Fatigue and recovery of central nervous system function following intermittent-sprint exercise

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Fatigue and recovery of central nervous system function following intermittent-sprint exercise

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ABSTRACT

Research into fatigue following sports characterised by intermittent sprint exercise has increased substantially in recent years. However, when investigating post-exercise impairments in muscle function and recovery thereof, studies have predominantly focused on peripheral perturbations. The aim of this thesis was to examine the aetiology of impairments in neuromuscular function which occur following intermittent sprint exercise, with a focus on both peripheral and central perturbations. Study 1 determined the optimal protocol for the measurement of corticospinal excitability (CSE), short-interval intracortical inhibition (SICI), and intracortical facilitation (ICF) in the *rectus femoris* using transcranial magnetic stimulation (TMS). Study 2 examined the reliability of single- and paired-pulse TMS measures, as well as the reliability of measures of neuromuscular and physical function and perceptual assessments, when measured within-day and on consecutive days. These studies informed the methodology of subsequent chapters, and demonstrated that these variables could be assessed reliability within- and between-days. Study 3 examined the aetiology and recovery of perturbations in neuromuscular function following competitive football match-play. The study demonstrated that football match-play elicited substantial post-match impairments in isometric maximal voluntary contraction (MVC) strength of the knee extensors (-14%, $P < 0.001$) that persisted for 48 h (-4%, $P = 0.01$), before recovering by 72 h post-exercise. In addition, match-play elicited protracted impairments in contractile function, as demonstrated through considerable post-match reductions in potentiated twitch force (−14%; $P < 0.001$), which persisted at 24 h (−6%; $P = 0.01$) before recovering by 48 h. Furthermore, match-play evoked prolonged impairment in central nervous system (CNS) function, with a decline in the capacity of the CNS to voluntarily activate muscle post-match (−8%; $P < 0.001$), which persisted at 24 h (−5%; $P = 0.01$) and required up to 48 h to recover. Study 3 implemented a novel method of cryotherapy using phase change material (PCM) in an attempt to accelerate recovery following match-play. The study showed that wearing cold PCM garments had no effect on recovery when compared with a placebo control. These studies provide novel insight into the aetiology of fatigue following intermittent sprint exercise, which elicits prolonged impairments in CNS function, requiring up to 48 h to resolve.

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LIST OF SYMBOLS AND ABBREVIATIONS

AP	Action potential
AMT	Active motor threshold
ATP	Adenosine triphosphate
AURC	Area under recruitment curve
Ca^{2+}	Calcium ion
CNS	Central nervous system
CMEP	Cervicomedullary motor evoked potential
Cl^{-}	Chloride ion
CV	Coefficient of variation
CWI	Cold water immersion
CS	Conditioning stimulus
CI	Confidence interval
CT	Contraction time
CSE	Corticospinal excitability
CMJ	Countermovement jump
CK	Creatine kinase
DHPRs	Dihydropyridine receptors
DJ	Drop jump
EMG	Electromyography
ERT	Estimated resting twitch
EPSP	Excitatory post-synaptic potential
EIMD	Exercise induced muscle damage
GABA	Gamma-aminobutyric acid
GPS	Global positioning system
$RT_{0.5}$	Half relaxation time
HR	Heart rate
HIR	High-intensity running
H^{+}	Hydrogen ion

IPSP	Inhibitory post-synaptic potential
IL-1β	Interleukin 1-beta
ISI	Inter-stimulus interval
ICC	Intraclass correlation coefficient
ICF	Intracortical facilitation
M_{max}	Maximum muscle compound action potential
MRFD	Maximum rate of force development
MRR	Maximum rate of relaxation
MSO	Maximum stimulator output
MVC	Maximum voluntary contraction
MEP	Motor evoked potential
PCM	Phase change material
K^+	Potassium ion
$Q_{tw,pot}$	Potentiated twitch force
$Q_{tw,}$	Quadriceps twitch force
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RSI	Reactive strength index
RMT	Resting motor threshold
RMS_{EMG}	Root mean square electromyography
SR	Sarcoplasmic reticulum
SICI	Short-interval intracortical inhibition
Na^+	Sodium
SD	Standard Deviation
SIT	Superimposed twitch
TD	Total distance
TMS	Transcranial magnetic stimulation
TE	Typical error
VA	Voluntary activation
VA_{TMS}	Voluntary activation measured with transcranial magnetic stimulation

Publications arising from this thesis

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GIBSON, NV., BROWNSTEIN, C.G., BALL, D., TWIST, C. 2017. Physiological, perceptual and performance responses associated with self-selected versus standardized recovery periods during a repeated sprint protocol in elite youth football players. *Paediatric Exercise Science.* 29, 186-193.

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 granted by the Faculty of Health and Life Sciences Ethics committee for each study.

Name: Callum Brownstein

Signature:

Date: 1st October 2018

CHAPTER 1 INTRODUCTION

1-1 Introduction

In recent years, scientific research pertaining to team sports such as rugby, field hockey and association football has increased substantially. In particular, due to its worldwide popularity and the large economic incentives associated with successful performance, a plethora of research has investigated a vast array of topics related to football performance. As such, the physical demands of competitive football match-play have been evaluated extensively (Akenhead *et al*., 2013, Bangsbo *et al*., 2006, Mohr *et al*., 2003). Typically played over two 45-minute halves interspersed with a 15-minute recovery interval, football match-play is characterised by prolonged bouts of intermittent sprint exercise interspersed with periods of low-to-moderate activity (Bangsbo *et al*., 2006). In addition, match-play is associated with a diverse range of physically demanding actions, such as jumping, tackling, accelerating, decelerating and changing direction, imposing significant disturbances on multiple physiological systems (de Hoyo *et al*., 2016). An inevitable consequence of these physical demands is fatigue, a universal and daily phenomenon characterised by sensations of tiredness and weakness during and following exercise, which is underpinned and/or modulated by a myriad of physiological and psychological processes (Enoka and Duchateau, 2016).

During match-play, fatigue manifests through transient reductions in work rate following the most demanding periods of a match and cumulative declines in work-rate during the latter stages of a match (Rampinini *et al*., 2011, Mohr *et al*., 2003). The fatigue induced by football match-play also persists post-exercise, and can take days to resolve (Rampinini *et al*., 2011). Given the high levels of mechanical (Akenhead *et al*., 2013), metabolic (Ekblom, 1986, Bangsbo *et al*., 2006) and cognitive stress (Impellizzeri *et al*., 2004) associated with football match-play, the ensuing fatigue is

likely to be a multi-factorial phenomenon (Nedelec *et al*., 2012). Previous research has related the fatigue experienced after football match-play to energy depletion (Ekblom, 1986), disturbances to peripheral homeostasis (Ispirlidis *et al*., 2008), and damage to muscle tissue, perturbations which manifest in reductions in the force generating capacity of muscle (Thomas *et al*., 2017a, Gandevia, 2001). In turn, reductions in physical function and voluntary force in response to match-play have been studied extensively (Rampinini et al., 2011, Thomas et al., 2017a), and are typically attributed to perturbations within the neuromuscular pathway, involving impairments in contractile function, and/or the capacity of the central nervous system (CNS) to activate muscle (Carroll *et al*., 2017).

Fatigue and recovery are particularly pertinent issues in modern day competitive football, where the fixture schedule is such that teams are required to play up to 50-80 games per season, often with only 3-4 days between successive games (Mohr *et al*., 2016). Consequently, the fatigue associated with match-play could be compounded if the recovery period between games is insufficient to allow restoration of homeostasis. This in turn could provoke chronic and acute fatigue, potentially compromising subsequent performance and/or increasing the risk of injury, as has been highlighted in elite level players (Ekstrand *et al*., 2004, Carling *et al*., 2016). As such, understanding the aetiology of fatigue and the time-course of recovery could have a number of important implications, including the optimisation of the training schedule, the use of squad rotation strategies during periods of congested fixtures, and the implementation of appropriate recovery interventions designed to alleviate fatigue and accelerate the natural time-course of recovery.

In recent years, the application of neurostimulation techniques in the study of fatigue has permitted greater insight into peripheral and central contributors to reductions in

voluntary force production (Sidhu *et al*., 2009a; Hureau *et al*., 2016a; Goodall *et al.*, 2015). Specifically, by measuring involuntary evoked responses to electrical and magnetic stimulation of nervous tissue at rest and during isometric muscle actions, peripheral and central contributors to reductions in the force generating capacity of the muscle can be assessed. Despite these advancements, research into the mechanisms which contribute to declines in muscle strength in the days post-football-match-play have typically been studied from a peripheral viewpoint, with studies focusing on disturbances at sites distal to the neuromuscular junction (Nedelec *et al*., 2012). However, there remains an apparent disconnect between the temporal pattern of recovery of physical function, and markers of peripheral physiology and muscle damage following intermittent-sprint exercise (Pointon and Duffield, 2012, Minett *et al*., 2014). This disconnect has led some to speculate that processes within the CNS could contribute towards post-match declines in muscle strength and the recovery thereof (Minett and Duffield, 2014), a proposition supported by recent findings (Thomas *et al*., 2017; Rampinini *et al*., 2011).

The primary aim of this thesis is to examine the aetiology of impairments of neuromuscular function and the time course of recovery following competitive football match play using novel assessments of contractile and CNS function. Concurrent to this a range of perceptual and physical performance indicators of fatigue will be assessed, with the aim to validate their use as indicators of neuromuscular function. The results of these series of studies will therefore advance our understanding of the mechanisms underpinning fatigue in intermittent-sprint sports, and provide practitioners with appropriate tools to optimise the training and recovery process.

CHAPTER 2 LITERATURE REVIEW

2-1 Overview

Research into the topic of exercise induced fatigue can be traced back as far as the 18th century. Using electrical stimulation techniques and ergographic tracings, Alessandro Mosso was able to characterise reductions in muscle strength and associate its occurrence with central and peripheral influences (Di Giulio *et al*., 2006). Since then, a number of notable scientists and researchers have advanced our understanding into the mechanisms which cause reductions in muscle strength during exercise, while technological advancements have enabled the development of more sophisticated techniques to quantify exercise-induced impairments in muscle strength and evaluate its causative factors.

Despite centuries of research, fatigue remains the subject of considerable interest, with over 3000 scientific publications on this topic in the last 20 years (Edwards, 1981; Gandevia, 2001; Enoka and Duchateau, 2016). This notwithstanding, a strict and unanimously accepted definition of fatigue has been difficult to establish, due in part to fatigue encompassing several different phenomena that are each the consequence of different physiological and perceptual processes (Enoka and Duchateau, 2016). In a clinical setting, the term fatigue is used to describe a non-specific but debilitating symptom in a range of chronic diseases, and is often assessed using psychometric questionnaires and scales (Twomey *et al*., 2017). In the exercise sciences, the term fatigue relates to a decline in performance capabilities induced by exercise (Enoka and Duchateau, 2016). In this context, fatigue was traditionally defined as "a failure to maintain the required or expected force" (Edwards, 1981), while others define fatigue as "a reduction in the maximum force generating capability of the muscle during exercise" (Gandevia, 2001). While these definitions view impairments in the muscle's ability to produce force as a consequence of fatigue, it has recently been suggested

that the term fatigue should encompass both the physical and cognitive processes that occur during and following exercise, both of which are interdependent (Enoka and Duchateau, 2016). Specifically, Enoka & Duchateau (2016) propose that the concept of fatigue should acknowledge its two attributes: 1) performance fatigability – which involves an acute exercise-induced reduction in force and power output of the involved muscles that dictate performance and; 2) perceived fatigability – changes in sensations that accompany fatigue (Enoka and Duchateau, 2016). In turn, each of these domains is dependent on the status of a myriad of modulating factors, with the level of fatigue experienced by an individual dependent on interactions between the two domains. An illustration of the taxonomy proposed by Enoka & Duchateau (2016) is displayed in Figure 2-1. For the purposes of this thesis, fatigue will be defined as a sensation of tiredness and weakness, underpinned and/or modulated by a myriad of physiological and psychological processes. The focus of this thesis is on decrements in the maximum force generating capacity of the muscle, and the adjustments in neuromuscular function that might contribute reductions in muscle strength and increases in the sensation of fatigue.

In an attempt to understand and improve human exercise performance, a plethora of research has investigated the mechanisms contributing to declines in muscle strength both during (Sidhu *et al*., 2017; Weavil *et al*., 2016) and following exercise (Husmann *et al*., 2017, Thomas *et al*., 2017a). The following literature review will provide a synopsis of the literature pertaining to the aetiology and methods of quantifying exercise-induced declines in muscle strength, before focusing specifically on the aetiology of reductions in the force generating capacity of the muscle and the recovery thereof following intermittent-sprint exercise.

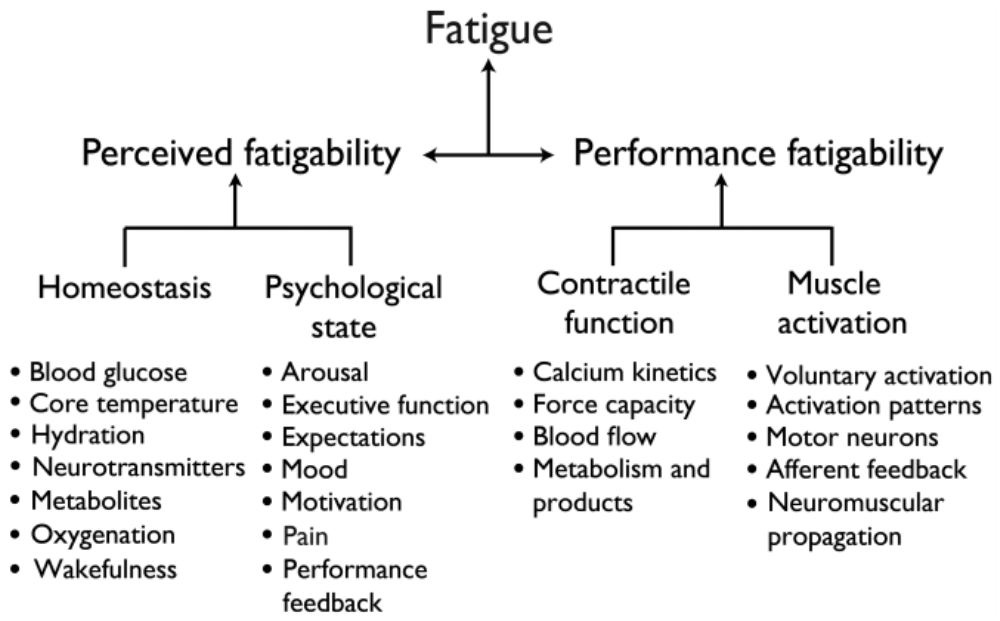


Figure 2-1. Fatigue taxonomy proposed by Enoka & Duchateua (2016), which states that fatigue can be defined as a self-reported disabling symptom derived from two interdependent attributes: perceived fatigability and performance fatigability, each of which are dependent on the status of a number of modulating factors. The focus of the present thesis is on reductions in the maximum force generating capacity of the muscle, a measure of performance fatigability, and the neuromuscular mechanisms that contribute to this impairment.

2-2 Mechanisms of voluntary force production

The voluntary production of force in the muscle is preceded by a complex chain of events beginning in the brain (Figure 2-2). Following command from supra-cortical structures, descending drive from the motor cortex activates spinal motor neurons which propagate the signal to the motor end plate, triggering acetylcholine (ACh) release across the neuromuscular junction. Acetylcholine subsequently binds to receptors on the sarcolemma, thereby altering its permeability and resulting in an action potential (AP) which induces depolarisation of the sarcolemma through movement of sodium (Na^+) and potassium (K^+) ions into and out of the cell, respectively. This AP propagates along the sarcolemma to the transverse tubules, stimulating the release of calcium (Ca^{2+}) ions from the lateral sacs of the sarcoplasmic

reticulum (SR) into the sarcomere. Calcium ions then binds to troponin, initiating the interaction between skeletal muscle proteins actin and myosin to liberate chemical energy to mechanical energy via the cross-bridge cycle. When neural stimulation ceases, Ca^{2+} moves back into the SR, causing muscle relaxation to occur. The link between the electrical AP and the mechanical muscle contraction is termed the excitation-contraction coupling.

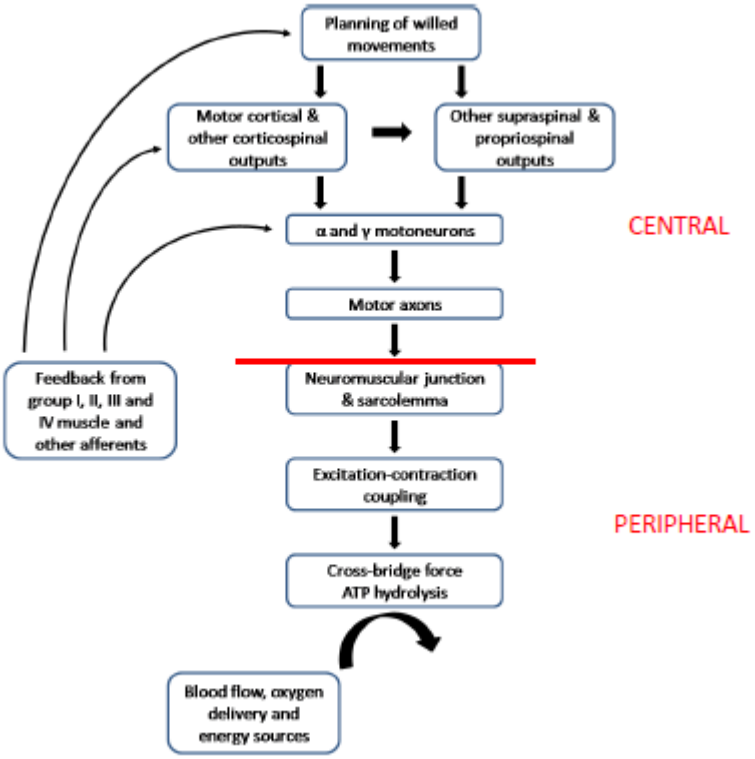


Figure 2-2. The chain of command involved in voluntary force production. Impairments in the force generating capacity of the muscle can potentially occur at any step along the brain-to-muscle pathway (from Gandevia, 2001).

2-3 Exercise-induced impairments in the force generating capacity of muscle

Impairments in maximum voluntary contraction (MVC) strength can occur at any site along the motor pathway; however, it is possible to identify this impairment as having either peripheral or central components (Figure 2-2). Peripheral factors which contribute to declines in muscle strength encompasses processes which occur at or distal to the neuromuscular junction, while central components involve processes which occur at points proximal to the neuromuscular junction (Gandevia, 2001). Traditionally, research investigating the mechanisms of reduced MVC has focused on events within the exercising muscle, with extensive mechanisms of contractile impairments proposed throughout the literature (Allen *et al*., 2008; Fitts, 2008). In contrast, the contribution of central factors to declines in MVC is less well understood (Twomey *et al*., 2018). The following sections will discuss the primary mechanisms associated with impairments in contractile and CNS function during exercise, methods of quantifying exercise-induced adjustments in neuromuscular function, and the relationship between task demands and adjustments in neuromuscular function.

2-4 Impairments in contractile function

Impairments in contractile function can arise due to a number of perturbations occurring at or distal to the neuromuscular junction (Fitts, 2008). A number of processes occurring beyond the neuromuscular junction precede cross-bridge formation and force production (Figure 2-3). In principle, any step in the chain of events that precedes voluntary contraction could be culpable for inhibitions in forceful

contractions, making localisation of the cause of contractile dysfunction difficult (Allen *et al*., 2008). The following section will outline the primary mechanisms purported to be responsible for impairments in contractile function.

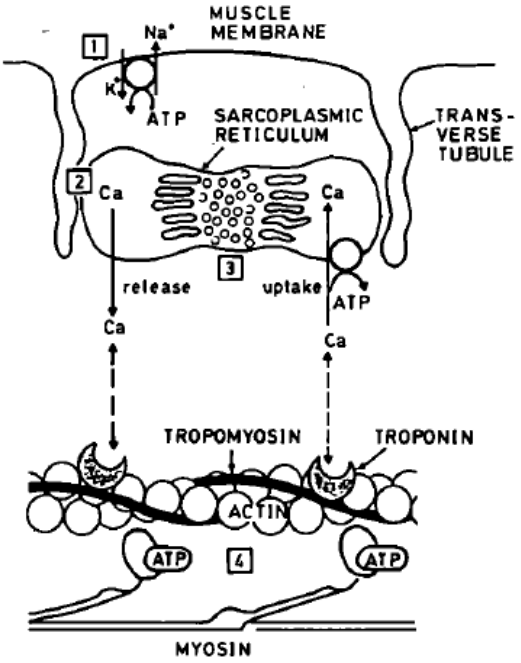


Figure 2-3. Diagrammatic representation of the excitation-contraction coupling: 1) Action potential propagates along the sarcolemma and transverse tubules. 2) L-type Ca^{2+} channels are activated, resulting in Ca^{2+} influx and opening of ryanodine receptors. 3) Ca^{2+} is released from the SR. 4) Ca^{2+} binds to troponin C to initiate cross-bride formation and force production. Relaxation occurs through reuptake of Ca^{2+} by the SR via the SR Ca^{2+} pump (from Sjogaard, 1990).

Failure of action potential propagation

In skeletal muscle cells, a resting membrane potential of approximately −75 to −85 mV is maintained as a result of electrochemical gradients between the intracellular and extracellular fluid. In particular, the selective permeability of the sarcolemma and the distribution of sodium (Na^+) and potassium (K^+) ions across the sarcolemma is integral in generating the resting membrane potential. The sodium-potassium (Na^+-K^+) pump plays a major role in maintaining these electrochemical gradients through active transport of Na^+ out of the cell and K^+ into the cell (Allen *et al*., 2008). During

an AP which precedes muscle contraction, the sarcolemma is depolarised and its permeability changes rapidly, causing passive movement of Na^+ into the cell and K^+ out of the cell. The rapid propagation of the AP along the sarcolemma is necessary to synchronously activate all parts of the muscle fibre during a contraction (Allen *et al*., 2008). In some cases, repeated activation of the muscle fibre causes K^+ to accumulate extracellularly, causing substantial depolarisation of the muscle membrane (Sjogaard, 1990). The increase in extracellular K^+ concentration which occurs during repeated activation has been implicated as a mechanism of contractile impairment (Sejersted and Sjogaard, 2000, Shushakov *et al*., 2007). Specifically, the substantial depolarisation which occurs during repetitive muscle action has been suggested to cause inhibition of the AP and a possible block in its propagation to the t-tubules (Sjogaard, 1990), resulting in a reduction in force (Allen *et al*., 2008). Thus, in certain circumstances, failure of AP propagation as a result of extracellular K^+ accumulation might contribute to impairments in contractile function.

Calcium (Ca^{2+}) handling and Inorganic Phosphate (Pi)

Force production in a muscle fibre depends on the AP triggering release of Ca^{2+} from the SR. When an AP arrives at the t-tubule, L-type Ca^{2+} channels known as dihydropyridine receptors (DHPRs) alter their conformation in response to the change in voltage, causing Ca^{2+} to move into the cell across the t-tubule membrane (Allen *et al*., 2008). Calcium release channels contained on the surface of the SR membrane, known as ryanodine receptors (RyR), lie adjacent to the L-type Ca^{2+} channels and open as a result of the charge movement, causing Ca^{2+} to be released from the SR into the myoplasm and allowing free Ca^{2+} to bind to troponin C to initiate muscle contraction.

The reduction in SR Ca^{2+} release in isolated muscle fibres has been proposed as a major contributor towards fatigue during repetitive muscle contractions (Allen and Westerblad, 2001, MacIntosh and Rassier, 2002). Evidence for a reduced Ca^{2+} transient being implicated as a cause of fatigue comes from studies applying caffeine to a fatigued muscle, causing a considerable increase in myoplasmic Ca^{2+} and force (Westerblad and Allen, 1991).

While it is well established that reduced SR Ca^{2+} release makes a substantial contribution to reductions in the force generating capacity of the muscle, the precise mechanisms responsible for this reduction are not fully understood (Allen *et al*., 2008). Several mechanisms have been proposed, including glycogen depletion following prolonged, exhaustive exercise (Gejl *et al*., 2014), direct inhibition of SR Ca^{2+} release by reduced adenosine triphosphate (ATP; Fitts, 2008), increases in the concentration of reactive oxygen and nitrogen species (Cheng *et al*., 2017) and the actions of inorganic phosphate (P_i) (Allen *et al*., 2008). During periods of intense skeletal muscle activity, P_i accumulates in the myoplasm due to the breakdown of phosphocreatine (PCr) through the creatine kinase (CK) reaction. Some of the myoplasmic P_i is then transported to the SR, were Ca^{2+} and P_i precipitate to form Ca^{2+}-P_i, thereby reducing the releasable pool of Ca^{2+} within the SR and in turn the free Ca^{2+} available to bind to troponin C (Chang *et al*., 2017).

Depletion of muscle glycogen stores during and following prolonged exercise could also contribute to impaired Ca^{2+} release. Previous work using skinned muscle fibres has shown the level of SR Ca^{2+} release in response to depolarisation is strongly correlated with muscle glycogen content (Stephenson *et al*., 1999). Using an in *vivo* model, Gejl *et al.* (2014) assessed SR Ca^{2+} release following 4 hours of glycogen depleting cycling exercise, before being given either water or a carbohydrate rich

drink. While muscle glycogen content and SR Ca^{2+} release were markedly reduced post-exercise in both trials, these variables recovered substantially at 4-hours post-exercise when a carbohydrate drink was provided. In contrast, when only water was provided, both glycogen depletion and SR Ca^{2+} remained depressed, and a significant correlation found between SR Ca^{2+} release rate and glycogen content (Gejl *et al*., 2014). While the mechanisms by which glycogen affects SR Ca^{2+} release remain speculative, it has been suggested that preferential glycogen depletion in the region of the t-tubular-SR junction could interfere with Ca^{2+} release (Ørtenblad *et al*., 2013). Thus, during and following prolonged, exhaustive exercise, depletion of glycogen could contribute to impaired contractile function by reducing SR Ca^{2+} release.

Another potential contributor to impaired Ca^{2+} handling is the activity of reactive oxygen species (ROS) and reactive nitrogen species (RNS), the production of which increases during exercise (Cheng *et al*., 2017, Powers and Jackson, 2008). Previous work has displayed a beneficial impact of reduced ROS and RNS on endurance performance (Reid *et al*., 1992, Powers *et al*., 2011). While the actions of ROS and RNS are complex, it is suggested that these could interference with Ca^{2+} release through redox modifications of RyR receptors, as well as reducing myofibrillar Ca^{2+} sensitivity (Cheng *et al*., 2017). In summary, impaired Ca^{2+} appears to contribute substantially towards contractile dysfunction, with a number of underlying mechanisms potentially responsible.

Acidosis

Acidosis has historically been suggested as the major cause of exercise-induced reductions in muscle strength (Fitts, 1994). During anaerobic glycolysis, excess

pyruvate is converted to lactic acid, which dissociates into lactate and H^+ and causes a reduction in intracellular pH by up to ~0.5 pH units during intense muscular activity (Stackhouse *et al*., 2001, Westerblad *et al*., 2002). Early lines of evidence linking reduced pH to contractile dysfunction cited the strong temporal correlation between muscle pH and the decline in muscle force (Dawson *et al*., 1978, Cady *et al*., 1989, Sahlin, 1986), and the reduced isometric force and shortening velocity of skinned muscle fibres under acidification (Westerblad *et al*., 2002). Before the 1990's, however, skinned muscle fibres used to investigate the causes of fatigue could not be kept stable above a temperature of approximately 15°C (Stackhouse *et al*., 2001), meaning that studies investigating the effects of acidosis on fatigue were performed below physiological temperatures. When methods allowed investigation at 25-30°C, the deleterious effects of acidosis on contractile function were attenuated. Pate and colleagues (1995) were the first to show that at physiological temperatures, acidosis has little effect on force production, findings which have since been corroborated (Westerblad *et al*., 1997). Furthermore, the temporal association between decrements in muscle pH and force is not always present in humans, with force often recovering more rapidly than pH following fatiguing contractions (Stackhouse *et al*., 2001).

Contrary to the belief that acidosis has a detrimental effect on contractile function, recent evidence has suggested that lactate and H^+ may in fact be ergogenic during exercise (Allen *et al*., 2008). Proposed benefits of lactate and H^+ production include greater release of O_2 from haemoglobin for working muscle fibres, stimulation of ventilation, and enhancement of blood flow (Cairns, 2006). Moreover, the "lactate shuttle hypothesis" proposes that lactate released by working muscle fibres is transported between and within cells and utilised as a metabolic fuel (Brooks, 2009). Thus, at a cellular level, increasing evidence suggests that acidosis is not detrimental

to contractile function, and could even be ergogenic. Nevertheless, induced acidosis during whole-body exercise has been shown to exacerbate fatigue (Kowalchuk *et al*., 1984) and alkalosis can improve performance in events lasting 1-10 minutes (Nielsen *et al*., 2002). Rather than a direct effect on contractile function, it has been hypothesised that the negative effects of acidosis on performance during whole-body exercise may be due to activation from group III and IV afferents in muscle, causing sensations of discomfort and reducing central drive from the CNS (Amann, 2011, Sidhu *et al*., 2017). Therefore, if acidosis is involved in exercise-induced fatigue, the effect might be indirect.

2-5 Impairments in central nervous system function

As well as peripheral perturbations, impairments in neuromuscular function can result from impairments in CNS function, involving a reduction in the capacity of the CNS to activate the muscle (Gandevia, 2001). While the mechanisms causing contractile impairments are well described, less is known about those causing impairments in CNS function. As displayed in Figure 2-2, the generation of force depends not only the intrinsic properties of the muscle, but also on activation from the CNS. A number of functional changes occurring within the CNS can contribute to fatigue during exercise. These include impairments in motor cortical output (Taylor *et al*., 2006, Goodall *et al*., 2015), reflex responses at the spinal cord (Gandevia, 2001) and intrinsic properties of the motoneurons (McNeil *et al*., 2011a). While, historically, limitations in measurement tools used to objectively assess CNS function likely contributed to the lack of research in this area, recent technological advancements have permitted a greater understanding of central processes that contribute to impairments in muscle

strength (Gandevia, 2001). In particular, the introduction of transcranial magnetic stimulation (TMS) to the study of fatigue has provided new insight into central processes which contribute to impairments in voluntary activation of skeletal muscle during and following exercise (Goodall *et al.*, 2015, Sidhu *et al.*, 2009b, Thomas *et al.*, 2017a). Investigations using TMS to assess CNS function, as well as other methods used to quantify impairments in muscle strength and evaluate its causative factors, are considered in section 2-6.

2-6 Quantifying fatigue

Since the 1800s, myographs and dynamometers have been used to measure muscle force. While it was established at an early stage that muscle force would gradually decline during sustained contractions despite maximal effort (Di Giulio *et al.*, 2006), limitations in the methods used to quantify impairments in muscle force generating capacity made localisation of its source difficult. As a result, a vigorous debate surrounded whether impairments in the capacity to produce force were a result of the intrinsic properties of the muscles themselves, or by the capacity of the CNS to drive the muscle (Gandevia, 2008). Since then, advancements in electrical and magnetic stimulation techniques alongside muscle force tracings have been used to elucidate on the contribution of central and peripheral mechanisms to exercise-induced declines in the maximum force generating capacity of muscle.

Evoked twitch force

Through electrical or magnetic stimulation of a motor nerve at rest, the contractility and excitability of the muscle can be investigated. By delivering a supramaximal stimulation of a nerve at rest following voluntary contraction of the corresponding muscle, the amplitude of the evoked potentiated twitch force ($Q_{tw,pot}$) can be used as a measure of contractile function. Reductions in $Q_{tw,pot}$ are indicative of impairments in contractile function and can result from both metabolic and mechanical disturbances which negatively influence the excitation-contraction coupling process and cross-bridge formation (Goodall *et al*., 2015). In addition, analysis of twitch characteristics can allow inferences to be made on changes in muscle shortening velocity (maximum rate of force development (MRFD) and contraction time (CT)) and muscular relaxation (maximum rate of relaxation (MRR) and half relaxation time ($RT_{0.5}$)) (Goodall *et al*., 2015). Reductions in MRFD are thought to reflect a decrease in the rate of cross-bridge formation (Parmiggiani and Stein, 1981), while reduced CT reflects reduced Ca^{2+} release from the SR (Allen *et al*., 2008). A reduction in the MRR and increase in $RT_{0.5}$ is indicative of slower removal of Ca^{2+} from the sarcoplasm and slower dissociation of Ca^{2+} from troponin following cross-bridge formation (Westerblad *et al*., 1997).

Using paired electrical stimulations at high (80-100 Hz) and low (10-40 Hz) frequencies permits greater insight into the mechanisms of peripheral fatigue (Keeton and Binder-Macleod, 2006). Low-frequency fatigue is characterised by a disproportionate loss of force elicited by stimulation at low- compared to high-frequencies (Janecki *et al*., 2016, Jones, 1996), and is thought to be related to reduced Ca^{2+} release from the SR (Keeton and Binder-Macleod, 2006). The differences between the effects of reduced Ca^{2+} release on force responses to low- and high-frequencies can be explained by the Ca^{2+}-tension relationship (Figure 2-4). Because

low-frequency stimulations correspond with the steep part of the Ca^{2+}-tension curve while Ca^{2+} at high frequencies is on the horizontal part of the curve, decreases in sarcoplasmic Ca^{2+} have a greater effect on force at low-frequencies (Westerblad and Allen, 1992, Keeton and Binder-Macleod, 2006).

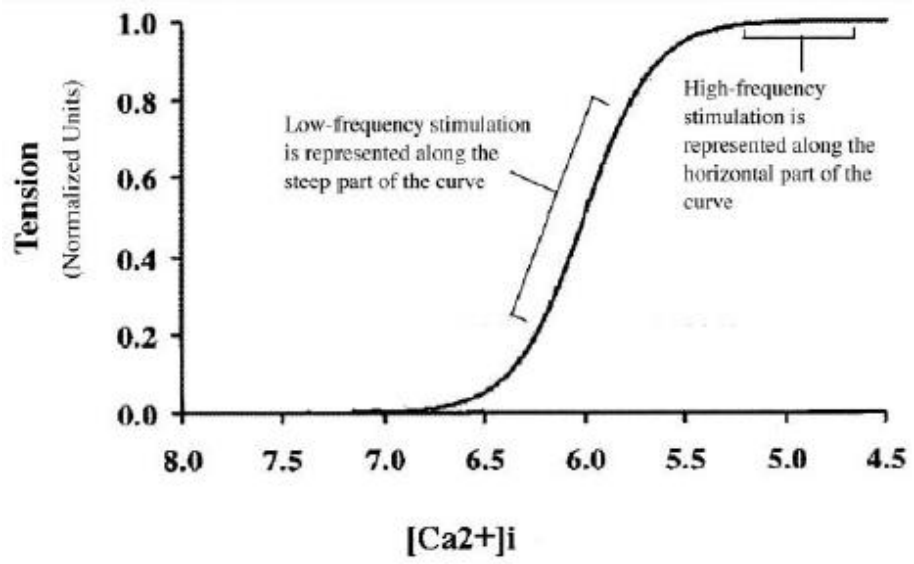


Figure 2-4. Hypothetical Ca^{2+}-tension relationship. At high stimulation frequencies, sarcoplasmic Ca^{2+} is increased such that moderate drops in Ca2+ have no effect on tension. In contrast, at low stimulation frequencies, Ca^{2+} is on the steep part of the curve, so falls in Ca^{2+} produce greater decreases in tension (from Keeton *et al*., 2006).

Muscle compound action potential (M-wave)

In addition to the mechanical response elicited by electrical or magnetic stimulation of the muscle nerve, electromyography (EMG) can be used to assess the electrical response in the muscle through the muscle compound action potential (M-wave). The

amplitude of the M-wave in response to electrical stimulation provides a measure of muscle membrane excitability (Baker *et al*., 1993). Maintenance of M-wave amplitude in the presence of peripheral fatigue indicates that the site of failure lies beyond the sarcolemma, most-likely due to impairments in the excitation-contraction coupling process and cross-bridge formation (Allen *et al*., 2008, Goodall *et al*., 2015).

Voluntary activation

The twitch interpolation technique is commonly employed to measure the completeness of muscle activation during a voluntary contraction (Shield and Zhou, 2004, Thomas *et al*., 2017a). This technique, first introduced by Merton (1954), involves delivering a supramaximal electrical or magnetic stimulation to a motor nerve during a voluntary contraction. If motor units have not been recruited, or are not firing fast enough during voluntary contraction, the stimulus will evoke a twitch-like increment in force, termed the superimposed twitch (Gandevia *et al*., 2013). The principal application of the interpolated twitch technique has been to study voluntary activation during fatiguing exercise. In a fatigued state, a decrease in voluntary activation is commonly referred to as "central fatigue". Twitch interpolation has been used extensively throughout the literature to demonstrate a reduction in voluntary activation in response to a range of tasks (Thomas *et al*., 2017a, Hartman *et al*., 2011, Gandevia *et al*., 2013). Despite its extensive use, twitch interpolation is not without its limitations. Recent research has suggested that peripheral factors alone could be responsible for the decrease in voluntary activation attributed to impairments in CNS function (Place *et al*., 2008, Neyroud *et al*., 2016, Contessa *et al*., 2016).

Paradoxically, Place *et al*. (2008) demonstrated the presence of "central fatigue" in an *in vitro* model using single mouse muscle fibres, and suggested that an intracellular mechanism in the form of an increased titanic Ca^{2+} might be responsible for the superimposed twitch evoked by stimulation rather than a central mechanism, an argument which has since been countered (Cheng *et al*., 2013). Furthermore, Taylor (2009) suggested that while twitch interpolation *does* give a measure of the capacity of the CNS to activate the muscle, it *does not* measure descending drive to the motoneurons. This is because any reduction in VA measured with twitch interpolation can occur due to perturbations within the neuromuscular pathway at both the spinal and/or supraspinal level, with the interpolated twitch technique unable to discern the site of impairment. Despite the apparent limitations, twitch interpolation remains an effective and widely used technique to measure voluntary activation in a range of muscle groups (Thomas *et al*., 2017a, Hartman *et al*., 2011, Gandevia *et al*., 2013).

Voluntary activation with transcranial magnetic stimulation (TMS)

In recent years, research has attempted to further elucidate on the aetiology of impaired voluntary activation through the application of TMS (Todd et al., 2003b, Jubeau et al., 2014). This technique involves delivering a magnetic stimulation to the area of the motor cortex corresponding with the muscle of interest during an MVC. If a superimposed twitch is elicited by TMS, this implies not only that some motor units are not recruited or are not firing fast enough, but also that motoneuron and/or motor cortical output is submaximal (Todd et al., 2016b). Thus, it has been suggested that measuring voluntary activation with TMS can provide additional information about neural drive compared with the interpolated twitch technique alone (Todd et al.,

2016b). Similar to the interpolated twitch technique, however, measuring voluntary activation with TMS is not without its limitations. The primary methodological concerns associated with measuring voluntary activation with TMS are the activation of the cortical representation of several muscles in the target limbs, including the antagonist muscles, inadequate activation of motoneurons which innervate the target muscle, and linearity (or non-linearity) of the voluntary force and superimposed twitch relation (Todd et al., 2016b). Nevertheless, the validity and reliability of this technique has been established in a range of muscle groups (Todd et al., 2003b, Goodall et al., 2009, Lee et al., 2008), and, used in conjunction with peripheral nerve stimulation, has the potential to provide further insight into the aetiology of exercise-induced impairments in neuromuscular function.

Motor evoked potential (MEPs)

Transcranial magnetic stimulation can also be used to assess the excitability (or responsiveness) of the human corticospinal tract (Kobayashi and Pascual-Leone, 2003). At a sufficient intensity, single-pulse TMS induces descending volleys which travel through pyramidal tract neurons to motor neurons and evoke an EMG response in the target muscle. The amplitude of this response, termed the motor evoked potential (MEP), can be used to quantify corticospinal excitability (CSE) (Goodall *et al*., 2014). As the excitability of corticospinal neurons increases during voluntary contraction, delivering TMS at the same intensity produces a larger MEP in contracting muscle compared with rest (Chen, 2000). This technique has been used to quantify changes in CSE during a range of isometric (Maruyama *et al*., 2006) and locomotor (Verin *et al*., 2004) exercise tasks. However, because changes in the size of the MEP can occur as

a result of alterations at both cortical and spinal level, it is not possible to ascribe alterations in MEP size to cortical or spinal excitability (Pitman and Semmler, 2012). In order to account for this limitation, and in an attempt to assess changes in excitability at a segmented level, recent studies have employed stimulation of the spinal cord at the cervicomedullary junction (McNeil *et al*., 2013). Changes in the size of the resultant cervicomedullary motor evoked potential (CMEP) reflect alterations in spinal excitability, and when used in concert with TMS, can provide additional information on the excitability of the CNS in response to fatiguing exercise.

Short-interval intracortical inhibition (SICI) & intracortical facilitation (ICF)

Within the motor cortex, there exists a diverse range of interneurons that play an important role in generating and shaping voluntary movement. These interneurons can be broadly defined as inhibitory or facilitatory based on their electrophysiological features and effects on the postsynaptic membranes of other neurons (Nakamura *et al*., 1997). Inhibitory interneurons release inhibitory neurotransmitters which increase the postsynaptic membrane's permeability to the efflux of K^+ and chloride (Cl^-) ions, thereby increasing the cell's resting membrane potential to create an inhibitory postsynaptic potential (IPSP). The efflux of K^+ and Cl^- ions hyperpolarises the postsynaptic neuron, making it less likely to reach the AP threshold in response to excitatory input. In contrast, facilitatory interneurons release neurotransmitters which excite the postsynaptic neuron to which it is connected, causing changes in membrane permeability so Na^+ ions can diffuse into the neuron. This generates an excitatory postsynaptic potential (EPSP), lowering the resting membrane potential and temporarily increasing its tendency to reach the threshold for AP. Gamma-

aminobutryic acid (GABA) and glutamate are the most abundant inhibitory and facilitatory interneurons in the CNS (Liepert *et al*., 1997).

Using paired-pulse TMS paradigms, the excitability of intracortical inhibitory and facilitatory circuits can also be assessed (Kujirai *et al*., 1993). When a stimulation below the threshold intensity required to elicit a response in the muscle (termed the conditioning stimulus; CS) is delivered 1-5 ms prior to a suprathreshold test stimulus, intracortical inhibitory circuits mediated by gamma-aminobutyric acid type A ($GABA_A$) interneurons are activated, resulting in a reduction in the size of the MEP elicited by the suprathreshold test stimulus (termed short-interval intracortical inhibition, SICI) (Kujirai *et al*., 1993). By contrast, paired-pulse TMS at an inter-stimulus interval (ISI) of 10-15 ms results in facilitation of the test pulse MEP (termed intracortical facilitation, ICF). While the mechanisms of ICF are less clear, 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). These methods have previously been used in response to fatiguing isometric (Maruyama *et al*., 2006, Hunter *et al*., 2016) and locomotor exercise (Tergau *et al*., 2000) to reveal fatigue induced changes in SICI and/or ICF, and permit insight into the activity of intracortical inhibitory and facilitatory interneurons that modulate cortical output.

