critical intensity and curvature constant (W′) were estimated from the force-impulse relationship of the four submaximal trials. A linear regression between force impulse at task failure from the four submaximal trials against time to task failure was plotted to determine the characteristics of the relationship. The slope of the regression determined critical intensity, and the *y*-intercept determined W′ (Burnley et al., 2009, 2012). Critical intensity was expressed in Newtons, and as %MVC to account for sex differences in absolute force production.

6.2.7. Transcranial Magnetic Stimulation

The procedure for aMT determination was carried out according to Section 3.3.2.6. Mean aMT was not different between males and females (39 ± 7 vs. 43 ± 10%, p = 0.379), or between visits (41 ± 9 vs. 41 ± 9%, p = 0.423). Similarly, for VA_{TMS}, the mean stimulator intensity used was not different between males and females (63 ± 6 vs. 66 ± 11%, p = 0.462) or between visits (66 ± 10 vs. 63 ± 7%, p = 0.218). The intensities used activated a large proportion of the motoneuron pool for the RF that was not different between trials at baseline (53 ± 13 vs. 53 ± 16% M_{MAX}, p = 0.920). The TMS pulse also avoided substantial activation of the antagonist (*biceps femoris*) with small incidental MEPs recorded at baseline (0.68 ± 0.52 vs. 0.70 ± 0.1 mV, p = 0.902).

6.2.8. Motor Nerve Stimulation

The procedure for determining optimal stimulus intensity was performed as described in Section 3.3.2.4. Mean stimulus intensity was not different between sexes (276 ± 142 vs. 190 ± 75 mA, p = 0.057) or between visits (241 ± 104 vs. 229 ± 107 mA, p = 0.492).

6.2.9. Near Infrared Spectroscopy

Data was collected according to the procedures outlined in section 3.5.1.

6.2.10. Haemodynamic Monitoring

Mean arterial blood pressure and heart rate were measured continuously throughout the final two testing visits using finger arterial pressure pulse wave analysis (Finometer Midi, Finapres Medical System, Arnhem, The Netherlands). This system was also used to estimate $\dot{Q}$ using the Modelfow equation (Wesseling *et al.*, 1993). An appropriately sized cuff was placed

between the distal proximal inter-phalangeal joint of the middle finger. To minimise the effect of arm and hand movement during the trials, arm position was maintained stationary throughout the trial. To account for hydrostatic pressure differences between the level of the hand and heart, a height correction unit was used. The Finapres was activated prior to the exercise tasks to allow calibration via the Physiocal function within the BeatScope software. This technique has previously been validated and shown to be reliable at rest and in exercise conditions (Parati *et al.*, 1989; Waldron *et al.*, 2017*b*). Signals were linearly interpolated and resampled at 1 Hz (Faisal *et al.*, 2009), then a 5 s rolling average was used to smooth the data (Beltrame *et al.*, 2017), before 30 s time intervals were taken pre-exercise, 25, 50, 75 and 100% of time to task failure. Pre-exercise, participants remained seated for five minutes to establish baseline values, with the final 30 s used as the baseline value.

6.2.11. Data Analysis

In order to achieve significant linearity ($r^2 > 0.80$, $p < 0.05$), a total of five out of 850 SITs across all trials were excluded (0.6%), which led to five regressions containing 9 data points rather than 10 (1 pre-exercise, 4 post-exercise). As a result, mean r^2 values for ERTs were linear throughout the study (0.93 ± 0.06). The NIRS (O_2Hb, HHb, TOI, and cHb) and Finapres (HR, $\dot{Q}$, MAP) data were expressed as a percentage of baseline, and the 30 s epochs throughout exercise are presented as Δ%. Despite a linear relationship between TTF and work done in the estimation trials ($r^2 = 0.98$), and a physiologically normal value for the critical intensity (22.7% MVC), one female participant demonstrated a large 95% confidence interval for the estimate of critical intensity (±13% MVC). As a result, there were no signs of fatigability (i.e. MVC did not decrease from baseline) during the +10% trial, thus the trial was terminated after 90 minutes, and the participant was excluded from further analyses. It was likely the case that this participant was exercising below the 'true' critical intensity. Similarly, one male was excluded due to a large 95% confidence intervals (±12% MVC), which resulted in the intensity-

duration relationship estimates residing >3 SDs from the mean value for males (critical intensity = 31.3% MVC, W′ = 2005 $N \cdot s^{-1}$), likely caused by premature task failure in the higher intensity estimation trial(s).

6.2.12. Statistical Analysis

Data are presented as mean ± SD within the text and figures. Normal Gaussian distribution of data was confirmed using the Kolmogorov–Smirnov test. If a violation was detected, the data was logarithmically transformed. This occurred for rmsEMG/M_{max} during the fatiguing tasks, therefore, statistical tests were run on the transformed data, but in text and figures the non-transformed data is presented. The alpha for all statistical tests was set at $P < 0.05$.

For variables assessed pre-, during, and post- exercise (MVC, VA_{MNS}, $Q_{tw.pot}$, rmsEMG, O_2Hb, HHb, TOI, HR, $\dot{Q}$, and MAP) a two-way (2×5) repeated measures ANOVA was used to assess differences between sex (male vs. female) and over time (Pre, 25, 50, 75% TTF, and Post). For variables that were assessed pre and post-exercise (ERT, VA_{TMS}, M_{MAX}, MEP/M_{max}, SICI) a two-way 2×2 repeated measures ANOVA was used to assess differences between sex (male vs. female) and over time (Pre vs. Post). For variables that were assessed during the recovery period (MVC, VA_{MNS}, $Q_{tw.pot}$, ERT, VA_{TMS}, M_{MAX}, MEP/M, SICI) a two way (2×4) repeated measures ANOVA was used to assess difference between sex (male vs. female) and over time (Post, and 15, 30 and 45 min post-exercise). If significant main or interaction effects were observed, these were followed up by *post-hoc* Bonferroni-corrected pairwise comparisons.

6.3. Results

6.3.1. Intensity-Duration Relationship

The trials to estimate the intensity-duration relationship ranged from 1.6 – 16.0 minutes in duration (Table 1). In order to match the TTFs between sexes, the trial intensities were required to be greater in females than the males (mean difference of 10-11% MVC for the four trials, all $p < 0.001$). Furthermore, the relationship between TTF and impulse across the four trials was linear (r^2 range: 0.89 – 1.00) for all participants (Figure 6-1A).

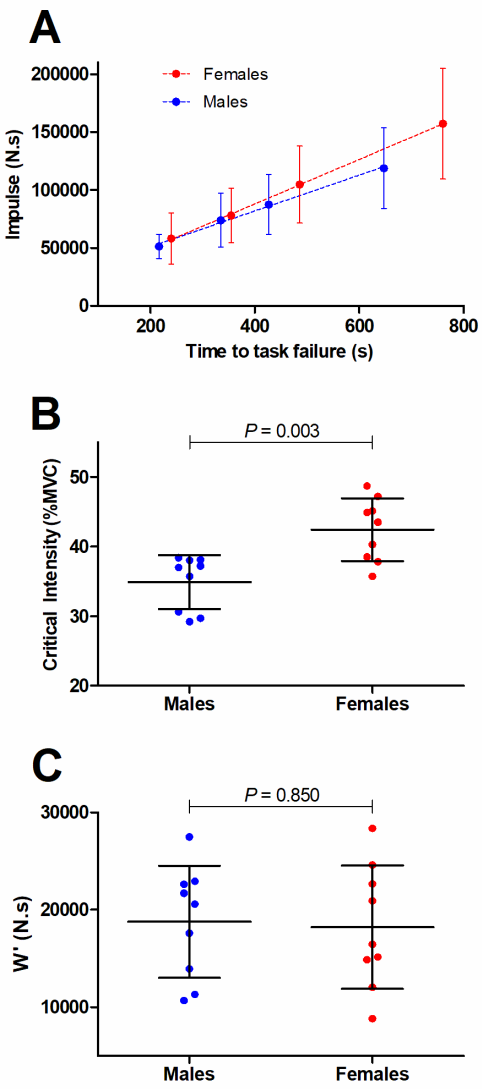

Figure 6-1: Characteristics of the intensity duration relationship for males and females. Panel A: The linear relationships between impulse and time to task failure across the four estimation trials. Panel B: Critical intensities expressed as a percentage of MVC. Panel C: W' in both sexes.

Maximal voluntary contraction was greater in males compared to females (708 ± 119 vs. 458 ± 59 N, $p < 0.001$); however, absolute critical intensity was not different (143 ± 26 vs. 123 ± 26 N, $P = 0.109$). When normalised to MVC, females had a greater critical intensity compared to males (24.7 ± 2.5 vs. 20.8 ± 2.3% MVC, $p = 0.003$, Figure 6-1B), however, there was no difference in W′ (18,206 ± 6,331 vs. 18,765 ± 5,762 N·s, $p = 0.850$, Figure 6-1C). Males and females demonstrated a consistent decline in MVC, $Q_{tw.pot}$, and VA_{MNS} across the four estimation trials (Figure 6-2, Trial × Time interactions $p \geq 0.144$).

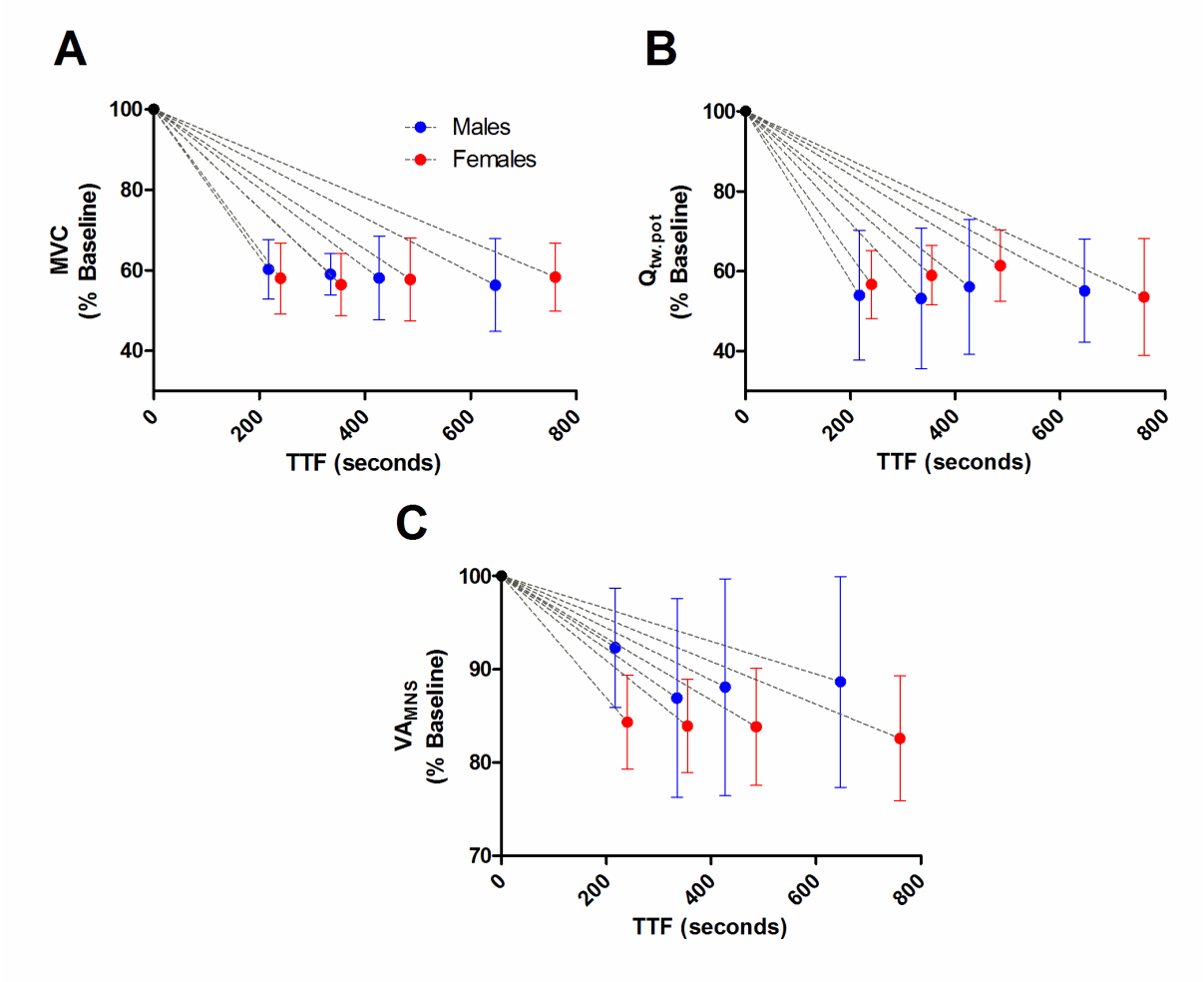

Figure 6-2: Pre-post changes in neuromuscular function across the four estimation trials. A: maximum voluntary contraction (MVC); B: potentiated quadriceps twitch force ($Q_{tw.pot}$); C: voluntary activation (assessed with motor nerve stimulation, VA_{MNS}).

Table 6-1: Intensity, times to task failure, impulse for the critical intensity estimation trials, and confidence intervals for critical torque. Values are mean ± SD

	Males			Females		
Trial	**% MVC**	**TTF (s)**	**Impulse (N·s^{-1})**	**% MVC**	**TTF (s)**	**Impulse (N·s^{-1})**
1	61 ± 2	217 ± 38	50,188 ± 10,600	71 ± 3*	216 ± 139	52,353 ± 22,164
2	56 ± 2	335 ± 102	74,185 ± 27,746	66 ± 3*	355 ± 158	70,105 ± 23,355
3	51 ± 2	427 ± 117	82,614 ± 22,044	61 ± 3*	486 ± 163	94,263 ± 33,207
4	46 ± 2	647 ± 186	120,318 ± 33,089	57 ± 4*	760 ± 148	141,504 ± 47,839
r^2		0.98 ± 0.03			0.99 ± 0.01	

95% CIs (±%MVC)	5.9 ± 4.3	6.8 ± 4.2

95% CIs: 95% confidence intervals for the linear regressions, MVC: maximal voluntary contraction, TTF: time to task failure; * = greater than males ($P < 0.001$)

6.3.2. Intensity-Duration Relationship (Further Considerations)

The intensity for the subsequent exercise trials was originally set as +10% and −10% from the critical intensity noted in the previous section. The intention was to achieve exercise intensities in the severe and heavy domains. However, as was noted by Denadai & Greco (2019), the calculation for critical intensity in both males and females was underestimated. Specifically, the rest period between contractions in the estimation trials was included in the linear regression between 'time to task failure' and 'impulse'. This led to an underestimation of the critical intensity in both sexes when compared to calculations that only incorporate 'contraction time' (Figure 6-3). As this was the case in both sexes, the sex difference in critical intensity remained when the calculation was adjusted (42.4 ± 4.5 vs. 34.9 ± 3.9% MVC*,* $p = 0.003$). However, importantly this meant that the trial intensities for subsequent exercise trials were both below the re-calculated critical intensity (−12 and −20%, respectively). Nevertheless, as described in the subsequent results section, the two trials resulted in 'unsustainable' and 'steady-state' work rates and associated physiological responses. For the purpose of clarity in the remainder of this results section, the +10% and −10% trials are referred to as the 'heavy+' and 'heavy−' intensity trials, respectively.

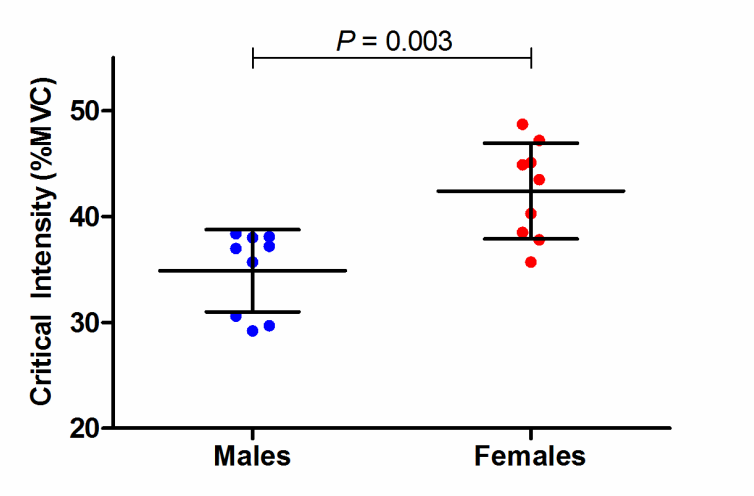

Figure 6-3: Critical intensities for males (blue) and females (red), re-calculated according to Burnley et al. (2009).

6.3.3. Heavy+ Trial

6.3.3.1. Fatigability

Throughout the heavy+ task and at task failure, MVC, $Q_{tw.pot}$, VA_{MNS}, VA_{TMS}, and MEP/M_{max} all decreased (all time effects $p < 0.001$, Figures 6-5 and 6-6), whilst rmsEMG/M_{max} increased ($p < 0.001$, Table 2). However, SICI ($p = 0.232$) and M_{max} ($p = 0.109$) did not change. When comparing the changes between sexes, MVC ($F_{2.2,34.5} = 4.36$, $p = 0.017$, $\eta_p^2 = 0.214$), and $Q_{tw.pot}$ ($F_{4,64} = 2.52$, $p = 0.049$, $\eta_p^2 = 0.136$) decreased more in males compared with the females (Figure 6-5, panel A & B), whilst the rmsEMG/M_{max} increased more in the males than the females ($F_{2.2,34.5} = 7.33$, $p = 0.002$, $\eta_p^2 = 0.314$). However, the change in VA_{MNS}, VA_{TMS}, MEP/M_{max}, and SICI were not different between the sexes ($p \geq 0.062$).

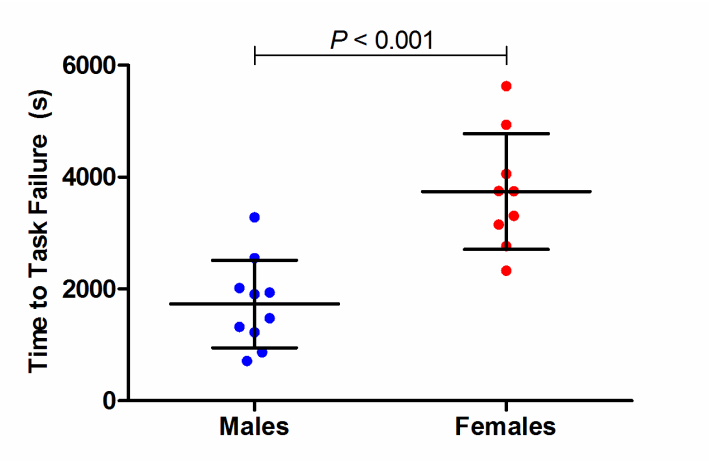

Figure 6-4: Time to task failure during intermittent, isometric knee extensor exercise in the heavy+ trial for males (blue) and females (red). Individual participants are represented as the dots, and group mean and standard deviations are illustrated by the horizontal bars.

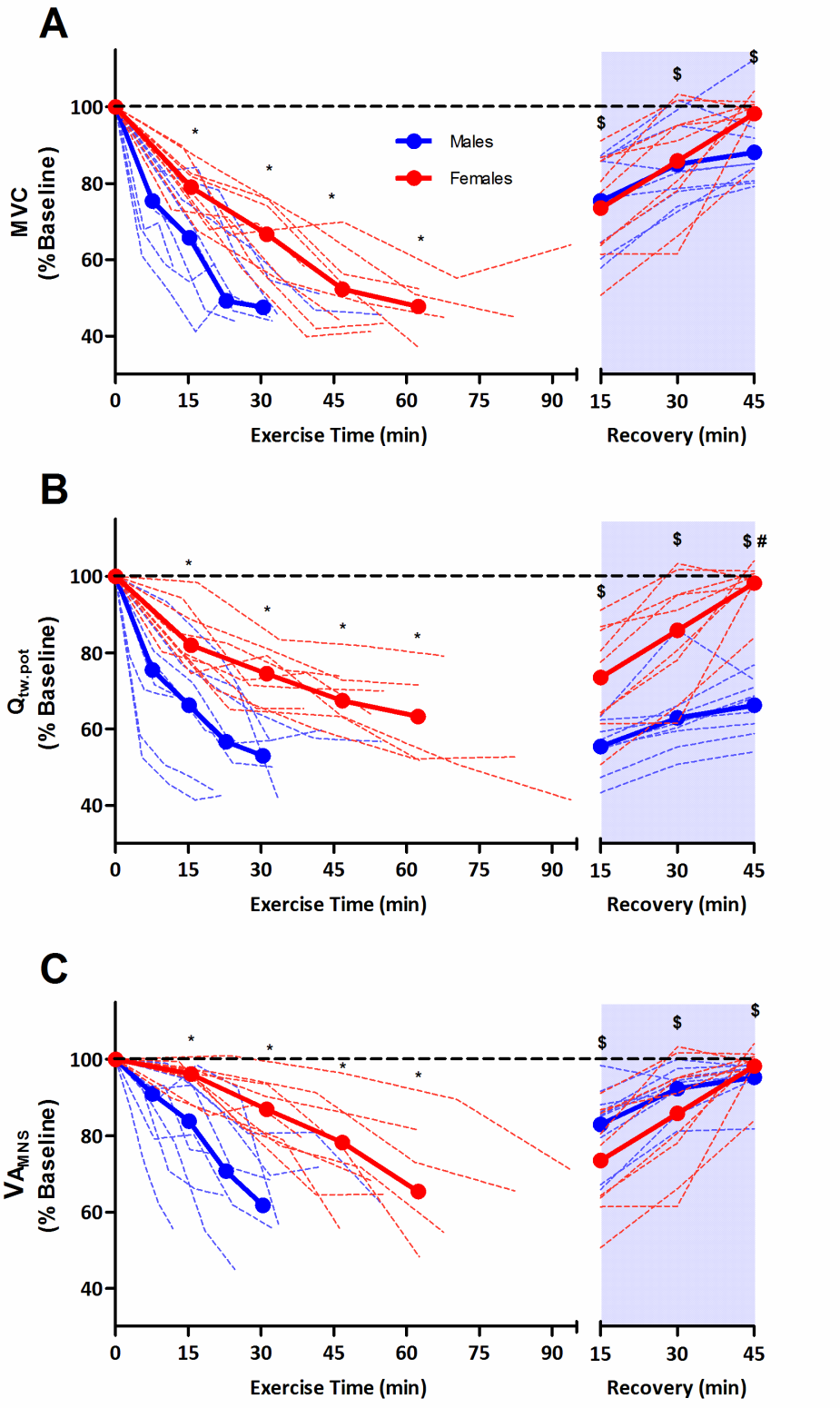

*Figure 6-5: Changes in neuromuscular parameters assessed during the heavy+ exercise task and recovery period. Panel A: maximum voluntary contraction (MVC); Panel B: potentiated quadriceps twitch force ($Q_{tw.pot}$); Panel C: voluntary activation assessed with motor nerve stimulation (VA_{MNS}). Filled lines and circles represent the group mean values, and the dashed lines represent individual participants. * = different from Pre (P < 0.05), $ = different from Post (P < 0.05), # = different between males and females (P < 0.05).*

6.3.3.2. Recovery

In the 45 minute recovery period, MVC, $Q_{tw.pot}$, VA_{MNS}, VA_{TMS}, and MEP/M_{max} all demonstrated a return towards baseline (recovery effects all $p < 0.001$, Figures 6-5 and 6-6). Females however, demonstrated a faster recovery for $Q_{tw.pot}$ ($F_{3,48} = 3.13$, $p = 0.034$, $\eta_p^2 = 0.164$), and VA_{TMS} ($F_{1.8,25.4} = 3.63$, $p = 0.045$, $\eta_p^2 = 0.206$), with no difference in recovery for MVC, VA_{MNS}, or MEP/M_{max} ($p \geq 0.096$).