Voluntary EMG

Surface electromyography (EMG) detects electrical signals transmitted through superficial muscle tissue, and is commonly used to assess both peripheral and central changes during voluntary contractions (Marco *et al*., 2017, Dimitrova and Dimitrov,

2003). At the peripheral level, decreases in sarcolemmal excitability, which can be implicated in declines in MVC strength, cause a reduction in EMG amplitude (Lepers *et al*., 2002). At the central level, alterations in motor unit firing rates and/or motor unit recruitment which occur during fatiguing contractions can produce concomitant changes in voluntary EMG. As a result, several studies have applied surface EMG normalised to the maximal M-wave as a surrogate measure of central motor drive during fatiguing isometric (Taylor and Gandevia, 2008) and locomotor exercise (Amann and Dempsey, 2008). During sustained maximal isometric contractions, EMG amplitude is reduced, likely due to decreases in motor unit firing rates, reduced motor unit recruitment and/or changes in muscle fibre-type recruitment (Sogaard *et al*., 2006, Taylor and Gandevia, 2008). In addition, changes in EMG amplitude during MVCs could also be attributable to changes in sarcolemmal excitability (Allen *et al*., 2008). Conversely, during sustained submaximal contractions, EMG amplitude increases concomitantly with impairments in the force generating capacity of the muscle, likely due to an increase in motor unit recruitment and/or firing rate as a compensatory mechanism (Taylor and Gandevia, 2008). Despite its widespread use, there are several limitations to surface EMG in the assessment of neuromuscular adjustments during exercise. For example, surface EMG amplitude cancellation, reflex effects at the spinal cord, and the inability of surface EMG to distinguish between changes in motor unit firing and recruitment are among several technical limitations associated with surface EMG (Weir *et al*., 2006, Keenan *et al*., 2005, Dimitrova and Dimitrov, 2003). These limitations notwithstanding, EMG provides a valuable tool for assessing neural control of human movement and central and peripheral changes in response to exercise.

Perception of exertion and effort

The sensation of fatigue is associated with cognitive elements, involving an increased sense of effort required to maintain or increase exercise intensity (the efferent component), and the perception of exertion based on the physical symptoms induced by exercise (the afferent component) (Swart *et al*., 2012). While the terms effort and exertion are often used interchangeably, it has been suggested that these describe two distinct psychological constructs, both of which contribute towards exercise-induced fatigue (Swart *et al*., 2012). Perceptions of exertion are thought to be the consequence of afferent feedback from multiple physiological systems as well as other complex psychological factors (Abbiss *et al*., 2015). In contrast, the sense of effort is derived from efferent commands, and is thought to be strongly dependent on centrally generated corticofugal motor demands giving rise to corollary discharge (Smirmaul Bde, 2012). This corollary discharge, described as a copy of the motor command that is sent to the muscle in order to produce movement, activates sensory areas within the brain and in turn influences perceptions of effort during exercise (Hureau *et al*., 2016b). It has been suggested that both of these constructs act to ensure that exercise intensity is regulated to ensure that homeostatic processes remain intact, and to prevent catastrophic system failure (Swart *et al*., 2012).

Summary

Fatigue is characterised by sensations of tiredness and weakness underpinned and/or modulated by a myriad of complex physiological and psychological processes. Exercise, and the consequent disruption to homeostasis, is a particularly potent stimulus to elicit fatigue. While the mediators of fatigue will vary depending on the exercise-task, decrements in the force generating capacity of the muscle are a frequent

contributor towards fatigue both during and following exercise. In turn, impairments in the force generating capacity of the muscle occur as a consequence of a multitude of processes, and are often attributed to impairments in neuromuscular function, occurring as a consequence of deficits in contractile function and/or the capacity of the CNS to activate muscle. The use of neurostimulation techniques, including nerve stimulation and TMS, have permitted greater insight into the aetiology of perturbations in neuromuscular function during a range of exercise tasks. The following section will discuss the research pertaining to the mechanisms of impaired neuromuscular function during specific exercise tasks.

2-7 Aetiology of impairments in neuromuscular function during exercise

As alluded to previously (Figure 2-2), muscle contraction is dependent on a complex chain of events occurring throughout the motor system. During exercise, the relative contribution of these processes is dependent on the nature of the task, with contraction intensity, duration, type of muscle action (i.e. concentric, eccentric, isometric and dynamic) and the nature of the exercise task (i.e. continuous or intermittent) influencing the stress imposed on sites within the neuromuscular system (Bigland-Ritchie *et al*., 1995). As such, the aetiology of neuromuscular adjustments during and following exercise is highly task-dependent (Thomas *et al*., 2015, Skurvydas *et al*., 2016, Millet and Lepers, 2004), with this task-dependency highlighted as a critical issue in the study of fatigue (Enoka and Duchateau, 2016, Hunter, 2017). As mentioned in the previous section, techniques involving neurostimulation have permitted insight into the relative contribution of neural and muscular processes which

contribute to impairments in neuromuscular function during a range of exercise challenges. The following section will discuss the literature pertaining to the aetiology of impaired neuromuscular function during different exercise tasks.

Sustained maximal contractions

The mechanisms of impaired neuromuscular function during sustained MVC's have been studied extensively, and can be attributable to both central and peripheral mechanisms (Schillings *et al*., 2003, Taylor and Gandevia, 2008, Kennedy *et al*., 2016). Schillings *et al.* (2003) assessed the development of impairments in contractile function and voluntary activation during a 2 min sustained MVC of the biceps brachii. The authors found that the decline in force during the early part of the MVC was predominantly a result of impaired contractile function, which displayed a nadir mid-way through the task. While a reduction in voluntary activation became evident during the latter part of the contraction, peripheral perturbations were primarily responsible for the reduction in force (Schillings *et al*., 2003), a finding also displayed elsewhere (Kent-Braun, 1999). The impaired contractile function which occurs during sustained maximal contractions manifests in a reduction in the evoked force response to electrical stimulation delivered at rest, while M-wave responses remained unchanged (Bigland-Ritchie *et al*., 1978). This suggests that the reductions in muscle strength during sustained MVCs is primarily a result of limitations within the contractile machinery, as appose to impairments in neuromuscular transmission.

The application of TMS has advanced scientific understanding of the contributors towards exercise-induced declines in muscle strength, as well as modulations in CNS function occurring during sustained MVCs. For example, Todd *et al.* (2005) measured

voluntary activation using TMS during a sustained MVC to demonstrate an increase in the size of the superimposed twitch evoked through magnetic stimulation, indicating submaximal motoneuron and/or motor cortical output. In addition, single-pulse TMS delivered to the motor cortex to evoke MEPs, and to the cervicomedullary junction to evoke CMEPs, has revealed fatigue induced changes in the excitability of the brain to muscle pathway during sustained MVCs (Taylor *et al*., 1996, Kennedy *et al*., 2016). Specifically, studies have noted an increase in the size of MEPs elicited during sustained MVCs with a concurrent decrease in the size of CMEPs (McNeil *et al*., 2009, McNeil *et al*., 2011a). These responses are indicative of a decrease in motoneuronal excitability, thought to occur as a consequence of changes in the intrinsic properties of motoneurons due to their repetitive discharge (McNeil *et al*., 2011b), and a compensatory increase in motor cortical excitability acting to preserve central drive during a MVC (Kennedy *et al*., 2016). Thus, while the decline in muscle strength which occurs during an MVC can primarily be attributed to peripheral perturbations, changes in cortical and motoneuronal excitability are also implicated in the reduced muscle force generating capacity.

Submaximal contractions

During submaximal isometric contractions, the aetiology of adjustments in neuromuscular function is dictated by the intensity of the contraction. At higher contraction intensities, peripheral perturbations primarily contribute to impaired neuromuscular function, while the relative contribution of central impairments increase at lower contraction intensities (Bigland-Ritchie *et al*., 1986). More specifically, distinct adjustments in neuromuscular function are observed above and

below the critical torque, defined as the asymptote of the torque-duration relationship (Burnley, 2009). As the contraction intensity increases above critical torque (generally found ~15-40% of MVC), there is a proportional increase in peripheral perturbations and a concomitant impairment in contractile function (Burnley *et al.*, 2012). The impaired contractile function which occurs at contraction intensities above critical torque is thought to be the consequence of a progressive loss of muscle metabolic homeostasis, such as the accumulation of P_i and H^+ (Jones *et al.*, 2008). At these higher contraction intensities, impairments in voluntary activation are modest or absent (Burnley *et al.*, 2012, Bigland-Ritchie *et al.*, 1986). Conversely, reductions in voluntary activation are more prevalent at contraction intensities below critical torque. For example, during an intermittent isometric contractions just below critical torque, Burnley *et al.* (2012) demonstrated a 24% reduction in voluntary activation following task completion, while only a 6% reduction in voluntary activation was found at the highest contraction intensity. The finding that central fatigue is more prevalent during prolonged, low-intensity contractions has been displayed in numerous other studies (Eichelberger and Bilodeau, 2007, Bigland-Ritchie *et al.*, 1986, Neyroud *et al.*, 2012).

In contrast to maximal voluntary contractions, motor unit recruitment is not maximal during submaximal voluntary contractions. Instead, as the force generating capacity of the muscle is reduced during sustained submaximal contractions, additional motor units are recruited to compensate for those that are less responsive (Taylor and Gandevia, 2008). The increase in motor unit recruitment during sustained submaximal contraction manifests as a rise in EMG activity. In addition, there is an increase in the size of CMEPs measured during sustained submaximal contractions, likely due to the increased excitatory drive to the motoneuron pool (Levenez *et al.*, 2008, Hoffman *et al.*, 2009). In order to assess the effect of sustained submaximal contractions on

motoneuron excitability independent of increased voluntary drive, McNeil *et al.* (2011a) had participants perform a contraction equal to 25% of maximum EMG measured in an unfatigued state, and recorded CMEPs during the TMS induced silent period. The results displayed that CMEPs were reduced to a greater extent when measured during the silent period compared with when they were measured with voluntary drive intact (~75 vs ~20% reduction in CMEP amplitude). This study reveals that motoneuronal excitability is diminished during sustained submaximal contractions, and, given the attenuated reduction in CMEP amplitude when voluntary drive was intact, highlights the importance of voluntary drive on motoneuron excitability. Another interesting finding from the study was that when conditioned CMEPs were assessed using strong test stimulus intensities (to evoke a CMEP of ~50% of Mmax), the reduction in CMEP amplitude was attenuated compared with when a weaker test stimulus was used (to evoke a CMEP of ~15% of Mmax), indicating that low-threshold motor units were more effected compared with high-threshold motor units. These findings were recently corroborated in the quadriceps muscles under the same conditions, and suggest that a mechanism related to repetitive activity of low-threshold motor units, which contribute predominantly during the sustained submaximal contraction, is responsible for the reduction in motoneuron excitability (Finn *et al*., 2018).

In addition to the effects of fatiguing exercise on motoneuronal excitability, studies utilising paired-pulse TMS paradigms have revealed fatigue induced changes in the excitability of intracortical circuits during submaximal isometric contractions (Maruyama *et al*., 2006, Hunter *et al*., 2016). During an intermittent fatiguing task in the first dorsal interosseous muscle, Maruyama *et al.* (2006) found a transient reduction in SICI measured both when the intensity of the test stimulus was kept

constant throughout the experiment, and when the test stimulus intensity was adjusted in order to keep constant the amplitude of the unconditioned MEP. Hunter *et al.* (2016) measured SICI during a sustained submaximal contraction of the elbow flexor, during which EMG was maintained at 25% of maximum EMG measured in an unfatigued state in order to mitigate any influence of changes in motoneuron activity. Similar to the findings of Maruyama *et al.* (2006), a transient reduction in SICI was found during the fatiguing protocol. In both studies, the reduction in SICI with fatigue was interpreted as a compensatory downregulation of intracortical inhibition acting in response to the large reduction in spinal motoneuron responsiveness (Maruyama *et al*., 2006, Hunter *et al*., 2016, McNeil *et al*., 2011a).

Locomotor exercise

As with isometric contractions, exercise intensity during locomotor exercise can be categorised into distinct domains demarcated by physiological thresholds (Jones *et al*., 2008). Specifically, three intensity domains have so far been established; moderate (power output below lactate threshold), heavy (power output between lactate threshold and critical power), and severe (power output above critical power that can be sustained until VO_{2max} is reached) (Burnley & Jones, 2018). Each intensity domain is characterised by differences in VO_2 kinetics, muscle metabolic and blood acid-base responses (Jones *et al*., 2008). In turn, the exercise intensity domain could have an influence on the mechanisms responsible for impairments in neuromuscular function. In order to elucidate the mechanisms contributing to impairments in neuromuscular function at different exercise intensities during locomotor exercise, studies have utilised constant–load exercise protocols set within different exercise domains

(Thomas *et al*., 2016; Martin *et al*., 2010; Black *et al*., 2017). In general, impairments in voluntary activation predominate during lower intensity exercise (e.g. moderate intensity), with a greater degree of contractile impairments at higher exercise intensities (e.g severe intensity) (Thomas *et al*., 2016; Burnley & Jones, 2012). This was recently demonstrated by Thomas *et al.* (2016), who had participants perform constant load cycling at severe exercise intensities (both at the power associated with VO_{2max} and 60% of the difference between gas exchange threshold and VO_{2max}) and the heavy exercise intensity domain (respiratory compensation point). The results displayed that contractile impairments increased in an intensity-dependent manner, with a greater reduction in potentiated twitch force at intensities within the severe domain compared with the heavy domain. Conversely, reductions in voluntary activation were greater following exercise within the heavy exercise domain compared with severe. Similarly, Black *et al.* (2017) assessed the metabolic and neuromuscular responses to constant load cycling in the moderate, heavy and severe intensity domains until task failure. Exercise within the severe intensity domain was associated with substantial perturbations to metabolic homeostasis, such as an increase in P_i and plasma K^+ concentration, and a decrease in muscle pH and ATP, with a concomitant reduction in M-wave amplitude indicative of a decrease in muscle excitability (Allen *et al*., 2008). These metabolic perturbations were greater than was observed during severe or moderate intensity exercise. In contrast, muscle voluntary activation and neural drive, measured through EMG RMS and RMS/M-wave amplitude, were lower at task failure following moderate and heavy intensity exercise compared with severe intensity exercise, further highlighting the influence of exercise intensity and duration on the aetiology of impairments in neuromuscular function (Black *et al*., 2017).

An increasing number of studies have employed TMS in an attempt to further elucidate the mechanisms of impairments in CNS function which occur during fatiguing locomotor exercise (Goodall *et al*., 2015; Sidhu *et al*., 2013; O'Leary *et al*., 2015). In particular, numerous studies have found reductions in voluntary activation measured using TMS following a range of locomotor exercise paradigms, indicative of submaximal motoneuron and/or motor cortical output (Thomas *et al*., 2015; Sidhu *et al*., 2009; Goodall *et al*., 2017; Ross *et al*., 2007). Additionally, studies have utilised single- and paired-pulse TMS to assess fatigue-induced changes in corticospinal and intracortical activity in response to locomotor exercise (O'Leary *et al*., 2015; Goodall *et al*., 2017). A common limitation associated with many of these studies, however, is the time-delay between exercise cessation and the post-exercise assessment procedure, particularly given that rapid recovery of TMS-evoked intracortical and CSE measures has been reported (i.e. < 10 mins; Carrol *et al*., 2017; Szubski *et al*., 2007). In an attempt to account for this limitation, a number of studies have devised innovative strategies which permit the assessment of neurophysiological function *during* locomotor exercise, rather than following (Sidhu *et al*., 2012; Sidhu *et al*., 2013; Weavil *et al*., 2016). These studies report that, in contrast to sustained isometric contractions, fatiguing locomotor exercise results in no increase in cortical excitability (Sidhu *et al*., 2012), with the lack of change potentially arising as a result of an increase in intracortical inhibition (Sidhu *et al*., 2013). Furthermore, Weavil *et al.* (2016) assessed CSE during fatiguing and non-fatiguing cycling bouts matched for increases in EMG. The data displayed that while motoneuronal excitability increased (as indicated by a similar ~40% increase in MEP and CMEP area) during non-fatiguing exercise, this effect was abolished at task failure following fatiguing exercise, with no change in MEP or CMEP area. These results indicate that fatigue induces a

disfacilitation of spinal motoneurons which diminishes the facilitating effects of voluntary drive on spinal excitability. The lack of overall change in CSE suggests that the excitatory influence associated with increased voluntary drive could help preserve the efficacy of the motor pathway to transmit neural drive from higher brain areas (Weavil *et al*., 2016), which is similar to exercise of a smaller muscle mass (McNeil *et al*., 2011a). Overall, the differences reported in these studies to that of sustained isometric contractions highlight the task-specific nature of intracortical and corticospinal responses to fatiguing exercise, and demonstrate the potential for TMS to provide further insight into the aetiology of fatigue during and following locomotor exercise.

Summary

An abundance of research has examined neuromuscular responses during and immediately following exercise. Studies have demonstrated that the aetiology of impairments in neuromuscular function is largely task-dependent, with the relative contribution of central and peripheral mechanisms dependent on the intensity, duration and mode of exercise. While research in this area has improved our understanding of the mechanisms contributing to fatigue *during* exercise, much less is known about the aetiology of fatigue during the recovery *following* exercise. In particular, research pertaining to the mechanisms of fatigue following exercise have focused predominantly on peripheral perturbations, with little attention given to the role of the CNS in recovery following fatiguing exercise. In light of the knowledge that factors within the CNS can contribute substantially towards impairments in neuromuscular function and muscle strength during exercise, recent reviews have suggested that

future research should focus more on central perturbations during fatigue and recovery following exercise (Minett and Duffield, 2014, Rattray *et al*., 2015, Carroll *et al*., 2017). The following section will discuss current literature concerning the recovery of fatigue after strenuous exercise.

2-8 Delayed recovery of fatigue following exercise

An inexorable consequence of sustained physical activity is a reduction in the capacity of the muscle to produce force, which contributes to the delayed recovery from fatigue. The reduction in MVC strength induced by strenuous physical activity also persists post-exercise, with the magnitude of impairment and the time-course of recovery of the force generating capacity of the muscle dependent on the intensity, duration and mode of exercise. Following the cessation of prolonged, high-intensity exercise, recovery of muscle function can remain incomplete for several hours or days. While peripheral perturbations such as substrate depletion (Ørtenblad *et al*., 2013, Gejl *et al*., 2014), ionic disturbances (Cheng *et al*., 2016, Cheng *et al*., 2017), muscle damage and the ensuing inflammatory response (Skurvydas *et al*., 2016, Ispirlidis *et al*., 2008) are implicated in contributing to post-exercise impairments in muscle function, growing evidence suggests that recovery of the capacity of the CNS to activate the previously active musculature can remain incomplete for a prolonged period following exercise (Carroll *et al*., 2017). This notwithstanding, the precise mechanisms behind this protracted activation deficit remain to be identified (Carroll *et al*., 2017). The following section will provide a synopsis of the literature pertaining to recovery of fatigue and the associated mechanisms driving recovery following a range of exercise tasks.

Recovery from maximal contractions

During a sustained MVC, force declines rapidly, typically falling to below 50% of initial MVC force within 1-2 minutes (Gandevia *et al*., 1996, Kennedy *et al*., 2014). As alluded to in the previous section, the fatigue induced by sustained MVCs is predominantly a consequence of limitations within the contractile machinery, while reductions in voluntary activation become evident in the latter stages of exercise (Schillings *et al*., 2003). Following an MVC, there is rapid partial recovery of voluntary force within the first 30 s, with re-perfusion of the exercised muscle suggested as a key factor in the initial stages of recovery (Gandevia *et al*., 1996). However, complete recovery of MVC force can take several minutes, reaching only ~80% of pre-exercise values after 4-5 minutes of rest (Gandevia *et al*., 1996).

By assessing the recovery time of voluntary activation and evoked force responses to electrical stimulation, it is possible to determine the contribution of central and peripheral impairments to fatigue and recovery following sustained MVCs. Several studies have shown that following sustained MVCs, voluntary activation (measured through motor nerve and/or motor cortical stimulation) recovers rapidly (Gandevia *et al*., 1996, Todd *et al*., 2005, Vernillo *et al*., 2018). In contrast, impairments in contractile function show a delayed time-course of recovery (Edwards *et al*., 1977, Senefeld *et al*., 2018, Vernillo *et al*., 2018). For example, following a 2 min sustained MVC in the knee extensors and elbow flexors, Vernillo *et al.* (2018) found that the potentiated twitch force remained below baseline following 8 min of recovery in both muscle groups. The delayed time-course of recovery of contractile function following sustained MVCs is likely a consequence of prolonged impairments in Ca^{2+} release

and/or reuptake from the sarcoplasmic reticulum, and/or reduced sensitivity to Ca^{2+} within the contractile apparatus (Edwards *et al*., 1977, Carroll *et al*., 2017). This is supported by evidence that shows that recovery of force responses to low-frequency stimulation remain depressed following several hours of recovery, while responses to high-frequency stimulation recover rapidly (Edwards *et al*., 1977). Accordingly, the prolonged reduction in the force generating capacity of the muscle following sustained MVCs is primarily a consequence of perturbations in contractile function, which persist following the cessation of exercise.

Recovery from sustained submaximal contractions

Similar to sustained maximal isometric contractions, there is an initial rapid recovery of MVC force following cessation of sustained submaximal contractions, but this remains incomplete following 20-30 minutes of recovery (Smith *et al*., 2007, Sogaard *et al*., 2006). As noted in the previous section, impairments in voluntary activation are more appreciable during sustained submaximal than maximal contractions (Smith *et al*., 2007). In addition, recovery of voluntary activation is delayed, and can take 20-30 minutes to return to pre-exercise values (Yoon *et al*., 2012, Keller *et al*., 2011). However, the mechanisms underpinning this prolonged activation deficit remain unknown (Carroll *et al*., 2017). Similarly, impairments in contractile function persist during the recovery period following sustained submaximal contractions, with minimal recovery in the first 20-30 minutes following exercise (Smith *et al*., 2007, Sogaard *et al*., 2006). For example, Smith *et al.* (2007) found that evoked force in response to motor point stimulation decreased from 14% to 12% of control MVC during a sustained contraction at 5% MVC, which did not recover significantly

following 30 mins of recovery. This delay in recovery of contractile function is thought to be a result of impaired intracellular Ca^{2+} handling or sensitivity (Carroll *et al*., 2017). Thus, reductions in voluntary activation and contractile function both contribute towards post-exercise fatigue and recovery following sustained submaximal contractions.

Recovery from locomotor exercise

While fatiguing protocols involving isometric contractions offer a convenient method of assessing fatigue and post-exercise recovery, recovery following locomotor exercise is a more complex field of study due to the varied nature of the mode of exercise, the type of contractions involved, and the intensity and duration of exercise involved in protocols employed throughout the literature. While systematic attempts to document the aetiology of fatigue and time-course of recovery as a function of exercise intensity and/or duration have not been made (Carroll *et al*., 2017), evidence suggests that impairments in contractile and CNS function can persist for prolonged periods following locomotor exercise of varying intensities and durations (Sidhu *et al*., 2009b, Thomas *et al*., 2017a, Booth *et al*., 1997, Millet, 2011). Following short duration, high-intensity exercise, in which muscle function is primarily limited by metabolic perturbations, there is a rapid initial recovery due to clearance of intramuscular metabolites (i.e. P_i and H^+) (Allen *et al*., 2008, Hureau *et al*., 2016a). For example, following high-intensity dynamic knee extension and cycling exercise, respectively, Froyd *et al*. (2013) and Hureau *et al*. (2016a) found rapid initial recovery of MVC force and $Q_{tw,pot}$ within the first two minutes of exercise cessation. However, both of these studies found incomplete restoration of contractile function following

six (Hureau *et al*., 2016a) and eight (Froyd *et al*., 2013) minutes of recovery, likely due to prolonged impairments in Ca^{2+} handling (Allen *et al*., 2008, Edwards *et al*., 1977). Following more prolonged exercise, impairments in voluntary activation and contractile function persisting for well over 30 minutes have been documented (Rampinini *et al*., 2011, Millet, 2011, Périard *et al*., 2014, Sidhu *et al*., 2009b). For example, following prolonged cycling exercise at 75% VO_{2peak} until exhaustion (72 ± 4 mins), Booth *et al.* (1997) found that reductions in MVC and evoked twitch force in the quadriceps persisted following 20 mins of recovery, and took 60 mins to return to baseline. Reductions in voluntary activation, which are heavily implicated during prolonged locomotor exercise, have also been shown to persist for at least 30-45 min following cycling exercise (Lepers *et al*., 2002, Sidhu *et al*., 2009b).

Recovery from exercise induced muscle damage

An additional complexity when assessing recovery of neuromuscular function following running based exercise, which involves eccentric muscle contractions associated with the stretch-shortening cycle, is the muscle damage induced by this form of activity. Eccentric based exercise has been shown to elicit impairments in contractile function and voluntary activation which persist for up to 7 days post-exercise (Endoh *et al*., 2005, Prasartwuth *et al*., 2005, Goodall *et al*., 2017a). The precise mechanisms of exercise induced muscle damage (EIMD) and recovery from eccentric exercise are complex, and not fully understood (Howatson and van Someren, 2008). It is beyond the scope of this thesis to comprehensively review all the potential mechanisms of muscle damage and the subsequent inflammatory response; for this the reader is referred to previous reviews on this topic (Peake *et al*., 2017a, Proske and

Morgan, 2001, Warren *et al*., 2002). However, muscle damage can be simplified into two general areas: the initial phase or primary muscle damage that occurs during exercise, and the secondary damage occurring as a consequence of processes associated with the inflammatory response (Howatson and van Someren, 2008).

Primary muscle damage

The most widely accepted proposal on primary muscle damage is that this initial event occurs due to the mechanical stress imposed on muscle fibres during eccentric contractions (Proske and Morgan, 2001, Armstrong *et al*., 1991). During active stretch of a muscle, the presence of sarcomere length inhomogeneities results in excessive strain being placed upon the weakest sarcomeres in myofibrils. On the descending limb of the length-tension relationship, these sarcomeres will undergo rapid lengthening to a point of no myofilament overlap, leading to "popped sarcomeres" (Peake *et al*., 2017a). During repeated eccentric contractions, such as those occurring during repetitive stretch-shortening cycles associated with running based exercise, this process is progressive, with additional sarcomeres becoming disrupted. With sufficient disruption, non-contractile and intermediate filaments, such as the sarcolemma and sarcoplasmic reticulum, can become damaged and torn (Morgan and Allen, 1999). The disruption to structural elements of the myofibril that occur due to mechanical stress imposed on the muscle during eccentric contractions leads to perturbations in intracellular Ca^{2+} homeostasis. Specifically, disruptions to sarcolemma and sarcoplasmic reticulum membrane integrity, as well as opening of mechanosensitive ion channels, lead to an influx of Ca^{2+} into the cytosol (Nielsen *et al*., 2005). In turn, the influx of Ca^{2+} initiates a cascade of events that induces further

damage to the myofibril by causing alterations in the sarcoplasmic reticulum, mitochondria and myofilaments (Howatson and van Someren, 2008). This secondary muscle damage is thought to occur as a result of Ca^{2+} mediated activation of proteolytic and lipolytic pathways that lead to the degradation of structural components of the myofibril, cell infiltration and subsequent activation, production of reactive oxygen species, and subsequent repair and regeneration of the muscle fibre (Proske and Morgan, 2001, Howatson and van Someren, 2008). As inflammation is believed to be the primary instigator of secondary muscle damage following exercise, and is thus an integral component of recovery following damaging exercise, this will be discussed in more detail in the following section.

Inflammation

The inflammatory responses which ensue following the occurrence of muscle damage are primarily mediated by a series of intracellular signalling molecules known as cytokines. Cytokines are a diverse family of intracellular signalling proteins that, upon binding to specific receptors on the surface of target cells, influence functions within the cell (Cannon and St Pierre, 1998). Following the perturbation of muscle tissue, damaged muscle cells synthesise a large number of inflammatory cytokines that are classed as pro-inflammatory or anti-inflammatory depending on their primary biological function within skeletal muscle and other cells (Calle and Fernandez, 2010). In particular, pro-inflammatory cytokines tumor-necrosis-factor-alpha (TNF-α) and interleukin 1-beta (IL-1β) play a pivotal role in attracting immune cells known as leukocytes from the circulation to damaged tissue (Butterfield *et al*., 2016).

While a number of immune cell types infiltrate damaged tissue during the inflammation, neutrophils are the first group of leukocytes implicated in the inflammatory response (Pizza *et al*., 2005). These cells typically arrive to damaged tissue immediately following the occurrence of muscle damage, and can remain elevated for several days post-exercise depending on the severity of the muscle damage incurred (Cannon and St Pierre, 1998). Following infiltration of damaged muscle tissue, neutrophils primary function is phagocytosis of damaged tissue through the secretion of proteolytic enzymes and cytotoxic molecules in order to facilitate the remodelling and regenerative process (Butterfield *et al*., 2006). Moreover, neutrophils compound the local inflammatory response through the secretion of an array of additional pro-inflammatory cytokines, which in turn attract more neutrophils to the damaged area to elicit further removal of damaged tissue (Tidball, 2005).

While neutrophil infiltration is a key step in the inflammatory process, it has been suggested that these cells could exacerbate muscle injury and have a deleterious effect on recovery of muscle function, at least during the early stages of muscle injury (Tidball, 2005, Pizza *et al*., 2005, Butterfield *et al*., 2006). Specifically, it has been suggested that neutrophils could hamper the recovery process by inhibiting the growth of new myofibrils or degrading undamaged ones, or through the release of cytotoxic compounds such as reactive oxygen species (Pizza *et al*., 2005). Indeed, studies have displayed a relationship between the early neutrophil response and the magnitude of muscle damage after eccentric based exercise (Brickson *et al*., 2003, Pizza *et al*., 2005). Furthermore, following a series of damaging contractions, Pizza *et al*. (2005) displayed that mice with a blunted capacity to produce neutrophils (due to integrin beta chain-2; CD18 deficiency) experienced attenuated muscle tissue damage and lower decrements in force in the days post-contraction compared with non CD18

deficient mice, while other studies have similarly demonstrated reductions in muscle damage when the phagocytic response is blunted (Brickson *et al*., 2003, Beaton *et al*., 2002). These findings have led to the suggestion that reducing the inflammatory response in the acute stages following damaging exercise could limit the secondary damage to muscle tissue and thereby enhance the rate of muscle recovery (Howatson and van Someren, 2008). Strategies aimed at reducing inflammation and accelerating recovery are discussed in section 2-12.

2-9 Fatigue and the need for recovery following intermittent sprint exercise

Post-exercise fatigue and recovery is a particularly pertinent issue in field-based team sports such as such as rugby, field hockey and association football (Minett and Duffield, 2014). In particular, due to the demanding nature of fixture schedules in modern day professional football, understanding the time-course of recovery following match-play is imperative in order to provide practitioners with information on how to optimise the training schedule around competitive fixtures and assist in decision making regarding player rotation strategies during congested fixture schedules, which are commonplace in modern day football (Nedelec *et al*., 2012). Moreover, understanding the aetiology of fatigue is critical when determining the potential efficacy of recovery interventions aimed at accelerating the natural time-course of recovery in an attempt to facilitate performance and reduce the likelihood of injury during subsequent activity. As such, a plethora of research has investigated the aetiology of fatigue and time-course of recovery following both simulated (Thomas *et al*., 2017a, Minett *et al*., 2014, Pointon and Duffield, 2012, Ingram *et al*., 2009) and

competitive match-play (Rampinini *et al*., 2011, Ascensao *et al*., 2008, Ispirlidis *et al*., 2008, Magalhaes *et al*., 2010). In order to gain insight into the mechanisms contributing to post-exercise fatigue and recovery, it is important to understand the extent of the load imposed on physiological and neuromuscular systems during the exercise bout. As such, the following section will provide a synopsis of the literature pertaining to the demands of competitive football match-play.

Physical and physiological demands of competitive football match-play

Since the proliferation in wearable technology and global positioning systems (GPS), the demands of competitive football match-play have been characterised extensively (Di Salvo *et al*., 2009, Bangsbo *et al*., 2006, Mohr *et al*., 2003, Akenhead *et al*., 2013). A myriad of factors can influence the demands of match-play, such as a player's physical capacity, playing position, standard of opposition, importance of game, and environmental factors (Mohr *et al*., 2003). However, during a typical 90 min match, time-motion analyses have demonstrated that elite male footballers generally cover a distance of 10-13 km (Mohr *et al*., 2003). Thus, the aerobic system is heavily taxed during match-play, with average heart rate and oxygen uptake values equating to 80-90% and 70% of maximum, respectively (Rampinini *et al*., 2007, Di Salvo *et al*., 2009). The type of exercise performed in football is intermittent in nature, with a change in activity every 4-6 s, with top-class players performing approximately 1500 activities during a match, covering 2-3 km at high speeds, and nearly 0.5 km covered during sprinting (Barnes *et al*., 2014). In addition, the high number of explosive actions during a game, such as jumping, tackling, accelerating, kicking and changing direction, which are often performed with incomplete recovery, indicate that anaerobic

energy turnover is also substantial, culminating in a significant utilisation of muscle glycogen and PCr concentrations (Nielsen *et al*., 2012). Due to the numerous activities throughout match-play which utilise the stretch-shortening cycle (e.g. jumping, sprinting and changing direction), as well as eccentric muscle contractions (e.g. decelerating), substantial muscle damage is also incurred (Ascensao et al., 2008). Finally, there is also a considerable cognitive demand associated with match-play, with players required to react and anticipate in an ever-changing and relatively unpredictable environment. Thus, it is evident that competitive football match-play imposes significant physiological, neuromuscular and cognitive demands on its participants.

Fatigue and recovery following competitive football match-play

An inevitable consequence of the demands of football match-play is fatigue, which manifests through transient reductions in work-rate following the most demanding periods of a match, and cumulative reductions in work rate towards the end of a match (Mohr *et al*., 2003). The fatigue induced by match-play also persists post-exercise, and can take days to resolve (Nedelec *et al*., 2012). The fatigue induced by match-play is concurrent with reductions in markers of physical function, with several studies assessing recovery of physical performance measures relevant to football performance, such as jump height, sprint time and reactive strength, have reported that at least 72 h of recovery are required to achieve pre-match values (Ispirlidis *et al*., 2008, Ascensao *et al*., 2008, Magalhaes *et al*., 2010). The mechanisms of fatigue following football match-play are multifactorial and complex, and have previously been related to energy depletion (Ekblom, 1986), perturbations to peripheral

homeostasis (Ispirlidis *et al*., 2008), and damage to muscle tissue (Magalhaes *et al*., 2010). For example, reductions in muscle glycogen, which is known to negatively influence contractile function (Ørtenblad *et al*., 2013), following match-play are substantial, and can take up to 72 h to return to baseline (Krustrup *et al*., 2011). Similarly, biochemical markers of muscle damage, inflammation and oxidative stress have been reported to take over 72 h to return to baseline. The prolonged nature of these perturbations highlights the importance of adequate recovery following match-play, particularly when taken in the context of congested modern day fixture schedules.

While football match-play induces peripheral perturbations which contribute toward declines in MVC, recent research has highlighted dissociated rates between the temporal pattern of recovery of muscle strength, and markers of EIMD following intermittent-sprint exercise (Minett and Duffield, 2014, Pointon *et al*., 2012). These findings have led to the suggestion that processes within the CNS could be contributing to the resolution of fatigue following football match-play (Minett and Duffield, 2014). In support of this suggestion, recent evidence demonstrated that impairments in CNS function (measured through reductions in voluntary activation, VA) were substantial following a simulated football match, and persisted for up to 72 h (Thomas *et al*., 2017a). Similarly, following competitive football match-play, Rampinini *et al*. (2011) found a significant decline in VA which persisted for up to 48 h post-match. While these studies suggest that central factors are likely to contribute to post-match fatigue, research pertaining to recovery of CNS function following competitive match-play remains limited (Thomas *et al*., 2017a), and recent reviews have highlighted the need for further work in this area (Minett and Duffield, 2014,

Rattray *et al*., 2015). The following section will discuss previous literature concerning the recovery of contractile and CNS function following intermittent sprint exercise.

2-10 Recovery of contractile function following intermittent sprint exercise

It is well established that intermittent sprint exercise, as encountered during team sports, results in prolonged impairments in contractile function (Thomas *et al*., 2017a, Rampinini *et al*., 2011). Studies measuring the electrically evoked force response of the quadriceps muscles at rest have demonstrated prolonged impairments in $Q_{tw,pot}$ in the days post-exercise (Thomas *et al*., 2017a, Rampinini *et al*., 2011). For example, following simulated football match-play, Thomas *et al.* (2017a) demonstrated that $Q_{tw,pot}$ remained below baseline following 72 h of recovery, while Rampinini *et al.* (2011) found that 48 h of recovery was sufficient to restore contractile function following competitive football match-play. The differences in the time-course of recovery in these studies could relate to differences in the neuromuscular and cognitive demands between a simulated (Thomas *et al*., 2017a) and actual match (Rampinini *et al*., 2011). For example, the study by Thomas *et al.* (2017a) included a high number of maximal sprints with forced decelerations, likely eliciting substantial muscle damage which could have contributed to the prolonged impairment in neuromuscular function compared with that reported by Rampinini *et al.* (2011). These protracted deficits in contractile function are proposed to be caused by a range of peripherally derived factors (Minett and Duffield, 2014), such as substrate depletion (Ekblom, 1986), muscle damage and inflammation (Ascensao *et al*., 2008, Ispirlidis *et al*., 2008), and ionic disturbances (Nielsen *et al*., 2012). The purported mechanisms of

impaired contractile function following intermittent sprint exercise will be discussed in the following section.

Substrate depletion

Intermittent sprint exercise incurs substantial oxidative and glycolytic strain, with declines in substrate availability thought to be a major contributor the fatigue which occurs towards the latter stages of matches (Mohr *et al*., 2003). In turn, limitations in energy supply are also thought to hinder the recovery process in the days post-exercise (Minett and Duffield, 2014, Krustrup *et al*., 2011). While PCr concentration might be depleted during demanding phases of play, it is unlikely that depleted PCr plays any role in post-exercise fatigue and recovery due to its' rapid rate of regeneration (Bogdanis *et al*., 1996). Conversely, intermittent sprint exercise induces considerable reductions in intramuscular glycogen, which can remain below baseline for 48-72 h post-exercise (Krustrup *et al*., 2011, Jacobs *et al*., 1982). For example, Krustrup *et al*. (2011) reported that muscle glycogen content was 43% below baseline values immediately following competitive football match-play in high level footballers, and 27% below baseline 24 h post-match. As alluded to in section 2-4, previous work has demonstrated a strong positive correlation between muscle glycogen content and SR Ca^{2+} release and reuptake (Gejl *et al*., 2014, Nielsen *et al*., 2009). Following competitive football match-play, Krustrup *et al*. (2011) demonstrated reduced SR Ca^{2+} reuptake and intramuscular glycogen, although the rates of recovery of these variables differed. Nevertheless, it is possible that the glycogen depletion associated with intermittent sprint exercise contributes to impairments in contractile function through interference with SR Ca^{2+} handling.

Muscle damage

Team sports involving intermittent sprint exercise involve repeated high-force eccentric contractions which incur considerable muscle damage. These events likely occur during decelerations, changes of direction, jumping and landing, and inter-player contacts throughout match-play (Akenhead *et al*., 2013). Following competitive football match-play, a number of studies have demonstrated the occurrence of muscle damage and inflammation which persists for as long as 72 h post-match (Ispirlidis *et al*., 2008, Ascensao *et al*., 2008). Indirect blood markers of muscle damage and inflammation, such as CK and c-reactive protein, generally peak around 24 h post-exercise, and return to baseline after 72-96 h of recovery (Magalhaes *et al*., 2010, Ispirlidis *et al*., 2008). As discussed in section 2-9, muscle damage and the ensuing inflammatory response and increase in oxidative stress can hinder contractile function through myofibrillar damage, disorganization of sarcomeres and interference with cellular Ca^{2+} handling (Cheng *et al*., 2016, Skurvydas *et al*., 2016). In addition, EIMD could indirectly impair contractile function through interference with glycogen synthesis post-exercise (Jentjens and Jeukendrup, 2003, Doyle *et al*., 1993). These mechanisms likely contribute to the prolonged impairments in the force generating capacity of the muscle known to occur following damaging exercise. Accordingly, muscle damage and the inflammatory response which ensues is likely a major contributing factor towards the prolonged impairments in contractile function following intermittent sprint exercise.

2-11 Recovery of central nervous system function following intermittent sprint exercise

The role of the CNS in fatigue and recovery following intermittent sprint exercise is poorly understood, and team sport performance recovery research remains limited in scope, with a specific focus on the periphery (Minett and Duffield, 2014). However, it has been demonstrated that during intermittent (Goodall *et al*., 2017b) and repeated sprint exercise (Goodall *et al*., 2015), perturbations in CNS function, as inferred through reductions in VA, contribute to impairments in neuromuscular function. For example, during 120 minutes of simulated football match-play, Goodall *et al.* (2017b) displayed a reduction in VA of the quadriceps by 15% when measured using both electrical nerve and TMS following 90 min of exercise. A number of other studies have similarly demonstrated a post-exercise activation deficit following intermittent sprint exercise (Rampinini *et al*., 2011, Pointon *et al*., 2012, Girard *et al*., 2015). Given the contribution of the CNS to impairments in neuromuscular function during and immediately following exercise, it is plausible that impaired CNS function could contribute to prolonged fatigue known to occur following intermittent sprint exercise (Nedelec *et al*., 2012).

In support of this posit, recent studies have demonstrated prolonged reductions in VA following simulated (Thomas *et al*., 2017a, Pointon and Duffield, 2012) and competitive football match-play (Rampinini *et al*., 2011). Following simulated football match-play, Thomas *et al* (2017a) found a reduction in VA measured using motor nerve stimulation which persisted for up to 72 h post-exercise, while VA_{TMS} took 48 h to return to baseline. Similarly, following competitive football match-play, Rampinini *et al* (2011) found a significant decline in VA which persisted for up to 48 h post-match. These studies demonstrate that the impairments in CNS function which

occur throughout intermittent sprint exercise could persist post-exercise, and contribute to the prolonged decrements in muscle function following football match-play.

The application of single- and paired-pulse TMS could provide further insight into fatigue and recovery of CNS function following intermittent sprint exercise. These methods have previously been applied during single-limb isometric (Kennedy *et al*., 2016), locomotor (Sidhu *et al*., 2017), and eccentric exercise (Pitman and Semmler, 2012) to reveal fatigue-induced changes in CSE and/or SICI. As such, it is possible that changes in the status of these variables could be implicated in impaired CNS function following competitive football match-play. A recent study found no change in SICI and a reduction in CSE 24 h following a simulated football match (Thomas *et al*., 2017a). However, while football match simulations are designed to replicate the physiological demands of competitive match-play (Nicholas *et al*., 2000), many aspects of a real match aren't fully replicated through laboratory simulations (Magalhaes *et al*., 2010). For example, laboratory simulations do not include the perceptual demands associated with decision making, reacting and anticipating, and the mechanical and neuromuscular demands associated with the diverse range of physically demanding activities involved during match-play (Williams, 2000, Magalhaes *et al*., 2010). Given that changes in CSE and SICI have been shown to be task specific (Kalmar, 2018, Giboin *et al*., 2018), it is unclear whether differences in the demands of a simulated and competitive football match could influence the responses of these variables. Thus, the role of CSE and SICI in post-exercise fatigue and recovery warrants further investigation.