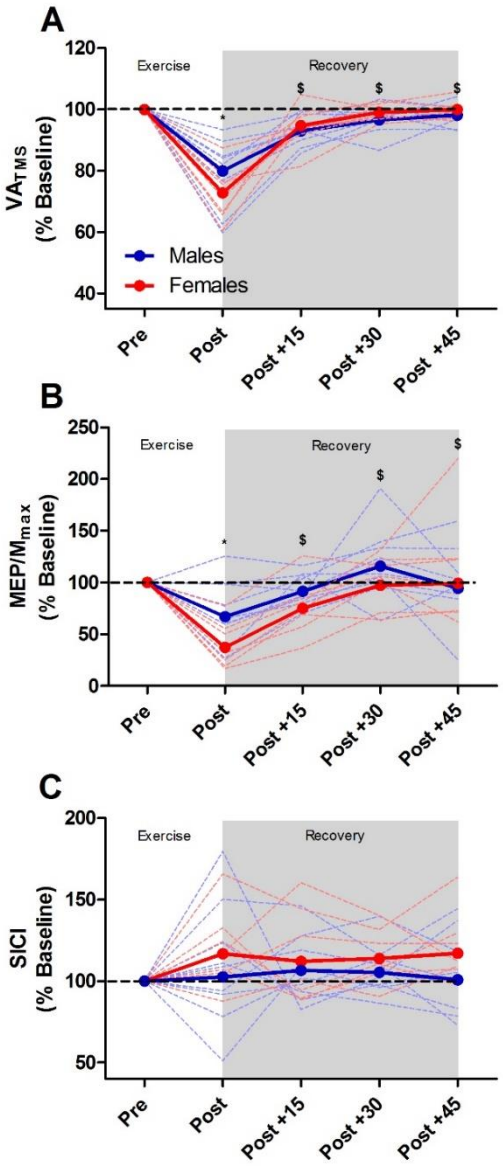

*Figure 6-6: Neuromuscular changes across the heavy+ fatiguing task and the recovery period. A: voluntary activation (transcranial magnetic stimulation, VA_{TMS}); B: motor evoked potentials (normalised to M_{max}, MEP/M_{max}), C: short interval intracortical inhibition (SICI). * = different from Pre ($P < 0.05$), $ = significantly different from Post ($P < 0.05$), # = different between males and females ($P < 0.05$).*

6.3.3.3. Oxygenation and Haemodynamics

Muscle oxygenation was altered during the heavy+ fatiguing task (Figure 6-7), with O_2Hb ($F_{1.4,22.5} = 7.00$, $p = 0.009$, $\eta_p^2 = 0.304$), HHb ($F_{1.4,22.5} = 11.53$, $p = 0.003$, $\eta_p^2 = 0.419$), and TOI ($F_{1.1,18.3} = 7.12$, $p = 0.004$, $\eta_p^2 = 0.393$) all demonstrating changes from baseline. For O_2Hb, females demonstrated an increase from baseline, whilst males decreased ($F_{1.4,22.5} = 8.05$, $p = 0.005$, $\eta_p^2 = 0.335$, Figure 6-7A). Females demonstrated a lesser increase in HHb ($F_{1.4,22.5} = 8.96$, $p = 0.007$, $\eta_p^2 = 0.359$), and decrease in TOI ($F_{1.2,18.3} = 7.12$, $p = 0.013$, $\eta_p^2 = 0.308$) than males (Figure 6-7B and C).

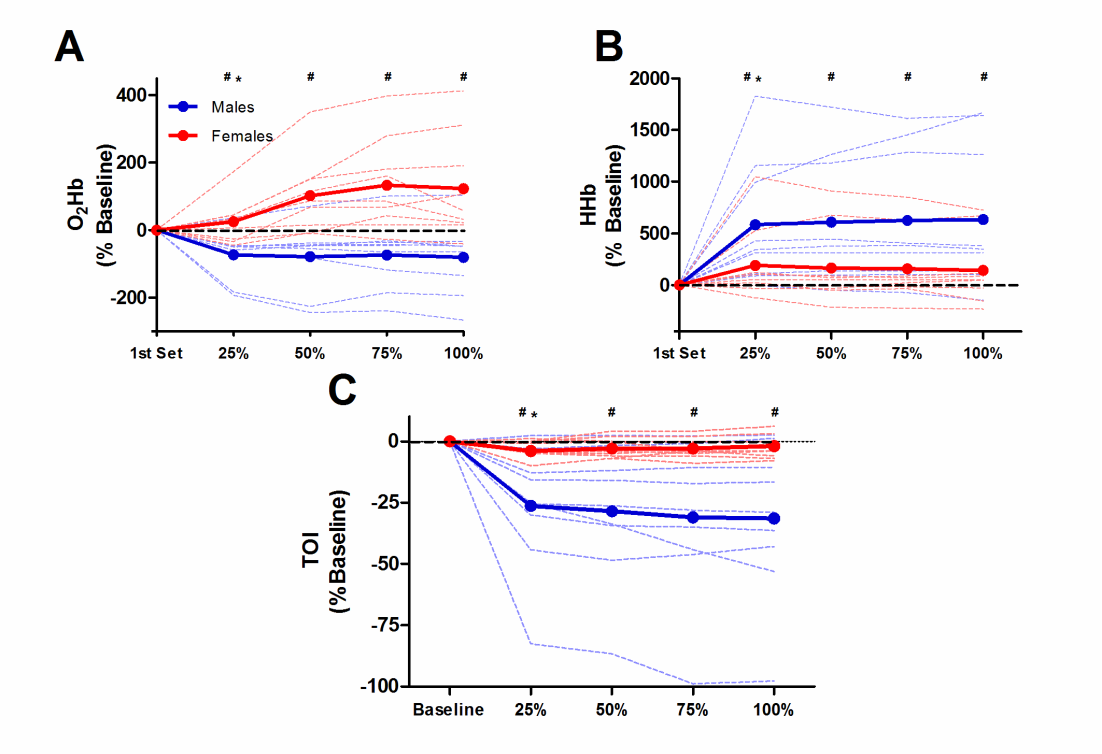

*Figure 6-7: Near Infrared Spectroscopy variables throughout the fatiguing task (heavy+). A: Oxyhaemoglobin (O2Hb), B: Deoxyhaemoglobin (HHb), C: Tissue oxygenation index (TOI). # = significantly different between males and females (P < 0.05), * = significantly different from Pre (P < 0.05).*

The heavy+ fatiguing task induced changes in cardiovascular function (Table 6.2) with HR ($F_{4,64} = 47.39$, $p < 0.001$, $\eta_p^2 = 0.748$), $\dot{Q}$ ($F_{4,64} = 19.70$, $p < 0.001$, $\eta_p^2 = 0.552$), and MAP ($F_{4,64} = 12.24$, $p < 0.001$, $\eta_p^2 = 0.433$) all increasing. Females demonstrated a lesser increase in HR ($F_{4,64} = 8.99$, $p < 0.001$, $\eta_p^2 = 0.360$) and $\dot{Q}$ ($F_{4,64} = 4.02$, $p = 0.006$, $\eta_p^2 = 0.201$), but not MAP ($p = 0.175$).

Table 6-2: Neuromuscular and cardiovascular function throughout the fatigue and recovery periods in both the higher and lower intensity trials.

		Heavy+					Heavy−				
						Neuromuscular Function					
		Pre	***Post***	***Post 15***	***Post 30***	***Post 45***	***Pre***	***Post***	***Post15***	***Post30***	***Post 45***
ERT (N)	Males	167 ± 66	130 ± 67*	135 ± 60$^{\#}$	133 ± 66	132 ± 63	167 ± 63	124 ± 52*	122 ± 47	120 ± 44	110 ± 39
	Females	151 ± 43	101 ± 23*	134 ± 27$^{\#}$	127 ± 23	129 ± 45	149 ± 33	131 ± 37*	123 ± 37	135 ± 39	132 ± 30
M_{MAX} (mV)	Males	6.55 ± 2.57	5.89 ± 2.54	5.86 ± 2.28	5.95 ± 2.51	6.41 ± 2.56	6.33 ± 2.44	5.52 ± 2.58*	5.61 ± 2.39	5.75 ± 2.48	5.19 ± 2.32
	Females	5.18 ± 2.95	4.75 ± 2.28	4.36 ± 2.32	4.47 ± 2.07	4.59 ± 2.80	4.98 ± 2.40	4.39 ± 1.66	4.43 ± 1.57	4.27 ± 1.63	4.2 ± 1.52
Pre-Stimulus rmsEMG (% M_{MAX})	Males	0.62 ± 0.27	0.65 ± 0.28	0.67 ± 0.28	0.70 ± 0.29	0.66 ± 0.30	0.64 ± 0.23	0.84 ± 0.34	0.80 ± 0.33	0.81 ± 0.32	0.90 ± 0.36
	Females	0.75 ± 0.34	0.8 ± 0.77	0.82 ± 0.56	0.87 ± 0.49	0.87 ± 0.64	0.73 ± 0.39	0.85 ± 0.53	0.79 ± 0.54	0.79 ± 0.46	0.77 ± 0.46
		1st Set	***25% TTF***	***50% TTF***	***75% TTF***	***100% TTF***	***1st Set***	***25% TTF***	***50% TTF***	***75% TTF***	***100% TTF***
rmsEMG during task (% M_{MAX})	Males	16 ± 8	22 ± 13*	24 ± 12*	25 ± 10*$^{\$}$	27 ± 11*$^{\$}$	8 ± 3	8 ± 3	8 ± 3	8 ± 4	8 ± 3
	Females	16 ± 5	19 ± 8	17 ± 5	17 ± 4	17± 4	8 ± 4	9 ± 3	9 ± 4	8 ± 4	9 ± 4
						Cardiovascular Function					
		Pre	***25% TTF***	***50% TTF***	***75% TTF***	***100% TTF***	***Pre***	***25% TTF***	***50% TTF***	***75% TTF***	***100% TTF***
Heart Rate (bpm)	Males	78 ± 5	95 ± 12*	99 ± 10*	108 ± 16*	116 ± 16*$^{\$}$	71 ± 11*	88 ± 20*	92 ± 18*	91 ± 19*	91 ± 17*
	Females	80 ± 13	91 ± 18*	94 ± 21*	94 ± 21*	96 ± 19*	81 ± 14	86 ± 13	87 ± 13	87 ± 12	87 ± 12
Cardiac Output ($L \cdot min^{-1}$)	Males	8.1 ± 2.2*$^{\$}$	10.0 ± 2.3*$^{\$}$	10.3 ± 2.0*$^{\$}$	10.6 ± 2.2*$^{\$}$	10.4 ± 2.2*$^{\$}$	6.8 ± 1.6	7.4 ± 1.8	7.5 ± 1.7*	7.6 ± 1.8*	7.6 ± 1.7*
	Females	6.0 ± 1.8	7.2 ± 1.9*	7.0 ± 1.6*	6.9 ± 1.6	6.8 ± 1.5	6.0 ± 1.3	6.2 ± 1.3	6.3 ± 1.4	6.3 ± 1.4	6.3 ± 1.2
Mean Arterial Pressure (mmHg)	Males	90 ± 13	98± 13	100 ± 15	104 ± 18*	107 ± 15*	94 ± 8	94 ± 10	95 ± 13	97 ± 10	101 ± 13
	Females	93 ± 11	104 ± 12*	104 ± 12	101 ± 12	105 ± 11*	93 ± 14	98 ± 15	99 ± 13	100 ± 13	100 ± 15

* = significantly different from Pre ($P < 0.05$), # = significantly different from Post ($P < 0.05$), $^{\$}$ = significantly greater than Females. ERT: estimated resting twitch; M_{max}: maximal compound action potential; rmsEMG: root mean squared EMG; TTF: time to task failure.

6.3.4. Heavy− Trial

6.3.4.1. Fatigability

All participants successfully completed the 45 minutes of exercise at the lower intensity and did not reach task failure. MVC, $Q_{tw.pot}$, M_{max}, VA_{MNS}, and VA_{TMS} all decreased (time effects: $p \leq 0.016$) throughout the intermittent isometric task, whereas rmsEMG/M_{max} ($p = 0.020$), and MEP/M_{max} ($P = 0.017$) increased. Short interval intracortical inhibition did not change ($p = 0.061$). Of these variables, a sex × time interaction was demonstrated for $Q_{tw.pot}$ ($F_{1.97,31.49} = 5.31$, $p = 0.011$, $\eta_p^2 = 0.249$) indicating a lesser decrease in females over the course of the intermittent isometric task. Post-hoc differences are displayed in Figure 6-8 and Table 6-2.

6.3.4.2. Recovery

In the 45 minute recovery period the MVC, $Q_{tw.pot}$, VA_{MNS}, and VA_{TMS} increased (recovery effects: $p \leq 0.032$). Conversely, M_{max} ($p = 0.267$), MEP/M_{max} ($p = 0.080$), and SICI ($p = 0.085$) demonstrated no recovery effects. Of the variables demonstrating recovery effects, VA_{TMS} demonstrated a sex × time interaction ($F_{1.45,20.26} = 4.57$, $p = 0.033$, $\eta_p^2 = 0.246$), indicating a faster recovery in females compared with males. No other variables (MVC, $Q_{tw.pot}$, and VA_{MNS}) demonstrated this sex by time interaction ($p \geq 0.069$).

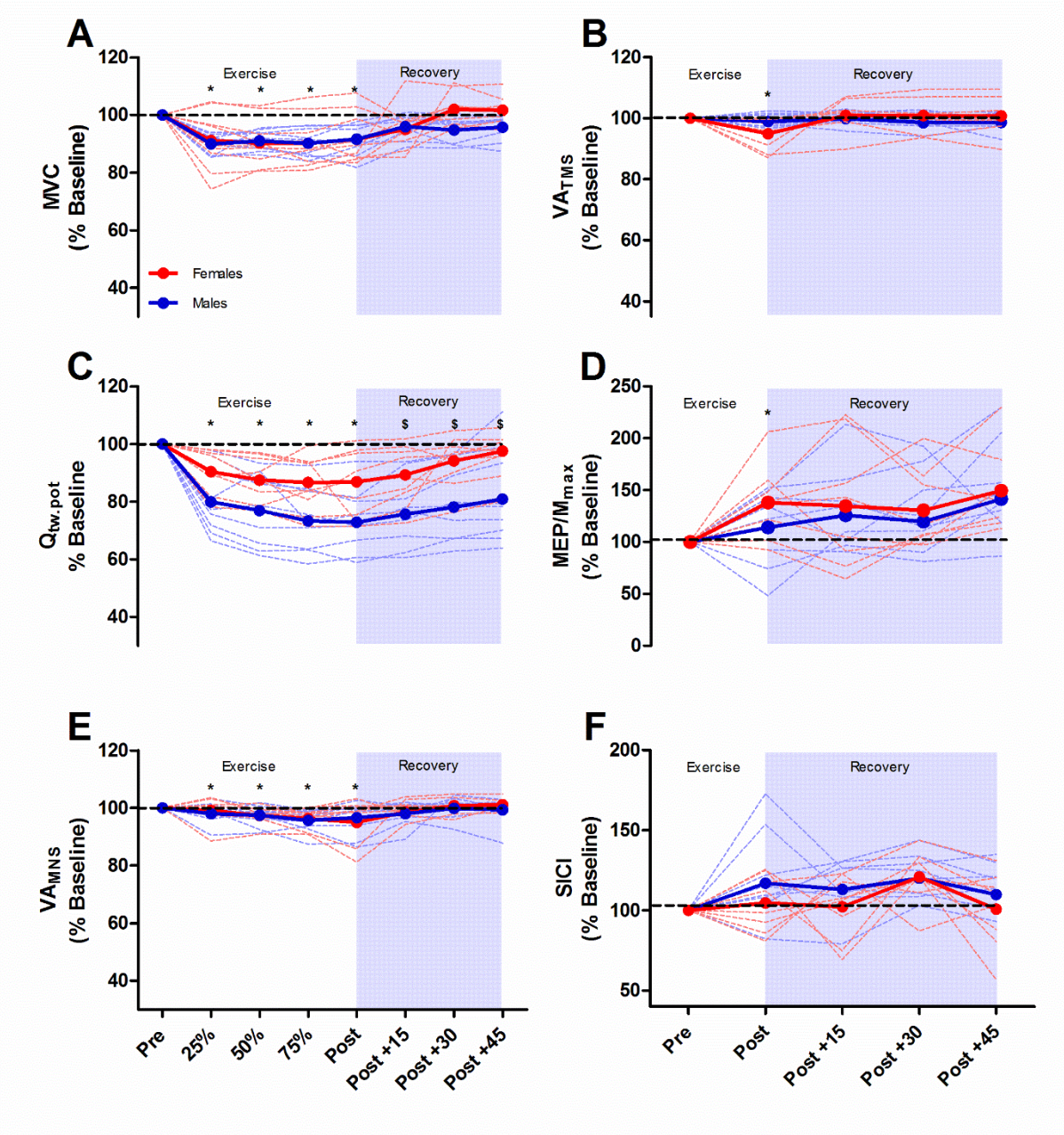

*Figure 6-8: Neuromuscular changes throughout the heavy- task and the recovery period. A: maximal voluntary contraction (MVC), B: voluntary activation measured using transcranial magnetic stimulation (VA_{TMS}), C: potentiated twitch force ($Q_{tw.pot}$), D: motor evoked potential amplitude normalised to M_{MAX}, E: voluntary activation measured using motor nerve stimulation (VA_{MNS}), F: short interval intracortical inhibition (SICI). * = significantly different from Pre ($P < 0.05$), $ = significantly different from Post ($P < 0.05$).*

6.3.4.3. Oxygenation and Haemodynamics

Muscle oxygenation was altered during the intermittent isometric task (Figure 6-9). Whilst O_2Hb ($F_{1.6,26.5} = 10.27$, $p = 0.001$, $\eta_p^2 = 0.391$) increased, HHb did not change ($p = 0.945$), and

TOI decreased ($F_{1.36,21.71}$ = 4.98, p = 0.027, η_p^2 = 0.237). Of these variables, O_2Hb demonstrated a sex × time interaction ($F_{1.64,26.25}$ = 3.77, p = 0.044, η_p^2 = 0.191), indicating a greater increase in females compared with males.

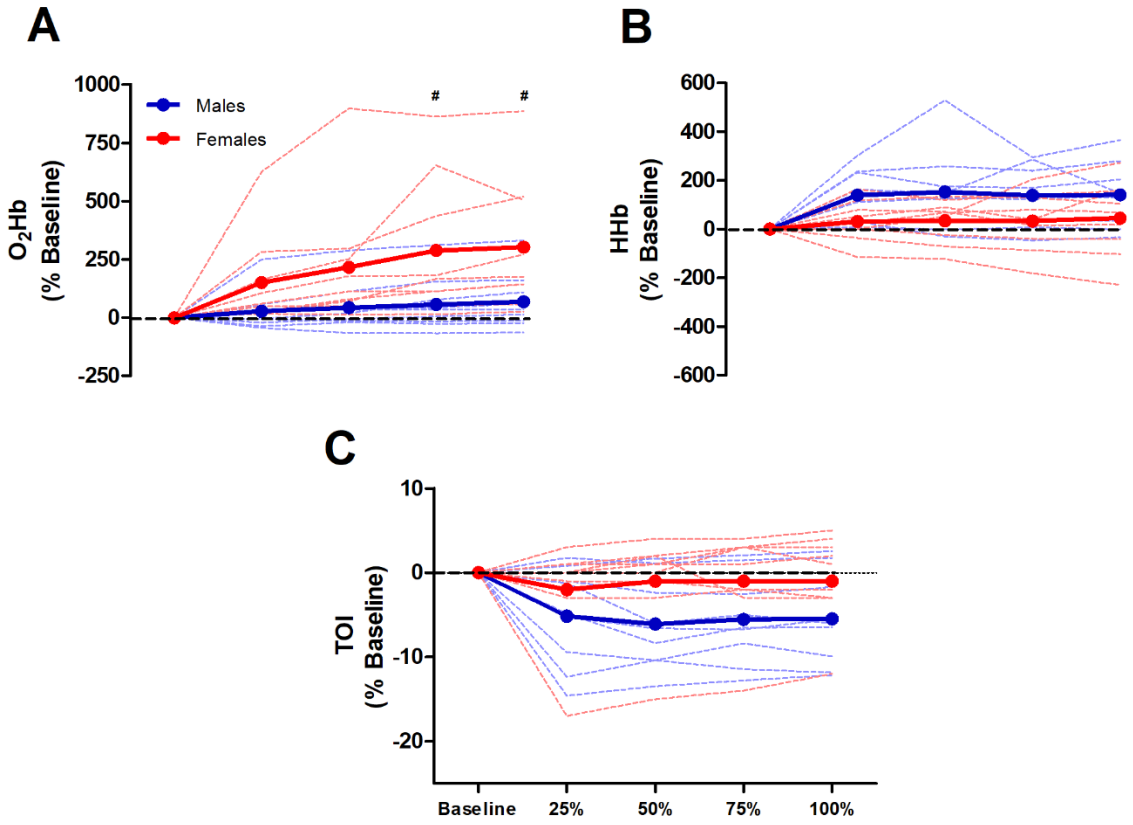

Figure 6-9: Near Infrared Spectroscopy variables throughout the lower intensity task. A: Oxyhaemoglobin, B: Deoxyhaemoglobin, C: Tissue oxygenation index. # = significantly different between males and females (P < 0.05).

Heart rate ($F_{2.6,41.5}$ = 18.42, p < 0.001, η_p^2 = 0.535), $\dot{Q}$ ($F_{2.83,45.23}$ = 4.06, p = 0.014, η_p^2 = 0.380) and MAP ($F_{1.96,31.37}$ = 6.72, p = 0.004, η_p^2 = 0.296) all increased throughout the intermittent isometric task (Table 6.2), with a sex × time interaction for HR ($F_{2.59,41.49}$ = 5.59, p = 0.004, η_p^2 = 0.259), indicating a greater increase in HR in males than females.

6.4. Discussion

The present study aimed to compare the intensity-duration relationship between males and females during intermittent, isometric knee extensor exercise, and assess whether a sex difference in fatigability and recovery existed when exercise was normalised to the critical intensity. Our data show that females demonstrated a greater relative critical intensity during intermittent isometric knee extensor exercise compared with males. Contrary to our hypothesis however, females lasted approximately twice as long than males for open-ended exercise normalised to this threshold. During two exercise trials at higher and lower relative intensities, females demonstrated less contractile impairment, and a faster rate of recovery following the higher intensity trial. Furthermore, these factors are likely related to the fact that females demonstrated lesser deoxygenation during exercise, which provides a plausible explanation for the observed sex differences in exercise tolerance and fatigability.

6.4.1. Intensity-Duration Relationship

Of the two parameters of the intensity-duration relationship, a sex difference was observed for critical intensity, but not W′. Females had a critical threshold ~7% MVC greater than males, due to a steeper slope in the TTF-impulse relationship (Figure 6-1A) and smaller absolute MVC. Critical intensity notes the maximal sustainable metabolic rate during exercise, at which oxidative energy provision is sufficient and reaches a steady state (Poole *et al.*, 2016*a*). Increasing the F_IO_2 during exercise increases critical intensity (Vanhatalo *et al.*, 2010), whereas decreasing F_IO_2 reduces the maximal sustainable intensity (Dekerle *et al.*, 2012). Similarly, complete blood flow occlusion reduces critical power to less than zero (Broxterman *et al.*, 2015*a*). Differences in skeletal muscle properties between males and females could explain the difference in critical intensity. It is well established that in the VL, females possess a greater relative proportion of type I muscle fibres (Simoneau & Bouchard, 1989; Staron *et al.*, 2000; Roepstorff *et al.*, 2006) and greater capillary density (Roepstorff *et al.*, 2006) than

males. When combined with a greater vasodilatory response of the femoral artery to exercise in females (Parker *et al.*, 2007), it is likely that these factors permit greater delivery of oxygenated blood to the muscle tissues of the knee-extensors, contributing to an ability to sustain greater relative rates of oxidative metabolism (i.e. critical intensity) than males. These observations could explain why females were able to attain a higher relative critical intensity than males in the present study. Indeed, recent evidence suggests that type I fibre % and muscle capillarisation are positively correlated with critical power during cycling exercise (Vanhatalo *et al.*, 2016; Mitchell *et al.*, 2018*a*). Mitchell *et al.* (2018) suggested that greater capillary supply likely leads to greater oxygen supply and extraction during exercise. To support this, during the higher intensity trial in the present study, a sex difference was observed for HHb, with females demonstrating lesser increases in deoxygenation (Figures 6-6 and 6-8). Therefore, the present data suggest that females are able to maintain elevated delivery of oxygen to the knee-extensors, leading to a greater relative rate of maximal sustainable oxidative metabolism.

The curvature constant of the intensity-duration relationship (W′), was not different between sexes. Whilst less is known about the origins and determinants of W′ (Poole *et al.*, 2016*a*), evidence suggests that there is no relationship between it and skeletal muscle properties (Vanhatalo *et al.*, 2016; Mitchell *et al.*, 2018*a*). More likely, W′ is related to the depletion of intramuscular energy stores (e.g. phosphocreatine, PCr) and accumulation of metabolites (e.g. Pi, H+, ADP; Vanhatalo *et al.*, 2010). This notion has been suggested to oversimplify such a concept, with the possibility of a different source in W′ between whole-body and single-muscle exercise (Poole *et al.*, 2016*a*). However, in single-muscle exercise, Broxterman *et al.* (2015*b*) suggested that W′ might be related to the maximum tolerable degree of neuromuscular dysfunction. Considering there was no difference in the Δ% in MVC, $Q_{tw.pot}$, and VA_{MNS} between males and females at task-failure in the estimation trials (Figure 6-5), this

notion could explain why W′ was not different between sexes in the intermittent, isometric model used in the present study.