2-12 Current strategies and interventions to attenuate fatigue and accelerate recovery following intermittent sprint exercise

Due to the congested nature of the fixture schedule in intermittent sprint team sports such as association football, ensuring players are sufficiently recovered between matches is of paramount importance in order to facilitate performance and reduce the risk of injury. As such, it is commonplace for teams to employ interventions aimed at accelerating the natural time-course of recovery following match-play (Nedelec *et al*., 2013). Recovery strategies can broadly be differentiated as being physiological (e.g. cryotherapy, massage, compression, electrical stimulation), pharmacological (e.g. non-steroidal anti-inflammatory medications) or nutritional (e.g. dietary supplements; Minett and Duffield, 2014). While a myriad of interventions have been applied, it is beyond the scope of this literature review to provide a comprehensive appraisal of recovery interventions; for this, the reader is referred to previous reviews on this area (Howatson and van Someren, 2008, Owens et al., 2018a, Nedelec et al., 2013). Rather, given that cryotherapy has been cited as the most commonly implemented recovery intervention following football match-play (Nedelec *et al*., 2013), a synopsis of the literature pertaining to the efficacy of cryotherapy as a recovery intervention will be provided.

Cryotherapy

Cryotherapy is the application of cold for therapeutic purposes, and is most commonly applied through whole body cryotherapy (dry air of −80°C to −110°C for 1–3 min), cold water immersion (CWI), ice or gel packs or ice massage (White and Wells, 2013a). While the mechanisms by which cryotherapy might facilitate recovery

following exercise are not fully understood, it is thought that the application of cold could facilitate recovery from metabolically or mechanically demanding exercise through vasoconstriction of the muscle vasculature, a decrease in muscle tissue temperature, and a subsequent reduction in cellular and capillary permeability (White and Wells, 2013a). In turn, fluid diffusion into the interstitial space is reduced, which could help negate muscle fibre oedema and the acute inflammatory response (Bongers *et al*., 2017), thereby reducing the secondary muscle damage induced by inflammation.

A reduction in local and systemic inflammation could positively impact recovery of the neuromuscular system through a number of mechanisms. Specifically, as discussed in section 2-4, the accumulation of reactive oxygen/nitrogen species has been shown to interfere with SR Ca^{2+} release, which has been attributed to redox modification of ryanodine receptors (Cheng *et al*., 2016). In addition, factors associated with inflammation have also been linked with compromised CNS function (Carmichael *et al*., 2006). For example, group III and IV muscle afferents, which provide inhibitory feedback to various sites within the CNS (Sidhu *et al*., 2017), are sensitive to various markers of muscle injury, such as the release of biochemical substrates (e.g., bradykinin, histamines, and prostaglandins) and factors associated with inflammation (Endoh *et al*., 2005, Pitman and Semmler, 2012, Sidhu *et al*., 2009b), while an increase in brain cytokines following eccentric exercise might also modulate recovery of CNS impairment (Carmichael *et al*., 2006). While cryotherapy has the potential to reduce impairments in neuromuscular function arising from perturbations in CNS and contractile function, evidence to support this posit is limited, and further research is warranted.

Evidence as to whether or not cryotherapy reduces inflammation remains equivocal (Peake *et al*., 2017a). For example, while studies in animals have displayed a reduction in markers of inflammation with the application of cold following damaging exercise (Schaser *et al*., 2007), evidence of the anti-inflammatory effects of cryotherapy in humans is insubstantial (Peake *et al*., 2017b). Likewise, mixed evidence exists concerning the efficacy of cryotherapy in accelerating muscle recovery. A recent Cochrane review demonstrated that cryotherapy in the form of CWI had beneficial effects on fatigue and muscle soreness 24 h post-exercise, with no beneficial effects on objective measures of maximal strength or power (Bleakley *et al*., 2012). The lack of agreement in the literature could be due, in part, to the high heterogeneity which exists between studies in regards to the fatiguing protocols used, the participant characteristics, and the method of CWI (e.g. temperature of water, time under immersion).

One potential limitation of common CWI protocols is the time spent in immersion due to the thermal discomfort associated with CWI. For example, in their systematic review, which included 17 studies, Bleakley *et al* (2012) reported that the average water temperature was 11 ± 4°C (range 5–15°C) and the average duration was 11.5 ± 7.4 min (range 3–30 min). Given that the inflammatory response initiates 2-6 h post-exercise (Armstrong *et al*., 1991), a more prolonged cooling period could be required to negate the injury proliferation during this period. A recent intervention which employs a more prolonged cooling period is the application of phase change material (PCM). The application of PCM has many logistical and practical benefits due to being easily transportable, the lower level of thermal discomfort compared with CWI, and due to its' high melting point and capacity to maintain low temperatures for a prolonged period of time (Clifford *et al*., 2018). A number of recent studies have

employed this method and produced encouraging results (McHugh *et al*., 2018, Clifford *et al*., 2018, Kwiecien *et al*., 2018). For example, Clifford *et al*. (2018) applied cold PCM to the quadriceps for 3 hours following competitive football match-play and found reduced muscle soreness and accelerated recovery of MVC strength (Clifford *et al*., 2018), findings which have since been corroborated (McHugh *et al*. 2018). Nevertheless, more evidence is required to substantiate these results and to gain mechanistic insight into the potential benefits of PCM on recovery.

2-13 Summary

The study of fatigue in sports characterised by intermittent sprint exercise has become the subject of considerable research attention in recent years. In particular, due to the high physical demands and the congested nature of fixture schedules in modern day professional football, scientific research has attempted to delineate the mechanisms of fatigue and the time-course of recovery in the days following football match-play in order to provide a scientific basis for recovery interventions aimed at attenuating fatigue and accelerating the natural time-course of recovery (Nedelec *et al*., 2012, Nedelec *et al*., 2013). Impairments in the force generating capacity of the muscle have been studied extensively following football match-play (Rampinini *et al*., 2011, Ascensao *et al*., 2008, Magalhaes *et al*., 2010), and are typically attributed to impairments in neuromuscular function, involving deficits in contractile function and/or the capacity of the CNS to activate muscle (Gandevia, 2001). However, despite an increased awareness of the contribution of the CNS towards altered neuromuscular function *during* exercise, less is known about the role of the CNS in fatigue and recovery *following* exercise, with current research on mechanisms and recovery

strategies primarily focused on peripheral perturbations (Minett and Duffield, 2014). Using methods of neurostimulation, such as electrical nerve and TMS, it is possible to gain insight into the central and peripheral contributors towards impairments in neuromuscular function and recovery thereof in the days following football match-play. Understanding the aetiology of impaired neuromuscular function and the time-course of recovery, with a particular focus on the role of the CNS, is the focus of this series of studies, the aims of which are outlined below.

2-14 Study aims

Study 1: An optimal protocol for measurement of corticospinal excitability, short intracortical inhibition and intracortical facilitation in the rectus femoris

Aims: To determine the optimal combination of stimulus variables (conditioning stimulus, inter-stimulus interval, and contraction strength) when measuring SICI and ICF and the minimum number of measurements required to obtain an accurate estimate of CSE, SICI and ICF when measuring responses in the *rectus femoris*.

Study 2: Reliability of neuromuscular, physical function, and perceptual assessment

Aims: To determine the within- and between-day reliability of neuromuscular, physical function and perceptual assessments to be used in later studies of the thesis.

Study 3: Aetiology and recovery of impairments in neuromuscular function following competitive football match-play

Aims: To examine the contribution and time-course of recovery of peripheral and central factors toward impairments in neuromuscular function following competitive

football match-play, and to assess the relationship between the temporal pattern of recovery of neuromuscular function and a range of physical and perceptual measures following match-play in order to provide practitioners with simple tools to monitor the physical and cognitive contributors to fatigue in the days post-match.

Study 4: The effect of phase change material on recovery of impairment neuromuscular function following competitive football match-play

Aims: To assess the effects of wearing cold PCM garments on the quadriceps and hamstring muscles for 3 h following football match-play on impairments in neuromuscular function, physical function and perceptual responses in the days post-match.

CHAPTER 3 GENERAL METHODS

3-1 Introduction

The general methods employed in this thesis are outlined in this chapter. Specific methods used in individual experimental chapters are discussed within the corresponding chapter.

3-2 Pre-test procedures

Institutional ethical approval was received from the Northumbria University Faculty of Health & Life Sciences Ethics committee in accordance with the ethical standards established in the *Declaration of Helsinki*. All participants were informed of the study procedures via an information sheet, which described the purpose of the study, before providing written informed consent to participate (Appendix 1). Participants were free of any cardiorespiratory, neurological or neuromuscular health disorders, had no metal plates in the head/brain, and were not taking any medication that might have interfered with the nervous system. All participants completed a TMS safety screening questionnaire prior to the data collection procedure (Keel *et al*. 2001). For each study, participants were asked to refrain from strenuous physical activity or alcohol consumption in the 48 hours prior to data collection, and from caffeine consumption on the day of testing.

3-3 Apparatus and procedures

3-3.1 Anthropometry

Prior to each experimental procedure, participants were assessed for stature, mass and date of birth. Stature was recorded to the nearest cm using a stadiometer (Seca, Bonn,

Germany). Briefly, participants placed their heels, buttocks and upper back in contact with the stadiometer, before being instructed to inhale and hold a deep breath while the investigator applied gentle upward lift through the mastoid process in order to ensure the head was kept in the Frankfort plane. At the same time, the headboard was placed firmly down on the vertex. Body mass was recorded on calibrated scales (Holitan, Crymych, Wales) to the nearest 0.1 kg, and age was recorded to the nearest year.

3-3.2 Competitive football match play

Chapters five and six used competitive football match-play to assess recovery of neuromuscular function in the days post-match. The competitive matches consisted of two 45 min halves interspersed by a 15 min recovery interval. All games consisted of twenty-two players (two goalkeepers and twenty outfield players), were registered as official matches under the English Football Association, and were refereed by officials from the Northumberland Football Association.

3-3.3 Perceptual measurements

In chapters five and six, participants completed the *"Elite Performance Readiness Questionnaire"* (Dean *et al*., 1990) (see Appendix 2), a measure of performance readiness consisting of 10 subjective measures of fatigue, soreness, motivation to train, anger, confusion, depression, tension, alertness, confidence and sleep. The Elite Performance Readiness Questionnaire is a shortened version of the Profile of Mood States Questionnaire (McNair *et al*., 1971), and has previously been validated in a sporting context (Dean *et al*., 1990). Participants were given verbal instructions on

how to use the scales, and were familiarised with its use during practice sessions prior to the experimental trials. During each trial, participants drew a vertical line on a 100 mm horizontal line in response to questions used for each measure, such as "how fatigued do you feel?", "how sore do your muscles feel?" and "how motivated to train do you feel?" Each scale was anchored with verbal descriptors "not at all" to "extremely" (Appendix 2). Perceptual measures were assessed prior to commencing the warm-up at each time-point.

3-3.4 Assessments of physical function

Following intermittent sprint team sport activity, it is commonplace for practitioners to utilise tests of physical function in order to monitor fatigue and recovery (Halson, 2014, Twist and Highton, 2013). In particular, sprint and jump tests are regularly used as a means of monitoring fatigue due to their simplicity of administration and the minimal amount of additional fatigue induced (Halson, 2014). These tests can provide information on fatigue-induced impairments in stretch-shortening cycle function, which in turn can have implications for performance during training or competition (Oliver *et al*., 2008). The sensitivity of these tests to detecting impairments in neuromuscular function, however, remains unclear, with conflicting findings on the magnitude of decrements in physical performance tests and the time-course of recovery post-intermittent sprint exercise (Gathercole *et al*., 2015b, Andersson *et al*., 2008, Ascensao *et al*., 2008).

In chapters five and six, participants completed a battery of assessments to measure physical function in variables relevant to optimal football performance. Jump height (cm) during a countermovement jump (CMJ) and reactive strength index (RSI) during

a drop jump (DJ) were measured using an optical timing system (Optojump Next, Microgate, Milan, Italy). For CMJ, participants started from a standing position with hands akimbo. On verbal command, participants made a downward countermovement before jumping vertically for maximum height. For reactive strength index (DJ-RSI), participants were instructed to step off a 30 cm box with hands akimbo, before jumping vertically for maximum height as soon as possible after landing. To ensure the DJ-RSI was assessing fast stretch-shortening cycle, a maximum ground contact time of 200 ms was allowed during each jump, with participants given visual feedback on each ground contact time and jump height after each jump. Reactive strength index ($cm \cdot s^{-1}$) was calculated as the ratio between jump height (cm) and ground contract time (s). All participants were given three attempts at each jump with 60 s between each repetition. In chapter five, linear speed (20 m sprint with 10 m splits) during three maximal effort sprints was recorded using electronic timing gates (TC Timing Systems, Brower Timing Systems, Draper, USA). Sprints were self-initiated from a standing start 30 cm behind the first timing gate, with participants encouraged to sprint maximally through the timing gate at 20 m.

3-3.5 Match play physical performance and intensity measurements

In chapters five and six, the activity profile and heart rate (HR) of the players during match-play were measured using GPS with built in HR monitors (Polar Team Pro, Polar Electro Oy, Finland). The GPS units were placed on the sternum and secured with an elastic strap around the chest and back. The data from the GPS units was sampled at 10 Hz, and included microelectronic motion sensors sampled at 200 Hz for accelerometery measurements. Following the matches, the GPS data was extracted and analysed offline. From the GPS data obtained, total distance (TD), high-intensity

running (HIR, distance covered at running velocities higher than 15 $km \cdot h^{-1}$), total accelerations (> 1 $m \cdot s^{-2}$), total decelerations (> −1 $m \cdot s^{-2}$) and mean and peak HR were chosen for further analysis. These variables were chosen as they provide an indication of both the internal and external work load during match-play, and previously been shown to be suitable parameters of match-related fatigue in football (Rampinini *et al*., 2011, Mohr *et al*., 2003, Akenhead *et al*., 2013). Moreover, in chapter five, GPS and heart rate variables collected were compared with data from the same players throughout the competitive season in order to ensure the demands of the matches were similar to that of a competitive fixture.

3-3.6 Neuromuscular function

In chapters five and six, the evoked force responses of the knee extensors and EMG responses of the *rectus femoris* to TMS of the primary motor cortex, and electrical stimulation of the femoral nerve, were used to assess fatigue and recovery of neuromuscular function from both central and peripheral origins. The quadriceps were studied as this muscle group incurs significant fatigue and decrements in function as a result of intermittent sprint exercise (Rampinini *et al*., 2011). Additional detail on these procedures are provided below.

Force & EMG recordings

A calibrated load cell (MuscleLab force sensor 300, Ergotest technology, Norway) recorded muscle force in Newton's (N) during an isometric MVC of the knee extensors. During contractions, participants sat with hips and knees at 90° flexion,

with a load cell fixed to a custom-built chair and attached to the participants right leg, superior to the ankle malleoli, with a noncompliant cuff. Knee and hip angle were measured using a goniometer at 90° flexion prior to each experiment and maintained during contractions. Participants were instructed to grasp the handles on the side of the chair for support during MVCs. The force trace was displayed on a computer screen directly in front of participants in order to assist in providing maximal efforts during MVC (Baltzopoulos *et al*., 1991) and to provide the target force during submaximal contractions. Each MVC lasted 3 s. The height of the load cell was adjusted at the beginning of each trial to ensure the force was applied in a direct line. The load cell was calibrated across the physiological range by suspending known masses (kg), with regression analysis used to convert raw analogue signals (mV) to force (N).

Electrical activity from the *rectus femoris* and *bicep femoris* were recorded from surface electrodes (Ag/AgCl; Kendall H87PG/F, Covidien, Mansfield, MA, USA) placed 2 cm apart over the belly of each muscle, with a reference electrode placed on the patella. Although the *vastus lateralis* has been studied when measuring responses to electrical nerve and TMS during and following locomotor exercise (O'Leary *et al*. 2016; Sidhu *et al*. 2013b), this muscle is uni-articular and is involved in knee extension exclusively. Given that studies measuring responses to nerve and TMS in the knee extensors are most commonly conducted in response to activities involving locomotion (Thomas *et al*. 2017b; Weier *et al*. 2012), the *rectus femoris* was believed to be a more suitable muscle to study due to its biarticular make up and significant contribution to both hip flexion and knee extension, movements which are heavily involved in locomotion and actions associated with football match-play, such as sprinting, jumping, kicking and changing direction (Mendiguchia *et al*., 2013). A

number of studies have monitored the *rectus femoris* when studying fatigue in the knee extensors in response to intermittent sprint exercise (Thomas *et al*., 2017a, Goodall *et al*., 2017b, Thorlund *et al*., 2009). Activity of the *biceps femoris* was measured to ensure low antagonist co-activation, as this may cause superimposed twitch underestimation during measurement of voluntary activation (Temesi *et al*., 2014). The placement of the EMG electrodes on the *rectus femoris* and *biceps femoris* were based on Seniam guidelines. Specifically, for the *rectus femoris*, electrodes were placed at 50% on the line from the anterior spina iliaca superior to the superior part of the patella, while for the *biceps femoris*, electrodes were placed at 50% on the line between the ischial tuberosity and the lateral epicondyle of the tibia. Electrode placement was marked with indelible ink to ensure consistent placement during repeated trials, with the areas shaved and cleaned with an alcohol swab before being left to dry prior to electrode placement. The electrodes recorded the root-mean-square (RMS) amplitude for sub-maximal and maximal voluntary contractions, the compound muscle action potential (M-wave) from the electrical stimulation of the femoral nerve, and the motor evoked potential (MEP) elicited by TMS. Signals were amplified: gain ×1,000 for EMG and ×300 for force (CED 1902; Cambridge Electronic Design, Cambridge, UK), band-pass filtered (EMG only: 20–2000 Hz), digitized (4 kHz; CED 1401, Cambridge Electronic Design) and analysed offline. Further details on these methods are provided below.

Motor nerve stimulation

Motor nerve stimulation was used for the measurement of contractile function, muscle membrane excitability and estimated VA. Single and paired electrical stimuli (100 Hz)

were administered using square wave pulses (200 μs) via a constant-current stimulator (DS7AH, Digitimer Ltd., Hertfordshire, UK) using self-adhesive surface electrodes (CF3200, Nidd Valley Medical Ltd., North Yorkshire, UK). The cathode was placed over the nerve high in the femoral triangle, with the anode placed between the greater trochanter and the iliac crest (Weavil *et al*., 2015). To ensure correct positioning, the stimulation response that elicited the maximum quadriceps twitch amplitude (Q_{tw}) and M-wave (M_{max}) was assessed. Once located, the area was marked with indelible ink to ensure consistent placement between trials. Subsequently, stimulation intensity was determined by administering electrical stimuli to the motor nerve rest in 20 mA step-wise increments from 20 mA until Q_{tw} and M_{max} plateaued. To ensure a consistent, supramaximal stimulus and account for any activity-induced changes in axonal excitability, the resulting stimulation intensity was increased by 30%. Further details on the evoked responses following femoral nerve stimulation are provided below.

Voluntary activation

Voluntary activation is defined as the level of neural drive to the muscle during exercise (Gandevia *et al*., 1995). During a MVC, if motor units have not been recruited or are not firing fast enough, delivering a supramaximal electrical or magnetic stimulation to the corresponding nerve or muscle will evoke a twitch-like increment in force, termed the superimposed twitch (SIT). This method, known as the twitch interpolation technique, was first described by Merton (1954), and quantifies the completeness of muscle activation during a voluntary contraction (Shield and Zhou, 2004).

In Chapters 5 and 6, the interpolated twitch technique was used to assess voluntary activation of the knee extensors. Paired electrical stimulation (100 Hz) of the femoral nerve was delivered during and 2 s following a MVC. Previous work has suggested the using paired electrical stimuli provides the most valid means of assessing voluntary activation based on the twitch interpolation technique (Place *et al*., 2007). Specifically, using paired stimuli is thought to be advantageous compared with single stimuli due to higher degree of certainty that motor units which are in a refractory state during the first stimuli will be nonrefractory upon delivery of the second stimuli, thereby increasing the size of the motor unit pool stimulated (Herbert and Gandevia, 1999). Voluntary activation was assessed by comparing the amplitude of the potentiated twitch at 100 Hz ($100HzQ_{tw,pot}$) with the SIT evoked during a MVC using the following equation:

$$\text{Voluntary activation (\%)} = (1 - (100HzQ_{tw,pot}/\text{potentiated resting paired stimulation})) \times 100$$

Contractile function

The evoked force and twitch characteristics in response to single- and paired-pulse (100 Hz) electrical stimulation were used to study contractile function in response to intermittent sprint exercise. The amplitude of the evoked twitch response at rest is sensitive to the preceding contraction history, such that a prior strong muscle contraction results in a marked increase in the twitch amplitude, a phenomenon known as twitch potentiation (Kufel *et al*., 2002). The proposed mechanism responsible for twitch potentiation is phosphorylation of myosin regulatory light chains which increase the sensitivity of the actin-myosin complex to Ca^{2+} released from the sarcoplasmic reticulum (Hodgson *et al*., 2005). In turn, augmented Ca^{2+} sensitivity

increases the rate by which myosin-light chains move from a non-force producing state to a force producing state, thereby increasing the amplitude of the evoked twitch response (Allen *et al*., 2008). Because the biochemical changes within the muscle cell purported to be responsible for impairments in contractile function could interfere with the mechanisms responsible for twitch potentiation, the sensitivity of the potentiated twitch to perturbations in muscle function is higher than that of the unpotentiated twitch (Kufel *et al*., 2002). As such, potentiated twitch force was evaluated throughout this thesis when assessing contractile function.

Along with the twitch amplitude, a number of parameters can be examined from the mechanical response to single-pulse stimulation to allow inferences to be made on the aetiology of contractile impairments. Namely, contraction time (CT), maximum rate of force development (MRFD), maximum relaxation rate (MRR) and one-half relaxation time ($RT_{0.5}$) can be derived from the potentiated twitch response. The methods by which these variables were determined is depicted in Figure 3-1 from a representative resting twitch. Specifically, CT was defined as the time between stimulus delivery (including mechanical delay) and the peak twitch force, while $RT_{0.5}$ is calculated as the time taken for the force to decrease to half the peak twitch amplitude. MRFD and MRR are defined as the maximal and the lowest value of the first derivate of the force signal, respectively. Since MRFD and MRR are dependent on the size of the twitch, they were both normalised with twitch amplitude to allow twitches of varying intensity to be compared.

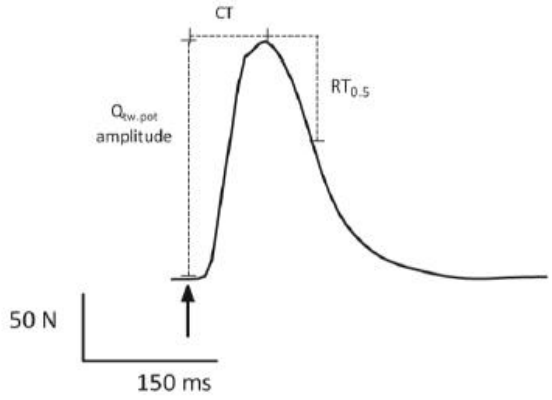


Figure 3-1. Potentiated twitch from representative participant showing twitch amplitude, contraction time (CT) and half-relaxation time (RT0.5). The arrow indicates when the electrical sitmulation was delivered.

Transcranial magnetic stimulation of the motor cortex

Transcranial magnetic stimulation (TMS) is based on the principle of electromagnetism, first proposed by Michael Faraday in the 19th century. When a changing magnetic field is delivered in electrically conductive regions, such as the human brain, an electromotive force is produced – a phenomenon known as electromagnetic induction (Auvichayapat and Auvichayapat, 2009). In 1985, Barker and colleagues applied this technique to stimulate the human motor cortex, which subsequently led to the development of TMS (Barker *et al.*, 1985, Goodall *et al.*, 2014). With TMS, a brief, large current is delivered over the scalp through a wire coil, producing a changing magnetic field and inducing electrical currents in the underlying brain (Chen, 2000). By stimulating specific regions of the motor cortex corresponding with different muscle groups, TMS can evoke mechanical and electrical responses in the target muscle when delivered at a sufficient intensity. The force and electrical responses to TMS can be used to quantitatively assess intracortical and corticospinal activity and voluntary activation in response to various interventions, such as fatiguing exercise.

In each experimental chapter, single- and/or paired-pulse TMS of 1 ms duration were delivered using a concave double cone coil using two linked monopulse magnetic stimulators (Magstim 200, The Magstim Company Ltd, Whitland, UK). The junction of the double cone coil was aligned tangentially to the sagittal plane, with its centre 1-2 cm to the left of the vertex. The optimal coil placement was determined at the start of each trial as the position that elicited the largest MEP in the RF. The position was marked with indelible ink for consistent placement. In chapters 4 and 5, the stimulator intensity was based on active motor threshold (AMT) measured during a light voluntary contraction (Thomas *et al.*, 2017a). In order to determine AMT, the stimulator intensity was increased in 5% steps beginning at 35% of stimulator output until a consistent MEP with peak-to-peak amplitudes of >200 μV was found. Thereafter, stimulus intensity was reduced in 1% step until an MEP of >200 μV was found in 50% of stimulations. Single- and paired-pulse TMS paradigms were used to assess intracortical and corticospinal activity and voluntary activation in the knee extensors throughout the studies of this thesis (discussed below).

Voluntary activation with transcranial magnetic stimulation

Voluntary activation can be measured with TMS to provide additional information on the aetiology of impaired neuromuscular function (Todd et al., 2003b). This technique involves delivering a magnetic stimulation to the area of the motor cortex corresponding with the muscle of interest during an MVC. If a superimposed twitch is elicited by TMS, this implies not only that some motor units are not recruited or are not firing fast enough, but also that motoneuron and/or motor cortical output is submaximal (Todd et al., 2016a). In contrast to measuring voluntary activation with

motor nerve stimulation, it is inappropriate to normalise the SIT elicited by TMS with a response evoked at rest. This is because the excitability of the motoneuron pool is lower at rest than during a voluntary contraction (Rothwell *et al*., 1991), resulting in activation of fewer motoneurons at rest compared with contraction (Todd et al., 2003b). Instead, the resting twitch is estimated using linear extrapolation based on the SIT evoked at contraction intensities between 50% and 100% MVC (Figure 3-2). The estimated resting twitch (ERT) is then entered into an equation in order to quantify voluntary activation as shown in the equation below:

$$\text{Voluntary activation (\%)} = (1\ (-\ \text{SIT/ERT})) \times 100$$

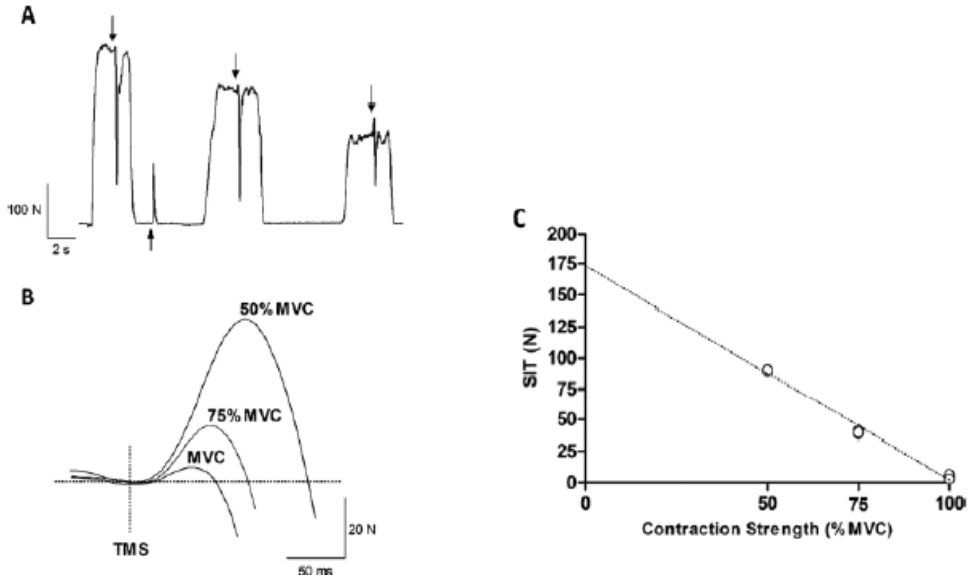


Figure 3-2. Measurement of voluntary activation using transcranial magnetic stimulation (TMS). A, force trace illustrating measurement of the superimposed twitch (SIT) during voluntary contractions at 100%, 75% and 50% MVC using single-pulse TMS (downward arrows). B, illustrates the amplitude of the SIT elicited by TMS at 100%, 75% and 50% MVC, subsequently used to calculate the estimated resting twitch (ERT) via linear extrapolation, C. From Goodall *et al*. (2014).

Prior to measuring voluntary activation with TMS, the appropriate stimulation intensity was determined as the stimulator intensity that elicited the maximum superimposed twitch (SIT) during a 50% MVC (Thomas *et al*., 2017a). This was determined by increasing the stimulator intensity in 5% increments beginning from

50% until a plateau in the SIT was found. The methods used to assess voluntary activation in the knee extensors have been previously validated to demonstrate impaired voluntary activation in response to fatiguing exercise (Goodall *et al*., 2009, Sidhu *et al*., 2009a).

Evoked potential responses electrical and magnetic stimulation

Evoked responses to electrical stimulation of the femoral nerve and TMS of the motor cortex were recorded using surface EMG and analysed offline post-test (Signal and Spike 7, Cambridge Electronic Design, Cambridge, UK). The peak-to-peak amplitude and area of the electrically evoked maximal compound action potential (M_{max}) and the motor evoked potential (MEP) induced by TMS were used as measures of membrane excitability, and CSE, respectively. When assessing CSE, the MEP was normalized relative to M_{max} in order to account for activity dependent changes in membrane excitability. In addition, paired-pulse TMS paradigms were used to examine the excitability of intracortical inhibitory and facilitatory circuits. Specifically, when a subthreshold CS precedes a suprathreshold tests stimulus by an interval of 1-5 ms, inhibitory circuits mediated by $GABA_A$ interneurons are activated, resulting in a reduction in the size of the MEP elicited by the suprathreshold test stimulus (termed short-interval intracortical inhibition, SICI) (Kujirai *et al*., 1993). By contrast, paired-pulse TMS at an inter-stimulus interval (ISI) of 10-15 ms results in facilitation of the test pulse MEP (termed intracortical facilitation, ICF), likely mediated by glutamate mediated N-methyl-D-aspartate excitatory interneurons (Nakamura *et al*., 1997, Liepert *et al*., 1997). The stimulus variables used to measure SICI and ICF, such as the conditioning stimulus intensity, ISI, and contraction strength during measurement,

are known to influence the degree of inhibition and facilitation (Ortu *et al*., 2008). While the stimulus variables have been optimised in order to elicit the highest degree of inhibition and facilitation when examining responses in the upper limb (Ortu *et al*., 2008), the optimal approach to measuring SICI and ICF in the knee extensors is unclear. Currently, significant heterogeneity exists in terms of the stimulus variables used in studies measuring responses to paired-pulse TMS in the knee extensors (O'Leary *et al*., 2016; Thomas *et al*., 2017b; Weier *et al*., 2012), making comparisons between studies problematic. As such, further research is warranted in order to determine the optimal approach to measuring SICI and ICF in the knee extensors in order to provide methodological guidance for future investigations.

CHAPTER 4 AN OPTIMAL PROTOCOL FOR MEASUREMENT OF CORTICOSPINAL EXCITABILITY, SHORT INTRACORTICAL INHIBITION AND INTRACORTICAL FACILITATION IN THE *RECTUS FEMORIS*

Publication arising as a result of this chapter:

BROWNSTEIN, C.G., ANSDELL, P., ŠKARABOT, J., HOWATSON, G., GOODALL, S., THOMAS, K. 2018. An optimal protocol for measurement of corticospinal excitability, short intracortical inhibition and intracortical facilitation in the *rectus femoris*. *The Journal of Neurological Sciences*, 39, 45-56.

4-1 Introduction

Transcranial magnetic stimulation (TMS) over the motor cortex is a safe and non-invasive technique that permits the quantitative assessment of intracortical and corticospinal activity in humans (Kobayashi and Pascual-Leone, 2003). At a sufficient intensity, single-pulse TMS induces descending volleys which travel through pyramidal tract neurons and spinal motor neurons to evoke an EMG response in a target muscle (Goodall *et al*., 2014). The amplitude of the compound EMG response, termed the motor evoked potential (MEP), can be used to quantify CSE. Paired-pulse TMS paradigms can be used to examine intracortical inhibitory and facilitatory circuits (Kujirai *et al*., 1993). Specifically, when a subthreshold CS precedes a suprathreshold test stimulus by an interval of 1-5 ms, inhibitory circuits mediated by $GABA_A$ interneurons are activated, resulting in a reduction in the size of the MEP (short-interval intracortical inhibition, SICI) (Kujirai *et al*., 1993). In contrast, paired-pulse TMS at a longer inter-stimulus interval (ISI; 10-15 ms) facilitates the MEP response (intracortical facilitation, ICF) (Kujirai *et al*., 1993). While the mechanisms of ICF are less clear, it has been suggested that MEP facilitation could be due to activation of glutamate mediated N-methyl-D-aspartate excitatory interneurons (Nakamura *et al*., 1997, Liepert *et al*., 1997).

The stimulus variables used to measure SICI and ICF can be manipulated in order to maximise activation of inhibitory and facilitatory intracortical interneurons and thereby augment the level of inhibition and facilitation induced by paired-pulse TMS. Specifically, the subthreshold CS intensity (Sidhu *et al*., 2013b, O'Leary *et al*., 2015, Vucic *et al*., 2009), suprathreshold test pulse intensity (Temesi *et al*., 2017), ISI (Ortu *et al*., 2008) and the contraction strength used during paired-pulse TMS measurements (Ridding *et al*., 1995, Zoghi and Nordstrom, 2007, Ortu *et al*., 2008) have all been

shown to influence the degree of inhibition and/or facilitation. While these stimulus variables have been systematically optimised in upper limb muscle groups (Ortu *et al*., 2008), no study exists examining the optimal configuration used to elicit SICI and ICF in the knee extensors. Given the differences in intracortical circuits between upper and lower limb muscles (Chen *et al*., 1998), using stimulus variables optimised in the upper limb might not be appropriate when investigating responses to paired-pulse TMS in lower limb locomotor muscles. At present, much heterogeneity exists between studies in the stimulus variables applied when measuring SICI and ICF in the knee extensors. For example, the CS intensity applied when taking measures of SICI and ICF varies between studies, with some studies applying a CS intensity of 70% (Thomas *et al*., 2017b) AMT or 90% (O'Leary *et al*., 2016, Latella *et al*., 2017) resting motor threshold (RMT) when measuring both SICI and ICF. Similarly, inconsistencies exist in the ISI used when measuring SICI, with studies using either a 2 (Goodall *et al*., 2018) or 3 ms (Thomas *et al*., 2017b, O'Leary *et al*., 2016) ISI for SICI, and an ISI of, 12, (Latella *et al*., 2017) 13 (Thomas *et al*., 2017b) or 15 ms for ICF (O'Leary *et al*., 2016, Luc-Harkey *et al*., 2017). Such methodological issues make comparisons between investigations problematic.

Another pertinent question when attempting to optimise single- and paired-pulse TMS in the knee extensors is the number of pulses required to obtain an accurate estimate of CSE, SICI and ICF. During single- and paired-pulse TMS, the amplitude of the MEP demonstrates significant pulse-to-pulse variation due to constant fluctuations in CSE (Kiers *et al*., 1993, Heroux *et al*., 2015), as well as randomness in the firing of pyramidal tract neurons and spinal motor neurons (Pitcher *et al*., 2003) and desynchronization of APs (Magistris *et al*., 1998). This variability can be reduced by taking measurements when the muscle is in an active state (Darling *et al*., 2006).

Nonetheless, consecutive measurements are required in order to obtain a reliable and accurate estimation of CSE, SICI and ICF. Cuypers *et al*. (2014) and Bashier *et al*. (2017) suggested that at least 30 consecutive stimuli are required to obtain an accurate estimate of CSE in the relaxed first dorsal interosseous muscle. However, it is known that the variability of MEP amplitude differs according to the muscle under investigation (Malcolm *et al*., 2006, Brasil-Neto *et al*., 1992), and differences in corticospinal projections between upper and lower limbs could influence the pulse-to-pulse variability in MEP amplitude (Brouwer and Ashby, 1990). Currently, the appropriate number of pulses in the active knee extensors remains unclear, with the majority of studies arbitrarily using 10-15 responses (O'Leary *et al*., 2015, Weier *et al*., 2012). Understanding the appropriate number of stimuli required during single- and paired-pulse TMS in the knee extensors is an important consideration in order to maximise the accuracy of intracortical and corticospinal measurements when assessing the neurophysiological effects of various acute and chronic interventions, such as fatiguing exercise, repetitive TMS, or strength training.

Assessing intracortical and corticospinal activity in the knee extensors is conceptually appealing given the key role of this muscle group in locomotion and sporting activity. Indeed, an increasing number of studies have used paired-pulse TMS to examine intracortical mechanisms involved in locomotion (Sidhu *et al*., 2013b), fatigue-induced alterations in intracortical activity (Verin *et al*., 2004, Thomas *et al*., 2017a, O'Leary *et al*., 2016), and neural adaptations to strength training (Weier *et al*., 2012), as well as the neurophysiology of movement disorders (Cantello, 2002). Using single- and paired-pulse TMS will permit greater insight into effect of intermittent sprint exercise on neuromuscular function in the days post-exercise in subsequent chapters. As such, understanding the optimal methods used to measure CSE, SICI and ICF and

the reliability of these measures will provide guidance for the design of experimental protocols used in subsequent chapters of this thesis, discern the sensitivity of these measures in detecting any potential changes in intracortical and corticospinal activity in response to intermittent sprint exercise, whilst also mitigating the heterogeneity that exists between studies. Accordingly, the aims of the study were twofold: 1) to establish the optimal combination of stimulus variables (CS intensity, ISI and contraction strength) when measuring SICI and ICF in the knee extensors and 2) to determine the minimum number of stimuli required to obtain an accurate estimation of CSE, SICI and ICF.

4-2 Methods

4-2.1 Participants

Twenty-nine young male adults participated in at least one experiment of the study. Participants were free of any cardiorespiratory, neurological or neuromuscular health disorders, had no metal plates in the head/brain, and were not taking any medication that might have interfered with the nervous system. All participants completed a TMS safety screening questionnaire prior to the data collection procedure (Keel *et al*., 2001).

4-2.2 Design

The study was divided into four experiments (Figure 4-1). During all experiments within the study, single- and paired-pulse TMS was delivered during tonic contractions. This is because studies applying single- and paired-pulse TMS paradigms in the knee extensors are commonly related to locomotor activities (Sidhu

et al., 2013b, Thomas *et al*., 2017b, Thomas *et al*., 2017a), and it is thus recommended that assessment of corticospinal and intracortical activity be conducted during contraction in order to provide a better reflection of neurophysiological processes occurring during motor activity (Gruet *et al*., 2013, Kalmar, 2018).

Experiments 1-3 aimed to determine the optimal stimulus variables used to measure SICI and ICF in the *rectus femoris* by investigating the effects of CS intensity, contraction strength and ISI, respectively, on the level of inhibition and facilitation. Experiment 4 assessed the minimum number of measurements required to obtain an accurate estimate of CSE, SICI and ICF using the optimal stimulus variables determined from Experiments 1-3. Each experiment was separated by between three and five weeks.

4-2.3 Instrumentation

Force & EMG recordings

Three MVCs of 3 s duration were performed prior to each trial, with 60 s between each contraction. The maximum force from the three MVCs was recorded in order to calculate the submaximal contraction values. As the methods utilised in the present study were implemented in the subsequent chapter, responses were recorded in the

rectus femoris due to their integral role in locomotion and heavy involvement during activities associated with competitive football match-play (Mendiguchia *et al*., 2013).

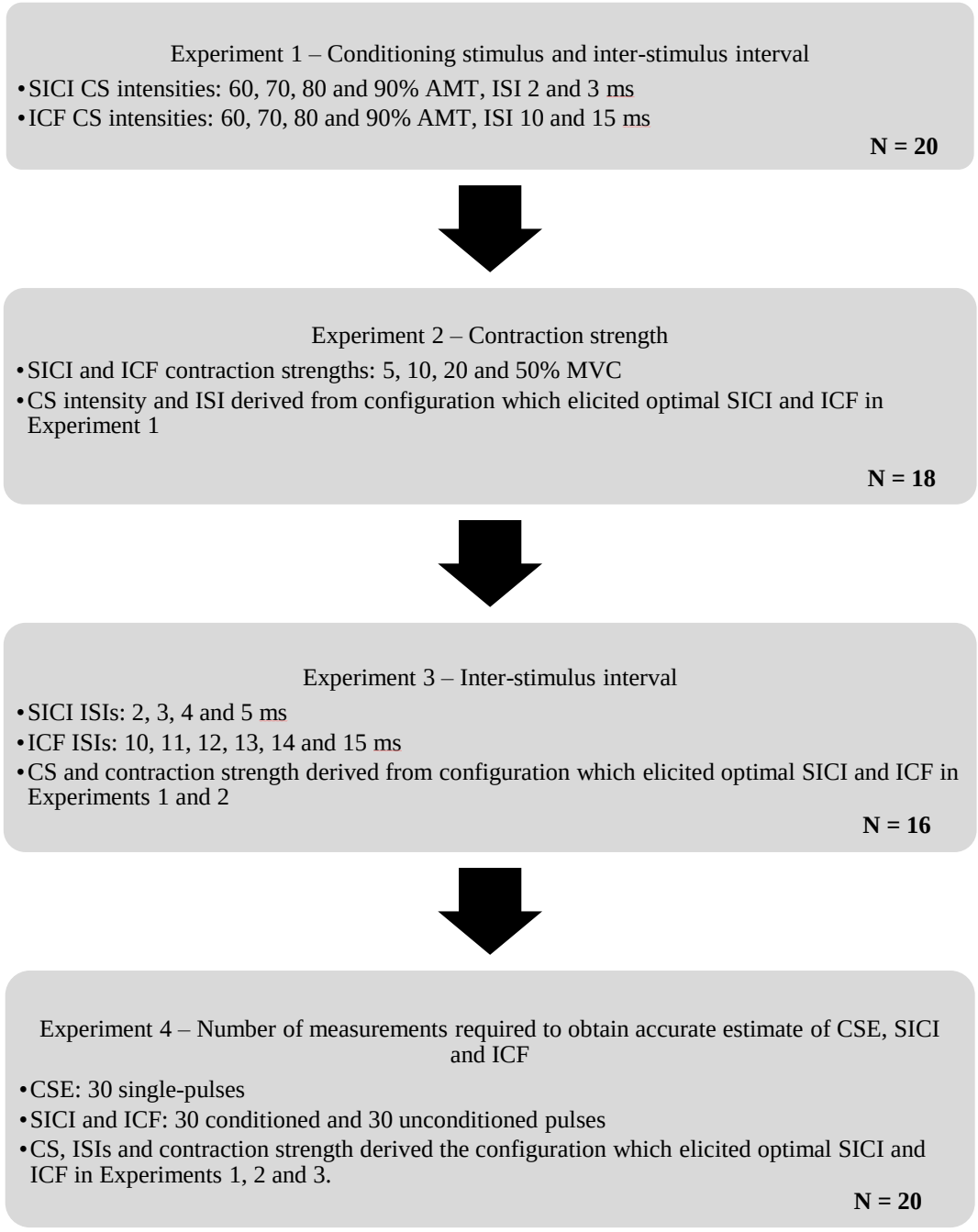


Figure 4-1. Flow chart displaying study design. Experiments 1-3 aimed to determine the optimal stimulus variables used to measure short-interval intracortical inhibition (SICI) and intracortical facilitation (ICF) in the rectus femoris by investigating the effects of conditioning stimulus (CS) intensity, contraction strength and inter-stimulus interval (ISI), respectively, on the level of inhibition and facilitation. Experiment 4 assessed the minimum number of measurements required to obtain an accurate estimate of CSE, SICI and ICF using the optimal stimulus variables determined from Experiments 1-3.

Transcranial magnetic stimulation

For all experiments, the single-pulse and test-pulse intensity was set at 120% of AMT, as this intensity lies on the middle portion of the ascending part of the stimulus-response curve (Han *et al*., 2001), and is thus sensitive to changes in CSE. During Experiments 1-3, the order in which SICI, ICF and/or CSE, and each stimulus variable was assessed was pseudo-randomised and counterbalanced using Latin square randomisation, while the order in which single- and paired-pulses were delivered was randomised using an online randomiser (www.randomizer.org).

4-2.4 Experimental procedures

Experiment 1 – Influence of conditioning stimulus intensity on SICI and ICF

Twenty participants (aged: 25 ± 4 years; stature: 181.4 ± 6.6 cm; mass: 84.2 ± 13.3 kg) took part in this experiment. SICI and ICF were assessed using a subthreshold CS, followed by a suprathreshold test stimulus as described by Kujirai *et al*. (1993). Subthreshold CS intensities of 60, 70, 80 and 90% AMT were applied. Inter-stimulus intervals of 2 (Goodall *et al*., 2018) and 3 ms (Thomas *et al*., 2017b, O'Leary *et al*., 2016) for SICI and 10 (Volz *et al*., 2012, Di Lazzaro *et al*., 2006) and 15 ms (Chen *et al*., 1998, Orth *et al*., 2003) for ICF were examined at each CS intensity since these ISIs successfully elicited inhibition and facilitation in a number of previous studies. The order of conditions was pseudo-randomised and counterbalanced. During each experimental condition, a total of 24 pulses (12 single and 12 paired) were delivered in a randomised order in 4 sets of 6 during a submaximal contraction set at 10% of the MVC force (total of 96 single- and 96 paired-pulses across all conditions). A short rest

(30 s) was given in between each set of pulses to minimise the development of fatigue. The CS intensity and ISI that elicited maximum SICI and ICF was used in Experiment 2.

Experiment 2 – Effect of different levels of muscle contraction on SICI and ICF

Eighteen participants participated in Experiment 2 (25 ± 4 years; stature: 182.3 ± 6.1 cm; mass: 85.9 ± 13.4 kg), which aimed to assess the effects of four different contraction strengths (5, 10, 20 and 50% MVC) on SICI and ICF. Based on the results from Experiment 1, the CS and ISI were 70% AMT and 2 ms for SICI, and 60% AMT and 10 ms for ICF, respectively. During the 5% and 10% MVCs, AMT was defined, as above, the lowest stimulator intensity required to produce MEPs >200 μV in 3 out of 5 stimulations. During the 20% and 50% MVCs, AMT was defined as the minimum stimulator intensity that produced a discernible MEP which was 200 μV greater than the pre-stimulus EMG. This approach was employed due to background EMG activity being greater than 200 μV at contraction intensities of 20% and 50% MVC. At lower contraction strengths (5, 10 and 20% MVC), 24 pulses (twelve single and twelve paired) were randomly delivered in sets of six, with a short rest (30 s) given between sets. At 50% MVC, 16 pulses (eight single and eight paired) were randomly delivered in groups of four, with a longer rest interval (1 min) given between sets in order to minimise fatigue (total of 44 single- and 44 paired-pulses across all conditions). The order of the 5, 10 and 20% MVC conditions were pseudo-randomised and counterbalanced, whilst the 50% MVC was always performed last because of the higher potential to induce fatigue. The contraction strength that elicited maximum SICI and ICF was used in Experiment 3.

Experiment 3 – Effect of inter-stimulus interval on SICI and ICF

Sixteen participants took part in Experiment 3 (aged: 24 ± 3 years; stature: 181.3 ± 6.5 cm; mass: 84.4 ± 10.2 kg). Using a CS of 70% AMT for SICI and 60% AMT for ICF and a contraction strength of 10% MVC based on the results from Experiments 1 and 2, this experiment assessed the influence of using different ISIs on SICI and ICF. For SICI, ISIs included 2, 3, 4 and 5 ms, while ICF ISIs included 10, 11, 12, 13, 14 and 15 ms. The order of conditions was pseudo-randomised and counterbalanced. At each ISI, 24 pulses (twelve single and twelve paired) were randomly delivered in four sets of six, with a short rest (30 s) given between sets (total of 60 single- and 60 paired-pulses across all conditions).

Experiment 4 – Assessment of the minimum number of measurements required to obtain an accurate estimation of CSE, SICI and ICF

Experiment 4 was conducted on twenty subjects (aged: 24 ± 4 years; stature: 180.4 ± 7.1 cm; mass: 79.7 ± 12.8 kg). Based on the results from Experiments 1, 2 and 3, SICI was elicited with a CS of 70% AMT, contraction strength of 10% MVC, and an ISI of 2 ms. For ICF, the stimulus variables incorporated a CS of 60% AMT, contraction strength of 10% MVC, and an ISI of 10 ms. For SICI and ICF separately, 60 pulses (30 single and 30 paired) were delivered in a randomised order, with 30 single pulses delivered for assessment of CSE separate from the assessment of SICI and ICF (total of 90 single- and 60 paired-pulses across all conditions). All pulses were delivered in

sets of 6, with a short rest between each set. The order of the conditions was pseudo-randomised and counterbalanced.

4-2.5 Data analysis

The peak-to-peak amplitude of the EMG responses to motor nerve stimuli and TMS were analysed offline. The root mean square EMG amplitude (RMS_{EMG}) and average force were calculated in the 80 ms prior to each TMS stimulus to ensure a similar level of background muscle activity during each stimulation, and excluded if pre-stimulation force was > 5% above or below the average force calculated from all stimulations in the set (< 1% excluded). To quantify SICI and ICF, the percentage of the average conditioned paired-pulse MEP amplitude was expressed relative to the average unconditioned MEP amplitude at 120% AMT. A percentage < 100% indicates inhibition, and a percentage > 100% indicates facilitation. Throughout the study, the stimulus variables which elicited the greatest degree of inhibition and facilitation and/or produced inhibition and facilitation in the highest number of participants were used in the subsequent experiments of the study. While the average degree of inhibition and facilitation was prioritised as the most important factor in determining which stimulus variable was used in subsequent experiments of the study, the number of participants that exhibited inhibition and facilitation at each configuration was considered if the configuration which produced the highest average degree of inhibition or facilitation produced inhibition or facilitation in a substantially fewer number of participants (≤10%) than other configurations. In Experiment 4, the average MEP for CSE was calculated for subsets of consecutive stimuli as follows:

$$\overline{MEP_n} = \frac{MEP_1 + \ldots + MEP_n}{n}$$

where n = 2 to 30 consecutive MEPs for CSE (Cuypers *et al*., 2014). This procedure was also conducted for subsets of consecutive pairs of conditioned/unconditioned MEPs for SICI and ICF. For this experiment, the average of 30 consecutive measurements was considered as the true value for CSE, SICI and ICF. A 95% confidence interval (CI) was then calculated using all 30 measurements for each participant. Based on the CSE, SICI and ICF n value and the CI, it was determined whether the value for subsets of stimuli were included in the CI, yielding a binary variable (0 = not included in the CI, 1 = included in the CI). Subsequently, the number of consecutive measurements required as a probability of falling within the 95% CI was determined (Cuypers *et al*., 2014).

4-2.6 Statistical analysis

All data are presented as mean ± SD. Statistical analysis was performed using Statistical Package for Social Sciences (SPSS, v22.0). Normality of the data was assessed using the Shapiro-Wilks test. If the assumption of normality was violated, appropriate transformations were performed, with common logarithm used for strongly positively skewed ICF and SICI data in Experiments 1 and 2, respectively, and reciprocal transformation used for extremely positively skewed ICF data in Experiment 2 (Bulmer, 1979). For repeated measures ANOVA, sphericity was assessed using Mauchly's test. The Greenhouse-Geisser correction was used to compensate for non-spherical data. In the event of a significant main effect, *post hoc* pairwise comparison with Bonferroni corrections for multiple comparisons was applied. Statistical significance was accepted at $P < 0.05$. For Experiment 1, the effect of CS intensity (60, 70, 80, 90%) and ISI (2, 3, 10, 15 ms) on SICI and ICF was tested using a two-way repeated measures ANOVA. For Experiment 2, the effect of

contraction strength (5, 10, 20, 50% MVC) on SICI and ICF was assessed using a one-way repeated measures ANOVA. For Experiment 3, a one-way repeated measures ANOVA was used to assess the effect of the ISI (2, 3, 4, 5 ms for SICI and 10, 11, 12, 13, 14, 15 ms for ICF) on SICI and ICF.

For Experiment 4, a linear regression was performed on the data of each participant to assess for change (slopes) in CSE, SICI or ICF over time. If the slope of the regression was statistically significant ($P < 0.05$), which would indicate a trend for scores to increase or decrease over time, the data from the corresponding participant was removed from the analysis of the specific condition. Although participants were given a rest period between each set throughout the experiment in order to prevent fatigue, this analysis was performed in order to ensure the results were not confounded by fatigue-induced alterations in CSE, SICI or ICF. After excluding 4 participants from the CSE analysis, 2 participants from the SICI analysis, and 1 participant from the ICF analysis, 16 (CSE), 18 (SICI) and 19 (ICF) participants were included in the final analysis.

4-3 Results

4-3.1 Experiment 1 − Influence of conditioning stimulus intensity on SICI and ICF

Figure 4-2A and B, respectively, display the percentages of the conditioned to unconditioned pulses for SICI and ICF at different CS intensities and ISIs. A two-way ANOVA comparing SICI and different CS intensities and ISIs showed no main effect for CS ($F_{1.77,33.65} = 3.191$, $P = 0.059$), ISI ($F_{1,19} = 2.111$, $P = 0.163$) or CS*ISI ($F_{1.81,34.29} = 2.879$, $P = 0.075$). Similarly, for ICF, there was no main effect for CS ($F_{1.96,37.14} =$

1.011, P = 0.372), ISI ($F_{1,19}$ = 0.416, P = 0.572) or CS*ISI ($F_{2.55,48.37}$ = 0.848, P = 0.473). Although there were no statistically significant differences between stimulus variables, a CS of 70% with an ISI of 2 ms elicited the greatest degree of inhibition on average (67 ± 17% of unconditioned MEP), with 19 out of 20 participants displaying a conditioned/unconditioned MEP percentage < 100%. For ICF, although a CS intensity of 80% AMT with an ISI of 10 ms produced the highest level of ICF on average (132 ± 40% of unconditioned MEP), only 16 out of 20 participants displayed a conditioned/unconditioned MEP percentage > 100%. In contrast, a CS of 60% AMT with an ISI of 10 ms induced facilitation (125 ± 20% of unconditioned MEP) in 18 out of 20 participants. Consequently, stimulus variables consisting of a 70% CS AMT with an ISI of 2 ms for SICI, and a CS of 60% AMT with an ISI of 10 ms for ICF, were applied in the subsequent parts of the study.

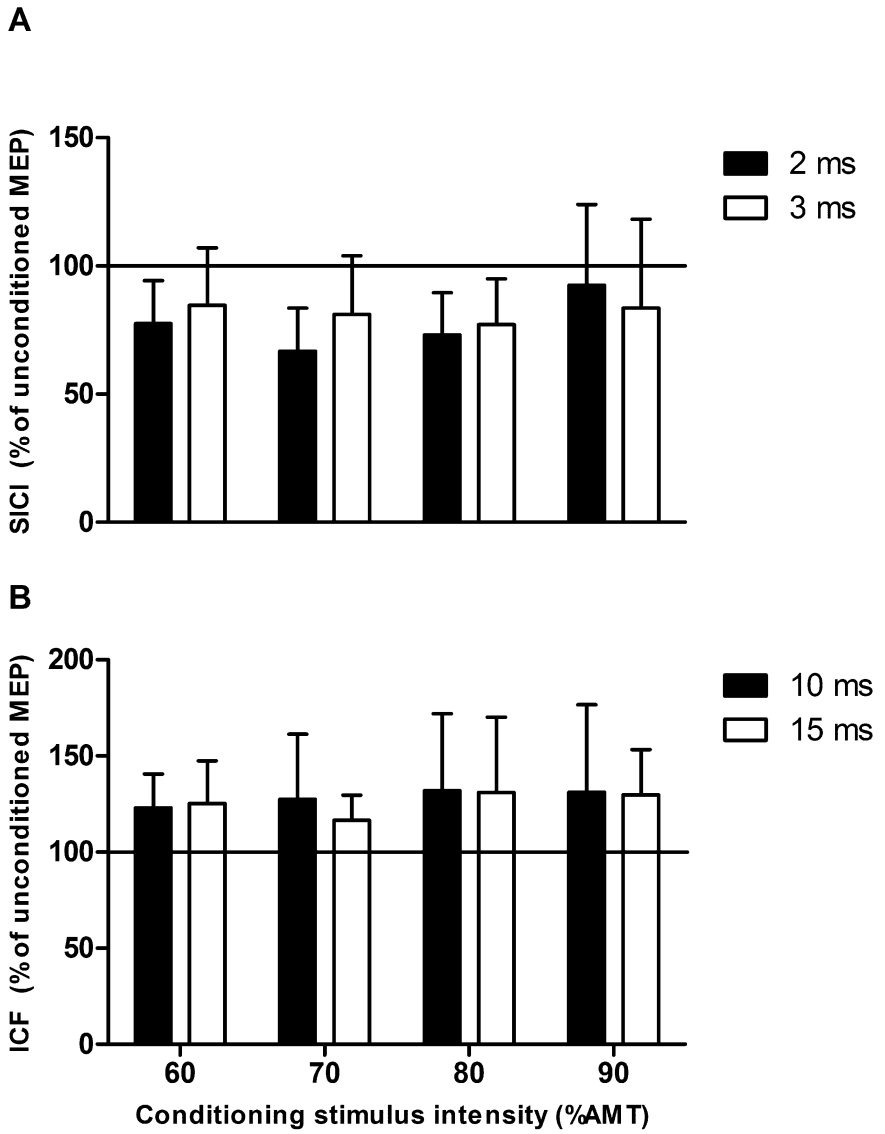


Figure 4-2. Effect of conditioning stimulus intensity relative to active motor threshold (AMT) and inter-stimulus interval (ISI) on short-interval intracortical inhibition (SICI) and intracortical facilitation (ICF) measured in the *rectus femoris* (n = 20) during a 10% MVC. Solid horizontal line represents threshold between inhibition (< 100%), and facilitation (> 100%). Values are mean ± SD.

4-3.2 Experiment 2 − Effect of different levels of muscle contraction on SICI and ICF

Figure 4-3 displays the percentages of the conditioned to unconditioned MEP at different contraction strengths. A main effect for contraction strength on SICI was

observed ($F_{2.196,37.325}$ = 21.604, P < 0.001). *Post hoc* analysis showed that there was more inhibition of the conditioned MEP at 5% MVC compared with 20% MVC (P = 0.021) and 50% MVC (P < 0.001). Similarly, there was more inhibition at 10% MVC compared with 20% MVC (P = 0.037) and 50% MVC (P < 0.001), with no differences between 5% and 10% MVC (P = 1.000), and more inhibition at 20% than 50% MVC (P = 0.005). For ICF, there was a main effect for contraction strength ($F_{3,51}$ = 4.741, P = 0.005), with *post hoc* analysis showing more facilitation of the conditioned MEP at 10% MVC compared with 50% MVC (P = 0.012), and more facilitation at 20% than 50% MVC (P = 0.006), with no other differences (P > 0.05). A contraction strength of 10% MVC was chosen for further analysis during SICI and ICF measurements.

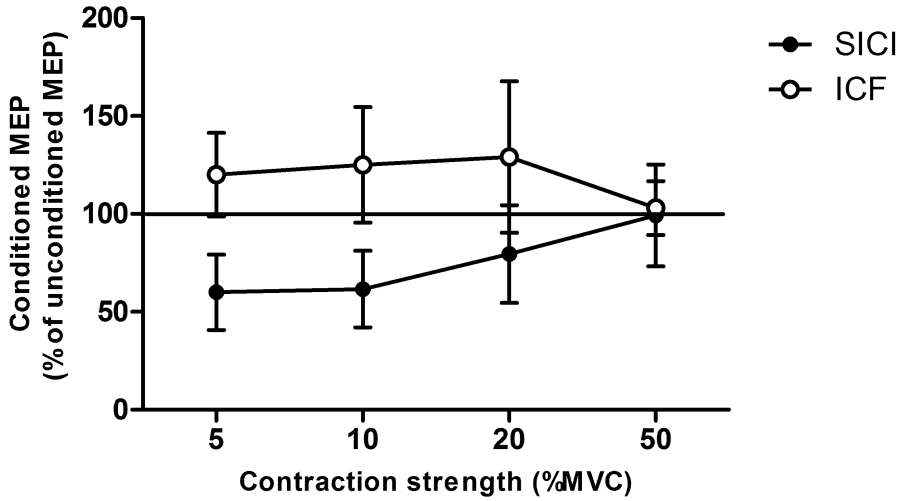


Figure 4-3. Effect of contraction strength relative to maximal voluntary contraction (MVC) on short-interval intracortical inhibition (SICI) and intracortical facilitation (ICF) measured in the *rectus femoris* (n = 18). Solid horizontal line represents threshold between inhibition (< 100%), and facilitation (> 100%). Values are mean ± SD.

4-3.3 Experiment 3 – Effect of inter-stimulus interval on SICI and ICF

Figure 4-4 displays the percentages of the conditioned to unconditioned MEP at different ISIs. A one-way ANOVA displayed a main effect for SICI ($F_{1.80,25.22}$ =

17.675, $P < 0.001$). *Post hoc* analysis revealed that a 2 ms ISI resulted in more inhibition of the conditioned MEP than 4 ms ($P = 0.001$) and 5 ms ($P < 0.001$), with no difference between 2 and 3 ms ($P = 0.092$). An ISI of 3 ms induced more inhibition than 5 ms ($P = 0.023$) with no difference between 3 and 4 ms ($P = 0.286$). No difference was found between inhibition at 4 and 5 ms ($P = 0.063$; Cohen's *d* effect size = 0.85). For ICF, there was a main effect for ISI ($F_{2.87,40.17} = 4.355$, $P = 0.011$), however, *post hoc* comparison revealed no differences between facilitation of the conditioned MEP at any ISI ($P > 0.05$). Although differences between SICI at ISIs of 2 and 3 ms were not observed, an ISI of 2 ms induced the greatest mean inhibition (59 ± 21% vs. 75 ± 31% of unconditioned MEP for 2 and 3 ms, respectively), and induced inhibition in more participants (16 at 2 ms vs. 14 at 3 ms). Similarly, the highest degree of facilitation on average was induced at 10 ms (120 ± 9% of unconditioned MEP), with the highest number of participants facilitated (13). As such, an ISI of 2 ms for SICI and 10 ms for ICF were used for the subsequent parts of the study.

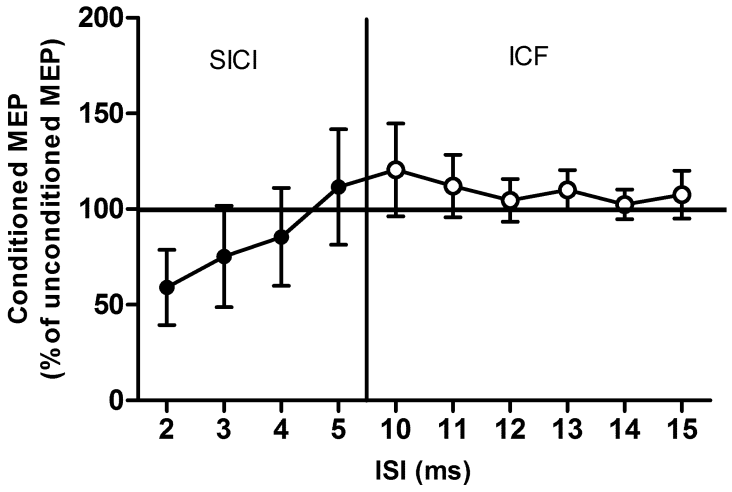


Figure 4-4. Effect of inter-stimulus interval (ISI) on short-interval intracortical inhibition (SICI) and intracortical facilitation (ICF) in the *rectus femoris* (n = 16) during a 10% MVC. Solid horizontal line represents threshold between inhibition (< 100%), and facilitation (> 100%). Solid vertical line represents cut off between ISIs used to measure SICI (2-5 ms) and ICF (10-15 ms). Values are mean ± SD.

4-3.4 Experiment 4 − Assessment of the minimum number of measurements required to obtain an accurate estimation of CSE, SICI and ICF.

The probability that MEP_n, $SICI_n$ and ICF_n fell within the 95% CI based on 30 TMS pulses or pairs of conditioned/unconditioned pulses increased with successive stimulations (Figure 4-5). At least 21, 18 and 17 stimuli were required for CSE, SICI and ICF, respectively, to reach a 100% probability that the average MEP fell within the 95% CI for all participants (Figure 4-6).

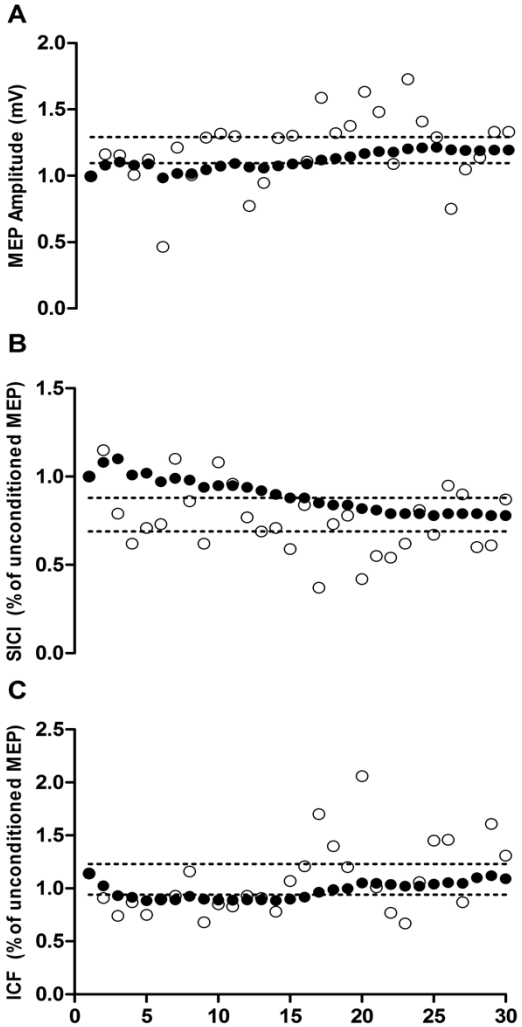


Figure 4-5. Corticospinal excitability (CSE, A), short-interval intracortical inhibition (SICI, B) and intracortical facilitation (ICF, C) during consecutive TMS stimuli from a representative participant measured during a 10% MVC. White dots represent the individual (raw) MEP (A) or ratio of conditioned to unconditioned MEPs (B and C), while black dots represent the average of consecutive MEPs or SICI and ICF ratios. Dashed lines represent the 95% confidence interval (CI), which is based on 30 stimuli. For this particular participant, 17, 16 and 17 consecutive stimuli for CSE, SICI and ICF, respectively, were sufficient to enter the 95% CI.

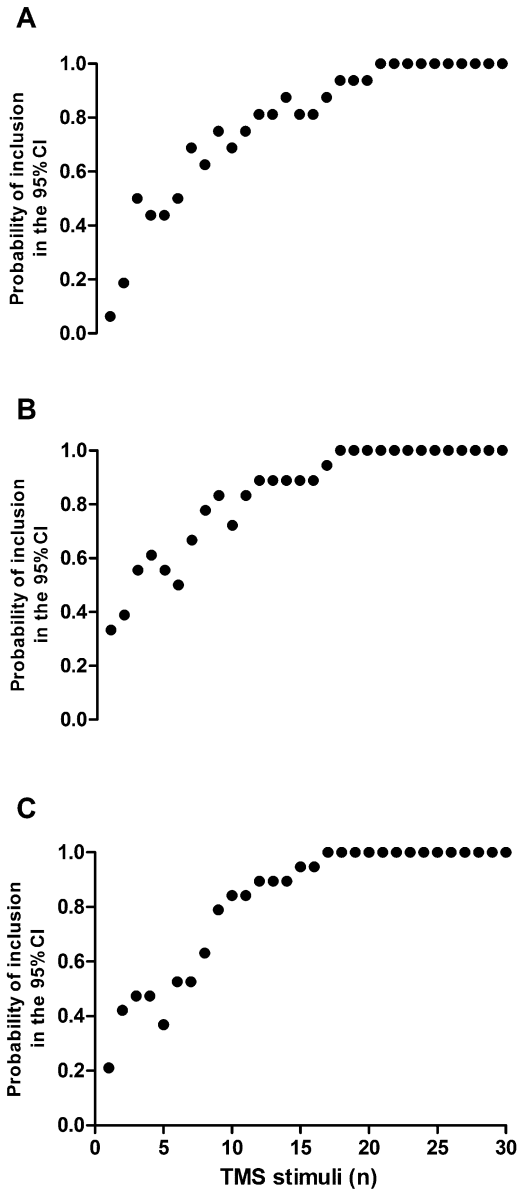


Figure 4-6. Probability that the motor evoked potential (MEP) during single-pulse measures of corticospinal excitability (CSE, A) or the ratio of conditioned to unconditioned MEP during measures of short-interval intracortical inhibition (SICI, B) and intracortical facilitation (ICF, C) for averaged consecutive stimuli and pairs of stimuli will fall within the 95% confidence interval (CI) based on 30 stimuli. 21, 18 and 17 measurements were required to a probability of 1 for inclusion in the 95% CI for CSE, SICI and ICF, respectively (CSE n = 16, SICI n = 18, ICF n = 19).

4-3.5 Supplementary experiment – Comparison of number of measures used in Experiments 1-3 with optimal number derived from Experiment 4

The results from Experiment 4 displayed that the minimum number of measurements required to obtain an accurate estimate of SICI and ICF were 18 and 17, respectively. However, in Experiments 1-3, 12 measurements were used to determine the optimal combination of stimulus variables used to measure SICI and ICF. In order to determine whether using a suboptimal number of measurements in Experiments 1-3 could have had any bearing on the results, the level of uncertainty (assessed using 95% CIs) associated with using 12 and 17 (for ICF) and 18 measurements (for SICI) was determined using random sampling without replacement. This procedure involved taking 12 and 17 (for ICF), and 12 and 18 (for SICI) random conditioned/unconditioned MEP percentages (without replacement) derived from the 30 measurements taken in Experiment 4, and calculating the mean and 95% CIs from each sample. One thousand replicates of 12, 17 and 18 random samples were generated, with the average of the thousand means and upper and lower bound CIs calculated. The width of the 95% CIs were compared between 12 measurements and 17 (for ICF) and 18 measurements (for SICI).

The distribution of mean values derived from 1000 resamples of 12 and 18 measures (for SICI) and 17 measures (for ICF) are displayed in Figure 4-7. Differences in mean and 95% CIs between the number of measures used in Experiments 1-3 and the optimal number derived from Experiment 4 were negligible. For SICI, using 12 measurements produced a mean inhibition of the conditioned MEP of 71%, with 95% CIs spanning 67-75%, while using 18 measurements produced a mean inhibition of the conditioned MEP of 70%, with 95% CIs spanning 67-74%. For ICF, using 12 measures produced a mean facilitation of the conditioned MEP of 125%, with 95%

CIs spanning 115-134%, while using 17 measures produced a mean facilitation of the conditioned MEP of 124%, with 95% CIs spanning 116-132%.

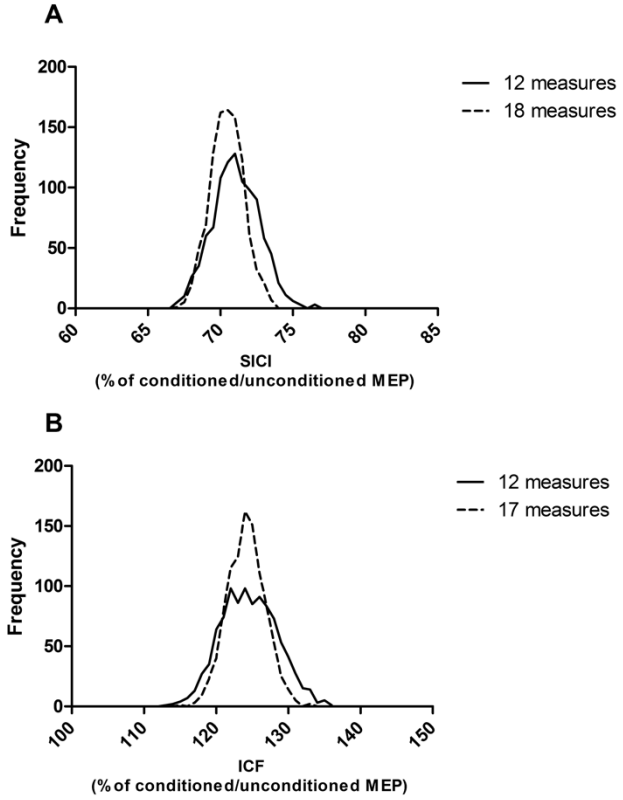


Figure 4-7. Histogram displaying distribution of mean values derived from 1000 resamples of 12 (solid line) and 18 measurements (dashed line) of SICI (A) and of 12 (solid line) and 17 measurements (dashed line) of ICF (B).

4-4 Discussion

The aims of the present study were: 1) to establish the optimal combination of stimulus variables when measuring SICI and ICF in the *rectus femoris* and, 2) to determine the minimum number of stimuli required to obtain an accurate estimation of CSE, SICI and ICF. The study demonstrates that a number of stimulus variables can be used to induce inhibition and facilitation in the evoked responses from *rectus femoris*. For SICI, a CS intensity of 70% AMT, and ISI of 2 ms, with a contraction strength of 5 or 10% MVC induced the highest degree of inhibition, suggesting that these stimulus variables are favourable when assessing SICI in the *rectus femoris*. Intracortical

facilitation was induced using most combinations of stimulus variables, with large inter-subject variability evident across configurations. For accurate estimates of CSE, SICI and ICF, the results indicate that 21, 18 and 17 evoked responses are required, respectively. Given the role of the knee extensors in locomotion and activities of daily living, an increasing number of studies are applying single- and paired-pulse TMS in the knee extensors in response to various acute and chronic interventions (Thomas *et al*., 2017a, Weier *et al*., 2012). As such, the results of the study could inform future investigations of this nature, and provide a standardised approach to the stimulus variables used when taking TMS measures in the active knee extensors in order to facilitate comparisons between studies.

Effect of conditioning stimulus intensity on SICI and ICF

While there was no statistically significant effect of CS intensity on SICI, a CS of 70% AMT induced the highest level of inhibition on average, with 19 out of 20 participants exhibiting inhibition at this intensity with an ISI of 2 ms. Contrasting results exist throughout the literature concerning the influence of CS on SICI, with a range of CS intensities suggested as producing optimal SICI in muscles of both the upper and lower limb. For example, in the active knee extensors, studies have reported that a CS of 90% AMT elicits the greatest degree of SICI (O'Leary *et al*., 2015, Sidhu *et al*., 2013b), corroborating the findings of Ridding *et al*. (1995) in the upper limb muscles. Our findings are in agreement with those of Ortu *et al*. (2008), who similarly reported that a CS of 70% elicited optimal SICI during a 10% MVC in the first dorsal interosseous muscle. While it is unclear why SICI was reduced at CS intensities above 70% AMT, it is possible that higher CS intensities lead to the concurrent recruitment

of both inhibitory and facilitatory interneurons, thereby reducing the magnitude of inhibition even at short ISIs. Indeed, previous work has shown that during a light, voluntary contraction (10% MVC), superimposed recruitment of intracortical facilitatory circuits during paired-pulse TMS at short intervals (1-5 ms) reduces the degree of SICI at specific CS intensities, due to concurrent activation of both inhibitory and facilitatory interneurons (Ortu *et al*., 2008). This facilitatory input, termed short-interval intracortical facilitation (SICF), overlaps in time with SICI, and can be assessed using a CS and test stimulus intensity which are both near AMT (Ziemann *et al*., 1998). By assessing both SICI and SICF during a 10% MVC, Ortu *et al.* (2008) found that a CS of 70% induced optimal SICI in the first dorsal interosseous because this intensity was not strong enough to simultaneously activate intracortical interneurons which mediate SICF. While previous work investigating SICF has shown that facilitation occurs at discrete ISIs (1.1-1.5, 2.3-2.9 and 4.1-4.4 ms) (Hanajima *et al*., 2002, Ziemann *et al*., 1998, Ortu *et al*., 2008), these studies have been conducted exclusively in the upper limb muscles. As such, it is possible that differences in cortical circuitry between upper and lower limbs (Chen *et al*., 1998) could influence the interaction between SICI and SICF, providing a potential mechanistic explanation as to why a CS of 70% AMT induced the greatest degree of inhibition in our study. However, as SICF was not measured in the present study, this interpretation should be viewed with caution. While it is unclear why discrepancies exist in the optimal CS intensity found between studies, methodological differences such as differences in the test-pulse intensity, contraction strength, ISI and the muscle being investigated could all contribute to the observed disparities between studies. Therefore, caution should be aired when attempting to extrapolate the optimal CS intensity for SICI identified in the present study when used in combination with other paired-pulse TMS variables.

Another important finding from Experiment 1 was the substantial inter-subject variability in the optimal CS intensity used when measuring SICI and ICF. Although a CS of 70% AMT with a 2 ms ISI produced the highest level of SICI on average, only 7 out of 20 (35%) participants exhibited optimal SICI using these stimulus variables. Previous work has displayed comparable inter-subject variability in SICI when assessing individual responses to different CS intensities in the upper limb (Ortu *et al*., 2008, Orth *et al*., 2003). Similarly, a high degree of inter-subject variability was found in ICF, with negligible differences in the mean level of facilitation using different stimulus variables. While a CS intensity of 80% AMT produced the highest level of ICF on average, corroborating the findings of previous work (Hunter *et al*., 2016), only 16 out of 20 participants displayed facilitation at this intensity, with a high degree of inter-subject variability found in the level of facilitation induced at this intensity. Although a CS of 60% AMT did not produce the highest level of ICF on average, the inter-subject variability in facilitation at this intensity was low, with ICF elicited in the highest number of subjects when used in combination with an ISI of 10 ms, with 18 out of 20 participants displaying some degree of facilitation, albeit a smaller magnitude. Furthermore, that ICF was induced using this CS intensity in combinations with different contraction strengths and inter-stimulus intervals in Experiments 2 and 3 suggests that, while this intensity might not elicit maximal levels of facilitation, it consistently induces ICF in the vast majority of participants. While these results suggest a high degree of inter-subject variability in the optimal CS intensity to elicit inhibition and facilitation, the differences noted between subjects could be a consequence of the variability inherent in measures of SICI and ICF. Alternatively, it is possible that differences in the electrophysiological properties of inhibitory and

facilitatory interneurons between-subjects might have contributed to the inter-subject variability (Orth *et al*., 2003).

Effect of contraction strength on SICI and ICF

Although it is well established that the magnitude of SICI is reduced during voluntary contraction (Ridding *et al*., 1995, Kujirai *et al*., 1993), it is recommended that assessments of corticospinal and intracortical activity should be conducted with the muscle in an active state when assessing responses in relation to locomotor activity (Gruet *et al*., 2013, Kalmar, 2018), as this is thought to be more reflective of motor cortical behaviour during locomotion (Sidhu *et al*., 2013a). Given the key role of this muscle group in locomotion and athletic activity, the majority of studies using single- and paired-pulse TMS in the knee extensors relate to locomotor activities, such as fatiguing exercise (Thomas *et al*., 2017a), neural adaptations to strength training (Weier *et al*., 2012, Thomas *et al*., 2017b), and the assessment of movement disorders (Cantello, 2002). As such, it was considered that because of the muscle group under investigation, it was more appropriate to assess responses to TMS with the muscle in an active state, and to examine the effects of varying contraction intensities on SICI and ICF. The results displayed that SICI was elicited at contraction strengths of 5%, 10% and 20% MVC, but was progressively reduced with higher contraction strengths (Figure 3). Although a contraction strength of 5 and 10% MVC induced a similar degree of SICI on average (60 ± 19% and 62 ± 20% of unconditioned MEP for 5 and 10% MVC, respectively), a contraction strength of 10% MVC was chosen because it was believed that using this contraction strength is more representative of the recruitment of neural pathways involved in locomotion (where single- and paired-

pulse TMS paradigms are regularly applied when assessing responses in the knee extensors) when compared with a 5% MVC due to the higher level of neural drive required during higher contraction strengths.

Previous work has similarly displayed a progressive reduction in SICI with stronger contraction strengths (Zoghi and Nordstrom, 2007, Ortu *et al*., 2008). The release of inhibition during contraction has been attributed to modulation of corticospinal neurons by GABAergic circuits (Zoghi and Nordstrom, 2007), and concomitant superimposition of facilitation during voluntary contraction (Ortu *et al*., 2008). From a functional perspective, it has been suggested that the reduction in SICI during voluntary contraction represents a transient compensatory down-regulation of inhibitory processes, such that there is a gradual reduction in SICI with increasing contraction strengths in order to preserve cortical output to the target muscle (Vucic *et al*., 2011, Maruyama *et al*., 2006).

Intracortical facilitation was also induced at contraction strengths of 5%, 10% and 20% MVC, with no ICF at 50% MVC. Limited evidence exists on the effect on contraction strength on ICF; however, contrasting evidence has suggested during voluntary contraction, ICF is reduced compared with rest (Ridding *et al*., 1995, Hanajima *et al*., 2002, Kujirai *et al*., 1993), with others reporting an increase in glutamate mediated SICF during contraction compared with rest (Ortu *et al*., 2008). Furthermore, it is unclear why ICF was abolished at 50% MVC. Ortu *et al.* (2008) suggested that at high contraction intensities, a 'busy line' phenomenon might occur, whereby there is too much activity within glumatergic circuits for facilitation to be observed. Alternatively, given that the largest MEPs are commonly evoked during a 50% MVC in the knee extensors (Goodall *et al*., 2014), it is possible that a ceiling

effect exists in MEP amplitude, whereby no increase in the conditioned MEP amplitude can be observed.

While previous authors have advocated taking measures of SICI and ICF with the muscle in an active state in order to better reflect motor cortical behaviour compared with taking measures at rest (Kalmar, 2018, Gruet *et al*., 2013), the limitations associated with taking measurements of paired-pulse TMS in relation to locomotor activities should be acknowledged. Specifically, because SICI and ICF are abolished at higher contraction intensities, the capacity to capture these measures at higher contraction intensities consistent with those used during and following high-intensity locomotor exercise, to which they are commonly applied (Thomas *et al*., 2017b, O'Leary *et al*., 2016, Weier *et al*., 2012), is precluded. These limitations were highlighted in a recent review by Kalmar (2018), who suggested that in an ideal scenario, measures of CSE, and in this case SICI and ICF, would be taken across a range of time points and contraction intensities that reflect the planning or execution phases of motor output that we consider most pertinent to the questions is posed. However, due to the constraints associated with taking such measures, this is of course not possible. Consequently, some degree of ecological validity must be sacrificed in order to ensure measures are taken in a controlled and reproducible environment. As a compromise, taking measures under conditions which more closely replicate the 'real-life' motor task has been advocated (Kalmar, 2018). Despite their limitations, measuring SICI and ICF during light voluntary contractions has previously been shown be responsive to changes in intracortical excitability following locomotor exercise interventions such as fatiguing exercise, acute and chronic strength training interventions involving high force contractions. Taking these considerations into account, we believe that measuring SICI and ICF during a low intensity voluntary

contraction offers a reasonable compromise when attempting to assess changes in response to muscular exercise.

Effect of inter-stimulus interval on SICI and ICF

The level of SICI was influenced by the ISI, with significant inhibition at 2 and 3 ms and no inhibition at 4 and 5 ms. Previous work has found that SICI is most prominent at 1 ms and 2.5 ms ISIs, with inhibition at 1 ms attributed to the refractory period of the interneurons activated by the preceding CS, and inhibition at 2.5 ms mediated by $GABA_A$ interneurons (Fisher *et al*., 2002, Hanajima *et al*., 2003). It is now generally accepted that all SICI occurring at 2-5 ms is a consequence of the activity of GABAergic inhibitory interneurons acting via $GABA_A$ receptors (Vucic *et al*., 2011). While no statistically significant difference in SICI was found between 2 and 3 ms, a 2 ms ISI induced the most inhibition on average, and the highest level of MEP suppression in 12 out of 16 participants. These results are in contrast to Hanajima *et al.* (2003), who found no suppression of late indirect waves (I-waves; descending volleys produced by indirect activation on pyramidal tract neurons), which are normally susceptible to inhibition, in the active first dorsal interosseous at an ISI of 2 ms, while 3-5 ms produced substantial inhibition. Moreover, previous studies investigating responses in the upper-limb have successfully induced SICI at ISIs of 4 and 5 ms (Kujirai *et al*., 1993, Ortu *et al*., 2008, Beck *et al*., 2007). While it is unclear why these discrepancies exist, the disparity between the studies highlight that the optimal stimulus variables for inducing SICI in one muscle group cannot necessarily be generalised across all muscle groups.