6.4.2. Sex Differences in Fatigability and Recovery

Despite normalising exercise to the intensity-duration relationship, which is a key step when modelling fatigability (Burnley & Jones, 2018), females outlasted males during the open-ended isometric intermittent contraction task (Figure 6-4). In both trials below critical intensity, females experienced a slower rate of decline in MVC, $Q_{tw.pot}$, and VA_{MNS} (Figures 6-5 and 6-8). A similar study in males (Burnley *et al.,* 2012) speculated that the origins of contractile dysfunction below this threshold might be related to the effects glycogen depletion had on calcium transients in skeletal muscle (Ørtenblad *et al.*, 2013). During whole-body exercise, females oxidise relatively more fat than carbohydrate compared to males (Roepstorff *et al.*, 2002, 2006); when combined with the more fatigue-resistant calcium properties in female muscle (Harmer *et al.*, 2014), this could explain why the post-exercise Δ% in $Q_{tw.pot}$ was less (Figure 6-8C). Furthermore, females were better able to maintain oxygen availability within the working muscles (Figure 6-7A), however, this is not thought to be a limiting factor to exercise performance below critical intensity (Poole *et al.*, 2016*a*), as oxidative metabolism is not at maximal rates.

This sex difference in fatigability observed in the present study helps to explain previous findings that showed females outlasting males during intermittent isometric exercise (Ansdell *et al.* 2017). For example, in the 50% MVC task in Ansdell *et al.* (2018), it is likely the case that females were working at ~117% of critical intensity, whereas males were working at ~143%. This greater magnitude of exercise intensity above critical intensity could explicate why females lasted over twice as long as males. Furthermore, during the 30% MVC task in Ansdell *et al.* (2018), females were likely exercising at a lower relative exercise intensity, as

well as developing both peripheral and central fatigue at slower rate within the heavy intensity domain, as the present study demonstrates. Collectively, these factors would explain why after 60 minutes at 30% MVC in Ansdell *et al.* (2018), females demonstrated less than half the reduction in MVC as males.

Following the higher intensity trial, females demonstrated a faster rate of recovery for $Q_{tw.pot}$ (Figure 6-4B), which supports the conclusions of Senefeld *et al.* (2018), who demonstrated a similar pattern following a fixed-duration dynamic fatiguing task. Rapid recovery of contractile function is likely related to the removal of potassium ions from the T-tubules, permitting repolarisation (Allen *et al.*, 2008*a*). Whereas further recovery of contractile function following long-duration isometric exercise is related predominantly to restoration of intracellular calcium handling/sensitivity, rather than metabolite clearance (Carroll *et al.*, 2016). Female skeletal muscle demonstrates a 24% lower maximal rate of Ca^{2+}ATPase activity (Harmer *et al.*, 2014), which has previously been suggested to lead to lower calcium-related impairments during exercise, and create a more fatigue-resistant muscle compared to males (Hunter, 2014). Thus, it could be the case that differences in calcium handling in female skeletal muscle translated to better post-exercise recovery kinetics. Although somewhat speculative, calcium handling has been studied *in vitro* to support the sex difference in fatigability (Harmer *et al.*, 2014), but no similar data *in vivo* exists to assess recovery of calcium handling between males and females after exercise. Therefore, calcium-related properties of skeletal muscle could help explain why female contractile function recovered quicker in the present study, but further research to support this proposition is warranted.

6.4.3. Further Considerations

Despite both exercise trials being below critical intensity, differential profiles of neuromuscular fatigue were observed. Whilst the higher intensity trial was not in the intended ‘severe’ intensity

domain, it did induce an unsustainable work rate, evidenced by progressive declines in MVC, potentiated twitch amplitude ($Q_{tw.pot}$), and voluntary activation (VA_{MNS}), along with increased EMG activity. Considering these responses, and the exercise time of this trial (~30 mins in the male group), it is likely that participants were exercising in the heavy exercise domain (Brickley *et al.,* 2002; Thomas *et al*., 2016). In contrast, the neuromuscular responses to the lower intensity trial indicate a sustainable exercise intensity consistent with the moderate exercise domain, where indices of neuromuscular function reached a far smaller nadir in comparison (Figure 6-8). Thus, our data demonstrate that females are more fatigue-resistant during distinct, metabolically matched exercise at sustainable and unsustainable intensities in the heavy and moderate exercise domains. When these data are considered in the context of the re-calculated critical intensity, the decline in neuromuscular function exhibited in the original "−10%" trial is similar to the CT−20% trial in Burnley *et al.* (2012). Another similarity between the two data sets is that when below critical intensity, neuromuscular function does indeed progressively decline, with both central and peripheral contributing factors. However, this decline occurs at a slower rate than exercise performed above critical intensity, indicating that task failure can indeed occur below this metabolic threshold.

One method to assess fatigability in the 'severe' intensity domain from the present study is to consider the data collected during the estimation trials when determining the critical intensity. By sex-matching the percentage of critical intensity *post-hoc*, we can identify trials at which males (n=8) and females (n=5) were exercising at ~160% critical intensity (Table 6.3). Using this approach, it is clear that when exercise is metabolically matched above critical intensity in the severe domain, females remain more fatigue resistant than males; not only was the time to task failure longer, the rate of decline in $Q_{tw,pot}$ was lower.

Table 6.3: Intensity-matched data demonstrating a sex difference in fatigability at 160% of critical intensity.

Post-hoc matched severe trials							
% critical intensity		**TTF (s)**		**$Q_{tw.pot}$ change (%)**		**Rate of change (%·min^{-1})**	
Male	Female	Male	Female	Male	Female	Male	Female
158 ± 4	159 ± 2	328 ± 142	624 ± 255	−47 ± 17	−31 ± 5	−9 ± 4	−3 ± 1
P value:	0.732		**0.020**		0.066		**0.009**

TTF: time to task failure; $Q_{tw.pot}$ = potentiated quadriceps twitch

To further support the notion that females possess more fatigue-resistant knee extensors, the rise in rmsEMG/M_{max} was smaller compared to males during the higher intensity task (Table 6.2). Despite the known limitations (Farina *et al.*, 2014; Enoka & Duchateau, 2015) associated with surface EMG, increases are suggested to reflect additional neural drive and recruitment of further motor units, as the contractile apparatus become fatigued (Gandevia, 2001). Therefore, the smaller increase in rmsEMG/M_{max} could suggest that female musculature was able to sustain the required intensity with a reduced need for additional neural drive and motor unit activation. This could also explain the smaller decrease in $Q_{tw.pot}$ experienced during the tasks, further supporting the notion that the sex differences in skeletal muscle properties influence fatigability during intermittent isometric exercise. Further research could employ the use of high density EMG, which is capable of discerning motor unit properties (Merletti *et al.*, 2008), without the limitations associated with bipolar surface EMG (Farina *et al.*, 2014; Enoka & Duchateau, 2015).

6.5. Conclusions

The present study is the first to demonstrate that females can sustain a greater relative work intensity compared with males during single limb exercise, as shown by the greater critical intensity. Importantly, when exercise intensity was normalised to this threshold, females out-performed males during the open-ended task, and showed reduced fatigability following exercise performance at a fixed workload. These sex differences in the intensity-duration relationship and fatigue resistance, are likely related to a greater ability to preserve oxygen availability within the knee-extensors during exercise, as demonstrated by the NIRS data. Following exercise, a faster rate of recovery was observed for contractile function in females, suggesting that, in addition to possessing more fatigue-resistant skeletal muscle, females are able to resolve exercise-induced dysfunction at a faster rate. These data explain previous findings related to sex differences in fatigability tasks, whilst providing the first sex-comparison of fatigability during work normalised to a metabolic threshold. Furthermore, the difference between sexes highlights the importance of individualising exercise and recovery prescription to males and females, rather than generalising from previously generated male-only data within the literature. The final experimental chapter of this thesis (Chapter 8) will employ a similar study design, but with cycling as the modality of exercise, in an attempt to see if the present conclusions translate to whole-body exercise.

CHAPTER 7 – METHODS DEVELOPMENT: ELECTRICAL STIMULATION OF CORTICOSPINAL AXONS AT THE LUMBAR SPINAL SEGMENTS

7.1. Introduction

As outlined in Chapter 2, investigating the responsiveness of the CNS is of interest to researchers wishing to discern the central contributions to fatigue. Indeed, as used in previous Chapters of this Thesis (Chapters 4-6), TMS is commonly employed to assess the behaviour of the corticospinal pathway, with changes in the size of the evoked MEP used to form conclusions about exercise-induced changes within the motor pathway. However, any change in MEP size might be due to changes in the excitation or inhibition of cortical neurons or spinal motoneurons (Rossini *et al.*, 2015). Being able to discern the influence of the spinal motoneurons permits researchers to draw conclusions about the 'final common path' of the nervous system (Sherrington, 1906), and differentiate cortical and subcortical modulations of evoked responses.

Typically, subcortical stimulation is performed at the cervicomedullary or thoracic levels of the spinal column, but this can be painful and responses can be difficult to ascertain, particularly when the lower limbs are targeted (McNeil *et al.*, 2013). Indeed, stimulation over the thoracic spinous processes has been shown to evoke responses in quiescent lower limb muscles, but not in all muscles and all participants (Martin *et al.*, 2008). Furthermore, even if responses are evoked, they tend to be small (≤10% of M_{max}; Martin *et al*., 2008). Notably, stimulation of the descending tracts at the mastoid or thoracic level also stimulates motoneurons associated with control of upper limb and trunk musculature (Nathan & Smith, 1982; Nathan *et al.*, 1996*a*) therefore the current applied is likely shared between the motoneuron pool of multiple muscle groups (Kendall *et al.*, 2005). Excitable tissues such as muscle and upper limb nerve roots likely also become depolarised by the large current applied (Taylor, 2006), resulting in contraction of back, neck, shoulder and arm muscles (Martin *et al.*, 2008). These reasons could potentially explain the relative lack of studies investigating changes in spinal cord properties during or following locomotor exercise. Thus, an alternative paradigm that mitigates

the aforementioned technical challenges would be advantageous for investigation of corticospinal behaviour in lower limb muscles.

One alternative technique to discern spinal contributions is the stimulation of peripheral Ia afferent neurons (H-reflex, Nielsen *et al.*, 1999). However, the H-reflex is known to be influenced by pre-synaptic inhibition (Zehr, 2002), which means changes in this variable might not necessarily be due to changes in corticospinal properties. For this reason, direct stimulation of the spinal cord is considered a more appropriate method for assessment of the spinal contribution to the overall corticospinal response. This is reinforced by the fact that the action potentials descending from the motor cortex are attenuated by antidromic collision of those originating from spinal stimulation, indicating activation of some of the same corticospinal axons (Ugawa *et al.*, 1991; Maertens De Noordhout *et al.*, 1992; Taylor *et al.*, 2002; Martin *et al.*, 2008).

A potential solution to the methodological challenge of subcortical stimulation of the corticospinal axons when lower limb muscles are targeted could be stimulation of the lower spinal column. At the lumbar level, the descending tracts contain a greater relative density of motoneurons projecting to lower limb muscles (Sayenko *et al.*, 2015*a*). Stimulation applied closer to these projections will likely result in a higher current density in the motoneuron pool of the lower limbs when compared to mastoid or thoracic stimulation. Indeed, Kuck *et al.* (2017) and Fernandes *et al.* (2018) have shown, via modelling techniques, that when the cathode and anode are placed over the lower thoracic and lumbar spinous processes (T8 – L2), respectively, the highest density of electrical field is concentrated around the spinal cord segments associated with lower limb projections. Furthermore, these modelling studies also indicated that electric field magnitude is likely to be higher in the lateral spinal cord white matter where the lateral corticospinal tract is located. Thus, when targeting the lower limb muscles,

stimuli delivered lower on the spinal tract might provide an alternative methodological paradigm to activate the descending corticospinal axons.

One further consideration for assessing lower limb corticospinal excitability is that responses are commonly evoked during muscle contraction (Sidhu *et al.*, 2011; Brownstein *et al.*, 2018*a*). This is recommended when responses during or following locomotion are of interest (Gruet *et al.*, 2013; Kalmar, 2018*b*; Weavil & Amann, 2018). Thus, it is important to discern how responses to lumbar stimulation behave during different levels or neural drive (i.e. contraction intensity). Similar to MEPs, if lumbar evoked responses (LEPs) change with increasing contraction intensity (Sidhu *et al.*, 2015), it would indicate that the response is primarily mediated by activation of corticospinal tract, and is an appropriate index of excitability during locomotor muscle contraction. When considered within the context of exercise-induced fatigue, excitability of the spinal cord appears to play a role in the development of lower-limb central fatigue (Finn *et al.,* 2018). Therefore, ascertaining the locus of CNS dysfunction following locomotor exercise can provide novel insight into the mechanisms of central fatigue in different populations and/or exercise modalities.

Therefore, this study aimed to explore whether a single electrical stimulus over the first lumbar spinous process (LS) activates descending corticospinal axons innervating lower limb muscles by pairing LS with TMS of the motor cortex at appropriately timed ISIs. It was hypothesised that when the stimuli are paired at intervals shorter than the difference in latencies of each stimulus alone, there will be an occlusion of the response to paired stimulation relative to the response to TMS alone. This technique has been employed previously with cervical and thoracic stimulation to explore similar hypotheses (Taylor *et al.*, 2002; Martin *et al.*, 2008). The responses to TMS and LS alone were also compared during

different contraction intensities. It was hypothesised that the size of responses would be similar with increasing contraction intensities.

7.2. Methods

7.2.1. Participants

Ten healthy, young volunteers (24 ± 4 years, 179 ± 8 cm, 77 ± 12 kg; seven males) participated in the study. As the study was not a repeated measures design, menstrual cycle phase or contraceptive usage was not controlled for in the three female participants.

7.2.2. Experimental Design

Participants visited the laboratory on two separate occasions: a familiarisation visit, then an experimental visit. These trials were separated by a minimum of 24 hours. Participants avoided the consumption of caffeine and performance of intense exercise for 24 h prior to the experimental visit.

7.2.3. Experimental Visit

In the first part of the experiment, with the muscle at rest, TMS and LS were either delivered separately, or paired with different ISIs. Initially, the responses to individual TMS and LS were standardised to elicit a response that was ~10%–15% of the resting M_{max}, and the stimulus intensities required to produce these outputs were then applied during paired stimulation. Paired TMS and LS were delivered either with TMS preceding LS (ISIs from −16 to −2 ms, every 2 ms), both stimuli occurring at the same time (ISI of 0 ms), LS preceding TMS (ISIs from 2 to 14 ms, every 2 ms) or LS and TMS delivered independently of each other (Figure 7-1). Therefore, there were a total of 18 different stimuli, delivered separately in 10 sets, totalling 180 stimulations. The order of each set of stimuli was randomised, with pulses within each set delivered every 5–10 s.

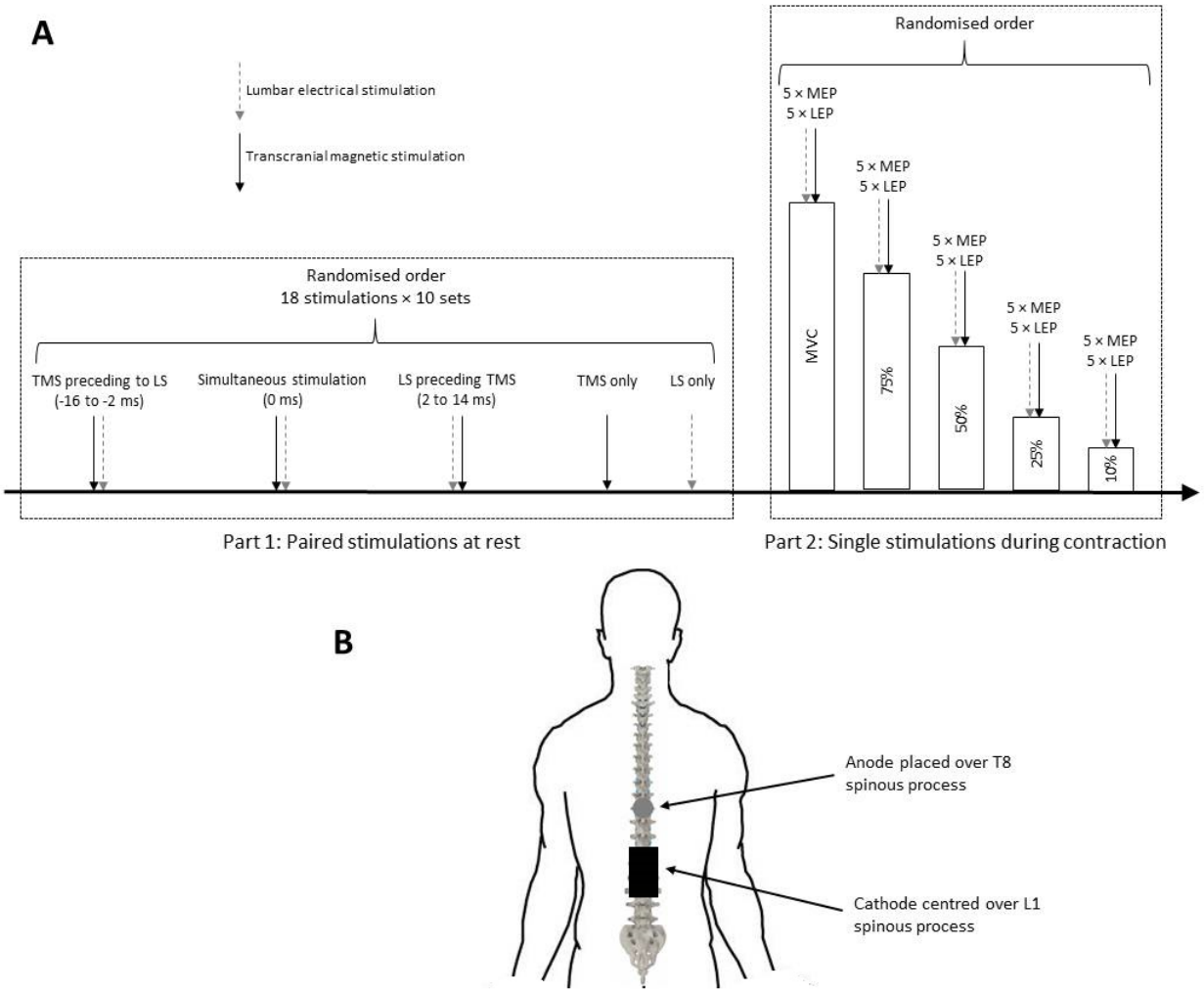

Figure 7-1: A, Experimental approach involved two parts, the first part being comprised of paired stimulation (transcranial magnetic stimulation and lumbar electrical stimulation) at rest at 16 different interstimulus intervals, and the second part consisting of single pulse magnetic and electrical stimulations at 10. 25. 50. 75 and 100% maximal voluntary contraction. B, Lumbar electrical stimulation was performed with cathode centred over L1 and anode placed over T8 spinous process. Based on modelling literature, this configuration is likely to produce the greatest electric field around the area of T10-T12 spinal segments. MEP, motor evoked potential; LEP, lumbar evoked potential.

In the second part of the experiment, participants performed two MVCs, of which the greatest instantaneous force was used to set guidelines for subsequent contractions. TMS and LS were then delivered separately at 10, 25, 50, 75 and 100% MVC (Figure 7-1A). The order of the type of stimulation and the contraction strength was randomised. Five stimuli were performed at each contraction intensity for each type of stimulation, to avoid the influence of decreases in muscle function at higher contraction intensities. Stimulations were delivered once the force had plateaued at the target line. At least 60 s rest was given between each contraction. Initially,

the responses to individual TMS and LS were standardised to ~50% M_{max} during a contraction at 50% MVC, and the stimulus intensities required to produce these outputs remained constant for all contraction intensities. Standardising evoked responses during contraction to a higher percentage of M_{max} than that used when evoking responses at rest was chosen to distinguish the evoked response from background EMG activity, and because this size response is known to be sensitive to change with contraction strength (Sidhu *et al.*, 2015).

7.2.4. Motor Nerve Stimulation

The femoral nerve was stimulated according to the methods outlined in Chapter 3; the optimum stimulus intensity for the experimental visit was 249 ± 88 mA.

7.2.5. Transcranial Magnetic Stimulation

Single-pulse TMS was delivered according to the methods outlined in Chapter 3. In the first part of the experiment, stimuli were delivered during rest at the intensity eliciting a MEP of 10-15% M_{max} (53 ± 19% MSO). In the second part of the experiment, stimuli were delivered at a 50% contraction at the intensity eliciting a MEP of 50% M_{max} (55 ± 10% MSO).

7.2.6. Electrical Stimulation of the Lumbar Spinous Process

Lumbar-evoked potentials were elicited with a constant current stimulator (1 ms pulse duration; Digitimer DS7AH, Hertfordshire, UK) via self-adhesive electrodes (Nidd Valley Medical Ltd., Bordon, UK). The cathode electrode (5 × 9 cm) was centred over the first lumbar (L1) spinous process, with the long axis of the electrode aligned to the centre of the vertebral column (Figure 7-1B). The surface area of the cathode covered two spinous processes above and below the centre point (T11–L3). A cathode of large area was chosen as it produces less discomfort and greater tolerance by participants (Ugawa *et al.*, 1995*b*; Kuhn *et al.*, 2010). The

bottom of the anode (circular shape; 3.2 cm diameter) was placed in the midline of the vertebral column 5 cm above the upper edge of the cathode (Ugawa *et al.*, 1995*b*), corresponding to the level of the eighth thoracic spinous process (T8). Based on modelling studies (Kuck *et al.,* 2017, Fernandes *et al.,* 2017), this electrode configuration was chosen as it is likely to induce the greatest electric field magnitude between T10 and T12 spinous processes due to electric field being highest between the stimulating electrodes (Kuck *et al.*, 2017). As such, the site of greatest spinal cord activation is likely to occur between the L1–L5 spinal segments, corresponding to the motoneuron pools of the *rectus femoris* (Sharrard, 1964; Sayenko *et al.*, 2015*b*). Similar to TMS, the intensity of stimulation was standardised to ~10%–15% M_{max} evoked in the resting position (168 ± 69 mA) and to ~50% M_{max} during a contraction at 50% MVC (145 ± 58 mA).

To ensure ventral roots were not stimulated, responses were monitored for a lack of an abrupt decrease in latency and increase in response size with voluntary contraction (Taylor, 2006). Paired LS was also performed at the target stimulus intensity at an ISI of 50 ms before the start of the main recording session, with the lack of depression of the second response excluding the possibility of stimulation of dorsal roots (Roy *et al.*, 2012; Danner *et al.*, 2016).

7.2.7. Data Analysis

In the experiment assessing the interaction of LS and TMS, the data analysis was similar to that described previously by Taylor *et al.* (2002). Briefly, using a customised script in Spike2 (v8, CED, UK), the waveforms of individual responses to LS alone, TMS alone and paired stimulation were averaged. These are depicted in the example responses (Figure 7-2) during selected ISIs from one individual in the top three rows. The averaged response waveform to LS alone was then temporally aligned to the LS stimulus time point of the averaged response waveform to paired stimulation and graphically subtracted from the latter (Paired—LEP; Figure

7-2, bottom row). After that, the peak-to-peak amplitude of the paired—LEP waveform was calculated and compared to the amplitude of the averaged response to TMS alone ([paired—LEP]/MEP). It has previously been suggested that the subtraction might reveal an inverted potential resulting in negative values, such as in the cases when response to LS is larger than the response to paired stimulation (Taylor *et al.*, 2002), however, this was never the case in the present study.

7.2.8. Statistical Analysis

Paired sample T-tests were used for assessing the statistical significance of the differences in the paired—LEP amplitude relative to the MEP alone amplitude. It should be noted that individuals of different height and thus, different lengths of neural pathways along with reported differences in conduction velocity between individuals (Andreassen & Arendt-Nielsen, 1987; Sadoyama *et al.*, 1988) could confound the interpretation of the interaction of LS and TMS. For that reason, additional analyses were performed to account for this potential disparity. Firstly, the difference in MEP and LEP latency was calculated estimating the time required for the first volley elicited by TMS to reach the segmental level activated by LS. This time (rounded to the nearest 2 ms) was referred to as normalised ISI of 0 ms. Subsequently, the positive and negative normalised ISI values are indicative of the first volley evoked by TMS not having arrived at or having passed the site of descending axon activation by LS respectively (Martin *et al.*, 2008). Due to incomplete number of samples ($n < 10$), statistical analysis using paired sample T-test was not performed for this part of the analyses at normalised ISIs of –6, –4 , 26 and 28 ms.