Although no significant differences between the level of ICF were found between different ISIs in the present study, we maintained an ISI of 10 ms when assessing ICF in Experiments 4 and 5, because this ISI induced the highest level of facilitation on average and in the greatest number of participants (14 out of 16) in comparison with other stimulus variables. However, even when using these stimulus variables, substantial inter-subject variability existed in the level of facilitation induced (average conditioned/unconditioned MEP percentage: 120 ± 10%, range: 98 to 169%). Furthermore, a high degree of inter-subject variability existed in the ISI which induced the highest level of ICF, with only 4 of 16 participants displaying the highest conditioned/unconditioned MEP percentage at this ISI. The erratic nature of ICF in the present study is in line with previous studies attempting to elicit ICF in the knee extensors (O'Leary *et al*., 2015, Brownstein *et al*., 2018). For example, a recent study from our laboratory attempting to compare intracortical and corticospinal responses between isometric squat and knee extension exercise found that only a limited number of participants exhibited facilitation in the *vastus lateralis* during both exercise modalities (Brownstein *et al*., 2018), and the measure was consequently omitted from the analysis due to the small number of valid cases. Similarly, O'Leary *et al.* (2015) displayed an average percentage of conditioned/unconditioned MEP amplitude below 1.0 in a cohort of 16 participants when assessing the reliability of ICF. While ICF is thought to reflect the excitability of glutamate mediated N-methyl-D-aspartate excitatory interneurons, the lack of facilitation suggests that using a subthreshold CS with an ISI of 10-15 ms fails to activate these interneurons in some participants. Consequently, future studies should exercise caution when attempting to measure and interpret ICF when assessing responses in the knee extensors. A prudent approach when assessing ICF could be to exclude participants who do not exhibit a

conditioned/unconditioned MEP percentage > 100% from the analysis, and to only proceed with the analysis if a sufficient number of participants exhibit facilitation.

Assessment of the minimum number of measurements required to obtain an accurate estimation of CSE, SICI and ICF

The number of measurements required to obtain an accurate estimate of CSE, SICI and ICF, i.e. the number of measurements required to fall within the 95% CI, was 21, 18 and 17, respectively. Responses to single- and paired-pulse TMS are inherently variable, with a high degree of pulse-to-pulse fluctuation in the MEP amplitude. As such, it is important to understand the optimal number of pulses required to obtain a 'true' estimate of CSE, SICI and ICF in order to maximise the reliability of these measurements. A number of recent studies have similarly assessed the minimum number of pulses required to obtain an accurate estimate of CSE; Bashir *et al*. (2017) and Cuypers *et al*. (2014) reported that a minimum of 30 stimuli were required, while Chang *et al*. (2016) reported that at least 20 and 25 pulses were required to obtain an accurate estimate of SICI and ICF, respectively. However, all of these studies measured responses in the resting first dorsal interosseous, while the present study was conducted in the active knee extensors. Given that it has previously been shown the variability of MEPs are reduced when measurements are taken during muscle contraction (Darling *et al*., 2006), this likely explains the lower number of pulses required to fall within the 95% CI in comparison with previous work (Cuypers *et al*., 2014, Chang *et al*., 2016, Bashir *et al*., 2017). In the majority of studies assessing responses in the knee extensor musculature, 10-15 measurements are arbitrarily applied when assessing CSE, SICI and/or ICF (O'Leary *et al*., 2016, Weier *et al*., 2012,

Thomas *et al*., 2017b). Based on the results from the present study, using 10-15 pulses would reduce the probability of the value for averaged consecutive measurements falling within the 95% CI based on 30 stimuli for CSE (0.60-0.75), SICI (0.65-0.90) and ICF (0.80-0.90). As such, the degree of error in the estimate of CSE, SICI and ICF is reduced considerably when using the number of stimuli commonly employed when measuring responses in the knee extensors (O'Leary *et al*., 2016, Thomas *et al*., 2017b, Weier *et al*., 2012). Thus, the information provided from this study on the optimal number of pulses required during single- and paired-pulse TMS measurement provides important practical information when assessing responses in the active knee extensors.

Limitations

While the present study provides important methodological information which can be used to guide future investigations employing single- and paired-pulse TMS in the knee extensors, the study is not without its limitations. Specifically, in Experiments 1-3, 12 measurements were used to assess the effect of each combination of stimulus variables on SICI and ICF. However, in Experiment 4, it was determined that 18 and 17 measurements were required to ensure 100% probability of falling within the 95% CI based on 30 measurements for SICI and ICF, respectively. Consequently, the number of stimuli used in Experiments 1-3 was below the minimum required to ensure the SICI or ICF value fell within the 95% CI for all participants. However, had the sequence of the experiments been such that Experiment 4 was conducted before Experiment 1, the optimal configuration used to assess SICI and ICF would not yet have been determined. As such, it is possible that performing the experiments in this sequence would have resulted in using a different set of stimulus variables for

measurements of SICI and ICF then would subsequently be determined in the next three experiments. In turn, using different stimulus variables could have influenced the variability in responses to paired-pulse TMS if a different population of inhibitory or facilitatory interneurons were activated, potentially invalidating the results of the experiment. To account for this limitation, we performed statistical resampling in order to establish the uncertainty (measured through 95% CIs) associated with using 12 measurements (i.e. the number used in Experiments 1-3) to quantify the level of SICI and ICF, compared with the level of uncertainty associated with using the 'optimal' number of measurements derived from Experiment 4, i.e. 18 for SICI and 17 for ICF. The results displayed that differences between the mean values and 95% CIs derived from using 12 measurements compared with the 'optimal' number were negligible. Specifically, 95% CIs were 1 and 3% wider when using 12 measurements compared with using 18 and 17 for SICI and ICF, respectively, suggesting that it is unlikely that using a suboptimal number of measurements in Experiments 1-3 had bearing on the results of the study.

Conclusion

The present study demonstrates that a number of stimulus variables can be used to assess short-interval intracortical inhibition and intracortical facilitation in the active *rectus femoris*. For measurements of short-interval intracortical inhibition, a CS of 70% AMT with an inter-stimulus interval of 2 ms during a contraction (5 or 10% maximum voluntary contraction) was the optimal combination of stimulus variables to elicit maximum inhibition. For intracortical facilitation, there appeared to be no optimal combination of stimulus variables to maximise facilitation, with low levels of

facilitation induced using most stimulus variables, and large inter-subject variability evident across all combinations of stimulus variables. A minimum of 21, 18 and 17 measurements were required to obtain an accurate estimate of CSE, short-interval intracortical inhibition and intracortical facilitation, respectively. The overall aim of this thesis is to examine the aetiology and recovery of impairments in neuromuscular function following intermittent sprint exercise. The application of single- and paired-pulse TMS paradigms has the potential to provide insight into the role of corticospinal and intracortical excitability in post-exercise fatigue and recovery. As such, this chapter informs the methodological approach in Chapter 6 of this thesis, which examines the aetiology and recovery of impaired neuromuscular function in the days following competitive football match-play.

CHAPTER 5 – RELIABILITY OF NEUROMUSCULAR, PHYSICAL FUNCTION, AND PERCEPTUAL ASSESSMENT

5-1 Introduction

The number of studies applying neurostimulation techniques in the study of fatigue during and following locomotor exercise has increased substantially in recent years (Sidhu *et al*., 2017; Goodall *et al*., 2017b; Hureau *et al*., 2016a). The application of electrical and magnetic stimulation permits the assessment of neuromuscular function at both the central and peripheral level. Specifically, peripheral contributors to the force loss observed after exercise can be assessed by measuring involuntary evoked responses to electrical stimulation at rest. Additionally, central adjustments can be examined through evoked responses to electrical and magnetic stimulation during voluntary contractions to assess voluntary activation, and provide greater insight into the neuromuscular determinants of impaired muscle function during and following exercise. These techniques have advanced understanding of the aetiology of impairments in neuromuscular function, and thereby the biological basis of fatigue, during a range of locomotor exercise tasks (Goodall *et al*., 2015; Thomas *et al*., 2017b; Sidhu *et al*., 2009b).

The application of single- and paired-pulse TMS can provide further insight into exercise-induced perturbations in neuromuscular function. In Chapter 4, the optimal combination of stimulus variables and the number of measurements required to accurately quantify SICI, ICF and CSE were determined. Another important consideration when taking measures using single- and paired-pulse TMS is the sensitivity of these measures in detecting neurophysiological changes. This is of particular relevance when considering that studies frequently assess changes in measures of neuromuscular function in response to interventions both within- and between-day (Weier *et al*., 2012; O'Leary *et al*., 2016). Although two recent studies showed good reliability of measures of single- and paired-pulse TMS in the active

knee extensors (O'Leary et al., 2015, Temesi et al., 2017), both of these studies showed that the configuration used when employing paired-pulse TMS influences the reliability of SICI and/or ICF. Insight into the within- and between-day reliability of single- and paired-pulse TMS can allow inferences to be made on the sensitivity of these measures in detecting changes in intracortical and corticospinal activity.

In addition to taking measures of neuromuscular function during and immediately following exercise, a number of studies have sought to determine the aetiology of impaired neuromuscular function and the time-course of recovery in the days post-exercise (Thomas *et al*., 2017a; Rampinini *et al*., 2011; Pointon and Duffield, 2012). These neuromuscular assessments are often accompanied by a range of measures of physical function, such as jump height and/or sprint speed, and perceptual responses relating to fatigue, muscle soreness, and/or readiness to train (Thomas *et al*., 2017a; Rampinini *et al*., 2011). Given that the assessment of neuromuscular and physical function involves performing actions at maximal intensity, it is feasible that such activities could themselves elicit adjustments in neuromuscular function that could confound the results from studies measuring fatigue and recovery on consecutive days pre- and post-exercise. As the subsequent chapters in this thesis will examine recovery of neuromuscular function in the days following competitive football match-play, quantifying the reliability of the measures used in the testing battery is integral in order to understand the sensitivity of these measures in detecting change.

The aim of the present study was twofold: 1) to determine the within- and between-day reliability of measures of CSE, SICI, and ICF using the stimulus variables and number of measurements optimised in the previous chapter and, 2) to determine the within- and between-day reliability of the assessments of neuromuscular, physical

function, and perceptual responses which will be used in the subsequent chapter of this thesis.

5-2 Methods

5-2.1 Participants

Twenty male adult participants took part in Experiment 1 (aged: 24 ± 4 years; stature: 1.83 ± 0.06 m; mass: 81 ± 10 kg), and 10 male adult participants took part in Experiment 2 (age: 22 ± 2 years; stature: 1.81 ± 0.07 m; body mass: 79 ± 9 kg). All participants gave written informed consent to participate in the study. Participants were free of any cardiorespiratory, neurological or neuromuscular health disorders, had no metal plates in the head/brain, and were not taking any medication that might have interfered with the nervous system. All participants completed a TMS safety screening questionnaire prior to the data collection procedure (Keel *et al*., 2001). For Experiment 2, all participants regularly participated in intermittent sprint sports such as football, rugby or basketball.

5-2.2 Experimental procedures

Experiment 1 – Within-day and between-day reliability of CSE, SICI and ICF

Experiment 1 assessed the within-day and between-day reliability of CSE, SICI and ICF using the optimal stimulus variables obtained from Chapter 4 (CS of 70% AMT, ISI of 2 ms, and contraction strength of 10% MVC for SICI, CS of 60% AMT, ISI of 10 ms, and contraction strength of 10% MVC for ICF). The instrumentation used (Force, EMG and TMS) replicated that which is described in Chapter 4. Twenty conditioned and 20 unconditioned pulses were delivered in sets of 6 to determine SICI

and ICF separately, with 20 single pulses delivered in sets of 5 for CSE separate from the assessment of SICI and ICF (total of 60 single-pulses and 40 paired-pulses across all conditions). For within-day reliability, participants visited the laboratory on two occasions in the morning and afternoon, separated by 4 h (e.g. 0900 and 1300). For between-day reliability, participants visited the laboratory on one further occasion at the same time of day as their previous morning session. In order to account for any within- or between-day fluctuations in peripheral muscle excitability, femoral nerve stimulation was administered at the beginning of each visit in order to assess M_{max}. In order to ensure consistent placement of electrodes during each visit in Experiment 5, electrodes were marked with indelible ink during each trial.

Experiment 2 – Reliability of neuromuscular, physical function, and perceptual assessments

Experiment 2 was conducted in order to determine the reliability of the battery of neuromuscular, physical and perceptual tests employed in Chapter 6 of this thesis, when measures were taken pre- and post-football match-play, and on the following three consecutive days. Participants attended a familiarisation visit for habituation with the experimental procedures prior to the main experimental trials. For the experimental trial, participants were required to attend the laboratory five times on four consecutive days, separated by 24 h. The experimental trials consisted of baseline, 2, 24, 48 and 72 h visits. The experimental procedures precisely replicated that of Chapter 6 of this thesis, apart from participation in a competitive football match. Specifically, the neuromuscular assessment consisted of VA with motor nerve stimulation and VA_{TMS} (described in Chapter 3), $Q_{tw,pot}$ and twitch characteristics

(MRFD, CT, MRR and $RT_{0.5}$; described in Chapter 3). Single-pulse TMS was used to assess CSE using a recruitment curve, while paired-pulse TMS was used to measure SICI (described in Chapter 6). Measures of physical function included CMJ, DJ-RSI, and linear speed (20 m sprint with 10 m splits; described in Chapter 3).

5-2.3 Data analysis

In Experiment 1, CSE was assessed by averaging single MEP amplitudes across 20 pulses and normalizing the value relative to the M_{max}. Additionally, to investigate the influence of the number of measurements taken for the within- and between-day reliability of CSE, SICI and ICF, subsets of 5, 10, 12 and 15 stimuli (for CSE) or pairs of conditioned/unconditioned stimuli (for SICI and ICF) were calculated.

For Experiment 2, a 9-point regression between SIT amplitude and contraction intensity was extrapolated to the *y*-intercept to obtain an ERT as described in Chapter 3 (ERT, Todd *et al*. 2003). The regression analysis confirmed a linear relationship between contraction strength and SIT evoked by TMS (r^2 range = 0.91 ± 0.06 to 0.93 ± 0.08). Recruitment curves were constructed by plotting the TMS stimulation intensity relative to AMT against the MEP amplitude averaged from the five stimulations at each intensity, expressed relative to M_{max}. The ratio of the MEP amplitude to the maximum M-wave was used as an index of CSE. In order to provide a summary measure of CSE, the summated area under the recruitment curve (AURC) was calculated for each participant at each time point using the trapezoid integration method, which has been shown to have excellent reliability (Carson *et al*., 2013). The root mean square EMG amplitude (RMS_{EMG}) and average force were calculated in the 80 ms prior to each TMS to ensure a similar level of background muscle activity was present during the recruitment curve and SICI measurements.

5-2.4 Statistical analysis

Data are presented as mean ± SD. Normality of the data was assessed using the Shapiro-Wilks test. Assumptions of sphericity were explored and controlled for all variables using the Greenhouse-Geisser adjustment, where necessary. For Experiment 1, a one-way repeated measures ANOVA was performed on all TMS variables (CSE, SICI and ICF) to assess for any within- or between-day differences using 20, 15, 12, 10 and 5 responses. Relative reliability of all TMS measures was assessed using intraclass correlation coefficient ($ICC_{3,1}$), while absolute reliability was assessed using typical error (TE) expressed in raw units (Hopkins, 2000), and variability assessed through coefficient of variation (CV) determined using the formula: standard deviation/mean × 100. As per the guidelines recommended by Koo and Li (2016), ICCs between 0.5 and 0.75 were considered moderately reliable, values between 0.75 and 0.9 were considered of good reliability, and values above 0.9 considered of excellent reliability.

For Experiment 2, a repeated measures ANOVA was used to assess changes in each outcome measure over time to determine whether there was a fatiguing effect associated with repeated performance of the neuromuscular and physical function measures. In the event of a significant main effect, Dunnett's multiple comparison procedure was employed with the pre-trial score used as the control category. Relative reliability of neuromuscular and physical function measures was assessed using intraclass correlation coefficient ($ICC_{3,1}$), while absolute reliability was assessed using typical error (TE) expressed in raw units (Hopkins, 2000), and variability assessed through CV determined using the formula: standard deviation/mean × 100. This

analysis was performed for within-day (i.e. baseline and 2 h post-baseline) and between-day measurements (i.e. baseline, 24, 48 and 72 h post-baseline).

5-3 Results

5-3.1 Experiment 1 – Within-day and between-day reliability of CSE, SICI and ICF

The individual within- and between-day data points for single- and paired-pulse variables are displayed in Figure 5-1, within- and between-day reliability of CSE, SICI and ICF can be viewed in Table 5-1. There were no within- or between-day differences for any of the TMS measures using 5, 10, 12, 15 or 20 measurements (AMT, CSE, SICI or ICF) ($P > 0.05$). Based on 20 MEPs (CSE) or pairs of conditioned/unconditioned MEPs (SICI and ICF), within-day measures of SICI and ICF were good (ICC ≥ 0.77), while within-day measures of CSE and AMT were excellent (ICC ≥ 0.91). Between-day reliability analysis showed moderate reliability for ICF and SICI (ICC ≥ 0.61). Measures of CSE displayed good reliability (ICC = 0.87), while AMT demonstrated excellent reliability (ICC = 0.99). When comparing the reliability of CSE, SICI and ICF when taking 5, 10, 12, 15 and 20 measures, the ICCs were higher and the CVs lower the more measurements were taken (Table 5-1). For CSE, ICC values were excellent when using 10 or more stimuli for within-day measurements (≥ 0.90), and were good when using 5 or more stimuli for between-day measurements (≥ 0.87). For within-day measurements of SICI, reliability was good when using 5 or more measurements (≥ 0.78), while a minimum of 10 measurements were required to obtain moderate reliability between-days (≥ 0.59). For ICF, a minimum of 15 measurements were required to obtain moderate reliability both within- (ICC ≥ 0.71) and between-days (ICC ≥ 0.70).

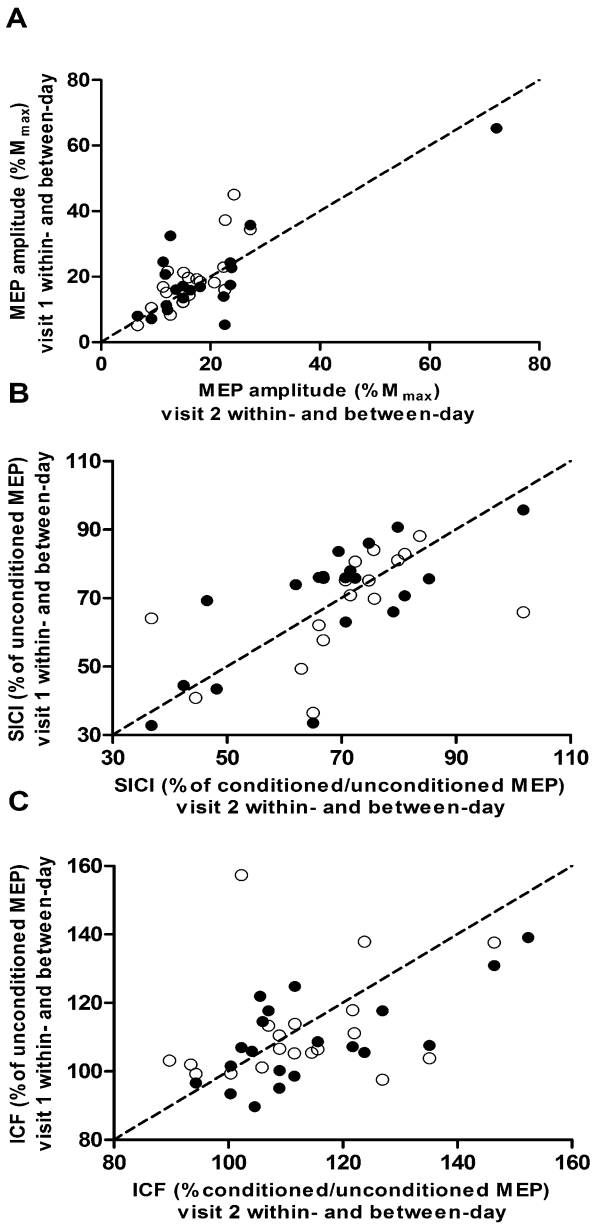


Figure 5-1. Individual data points for within- and between-day measures of corticospinal excitability (CSE, A), short-interval intracortical inhibition (SICI, B) and intracortical facilitation (ICF, C) measured during a 10% MVC. White dot represents between-day measurements, while black dots represent within-day measurements. The dashed lines represent lines of agreement (n = 20).

Table 5-1. Intraclass correlation coefficients, typical error expressed in raw units (CSE: % of M_{max}, SICI and ICF: % of unconditioned MEP), and coefficient of variation (%) for within- and between-day measures of single- and paired-pulse transcranial magnetic stimulation (n = 20).

	20 measurements			15 measurements			12 measurements			10 measurements			5 measurements		
Within-day															
	ICC	TE	CV	ICC	TE	CV	ICC	TE	CV	ICC	TE	CV	ICC	TE	CV
CSE	0.91	6	17.9	0.90	6	20.3	0.87	6	22.8	0.90	6	21.3	0.87	6	24.8
SICI	0.84	9	10.9	0.84	9	11.3	0.78	11	12.7	0.78	11	12.9	0.80	11	12.1
ICF	0.77	15	6.9	0.71	13	7.1	0.80	10	7.3	0.36	17	9.6	0.30	30	14.2
Between-day															
CSE	0.87	5	18.3	0.84	5	18.0	0.77	5	17.0	0.78	6	19.6	0.77	7	20.2
SICI	0.74	11	10.6	0.70	10	13.1	0.68	11	13.3	0.59	12	14.3	0.23	17	21.1
ICF	0.61	15	8.2	0.70	13	7.8	0.78	15	7.8	0.67	17	8.0	0.11	30	15.1

ICC = intraclass correlation coefficient, TE = typical error, CV = coefficient of variation, CSE = corticospinal excitability, SICI = short-interval intracortical inhibition, ICF = intracortical facilitation

5-3.2 Experiment 2 – Reliability of neuromuscular, physical function, and perceptual assessments

Perceptual responses

Perceptual responses from the Elite Performance Readiness Questionnaire can be viewed in Table 5-2. No main effects of time were found for any of the perceptual variables ($F_{4,32} \geq 1.203$, $P \geq 0.33$).

Table 5-2. Perceptual responses measured via visual analogue scales (mm) at baseline, 2, 24, 48, and 72 h post-baseline ($n = 10$).

	Baseline	2 h	24 h	48 h	72 h
Fatigue	13.9 ± 9.1	17.0 ± 8.2	20.0 ± 19.4	22.0 ± 16.7	17.6 ± 9.9
Soreness	15.9 ± 15.3	17.4 ± 17.4	20.6 ± 16.3	23.8 ± 15.0	25.8 ± 24.4
Motivated to train	63.6 ± 20.4	58.4 ± 20.2	62.9 ± 20.4	56.7 ± 26.1	58.1 ± 21.9
Anger	4.9 ± 4.1	4.9 ± 3.9	7.0 ± 7.4	4.3 ± 3.5	5.0 ± 4.7
Confusion	5.4 ± 4.4	5.6 ± 4.2	5.9 ± 4.1	4.3 ± 4.0	6.7 ± 6.1
Depression	5.8 ± 5.4	5.4 ± 4.8	6.8 ± 4.5	5.2 ± 5.1	6.8 ± 6.6
Tension	8.8 ± 6.1	12.0 ± 12.9	11.4 ± 8.1	12.2 ± 12.3	9.6 ± 7.9
Alertness	59.1 ± 21.1	58.1 ± 23.4	47.0 ± 27.1	68.2 ± 21.8	59.7 ± 28.0
Confidence	62.8 ± 21.6	56.7 ± 24.7	63.1 ± 16.0	70.2 ± 20.5	69.4 ± 17.5
Sleep	67.3 ± 16.6	N/A	62.3 ± 25.9	69.0 ± 18.7	59.1 ± 23.8

Neuromuscular responses

The within- and between-day reliability of neuromuscular and physical function variables is displayed in Table 5-2. Force derived variables, including MVC, VA measured with motor nerve stimulation, $Q_{tw,pot}$, MRFD, MRR, CT, $RT_{0.5}$, displayed

moderate to excellent reliability within-day (ICC ≥ 0.60), and moderate to excellent reliability between day (ICC ≥ 0.62) for every variable apart from MRR (ICC = 0.30). No main effects of time were found for MVC ($F_{4,32}$ = 0.217, P = 0.93; Figure 5-2A), VA measured with motor nerve stimulation ($F_{4,32}$ = 0.567, P = 0.69; Figure 5-2B), or VA_{TMS} ($F_{4,32}$ = 0.108, P = 0.98; Figure 5-2C). A main effect of time on $Q_{tw,pot}$ was found ($F_{4,32}$ = 2.938, P < 0.05; Figure 5-2D), however *post-hoc* analysis revealed no significant differences relative to baseline (P ≥ 0.14).

Table 5-3. Intraclass correlation coefficients, typical error expressed as raw units, and coefficient of variation (%) for within- and between-day measures of neuromuscular and physical function (n = 10).

	Within-day			Between-day		
	ICC	TE	CV	ICC	TE	CV
Force derived variables						
MVC	0.98	22.5	2.6	0.98	20.6	3.4
Motor nerve VA	0.86	1.8	1.8	0.88	1.8	2.0
$Q_{tw,pot}$	0.79	13.2	8.8	0.86	10.1	7.8
MRFD	0.85	640.4	9.4	0.87	605.4	13.4
MRR	0.60	349.0	12.5	0.30	830.3	24.4
CT	0.75	5.6	4.1	0.62	6.0	5.1
$RT_{0.5}$	0.94	3.55	12.5	0.89	4.8	21.7
EMG derived variables						
VA_{TMS}	0.79	1.5	1.4	0.85	1.9	2.4
AURC	-	-	-	0.71	266.3	19.8
SICI	-	-	-	0.84	10	21.7
Physical function variables						
CMJ height	0.99	1.1	2.4	0.99	1.1	3.2
DJ-RSI	0.96	8.7	5.0	0.93	12.6	9.9
10 m sprint	0.95	0.0	1.7	0.95	0.0	1.7
20 m sprint	0.75	0.1	1.7	0.57	0.1	1.7

ICC = Intraclass correlation coefficient, TE = typical error, CV = coefficient of variation, MVC = maximal voluntary contraction, VA = voluntary activation, $Q_{tw,pot}$ = potentiated twitch force, MRFD = maximum rate of force development, MRR = maximum rate of relaxation, CT = contraction time, $RT_{0.5}$ = half relaxation time, EMG = electromyography, VA_{TMS} = voluntary activation measured with transcranial magnetic stimulation, AURC = area under recruitment curve, SICI = short interval intracortical inhibition, CMJ = countermovement jump, DJ-RSI = drop jump reactive strength index

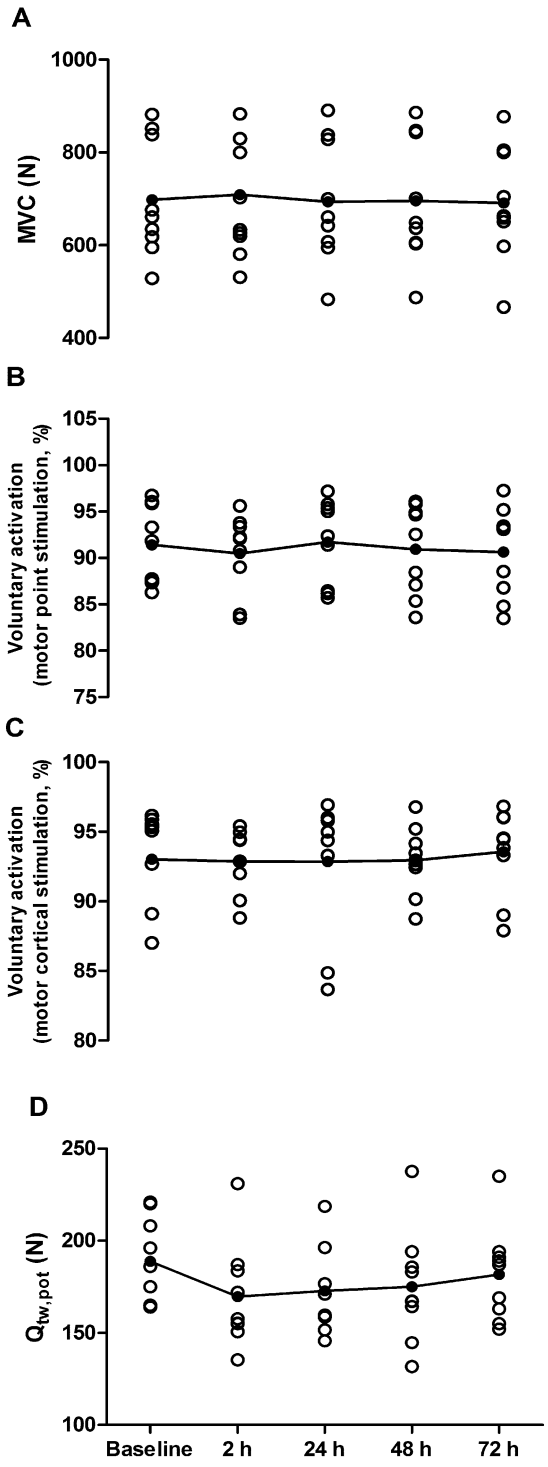
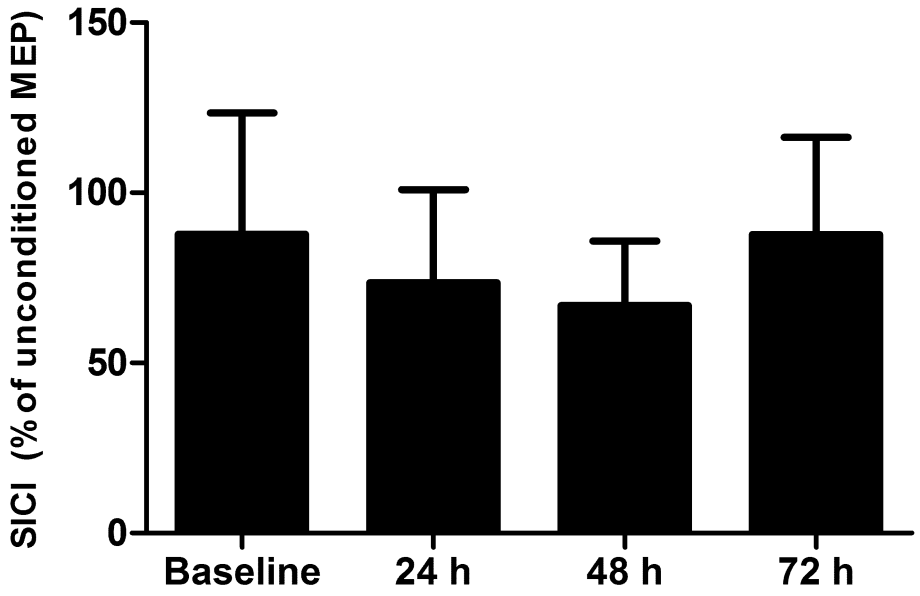


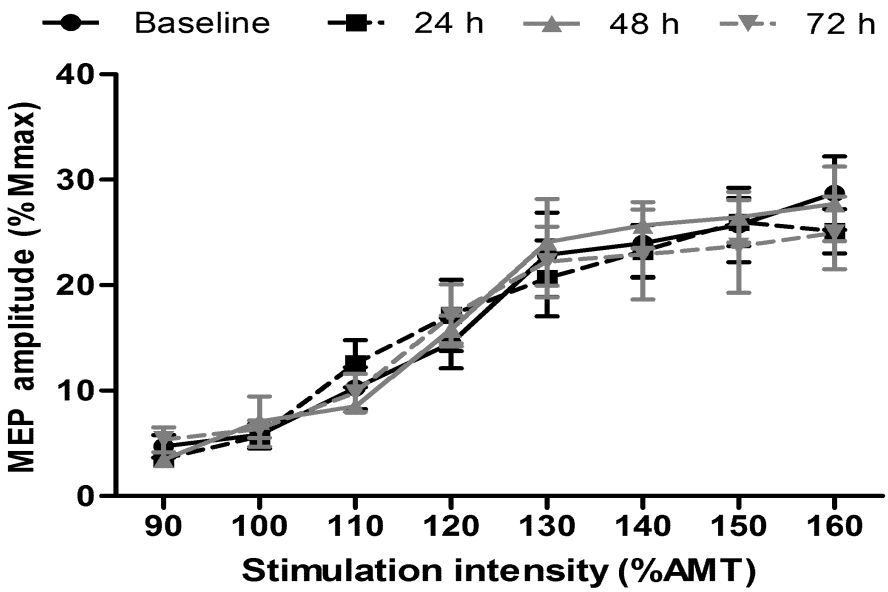
Figure 5-2. Maximal voluntary contraction force (MVC, A), voluntary activation measured with femoral nerve stimulation (B), voluntary activation measured using motor cortical stimulation (C), and quadriceps potentiated twitch force (Qtw,pot,D) measured at baseline, 2, 24, 48 and 72 h post-baseline (n = 10). Individual responses are plotted, with lines representing the mean scores.

Central nervous system excitability and inhibition

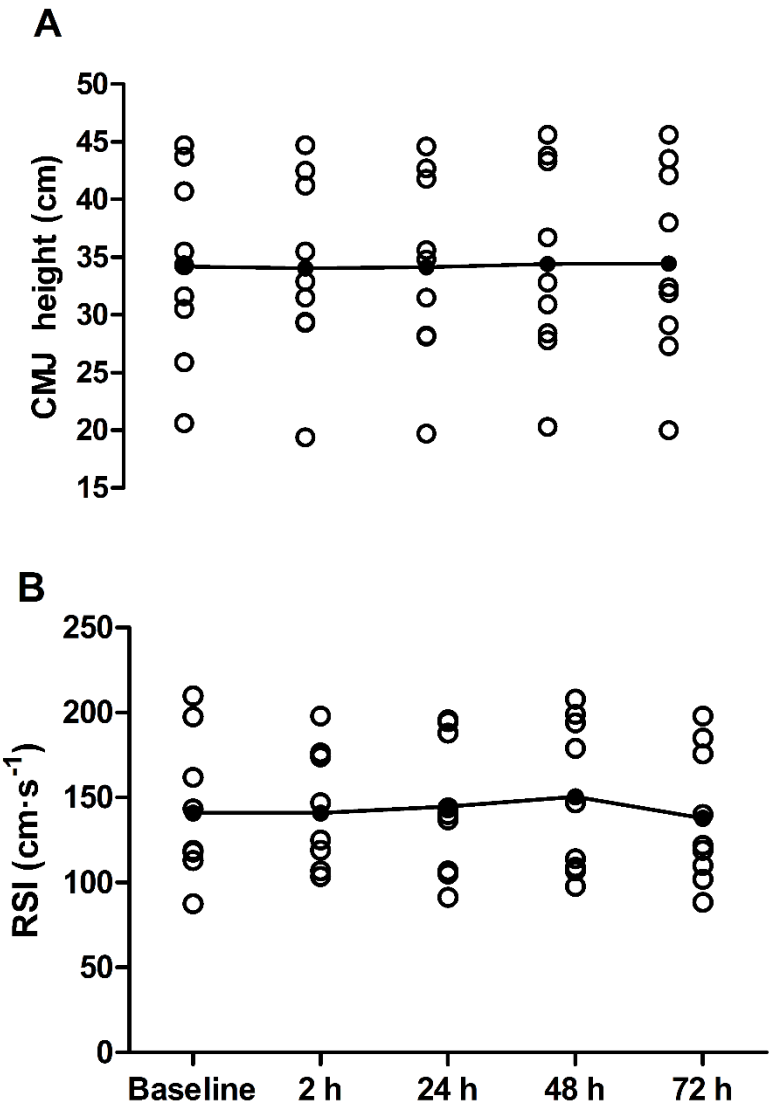
There were no differences in AMT at any time point ($P \geq 0.61$). No main effect of time on short interval intracortical inhibition ($F_{3,32} \geq 1.988$, $P = 0.14$; Figure 5-3) or AURC were found ($F_{3,32} \geq 0.645$, $P = 0.59$; Figure 3-4).



Physical function



Physical function variables displayed good to excellent reliability within-day (ICC ≥ 0.75) and moderate to excellent reliability between-day (ICC ≥ 0.57; Table 5-4). No main effects of time were found for CMJ height ($F_{4,32}$ = 0.223, P = 0.92), DJ-RSI ($F_{4,32}$ = 0.704, P = 0.59), 10 m ($F_{4,32}$ = 1.674, P = 0.18) or 20 m sprint time ($F_{4,32}$ = 2.094, P = 0.11).



5-4 Discussion

The aims of the present study were: 1) to determine the within- and between-day reliability of measures of CSE, SICI, and ICF using the stimulus variables and number of measurements optimised in Chapter 4 and, 2) to determine the within- and between-day reliability of the assessment of neuromuscular, physical function, and perceptual responses which will be used in Chapter 6 of this thesis. Experiment 1 of the study demonstrated that measures of CSE, SICI and ICF can be measured reliably both within- and between-days when measuring responses in the *rectus femoris*. Experiment 2 demonstrated that measures of neuromuscular and physical function and perceptual responses display low variability and good reliability when measurements are taken on four consecutive days. Thus, the testing battery employed in Experiment 2, which involved performing maximal intensity isometric and explosive contractions, does not appear to elicit fatigue or impairments in neuromuscular or physical function which could confound the results from studies measuring recovery on consecutive days pre- and post-exercise. The results from the present study provide valuable information on the reliability of a battery of neuromuscular, physical function and perceptual tests similar to that employed in previous studies (Thomas *et al*., 2017a; Thomas *et al.*, 2018; Rampinini *et al*., 2011; Pointon *et al*., 2012), and replicating that of the subsequent chapter of this thesis.

Within-day and between-day reliability of single- and paired-pulse TMS

Using the optimal number of measurements established in Chapter 4, reliability analyses revealed that CSE, SICI and ICF can be measured with moderate-to-excellent relative reliability both within- and between-days. CSE was highly reproducible both

within- and between-days, corroborating findings from previous studies in the active *rectus femoris* (Temesi *et al*., 2017). The level of within- and between-day reliability of CSE was slightly higher than reported by O'Leary *et al*. (2015) (ICC = 0.85 and 0.82, respectively). However, their study investigated responses in the *vastus lateralis*, and was based on averaged responses from 10 measurements rather than the 20 used in the present study, possibly contributing to the differences in ICCs. Despite the high reproducibility of CSE in the present study, there was also a higher degree of variability for within- and between-day measurements when compared with SICI and ICF measurements, which should be taken into account when taking multiple measures of CSE throughout an intervention. Based on 20 measurements, both SICI and ICF displayed good reliability within-day, and moderate reliability between-days, similar to previous findings in the *vastus lateralis* (O'Leary *et al*., 2015). Furthermore, the excellent reliability of MVC and M_{max} suggest that the variability in CSE, SICI or ICF was not a result of changes in contraction strength or neuromuscular transmission.

While Chapter 4 identified the optimal number of measurements as 21, 18 and 17 when assessing CSE, SICI and ICF, respectively, many studies require responses to single- and paired-pulse TMS to be captured in a more timely fashion. For example, several studies have measured CSE and SICI during and following exercise interventions in order to assess fatigue-induced alterations in corticospinal or intracortical activity (Thomas *et al*., 2017a, Sidhu *et al*., 2013b). As such, it is often impractical to employ a prolonged testing battery during which intervention-induced changes in CNS activity could dissipate, and using a lower number of stimuli might be more appropriate in order to reduce the time required for assessment. In these circumstances, it is important to understand the reliability and sensitivity of single- and paired-pulse TMS in detecting changes when a suboptimal number of stimuli have

been used. In general, using a higher number of measurements resulted in greater relative and absolute reliability and lower variability, particularly for between-day measurements. Despite this, the reliability and variability for measurements of CSE and SICI were not markedly impaired between 20 and 5 measurements when assessed within-day. In contrast, SICI and ICF displayed a substantial drop in between-day reliability and increase in variability when taking under 15 measurements. Based on these results, we suggest that taking 20 measurements of CSE, SICI and ICF will improve the accuracy and reliability of results both within- and between-days.

Within- and between-day reliability of neuromuscular, physical function and perceptual responses

In order to substantiate the neuromuscular, physical function, and perceptual responses to competitive football match-play, it is integral to determine the magnitude of change against the reliability of the measurements. The present study employed measures of absolute and relative reliability to provide a comprehensive assessment of the repeatability and variability of the measures to be used in the subsequent chapter of this thesis. Measures of neuromuscular function displayed moderate to excellent reliability when measured within-day separated by 2 h, with ICCs ranging from 0.60 to 0.98, with CVs ranging between 1.2 and 12.0%. Similarly, for between-day measures of neuromuscular function measured on four consecutive days (i.e. baseline, 24, 48 and 72 h post-baseline), reliability was moderate to excellent for all measures apart from MRR (ICC 0.30, CV 24.4%), with all other ICCs ranging between 0.61 and 0.98, and CVs ranging between 2.0 and 21.7%. Physical function and EMG derived measures displayed good to excellent reliability within- and between-days, with

negligible variability. While previous work has reported similar levels of reliability of measures of neuromuscular (Thomas *et al*., 2015; 2016) and physical function variables (Rampinini *et al*., 2011), it is important to understand the reliability of these measures when taken on consecutive days to determine whether performing maximal intensity isometric contractions and explosive movements involving the stretch-shortening cycle elicits fatigue. The lack of change in any of the variables when measured on consecutive days, concurrent with the moderate to excellent level of reliability of measures of neuromuscular and physical function, demonstrate the robust nature of these measures and suggest that any confounding influence of testing-induced fatigue in intervention studies of this nature is negligible.

Conclusion

The present study demonstrates that measures of single- and paired-pulse TMS can be measured reliably both within- and between-days, and that performing a testing battery consisting of neuromuscular, physical function and perceptual assessments on consecutive days does not elicit change any of the variables assessed. Given that subsequent chapters of this thesis will implement these measures on consecutive days in order to examine the neuromuscular response to competitive football match-play, the results of this chapter provide important information in the overall context of the thesis.

CHAPTER 6 AETIOLOGY AND RECOVERY OF IMPAIRMENTS IN NEUROMUSCULAR FUNCTION FOLLOWING COMPETITIVE FOOTBALL MATCH-PLAY

Publication arising as a result of this chapter:

BROWNSTEIN, C. G., DENT, J. P., PARKER, P., HICKS, K. M., HOWATSON, H., GOODALL, S., THOMAS, K. 2017. Etiology and recovery of neuromuscular fatigue following competitive soccer match-play. *Frontiers in Physiology*, 8, 831

6-2 Introduction

Association football (soccer) is characterised by intermittent bouts of high-intensity activity interspersed with periods of low-to-moderate-intensity exercise (Mohr *et al*., 2003). During competitive match-play, players perform a diverse range of physically demanding actions, such as sprinting, jumping, accelerating, decelerating and changing direction, imposing significant disturbances on multiple physiological systems (de Hoyo *et al*., 2016). An inevitable consequence of these physical demands is fatigue, a sensation of tiredness and weakness during and following exercise, which is underpinned and/or modulated by a myriad of physiological and psychological processes. During match-play, fatigue manifests through transient reductions in work rate following the most demanding periods of a match and cumulative declines in work-rate during the latter stages of a match (Rampinini et al., 2011, Mohr et al., 2003). The fatigue induced by football match-play also persists post-exercise, and can take days to resolve (Nedelec *et al*., 2012). This notwithstanding, the competitive schedule in modern day football is such that teams are frequently required to play multiple games with short recovery periods between successive matches. As such, understanding the etiology of fatigue can provide important information for research and practice concerning recovery interventions aimed at alleviating fatigue and accelerating recovery.