The variability of individual responses to TMS and LS was assessed by calculating a coefficient of variation for each series of 5 evoked potentials (MEPs or LEPs) for each

individual (CV = standard deviation of 5 evoked potentials ÷ mean of 5 evoked potentials × 100%).

In the experiment assessing the responses with increased contraction strength, peak-to-peak amplitudes of the single pulse evoked responses were calculated, averaged and normalised to M_{max}. Background rmsEMG activity was quantified as the 100-ms epoch prior to stimulus and normalised to MVC rmsEMG. A 2 × 5 repeated measures ANOVA was performed to determine whether contraction strength-response curves and background EMG activity were different. The effect of contraction strength on MEP and LEP latencies was assessed via a one-way repeated-measures ANOVA. If F-values were found to be statistically significant, analysis was continued using pairwise comparison with Bonferroni correction. All statistical analyses were performed in SPSS (v20, SPSS Inc., Chicago, IL, USA).

All data are reported as means ± standard deviations. Significance was set at alpha level of 0.05. To allow for a more nuanced interpretation of the data, Cohen's dz were calculated as an effect size measure for statistical procedures involving paired sample T-tests. Cohen's dz was calculated as the ratio of mean difference and standard deviation of differences, which slightly differs from traditional Cohen's d calculation in that it is better suited for within-subject, rather than traditional between-subject differences (Becker, 1988; Smith & Beretvas, 2009). Partial eta squared (ηp^2) were calculated as a measure of effect size for statistical procedures involving ANOVA.

7.3. Results

7.3.1. Latencies and Variability of Responses

Latencies of MEPs and LEPs at rest and across contraction intensities remained unchanged ($p = 0.081$; Table 7-1). The response variability was greater for MEP compared to LEPs and was reduced in an active muscle compared to rest for both evoked responses (Table 7-1).

Table 7-1: Latencies of evoked potentials in milliseconds, and variability of evoked potentials expressed as coefficients of variations (mean ± SD).

	Latencies (ms)	
	LEP	**MEP**
Rest	9.5 ± 1.1	22.1 ± 1.9
10% MVC	9.0 ± 1.0	20.8 ± 1.9
25% MVC	9.2 ± 1.2	20.4 ± 1.7
50% MVC	9.1 ± 0.9	20.4 ± 1.7
75% MVC	9.3 ± 0.8	20.8 ± 2.0
MVC	9.5 ± 1.2	20.4 ± 1.5
	Variability (%)	
	LEP	**MEP**
Rest	31 ± 22	38 ± 11
10% MVC	13 ± 8	20 ± 13
25% MVC	13 ± 9	30 ± 15
50% MVC	13 ± 6	24 ± 16
75% MVC	16 ± 10	20 ± 13
MVC	17 ± 4	22 ± 7

MVC = maximal voluntary contraction, LEP = lumbar evoked potential, MEP = motor evoked potential.

7.3.2. Interaction of LS and TMS

Representative traces recorded from one individual assessing the interaction of LS and TMS are shown in Figure 7-2. Similar individual behaviour was observed across all participants.

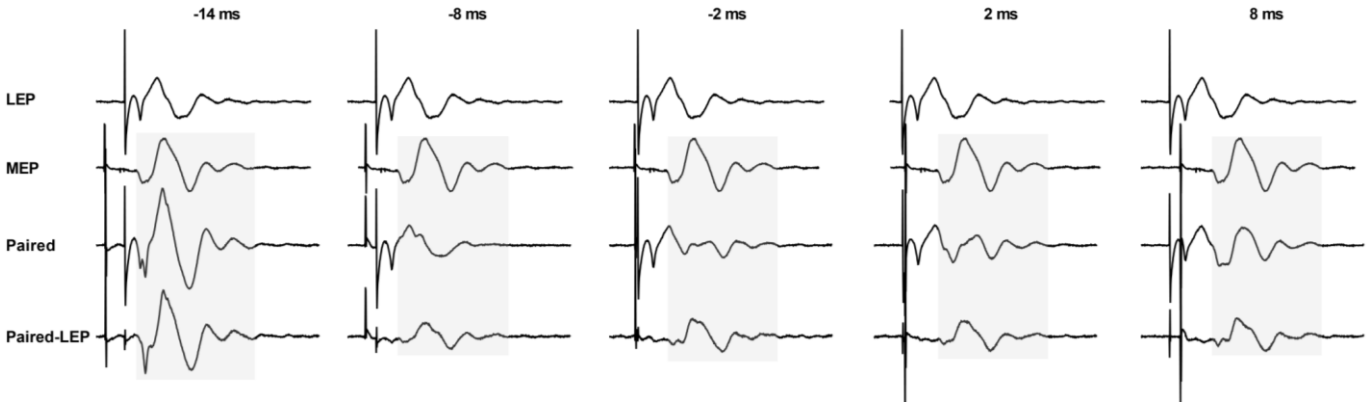

Figure 7-2: Individual traces from a participant best representing the sample mean. Evoked responses to electrical stimulation of the first lumbar spinous process alone (LEP), magnetic stimulation of the cortex alone (MEP), paired stimuli (Paired), and a subtracted response (Paired – LEP) for ISIs of −14, −8, −2, 2 and 8 ms are shown. Each trace is an average waveform of 10 responses. Shaded grey area is drawn for better visualisation of differences.

Transcranial magnetic stimulation alone evoked responses of 15.3 ± 5.8% M_{max}, and LS alone evoked responses of 13.3 ± 3.3% M_{max}. The interaction of TMS and LS resulted in occlusion of responses between ISIs of −8 and 14 ms (*p* value range = 0.001 – 0.049, dz range = 0.5 – 1.4; Figure 7-3A) and facilitation at ISIs of −16 and −14 ms (*p* = 0.031 & 0.011; dz = 0.5 & 0.7). At ISIs of −12 ms, no facilitation was observed at the group level (*p* = 0.119, dz = 0.5); however, on an individual level, 6 participants exhibited facilitation ([Paired – LEP]/MEP > 1.0).

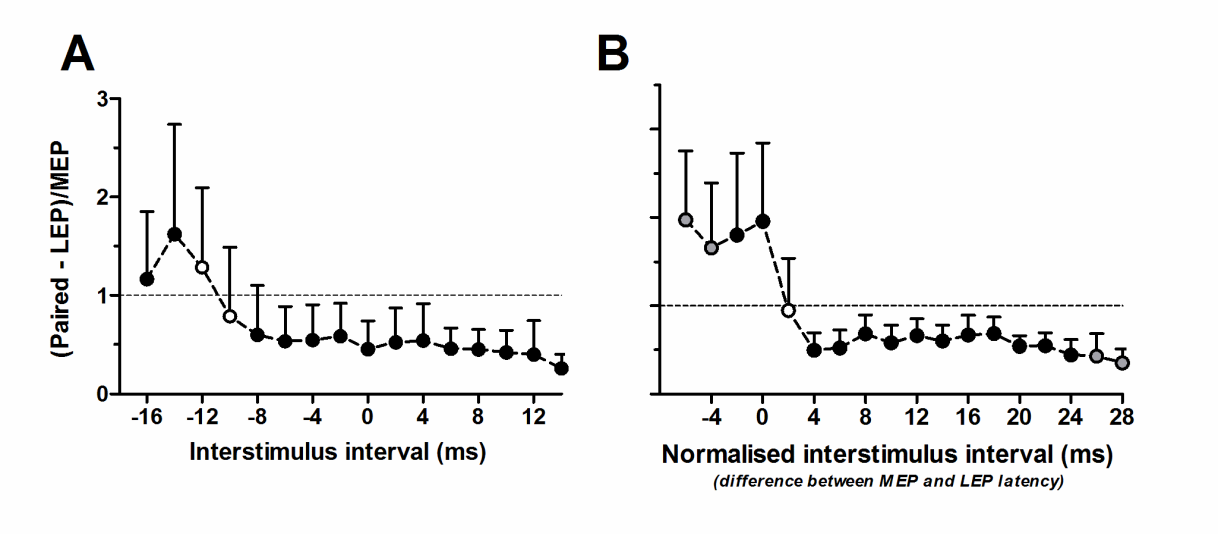

Figure 7-3: Temporal relationship of the interaction between transcranial magnetic stimulation and electrical stimulation over the first lumbar spinous process. Means and standard deviations are shown for the differences in peak-to-peak amplitudes of evoked responses between paired stimulation and electrical stimulus alone and expressed relative to the response to magnetic stimulation for 16 different interstimulus intervals ranging from −16 to 14 ms every 2 ms (Panel A) and normalised interstimulus intervals (Panel B) when lumbar stimulation was delivered before (positive interstimulus intervals), at (0 ms) or after (negative interstimulus intervals) the first volley evoked by transcranial magnetic stimulation was expected to arrive at the lumbar level. Horizontal dashed line represents the size of the response that would be expected if there was no physiological interaction. The black filled circles indicate the response is significantly different from the MEP alone ($p < 0.05$). For interstimulus intervals denoted by the grey filled circles statistical analyses was not performed due to incomplete number of samples ($n < 10$).

7.3.4. The effect of timing of stimuli on interaction of LS and TMS

The responses to paired stimulation were occluded at >2 ms before the expected arrival of the first descending volley evoked by TMS to the segmental level of LS (*p* value range = 0.001 – 0.012, dz range = 0.6 – 1.2; Figure 7-3B). The paired responses were also facilitated when the first descending volley evoked by TMS was at the same level as LS (0 ms, $p = 0.010$; dz = 0.7), and when LS was delivered 2 ms after the expected arrival of the first descending volley evoked by TMS (−2 ms, $p = 0.038$, dz = 0.6).

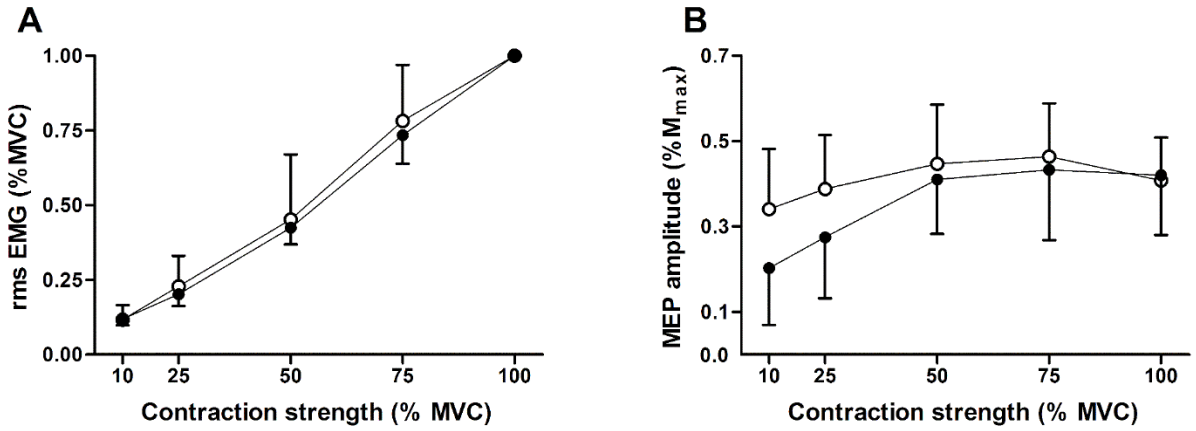

Figure 7-4: Root-mean-square EMG activity during 100-ms epoch prior to stimulus normalised to root-mean-square EMG activity during maximal voluntary contraction (A and B) and the amplitude of responses to motor (filled circles) and lumbar (open circles) evoked potentials at different contraction strengths (C and D).

7.3.5. Responses with increases in contraction strength

Background muscle activity increased progressively from 10 – 100% MVC ($F_{2.1, 18.9} = 318.4$, $p < 0.001$, $\eta p^2 = 0.97$; Figure 7-4A). There was also a contraction intensity effect on MEPs and LEPs ($F_{1.6, 7.8} = 11.9$, $p = 0.001$, $\eta p^2 = 0.57$) insofar as the responses peaked at 50% MVC (Figure 7-4B). There was no interaction between contraction strength and type of stimulus ($F_{1.2, 5.8} = 1.9$, $p = 0.125$, $\eta p^2 = 0.18$).

7.4. Discussion

The results of this experiment show that the response to paired magnetic cortical and electrical stimulation of the lumbar spinal segments is occluded at appropriate interstimulus intervals and that responses to TMS and LS similarly increase with increases in contraction strength with no change in onset latency. This behaviour suggests that LS and TMS activate some of the same corticospinal axons and that responses to LS are evoked transsynaptically with a monosynaptic component. Thus, this stimulation technique has applicability as an alternative

paradigm for investigating the contribution of spinal motoneuron excitability to the overall corticospinal response when lower limb muscles are targeted.

7.4.1. Evidence for stimulation of descending tracts

The occlusion observed with TMS being delivered before LS at intervals shorter than the difference in latencies of each stimulus alone corroborates previous findings when electrical stimulation was performed over the mastoids with arm and hand muscles targeted (Ugawa *et al.*, 1991; Taylor *et al.*, 2002). Similarly, when LS preceded TMS, the responses were occluded to the same degree, again confirming the findings seen when electrical stimulation was performed over the mastoids and targeting the muscles of the arm (Taylor *et al.*, 2002). This occlusion corresponded to the timing of the stimuli when LS was delivered more than 2 ms before the expected arrival of the first descending volley evoked by TMS (Martin *et al.*, 2008), which is consistent with collision of the descending cortical volleys of TMS with antidromic volley originating from LS. These findings indicate that LS activates some of the same axons as TMS, likely the pyramidal cells in the corticospinal tract (Ugawa *et al.*, 1991; Taylor *et al.*, 2002). Whilst the occluded response could have emerged due to disynaptic inhibition originating from LS-induced activation of inhibitory interneurons via cutaneous receptors of the lumbosacral region (Frigon *et al.*, 2012), this is unlikely given the facilitation that was observed at longer ISIs when TMS preceded LS (Taylor *et al.*, 2002). Facilitation corresponded to the first descending volley evoked by TMS having passed the segmental level of LS by more than 2 ms. This facilitation, which has been consistently shown for the aforementioned timing (Ugawa *et al.*, 1991; Taylor *et al.*, 2002; Martin *et al.*, 2008), is the result of the descending volley evoked by LS arriving at the motoneuron pool that is already excited by TMS descending volleys (Martin *et al.*, 2008).

In theory, it was expected that when LS was delivered prior to TMS at ISIs longer than the difference in latencies of individual stimuli, the antidromic volley would reach the cortex prior to its excitation by the magnetic stimulus, resulting in a response similar to a single- pulse TMS. However, we found that at longer ISIs (LS preceding TMS), responses remained occluded in both muscles. This behaviour is in agreement with Taylor *et al.* (2002), but differs to that of Ugawa *et al*. (1991). However, the latter employed electrical stimulation of the cortex, whilst the former stimulated the cortex with TMS, similar to the present study, suggesting that the origin of the observed depression at these longer ISIs is cortical, possibly through inhibition via collaterals of corticospinal axons (Krnjević *et al.*, 1966; Ghosh & Porter, 1988).

There are certain factors that complicate the interpretation of the interaction between electrical stimulation of the spinal tracts and responses evoked by TMS, even if it is assumed that the pathway is purely monosynaptic (Petersen *et al.*, 2002). Firstly, magnetic and electrical stimulation differ in their mechanism of activation of neurons, such that TMS evokes multiple descending volleys, whereas LS only elicits a single descending volley (Nakamura *et al.*, 1996; Houlden *et al.*, 1999; Terao *et al.*, 2000). This makes it likely that only the first volley of TMS is affected by the collision originating from LS. Consequently, comparison of responses to paired stimulation to a single TMS response actually underestimates the occlusion as a result of collision (Martin *et al.*, 2008). However, despite the interaction of the stimuli being complex, our data, in conjunction with previous work in the area (Taylor *et al.*, 2002; Martin *et al.*, 2008), suggests that the single volley produced by LS can occlude the response to TMS. Secondly, the observed occlusion could be a result of descending action potentials being in a refractory state. However, this is an unlikely contributor given the observed facilitation of responses when the first descending volley evoked by TMS had passed the segmental level of LS and since occlusion occurred at ISIs far longer than the refractory period of motoneurons (>3 ms; Day *et al.*, 1989). Lastly, if the motoneurons are not activated monosynaptically, the paired response could be influenced by excitatory and inhibitory interneurons. However, had there

been multiple synapses involved in the present study, increased excitability of motoneurons with increased contraction strength would have likely shortened the activation time of each postsynaptic cell and thus reduced the onset latency of evoked potentials (Petersen *et al.*, 2002). This was not the case as responses to individual TMS and LS increased similarly with increased contraction strength with no change in onset latency.

The increase in response amplitude with increased contraction strength is an indicator that the responses were evoked transsynaptically as opposed to distal to the cell bodies (Martin *et al.*, 2008). It is also worth noting that both LEPs and MEPs increased at a similar rate as shown previously (Maertens De Noordhout *et al.*, 1992; Sidhu *et al.*, 2015) and peaked at 50% consistent with the relationship between motor unit recruitment and firing frequency of the muscles investigated (Gelli *et al.*, 2007), which determines the probability of an evoked response (Brouwer *et al.*, 1989; Bawa & Lemon, 1993; Jones & Bawa, 1999). Similar behaviour of LEPs and MEPs with increased contraction strength is a good indicator that segmental responses can be assessed during a voluntary contraction which is of importance for the lower limbs, where exercise-induced alterations in corticospinal excitability are of interest (Lévénez *et al.*, 2007; Finn *et al.*, 2018).

7.4.2. The possibility of stimulation of other neural structures

Whilst the present data provide evidence that LS and TMS activate similar axons, i.e. the pyramidal cells in the corticospinal tract, there remains the possibility that other descending tracts might also be excited and hence be contributing to the observed effects (Ugawa *et al.*, 1991). Of particular consideration would be those tracts located in the lateral white matter, such as rubrospinal and reticulospinal tracts (Nathan & Smith, 1982; Nathan *et al.*, 1996*b*), as modelling studies indicate that electric field magnitude, due to LS, is likely higher at the lateral aspects of the spinal cord where these tracts are located (Fernandes *et al.*, 2018). Any

potential effects from the rubrospinal tract can be discounted as this tract does not project below the cervical region in humans (Nathan & Smith, 1982). The reticulospinal tract does project down to the lumbar region, however, its contribution to the effects observed is likely small due to lower density of the axons compared to corticospinal tract (Nathan *et al.*, 1996*b*). Thus, it appears unlikely that descending tracts other than corticospinal tract were stimulated with LS.

Though the aforementioned observations relating to a lack of changes in onset latency with increased contraction strength provide support for the monosynaptic nature of the pathway, the data from the present experiment does not completely exclude the influence of non-monosynaptic pathways. Indeed, a large propriospinal system has been shown to exist in humans that might influence corticospinal responses (Pierrot-Deseilligny, 2002). It is important to note that the corticospinal pathway as a whole encompasses not only cortical circuitry and the motoneuron pool, but also any spinal interneuronal connections (Devanne *et al.*, 1997). TMS might activate inhibitory interneurons due to their lower threshold for activation in some muscles (Nielsen et al., 1993), reducing the excitability at the level of the motoneuron pool leading to reduced temporal summation of the responses. Though supra- additive facilitation observed in the present experiment makes this possibility less likely, it should be noted that the lower limb muscles investigated in these experiments have been demonstrated to have di- and polysynaptic pathways (Nielsen *et al.*, 1993; Simonetta-Moreau *et al.*, 1999). Thus, further work is required to elucidate whether responses to LS are evoked purely monosynaptically, or whether they involve an interneuronal component.

When LS is performed, there is always the possibility that nerve roots are stimulated in addition to the spinal tract. Ventral roots were unlikely to have been activated in the present experiments due to the fact that when dorsal roots are stimulated, the occlusion of responses

to paired TMS and dorsal root is absent, and responses to dorsal root stimulation are not facilitated by voluntary muscle contraction (Roy *et al.*, 2014), the opposite of which was observed in the present experiments. Thus, the possibility of having activated ventral or dorsal roots with electrical stimulation is minimal.

7.4.3. Variability of responses

The present data show that the CVs for LEPs at rest and during contraction are lower than MEPs (see Table 7-1). As is well established, MEPs are inherently variable due to the fluctuating nature of corticospinal and motoneuronal excitability (Kiers *et al.*, 1993; Ellaway *et al.*, 1998), randomness in the firing of pyramidal tract neurons and spinal motoneurons (Pitcher *et al.*, 2003) as well as desynchronization of action potentials (Magistris *et al.*, 1998). The variability of cortically-evoked responses observed in the present study is comparable to that reported previously when similar numbers of pulses were employed (Brownstein *et al.*, 2018*b*). A greater variability in MEPs compared to LEPs can perhaps be explained by differences in the complexity of the responses to TMS as opposed to LS as discussed above. In addition to moment-to-moment fluctuations in motor cortex excitability (Ellaway *et al.,* 1998), some of the multiple volleys evoked by TMS, particularly the later, indirect waves, can fire multiple times (Edgley *et al.*, 1997), which might contribute to the greater variability. Furthermore, greater variability of MEPs might also stem from interference signals from other cortical networks. The variability of evoked responses can be reduced by eliciting responses during a contraction (Darling *et al.*, 2006), which is shown in the present data for both MEPs and LEPs. Due to the inherent variability of evoked responses a large quantity of evoked responses are recommended to ascertain a stable index of corticospinal excitability (Brownstein *et al.*, 2018*b*). The present data suggests that when using LS to evoke LEPs, fewer responses might be required compared to TMS evoked MEPs. However, further work is needed to elucidate the optimal number of LS stimuli to obtain a reliable average response, and whether the inherent lower variability of LEPs relative to MEPs results in greater repeatability of responses.

7.5. Conclusions

Based on the occlusion of responses when the first descending volley evoked by TMS had not arrived at the segmental level, it can be concluded that electrical stimulation of the first lumbar spinous process activates some of the same corticospinal axons projecting to lower limb muscles as transcranial magnetic stimulation of the motor cortex. These responses at rest were standardised to 10-15% M_{max} and were elicited with ease in all participants. Furthermore, responses to LS grew similarly to TMS with increasing contraction strength, suggesting transsynaptic activation. All participants found stimulations to be tolerable and whilst muscle activity of upper body muscles was not measured, the experimenters did not observe shoulder and arm movements in response to stimulation during the trials. Thus, electrical stimulation over the first lumbar spinous process can be used as an alternative method to assess corticospinal excitability at the segmental level and might be better suited when targeting lower limb muscles due to the proximity of the motoneuronal projections to the stimulating site and the ability to evoke responses in leg musculature at rest. Therefore, the subsequent Chapter will utilise this technique to discern segmental changes in corticospinal excitability following whole-body exercise.

CHAPTER 8 – A SEX COMPARISON OF FATIGABILITY FOLLOWING EXERCISE NORMALISED TO THE POWER-DURATION RELATIONSHIP

8.1. Introduction

As established in Chapter 6, the intensity-duration relationship differs between males and females for intermittent, isometric exercise. The underpinning mechanism for this is likely related to a better ability to maintain oxygenation of the knee-extensors during exercise in females. Whilst these data provide mechanistic insight into the sex difference in fatigability during single-limb exercise, a phenomenon previously described (Hunter *et al.*, 2006*a*; Yoon *et al.*, 2007*b*; Russ *et al.*, 2008; Ansdell *et al.*, 2018*b*), it does not fully explain why a similar sex difference is demonstrated during whole-body exercise (Glace *et al.*, 2013; Temesi *et al.*, 2015). To date, it remains unclear whether the power-duration relationship is different between sexes, and whether a sex difference in fatigability exists if exercise to exhaustion is performed relative to a critical intensity for whole body exercise.

The underpinning mechanisms of fatigability differ for whole-body and single-limb exercise (Hureau *et al.*, 2018; Thomas *et al.*, 2018). Indeed, Poole *et al.* (2016) suggest that parameters of the intensity-duration relationship, such as W', are likely influenced by different factors in the two modalities of exercise. For example, termination of exercise above critical power (whole-body exercise) coincides with the attainment of maximum oxygen consumption and cardiopulmonary responses (e.g. Vanhatalo *et al.*, 2010; Murgatroyd *et al.*, 2011), whereas for single-limb exercise, equivalent variables do not reach maximal values (e.g. Chapter 6). As described by Hureau *et al.* (2018) and Thomas *et al.* (2018), during whole-body exercise afferent feedback from other physiological systems (e.g. respiratory) contributes to the attainment of a 'sensory tolerance limit', in addition to accumulation of intramuscular metabolites and depletion of energy stores (Broxterman *et al.*, 2015*b*). Therefore conclusions based on data from single-limb exercise are not appropriate to explain exercise limitation in a whole-body model.

The question of sex differences in the whole-body power duration relationship differs from that of single-limb exercise. As demonstrated in Chapter 6, females have more fatigue-resistant locomotor muscles, and additionally females demonstrate more fatigue resistant respiratory musculature (Guenette *et al.*, 2010; Welch *et al.*, 2018). These factors alone might suggest that females would be able to sustain a greater relative exercise intensity compared to males, however, morphological sex differences within the respiratory system have the potential to reduce high-intensity exercise tolerance. For example, even when height-matched, females have smaller lung volumes and airway size, weaker respiratory muscles, and smaller alveolar surface area for gas exchange compared to males (Mead, 1980; Crapo *et al.*, 1982; Martin *et al.*, 1987; Sheel *et al.*, 2009). In response to exercise, these factors amalgamate into a greater expiratory flow limitation in females at near-maximal ventilatory capacity (Guenette *et al.*, 2007). Furthermore, females demonstrate a greater work of breathing (W*b*) than males (Witt *et al.*, 2007), and at peak exercise, the oxygen cost of breathing (expressed at a fraction of whole-body $\dot{V}O_2$), is greater in females compared to males (14 vs. 9%, Dominelli *et al.*, 2015). These potentially deleterious factors could counter the greater fatigue-resistance of locomotor and respiratory muscle, and negate the sex difference observed in Chapter 6, when a similar research design is implemented in whole-body exercise.

Accordingly, the present Chapter had two primary aims: 1) to compare the power-duration relationship between males and females; 2) determine whether a sex difference in fatigability exists when exercise intensity is normalised to the power-duration relationship. It was hypothesised that: 1) no sex difference in the power-duration relationship would exist when expressed relative to maximum exercise performance, and 2) females would exhibit greater fatigue resistance of the knee-extensors compared to males in both heavy and severe intensity domains.

8.2. Methods

8.2.1 Participants

Using the effect size from Ansdell et al. (2018) for the sex difference in fatigability, a power calculation (alpha = 0.05, power = 0.80) determined that a sample size of 20 participants was required. Ten males (mean ± SD age: 25 ± 5 years, stature: 178 ± 9 cm, mass: 67.0 ± 8.8 kg) and eight females (age: 25 ± 6, stature: 169 ± 9 cm, mass: 63.3 ± 7.2 kg) gave written informed consent to participate. The females that volunteered were all using monophasic hormonal contraceptives (>6 months), and those using contraceptive pills were tested in the 21-day consumption period of the pill cycle in order to negate the effects of endogenous hormones on neuromuscular function and fatigability (see Chapter 4). To ensure homogeneity in the training status of participants, minimum criteria were set for relative $\dot{V}O_{2max}$ and maximal ramp test power (P_{max}) attained in the first visit (see section 8.2.3.1). These values were based upon recommendations by De Pauw *et al.* (2013) for males, and Decroix *et al.* (2016) for females and were as follows: minimum $\dot{V}O_{2max}$ of 55 and 48 $mL \cdot kg^{-1} \cdot min^{-1}$, and P_{max} of 4.6 and 3.8 $W \cdot kg^{-1}$ for males and females, respectively. Participants had to achieve one of the aforementioned criteria in order to proceed to the subsequent experimental visits. In total, 18 males and 16 females were screened to achieve the resultant sample size.

8.2.2. Experimental Design

All participants visited the laboratory six or seven times, completing a familiarisation visit, three or four constant intensity trials to estimate critical intensity, then trials 10% above and below critical power (see Section 8.2.3.1 for more detail). Testing took place over a three to five week period, with a minimum of 48 h between visits to permit recovery (Carroll *et al.*, 2016). The time of day for each testing session was replicated (± 1 h) to account for diurnal variations in maximal force generating capacity and corticospinal excitability (Tamm *et al.*, 2009). For all visits, environmental conditions remained constant (20°C, 40% relative humidity).

8.2.3. Experimental Protocol

8.2.3.1. Familiarisation and Incremental Exercise Test

Upon giving informed consent, participants performed a 5-minute warm up (80-100 W) on a cycle ergometer (Velotron Pro, RacerMate Inc, Seattle, Washington, USA) at a self-selected cadence (60-100 rpm). Participants were then provided 2 minutes of rest, during which they remained stationary on the cycle ergometer, before an incremental exercise test was initiated. For both sexes, the test began at 100 W, then for males the intensity increased gradually by 25 $W \cdot min^{-1}$ (0.416 $W \cdot sec^{-1}$), and for females by 20 $W \cdot min^{-1}$ (0.333 $W \cdot sec^{-1}$). The different rate of intensity increase was intended to produce ramp tests of similar duration in both sexes, due to lower absolute power outputs demonstrated in females (Sundberg *et al.*, 2016), in an attempt to negate the effects of test duration on cardiopulmonary outcomes (Yoon *et al.*, 2007*a*). Mean ramp test duration was not different between males and females (males: 10.5 ± 1.2 vs. females: 9.1 ± 2.1 mins, $p = 0.103$). The test was terminated once the participant's self-selected cadence decreased by 10 rpm despite strong verbal encouragement. During the test, expired gas was analysed breath-by-breath using an online system (Vyntus CPX, Jaeger, CareFusion, Germany). The outcome variables from the ramp test were $\dot{V}O_{2max}$ and maximal ramp test power (P_{max}).

Following the incremental exercise test, participants rested for 15 minutes, before a neuromuscular familiarisation was performed. For this, participants were sat in the isometric dynamometer with hip and knee angles at 90˚. This set up was replicated for all visits. Electrical nerve stimulation threshold was determined, followed by TMS hotspot, aMT, and VA_{TMS} stimulator intensity determination (as described in Chapter 3). Following this, a baseline neuromuscular function assessment was performed.

8.2.3.2. Critical Power Estimation Trials

To estimate critical power, participants completed a minimum of three constant-load exercise trials to task failure. The intensities for the initial three trials were set at 110, 90, and 80% of P_{max} and were performed in a randomised order, designed to elicit task failure within 2-15 minutes (Poole *et al.*, 1988). Time to task failure, recorded in seconds, was taken as the first time at which participants' cadence fell by 10rpm. No feedback was provided to participants regarding power output and time elapsed during the trials. A criterion of an end-exercise $\dot{V}O_2$ of >95% $\dot{V}O_{2max}$ was set, and all trials used for estimation achieved this.

The parameters of the power-duration relationship (CP and W') were estimated using the inverse linear model (equation 9.1), the linear work-time model (equation. 9.2), and the hyperbolic model (equation 9.3). The equation with the highest r^2 and lowest SE was selected for each individual and used for all further analysis (Mitchell *et al.*, 2018*a*):

$$9.1.\quad P = W' \cdot \left(\frac{1}{\mathrm{t}}\right) + CP$$

$$9.2.\quad W = CP \cdot t + W'$$

$$9.3.\quad t = W'/(P - CP)$$

Where t is time to task failure, P is power output, and W is total work done. If three estimation trials did result in a large SE for CP (>%5 of the mean) and W' (>10%), a fourth trial was performed (Mitchell *et al.*, 2018*a*). This occurred for three out of the 18 participants (two males, one female).

8.2.3.3. Severe and Heavy Intensity Trials

Once CP and W' were estimated, severe (110% CP) and heavy (90% CP) intensity trials were performed in a randomised order. These visits began with electrical nerve stimulation and TMS thresholds being determined. Participants then completed a standardised isometric warm up (Gruet *et al.*, 2014), before a baseline assessment of neuromuscular function. Following this, the NIRS optodes were attached to the VL and baseline measures were recorded for five minutes on the cycle ergometer with the right leg relaxed in the fully extended position (crank angle 180° from top dead centre). Resting measures of gas exchange were also recorded in this period, then both NIRS and gas exchange were continuously sampled until task failure. Participants completed a five-minute warm up (80-100 W), followed by one minute of seated rest on the ergometer. In the 5-10 s prior to the trial, participants were instructed to obtain their self-selected cadence against no resistance, then when achieved, the resistance was applied in a square wave fashion. Time to task failure was recorded for the severe intensity trial, whereas for the heavy intensity trial participants cycled to task failure, or for 60 minutes, whichever occurred sooner. Immediately upon task failure (<20 s) participants transitioned from the ergometer to the dynamometer and commenced a neuromuscular assessment which was completed within 2.5 min post-exercise.

8.2.4. Pulmonary Gas Exchange

Breath-by-breath pulmonary gas exchange and ventilation were measured continuously during all exercise tests. Prior to each visit, the Vyntus CPX was calibrated for oxygen (O_2) and carbon dioxide (CO_2) with gas of known concentration (16% O_2 and 4.97% CO_2) using an electrochemical fuel cell and non-dispersive infrared cell, respectively. Ventilatory volumes were calibrated using a digital turbine transducer at high (2 $L \cdot s^{-1}$) and low (0.2 $L \cdot s^{-1}$) flow rates. The associated measurement errors provided by the manufacturer are as follows: O_2 = 0.2%, CO_2 = 1%, volume = 2%, and flow = 3%.

8.2.5. Transcranial Magnetic Stimulation

Mean aMT was not different between males and females (43 ± 6 vs. 40 ± 5%, *P* = 0.392), or between visits (42 ± 5 vs. 43 ± 7%, *P* = 0.245). Similarly, for VA_{TMS}, mean stimulator intensity was not different between males and females (65 ± 6 vs. 64 ± 5% MSO, *p* = 0.791) or between visits (66 ± 6 vs. 64 ± 6% MSO, *p* = 0.100). The intensities used activated a large proportion of the motoneuron pool for the RF that was not different between trials at baseline (51 ± 15 vs. 53 ± 11% M_{MAX}, *p* = 0.314). The TMS pulse also avoided substantial activation of the antagonist (BF) with small incidental MEPs recorded at baseline (0.44 ± 0.23 vs. 0.47 ± 0.23 mV, *p* = 0.476).

8.2.6. Lumbar Electrical Stimulation

Lumbar stimulation was performed as described in Chapter 7, with the pre-exercise LEP standardised to 15-25% of M_{MAX}. Lumbar stimulation was performed during a 10% MVC contraction alone (unconditioned), and 100 ms into a 200 ms SP (conditioned). The mean stimulus intensity for unconditioned LEPs was 172 ± 47 mA for males and 166 ± 24 mA for females, (*p* = 0.732). For conditioned LEPs (SP-LEPs), the TMS intensity to produce a SP of 200 ms was not different between males and females (49 ± 8 vs. 51 ± 6% MSO, *p* = 0.605), likewise the intensity of subsequent lumbar stimulation was not different between males and females (176 ± 46 vs. 172 ± 22 mA, *p* = 0.747).

8.2.7. Motor Nerve Stimulation

Mean stimulus intensity was not different between sexes (189 ± 62 vs. 210 ± 57 mA, *p* = 0.438) or between visits (194 ± 61 vs. 202 ± 61 mA, *p* = 0.620).

8.2.8. Near Infrared Spectroscopy

Data was collected according to the procedures outlined in section 3.5.1.

8.2.9. Data Analysis

In order to achieve significant linearity ($r^2 > 0.80$, $p < 0.05$), a total of three out of 720 SITs across all trials were excluded (0.4%), which led to three regressions containing 9 data points rather than 10 (all post-exercise). As a result, mean r^2 values for ERTs were linear throughout the study (0.92 ± 0.07).

The NIRS (O_2Hb, HHb, and TOI) and gas exchange ($\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}_E$, and RER) data were expressed as a percentage of baseline, and the 30 s epochs throughout exercise are presented as Δ%. Gas exchange data was also expressed as a % of final ramp test values, to facilitate comparisons between sexes.

Data are presented as mean ± SD within the text and figures. Normal Gaussian distribution of data was confirmed using the Kolmogorov–Smirnov test. If a violation was detected, the data was logarithmically transformed. The alpha for all statistical tests was set at $P < 0.05$. For variables assessed prior to and during exercise (NIRS and gas exchange) a two-way (2×5) repeated measures ANOVA was used to assess differences between sex (male vs. female) and over time (Pre, 25, 50, 75, and 100% TTF). For variables assessed only during exercise (rmsEMG) a two-way (2×5) repeated measures ANOVA was used to assess differences between sex (male vs. female) and over time (Start, 25, 50, 75, and 100% TTF). For variables that were assessed pre and post-exercise (neuromuscular function) a two-way 2×2 repeated measures ANOVA was used to assess differences between sex (male vs. female) and over

time (Pre vs. Post). If significant main or interaction effects were observed, these were followed up by post-hoc Bonferroni-corrected pairwise comparisons.

8.3. Results

8.3.1 Incremental Ramp Test

The variables recorded during the ramp test are displayed in Table 8.1. As shown, males recorded greater values for $\dot{V}O_{2max}$, and Pmax when expressed in absolute units and also when normalised to body mass (all $p < 0.001$).

Table 8-1: Comparison of the results from the incremental exercise test, and power-duration relationship modelling in males and females.

	Males	Females	P value
	Incremental Test		
$\dot{V}O_{2max}$ ($L \cdot min^{-1}$)	4.02 ± 0.47	2.85 ± 0.51	*< 0.001*
$\dot{V}O_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	60.5 ± 8.2	45.1 ± 6.3	*< 0.001*
P_{max} (W)	362 ± 29	241 ± 42	*< 0.001*
P_{max} ($W \cdot kg^{-1}$)	5.5 ± 0.6	3.8 ± 0.5	*< 0.001*
	Power-Duration Relationship		
CP (W)	260 ± 28	179 ± 32	*< 0.001*
CP (% P_{max})	72 ± 5	74 ± 2	0.210
W' (J)	18,515 ± 4,831	12,684 ± 3,155	*0.009*
W' ($J \cdot P_{max}^{-1}$)	51 ± 11	52 ± 10	0.733

8.3.2. Power-Duration Relationship

The parameter estimates for the power-duration relationship are presented in Table 9.1. The range of times to task failure for the shortest estimation trial was 105-185 s, while the range for the longest trial was 568-1192 s. Once more, when data were expressed in absolute units, males demonstrated greater values than females ($p \leq 0.009$), however, when CP and W' were normalised to P_{max}, no difference was observed ($p \geq 0.210$).

8.3.4 Severe Intensity Exercise

8.3.4.1. Fatigability

There was no difference in time to task failure between sexes during the trial at 110% CP (males: 752 ± 329 s, females: 681 ± 277 s, $p = 0.645$).

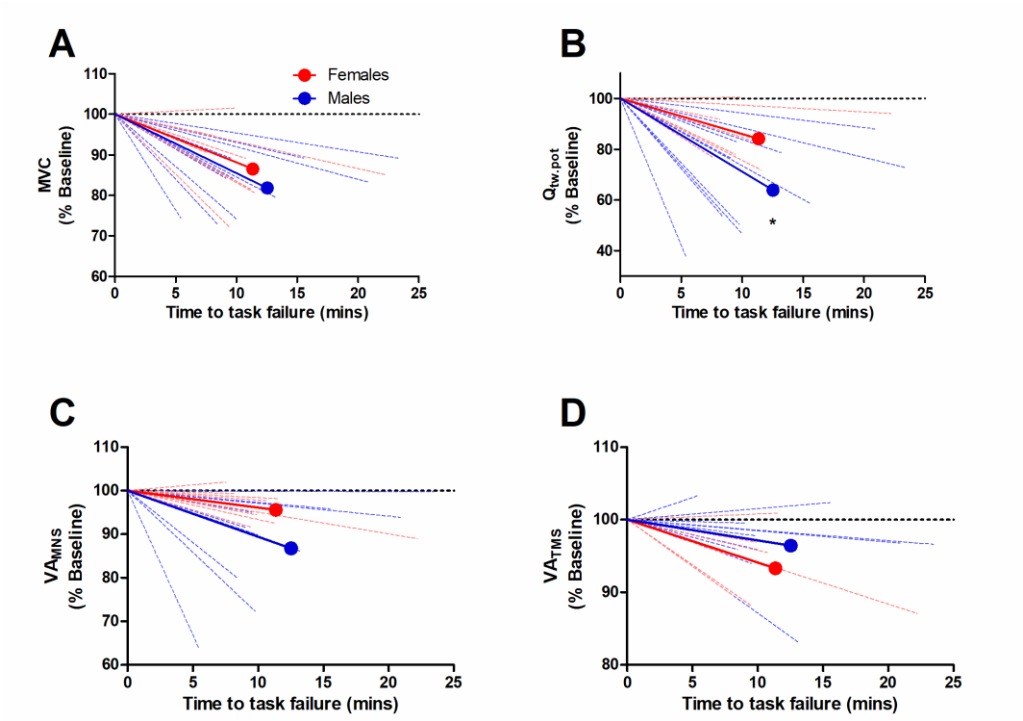

*Figure 8-1: Pre-post exercise changes in neuromuscular function following exercise at 110% CP. Panel A: MVC, Panel B: $Q_{tw.pot}$, Panel C: VA_{MNS}, Panel D: VA_{TMS}. * = a greater decrease in males (*P < 0.05).Male data is presented in blue, and female data in red. Dashed lines indicate individual participants, and the solid lines indicate the group mean.

The change in neuromuscular variables is displayed in Figure 8-1. As demonstrated, MVC, $Q_{tw.pot}$, VA_{MNS}, and VA_{TMS} decreased pre-post the exercise trial at 110% CP ($p \leq 0.002$), and this decrease was less in females compared to males for $Q_{tw.pot}$ (−36 ± 17 vs. −15 ± 10%, $F_{1,16} = 8.4$, $p = 0.010$, $\eta p^2 = 0.344$). The amplitude of MEPs, LEPs, and SP-LEPs did not change from pre-post exercise ($p \geq 0.094$, Table 8.2). Maximum inspiratory and expiratory pressures decreased from pre-post exercise ($p \leq 0.005$), with no sex difference in the magnitude of decrease ($p \geq 0.565$, Table 8.2).

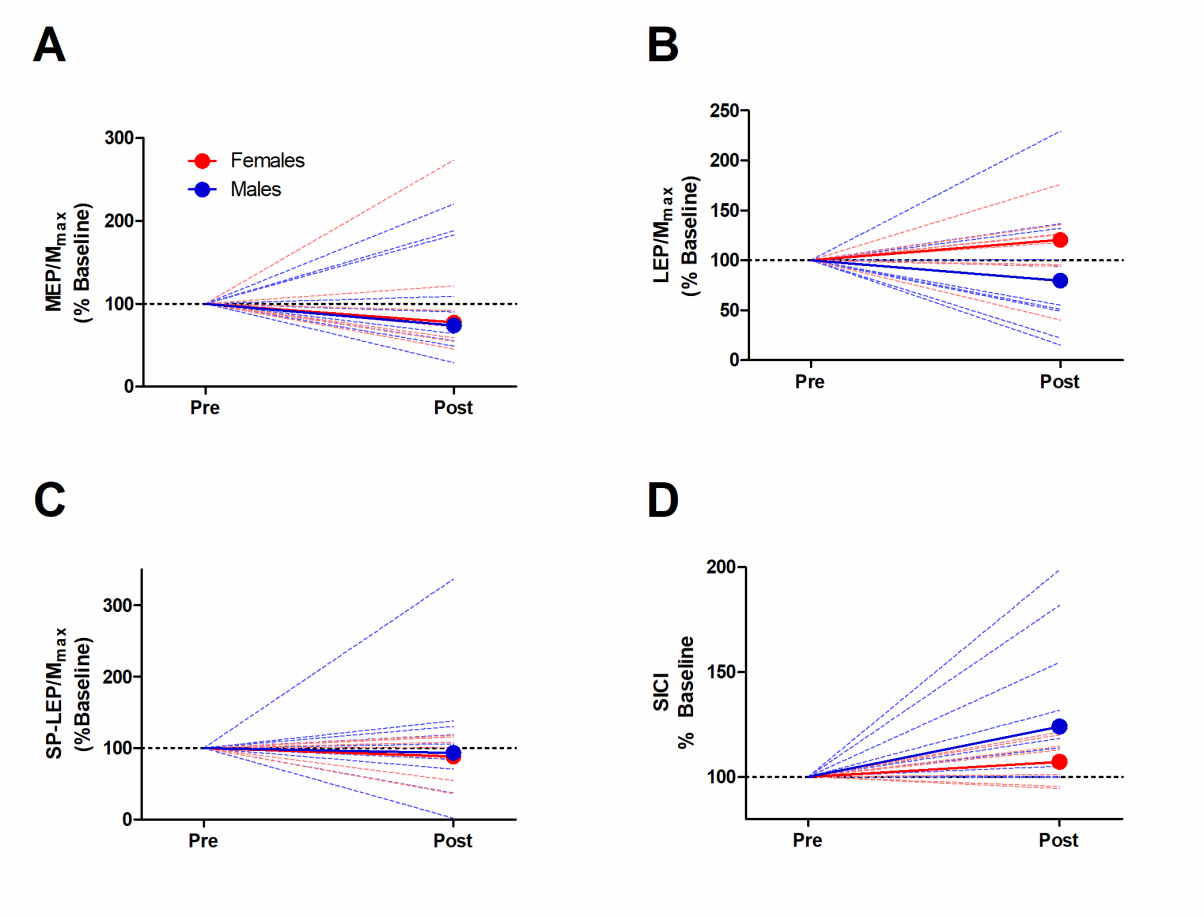

Figure 8-2: The change in indices of neural excitability following exercise at 110% CP. Panel A: MEP, Panel B: LEP, Panel C: SP-LEP, Panel D: SICI

8.3.4.2. Oxygenation

Both O_2Hb and TOI decreased throughout exercise (Figure 8-2, time effects $P < 0.001$), whilst HHb increased ($P < 0.001$). A lesser decrease in O_2Hb ($F_{1.2, 20.6} = 8.8$, $P = 0.005$, $\eta p^2 = 0.355$)

and TOI ($F_{1.3,\ 21.1}$ = 36.2, P < 0.001, ηp^2 = 0.656) was observed for females, as well as a reduced increase in HHb ($F_{1.7,\ 26.5}$ = 10.1, P = 0.001, ηp^2 = 0.387).

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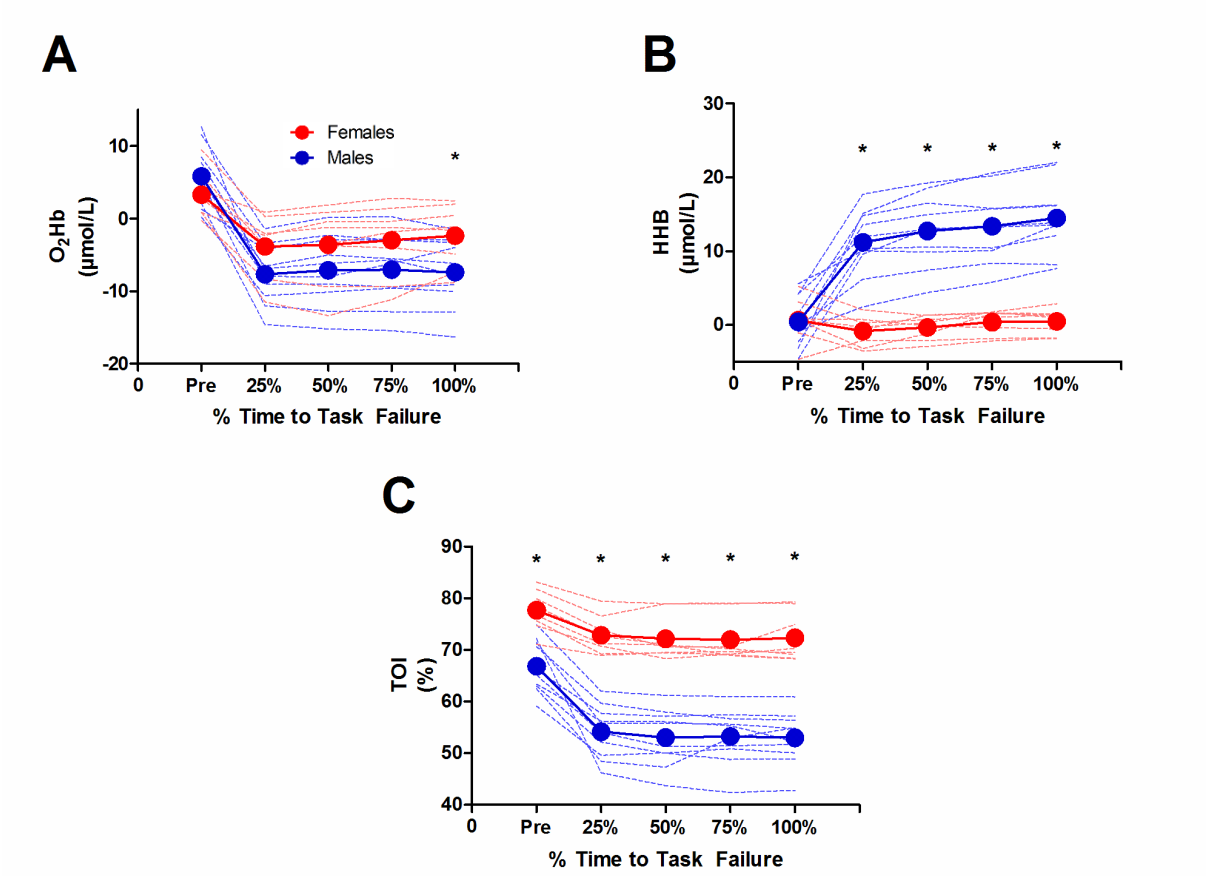

*Figure 8-3: Changes in indices of muscle oxygenation throughout exercise at 110% CP. Panel A: O_2Hb, Panel B: HHb, Panel C: TOI. * = greater in males than females (P < 0.05).*

8.3.4.3. Respiratory and Locomotor Muscle Electromyography

The rmsEMG signal from the VL, sternocleidomastoid (SCM), and external intercostal (IC) all increased throughout the task (Figure 8-3, all time effects P < 0.001). Females demonstrated a lesser increase in rmsEMG for the VL ($F_{4,64}$ = 2.7, P = 0.041, ηp^2 = 0.142), but not the SCM (P = 0.079), or IC (P = 0.255).