The contributors to the fatigue experienced after football match-play have been previously related to energy depletion (Ekblom, 1986), perturbations to peripheral homeostasis (Ispirlidis *et al*., 2008), and damage to muscle tissue (Nédélec *et al*., 2012), which manifest in reductions in the force generating capacity of the quadriceps muscles (Thomas *et al*., 2017a). Reductions in MVC strength and performance during field tests of physical function (e.g. vertical jump and sprint tests) in response to

match-play have been studied extensively (Nédélec *et al*., 2012, Rampinini *et al*., 2011, Thomas *et al*., 2017a). Decrements in MVC post-exercise are typically attributed to impairments in neuromuscular function; that is a consequence of impairments in contractile function, and/or the capacity of the CNS to activate muscle (Gandevia, 2001). Peripheral contributors to reductions in MVC can be assessed by measuring involuntary evoked responses to electrical stimulation at rest. Additionally, central contributors can be examined through evoked responses to electrical and magnetic stimulation during voluntary contractions, thereby permitting greater insight into the neuromuscular determinants of reductions in MVC strength following competitive football match-play. The physiological mechanisms that contribute to fatigue in the days post-football-match-play, however, have typically been studied from a peripheral viewpoint, with research focusing on disturbances at sites at or distal to the neuromuscular junction. In turn, the theoretical framework of recovery interventions in football has targeted events within the exercised muscle, such as EIMD and substrate depletion (Nedelec *et al*., 2013).

While football match-play induces peripheral perturbations which contribute towards impairments in the force generating capacity of the muscle (Nédélec *et al*., 2012), recent research has highlighted dissociated rates between the temporal pattern of recovery of MVC strength, and markers of EIMD following intermittent-sprint exercise (Minett and Duffield, 2014, Pointon *et al*., 2012). These findings have led to the suggestion that processes within the CNS could be contributing to the resolution of fatigue following football match-play (Minett and Duffield, 2014). In support of this suggestion, recent evidence demonstrated that impairments in CNS function (measured through reductions in VA) were substantial following a simulated football match, and persisted for up to 72 h (Thomas *et al*., 2017a). Similarly, following

competitive football match-play, Rampinini *et al.* (2011) found a significant decline in VA which persisted for up to 48 h post-match. While these studies suggest that central factors are likely to contribute to post-match fatigue, research pertaining to recovery of CNS function following competitive match-play remains limited (Thomas *et al*., 2017a), and recent reviews have highlighted the need for further work in this area (Minett and Duffield, 2014, Rattray *et al*., 2015).

The application of single- and paired-pulse TMS paradigms has the potential to provide additional information on the fatigue and recovery of CNS function following competitive match-play. These methods have previously been applied during single-limb isometric (Kennedy *et al*., 2016), locomotor (Sidhu *et al*., 2017) and eccentric exercise (Pitman and Semmler, 2012) to reveal fatigue-induced changes in CSE and/or SICI. As such, it is possible that changes in the status of these variables could be implicated in impaired CNS function following competitive football match-play. A recent study from our laboratory found no change in SICI and a reduction in CSE 24 h following a simulated football match (Thomas *et al*., 2017a). However, while football match simulations are designed to replicate the physiological demands of competitive match-play (Nicholas *et al*., 2000), many aspects of a real match are not fully replicated through laboratory simulations (Magalhaes *et al*., 2010). For example, laboratory simulations do not include the perceptual demands associated with decision making, reacting and anticipating, and the mechanical and neuromuscular demands associated with the diverse range of physically demanding activities involved during match-play (Magalhaes *et al*., 2010). Given that changes in CSE and SICI have been shown to be task specific (Sidhu *et al*., 2012, Maruyama *et al*., 2006), it is unclear whether differences in the demands of a simulated and competitive football match

could influence the responses of these variables. Thus, the role of CSE and SICI in post-exercise fatigue and recovery warrants further investigation.

Insight pertaining to the mechanisms of impaired neuromuscular function and the time-scale of recovery thereof could have important practical implications in regards to the optimal management of the training and recovery process, player rotation strategies during congested competitive schedules, and for those involved in the scheduling of matches. The primary aim of the present study was to examine the contribution and time-course of recovery of peripheral and central factors towards impairments in neuromuscular function following competitive football match-play. Furthermore, while practitioners regularly implement physical and perceptual measures in order to assess readiness to train/compete, the sensitivity of these measures to perturbations in neuromuscular function is unclear. As such, a secondary aim of the study was to assess the relationship between the temporal pattern of recovery of neuromuscular impairments and a range of physical and perceptual measures following match-play in order to provide practitioners with simple tools to monitor the physical and cognitive contributors to fatigue in the days post-football-match-play. It was hypothesised that competitive football match-play would elicit substantial impairments in neuromuscular function from both central and peripheral origin which would persist in the days post-exercise, with concurrent decrements in measures of physical performance.

6-2 Methods

6-2.1 Participants

Sixteen male semi-professional Association Football players (six defenders, seven midfielders and three forwards; age: 21 ± 1 years; stature: 1.77 ± 0.06 m; body mass: 78 ± 8 kg) from Level 9 of the English Football League gave written informed consent to participate in the study. Players trained three to four times a week in addition to at least one competitive match. Participants were required to refrain from physical activity and alcohol consumption for the duration of the study and in the 48-hours prior to data collection, and abstain from caffeine consumption for the 12-hours prior to each experimental visit. The study was conducted one week after the completion of the competitive season while players were still accustomed to their normal training and game load.

6-2.2 Design

A practice visit preceded the main trial for habituation to the measurement tools employed in the study. For the experimental trial, participants were required to attend the laboratory on four consecutive days, separated by 24 h at the same time of day. On the first day, participants completed a 90-minute competitive football match (two 45 minute halves with a 15 minute rest interval). Pre-, post- and on subsequent days at 24, 48 and 72 h post-exercise, participants completed assessments of neuromuscular, physical and perceptual function to ascertain the time-course of recovery of these variables following competitive football match-play. A schematic of the experimental design is depicted in Figure 6-1.

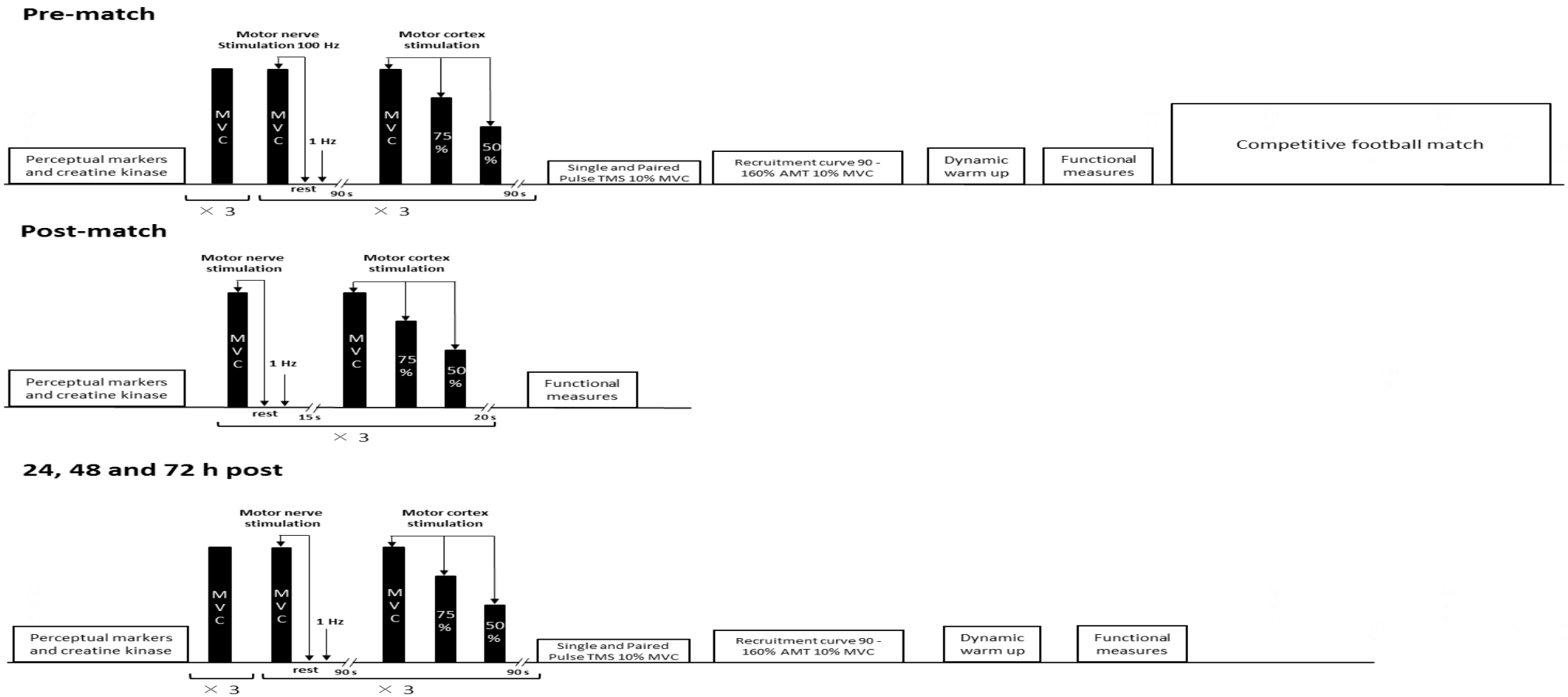


Figure 6-1. Schematic of experimental protocol. Pre-match and at 24, 48 and 72 h post participants completed a battery of perceptual, neuromuscular and functional assessments in the same order. After the pre-match assessment, participants completed a 90 min competitive soccer match consisting of two 45 minute halves interspersed by a 15 min rest interval. For the post-match assessment, a "conveyer belt" system was applied, whereby one player finished one set of tests, and the subsequent player began testing. Single- and paired-pulse TMS were administered in 2 sets of 10 stimuli during a light voluntary contraction (10% maximal voluntary contraction (MVC)). For the recruitment curve, five stimuli were delivered at each of 90%, 100%, 110%, 120%, 130%, 140%, 150% and 160% of AMT in a randomized order during a 10% MVC.

6-2.3 Procedures

Practice trial

Prior to the data collection, participants attended a practice visit during which they were habituated with the measurement tools and study protocol. The procedures used during the main trial were explained, before participants performed a practice trial consisting of the neuromuscular, physical and perceptual measures employed in the study (described below).

Competitive football match-play

On the day of the first experimental trial, participants attended the laboratory for baseline measurements (described in detail below). Subsequently, the players completed a 90 minute competitive football match consisting of two 45 minute halves interspersed by a 15 minute recovery interval. The study took place across two games separated by one week, with eight players investigated following game one and eight following game two. Both games took place on an outdoor synthetic pitch at the same time of day (13:00), with ambient temperatures 15 and 18°C and air humidity of 57 and 72% for games one and two, respectively. The games consisted of twenty-two players (two goalkeepers and twenty outfield players) and five substitutes, with players assigned to one of two different teams, of the same level, competing against each other. Players retained their normal playing positions during the games. The 16 participants being investigated participated in the full match and were not substituted. In order to ensure the match was competitive in nature and to create the physical and psychological environment of a normal competition, coaches and managers were present at both games and provided verbal encouragement throughout. The games

were refereed by officials from the Northumberland Football Association, and were registered as official matches under the English Football Association. During the game, players were allowed to drink water *ad libitum*. The activity profiles and heart rates of the players were measured throughout the games using GPS with built in heart rate monitors (Polar Team Pro, Polar Electro Oy, Finland), as described in Chapter 3. These variables were compared between games, and with the season averages recorded during competitive matches in the sample group to ensure the matches elicited a physical demand comparable to that experienced during normal competition.

Outcome measures

A range of neuromuscular, physical and perceptual measures were assessed pre- and post-match, and at 24, 48 and 72 h post-match. A brief overview of these measures are provided below, alongside further details outlined in Chapter 3 of this thesis. Perceptual responses were assessed using the *"Elite Performance Readiness Questionnaire"* (Dean et al., 1990). The neuromuscular assessment consisted of VA with motor nerve stimulation and VA_{TMS} (described below), $Q_{tw,pot}$ and twitch characteristics (MRFD, CT, MRR and $RT_{0.5}$). Single-pulse TMS was used to assess CSE using a recruitment curve (described below), while paired-pulse TMS was used to measure SICI (described below). Measures of physical function included CMJ, DJ-RSI, and linear speed (20 m sprint with 10 m splits).

Motor evoked potential recruitment curve (stimulus-response curve)

Once AMT was established (mean stimulation intensity, 37 ± 5%), the stimulator intensities required to assess the MEP response to varying TMS intensities were determined. The recruitment curve (or stimulus-response curve) was constructed by delivering TMS at a range of intensities relative to AMT, and assessing average MEP amplitude at each intensity (Carson *et al*., 2013). While previous studies have assessed changes in CSE using a single stimulus intensity (e.g. 120% AMT; Thomas *et al*., 2017a), given that there exists considerable variations across individuals in the input-output relationship between TMS intensity and MEP amplitude, it is recommended that using a stimulus-response curve should be obtained in order to provide a more comprehensive measure of CSE (Carson *et al*., 2013). This method has been deemed the most sensitive measure of motor system excitability (Carson *et al*., 2013) Participants held a light voluntary contraction (10% MVC) with one set of five stimuli delivered at each of 90%, 100%, 110%, 120%, 130%, 140%, 150% and 160% of AMT in a randomized and counterbalanced order, with 4-6 s between each stimuli and 15 s between each set.

Short-interval intracortical inhibition (SICI)

Ten single and ten paired-pulse TMS stimuli were administered in two sets of 10 stimuli during a 10% MVC, for measurement of unconditioned and conditioned MEP amplitude respectively. Paired-pulse TMS consisted of a subthreshold conditioning pulse at 70% of AMT, and a suprathreshold test pulse at 120% AMT, with an ISI of 2 ms. These stimulus variables were derived from the optimal configuration used to elicit SICI as determined in Chapter 4 of this thesis. However, it should be noted that the number of measurements of SICI (and CSE) in the present study were below that

which was deemed optimal in the previous chapter (18 measurements for SICI and 21 measurements for CSE). This was due to the time constraints associated with taken neuromuscular, physical and perceptual measures in a high number of participants within a confined time-frame. Single- and paired-pulses were delivered in a pre-determined randomised order, with 4-6 s between each stimulation and a short rest between each set. The percentage of conditioned to unconditioned MEP amplitude was used as a measure of SICI.

Voluntary activation with TMS

Single pulse TMS was delivered during brief (3-5 s) contractions at 100%, 75% and 50% MVC, separated by 5 s of rest, for determination of VA_{TMS}. This procedure was repeated 3 times with 15 s rest between each set. The stimulation intensity was set at the stimulator output that elicited the maximum superimposed twitch force (68 ± 8% MSO) during a 50% MVC at the beginning of each trial apart from the post-match assessment, which used the same intensity as pre-match (Thomas *et al*., 2017a). The stimulation intensity did not differ across the 4 time-points (P = 0.62). The stimulator output activated a large proportion of the knee extensor motoneuron pool at baseline (66 ± 19% M_{max}), with no differences across time-points (P = 0.48). Small co-activation of the antagonist muscle (BF) was observed in response to TMS (0.81 ± 0.46 mV), with no differences across time-points (P = 0.61).

Creatine kinase

Fingertip samples of capillary blood were obtained at each time point and immediately assayed for CK concentration (Reflotron, Roche Diagnostics, Germany).

6-2.4 Data analysis

For VA_{TMS}, a 9-point regression between SIT amplitude and contraction intensity was extrapolated to the *y*-intercept to obtain an ERT as described in Chapter 3 (ERT, Todd *et al*. 2003). The regression analysis confirmed a linear relationship between contraction strength and SIT evoked by TMS (r^2 range = 0.89 ± 0.06 to 0.92 ± 0.04). Recruitment curves were constructed by plotting the TMS stimulation intensity relative to AMT against the MEP amplitude averaged from the five stimulations at each intensity, expressed relative to M_{max}. The percentage of the MEP amplitude to the maximum M-wave was used as an index of CSE. In order to provide a summary measure of CSE, the summated area under the recruitment curve (AURC) was calculated for each participant at each time point using the trapezoid integration method, which has been shown to have excellent reliability (Carson *et al*., 2013). The root mean square EMG amplitude (RMS_{EMG}) and average force were calculated in the 80 ms prior to each TMS to ensure a similar level of background muscle activity was present during the recruitment curve and SICI measurements.

6-2.5 Statistical analysis

Data are presented as mean ± SD. Repeated measures ANOVA was used to assess changes in each outcome measure over time (pre-, post-, 24 h, 48 h & 72 h). Normality of the data was assessed using the Shapiro-Wilks test. Assumptions of sphericity were explored and controlled for all variables using the Greenhouse-Geisser adjustment,

where necessary. In the event of a significant main effect, Dunnett's multiple comparison procedure was employed with the pre-trial score used as the control category. The assumptions underpinning these statistical procedures were verified as per the guidelines outlined by Newell *et al.* (2010). Paired sample *t*-tests were used to assess differences in match-running variables between the first and second halves of the competitive matches, as well the match-running data from the study, and normative data gathered throughout the season. Independent sample *t*-tests were used to assess differences between the match demands of the two competitive matches in the study. Pearson product-moment correlations coefficients were calculated to determine relationships between pre-post changes in MVC, contractile ($Q_{tw,pot}$) or CNS function (motor point and VA measured with) and match-running variables, as well as the association between the proximity of the post-match assessment to the end of the match and the pre-post changes in these variables. Spearman's rank-order correlation was used to assess the relationship between the temporal pattern of recovery of neuromuscular variables ($Q_{tw,pot}$, motor point and VA measured with TMS), physical function tests (CMJ, DJ-RSI and 20 m sprint) and perceptual responses (fatigue and soreness). The relationship between the time-point (post-, 24, 48, 72 or > 72 h post-match) at which neuromuscular function, physical function or perceptual responses recovered was then assessed. Standardised effect sizes (Cohen's *d*) were calculated for focussed pairwise comparisons and interpreted as small (≥0.2), moderate (≥0.6) and large (≥1.2). All data was analysed using Statistical Package for Social Sciences (SPSS version 22.0). Statistical significance was accepted at $P < 0.05$.

6-3 Results

6-3.1 Match performance and intensity

Match activity and heart rate variables are presented in Table 6-1. In both games, reductions in total distance (game 1: −26 ± 18%, $P < 0.001$; game 2: −14 ± 6%, $P = 0.01$) and HIR (game 1: −22 ± 19%, $P < 0.001$; game 2: −35 ± 21%, $P < 0.001$) were found between the first and second halves. No differences were found in time-motion or heart rate variables between games 1 and 2, or with data gathered throughout the competitive season (all $P > 0.11$). No correlations were found between any of the match-running variables and pre-post changes in measures of neuromuscular function (all $P > 0.16$).

Table 6-1. Match activity and heart rate variables during competitive football match-play. The study data was gathered across two competitive matches, while normative data from the same players was gathered throughout the competitive season (n = 16). Values are mean ± SD.

	TD (m)	HIR (m)	Accels (no.)	Decels (no.)	Mean HR (bpm)	Max HR (bpm)
Pooled data	10041 ± 626	1211 ± 257	315 ± 64	208 ± 56	164 ± 11	193 ± 10
Game 1	10037 ± 552	1286 ± 199	301 ± 64	197 ± 49	170 ± 11	197 ± 12
Game 2	10046 ± 770	1126 ± 303	329 ± 64	218 ± 64	158 ± 7	189 ± 5
Season average	10076 ± 1363	1456 ± 143	289 ± 97	204 ± 63	158 ± 12	194 ± 12

TD = total distance; HIR = high-intensity running; Accels = accelerations; Decels = decelerations, HR, = heart rate.

6-3.2 Perceptual responses

Perceptual responses from the Elite Performance Readiness Questionnaire can be viewed in Table 6-2. A main effect for time on fatigue ($F_{4,60}$ = 50.85, P < 0.001), soreness ($F_{4,60}$ = 44.50, P < 0.001), motivation to train ($F_{4,60}$ = 17.60, P < 0.001) tension ($F_{4,60}$ = 6.26, P < 0.001), and post-warm-up readiness to train ($F_{3,27}$ = 6.85, P = 0.01) was noted. *Post-hoc* comparisons showed that fatigue was higher than pre-match at post-, 24 and 48 h (all P < 0.001) and at 72 h (P = 0.001). Muscle soreness was higher than pre-match values at post-match, 24 and 48 h (P < 0.001) before recovering at 72 h (P = 0.16). Motivation to train was reduced at all-time points post-match (P < 0.001 at post-match and 24 h, P = 0.001 at 48 h and P = 0.01 at 72 h), while post-warm-up readiness to train was reduced at 24 h (P = 0.02) before recovering by 48 h (P = 0.16).

Table 6-2. Perceptual responses measured via visual analogue scales (mm) pre-, post-, and 24, 48 and 72 h post- competitive soccer match-play (n = 16). Values are mean ± SD. Significant differences in comparison with baseline indicated by * = p < 0.05, ** = p < 0.01 and *** = p < 0.001.

	Pre-	Post-	24 h	48 h	72 h
Fatigue	10.9 ± 9.7	73.1 ± 16.8***	61.7 ± 16.0***	41.8 ± 16.6***	26.0 ± 16.7**
Soreness	17.2 ± 16.7	70.5 ± 16.0***	64.8 ± 17.3***	50.3 ± 15.6***	23.3 ± 13.2
Motivated to train	68.9 ± 17.6	29.9 ± 25.8***	51.6 ± 24.5***	54.4 ± 21.6**	60.6 ± 18.4*
Anger	6.3 ± 8.6	14.5 ± 16.7	5.1 ± 6.4	12.4 ± 20.5	5.4 ± 6.7
Confusion	5.1 ± 6.9	7.5 ± 7.9	4.5 ± 5.5	4.4 ± 4.7	5.6 ± 6.2
Depression	3.4 ± 4.4	7.2 ± 14.6	4.4 ± 5.6	4.5 ± 5.2	5.3 ± 6.9
Tension	10.0 ± 11.3	38.4 ± 31.8**	25.3 ± 22.9**	27.6 ± 19.7***	17.4 ± 14.8
Alertness	62.5 ± 23.9	45.6 ± 26.5*	57.8 ± 23.0	56.1 ± 18.9	57.1 ± 22.9
Confidence	69.9 ± 18.4	68.9 ± 27.2	69.5 ± 23.9	69.8 ± 20.5	72.7 ± 21.6
Sleep	58.3 ± 20.2	N/A	67.7 ± 22.2	57.3 ± 16.3	57.9 ± 24.8
Post warm-up readiness to train	76.9 ± 19.7	N/A	51.7 ± 31.9*	67.6 ± 26.9	69.8 ± 29.9

6-3.3 Neuromuscular function

A main effect for time on MVC ($F_{4,60}$ = 21.36, P < 0.001), VA measured with motor nerve stimulation ($F_{4,60}$ = 13.29, P < 0.001), $Q_{tw,pot}$ ($F_{4,60}$ = 22.06, P < 0.001), VA_{TMS} ($F_{4,60}$ = 6.36, P < 0.001), MRFD ($F_{4,60}$ = 3.69, P = 0.009), and CT ($F_{4,60}$ = 6.89, P < 0.001) was found. Maximal voluntary contraction force was reduced by 14 ± 9% from pre- to post-match (726 ± 109 N vs. 621 ± 106 N, P < 0.001, d = 0.89, Figure 6-2A), remained depressed at 48 h (695 ± 100 N, P = 0.01, d = 0.31), but recovered by 72 h (709 ± 94 N, P = 0.27, d = 0.17). Voluntary activation measured with motor nerve stimulation decreased by 7.1% from pre- to post-match (92.0 ± 3.7% vs 84.9 ± 6%, P < 0.001, d = 1.16), remained depressed at 24 h by 4.7% (87.3 ± 6.0%, P = 0.01, d = 0.86), but recovered by 48 h (91.5 ± 3.1%, P = 0.61, d = 0.12, Figure 6-2B). Voluntary activation measured with motor cortical stimulation was reduced by 5.3% from pre- to post-match (91.4 ± 3.7% vs. 86.1 ± 4.03%, P < 0.001, d = 1.09), but had recovered by 24 h (89.3 ± 5.3%, P = 0.08, d = 0.41, Figure 6-2C). Potentiated twitch force was reduced by 14 ± 6% from pre- to post-match (214 ± 45 N vs. 183 ± 37 N, P < 0.001, d = 0.71), remained depressed by 6 ± 6% at 24 h (201 ± 37 N, P = 0.01, d = 0.32), but recovered by 48 h (210 ± 41 N, P = 0.19, d = 0.08, Figure 6-2D). The pre- to post-match decline in $Q_{tw,pot}$ was accompanied by changes in peripherally derived measures of muscle contractility. Specifically, MRFD and CT were both reduced by 10 ± 15% (P = 0.008) and 8 ± 11% (P = 0.006), respectively, with both recovering by 24 h (P > 0.65). Maximum M-wave and RMS/M_{max} did not differ from baseline values at any time point (all P > 1.00). No significant correlation was found between the proximity of the post-match neuromuscular assessment to the end of the match and the magnitude of change in MVC (r = 0.24, P = 0.36), $Q_{tw,pot}$ (r = 0.21, P = 0.43), VA measured with motor nerve (r = −0.18, P = 0.52) and motor cortical stimulation (r = 0.18, P = 0.51).

5-3.4 Central nervous system excitability and inhibition

There were no differences in AMT at any time-point. No main effects of time on SICI ($F_{3,42}$ = 0.75, P = 0.531; Figure 6-7) or AURC ($F_{3,45}$ = 1.22, P = 0.312; Figure 6-8) were found.

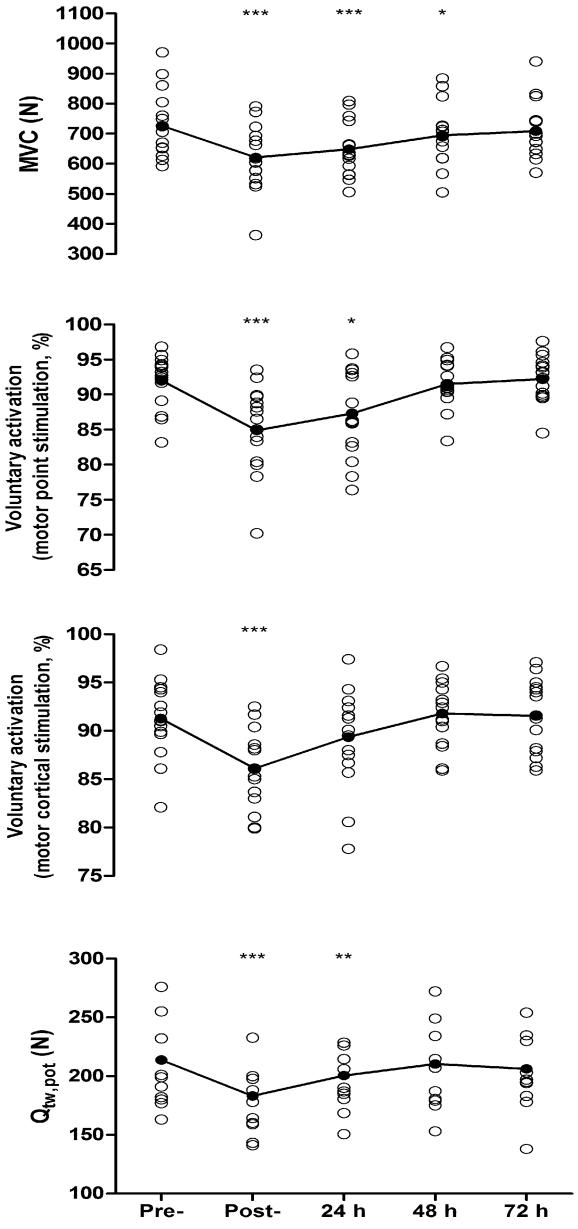


Figure 6-2. Maximal voluntary contraction force (MVC, A), voluntary activation measured with femoral nerve stimulation (B), voluntary activation measured using motor cortical stimulation (C), and quadriceps potentiated twitch force ($Q_{tw,pot}$,D) measured pre-, post- and at 24, 48, and 72 h post-competitive football match-play (n = 16). Significant differences in comparison with baseline indicated by *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001. Individual responses are plotted, with lines representing the mean scores.

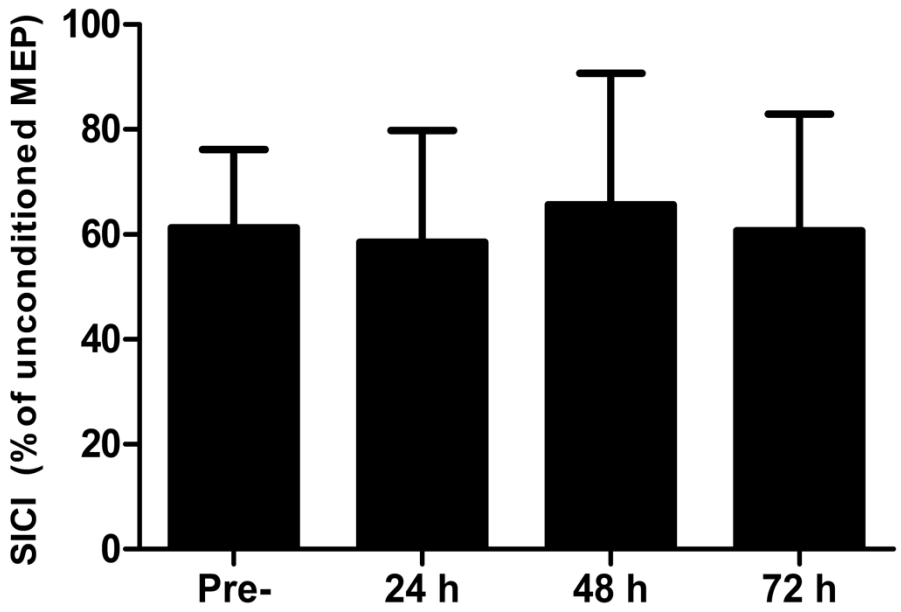


Figure 6-3. Short-interval intracortical inhibition (SICI) measured in the *rectus femoris* pre-, 24, 48, and 72 h post- competitive soccer match-play (n = 16). Values are mean + *SD.*

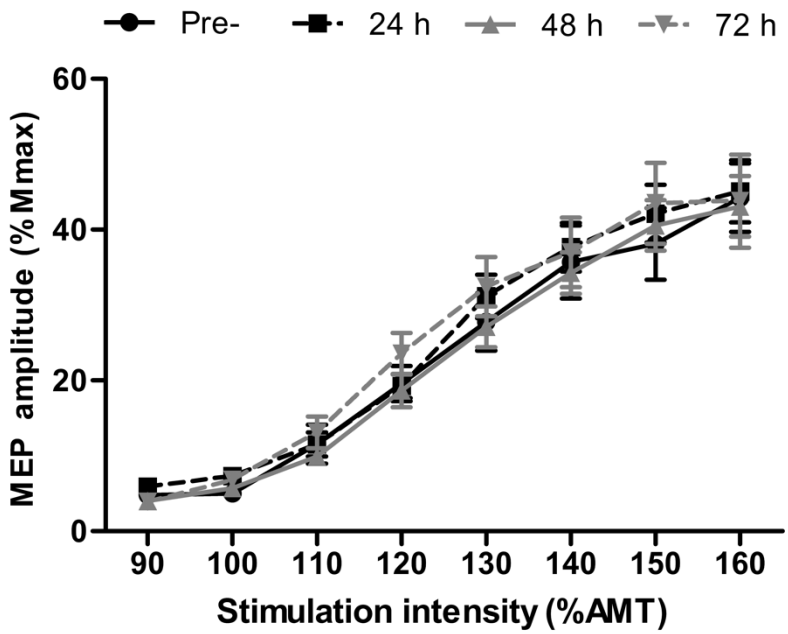


Figure 6-4. Recruitment curve displaying motor evoked potential (MEP) amplitude relative to the maximal compound muscle action potential (M_{max}) in the *rectus femoris* at stimulation intensities relative to active motor threshold (AMT) at pre-, 24, 48, and 72 h post- competitive football match-play (n = 16). Values are mean ± *SD.*

5-3.5 Physical function

A main effect for time on CMJ height ($F_{4,60}$ = 4.066, P = 0.006), DJ-RSI ($F_{4,60}$ = 14.903, P < 0.001), and 20 m sprint time ($F_{4,60}$ = 11.049, P < 0.001) was found. *Post-hoc* comparisons showed that CMJ height was reduced from pre- to post-match by 5 ± 8% (43.4 ± 5.1 vs. 41.0 ± 4.6 cm, P = 0.03, d = 0.47) and at 24 h by 4 ± 6% (41.5 ± 4.6 cm, P = 0.02, d = 0.39) but recovered by 48 h (42.8 ± 5.2 cm, P = 0.34, Figure 6-9A). For DJ measurements, contact time was 169 ± 16 ms at baseline, and was successfully maintained on subsequent days (range 169–173 ms). A reduction in RSI was found from pre- to post-match by 17 ± 8% (189 ± 35 vs. 158 ± 35 cm·s^{-1}, P < 0.001, d = 0.83) that remained below baseline at 24 h post by 7 ± 9% (176 ± 41 cm·s^{-1}, P = 0.01, d = 0.33), but recovered by 48 h (188 ± 40 cm·s^{-1}, P = 0.88, Figure 6-9B). Sprinting performance over 20 m was reduced from pre- to post-match by 4 ± 2% (3.11 ± 0.11 vs. 3.23 ± 0.11 s, P < 0.001, d = 0.93) but recovered thereafter (all P > 0.45), while 10 m sprint time showed no decrease between baseline and any other time-point (all P > 0.07).

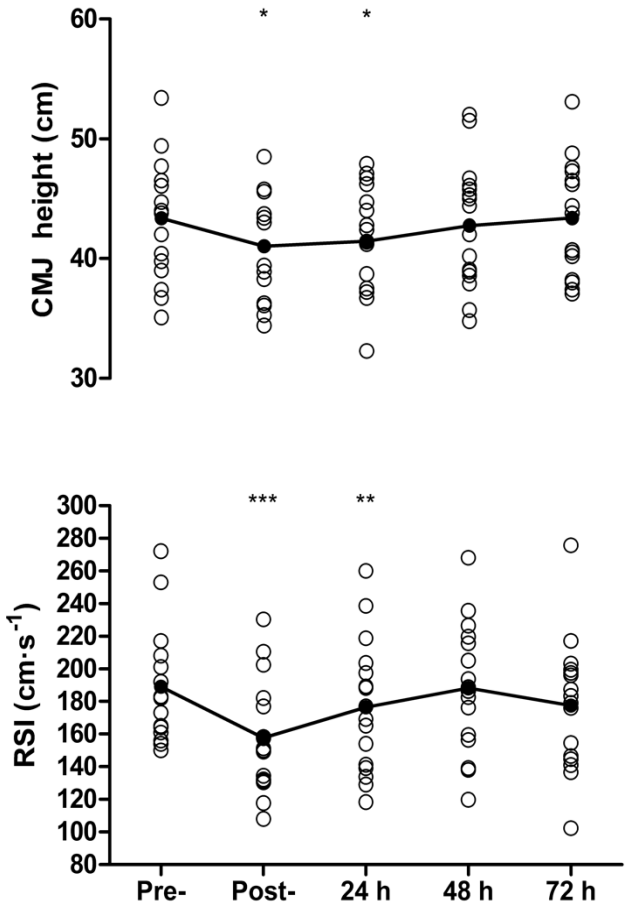


Figure 6-5. Countermovement jump height (CMJ, A), and reactive strength index (RSI, B) measured pre-, post-, and 24, 48, and 72 h post- competitive football match-play (n = 16). Significant differences in comparison with baseline indicated by *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001. Individual responses are plotted, with lines representing the mean scores.

5-3.6 Relationship between recovery of neuromuscular variables and physical and perceptual measures

Table 6-3 displays the relationships between the temporal pattern of recovery of neuromuscular function and physical and perceptual measures. A significant correlation was found between recovery of motor point VA and CMJ (r = 0.831, P < 0.001), with no other significant correlations found with any other physical function or perceptual variable (all $P \geq 0.07$).

Table 6-3. Spearman's rank-order correlation coefficients between the temporal pattern of recovery neuromuscular function indicators and physical function and perceptual measures. Significant correlation indicated by *** = p < 0.001.

	$Q_{tw,pot}$		Motor point VA		VA measured with TMS	
	R^2	*P*	R^2	*P*	R^2	*P*
CMJ	0.31	0.59	0.83***	<0.01	−0.13	0.66
DJ-RSI	0.41	0.15	−0.08	0.78	0.14	0.62
10 m sprint	−0.28	0.29	0.34	0.19	−0.17	0.56
20 m sprint	−0.24	0.41	−0.10	0.71	0.44	0.10
Fatigue	0.46	0.07	0.08	0.77	−0.15	0.56
Soreness	−0.12	0.66	0.34	0.19	0.06	0.88

$Q_{tw,pot}$, quadriceps potentiated twitch force, VA, voluntary activation, TMS, transcranial magnetic stimulation, CMJ, countermovement jump, DJ-RSI, drop-jump RSI.

5-3.7 Creatine Kinase

Creatine kinase ($IU \cdot L^{-1}$) increased from pre- to post-match (344 ± 319 vs. 872 ± 423 $IU \cdot L^{-1}$, $P < 0.001$), peaked at 24 h post (1,059 ± 571 $IU \cdot L^{-1}$, $P < 0.001$), and remained elevated at 48 h (763 ± 477 $IU \cdot L^{-1}$, $P = 0.001$) and 72 h post (537 ± 349 $IU \cdot L^{-1}$, $P = 0.02$).

6-4 Discussion

The aim of this study was to examine the contribution and time-course of recovery of central and peripheral factors towards impairments in neuromuscular function following competitive football match-play. The data indicate that competitive match-play elicits considerable impairments in neuromuscular function that require 48-72 h to resolve. Impairments in both VA and $Q_{tw,pot}$ were substantial post-match, and persisted for up to 48 h following exercise, while reductions in the voluntary force generating capabilities of the knee extensors took 72 h to recover. A secondary aim of

the study was to ascertain the relationship between the temporal pattern of recovery of impaired neuromuscular function and physical and perceptual measures following match-play in order to provide practitioners with simple tools to monitor fatigue and recovery. While a significant correlation was found between recovery of motor point VA and CMJ, no other significant associations were found, suggesting that the physical and perceptual measures used in the present study cannot be used as surrogate measures of neuromuscular function in the quadriceps. Collectively, these data add to growing evidence that prolonged impairments in the capacity of the CNS to activate the quadriceps muscles are implicated in fatigue following intermittent sprint exercise (Thomas *et al*., 2017a, Rampinini *et al*., 2011), and could have important implications for the optimization of the training process and the implementation of appropriate recovery interventions.

Match performance and intensity

The performance indices and physiological demands of the competitive match intervention were similar to data gathered from the same team throughout the competitive season, and not different between matches. Total running distance and mean and peak HR were comparable to values reported in elite level professional footballers (Mohr *et al*., 2003, Ekblom, 1986). High-intensity running distance, which has previously been shown to be a distinguishing factor between top-class players and those at a lower level (Mohr *et al*., 2003), was similar to that described for players of the same level (O'Donoghue *et al*., 2001), and lower than values reported in elite players (Mohr *et al*., 2003, Rampinini *et al*., 2011). The significant decline in TD and HIR between the first and second halves indicates that players were experiencing

match-related fatigue during the fixtures, suggesting that the physical demands imposed on the players were similar to those during real competition.

Impairments in neuromuscular function following competitive match-play

Following competitive football match-play, the players in the present study exhibited substantial reductions in VA and $Q_{tw,pot}$. While previous investigations concerning the etiology of fatigue following match-play have predominantly focused on peripheral perturbations (Ispirlidis *et al*., 2008, Mohr *et al*., 2003, Nédélec *et al*., 2012), the results from the present study suggest that competitive match-play elicits impairments in CNS function which take days to resolve. Specifically, VA measured through motor point stimulation was significantly reduced post-match (7.1%) and remained depressed at 24 h (4.7%), before recovering by 48 h, while VA_{TMS} was reduced post-match (5.3%) before recovering by 24 h. The magnitude of impairments and time-course of recovery of VA was similar to that reported elsewhere following both competitive (Rampinini *et al*., 2011) and simulated match-play (Thomas *et al*., 2017). Accordingly, these results suggest that football match-play elicits prolonged impairments in the capacity of the CNS to activate the quadriceps muscles. While the functional relevance of this activation deficit cannot be accurately quantified, the results support recent suggestions that future research should place more emphasis on the recovery of CNS function following intermittent sprint exercise (Minett *et al*., 2014, Thomas *et al*., 2017a).

While the precise mechanisms underpinning the prolonged impairments in the capacity of the CNS to activate muscle following long-duration locomotor exercise are unknown (Carroll *et al*., 2017), a number of potential factors might have

contributed towards the residual activation deficit which persisted at 24 h. Group III and IV muscle afferents, that provide inhibitory feedback to various sites within the CNS (Sidhu *et al*., 2017), are sensitive to various markers of muscle injury, such as the release of biochemical substrates (e.g. bradykinin, histamines and prostaglandins) and factors associated with inflammation (Sidhu *et al*., 2009b, Pitman and Semmler, 2012, Endoh *et al*., 2005). Furthermore, there is some indication that elevations in the concentration of brain cytokines following eccentric exercise might also modulate recovery of CNS impairment (Carmichael *et al*., 2006). Given that the repeated eccentric contractions associated with match-play are likely to have induced muscle damage, as evidenced by the increase in CK in the days post-exercise, and a subsequent inflammatory response (Ispirlidis *et al*., 2008), it is possible that the inflammatory response which ensues following match-play could have contributed to the residual activation deficit which persisted for up to 48 h post-match.

In addition to impairments in VA, substantial impairments in contractile function, assessed via $Q_{tw,pot}$, were evident post-match and persisted at 24 h before recovering by 48 h. A decrease in the force output of the muscle in response to electrical stimulation can be attributed to metabolic and mechanical factors that negatively influence the excitation-contraction coupling process, as well as impairments in neuromuscular transmission at the sarcolemma (Allen *et al*., 2008). The lack of change in M_{max}, a measure of neuromuscular transmission, combined with the significant reductions in peripherally derived measures of contractility (CT and MRFD), suggest that the contractile impairments demonstrated post-match were a result of disruptions occurring beyond the sarcolemma. Furthermore, many of the metabolic mechanisms thought to interfere with the excitation-contraction process, such as the accumulation of intramuscular metabolites (e.g., P_i and H^+) and the depletion of cellular ATP levels,

recover rapidly following exercise (Allen *et al*., 2008) and had likely returned close to pre-exercise levels by the time of the post-match assessment. The more prolonged reductions in $Q_{tw,pot}$ evident in the present study were more likely the consequence of the large mechanical stress imposed on muscle fibres during match-play, which can lead to myofibrillar damage, disorganization of sarcomeres and interference with cellular Ca^{2+} handling (Skurvydas *et al*., 2016). This supposition is further supported through comparisons with previous data on recovery of $Q_{tw,pot}$ following cycling exercise, which imposes large metabolic but little mechanical stress on the muscles and consequently leads to a more hastened recovery of contractile function (Blain *et al*., 2016). For example, following a 5 km cycling time-trial, Blain *et al*. (2016) found that $Q_{tw,pot}$ had recovered 5 h post-exercise despite the immediate post-exercise reduction (~30%) being substantially higher than the present study. Thus, it is likely that the prolonged reduction in $Q_{tw,pot}$ was primarily a result of mechanical damage incurred during match-play.