8.3.4.4. Pulmonary Gas Exchange

Oxygen consumption, $\dot{V}CO_2$, and $\dot{V}_{E,}$ all demonstrated increases while RER decreased throughout the task (Table 8-2, $P < 0.001$). The sex effect for V_E was not significant ($P = 0.052$), and no other sex differences were observed ($P \geq 0.114$). End-exercise $\dot{V}O_2$ was not significantly different from the $\dot{V}O_{2max}$ measured during the incremental test ($P = 0.442$).

*Table 8-2: Changes throughout exercise above critical power for pulmonary gas exchange, EMG, and pulmonary function variables. * = significantly different from pre-exercise (P < 0.05). $ = significantly different from females.*

		Severe Intensity					**Heavy Intensity**				
Time to Task Failure/Termination	Males			752 ± 329					3073 ± 835		
(s)	Females			681 ± 277					2937 ± 964		
						Pulmonary Gas Exchange					
		Pre-Exercise	*25% TTF*	*50% TTF*	*75% TTF*	*100% TTF*	*Pre-Exercise*	*25% TTF*	*50% TTF*	*75% TTF*	*100% TTF*
$\dot{V}O_2$	Males	19 ± 4	87 ± 6*	93 ± 5*	95 ± 7*	98 ± 4*	17 ± 3	76 ± 6*	78 ± 6*	78 ± 5*	81 ± 5*
(%$\dot{V}O_{2max}$)	Females	18 ± 3	82 ± 6*	87 ± 5*	93 ± 6*	98 ± 4*	18 ± 3	76 ± 7*	79 ± 7*	81 ± 7*	84 ± 6*
$\dot{V}CO_2$	Males	15 ± 2	76 ± 12*	78 ± 12*	79 ± 12*	81 ± 12*	15 ± 2*	64 ± 9*	63 ± 9*	64 ± 9*	65 ± 9*
(%$\dot{V}O_{2max}$)	Females	16 ± 2	77 ± 6*	82 ± 6*	84 ± 7*	86 ± 6*	16 ± 4*	63 ± 8*	63 ± 6*	66 ± 6*	68 ± 6*
$\dot{V}_E$	Males	15 ± 3	76 ± 12*	78 ± 12*	79 ± 12*	81 ± 12*	14 ± 3	52 ± 8*	56 ± 8*	59 ± 7*	64 ± 10*
(%$\dot{V}O_{2max}$)	Females	16 ± 2	77 ± 6*	82 ± 6*	84 ± 7*	86 ± 6*	20 ± 2	66 ± 5*	68 ± 8*	72 ± 7*	77 ± 7*
RER	Males	0.92 ± 0.04	1.01 ± 0.11	0.98 ± 0.09	0.95 ± 0.06	0.95 ± 0.07	1.00 ± 0.10	0.96 ± 0.05$^{\$}$	0.94 ± 0.07	0.95 ± 0.07	0.93 ± 0.05$^{\$}$
($\dot{V}CO_2$ / $\dot{V}O_2$	Females	0.95 ± 0.06	1.02 ± 0.06	1.00 ± 0.05	0.97 ± 0.06	0.94 ± 0.05	0.97 ± 0.14	0.90 ± 0.05	0.88 ± 0.06	0.89 ± 0.05	0.88 ± 0.05
						Muscle Activation					
		Start-Exercise	*25% TTF*	*50% TTF*	*75% TTF*	*100% TTF*	*Start-Exercise*	*25% TTF*	*50% TTF*	*75% TTF*	*100% TTF*
Vastus Lateralis	Males	3.3 ± 1.6	4.2 ± 2.0*	4.5 ± 2.2*	5.2 ± 2.0*	5.7 ± 1.9*	4.3 ± 3.8	4.7 ± 3.8	4.6 ± 3.3	5.3 ± 4.2*	5.1 ± 3.7*
(rmsEMG·M_{max}^{-1})	Females	3.7 ± 1.4	4.5 ± 1.6*	5.0 ± 1.6*	5.0 ± 1.7*	5.2 ± 1.9*	2.9 ± 1.1	3.2 ± 1.2	3.4 ± 1.2	3.4 ± 1.5	3.4 ± 1.4
Sternocleidomastoid	Males	11.6 ± 8.8	18.4 ± 9.4	26.2 ± 14.1	33.3 ± 16.0	44.5 ± 22.2	10.6 ± 8.0	11.6 ± 8.2	11.9 ± 8.8	15.1 ± 13.6	15.1 ± 11.1
(% rmsMIP)	Females	16.9 ± 10.5	23.4 ± 12.3	24.9 ± 10.0	28.4 ± 11.7	36.2 ± 11.7	12.9 ± 5.0	19.9 ± 12.8	19.9 ± 12.3	17.9 ± 9.4	19.5 ± 10.8
External Intercostal	Males	19.5 ± 9.9	27.9 ± 13.4	34.4 ± 16.3	37.1 ± 17.9	49.6 ± 26.1	10.3 ± 6.5	12.6 ± 13.5	13.5 ± 6.5	13.6 ± 7.8	15.2 ± 7.1
(% rmsMEP)	Females	38.5 ± 18.4	55.5 ± 38.9	54.9 ± 38.9	62.15 ± 38.6	61.0 ± 34.7	24.9 ± 8.3	31.0 ± 11.7	30.2 ± 12.5	29.5 ± 14.2	33.9 ± 17.6
						Maximal Pulmonary Pressures					
		Pre-Exercise			*Post-Exercise*		*Pre-Exercise*			*Post-Exercise*	
Maximum Expiratory Pressure	Males	197 ± 52			171 ± 48*		174 ± 39			157 ± 31	
(mmHg)	Females	143 ± 37			129 ± 38*		138 ± 34			136 ± 40	
Maximum Inspiratory Pressure	Males	130 ± 37			118 ± 33*		140 ± 53			135 ± 50*	
(mmHg)	Females	113 ± 29			104 ± 25*		138 ± 34			136 ± 40	

8.3.5. Heavy Intensity

8.3.5.1. Fatigability

Three males and three females reached task failure prior to the 60 min cut-off (mean duration: 3073 ± 835 vs. 2937 ± 964 s, p = 0.758).

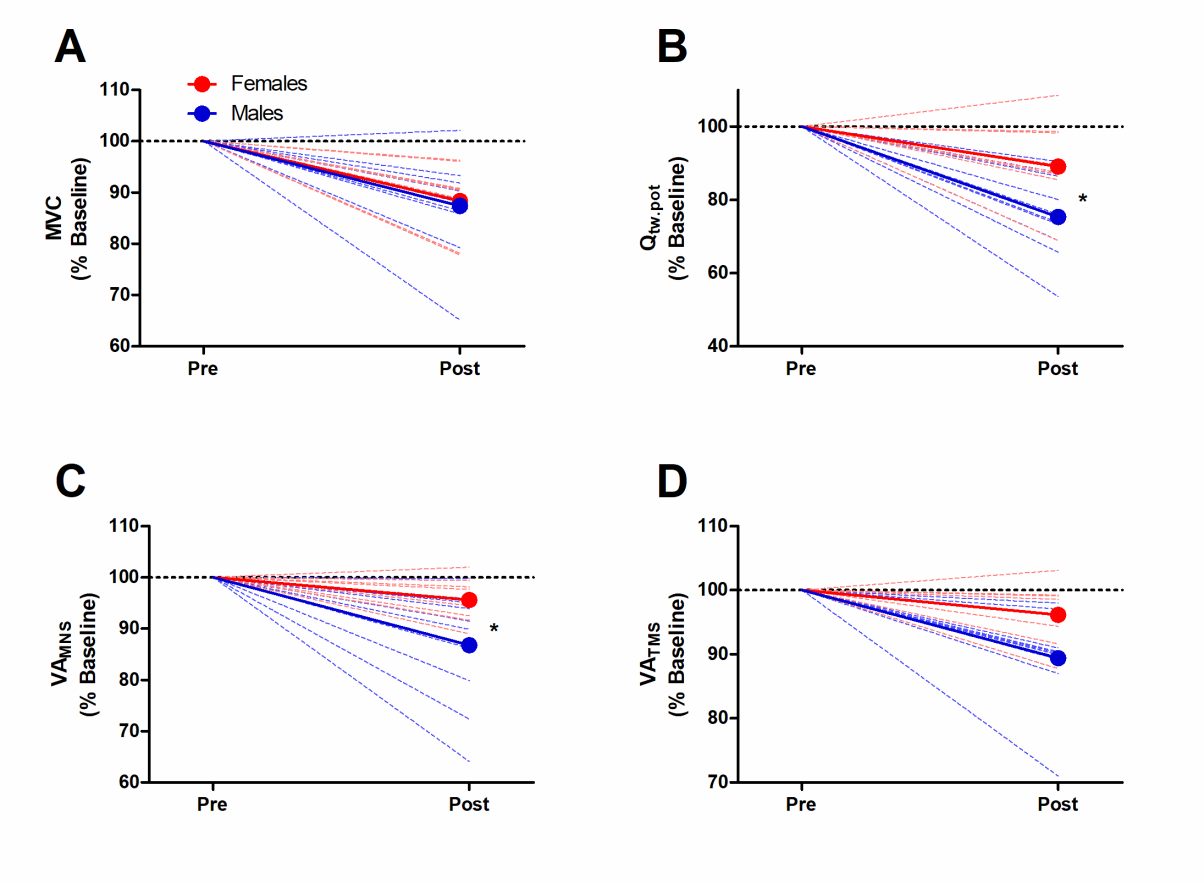

*Figure 8-4: Neuromuscular changes from pre-post exercise at 90% CP. Panel A: MVC, Panel B: $Q_{tw.pot}$, Panel C: VA_{MNS}, Panel D: VA_{TMS}. * = a greater decrease in males* (P < 0.05).

From pre-post exercise, decreases in MVC, $Q_{tw.pot}$, VA_{MNS}, VA_{TMS}, MEP, and SP-LEP were observed ($p \leq 0.039$, Figure 8-4). Females demonstrated less of a decrease in $Q_{tw.pot}$ (−24 ± 11 vs. −10 ± 11%, $F_{1,16} = 31.8$, $p = 0.020$, $\eta p^2 = 0.655$) and VA_{MNS} (−9 ± 6 vs. −4 ± 3%, $F_{1,16} = 5.2$, $p = 0.036$, $\eta p^2 = 0.246$), but no sex difference was demonstrated for VA_{TMS}, MEP, and SP-LEP ($p \geq 0.051$) Maximum inspiratory pressure decreased from pre-post exercise ($p = 0.001$),

whereas maximum expiratory pressure did not (p = 0.063; see Table 8-2). No sex difference in the magnitude of decrease for the former was observed (p = 1.000).

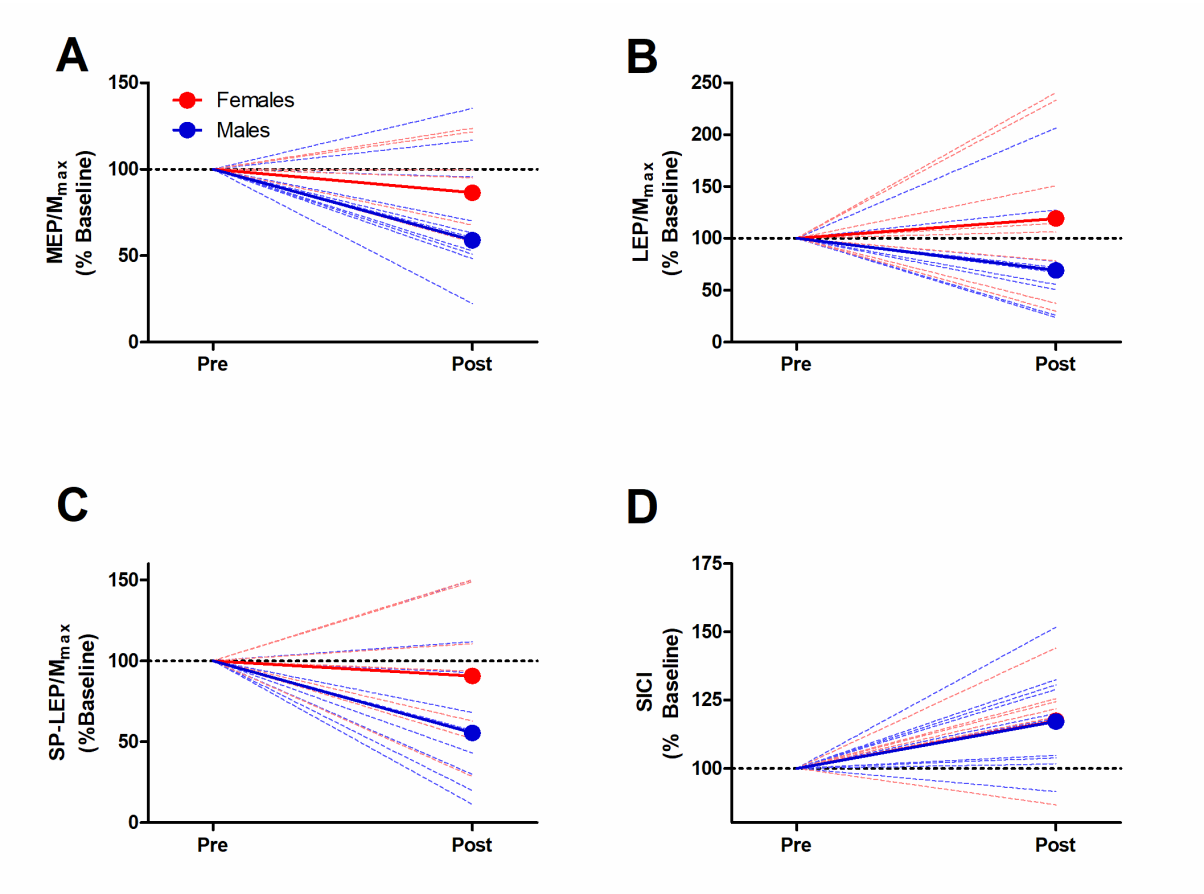

Figure 8-5: The change in indices of neural excitability from pre-post exercise at 90% CP. Panel A: MEP, Panel B: LEP, Panel C: SP-LEP, Panel D: SICI

8.3.5.2. Oxygenation

Decreases in O_2Hb (p < 0.001) and TOI (p < 0.001) were observed during exercise, with females demonstrating less of a decrease for both (O_2HB: $F_{1.8,30.0}$ = 7.4, p = 0.003, ηp^2 = 0.315; TOI: $F_{1.7,26.9}$ = 29.7, p < 0.001, ηp^2 = 0.650). An increase in HHb was observed for both sexes (p < 0.001), with females demonstrating less of an increase (Figure 8-6, $F_{1.4,22.8}$ = 33.7, p < 0.001, ηp^2 = 0.678).

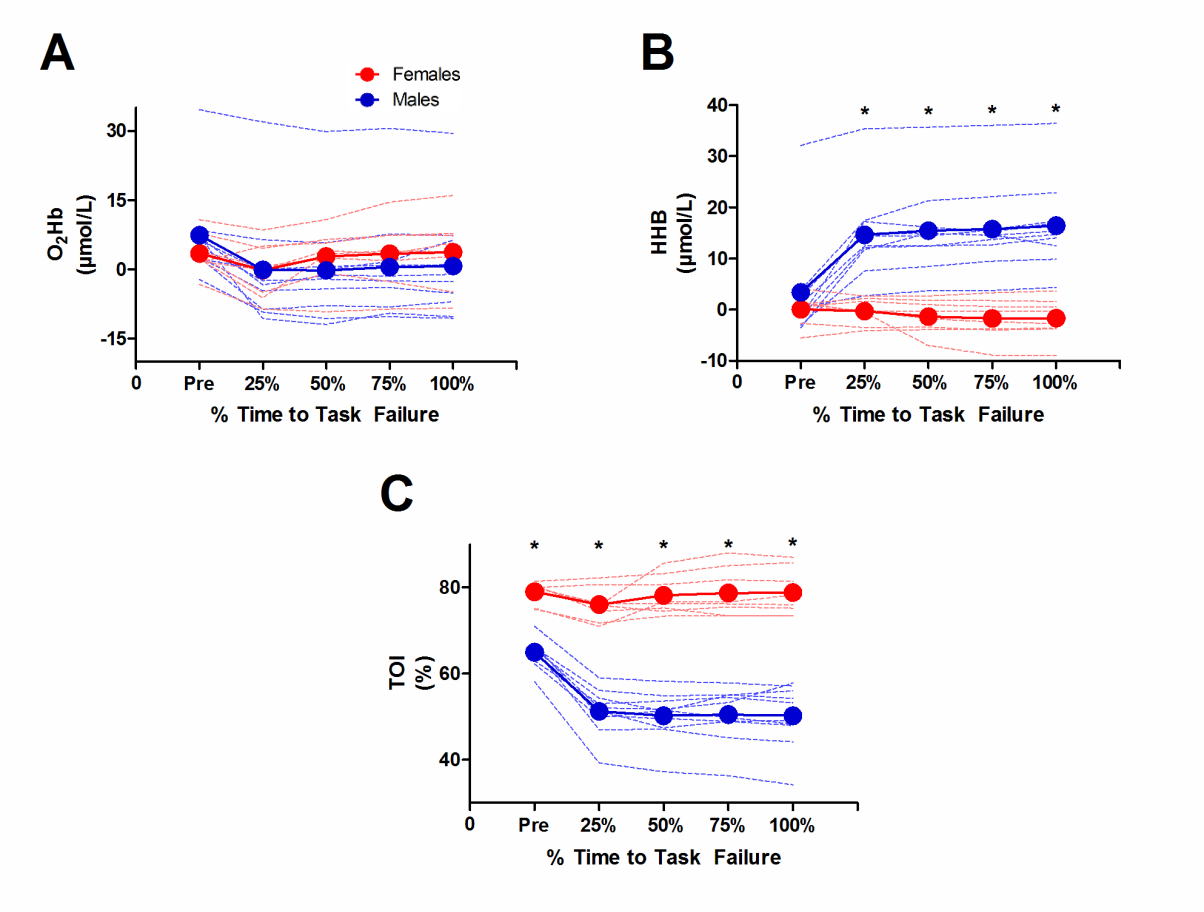

*Figure 8-6: Changes in muscle oxygenation throughout exercise at 90% CP. Panel A: O_2Hb, Panel B: HHb, Panel C: TOI. * = greater in males than females ($P < 0.05$)*

8.3.5.3. Respiratory and Locomotor Muscle Electromyography

The rmsEMG signal from the VL, SCM, and IC all increased throughout the task (Table 8-2, all time effects $p \leq 0.033$). However, there were no sex differences in the rate of increase for any muscle ($p \geq 0.063$).

8.3.5.4. Pulmonary Gas Exchange

Oxygen consumption, $\dot{V}CO_2$, and $\dot{V}_E$ all increased throughout the task ($p < 0.001$) while RER decreased ($p = 0.001$). A sex effect was not observed for $\dot{V}_E$ ($p = 0.052$), but was for RER ($F_{1,16} = 5.08$, $p = 0.039$, $\eta p^2 = 0.241$; see Table 8-2). *Post-hoc* tests indicated that females had

a lower RER (−0.05) throughout the trial and end-exercise $\dot{V}O_2$ was 19% lower than $\dot{V}O_{2max}$ ($p < 0.001$).

8.4. Discussion

The present study aimed to investigate the sex difference in fatigability during locomotor exercise by comparing the power-duration relationship, then assessing oxygenation and neuromuscular responses to metabolically-matched exercise intensities. In absolute terms, males demonstrated a greater critical power and W', however, when these parameters were normalised to P_{max} no sex difference was observed. In both severe and heavy intensity domains, females demonstrated smaller reductions in knee-extensor muscle function post-exercise. These sex differences are likely related to the fact that females demonstrated greater VL oxygenation during exercise in both intensity domains. The change in corticospinal excitability appeared to be domain- but not sex-specific, with a decrease in MEP and SP-LEP amplitude observed below CP only. Together, these data present an integrative profile of the sex difference in fatigability and suggest that the mechanism(s) for greater neuromuscular fatigue-resistance in females reside within the musculature.

8.4.1. Incremental Test & Power-Duration Relationship

As expected, males were capable of producing greater absolute power outputs for variables such as P_{max} and CP. Similar to Sundberg *et al.* (2016), who demonstrated that female P_{max} was 68% of male equivalents, the present study observed that female P_{max} was 66% of male values. This sex difference was still evident when P_{max} was normalised to body mass ($W \cdot kg^{-1}$), which is likely a result of differences in body composition (Pate & O'Neill, 2007). A similar sex difference was observed for $\dot{V}O_{2max}$, with males having greater absolute and relative values

(Pate & O'Neill, 2007). However, as discussed by Joyner (2017), allometric scaling of $\dot{V}O_{2max}$ to fat-free mass eliminates this sex difference.

As cycling power output is associated with body mass, the normalisation of CP with P_{max} presents a method of making inter-individual comparisons of the power-duration relationship. Indeed, when relative CP (% P_{max}) was compared, no sex difference was demonstrated. The present study is the first to directly compare males and females in this way, and conflicts data obtained in an isometric exercise setting where females had a ~7% greater critical intensity compared to males (Chapter 6). This discrepancy is likely explained by the modality of exercise; single-limb intermittent isometric exercise is not limited by blood flow to the exercising limb (Duner 1959), whereas leg blood flow during two-leg exercise is associated with a plateau at high intensities (Calbet, 2000). Critical power is considered to be limited by oxygen delivery to the working muscle (Vanhatalo *et al.*, 2010; Dekerle *et al.*, 2012; Broxterman *et al.*, 2015*a*), therefore, during single-limb exercise, where blood flow is not a limiting factor (i.e. Chapter 6), the sex difference in critical intensity could be a result of greater diffusive capacity of the muscle. For example, it is well established that females have greater capilliarisation and type I fibre proportional area of the knee-extensors (Roepstorff *et al.*, 2006), which could permit a greater rate of oxygen extraction, and a greater relative critical intensity. Whereas in the present study, knee-extensor blood flow is limited due to the modality of exercise, therefore, this becomes the primary determinant of CP, rather than diffusive capacity of the muscle, leading to the lack of sex difference. Other contributing factors to the lack of sex difference in CP could be that haemoglobin concentrations are typically ~12% lower (Murphy, 2014), and lung volumes are smaller (Schwartz *et al.*, 1988) in females. The negative consequence of these factors is that females are more prone to exercise-induced arterial hypoxemia (Harms *et al.*, 1998), meaning that when cardiac output is at its maximum (i.e. the severe intensity domain), there is no possibility for increased oxygen extraction. This leads to a reduced arterio-venous oxygen difference, which has recently been suggested to

negate the sex difference in muscle fatigability (Dominelli *et al.*, 2017) and could conceivably oppose the positive aspects of greater type I muscle fibre proportion on CP (Mitchell *et al.*, 2018*a*), leading to a lack of sex difference.

8.4.2. Severe Intensity Exercise

The power-duration relationship successfully predicted time to task failure in the exercise trial at 110% CP (Table 8-2), and the $\dot{V}O_2$ response to exercise confirmed that exercise was indeed in the severe intensity domain. The end-exercise $\dot{V}O_2$ was >95% $\dot{V}O_{2max}$ and demonstrated a gradual increase throughout exercise, indicating the presence of a considerable slow component, that drove oxygen consumption to $\dot{V}O_{2max}$ at task failure. Fatigability within the severe intensity domain is typically associated with depletion of high-energy phosphates and an accumulation of deleterious metabolites within the exercising musculature (Jones *et al.*, 2008; Black *et al.*, 2016; Vanhatalo *et al.*, 2016), which consequently reduce the contractile capacity of exercised musculature until the attainment of a limiting degree of disruption (Amann, 2011; Burnley & Jones, 2018). In the present study this can be demonstrated by the large decrease in $Q_{tw.pot}$ post-exercise (Figure 8-2B), of which the male data (−36%) is comparable to previous studies assessing contractile impairment following severe intensity cycling (~30-40%, Amann & Dempsey, 2008; Amann *et al.*, 2011; Thomas *et al.*, 2016). Although central fatigue occurred during this trial, excitability of the corticospinal tract was unaltered at the cortical and spinal levels, suggesting that responsiveness descending neurons did not change post-exercise (Weavil & Amann, 2018). This central fatigue might have been a result of impaired neural drive, or synaptic input into the corticospinal tract (Amann, 2011). Regardless, central fatigue is not considered to be the limiting factor to exercise within the severe intensity domain (Burnley & Jones, 2018). One caveat of this Chapter, and other locomotor neuromuscular fatigue studies is that responses were assessed pre-post exercise in an isometric setting. Responses evoked *during* exercise could elucidate further details about the time-course and magnitude of change.

In contrast to our hypothesis, when exercise intensity was metabolically-matched, females demonstrated greater fatigue-resistance of the knee-extensors compared to males (−15% $Q_{tw.pot}$ decline). There are multiple factors that could explain this occurrence from the present study and previous data. For example, as previously mentioned, females typically have a greater proportional area of type I muscle fibres (Staron *et al.*, 2000; Roepstorff *et al.*, 2006), and whilst in the context of this study it might not contribute to differences in the power-duration relationship, it could provide females with the capacity to tolerate deleterious metabolites when exercising above CP. Indeed, previous studies using ^{31}P-MRS have shown lower decreases in muscle pH, PCr, and attenuated increases in ADP (Russ *et al.*, 2005; Willcocks *et al.*, 2010) at high exercise intensities. Furthermore, slower calcium handling kinetics in females (Harmer *et al.*, 2014) could produce more fatigue-resistant contractile apparatus. Additionally, females demonstrate a lesser increase in rmsEMG during the severe intensity task, and whilst a limited measure of neural activity (Farina *et al.*, 2014), could represent less of a compensatory increase in motor unit recruitment as a result of less peripheral fatigue within the already-recruited motor units. Indeed, this finding mirrors that shown in Chapter 6, and exists when rmsEMG is normalised to M_{max} to negate the influence of subcutaneous fat on electromyographic signal.

Another potential contributing factor to the sex difference in fatigability could be the greater oxygenation within the VL for females during exercise (Figure 8-3). In both Chapter 6 and the present study, this manifested predominantly as a lesser rise in HHb concentration for females during exercise, which could be a result of the fibre type difference between males and females. Specifically, the greater HHb increase in males could be a result of greater oxygen extraction (Grassi *et al.*, 2003), which could be related to a greater oxygen cost within the muscle ($m\dot{V}O_2$) compared to females. Indeed, when assessed at a pulmonary level, individuals with greater type I fibre proportion of the VL demonstrate a lower $\dot{V}O_2$ for a given exercise intensity (Coyle *et al.*, 1992). This is speculative, although potentially fertile ground

for future research as m$\dot{V}O_2$ can be non-invasively quantified with a combination of NIRS and muscle occlusion (Ryan *et al.*, 2012). One might expect pulmonary $\dot{V}O_2$ to reflect a potential sex difference in m$\dot{V}O_2$, however, females experienced a similar $\dot{V}_E$ to males during severe intensity cycling, which is linked with a greater oxygen cost of breathing in females (Witt *et al.*, 2007). When measured at the pulmonary level, the $\dot{V}O_2$ response to exercise is an amalgamation of all physiological systems, therefore the elevated Wb might have counterbalanced reduced m$\dot{V}O_2$.

Together, the aforementioned data present compelling evidence that whilst exercise performance (time to task failure) in the severe intensity domain is not affected by sex, the integrative response differs between males and females. Females experience less peripheral fatigue, potentially as a result of intramuscular differences in muscle oxygenation and contractile differences, however, likely have a greater Wb at a metabolically-matched exercise intensity. Therefore implying that the mechanisms of task failure might differ between the two sexes.

8.4.3. Heavy Intensity Domain

The $\dot{V}O_2$ response to exercise at 90% CP was typical of heavy intensity exercise. The $\dot{V}O_2$ response exhibited a slow component, however only reached ~83% $\dot{V}O_{2max}$ at task termination, indicating that energy provision from aerobic sources was not maximal (i.e. exercise intensity was less than critical power).

In terms of the pre-post exercise change in neuromuscular function, the fatigue observed was not due to an accumulation of disruptive metabolites, or an exhaustion of high-energy phosphates as substrate-level phosphorylation reaches a steady-state (Black *et al.*, 2016;

Vanhatalo *et al.*, 2016). Rather, neuromuscular fatigue in the heavy intensity domain is a result of both central and peripheral contributions, with the latter occurring in response to depletion of intramuscular glycogen concentration and the negative consequences for excitation-contraction coupling (Ørtenblad *et al.*, 2013). Furthermore, reactive oxygen species generation, and extracellular accumulation of K^+ might also impair contractile function (Allen *et al.*, 2008*a*). The net result in the present study is a decrease in $Q_{tw.pot}$ (Figure 8-4), that was less profound in females. Given that the mechanisms of peripheral fatigue differ above and below CP, this fatigue-resistance of female knee-extensors below CP must be a result of different physiological processes as well. One explanation could be that, given RER was lower in females during the 90% CP trial, the rate of fatty acid utilisation was greater, eliciting a glycogen-sparing effect. This notion is supported by previous evidence demonstrating that males utilise ~25% more muscle glycogen at exercise intensities matched below CP (Tarnopolsky *et al.*, 1990; Roepstorff *et al.*, 2002, 2006). Similarly to 110% CP, the decrease in muscle oxygenation was less in females at 90% CP, again, potentially reflecting a lower oxygen cost of contraction as a result of greater type I muscle fibre proportion.

The central fatigue occurring below CP is thought not to be a result of group III/IV afferent feedback, as there is no progressive metabolite accumulation beneath CP. Instead, repetitive activation of descending fibres can alter their intrinsic properties, rendering them less responsive to activation (Carpentier *et al.*, 2001). This phenomenon is reflected in the present study as a decrease in VA_{MNS} and VA_{TMS}, with a greater decline in VA_{MNS} only for males. This discrepancy might indirectly suggest that the aetiology of the sex difference in central fatigue would be located at a sub-cortical level. Indeed, a decrease in MEP and SP-LEP was observed (Figure 8-5), and is likely a result of reduced strength of persistent inward currents (Heckman *et al.*, 2008). However, the sex × time interaction for these evoked variables was not different ($p \geq 0.132$). Multiple studies have provided evidence to show reduced motoneuronal excitability with fatigue in single-limb (Kennedy *et al.*, 2016; Finn *et al.*, 2018) and whole-body exercise

modalities (Weavil *et al.*, 2016; Sidhu *et al.*, 2017), however the present study is the first to match exercise intensity to critical power and assess the neural response. Interestingly, the decrease in LEP was only evident during the SP, with no change in unconditioned LEP (Figure 8-5). Finn *et al.* (2018) demonstrated a similar phenomenon in an isometric modality and suggested that SP-LEPs were more sensitive to intrinsic changes in motoneuronal properties, as inhibiting descending drive from the motor cortex removes a confound of excitatory synaptic input to the motoneuron. The unconditioned LEPs do not change, as neural drive is capable of increasing (seen in Table 8-2 of the present study) and maintaining net motoneuronal output. Therefore, as only SP-LEPs changed, the central fatigue observed at 90% CP in the present study is likely a result of a change in intrinsic properties of motoneurons, rendering them less responsive to synaptic input. As mentioned, this occurred independently of sex, with no sex × time interaction observed for any evoked potential. It is possible that due to far smaller measurement error for VA_{MNS} compared to evoked potentials (Chapters 4 and 8), a sex difference in motoneuronal excitability was not able to be discerned due to statistical under powering.

Collectively, these data suggest that the neuromuscular response to cycling at 90% CP is underpinned by decreases in central nervous system function and contractile impairment. Similar to severe intensity exercise, performance was not altered, but the neuromuscular adjustments were different between males and females.

8.4.4. Further Considerations

Fatigability of both inspiratory and expiratory muscles was demonstrated above CP, which was not sex-dependent. This contrasts previous evidence suggesting that the diaphragm is a more fatigue-resistant muscle in females (Guenette *et al.*, 2010; Welch *et al.*, 2018), however, the assessment modality employed in the present study was less precise, and could not

discern individual muscle fatigability. The rise in rmsEMG for respiratory musculature was similar between sexes in both trials, which also contradicts previous findings suggesting females activate 'accessory' respiratory muscle such as the SCM to reduce the diaphragmatic load (Mitchell *et al.*, 2018*b*). Whilst no sex difference in respiratory muscle fatigability or gas exchange were observed, the similar V_E during both severe and heavy intensity cycling likely led to females experiencing a greater work of breathing during both trials (Dominelli *et al.*, 2015), which could contribute to greater exertional dyspnea (Schaeffer *et al.*, 2014; Cory *et al.*, 2015). When taken into consideration with the reduced peripheral fatigue in locomotor muscles, it could conceivably be suggested that the 'sensory tolerance limit' consists of different magnitudes of afferent feedback from different physiological systems in males and females (Hureau *et al.,* 2018, Thomas *et al.,* 2019). Such that the locomotor muscle component is lesser, but the respiratory component is greater in females.

To compare fatigability in different populations, it is necessary to match both the intensity of exercise and the training status of the populations. The former was addressed in the present study by normalising exercise intensity to CP. Attempts were made to recruit populations of males and females of equivalent training status (De Pauw *et al.*, 2013; Decroix *et al.*, 2016), which resulted in similar average performance levels between groups. However, the sex difference in relative $\dot{V}O_{2max}$ was ~25%. This is larger than the sex difference suggested for sexes of equivalent training status (~10%, Joyner 2017), although this was based off a mixture of studies and a small sample of n = 8 male and 15 female elite distance runners (Pate & O'Neill, 2007). Other sources have previously described larger magnitudes in this sex difference (e.g. 17%, Froberg & Pedersen, 1984), although similarly rely on small sample sizes (n = 6 females and n = 7 males). Indeed, a meta-analysis of 440 male and 381 female participants that demonstrated an average sex difference of 28% in $\dot{V}O_{2max}$ when expressed relative to body mass; this difference remained in trained vs. untrained populations when body composition was accounted for (Sparling, 1980). Nevertheless, there appears to be a

discrepancy in what researchers deem to be an appropriate magnitude for the sex difference in $\dot{V}O_{2max}$. The present study used a minimum performance level (De Pauw *et al.*, 2013; Decroix *et al.*, 2016) to account for $\dot{V}O_{2max}$, relative P_{max}, as well as training history (hours·week^{-1}), and the sex differences demonstrated are therefore assumed to be independent of training status. However, as is well established, training status influences aerobic fitness, therefore this discrepancy in the appropriate magnitude of sex difference in $\dot{V}O_{2max}$ highlights a potential limitation in the present study, if the differences in indices of aerobic fitness are considered to be of too great a magnitude. The precise measurement of, and normalisation of values to fat-free mass could be an area for future research in order to uncouple the effects of sex and muscle mass in the field of integrative exercise physiology.

8.5. Conclusions

This study demonstrated that the power-duration relationship for cycling did not differ between males and females when expressed relative to P_{max}. Subsequent exercise performance in the severe and heavy intensity domains was not different, however the integrative response of cardiopulmonary, respiratory, and neuromuscular systems differed between males and females. Specifically, muscle oxygenation was greater and locomotor muscle fatigue was less in females during both tasks, additionally, the decline in CNS function was attenuated for females in the heavy intensity domain. Conversely, females experienced a greater ventilatory rate during both trials. Collectively, the present data suggests that the underpinning mechanisms of the sensory tolerance limit differ between males and females, which has important implications for acute and chronic exercise prescription. These considerations are discussed in Chapter 9.

CHAPTER 9 – GENERAL DISCUSSION

9.1. Experimental Recap

The overall aim of this Thesis was to investigate the sex difference in fatigability in healthy young adults. The focus was placed on the knee extensor muscles, due to their particular importance for athletic and locomotor activities. Chapter 4 aimed to investigate how the changing hormonal environment across the eumenorrheic menstrual cycle affects neuromuscular function and fatigability. Non-invasive neurostimulation was performed before and after an open-ended intermittent, isometric exercise task to discern the mechanisms behind hormonally-induced and exercise-induced changes, and the interaction between the two factors. A reliability study was also conducted (Chapter 5) in a hormonally-constant group of females using the monophasic oral contraceptive pill; the tasks performed were identical to the eumenorrheic group, which permitted the degree of measurement error of the tasks and techniques to be discerned. Chapter 6 then studied the intensity-duration relationship during intermittent, isometric exercise in males and females, whilst assessing fatigability and recovery during and after two subsequent exercise tasks to failure normalised to the critical intensity. Chapter 7 developed a novel method of non-invasive electrical stimulation for the assessment of the spinal component of the corticospinal tract. Collision experiments with TMS were conducted to study the interaction between the two forms of stimulation, and the size of responses was monitored with increasing contraction intensity. Finally, Chapter 8 employed a similar design as Chapter 6, but utilised cycling as the modality of exercise. The power-duration relationship was profiled in both sexes, and then exercise trials in the severe and heavy intensity domains, normalised to critical power, were performed to task failure.

9.2. Summary of Main Findings

In Chapter 4, it was shown that the menstrual cycle induces changes in neuromuscular function and fatigability. Specifically, pre-exercise voluntary activation was greatest in the late follicular phase, when oestrogen concentrations were greatest and progesterone lowest.

Based upon the absence of change in corticospinal excitability, but reduced intra-cortical inhibition at this time point, the aetiology of this change was inferred to be related to GABA-ergic neurotransmission within the motor cortex. Thereafter, during exercise a similar profile of neuromuscular fatigue was demonstrated between all three phases, however, in the luteal phase, females were able to exercise for longer upon reaching a nadir in neuromuscular function, leading to a ~30% longer time to task failure. Thus, the rise in progesterone concentration in the luteal phase seemed to positively affect fatigability. The aforementioned changes in neuromuscular function and fatigability led to the conclusion that if females were to be compared to males, hormonal concentrations would need to be controlled. Chapter 5 therefore assessed the repeatability of neuromuscular function and fatigability in a hormonally-constant population of healthy, young females – monophasic combined oral contraceptive users. It was demonstrated that the measures and tasks were repeatable, with similar reliability indices to previously reported male data. Thus, consequent studies in this thesis only recruited females using monophasic hormonal contraceptives.

Chapter 6 then demonstrated that in an intermittent, isometric exercise modality, females are capable of sustaining a greater relative exercise intensity (termed the critical intensity). Subsequently, during open-ended exercise at intensities normalised to critical intensity, females had a greater time to task failure than males. This was accompanied by slower declines in neuromuscular function, including central and peripheral components. During these normalised exercise trials, muscle oxygenation was monitored and demonstrated to be greater in females during both tasks, which likely contributed to the differences in fatigability. Following exercise, the rate of recovery for peripheral fatigue was greater for females, which, similar to the slower rate of fatigue, is likely a result of differences within the contractile apparatus.

Chapter 7 served to develop a novel assessment of corticospinal excitability at the spinal level. Delivering electrical stimulation to the lumbar spinal cord segments was shown to attenuate descending action potentials from the motor cortex, one line of evidence to suggest that it activated corticospinal neurons. Additionally, the lumbar evoked responses demonstrated a change in amplitude with contraction intensities. This method of spinal cord activation did not require high stimulus intensities to evoke responses at rest or during contraction, therefore could be considered an alternative to techniques such as CMEPs and TMEPs, which require much higher intensities. Thereafter, this technique was used to explore the spinal contribution to CNS adjustments following whole-body exercise in Chapter 8.

The final experimental section (Chapter 8) used a similar experimental design to Chapter 6, in that the maximum sustainable exercise intensity and subsequent exercise performance was assessed in males and females. In this instance, the exercise modality was cycling, which presented different limiting factors than the isometric study. In Chapter 8, no sex difference was observed for critical power or subsequent exercise performance, however, the mechanisms owing to task-failure were different between males and females. Specifically, females exhibited less peripheral fatigue of the knee-extensors, as well as greater muscle oxygenation. It is speculated that this greater fatigue-resistance of the locomotor muscles counteracts known central limitations of oxygen delivery and ventilation for females, leading to comparable exercise performance. The explanations for the aforementioned findings are presented below in greater detail, and considerations for acute and chronic exercise prescription in males and females are discussed.

9.3. The difference between single-limb and whole-body exercise

Chapter 6 demonstrated a sex difference in the intensity-duration relationship during intermittent, isometric exercise, whereas Chapter 8 did not observe a sex difference in the

power-duration relationship during cycling. This discrepancy can more than likely be explained by the limiting factors of both modalities of exercise. As has been established in fatigue-related literature for decades, the mechanisms of fatigue are specific to the exercise task, the performer, and the external conditions. This thesis has manipulated the first two, in order to explore the interaction between exercise task and performer on fatigability.

Critical power is the greatest metabolic rate that results in wholly-oxidative metabolism, and as a result is sensitive to oxygen-related interventions such as manipulation of inspired oxygen concentration (Vanhatalo *et al.*, 2010; Dekerle *et al.*, 2012). During whole-body exercise, two elements influence the use of oxygen within working musculature: 1) convective factors, such as ventilation, cardiac output, haemoglobin concentration, etc; and 2) diffusive factors such as muscle capillarity, mitochondria density, and the arteriovenous oxygen difference (Wagner, 1988, 1996). As evidenced previously (Rossman *et al.*, 2012, 2014) and in the present thesis (Chapter 6), the cardiovascular response to single-limb exercise does not reach near-maximal values, implying that the limitation to oxygen-dependent variables (e.g. CP) would lie within the diffusive capacity of exercising muscle(s). This puts females at an advantage when compared with male counterparts during exercise of this modality with the knee-extensors. Ample evidence exists to suggest that females have a greater proportional area of type I muscle fibres in the VL (Staron *et al.*, 2000; Roepstorff *et al.*, 2006), which possess a superior phenotype for oxidative metabolism and fatigue resistance compared to type II fibres (Schiaffino & Reggiani, 2011). For instance, in the internal muscle structure, type I fibres demonstrate a greater density and volume of mitochondria, and greater rate of oxidative enzyme activity (e.g. succinate dehydrogenase, Rivero *et al.*, 1998); and with regard to the extracellular surroundings, type I fibres have a greater capillary-to-fibre ratio (Andersen, 1975). These factors combine to permit metabolic differences between fibres, such as a greater ability to regenerate ATP via the TCA (Krebs) cycle and therefore an ability to match ATP consumption with regeneration in type I fibres, an occurrence that is not possible in type

II fibres (Schiaffino & Reggiani 2011). This matching of consumption and aerobic regeneration is the underlying physiological principle behind critical power; thus it is conceivable that due to a greater proportion of type I fibres, critical power, in tasks not limited by oxygen delivery, is greater in females (Chapter 6).

During high intensity whole-body exercise, the cardiovascular demand is elevated as more active muscle mass is recruited. When multiple muscle groups are active, the required skeletal muscle blood flow can exceed the pumping capacity (cardiac output) of the heart (Calbet *et al.*, 2004). Blood flow is distributed to different muscles and physiological systems during exercise by sympathetically-mediated vasoconstriction in an attempt to maintain adequate perfusion pressures (Sheel *et al.*, 2018). As a result, the physiological challenges during whole-body exercise are multi-factorial, when compared to single-limb exercise where the quantity and magnitude of muscles recruited is far less. As mentioned, critical power during whole-body exercise is dependent on oxygen delivery, as well as the capacity for consumption (Monod & Scherrer, 1965; Jones *et al.*, 2010). This is demonstrated with careful experimental work that shows complete blood flow occlusion reduces CP to zero (Broxterman *et al.*, 2015*a*), whilst manipulating the degree of occlusion via changing contraction duty cycle shows a positive association between rest period duration (non-occlusion) and CP (Broxterman *et al.*, 2014). Additionally, individuals with impaired oxygen delivery (e.g. those with central limitations such as chronic obstructive pulmonary disorder patients) exhibit a severely reduced CP (Van Der Vaart *et al.*, 2014). When this information is considered in the context of sex differences, it is important to consider the fact that females have smaller lung volumes (Schwartz *et al.*, 1988), and lower haemoglobin concentrations (Cureton *et al.*, 1986) compared to males. These factors amalgamate to a greater risk of exercise-induced arterial hypoxemia in females during high-intensity whole-body exercise (Harms *et al.*, 1998), reducing oxygen delivery to the working muscle(s). It is therefore possible, that the beneficial fatigue-resistance and metabolic properties of female skeletal muscle (diffusive capacity) are

counteracted by an impaired ability to deliver oxygen (convective capacity), and when both factors are considered, CP is equal between sexes, when expressed relevant to a maximum.

Despite no difference in the whole-body power-duration relationship, females demonstrated less muscle fatigue during exercise normalised to CP (Chapters 6 and 8). As explained, the contractile and metabolic properties of type I muscle fibres likely contribute to this phenomenon; however, what is of interest is that this greater peripheral fatigue-resistance did not elicit any performance enhancement in females compared to males. The sensory tolerance limit (Hureau *et al.,* 2018), and critical threshold theory (Amann, 2011) would suggest that in the severe intensity domain, exercise performance is terminated at the point at which metabolic disturbance in the skeletal muscles reaches an intolerable point. Indeed, W' is also considered to be an amount of work do-able before this intolerable metabolic environment is attained. However, as stated by Poole *et al.* (2016), a physiological equivalent of W' is unknown, and the nature of W' cannot be limited to a single physiological process. It is possible that the depletion of W' reflects a balance of cardiopulmonary and neuromuscular 'strain', which is akin to the sensory tolerance limit in suggesting that the maximum tolerable sensation of fatigue is composed of afferent information from multiple physiological systems. As Poole *et al.* (2016, p2331) suggested, "*W' will depend on the mode and conditions of the exercise and the participants involved"*. Therefore, based upon the data presented in Chapter 8, it could be postulated that the magnitude of the constituent parts of W' differ between males and females during whole-body exercise. As visually demonstrated in Figure 9-1, the muscular component of W' (or sensory tolerance limit) is reduced in females compared to males, however, the elevated Wb and concurrent sensations of dyspnea (Schaeffer *et al.*, 2014; Cory *et al.*, 2015; Dominelli *et al.*, 2015) are greater in females. When combined, the quantity of W', or the maximum tolerable sensation of fatigue is similar between sexes as demonstrated by equal performance during metabolically matched whole-body exercise (Chapter 8). The implications of this are subsequently discussed.

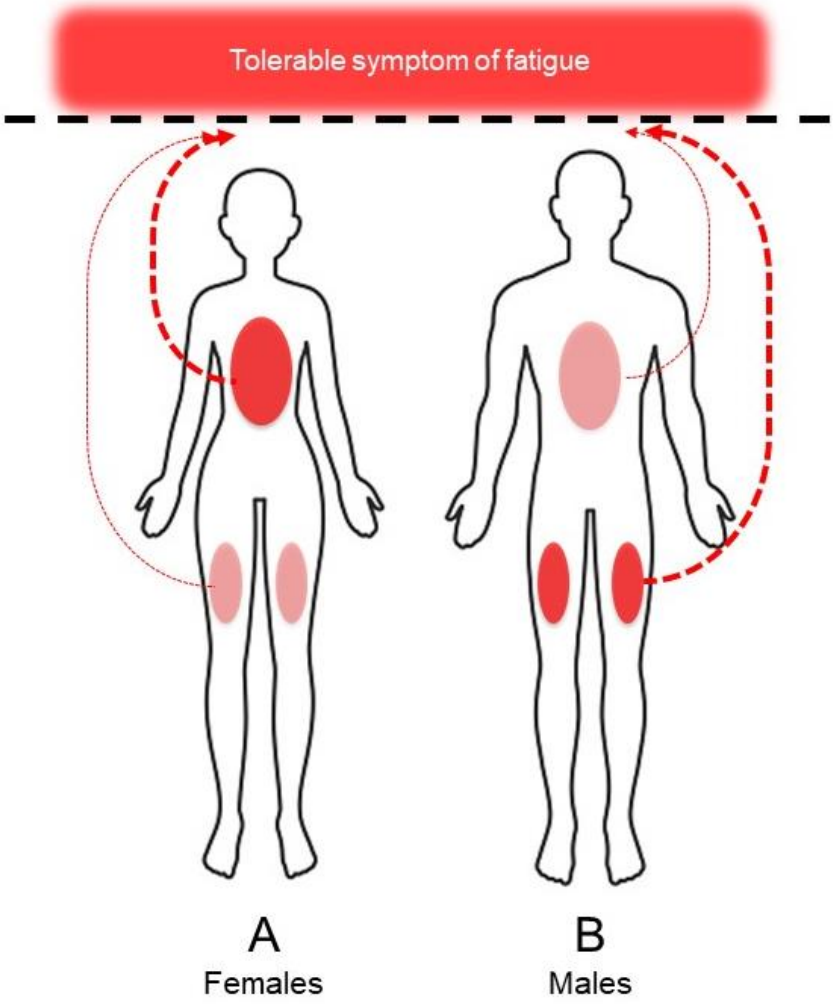

Figure 9-1: A conceptual model to demonstrate that whilst the maximum tolerable degree of fatigue is similar between males and females, the magnitudes of its constituent parts are different. Figure adapted from Thomas et al. (2018).