Maximum voluntary contraction force remained below baseline at 48 h, despite there being no statistically significant difference in VA or $Q_{tw,pot}$ between baseline and 48 h. Although not statistically significant, both $Q_{tw,pot}$ and motor point VA remained 1.8% and 0.5% below baseline 48 h post-match, respectively, with 7 of the 16 participants displaying a reduction in VA or $Q_{tw,pot}$ at 48 h above the measurement error obtained from previous work from our laboratory (2.2-3.1% VA, 4.8-5.3% $Q_{tw,pot}$; (Goodall *et al*., 2017b, Thomas *et al*., 2017a)). It is possible that these small decrements combined might explain the reduced MVC at 48 h. The MVC force loss at 48 h could also be explained by impairments in neuromuscular function that were not fully captured by measures of VA and $Q_{tw,pot}$, or a contribution of other physiological or psychological factors that could contribute to impairments in the force generating capacity of the

muscle, such as substrate depletion, inflammation or perceptions of muscle soreness. Furthermore, although MVC remained below baseline at 48 h, reductions were small, with absolute decrements equating to −32 ± 43 N, or 4 ± 6%; a value similar to the measurement error of this variable in our lab (4.0% & 4.4%; ((Thomas *et al*., 2015, Thomas *et al*., 2016). As such, the functional relevance and meaningfulness of such small impairments could be questioned.

The magnitude of post-match decrements in neuromuscular function in the present study was similar to that of previous work using a simulated football match protocol (Thomas *et al*., 2017a). However, the time-course of recovery in the days post- was markedly faster in the present study. While MVC force had recovered by 72 h in the present study, small impairments in MVC persisted at this time-point after a simulated match (Thomas *et al*., 2017). Furthermore, although the post-match reduction in $Q_{tw,pot}$ was similar to that observed after simulated match-play, the time-course of recovery of contractile function was substantially faster in the present study. Specifically, at 24 h following simulated football, recovery of $Q_{tw,pot}$ was negligible (−14 ± 10% post-match to −13 ± 5% at 24 h) and remained below baseline at 72 h (Thomas *et al*., 2017a). In contrast, recovery of $Q_{tw,pot}$ at 24 h in the present study after a competitive match was substantial (−14 ± 6% post-match to −6 ± 6% at 24 h), and returned to baseline by 48 h. Two integral differences between the studies might explain the disparity between the results. Namely, the study by Thomas *et al*. (2017) was primarily conducted during the late off-season and early pre-season phase, while the current study was conducted a week following the competitive season, when players were conceivably in better physical condition and more accustomed to the demands of football match-play. The more rapid recovery of $Q_{tw,pot}$ might reflect a mechanical adaptation of skeletal muscle acquired throughout the competitive season, acting to

provide greater protection against the muscle damage sustained during match-play (Silva *et al*., 2014, Hoffman *et al*., 2005). Differences between match-related fatigue and the time course of recovery during different phases of training throughout the competitive season presents an interesting area for future investigation. In addition, Thomas *et al*. (2017) employed a simulated-match protocol with forced decelerations which, in contrast to the self-paced nature of competitive match-play, requires players to match running speeds with externally controlled stimuli. Although competitive match-play includes an array of actions associated with eccentric contractions which subsequently lead to muscle damage, it is possible that players were less accustomed to the specific demands of the simulated match-protocol, resulting in greater muscle damage than during a competitive match, possibly contributing towards the slower time-course of recovery of muscle function and fatigue. Thus, caution should be exercised when making comparisons between simulations and competitive football matches, or using the two protocols interchangeably.

Corticospinal excitability and short-intracortical inhibition

No changes were found in CSE or SICI at any time-point throughout the study. We implemented a recruitment curve (or stimulus-response curve), which measures MEP amplitude normalised to the maximal M-wave in response to varying stimulation intensities relative to AMT, and has been suggested as the most sensitive measure of motor system excitability (Carson *et al*., 2013). However, no differences were found in the summated AURC, suggesting that CSE does not change in the days following competitive football match-play. Furthermore, while changes in MEP amplitude in response to fatigue might depend on the level of force at TMS delivery (Gruet *et al*.,

2013), no changes were found in MEP amplitude elicited during measurement of cortical VA at 50, 75 or 100% MVC, further indicating a lack of change in CSE. The excitability of corticospinal cells to fatiguing exercise seems to be task specific, with several studies reporting altered CSE in response to fatiguing isometric exercise in isolated upper (Maruyama *et al*., 2006) and lower limb (Mileva *et al*., 2012) models, whereas numerous studies report no change in response to various modes of locomotor exercise (Sidhu *et al*., 2012, Goodall *et al*., 2015). Discrepancies between studies involving isometric and locomotor exercise can likely be explained by differences in the systemic and local responses between the two types of exercise, which might differentially influence the responsiveness of corticospinal cells (Sidhu *et al*., 2012). The lack of change in CSE in the present study is thus consistent with previous findings following locomotor exercise (Sidhu *et al*., 2012, Goodall *et al*., 2015). Similarly, no changes were found in SICI, which reflects intracortical inhibition mediated by $GABA_a$, following competitive match-play, corroborating the findings of Thomas *et al.* (2017a). Previous studies which have found changes in SICI in response to locomotor (Sidhu *et al*., 2012) and isometric exercise (Hunter *et al*., 2016) have noted that exercise-induced changes in the excitability of inhibitory circuits are short lasting, and dissipate within minutes of exercise cessation. Thus, the lack of change in SICI post-exercise might not fully reflect modulations in SICI that could occur during exercise. While it is possible that any change in SICI and CSE would have resolved by the time measurements were taken, the lack of change in these measures in the days post-match suggests that this measure plays a negligible role in the residual perturbations in CNS function which occurs following competitive match-play.

Recovery of physical function

Jump performance (CMJ and drop jump for RSI) was significantly impaired post-match and at 24 h, before recovering by 48 h. The time-course of recovery in jump performance is similar to that reported following competitive match-play (Nédélec *et al*., 2012, Ispirlidis *et al*., 2008). In contrast to the CMJ and DJ, 20 m sprint performance was impaired post-match but recovered thereafter. The superior sensitivity of CMJ and DJs to altered neuromuscular function compared with 20 m sprint time has been reported elsewhere (Gathercole *et al*., 2015a, Thomas *et al*., 2017a). Although the temporal pattern of recovery of vertical jump performance and decrements in measures of neuromuscular function was similar on a group level, correlation analysis showed only one significant relationship between recovery of motor point VA and CMJ height, with no other significant associations between any of the neuromuscular and physical or perceptual measures. A number of possible explanations could account for the discrepancies between recovery of neuromuscular function and physical and perceptual measures. Namely, there is an inherent level of variability associated with measures of voluntary performance, with individuals able to alter their jump or sprint mechanics in an attempt to maximise performance (Ratel *et al*., 2006). For example, it has previously been suggested that individuals alter their jump mechanics when fatigued in order to help maintain jump height (Gathercole *et al*., 2015a). In addition, measures of neuromuscular function target the knee extensors under isometric conditions, while jump performance involves multi-joint dynamic movements, which could further contribute to the discrepancies. Thus, the divergence in the temporal pattern of recovery suggests that using the physical function and perceptual measures employed in the present study as surrogate measures of neuromuscular function in the quadriceps is inappropriate.

Recovery of perceptual responses

Competitive match-play resulted in fatigue, perceptions of soreness and tension, and decreases in alertness and motivation to train in the hours and days post-exercise. Despite neuromuscular function and physical performance measures having returned to baseline, differences in fatigue and motivation to train persisted at 72 h post-exercise. A lack of association between subjective and objective indicators of fatigue has previously been reported (Saw *et al*., 2016), and provides support for the inclusion of both when monitoring recovery following match-play to provide a more comprehensive understanding of an athletes physical and psychological readiness to train/compete. Although neuromuscular function and physical performance measures had returned to baseline by 72 h, it is possible that the inflammatory response, which ensues following match-play (Ispirlidis *et al*., 2008) and exacerbates fatigue (Smith, 2000), persisted at 72 h, potentially explaining the differences in recovery of perceptual and neuromuscular responses. The divergent recovery of sensations of fatigue compared to measures of neuromuscular function emphasises the aforementioned multi-factorial nature of fatigue elicited by the varied mechanical, metabolic and cognitive demands of soccer match-play. A possible alternative explanation for the self-reported fatigue which persisted at 72 h is that a bias effect might exist during measurements of perceptual responses, in which participants feel inclined to report elevated levels of fatigue relative to baseline at each post-match assessment. Similar to the findings of Thomas *et al.* (2017a), perceptions of readiness to train assessed after a standardised warm-up recovered faster than that assessed at rest. Specifically, readiness to train returned to baseline by 48 h when assessed post-warm up and remained below baseline at 72 h when measured at rest. This provides further support for the suggestion that a warm-up could mask perceptions of fatigue in

the days post-match (Thomas *et al*., 2017a), and practitioners should consider the timing of perceptual assessments when monitoring fatigue following match-play.

Limitations

The demands of competitive football match-play are inherently unpredictable, and a high degree of inter-individual variability in match activity exists. Consequently, the magnitude of fatigue and in turn the time-course of recovery can be highly variable between participants. Thus, although competitive match-play is the most valid model of investigating the mechanisms of fatigue and time-scale of recovery, one limitation of this method compared with a laboratory simulation is the lack of experimental control over the activity profiles of the players and the high inter-subject variability in match demands. In order to account for this limitation, the relationships between match-running variables and the changes in measures of neuromuscular function from pre-to-post match were assessed, with no significant associations found. Overall, the results of the present study provide valuable information on the fatigue and recovery following a real match; an ecologically valid stimulus that includes movements, skill and cognitive demands that aren't fully replicated by a laboratory simulation. Moreover, it should be noted that measurements of neuromuscular function were performed in the quadriceps muscles only. Other muscle groups of the lower extremity, such as the hamstrings, calves and hip adductors/abductors, also play an important role in many match-related actions and likely incur decrements in function following match-play. However, measuring neuromuscular function in many of these muscles groups is fraught with difficulties, such as targeting a specific muscle group through motor nerve and/or cortical stimulation, and participant discomfort associated

with receiving motor nerve stimulation. Additionally, the quadriceps muscles are heavily involved in actions associated with football match-play, and have previously been reported to incur significant fatigue as a result of match-play (Rampinini *et al*., 2011, Thomas *et al*., 2017a), thus making them a suitable choice when assessing match-related impairments in neuromuscular function. Due to the logistical constraints of taking multiple measures on a large group of participants in a short time frame, neuromuscular and physical assessments took place from 10-60 minutes post-match. In this time, some aspects of impaired neuromuscular function might have dissipated by the time of measurement, while it could also be argued that those who had neuromuscular measures taken at a closer proximity to the end of the match might have been more fatigued than those assessed later. However, correlation analysis showed no significant relationship between the proximity of the post-match neuromuscular assessment to the end of the match and the magnitude of reductions in MVC strength. Furthermore, the level of post-match reductions in CNS and contractile function were similar to or higher than that found in previous studies (Rampinini *et al*., 2011, Thomas *et al*., 2017a), demonstrating the robust nature of the data. Finally, due to the time constrains associated with taking neuromuscular, physical and perceptual measures in a high number of participants within a confined time-frame, the number of measurements used when assessing SICI and CSE was below the minimum required to ensure the CSE and SICI value fell within the 95% CI for all participants, as determined in Chapter 4. While using a suboptimal number of measurements is acknowledged as a limitation of the present study, it should be noted that the between-day reliability of SICI based on 10 measurements is moderate ($ICC_{3,1}$ = 0.59, CV = 14.3%, TE = 12% of unconditioned MEP), and good when using 5 measurements of CSE between-days ($ICC_{3,1}$ = 0.77, CV = 20.2%, TE = 7% of M_{max}).

Practical applications

The results of the study could have a number of important practical implications. Namely, understanding the time-course of recovery can allow practitioners to make more informed decisions when devising the training schedule around competitive fixtures, providing valuable ancillary information when prescribing training in the 72 hours following competitive match-play. In addition, understanding the magnitude of fatigue and the time-course of recovery can assist in decision making regarding player rotation strategies during congested fixture schedules, which are commonplace in modern day soccer. Furthermore, understanding the etiology of fatigue is critical when determining the potential efficacy of recovery interventions aimed at accelerating the natural time-course of recovery in an attempt to facilitate performance and reduce the likelihood of injury during subsequent activity. The results of the study could thus provide a scientific basis for research and practice concerning the implementation of recovery strategies following match-play. Finally, the lack of association and differential time-course of recovery between neuromuscular, physical function and perceptual measures in the present study suggests that practitioners should use a range of both subjective and objective measures when monitoring fatigue and recovery in order to provide a more comprehensive understanding of readiness to train/compete.

Conclusions

Competitive soccer match-play induced impairments in both VA and $Q_{tw,pot}$, requiring up to 48 h to return to baseline, while reductions in MVC took 72 h to recover. The

overall aim of this thesis was to examine the aetiology of impairments in neuromuscular function from both central and peripheral origins, and whether perturbations in CNS and/or contractile function were implicated in recovery following intermittent sprint exercise. While previous research on post-match fatigue and recovery has predominantly focused on the recovery of contractile function, the results of this chapter suggest that competitive match-play elicits substantial deficits in CNS function, requiring up to 48 h to resolve. Decrements in contractile function showed a similar time-course of recovery, and can likely be attributed to disturbances in excitation-contraction coupling as a result of the muscle damage incurred during match-play. While measures of vertical jump performance followed a comparable time-course of recovery to neuromuscular function on a group-level, only one significant association was found between recovery of neuromuscular function and measures of physical function or perceptual responses. Given the prolonged nature of impairments in neuromuscular function following competitive football match-play, a challenge for practitioners is to mitigate the magnitude of fatigue and attempt to expedite the recovery process through appropriate interventions in order to prepare players for the demanding fixture periods associated with modern day football and reduce the risk underperforming or of sustaining injuries. The final chapter of this thesis will implement an intervention aimed at attenuating post-match fatigue and accelerating the natural time-course of recovery by alleviating the symptoms of muscle damage and inflammation hypothesised to interfere with contractile and CNS function.

CHAPTER 7 THE EFFECT OF PHASE CHANGE MATERIAL ON RECOVERY OF NEUROMUSCULAR FUNCTION FOLLOWING COMPETITIVE FOOTBALL MATCH-PLAY

7-1 Introduction

In Chapter 7 of this thesis, significant fatigue and neuromuscular adjustments were demonstrated in response to competitive football match-play. Specifically, it was reported that deficits in MVC strength following match-play took up to 72 h to recover, while impairments in both contractile function and the capacity to voluntarily activate the quadriceps required 48 h to recover. Given that successive football matches can often be placed within these time-frames, a challenge for practitioners is to mitigate the magnitude of fatigue and attempt to expedite the recovery process through appropriate interventions in order to prepare players for the demanding fixture periods associated with modern day football and reduce the risk underperforming, or of sustaining injuries.

When implementing recovery strategies aimed at alleviating fatigue and accelerating recovery, it is imperative to understand the stressors causing reductions in performance and delayed recovery before applying the intervention (Howatson *et al*., 2016). In Chapter 5, it was hypothesised that the protracted impairments in contractile and CNS function were likely a consequence of the repeated eccentric contractions associated with match-play and the subsequent muscle damage and inflammatory response which ensues. A number of factors would support this suggestion. Firstly, it is known that football match-play induces considerable muscle damage and a prolonged inflammatory response which can persist for several days post-exercise (Fatouros *et al*., 2010, Ispirlidis *et al*., 2008). Secondly, while impairments in contractile and CNS function can also occur due to metabolic influences (Allen *et al*., 2008), many of the metabolic mechanisms thought to interfere with neuromuscular function dissipate rapidly following exercise cessation. For example, following exercise that imposes large metabolic but little mechanical demand, recovery is

substantially faster than exercise that is mechanically demanding (Skurvydas *et al*., 2016). In addition, the mechanical stress imposed on muscle fibres during eccentric based exercise has been shown to elicit prolonged impairments in the excitation-contraction coupling process (Souron *et al*., 2018), as well as residual deficits in voluntary activation which can take days to resolve (Goodall *et al*., 2017a). As such, it is a plausible assumption that the impaired neuromuscular function which persists for several days following football match-play is primarily a consequence of muscle damage and the associated inflammatory response, and strategies to alleviate the negative effects of muscle damage and inflammation could thus be suitable to accelerate recovery following competitive football match-play.

The precise mechanisms of EIMD are complex and remain to be fully elucidated. However, muscle damage has previously been simplified into two general areas; the initial event that occurs during the exercise bout (termed "primary damage"), and the secondary events that propagates damage through factors associated with inflammation (termed "secondary damage") (Owens et al., 2018b). While the inflammatory response that ensues following EIMD is thought to be crucial in orchestrating muscle repair and recovery (Butterfield *et al*., 2006), the secondary damage associated with inflammation is suggested to further exacerbate impairments in muscle function (Pizza *et al*., 2005). As such, a common target of interventions is to alleviate the negative effects associated with the inflammatory response in an attempt to expedite the recovery process (Howatson *et al*., 2010, Rowsell *et al*., 2011).

A common post-exercise recovery strategy is cryotherapy, which is regularly implemented following football match-play and is believed to attenuate post-exercise reductions in functional capacity and athletic performance (Nedelec *et al*., 2013). While the precise underlying mechanisms remain to be elucidated, cryotherapy is

purported to reduce muscle temperature and increase hydrostatic pressure, thereby reducing inflammation and oxidative stress (White and Wells, 2013b). A recently implemented form of cryotherapy that has produced encouraging results as a recovery aid is phase change material (PCM) (McHugh *et al*., 2018, Clifford *et al*., 2018, Kwiecien *et al*., 2018). Phase change material is a substance with a high heat fusion, which melts and solidifies at certain temperatures. When frozen PCM is heated, for example, through exposure to the human body, it will continuously absorb heat until all material has changed from solid to liquid. As such, PCM can maintain low temperatures for sustained periods. The application of PCM has many logistical and practical benefits due to being easily transportable, the lower level of thermal discomfort compared with CWI, and due to its' high melting point and capacity to maintain low temperatures for a prolonged period of time. A recent study applied cold PCM to the quadriceps for 3 hours following competitive football match-play and found reduced muscle soreness and accelerated recovery of MVC (Clifford *et al*., 2018), findings which have since been corroborated (McHugh *et al*. 2018).

Despite the promising results of recent studies (McHugh *et al*., 2018, Clifford *et al*., 2018, Kwiecien *et al*., 2018), more evidence is required to substantiate the efficacy of PCM as a recovery intervention and to gain mechanistic insight into the potential benefits of PCM on recovery. Accordingly, the aim of the present study was to examine the effect of wearing cold PCM garments on neuromuscular function, as well as physical and perceptual measures following football match-play.

7-2 Methods

7-2.1 Participants

Fifteen male semi-professional footballers from Level 8 of the English Football League gave written informed consent to participate in the study. Throughout the data collection period, 4 players sustained injuries which prevented them from completing the study, leaving 11 participants in total (three defenders, five midfielders, three attackers; 22 ± 1 years; stature 1.80 ± 10 m; mass 78 ± 8 kg). Players trained three to four times a week, in addition to at least one competitive match. The participants competitive season ran from August to May, with testing taking place in the mid-season phase of the players training year. Participants were required to refrain from physical activity and alcohol consumption for the duration of the study and in the 48 h prior to data collection, and abstain from caffeine consumption for the 12-h prior to each experimental visit.

7-2.2 Design

The study employed a randomised cross-over design to assess the effectiveness of PCM on recovery in the days following competitive football match-play. Participants visited the laboratory prior to commencement of the data collection period for habituation to the measurement tools employed in the study. For the experimental trials, participants were required to visit the laboratory prior to and 24, 48 and 72 h following two competitive football matches. The pre-assessment visit took place 24 h before the fixtures. On one occasion, players wore shorts fitted with PCM (Glacier Tek; USDA BioPreferred PureTemp, Plymouth, MN) that was either cooled (PCM_{cold}) or left ambient, which served as our sham control. The order of the conditions was

randomised using an online randomiser (www.randomizer.org). PCM was applied to the quadriceps and hamstring muscle groups, and was worn for 3 h post-match. To ensure compliance with the intervention, away matches in which the team were required to travel back for ≥ 3 h were selected. The two league matches were separated by 4-8 weeks. During each experimental visit, participants completed assessments of neuromuscular, physical, and perceptual function to ascertain the effect of PCM on recovery.

7-2.3 Procedures

Practice trial

Prior to the experimental trials, participants attended the laboratory for habituation with the study procedures. This involved an explanation of the methods employed in the study, before participants performed a practice trial consisting of the neuromuscular, physical and perceptual measures employed in the study. A brief overview of these measures are provided below, alongside further details outlined in Chapter 3 of this thesis.

Competitive football match

Participants visited the laboratory 24 h prior to each match for baseline measurements (described in detail below). On the subsequent day, players completed a 90 min football match within their competitive league consisting of two 45 min halves interspersed by a 15 min recovery interval. Each player competed in two matches as part of the study, with 4-8 weeks separating each match. In total, the study took place across six matches, with five participants investigated following games one and two,

three participants investigated following games three and four, and three participants investigated following games five and six. All fixtures took place on a grass pitch at either 13:00 (games one, two and six) or 14:00 (games three, four and five). Players were required to play a minimum of 70 min per match in order to be included in the experiment. The activity profiles and heart rates of the players were measured throughout the games using GPS with built in heart rate monitors (Polar Team Pro, Polar Electro Oy, Finland), and compared between games in order to ensure the physical and physiological demands of the matches in each condition were similar.

Phase change material

Phase change material is composed of derivatives of vegetable oil, with each block enclosed with a plastic sheeting, as displayed in Figure 6-1. When frozen PCM is exposed to heat, such as the human body, it will continuously absorb heat and change from solid to a liquid, with the temperature being held constant until all material has changed to liquid. Previous work has displayed PCM with a phase point of 15°C takes > 3 h to equilibrate to skin temperature ~33°C, with average skin temperature reduced to ~22°C whilst PCM is applied (Kwiecien *et al*., 2018). As such, PCM was applied for a 3 h period post-match. Prior to the matches, PCM_{cold} were cooled in a freezer to 15°C, while PCM_{amb} were stored > 22°C. The PCM blocks worn over the quadriceps were 32 cm in length and 13 cm in width, while the blocks worn over the hamstrings were 16 cm in length and 13 cm in width. Two blocks were worn on the quadriceps and hamstring muscles inside compression shorts, with blocks placed over the medial and lateral parts of both muscle groups. The PCMs were applied within 30 mins post-exercise, and were worn while travelling back from the matches on the team bus.

Figure 7-1. Phase change material composed of derivatives of vegetable oil enclosed by a plastic sheeting. When frozen PCM is exposed to heat, such as the human body, it will continuously absorb heat and change from solid to a liquid, with the temperature being held constant until all material has changed to liquid.

Outcome measures

A range of neuromuscular, physical and perceptual measures were assessed 24 h pre-match, and 24, 48 and 72 h post-match. An overview of these measures are provided below, with a detailed description outlined in Chapter 3 of this thesis.

Perceptual responses

Participants completed the "*Elite Performance Readiness Questionnaire"* (Dean et al., 1990) at each time point as described in Chapter 3. In addition, similar to a previous study (Clifford *et al*., 2018) participants completed a questionnaire in which they rated

how effective they felt the cold and ambient PCM were going to be for recovery prior to the intervention (pre-match), and how effective they felt they were in improving recovery at the end of the intervention (72 h post-match). The belief questionnaire consisted of a Likert scale from 1 “not effective at all” to 5 “extremely effective” (Appendix 3).

Assessment of neuromuscular function

The neuromuscular assessment consisted of measures of voluntary activation with motor nerve and motor cortical stimulation for the assessment of CNS function, and $Q_{tw,pot}$ for the assessment of contractile function. Details of these measures can be found in Chapter 3 of this thesis.

Assessment of physical function

Participants completed a battery of assessments to measure physical function in variables relevant to optimal football performance. Specifically, CMJ height and DJ-RSI were assessed at each time point. Details of these measures can be found in Chapter 3 of this thesis.

6-2.4 Data analysis

Voluntary activation measured with TMS was assessed during two sets of contractions at 100, 87.5, 75, 62.5 and 50% MVC according to Dekerle *et al*. (2018), and the regression between SIT amplitude and contraction intensity was extrapolated to the y

intercept to obtain an ERT (Todd *et al*. 2003). Whilst the method for assessment of VA_{TMS} employed in Chapter 6, whereby a three-contraction protocol (100, 75 and 50% MVC) repeated three times is the current gold standard, a recent study suggested that a five-contraction protocol (100, 87.5, 75, 62.5 and 50% MVC) repeated two times presents a method which offers improved accuracy when measuring the VA_{TMS} compared with the traditional 3 × 3 protocol (Dekerle *et al*., 2018). While many of the methodological concerns associated with measured VA_{TMS} using the 3 × 3 protocol relate to its' poor reliability when assessed immediately post-exercise, and when using a 3-point linear regression (rather than a 9-point linear regression as was used in Chapter 6; Dekerle *et al*., 2018), the more robust nature of the 2 × 5, 10-point linear regression suggest that this method is likely to become the new gold standard when measuring VA_{TMS}. As such, it was deemed appropriate to utilise this method when assessing VA_{TMS} in the present study. The regression analysis confirmed a linear relationship at each time-point (r^2 range = 0.89 ± 0.04–0.93 ± 0.06). CSE was determined by expressing MEP amplitude as a percentage of M_{max}, which was performed during the VA_{TMS} protocol.

6-2.5 Statistical analysis

Data are presented as mean ± SD. A two-way (treatment × time) repeated measures ANOVA with 2 treatment levels (PCM_{cold} vs PCM_{amb}), and 4 time points (Pre-, 24, 48 and 72 h post-match) was performed. Normality of the data was assessed using the Shapiro–Wilks test. Assumptions of sphericity were explored and controlled for all variables using the Greenhouse-Geisser adjustment, where necessary. In the event of a significant interaction effect (treatment × time), Bonferonni *post hoc* analysis was performed to locate where the differences lie. Paired sample *t*-tests were used to assess

differences in match-running and heart rate variables between the two conditions. The belief questionnaire was analysed using the Wilcoxon signed-rank test. Standardized effect sizes (Cohen's *d*) were calculated for focused pairwise comparisons and interpreted as small (≥0.2), moderate (≥0.6), and large (≥1.2). All data were analyzed using Statistical Package for Social Sciences (SPSS version 22.0). Statistical significance was accepted at $P < 0.05$.

7-3 Results

7-3.1 Match performance and intensity

Match activity and heart rate variables are displayed in Table 7-1. No differences in playing time or any time-point or heart variables were found between the two conditions ($P \geq 0.1$). Players were required to play at least 70 minutes in order to be included in the intervention; no players were excluded on this criteria. In terms of treatment order, six players wore PCM_{amb} first and five players wore PCM_{cold}.

Table 7-1. Match activity and heart rate variables during competitive football match-play for the two conditions (PCM_{cold} vs PCM_{amb}).

	Playing time (mins)	TD (km)	HIR (m)	Accels (no.)	Decels (no.)	Mean HR (bpm)	Max HR (bpm)
PCM_{cold}	83 ± 6	10.1 ± 1.3	1738 ± 478	373 ± 34	382 ± 31	167 ± 9	192 ± 7
PCM_{amb}	81 ± 4	9.9 ± 1.2	1795 ± 415	391 ± 137	369 ± 39	165 ± 5	197 ± 12

TD = total distance; HIR = high-intensity running; Accels = accelerations; Decels = decelerations; HR = heart rate

7-3.2 Perceptual responses

Perceptual responses from the Elite Performance Readiness Questionnaire can be viewed in Table 7-2. Football match-play elicited fatigue ($F_{3,30} = 18.62$, $P < 0.001$)

and soreness ($F_{3,30}$ = 17.99, P < 0.001) which persisted up to 72 h relative to baseline (all $P \leq 0.03$). No effects of PCM were observed for any of the perceptual responses ($F_{3,30} = \leq 0.65$, $P \geq 0.59$). Analysis of the belief questionnaire revealed no differences in the perceived effectiveness of the two treatments either pre- or post-intervention (P = 0.56; Table 7-2).

7-3.3 Neuromuscular function

Neuromuscular function variables are depicted in Figure 7-1. Soccer match-play elicited declines in MVC force ($F_{3,30}$ = 6.26, P < 0.01), VA measured with motor nerve stimulation ($F_{3,30}$ = 5.05, P < 0.01), and $Q_{tw,pot}$ ($F_{3,30}$ = 3.09; P = 0.03), with impairments in MVC and $Q_{tw,pot}$ persisting for up to 72 h post-match (all $P \leq 0.04$), and reductions in VA persisting for up to 48 h post-match (P = 0.03). Measures of VA_{TMS}, corticospinal excitability, or muscle contractility were not changed at any time-point. No treatment × time interactions were observed for any of the neuromuscular variables ($F \leq 2.73$, $P \geq 0.18$). However, MVC and VA measured with motor nerve stimulation were greater under the PCM_{cold} condition, as indicated by the treatment effect (MVC: $F_{1,10}$ = 6.254, P = 0.03; VA with motor nerve stimulation: $F_{1,10}$ = 5.47, P = 0.04). Measures of muscle contractility were not changed at any time-point for either condition.

Table 7-2. Perceptual responses measured through a visual analogue scale (mm) at pre-, and 24, 48 and 72 h post-match (n = 11) for two conditions (PCM_{cold} vs PCM_{amb}). Values are mean ± SD. Significant differences in comparison with baseline indicated by * = $p < 0.05$, ** = $p < 0.01$ and *** = $p < 0.001$.

	PCM_{cold}				PCM_{amb}			
	Pre-	24 h	48 h	72 h	Pre-	24 h	48 h	72 h
Fatigue	15.2 ± 11.8	55.5 ± 17.7**	37.3 ± 21.7*	23.7 ± 9.2	20.9 ± 18.0	51.7 ± 21.0**	41.0 ± 13.6*	24.0 ± 14.6
Soreness	18.6 ± 13.5	53.9 ± 17.7**	40.2 ± 16.1**	20.8 ± 18.3	23.5 ± 20.7	52.1 ± 19.6**	51.8 ± 18.2**	28.4 ± 19.1
Motivated to train	74.4 ± 20.2	51.6 ± 21.4	66.8 ± 14.4	67.6 ± 18.6	71.8 ± 23.6	45.2 ± 18.6	57.2 ± 24.5	64.8 ± 25.3
Anger	11.8 ± 9.4	12.9 ± 10.9	7.5 ± 4.5	7.7 ± 6.9	10.5 ± 9.7	14.6 ± 18.6	8.5 ± 7.1	7.1 ± 6.4
Confusion	18.6 ± 13.5	53.9 ± 17.7	40.2 ± 16.1	20.8 ± 18.3	23.5 ± 20.7	52.1 ± 19.6	51.8 ± 18.2	28.4 ± 19.1
Depression	8.5 ± 6.6	16.0 ± 17.2	8.1 ± 5.7	7.2 ± 4.8	7.7 ± 7.2	8.5 ± 6.6	8.9 ± 8.1	8.9 ± 6.2
Tension	20.5 ± 15.9	33.5 ± 25.1	17.6 ± 14.0	14.5 ± 8.6	18.9 ± 16.2	30.8 ± 22.9	25.0 ± 15.3	18.8 ± 15.6
Alertness	68.4 ± 16.7	46.5 ± 23.2	60.5 ± 17.0	63.9 ± 24.4	66.5 ± 22.4	54.5 ± 20.1	65.4 ± 18.8	65.6 ± 14.9
Confidence	65.6 ± 21.6	71.5 ± 12.3	66.8 ± 22.1	74.5 ± 10.6	71.9 ± 15.3	71.1 ± 12.0	70.7 ± 16.0	75.6 ± 12.5
Sleep	67.1 ± 18.1	63.5 ± 27.5	65.9 ± 18.1	64.2 ± 25.1	72.7 ± 24.4	56.5 ± 27.4	66.5 ± 15.2	63.5 ± 25.4

Table 7-3. Perceived effectiveness of the PCM garments for recovery before and after the intervention.

	PCM_{cold}	PCM_{amb}
Pre-match	3.6 ± 0.5	3.0 ± 0.6
72 h post-match	3.3 ± 0.9	3.0 ± 1.0

PCM = phase change material

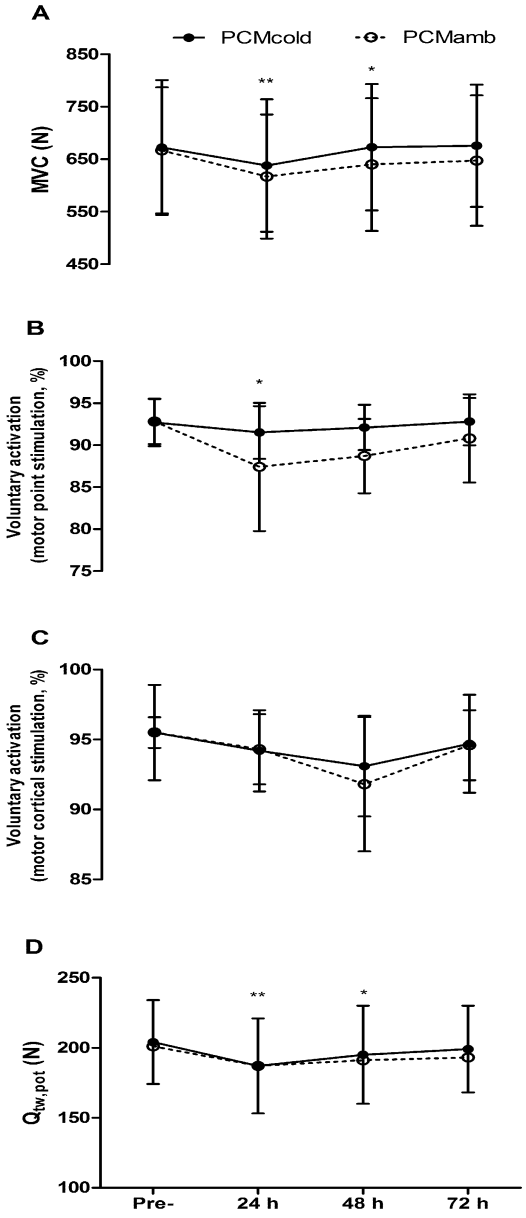


Figure 7-2. Maximal voluntary contraction force (MVC, A), voluntary activation measured with femoral nerve stimulation (B), voluntary activation measured using motor cortical stimulation (C), and quadriceps potentiated twitch force ($Q_{tw,pot,}$D) measured at pre-, 24 h, 48 h, 72 h post- competitive football match-play for two conditions (PCM_{cold} vs PCM_{amb}; n = 11). Values are mean ± SD. Significant differences in comparison with baseline indicated by * = $p < 0.05$ and ** = $p < 0.01$.

7-3.4 Physical function

Physical function variables are displayed in Figure 6-3. There was no effect of PCM on any of the physical function variables (treatment × time $F_{3,30} \geq 1.046$, $P \geq 0.201$). Although a main effect of CMJ height was observed ($F_{3,30} = 5.009$, $P < 0.01$), *post-hoc* analysis revealed no significant differences relative to baseline (Figure 6-3A). For both conditions, RSI was reduced at post-exercise (time effect F3,30 = 7.452, P < 0.01), with *post-hoc* analysis displaying a reduction in RSI at 24 h ($P < 0.05$) and 48 h ($P < 0.05$) relative to baseline, before recovering by 72 h ($P > 0.05$; Figure 6-3B).

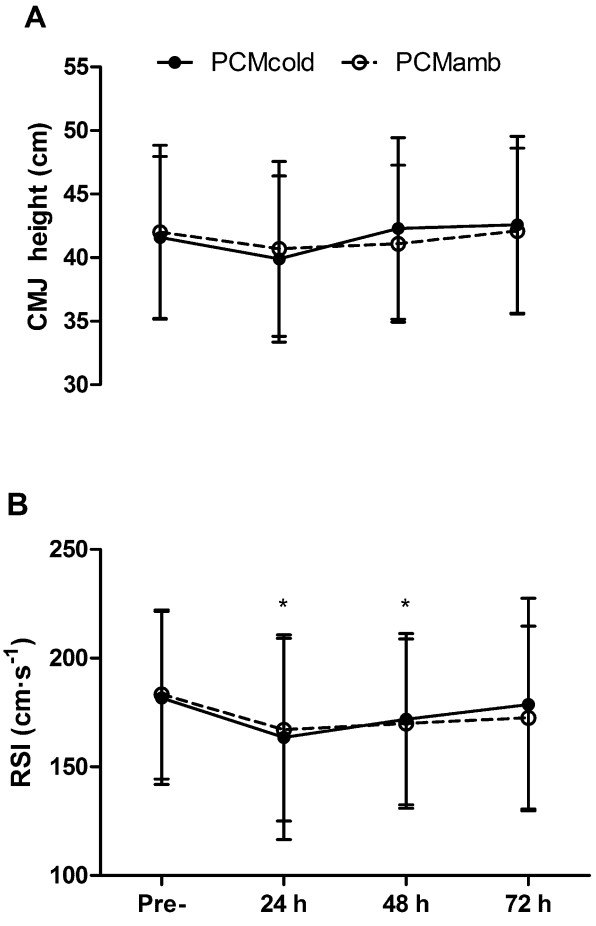


Figure 7-3. Countermovement jump height (CMJ, A) and reactive strength index (RSI, B) measured at pre-, 24 h, 48 h, 72 h post- competitive football match-play for two conditions (PCM_{cold} vs PCM_{amb}; n = 11). Values are mean ± SD. Significant differences in comparison with baseline indicated by * = p < 0.05.

7-4 Discussion

The aim of the present study was to examine the effect of wearing cold PCM garments on recovery of neuromuscular function, physical function and perceptual measures following football match-play. It was hypothesised that wearing cold PCM garments would expedite recovery of impaired neuromuscular function and attenuate muscle soreness, possibly by reducing the negative effects associated with the acute inflammatory response on contractile and CNS function. However, contrary to this hypothesis, the data indicate that wearing cold PCM garments did not favourably affect recovery of any of the neuromuscular, physical function or perceptual indices when compared with wearing ambient PCM garments. As such, the results of the study demonstrate that the prolonged application of cooling garments did not significantly enhance the recovery process following competitive football match-play. These results are in contrast to a number of recent studies that have demonstrated accelerated recovery of muscle function following the application of PCM_{cold} compared with PCM_{amb} (Clifford *et al*., 2018, Kwiecien *et al*., 2018, McHugh *et al*., 2018).

The magnitude of fatigue and the time-course of recovery in the present study was similar to that observed in Chapter 6 of this thesis, as well as previous studies conducted following competitive (Rampinini *et al*., 2011) and simulated football match-play (Thomas *et al*., 2017a). Specifically, MVC was reduced at 24 (PCM_{cold} 5.2%, PCM_{amb} 7.5%) and 48 h (PCM_{cold} 0.0%, PCM_{amb} 4.3%), before recovering by 72 h post-match. Similarly, $Q_{tw,pot}$ was reduced at 24 (PCM_{cold} 8.0%, PCM_{amb} 6.7%), and 48 h (PCM_{cold} 4.2%, PCM_{amb} 3.4%), before recovering by 72 h post-match. Voluntary activation measured with motor nerve stimulation was reduced at 24 h (PCM_{cold} 1%, PCM_{amb} 6%) before recovering by 48 h post-match. In addition, physical function measured through the DJ-RSI was impaired for up to 72 h post-match, while

analysis of perceptual responses indicate that fatigue and muscle soreness persisted for up to 72 h post-match. Thus, the level of fatigue and impairments in neuromuscular and physical function corroborate the findings from Chapter 6 of this thesis. In addition, the reduction in MVC, one of the most widely used indicators of EIMD (Goodall *et al*., 2017a), along with the increase in muscle soreness for up to 72 h post-match, indicates that the competitive football matches involved in the study elicited muscle damage. The occurrence of muscle damage was likely a consequence of the high volume of decelerations recorded throughout the matches along with the numerous other eccentric actions associated with football match-play. Given that recovery of contractile and CNS function has been shown to occur rapidly following exercise that is metabolically, but not mechanically demanding (Skurvydas *et al*., 2016), it is likely that the prolonged impairments in $Q_{tw,pot}$ and VA in the present study were a consequence of the muscle damage incurred during match-play along with the inflammatory response which ensues thereafter.

The lack of an interaction effect between treatment and time for any of the dependent variables indicate that PCM_{cold} had no effect on recovery of neuromuscular function, physical function or perceptual responses following football match-play. These results are in contrast to a recent study conducted by Clifford *et al.* (2018), who displayed a substantially accelerated recovery of MVC strength in the days following football match-play. There are, however, a number of important differences between the studies that could account for these discrepancies. Firstly, a comparison of the decline in MVC strength between the present study and that of Clifford *et al.* (2018) reveals that the reduction in MVC was substantially lower in the present study. For example, at 36 h post-match in the study by Clifford *et al.* (2018), MVC strength remained ~15% below baseline following the application of ambient PCM, while MVC strength was

reduced by just 8 ± 8% following 24 h and 4 ± 5% following 48 h following the application of the same garments in the present study. One possible explanation for the disparity in the recovery rate between the studies is that in the present study, participants refrained from physical activity in the 72 h post-match, while participants continued to train in the days post-match in the study by Clifford *et al*. (2018), potentially compounding the impairments in MVC strength. It is therefore possible that, although the competitive matches in the present study elicited prolonged reductions in the force generating capacity of the muscle, the small magnitude of decrements in MVC strength could have limited the ability to detect any subtle differences between groups. Another potentially important difference between the studies comes from the differences in the results from the belief questionnaires administered in both studies. Specifically, in the study by Clifford *et al*. (2018), players reported that they believed that the PCM_{cold} were more effective in improving their recovery compared with PCM_{amb}, while no differences were found in the present study. Consequently, it is possible that the results of the study by Clifford *et al*. (2018) were influenced by a placebo effect, as was acknowledged by the authors.