9.4. Considerations for Acute and Chronic Exercise Prescription

Whilst whole-body exercise performance did not differ between sexes in Chapter 8, this thesis has demonstrated that the mechanisms of task failure differ between males and females. The difference in the integrative response to acute exercise is of importance, particularly when exercise prescription is considered. Optimising the function of relevant physiological systems over the course of a training cycle is key to enhancing endurance exercise performance, but this process relies upon the delivery of consistent optimal adaptive stimuli. Evidence presented

in this thesis would suggest that for the same 'dosage' of exercise, the adaptive stimulus for different physiological systems is not equal for males and females.

Metabolic stress, the result of non-oxidative ATP synthesis, consists of an accumulation of metabolites, and depletion of high-energy phosphate stores (Allen *et al.*, 2008*a*). These metabolites and the associated intracellular hypoxia are potent stimuli for activation of AMP-activated protein kinase (AMPK) and hypoxia-inducible factor (HIF- α) pathways, that are crucial stages in mitochondrial biogenesis and angiogenesis (Marcinko & Steinberg, 2014). Indeed, a recent training study aiming to maximise metabolic stress using exercises such as sprint interval training combined with blood flow restriction demonstrated greater HIF-α activation following an acute bout of exercise, and greater $\dot{V}O_{2max}$ adaptation following a four-week training programme in when blood flow was restricted (Taylor *et al.*, 2016). Similarly, the production of ROS is thought to stimulate peroxisome proliferator-activated receptor gamma coactivator (PGC-1α) and p38 mitogen-activated protein kinase (p38MAPK) pathways (Ji, 2007), responsible for expression of genes involved in mitochondrial biogenesis, troponin I, and myoglobin content in mice (Lin *et al.*, 2002) and humans (Olesen *et al.*, 2010). Essentially, metabolic stress elicits an adaptive response that promotes the enhancement of oxidative capacity when repeated over time. In the context of this thesis, the utilisation of exercise normalised to the intensity-duration relationship allows confidence regarding the metabolic response to exercise. The severe intensity is associated with what is described above as metabolic stress, and disruption to the metabolic environment within the muscle could be inferred from the post-exercise reduction in $Q_{tw.pot}$. Consistently throughout Chapters 6 and 8, it was demonstrated that for an equivalent duration of high-intensity exercise, females experienced less peripheral fatigue than males, potentially indicative of less disruption to the muscle metabolic environment, and perhaps less of an adaptive stimulus for an equivalent amount of work. Indeed, evidence from ^{31}P-MRS techniques during isometric exercise modalities would support this notion (Russ *et al.*, 2005), however, equivalent data during

locomotor exercise is difficult to obtain, and as yet, it is unknown if this same phenomenon occurs.

The work incorporated in this thesis could help to inform recent suggestions that females are 'less trainable' than males (Howden *et al.*, 2015; Diaz-Canestro & Montero, 2019). When $\dot{V}O_{2max}$ increases in response to training are compared between sexes, males demonstrate greater absolute and relative increases. The authors speculated that a combination of genetic differences, lower blood volumes, and impaired cardiac adaptation in females contribute to this phenomenon (Diaz-Canestro & Montero, 2019). Perhaps one other explanatory factor could be that if females experience less metabolic stress for the same amount of high-intensity exercise, the aforementioned pathways leading to greater oxidative capacity might be less activated. When this is repeated over the course of a training programme, female $\dot{V}O_{2max}$ adaptation could be lower in comparison to males as a result of less mitochondrial biogenesis and angiogenesis within the skeletal muscle. Indeed, Scalzo *et al.* (2014) demonstrated greater mitochondrial biogenesis in males compared to females after 4 weeks of interval training. Future research could investigate the acute responses of these adaptive pathways, and further interrogate the plasticity of skeletal muscle in both sexes in response to both acute and chronic high-intensity exercise protocols.

Other considerations for acute exercise prescription stem from Chapter 6, specifically, females demonstrated a greater rate of recovery for peripheral fatigue post-exercise compared to males. Other evidence corroborates this finding, Senefeld *et al.* (2018) demonstrated quicker recovery of contractile function in the immediate (10 minute) post-exercise window, whilst Chapter 6 extended this finding to 45 minutes. Forms of interval exercise relying on repeated activity close to, or exceeding the intensity eliciting $\dot{V}O_{2max}$ are based upon the notion that repeatedly disrupting the metabolic environment of the exercising muscle(s) will augment

aerobic and anaerobic adaptation (Gibala, 2009). The possibility exists that females require less rest between intense interval exertions for adequate metabolic recovery; if standard training recommendations based predominately on male research are applied to both sexes, females might not be receiving an optimal stimulus for adaptation.

9.5. The Influence of the Menstrual Cycle on Acute and Chronic Exercise

To neglect the influence of the menstrual cycle on exercise physiology would be remiss of a thesis concerning sex differences. Chapter 4 specifically focussed on this inherent chronobiological occurrence. In terms of acute exercise, Chapter 4 demonstrated an influence of endogenous hormone concentrations on pre-exercise neuromuscular function, and fatigue-resistance during metabolically-challenging exercise. The literature surrounding these topics is equivocal, with certain physiological responses such as cardiovascular strain (Pivarnik *et al.*, 1992), substrate utilisation (Campbell *et al.*, 2001), and the ventilatory response (Dombovy *et al.*, 1987) all demonstrating menstrual cycle effects. However, data concerning high-intensity exercise performance is conflicting (Janse De Jonge, 2003). Chapter 4 adds to the body of literature on this topic, using quantification of endogenous hormone concentrations to validate menstrual cycle phases. Such a tight experimental control has recently been recommended as best practice when conducting female physiological research to attempt to reduce the ambiguity in understanding hormonal influences on physiological systems (Sims & Heather, 2018; Janse de Jonge *et al.*, 2019).

The conclusions from Chapter 4 were that high-intensity, isometric exercise performance was greatest in the luteal phase. The intensity-duration relationship was not profiled across the menstrual cycle, however, in the discussion of Chapter 4 we proposed that the elevated progesterone concentrations induced a state of ‘luteal analgesia’ (Vincent *et al.*, 2018). The

data in Chapter 4 would suggest that the sensory tolerance limit was increased in the luteal phase, as females were able to exercise for longer once a nadir in $Q_{tw.pot}$ had been reached. High levels of metabolic disturbance are associated with sensations of pain and fatigue (Pollak *et al.*, 2014), therefore if the sensory tolerance limit and W' are linked to a maximal tolerable degree of metabolic disturbance, the menstrual cycle could potentially augment W' in the luteal phase. The sensory tolerance limit in this scenario provides a more attractive explanation than the critical threshold hypothesis. Once a nadir in contractile function was reached, time to task failure was not immediate, indicating that a maximum degree of peripheral fatigue (Amann 2013) was not the primary determinant of task failure. Rather, it could be speculated that task failure/fatigability was ultimately determined by the perceptual aspects of fatigability, rather than the aforementioned neuromuscular adjustments. These assertions should be investigated directly, and the use of a five-minute 'all-out' test (Burnley, 2010) would permit immediate assessment of the isometric intensity-duration relationship in each phase of the menstrual cycle, something that is challenging with four repeated constant-load trials. Additionally, as highlighted in previous sections of this discussion, Chapter 4 identified changes in single-limb exercise performance, the conclusions of which do not necessarily translate to whole-body exercise. Therefore, it could be suggested that the current evidence base is not sufficient to propose manipulation of training intensity, duration and volume around the fluctuations in endogenous hormone concentrations.

Chapter 4 also identified large changes in neurotransmission (SICI), of a similar magnitude to anti-convulsant drugs, where this physiological change is the goal (Ziemann *et al.*, 1996*a*). Indeed, evidence from patients using these forms of pharmacological intervention demonstrate similar decreases in voluntary activation of the knee-extensors as Chapter 4, without changes in maximal strength (Cabibel *et al.*, 2019). To further probe this acute change in neuromuscular function, longitudinal tracking of motor neurons using high-density EMG (Del Vecchio & Farina, 2019) combined with non-invasive neurostimulation, could allow a more

detailed interrogation into the influence of hormonal concentrations on synaptic input and neural drive, as well as intrinsic motor neuron properties. This change in neurotransmission is also relevant to long-term adaptation of the CNS. Motor neuroplasticity, both in health and disease, is thought to be mediated by reduced inhibition within the motor cortex (Stagg *et al.*, 2011; Kolasinski *et al.*, 2018). This is thought to enable a necessary increase in excitability and neural firing rates, permitting the coding of motor skills. It has recently been suggested that cortical inhibition could present a 'barrier' to motor plasticity (Kolasinski *et al.*, 2018), and evidence shows that when cortical inhibition is pharmacologically upregulated, neuroplasticity is impaired (McDonnell *et al.*, 2007). In this context of Chapter 4, it could be the case that at time points of the menstrual cycle such as the luteal phase, when SICI is elevated, adaptation might be harder to achieve. How the chronic downregulation of endogenous hormone concentrations via the use of exogenous hormonal contraceptives affects neural function and plasticity is also unknown. Considering 73% of women in the general population have used hormonal contraceptives at some stage in their life (Cea-Soriano *et al.*, 2014), this information would be pertinent to those seeking to optimise neural health across the female lifespan.

9.6. Concluding Remarks

This thesis has studied the sex difference in fatigability by profiling the intensity-duration relationship across different modalities of exercise in an attempt to gain mechanistic insight into the underpinnings of the phenomenon. Combining non-invasive neurostimulation, haemodynamic, cardiopulmonary, and muscle oxygenation monitoring, has enabled the integrative physiological response to metabolically-matched exercise to be compared between males and females. The summaries of each study are presented above, however, the overarching theme of the individual Chapters' conclusions is that data regarding male responses to exercise should not be generalised to female populations. As discussed here, such generalisations could potentially be causing sub-optimal prescription of exercise for

females and as a result, poorer training outcomes. The data presented in this Thesis, including future comprehensive investigations into how biological context (i.e. sex and hormonal status) influence acute and chronic physiological responses, will enable the most optimal training patterns to be identified, promoting athletic excellence and health outcomes in all humans.

APPENDICES

Appendix 1 – Example of a participant information sheet

Faculty of Health & Life Sciences

Study Title: Is there a sex difference in fatigability following cycling exercise above and below critical power?

Investigator: Paul Ansdell

Participant Information Sheet

You are being invited to take part in this research study. Before you decide it is important for you to read this leaflet so you understand why the study is being carried out and what it will involve.

Reading this leaflet, discussing it with others or asking any questions you might have will help you decide whether or not you would like to take part.

What is the Purpose of the Study

Females have shown to be less fatigable than males during certain exercise conditions. Some research has shown that females have more aerobic, fatigue-resistant muscles, and that they can last longer on endurance tasks than males. The literature on this topic typically bases the intensity of endurance tasks on a percentage of maximum capacity. Very rarely do researchers take into account factors like anaerobic threshold/critical intensity (the point at which your muscles can't cope with the build-up of acid, and other negative by-products of exercise). Therefore, in order to create a more well-rounded picture of the sex difference in fatigability, we aim to work out your cycling 'critical power' and base two fatiguing tasks around it (+10% and -10%). We will use magnetic and electrical stimulation of the nervous system to assess how fatigued your muscles and central nervous system get during and following exercise; and we will also use non-invasive techniques to see how your cardiovascular system delivers oxygen to the muscles, and how the muscles utilise the oxygen.

Why have I been invited?

You have been invited to take part in this study as you are a male or female aged 18-35. If you are female, you have taking the monophasic hormonal contraceptive pill for the past 6

months. You have no history of neurological, cardiovascular or respiratory illness, and do not have any metal plates in the skull.

Do I have to take part?

Your participation is entirely voluntary. It is up to you whether you take part in the study. This information sheet is given to you to help you make that decision. If you decide to take part, you can stop testing and discontinue participation at any point without any need for explanation. You are completely free to decide whether or not to take part, or to take part and then leave the study before completion.

What will happen if I take part?

Prior to the data collection procedure, we will ask you to fill out pre-screening and health questionnaire in which you will be required to disclose information about your general health, history of contraceptive use and menstrual cycles (if female). Following this, you will be required to attend the laboratory 6 times. The first will be a familiarisation session, followed by 3 trials required to estimate your critical power. Then two final trials (+10% and -10%) based around your critical intensity. Sessions will take in the neurophysiology laboratory in Northumberland building in the centre of Newcastle. The times and dates of each laboratory session will be arranged with the principal investigator. In the 24-hours prior to each session, you will be asked to avoid strenuous exercise and the consumption of alcohol. On the day of each session, you will also be asked to avoid the consumption of caffeine.

Visit 1: Familiarisation & Ramp test

During this session, you will be habituated with the study protocol and the techniques used; these involve transcranial magnetic stimulation of the brain, and electrical stimulation of the spine, and nerve that causes your quadriceps to activate. Your maximal leg strength during an isometric knee extension exercise will be tested. This will involve you sitting in a chair with your ankle attached to a rigid cuff, and performing a maximal contraction of your quadriceps. You will also practice sub-maximal contractions, and maintaining force at a certain percentage of you maximum. The magnetic stimulations will involve single and paired pulses being delivered directly above the scalp. The spinal and peripheral nerve stimulation will involve an electrical current being discharged through electrodes placed towards the top of your groin, invoking an involuntary contraction causing the foot to kick out. A small amount of discomfort may be experienced during this procedure however; this is only short lived. Both electrical and magnetic stimulation are safe and painless techniques that are routinely used in our laboratories. You will also complete maximum inspiratory and expiratory manouvres, in which you maximal breathing force is measured. This gives us an indication of how the function of your lung muscles changes with fatigue. Following this you will perform a ramp test, which is a commonly used cycling test to work out the maximum power you can produce aerobically. During this test, we will measure your expired gas using a mask that will analyse the oxygen and carbon dioxide content of the air you breathe out. This test will involve gradually increasing the intensity you cycle at until you cannot maintain a constant cadence. The familiarisation session is estimated to take 60 minutes. Providing that you meet certain criteria of a maximum oxygen consumption at the end of the ramp test (VO2max) greater 55 $ml.kg^{-1}.min^{-1}$ for males and 48 $ml.kg^{-1}.min^{-1}$ for females, participants will move onto the remainder of the experimental visits.

Visits 2-4: Estimation trials

Using your maximum power from the ramp test, we will set three power outputs for you to cycle at (one per visit, separated by 24 hours minimum). Again, during these trials we will measure your expired gas using the mask. You will cycle to 'exhaustion' in these trials, meaning that you maintain the cadence until you physically cannot any longer. Using the duration of these trials and the amount of 'work' you perform, we can enter these variables into a formula that will predict your maximal sustainable intensity (critical power). These sessions will take approximately 30-45 minutes.

Visits 5 and 6: Supra- and sub-critical power trials

When you arrive in labs, you will complete a neuromuscular and respiratory assessment (as described above), then a cycling trial to exhaustion. During this trial, we will stimulate the nervous system at regular intervals to see how the brain and spinal cord respond to fatiguing cycling. Immediately following this, a post-exercise neuromuscular assessment will be performed. During the cycling your expired gas will be measured through the mask, your cardiovascular (e.g. heart rate, blood pressure) will be continuously monitored via an infrared cuff around your middle finger, and the amount of oxygen in your quadriceps will be assessed using an LED 'optode' attached to your leg. All procedures are non-invasive and pain-free. These final two visits will take 90-120 minutes.

*An example of the gas exchange mask and gas analysis setup during cycling. *Not the same equipment used in the present study.*

What are the possible disadvantages of taking part?

Aside from taking the time out of your life to participate, which we are grateful for, there are relatively few disadvantages. The discomfort associated with TMS and nerve stimulation may be unpleasant, but it is short lived and does not last longer than a few milliseconds. Should you experience too much discomfort and wish to withdraw then that is perfectly acceptable. Additionally, TMS carries a risk of seizures, fainting, or transient hearing alterations; however, the risk of adverse events is extremely rare in healthy individuals. A screening questionnaire

is used to check for anything that would prevent you from receiving TMS. The fatiguing tasks will be to 'exhaustion' or the point where you cannot maintain the required cadence. This will cause increases in heart rate, blood pressure, and you may feel breathlessness, as well as fatigue and discomfort in your quadriceps. There is potential risk of injury, however this is no greater than any other form of exercise, and a warm up will be performed prior to any exercise. Exhaustive exercise also carries a risk of adverse events such as cardiovascular event (e.g. cardiac arrest or heart attack), however the incidence rates are extremely low (1 per 50,000 athletes, Harmon et al. 2014. *British Journal of Sports Medicine*).

What are the possible benefits of taking part?

You will be adding to the under-researched area of sex differences in physiology. How females and males differ physiologically is not well understood and an emerging area of biomedical research. Data from the present study may also be used to inform athletic training or clinical rehabilitation.

How can I withdraw from the study?

If you do wish to withdraw then you can do so without any judgement or negative consequences. Simply email any of the research team informing us that you do not wish to continue with testing. You do not have to give any reason.

Will my taking part in this study be kept confidential and anonymous?

All data will be dealt with under the strictest of guidelines and according to the Data Protection Acts of 1984 and 1998. All data will remain anonymous other than to the researcher and supervisor. All data collected will be kept on a secure password protected computer system. Your name will not be written on any of the data we collect; the written information you provide will have an ID number, not your name. The consent form you have signed will be stored separately from your other data. The data collected from you in this study will be confidential.

How will my data be stored?

All data will be stored in accordance with University guidelines and the Data Protection Act (1998). Data will be stored on a USB stick that will be locked in a secure draw in the lead researcher's desk. Data will be stored on a password protected computer and backed up onto a password protected cloud storage service that will only be accessible to the lead researcher.

What will happen to the results of the study?

The results of the study will be used to formulate relevant conclusions. The general findings might be reported in a scientific journal or presented at a research conference, however the data will be anonymised and you or the data you have provided will not be personally identifiable.

Who is Organizing and Funding the Study?

The study will be organised and funded by Northumbria University.

Who has reviewed this study?

Before the study can begin, permission will be obtained from Northumbria University. The study and its protocol have received full ethical approval from the Chair of the Faculty of

Health and Life Sciences Ethics Committee. If you require confirmation of this, please contact the Chair of the Faculty of Health and Life Sciences Ethics Committee (Mick Wilkinson mic.wilkinson@northumbria.ac.uk) and stating the full title and principal investigator of the study.

Contact for further information:
Paul Ansdell (paul.ansdell@northumbria.ac.uk)
Dr Stuart Goodall (stuart.goodall@northumbria.ac.uk)

Appendix 2 – Example of an informed consent form

Faculty of Health & Life Sciences

INFORMED CONSENT FORM

Project Title: Is there a sex difference in fatigability following cycling exercise above and below critical power?

Principal Investigator: Paul Ansdell

	please tick or initial where applicable
I have carefully read and understood the Participant Information Sheet.	☐
I have had an opportunity to ask questions and discuss this study and I have received satisfactory answers.	☐
I understand I am free to withdraw from the study at any time, without having to give a reason for withdrawing, and without prejudice.	☐
I agree to take part in this study.	☐

Signature of participant..... Date.....……..   (NAME IN BLOCK LETTERS).....……
Signature of researcher..... Date.....……..   (NAME IN BLOCK LETTERS).....……

Appendix 3 – Example of the screening questionnaire

The questionnaire will take you approximately 15 minutes to complete. The questionnaire consists of tick boxes or one-sentence answers. If you would prefer not to answer certain questions, please leave them blank. You are allowed to take the questionnaire away with you to allow you to answer the questions in private. If you need any help answering the questions, please do not hesitate to contact the lead researcher. Please see contact details at the end of the questionnaire.

Weight: _____kg **Height:** _____ m
Date of Birth (dd/mm/yy): __/__/__

1. **How often do you take part in structured physical activity?**
   For example: Jogging, team sports, aerobics etc.

Please tick one:		How many hours?
a. Once a week	☐	_____
b. Twice a week	☐	_____
c. Three times a week	☐	_____
d. Four times a week	☐	_____
e. More than 5 times a week	☐	_____
f. Never	☐	

2. **Do you suffer from any medical conditions?**
   For example: Arthritis, Myositis, Fibromyalgia, Myopathy, Diabetes Mellitus, or Hypothyroidism

   Yes ☐ No ☐

   If yes, please elaborate:_____
   _____
   _____
   _____

3. **Are you currently taking any medication?**

   Yes ☐ No ☐

   If yes, please elaborate:_____
   _____

4. **Are you currently taking any supplements or vitamins?**
   For example: Protein supplements or vitamin e tablets?
   Yes ☐ No ☐

   If yes, please elaborate:_____
   _____

5. **Are you <u>currently</u> suffering from any musculoskeletal or tendon injury?**
   For example: Sore muscles, broken bones or sore tendons

   Yes ☐ No ☐

   If yes, please describe information regarding the type of injury, injury location, and when it occurred (dd/mm/yy):
   _____
   _____
   _____

6. **Have you <u>ever</u> severely controlled your diet to achieve a dramatic change in weight?**

   Yes ☐ No ☐

<u>IF MALE PLEASE GO TO QUESTION 19</u>

7. **Do you have any children?**

   Yes ☐ No ☐

   If no please go to question 9

   a. Please list the date/s you had your child/ children (dd/mm/yy):

   1) ___/___/___
   2) ___/___/___
   3) ___/___/___
   4) ___/___/___

8. **Have you breast fed in the last year?**

   Yes ☐ No ☐

9. **At what age did you start your period?**

   _____ years old

10. **On average how long does your menstrual cycle last (how many days between a period)?**

    _____days

11. **On average how long does your period last?**

    _____ days

12. **Have you had a regular period for the last six months (one period at least every month and no spotting in-between periods)?**

    Yes ☐ No ☐

13. **Have your periods stopped?**

    Yes ☐ No ☐

    If no, please go to question 14

    a. What was the date of your last period (dd/mm/yy)?
       ___/___/___

14. **Have you ever had a hysterectomy?**

    Yes ☐ No ☐

15. **Have you ever had your ovary / ovaries removed?**

    Yes ☐ No ☐

16. **Have you ever taken the oral contraceptive pill?**

    Yes ☐ No ☐

If no please go to question 17

a. What type of pill did / do you take (name and dose of oestrogen and progesterone)?
   Dose values can be found on the medication box. If you are unsure, please bring your medication with you.

_____

_____

b. When did you start taking the pill (mm/yy)? ____/___
c. If you have, when did you stop taking the pill (mm/yy)? ____/___

17. Have you ever used any other form of hormone-based birth control (injection, vaginal ring, etc.)?

Yes ☐ No ☐

If no please go to question 18

a. What type of hormone-based birth control did / do you take (name and dose of oestrogen and progesterone)?
Dose values can be found on the medication box. If you are unsure, please bring your medication with you.

_____

_____

b. When did you start using the contraception (mm/yy)? ____/___
c. If you have, when did you stop using the contraception (mm/yy)? ____/___

18. Have your ever used hormone replacement therapy?

Yes ☐ No ☐

19. Have you ever been diagnosed with a neurological disorder, i.e., epilepsy?

Yes ☐ No ☐

20. Have you ever been diagnosed with a brain disorder such as Parkinson's disease?

Yes ☐ No ☐

21. Have you ever had a stroke?

Yes ☐ No ☐

22. Do you have metal objects in your head?

Yes ☐ No ☐

23. Are you taking any medication that would affect neuronal conductions?

Yes ☐ No ☐

24. Do you have a pacemaker?

Yes ☐ No ☐

Thank you for your time and honesty whilst completing the questionnaire. All questionnaires will remain strictly confidential.

Contact details:
Paul Ansdell (Lead Researcher)
paul.ansdell@northumbria.ac.uk

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