A number of previous studies have shown that muscle damage leads to prolonged impairments in both contractile and CNS function, as evidenced through protracted reductions in $Q_{tw,pot}$ and VA . In regards to contractile function, it is likely that the prolonged reductions in $Q_{tw,pot}$ following eccentric based exercise are a consequence of direct myofibrillar damage, disorganization of sarcomeres and interference with cellular Ca^{2+} handling which inhibit the excitation-contraction coupling process (Skurvydas *et al*., 2016). However, events that occur secondary to the initiation of muscle damage have also been implicated in impairments in excitation-contraction coupling. Specifically, the accumulation of reactive oxygen/nitrogen species has been

shown to interfere with SR Ca^{2+} release, which has been attributed to redox modification of ryanodine receptors (Cheng *et al*., 2016). In addition, factors associated with inflammation have also been linked with compromised CNS function (Carmichael *et al*., 2006). For example, group III and IV muscle afferents, which provide inhibitory feedback to various sites within the CNS (Sidhu *et al*., 2017), are sensitive to various markers of muscle injury, such as the release of biochemical substrates (e.g., bradykinin, histamines, and prostaglandins) and factors associated with inflammation (Endoh *et al*., 2005, Pitman and Semmler, 2012, Sidhu *et al*., 2009b), while an increase in brain cytokines following eccentric exercise might also modulate recovery of CNS impairment (Carmichael *et al*., 2006). In this regard, it was thought that the application of cryotherapy, which has been suggested to inhibit the inflammatory response and limit the generation of reactive oxygen/nitrogen species (White and Wells, 2013b), could ameliorate the impairments in contractile and CNS function in the days following football match-play. However, the application of cold PCM had no effect on recovery of either $Q_{tw,pot}$ or VA. The lack of effect of PCM_{cold} on neuromuscular function could have been due to a number of factors. Firstly, whether or not cryotherapy actually reduces inflammation remains equivocal, despite its widespread application (Peake *et al*., 2017b, Broatch *et al*., 2014). Veritably, studies have neither been consistent nor produced compelling evidence to support the role of cryotherapy in reducing inflammation and improving aspects of recovery (Leeder *et al*., 2012), and it has been suggested that many of the previously reported benefits of cryotherapy could simply be due to a placebo effect, rather than any physiological occurrence (Broatch *et al*., 2014). Despite the promising findings from recent studies using cold PCM as a recovery aid (Clifford *et al*., 2018, McHugh *et al*., 2018), and that sporting these garments has been shown to reduce muscle temperature

(Kwiecien *et al*., 2018), there is no evidence to suggest that cold PCM reduces inflammation. As such, it is possible that PCM_{cold} had no effect on the inflammatory processes suggested to interfere with contractile and CNS function. Secondly, as alluded to previously, the magnitude of the impairments in $Q_{tw,pot}$ and VA were relatively small, potentially limiting the ability to detect subtle differences between groups. Indeed, it would be reasonable to assume that the benefits of cryotherapy on recovery would only be evident were the impairments in neuromuscular function more substantial than was seen in the present study. Further research to examine the effects of wearing cold PCM on recovery of neuromuscular function following exercise which elicits more substantial damage is warranted.

Limitations

This study used a competitive football match in order to study the effects of the application of cold PCM on recovery in the days post-match. While this approach provides the most ecologically valid means of investigating the effects of a recovery intervention following football match-play, one limitation of this method compared with a laboratory simulation is the lack of experimental control over the activity profiles of the players and the high inter-subject variability in match demands. Consequently, it is possible that differences between match-demands could have influenced the magnitude of fatigue and time-course of recovery following the two treatments. However, differences between the time-motion and heart rate variables between the matches were negligible. Furthermore, although simulated match protocols are designed to replicate the physiological demands of competitive matches, many of the neuromuscular, skill and cognitive demands associated with competitive

match-play cannot be replicated through match simulations, and the validity of using these protocols when assessing the efficacy of a recovery intervention could thus be questioned. Indeed, in the discussion of Chapter 6 of this thesis, discrepancies between the time-course of recovery of neuromuscular function between competitive match-play and a previous study using a simulated protocol (Thomas *et al*., 2017a) were highlighted. In addition, although no differences were found in the results from the belief questionnaires, on average, participants reported that they believed both PCM_{cold} and PCM_{amb} were "moderately effective" in improving recovery both before and following the intervention. As such, it is possible that a placebo effect could have influenced recovery under both conditions. However, the similar magnitude of fatigue and the time-course of recovery compared with Chapter 6 of this thesis, as well as previous studies following competitive match-play (Rampinini *et al*., 2011, Ascensao *et al*., 2008), suggests that any placebo effect on the results was negligible. Moreover, because local tissue temperature was not measured in the present study, it is unknown whether or not PCM_{cold} had the desired effect in regards to cooling the muscle. Nevertheless, previous work has displayed that PCM_{cold} reduced skin temperature to 22°C for 3 h following eccentric based exercise (Kwiecien *et al*., 2018). Thus, it is likely that the skin temperature was similarly decreased in the present study. Finally, another limitation of the present study was the 4-8 week gap between matches for each condition. Consequently, it is possible that players were in a different phase of the training cycle between the two matches, potentially influencing the magnitude of fatigue and time-course of recovery in response to competitive match-play. However, the majority of fixtures were separated 6 weeks or less, with only two matches separated by 8 weeks. As such, it is likely that the influence of the duration between conditions had a negligible effect on the results of the study.

Conclusion

The present study shows that applying cooled PCM to the quadriceps and hamstring muscles for 3 hours following football match-play has no effect on neuromuscular function, physical function or perceptual responses. It is possible that the lack of effect of these garments could be due to the relatively small impairments in neuromuscular and physical function in the days post-match. For example, the magnitude of the reduction in MVC induced in the present chapter was lower than that found in Chapter 5, with a 7% reduction in MVC compared with a 10% reduction in Chapter 6. Nevertheless, the present findings do not support the use of PCM_{cold} as a recovery intervention following competitive football match-play. The overall aim of this thesis is to examine the aetiology of impaired neuromuscular function following intermittent-sprint exercise. The results of this chapter support that of Chapter 6, which displayed that competitive football match-play elicits considerable reductions in neuromuscular function due to impairments in both contractile and CNS function.

CHAPTER 8 GENERAL DISCUSSION

8-1 Introduction

The overall aim of this thesis was to examine the aetiology of impaired neuromuscular function and the time course of recovery following competitive football match play using novel assessments of contractile and CNS function in order to better understand the mechanisms underpinning fatigue, and driving recovery in the days post-exercise. Chapter 4 determined an optimal protocol for measuring CSE, SICI and ICF in the *rectus femoris* in order to provide methodological guidance for the subsequent chapter when assessing the effect of competitive football match-play on CNS function. Chapter 5 assessed the reliability of measures of neuromuscular, physical function and perceptual measurements which were used in the subsequent chapters of the thesis. Chapter 6 examined the aetiology impaired neuromuscular function and the time-course of recovery thereof following competitive match-play from both central and peripheral origins. In Chapter 7, the effect of wearing cold PCM garments on recovery of neuromuscular function, physical function and perceptual responses following football match-play was examined. This chapter will discuss the main findings of this thesis in the context of existing literature, with specific focus on the prolonged impairment in CNS function following intermittent sprint exercise.

8-2 Main findings

8-2.1 Prolonged impairments in central nervous system function following intermittent sprint exercise

In the exercise sciences, early theorists suggested that a reduction in the maximum force generating capacity of the muscle was a result of physiological impairment, in which mechanisms originating in the periphery were responsible for inhibitions in

forceful muscle contractions, thereby reducing physical work capacity and performance (Edwards, 1981). Despite the focus of early studies, research into the aetiology of impaired muscle function over the last two decades has suggested that the mechanisms contributing to exercise induced reductions in muscle force generating capacity are more complex, and involve an interplay between both central and peripheral factors (Amann, 2011). Specifically, studies have suggested that rather than peripheral factors alone causing impaired muscle function, information regarding exercise-induced biochemical changes within the muscle are relayed to the CNS through metabosensitive thin-fibre muscle afferents (St Clair Gibson and Noakes, 2004, Amann, 2011, Sidhu *et al*., 2017). This afferent physiological feedback results in adjustments in the efferent, feedforward control of motor unit recruitment from the motor cortex of the brain, causing alterations in the type, rate and frequency of motor unit recruitment (St Clair Gibson and Noakes, 2004). It is suggested that inhibitory afferent feedback induces a reduction in motor unit recruitment and/or firing rate, culminating in a reduction in the capacity of the CNS to activate the active musculature (Gandevia *et al*., 1996), although whether afferent feedback directly effects central drive has been the topic of debate (Marcora, 2010, Amann and Secher, 2010). In addition, intrinsic changes within the CNS as a result of repetitive activation during exercise are suggested to contribute to declines in VA (McNeil *et al*., 2011b). Indeed, a number of studies have shown a progressive decline in VA during a range of exercise tasks over a range of intensities and durations (Thomas *et al*., 2016, Goodall *et al*., 2015, Ross *et al*., 2010, Rampinini *et al*., 2011). As such, it is now widely acknowledged that impairments in CNS function make a substantial contribution towards impaired muscle function during exercise. This notwithstanding, research into the mechanisms which contribute to protracted impairments in the force generating

capacity of the muscle which persist, sometimes for days following exercise, has focused almost exclusively on peripheral perturbations, with little or no attention paid to the role the CNS in post-exercise fatigue and recovery (Minett and Duffield, 2014). In Chapters 6 and 7 of this thesis, the application of neurostimulation techniques revealed insights in to the aetiology of impaired neuromuscular function from both central and peripheral origins following competitive football match-play, a sport where recovery is of paramount importance due to the congested nature of modern day fixture schedules.

In both Chapters 6 and 7, it was demonstrated that competitive football match-play elicited impairments in VA which took up to 48 h to recover. This prolonged reduction in VA is in line with previous research examining the aetiology of impaired neuromuscular function following competitive (Rampinini *et al*., 2011) and simulated football match-play (Thomas *et al*., 2017a). As highlighted in a recent review, the mechanisms by which impairments in VA persist following exercise cessation are unknown (Carroll *et al*., 2017). Reductions in the capacity of the CNS to activate the muscle can occur anywhere along the pathway from the motor cortex to the neuromuscular junction. Using single- and paired-pulse TMS, changes within the motor pathway at the corticospinal and intracortical level can be inferred. While a theoretically plausible link exists between exercise-induced alterations in intracortical and corticospinal activity and reductions in VA, whereby a reduction in CSE and/or increase in intracortical inhibition could contribute to impaired motor output and thereby limit voluntary drive to the muscle, an association between these measures has not been established. Nevertheless, previous work has displayed perturbations in CSE and/or SICI during and/or following fatiguing isometric (Kennedy *et al*., 2016), eccentric based (Pitman and Semmler, 2012) and locomotor exercise (Sidhu *et al*.,

2017), suggesting that these circuits could be implicated in the prolonged impairments in CNS function that occur following football match-play. The optimal approach to measuring SICI in the *rectus femoris* was determined in Chapter 4 and applied in the days following match-play in Chapter 6, along with a MEP recruitment curve, deemed the most sensitive measure of motor system excitability (Carson *et al*., 2013). The study found no changes in CSE or SICI in response to competitive match-play at any time-point. It appears that any change in CSE or SICI which might occur during fatiguing exercise is short-lasting, with previous studies displaying a rapid recovery of these variables following locomotor (Sidhu *et al*., 2012) and isometric exercise (Hunter *et al*., 2016). As such, it seems that the excitability of the corticospinal tract and inhibitory circuits play a negligible role in the residual perturbations in CNS function that persist in the days following match-play.

While the mechanisms behind the prolonged reduction in VA displayed in Chapters 6 and 7 cannot be deduced from the findings of this thesis, a number of potential explanations could account for the residual activation deficit. Previous work has shown that the occurrence of muscle damage following eccentric based exercise elicits prolonged reductions in VA, which requires days to resolve. One commonly cited explanation for the link between muscle damage and impaired VA is that 'noxious' biochemical substrates that infiltrate the muscle following EIMD stimulate group III/IV muscle afferents, which relay inhibitory afferent feedback to various sites within the CNS (Endoh *et al*., 2005, Pitman and Semmler, 2012) Indeed, biochemical substrates such as prostaglandins and bradykinins, and factors associated with inflammation such as neuropeptides and histamines, have been shown to activate group III/IV muscle afferents (Clarkson and Hubal, 2002, Smith, 1991). However,

evidence to suggest that afferent feedback directly effects motor output from the CNS is equivocal, and remains hotly debated (Marcora, 2010, Amann and Secher, 2010).

It is known that inflammatory cytokines produced following damaging exercise are a potent effector of CNS function (Carmichael *et al*., 2006). For example, increases in interleukin-1 beta (IL-1β) concentrations within the brain have been displayed following downhill running, concurrent with delayed recovery of running performance in the 48 h post-exercise (Carmichael *et al*., 2006), which is comparable with the recovery time of VA in Chapters 6 and 7 of the present thesis. Elevated concentrations of these cytokines have also been linked with impaired behavioural responses (Dantzer, 2004) and fatigue (Swain *et al*., 1998). Nevertheless, whether or not increases in inflammatory cytokines within the brain contribute to an impaired capacity of the CNS to activate the muscle is unknown, and warrants further investigation.

Although Chapter 6 found no change in the excitability of the corticospinal tract in the days following football match-play, a potential spinal contribution to the impairments in VA cannot be ruled out. Indeed, the mechanisms of reduced VA can be attributed to perturbations at both the spinal and supraspinal level (Gandevia, 2001). At the spinal level, it is possible that motoneurons become less responsive to descending drive from the motor cortex following fatiguing exercise. Indeed, it has been displayed that the responsiveness of motoneurons is impaired during fatiguing isometric contractions (Finn *et al*., 2018). It is unknown, however, whether impairments in the responsiveness of motoneurons persists post-exercise, and whether or not they contribute to a reduction in VA. Further work utilising methods such as cervicomedullary electrical stimulation to elicit MEPs in the hours and days following strenuous exercise could help to shed light on this issue.

Finally, it should also be highlighted that the validity of the activation deficit as a measure of decrements in CNS function remains disputed (Contessa *et al*., 2016). For example, it has been suggested that increases in SIT commonly observed when performing the interpolated twitch technique following exercise inducing fatigue could be solely attributed to peripherally derived factors (Contessa *et al*., 2016). Paradoxically, Place *et al*. (2008) demonstrated the presence of 'central fatigue' in an *in vitro* model using single mouse muscle fibres, and suggested that an intracellular mechanism in the form of an increased titanic Ca^{2+} might be responsible for the SIT evoked by stimulation rather than a central mechanism. Specifically, using single muscle fibres from mice, the study elicited repeated contractions at submaximal frequencies through electrical stimulation, and delivered an extra stimulation pulse during contractions to induce a SIT. The results showed an increase in SIT, which was explained by a shift in the force elicited by 70 Hz constant frequency stimulation to the steep part of the force-Ca^{2+} curve. As such, a small increase in myoplasmic Ca^{2+} concentration upon delivery of the superimposed stimulus resulted in an increase in SIT force. Consequently, the authors suggested that perturbations within the contractile machinery could confound the quantification of VA using the interpolated twitch technique (Place *et al*., 2008). Indeed, the contribution of intramuscular factors to measurement of the SIT cannot be ruled out given that the interpolated twitch technique depends intrinsically on the properties of skeletal muscle to generate force. In this regard, it could be proposed that peripheral factors could have contributed to the declines in VA displayed throughout this thesis. Indeed, the temporal pattern of recovery of $Q_{tw,pot}$ and VA was similar in Chapters 6 and 7. However, whether or not peripheral factors contribute to changes in SIT is disputed, and the findings of Place *et al.* (2008) were opposed in a similar experiment in which SITs were evoked during

involuntary sustained contractions if the human adductor pollicis muscle *in vivo* (Gandevia *et al*., 2013). While measurements of VA, both with electrical stimulation and TMS, have a number of limitations and potential confounding influences, these techniques are widely implemented and believed to be an effective technique for the assessment of VA (Cheng *et al*., 2013).

While it is evident from the findings of this thesis and previous work (Rampinini *et al*., 2011, Thomas *et al*., 2017a, Pointon *et al*., 2012) that association football match-play elicits prolonged reductions in VA, it is difficult to determine just how much impaired VA impacts the performance of football related activities, such as sprint, jump or skill performance, or whether or not impairments in VA would have any influence on running performance or physiological demands during match-play. Given that it is known that declines in MVC force can be largely attributed to reductions in VA, it is plausible that a reduced capacity of the CNS to activate muscles would impede the ability to perform tasks requiring maximal force production, such as when attempting to maximally jump, accelerate, or strike a ball. In Chapter 6, a strong correlation was found between the temporal pattern of recovery of VA and CMJ height, while previous research has reported a correlation between reductions in VA and sprint performance (Rampinini *et al*., 2011). However, these correlations do not imply causation, and given that current methods of determining VA are restricted to isometric or isokinetic contractions, it is not possible to accurately qualify the functional consequences of reduced VA on the performance of unconstrained physical tasks relevant to football performance. Furthermore, reductions in motor unit firing rate, which are known to contribute to exercise-induced declines in VA (Taylor, 2009), could also have implications for the performance of tasks requiring rapid intra- and inter-muscular coordination in order to stabilise joints in response to unexpected

balance perturbations (McLean and Samorezov, 2009). These tasks are commonplace during football match-play, such as when jumping and landing, changing direction, or in response to player-to-player contact, and are associated with the occurrence of injury, particularly during the latter stages of matches when players are in a fatigued state (McLean and Samorezov, 2009). In this respect, another potential implication of impaired CNS function in the days following football match-play could relate to an increased injury risk due to suboptimal muscle activation. While the functional consequences of impaired VA in the days following football match-play cannot be conclusively delineated, the studies of this thesis support previous work which has highlighted the need for a greater focus on the role of the CNS when examining recovery following intermittent sprint exercise and the implementation of appropriate recovery interventions (Minett and Duffield, 2014, Rattray *et al*., 2015).

8-2.2 Prolonged impairment in contractile function following intermittent sprint exercise

The disturbances that occur within the exercised muscle in response to football match-play have been studied extensively, and have been related to EIMD, substrate depletion, and ionic disturbances (Nedelec *et al*., 2012). These disturbances are interrelated, and manifest in a reduction in the capacity of skeletal muscle to produce force in response to neural input. Chapters 6 and 7 displayed that impairments in contractile function took 48-72 h to recover following competitive match-play. As alluded to in these chapters, a number of possible mechanisms could explain the prolonged reduction in $Q_{tw,pot}$. Glycogen depletion throughout football match-play is substantial, and can take 48-72 h to return to baseline (Krustrup *et al*., 2011). Reduced muscle glycogen has been linked with impaired SR Ca^{2+} handling, thought to occur

due to preferential glycogen depletion in the region of the t-tubular-SR junction (Gejl *et al*., 2014). However, impaired SR Ca^{2+} release is thought to occur below critical levels of muscle glycogen (250-300 mmol·kg^{-1} dw) (Ørtenblad *et al*., 2013). While previous studies have demonstrated that glycogen levels can fall to < 200 mmol·kg^{-1} dw in the *vastus lateralis* when measured immediately post-match, by 24 h post this value has been shown to exceed > 300 mmol·kg^{-1} dw (Krustrup *et al*., 2011). As such, while glycogen depletion could contribute to declines in $Q_{tw,pot}$ in the initial hours post-match, it is likely that the contribution of reduced muscle glycogen content to impaired contractile function in the days post-match are negligible. A more likely candidate which could explain the prolonged reductions in $Q_{tw,pot}$ is the muscle damage incurred during match-play and the inflammatory response which occurs thereafter. During the initial damaging event, or 'primary muscle damage', reductions in $Q_{tw,pot}$ can likely be explained by myofibrillar disintegrity and disorganisation of sarcomeres (Skurvydas *et al*., 2016), leading to a reduced ability of the contractile machinery to produce force. However, the long-lasting force depression in response to motor nerve stimulation could also be linked to increased ROS/RNS production and changes in cellular Ca^{2+} handling (Cheng *et al*., 2016). Increases in ROS and RNS occur during metabolically demanding exercise due to increase in the efflux of superoxide anion (O^{-2}) from the mitochondria and an increase in production of nitric oxide (NO) following increased nitric oxide synthase activity, while delayed increases in ROS have been observed following EIMD and have been linked with leukocyte infiltration and inflammation (Nikolaidis *et al*., 2008). A number of studies have displayed that ROS/RNS impede SR Ca^{2+} release, thought to be due redox modifications of RyR (Cheng *et al*., 2016, Cooper *et al*., 2013, Cheng *et al*., 2017). Furthermore, studies have demonstrated a clear benefit of decreased ROS/RNS concentrations on endurance performance when

preceded by fatiguing contractions (Powers and Jackson, 2008, Khawli and Reid, 1994). The detrimental impact of increased ROS/RNS concentrations are most apparent during submaximal contractions, which has been attributed to their marked effects on the steep part of the force-Ca^{2+} relationship, where small changes in SR Ca^{2+} release or myofibrillar Ca^{2+} sensitivity have considerable effects on force production (Allen *et al*., 2008). Given that football match-play has been shown to induce prolonged increases in oxidative stress (Ascensao *et al*., 2008), it is possible that higher concentrations of ROS/RNS could have contributed to the prolonged reduction in $Q_{tw,pot}$ displayed in Chapters 6 and 7 of this thesis.

Impairments in contractile function could have functional implications that are relevant to football performance. If the capacity of skeletal muscle to produce force in response to neural input is diminished, a higher level of neural drive would be required to achieve the same degree of force production. Consequently, the perceived effort for exercising at submaximal intensities would increase in line with the more substantial motor command required. Furthermore, the physiological responses to exercise at a given intensity could also be influenced by a reduced ability of recruited fibres to generate force. For example, recent work has displayed that the maximum sustainable power, or 'critical power', is reduced when its measurement is preceded by prolonged heavy-intensity exercise (Clark *et al*., 2018), possibly due to perturbations occurring within the exercised muscle. In turn, the power or speed below which steady-state values for muscle metabolism (PCr concentration and pH), blood lactate and VO_2 can be attained is reduced. This could be of particular relevance to football performance given that much of competitive match-play involves running at variable submaximal speeds. For example, if successive games are separated by short recovery intervals (i.e. < 72 h), and contractile function remains impaired going in to the subsequent

match, it is possible that the 'critical speed' of a player could be reduced. This could conceivably influence the amount of work done above critical speed, and in turn fatigue development throughout match-play (Jones and Vanhatalo, 2017). Alternatively, or additionally, a reduction in critical speed and concomitant decrease in the speed above which perturbations in muscle metabolic homeostasis occur could influence player pacing strategies during match-play. As such, impairments in contractile function, which were demonstrated to take 48-72 h to recover in Chapters 6 and 7 of the present thesis, could have implications for fatigue development when successive matches are interspersed with short recovery periods, as is often the case in modern day professional football.

8-2.3 Effect of prolonged cryotherapy on recovery from neuromuscular fatigue following intermittent sprint exercise

In Chapter 7, a novel method of cryotherapy known as PCM was applied following competitive match-play with an aim to ameliorate impairments in neuromuscular function and expedite recovery in the days post-match. However, the application of cold PCM on the quadriceps and hamstring muscles for 3 h post-match play had no effect on recovery of any of the neuromuscular, physical function, or perceptual indices when compared with the sham control. While decrements in $Q_{tw,pot}$ and VA measured with motor nerve stimulation were evident post-match, and took 72 h and 48 h to recovery, respectively, the magnitude of these decrements was relatively small. As such, it is likely the small magnitude of the decrements in neuromuscular function could have limited the ability to detect any subtle differences between groups. Despite the lack of difference in any of the variables in the study, the results from the belief questionnaires indicated that participants believed the PCM to be moderately effective

in improving their recovery following the intervention. This could be considered an important finding given that a growing body of evidence indicates that recovery is related to individual preference and perceptions of the intervention (Halson, 2014). Further investigations are warranted to assess whether cold PCM has any effect of neuromuscular function during periods of fixture congestion, when muscle damage could be compounded by the limited recovery periods.

8-2.4 Recovery of fatigue following intermittent sprint exercise

The studies of this thesis investigated recovery of neuromuscular function in the days following intermittent sprint exercise. Impairments in neuromuscular function, including perturbations within the contractile machinery and the CNS, are largely responsible for impairments in the force generating capacity of the muscle. Given that the ability of the muscle to produce force during sports characterised by intermittent sprint exercise, such as association football, is integral when performing activities associated with successful performance, such as sprinting, jumping, or kicking, understanding the contributors to reductions in the muscles maximum force generating capacity, and the time-course of their recovery, is a highly pertinent field of study. In this regard, the studies of this thesis provide valuable and relevant information relating to post-match fatigue and recovery following football match-play. Nevertheless, fatigue is a multifactorial and complex phenomenon, underpinned and/or modulated by a number of psychological and physiological factors (Enoka and Duchateau, 2016). As such, impairments in the force generating capacity of the muscle are just one component of fatigue. Other factors which might not influence the force generating capacity of the muscle, but contribute to fatigue, could also be implicated in fatigue and recovery in the days following football match-play. For example, factors such as

mood, motivation, pain and wakefulness could also be influential in contributing to post-exercise fatigue (Enoka and Duchateau, 2016). While the use of the *Elite Performance Readiness Questionnaire* in the present thesis attempted to measure and account for many of these potential influences, it is likely that other modulating factors could have contributed to fatigue following competitive football match-play. Indeed, in Chapter 5 of this thesis, it was demonstrated that self-reported fatigue persisted 72 h post-exercise, while impairments in MVC had returned to baseline by 48 h. As such, it is evident that when attempting to monitor fatigue in the days following football match-play, a range of fatigue-modulating outcomes should be assessed, including objective measures of physical performance, such as muscle strength and power, along with subjective assessments of athlete fatigue and well-being.

8-3 Practical implications

The results from this thesis could have a number of important practical implications. The topic of recovery in professional football has become increasingly scrutinised in recent years due to the ever-increasing demands of competitive match-play (Di Salvo *et al*., 2009, Barnes *et al*., 2014), and the congested nature of fixture schedules in many top professional leagues. In particular, due to the well-established finding that limited recovery periods between games predispose players to an increased risk of sustaining injuries (Ekstrand *et al*., 2004, Carling *et al*., 2016), understanding the aetiology of fatigue and the time-course of recovery following match-play has become an imperative field of study. For example, when devising the training schedule, understanding the time-course of recovery from fatigue induced by match-play can provide valuable ancillary information for practitioners and help inform training prescription, and when is most appropriate for players to return to training following

match-play. The findings from Chapters 6 and 7 suggest that 48-72 h of recovery are required for impairments in neuromuscular function and MVC force to dissipate following match-play. It should be noted that at 48 h, the reduction in MVC was relatively modest (4% in Chapter 6 and following the application of the sham control intervention in Chapter 7). As such, the functional relevance of such a small decrement in MVC could be questioned. Furthermore, whether or not players need to be fully recovered to produce the physical and technical demands placed on them through training has been questioned (Carling *et al*., 2018). In this regard, it could be suggested that 48 h of recovery are sufficient for players to return to training, despite the persistence of fatigue and reductions in MVC. A cautionary approach could be to ensure that training at this stage post-match is low intensity/volume to avoid compounding any residual impairments in neuromuscular function. Anecdotally, during a regular week of fixtures in which teams participate in 1 game, it is common practice for teams to employ their heaviest training load day 72 h post-match, before a gradual taper to ensure that players are adequately recovered for the subsequent match (Wrigley *et al*., 2012). The findings of this thesis would suggest that employing such a strategy would ensure that players are adequately recovered from the stress imposed by match-play.

Another potential practical implication that can be inferred from this thesis relates to the use of squad rotation strategies during congested fixture periods. During the most demanding stages of a competitive season, teams can be required to play in matches separated by as little as 48 h of recovery. The results from Chapters 6 and 7 would suggest that such a limited recovery period would be insufficient to allow restoration of neuromuscular and physical function, while fatigue and perceptions of muscle soreness also persist at this time-point. Consequently, performance could be

compromised and the stress imposed on the neuromuscular system compounded if players are exposed to such demands. Using squad rotation strategies during congested fixture periods is commonplace, with research displaying that clubs regularly competing in European club competitions spare its' players exposure to congested schedules despite high availability for selection (Carling *et al*., 2016). The evidence provided in this thesis lends support to this practice, particularly when games are separated by < 72 h.

Understanding the aetiology of fatigue is imperative when implementing interventions aimed at accelerating the natural time-course of recovery. Although teams frequently implement squad rotation strategies during congested fixture periods, there are likely to be periods within a season when players are required to play in successive matches with limited recovery periods. In these situations, it might be appropriate to implement recovery interventions in an attempt to facilitate subsequent performance and/or reduce the risk of sustaining injury. Based on the posit that the prolonged impairments in contractile and CNS function displayed in Chapters 6 and 7 were largely due to the damage incurred during match-play, interventions aimed at ameliorating muscle damage and supressing the inflammatory response could be a suitable approach. A number of reviews have summarised evidence for the effectiveness of interventions of recovery following EIMD, with nutritional interventions such as antioxidant and protein supplementation and physiological strategies such as cryotherapy and compression garments commonly implemented in research and practice to varying degrees of success (Howatson and van Someren, 2008, Nedelec et al., 2013, Owens et al., 2018a). There exists a paucity of research examining the effects of these interventions on neuromuscular function following damaging exercise, and this warrants further investigation.

Finally, it is common practice in professional football for practitioners to implement physical and perceptual measures in order to monitor readiness to train/compete (Halson, 2014). Given the impractical nature of implementing neurostimulation techniques in an applied setting, surrogate measures of neuromuscular function, such as CMJ, DJ-RSI and sprint speed are regularly employed (Halson, 2014). Chapter 6 displayed that 20 m sprint time was not sensitive to impaired neuromuscular function, and had recovered by 24 h despite impairments in contractile function and VA persisting. Measures of CMJ and DJ-RSI appeared to be more sensitive, requiring 48 h to recover. However, a lack of association was found between the temporal pattern of recovery of indices of neuromuscular and physical function, suggesting that it would be inappropriate to employ these measures exclusively when attempting to assess recovery of neuromuscular function in practice. Rather, given the multifactorial nature of the fatigue experienced following football match-play, which is likely to be influenced by both physiological and psychological processes, it might be more appropriate to implement both objective and subjective measures of recovery in order to provide a more comprehensive understanding of readiness to train/compete.

8-4 Directions for future research

The data from this thesis provides insight into the aetiology of impaired neuromuscular function following competitive football match-play, and opens up interesting avenues for future research. In Chapters 6 and 7, it was displayed that impairments in contractile and CNS function required 48-72 h to return to baseline. This notwithstanding, players are frequently required to play in successive matches within this time-period during the most demanding stages of the season in terms of fixture scheduling. Although research into the effects of congested fixture periods has general

shown no deleterious effects of fixture congestion on match running and technical performance, it is a well established finding that players are exposed to an increased injury risk during these periods (Ekstrand *et al*., 2004, Carling *et al*., 2016). As such, it is conceivable that fatigue is compounded when successive matches are interspersed with recovery periods which are inadequate for the restoration of normal homeostasis. However, whether or not the impairments in neuromuscular function associated with playing in a single-match are exacerbated during congested fixture periods is unknown, and warrants investigation.

While match-play elicited prolonged impairments in both VA and contractile function, individual differences exist in the magnitude of these impairments and the time-course of recovery. For example, certain players appear to be more prone to impairments in contractile and/or CNS function and thereby experience more profound decrements in neuromuscular function and require a more prolonged recovery period following match-play. An interesting area for future investigation would be to assess whether any association exists between the physiological and morphological profile of players and their neuromuscular response to competitive match-play. Previous work has displayed lower body strength to be negatively correlated with muscle damage incurred following football match-play (Owen *et al*., 2015). Similar research of this nature, whereby the physical profiles of players are assessed in relation to their recovery potential, could provide useful information for practitioners which could help inform training strategies. Furthermore, the effect of morphological characteristics, such as muscle fibre type, could also conceivably influence recovery of neuromuscular function in the days following match-play. Hamada *et al*. (2003) displayed that individuals with a higher predominance of type II muscle fibres exhibited a greater decline in $Q_{tw,pot}$ and a slower rate of recovery in the 5 minute period following

exercise cessation when compared with those with a predominance of type I fibres. Whether or not a similar pattern is observed in response to football match-play is unknown, but could also help to inform and individualise the recovery practices implemented by practitioners and coaches.

An interesting finding from Chapters 6 and 7 came from a comparison of the results with that of Thomas *et al*. (2017a), who assessed neuromuscular function following a simulated football match. Specifically, it was evident that the time-course of recovery in the days post-exercise was markedly faster in the study from Chapters 6 and 7 of this thesis. The magnitude of fatigue and time-course of recovery in Chapters 6 and 7, was markedly similar. For example, in Chapter 5, MVC was reduction by 7% and 4% at 24 and 48 h post-match, and 7% and 4% at the same time-points in Chapter 7. Similarly, $Q_{tw,pot}$ was reduced by 6% and 2% at 24 and 48 h in Chapter 6, and 6% and 4% at the same time-points in Chapter 7. Overall, the decrements in neuromuscular variables had recovered by 48-72 h post-match in both of these studies. In contrast, Thomas *et al*. (2017a) found that impairments in contractile function and the force generating capacity of the muscle persisted at 72 h post-match. An integral difference between these studies, which opens up a potentially interesting area for future research, was the stage of the season in which the two studies were conducted. In Chapters 6 and 7, fatigue and recovery was assessed following a matches in the mid-season and end-of-season phase, when players were conceivably in better physical condition and more accustomed to the demands of soccer match-play, while the study by Thomas *et al.* (2017a) was primarily conducted during the late off-season and early pre-season phase. As such, it is possible that differences exist in the magnitude of fatigue and the time-course of recovery in response to competitive match-play during different phases of training throughout the competitive season. Previous work has

displayed that the fatigue response during match-play is influenced by the stage of the competitive season, with players performing a greater high-intensity running distance during the final quarter of the season compared with the second and third quarters (Silva *et al*., 2013). Furthermore, it is well established that prior bouts of eccentric exercise elicit a prophylactic effect on subsequent bouts of potentially damaging exercise (Howatson and van Someren, 2008), which is mediated by adaptations within the muscle and CNS (Goodall *et al*., 2017a). As such, it is likely that players experience lower levels of muscle damage and a faster rate of recovery as they become more accustomed to the physiological and neuromuscular demands of match-play throughout a competitive season. Indeed, previous work analysing the biochemical and hormonal responses to match-play throughout a competitive season have displayed reduction in markers of muscle damage and inflammation during the latter stages of the season (Silva *et al*., 2014), findings which have been corroborated in American Football players (Hoffman *et al*., 2005). These findings also have implications for future research in terms of strictly defining when in the competitive season players are being tested. Accordingly, an interesting area for future would be to examine differences in the magnitude of decrements in neuromuscular function and the time-course of recovery at different training phases throughout the competitive season

Finally, as discussed previously, more work is required to assess the influence of different recovery interventions on contractile and CNS function. Contemporary recovery strategies, such as antioxidant supplementation, cryotherapy, or compression, focus predominantly on limiting post-exercise disturbances and inflammatory events within the exercise muscle cells. In contrast, little attention has been paid to attenuating decrements in CNS function following intermittent sprint

exercise, as has been highlighted in recent reviews (Minett and Duffield, 2014, Rattray *et al*., 2015). Although a residual activation deficit persisted for up to 48 h post-exercise in Chapters 6 and 7 of this thesis, the magnitude of this decrement was relatively small, while no other changes in measures of CNS function found. As such, it is debatable that recovery interventions should focus on restoration of CNS function in the days following football match-play, and it could be argued that focusing on peripheral perturbations is a more valuable endeavour than attempting to alleviate the small reductions in VA displayed in the days post-exercise. Considering that assessment of contractile function through $Q_{tw,pot}$ is involuntary, and thus devoid of any potential influence of a placebo effect, this offers a valid means of assessing the efficacy of recovery interventions on post-exercise impairments in contractility of the muscle.

APPENDICES

Appendix 1 – Example of participant information sheet and informed consent forms

Recovery of neuromuscular function following competitive football match-play

Investigator: Callum Brownstein

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?

Intermittent-sprint sports like football cause significant fatigue and can take days to recover from. Despite this knowledge multiple games are often scheduled in a week in close proximity. The purpose of this study is to investigate the fatigue experienced after a competitive game of football and study how long it takes the body to recover, with an emphasis on the role of the central nervous system in causing post-match fatigue.

Why have I been invited?

You have been selected as a possible participant in this investigation because you a male, semi-professional football player, aged 18-35 in regular training and competition (3-4 times per week), and are injury-free at the time of the study.

Do I have to take part?

No. It is up to you whether you would like to take part in the study. I am giving you this information sheet to help you make that decision. If you do decide to take part, you can discontinue your involvement in the study whenever you choose, without telling me why. 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?

If you agree to take part you will be required to play 2 x 90 min 11 vs. 11 football games with a group of players of a similar level. Of the 22 players, 16 players will be asked to fully take part in all of the measurements in the study. These are described below, and would involve you being measured before, immediately after, and at 24, 48 and 72 hours after one of the games. If you aren't one of these players you will just take part in the 11 vs. 11 games as a player. Everyone will be asked to fill out pre-screening and health

questionnaires before taking part in which you will be required to disclose information about your health. You will be required to bring football boots suitable for playing on artificial turf, and trainers suitable for performing physical measurements such as jumping and sprinting indoors. For those players taking part in the full study, you will be asked to complete 7 trials around the 11 vs. 11 game as follows:.

Trials 1 & 2 – Practice trials

These trials will require you to visit the lab on two separate occasions before the 11 vs. 11 games, and are designed to familiarise you with the neuromuscular, perceptual and physical assessments involved in the study protocol. Details of the measurements taken are provided below.

11 vs. 11 game: Pre- and post-assessment (trials 3 & 4)

On the first day of the investigation you will be required to take part in a 90-minute 11-a-side football match. A 15 minute break will be given at half time, with no substitutions allowed during the game unless an injury occurs. Before and immediately after the game you will complete tests that will measure your central nervous system and muscle function, your strength, power and speed and your perceptions of muscle soreness (passive and active) and readiness to train. The 90 minute football match as well as the pre- and post-match measures will take approximately 3 hours and 30 minutes.

24 h, 48 h and 72 h post-match (trials 5, 6 & 7)

For 3 subsequent days after the 11 vs. 11 match (24, 48 and 72 hours post-match) you will visit the lab to repeat the assessments of central nervous system and muscle function, your strength, power and speed and your perceptions of soreness and readiness to train, to see how you are recovering after the game. Each of these visits should take approximately 60 min.

DETAILS OF MEASURES

Central nervous system and muscle function measures. This will involve you sitting in a chair with your ankle attached to a rigid cuff. You will be asked to contract against this cuff while we stimulate your central nervous system. Specifically, an electrical stimulus will be delivered to the nerve that activates your quadriceps, and a magnetic stimulus will be delivered to the part of your brain that activates your quadriceps. These measurements will allow us to measure the degree of fatigue in response to the exercise, and the recovery of the central nervous system and muscle.

Physical function. After a standardised 10-15 min warm up you will complete a range of tests to measure your jumping performance, your strength and your speed.

Perceptual measures. You will be asked to mark on a range of scales your perceptions of muscle soreness, fatigue and readiness to train, and you will fill in a questionnaire about your recovery status.

Blood samples. Blood samples will be taken during each experimental visit to measure creatine kinase, which is an enzyme released by muscle when it

is damaged. An alcohol wipe will be used to disinfect the finger immediately prior to the blood sample being taken. In order to ensure that prior physical activity has no influence on the concentration of creatine kinase in the blood or on any of the neuromuscular or performance measures prior to the game, you will be required to abstain from strenuous physical exercise in the 48 hours prior to the game, and in the 72 hours following the game until the final assessment is complete to ensure there is no interference with the recovery process.

What are the possible disadvantages of taking part?

There are limited disadvantages to taking part in the study aside from the significant impact on your time, which we are very grateful for. There is some mild discomfort associated with transcranial magnetic stimulation and electrical stimulation of the nerve. Should you perceive excessive discomfort, you will be able to withdraw your involvement in the study at any time. The practice trials are designed to get you used to this procedure, which is unfamiliar to most people.

Maximal voluntary contractions, sprints and jumps involve some physical effort but are brief and any fatigue disappears after a few seconds. These physical tests also cause a brief increase in blood pressure, as is the case for any forceful muscle contraction. There is a potential risk for injury during the football match and the physical tests, but this is no greater than that associated with normal human movements of this nature. A number of first aiders will be on hand at the side of the pitch in the event that an injury occurs.

What are the possible benefits of taking part?

The 16 players who take part in all aspects of the study will receive £100 as payment for their time. The remaining players who take part in the two games will be paid £30. Your participation will help develop knowledge and understanding of the mechanisms of fatigue and the time scale of recovery following competitive football match-play.

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 collected will be kept on a secure password protected computer system. All paper data will be kept in locked storage. All data will be stored in accordance with University guidelines and the Data Protection Act (1998). All data will be destroyed 3 years following data collection.

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.

If I would like to make a complaint about the study, who can I contact?

If you are unsatisfied with any element of the study you can contact the principal investigator or their supervisor (contact details below), or the Department representative for the postgraduate research ethics committee, Dr Mick Wilkinson (mic.wilkinson@northumbria.ac.uk / 0191 243 7097), to discuss your concerns.

Who is Organizing and Funding the Study?

The study will be organised and funded by Northumbria University.

Who has reviewed this study?

The Faculty of Health & Life Sciences Ethics Committee at Northumbria University

Contact for further information:

Researcher email: callum.brownstein@northumbria.ac.uk

Supervisor email: kevin2.thomas@northumbria.ac.uk

Faculty of Health & Life Sciences

CONSENT FORM

Project Title: Recovery of central nervous system function following competitive football match-play

Principal Investigator: Mr. Callum Brownstein

	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).....…….

FOR USE WHEN TISSUE IS BEING REMOVED AND STORED

Principal Investigator: _____

I agree that the following tissue or other bodily material may be taken and used for the study:

Tissue/Bodily material	Purpose	Removal Method
e.g. saliva	*e.g. for cortisol analysis*	*e.g. via Salicaps*

I understand that if the material is required for use in any other way than that explained to me, then my consent to this will be specifically sought. I understand that I will not receive specific feedback from any assessment conducted on my samples, but should any kind of abnormality be discovered then the investigator will contact me.

I understand that the University may store this tissue in a Licensed Tissue Bank only for the duration of the study, it will then be destroyed.

Method of disposal:

Clinical Waste ☐

Other ☐

If other please specify.....

I consent to the University distributing this tissue to partners in this research study, outside of the University, for further testing (please tick the box if you agree). ☐

Signature of participant..... Date.....……..

Signature of Parent / Guardian in the case of a minor

..... Date.....……..

Signature of researcher..... Date.....……..

Participant Screening
Telephone / Personal Interview / Eligibility Checklist

Name: Date:

Please read the following statements; If you answer yes to any of the following questions you are not eligible to take part in the study. You do not have to disclose any information to the investigator.

Have you ever broken a bone in your arm and/or hand?

Do you have pain in your arms and your hands?

Have you ever been diagnosed with a neurological disorder?

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

Have you ever had a stroke?

Do you have any metal objects in your head?

Are you taking any medications that you know would affect neuronal conduction?

Do you have a pacemaker?

Have you had any operations involving your heart?

Do you have a metal plate in the skull, metal objects in the eye or skull (for example after brain surgery or shrapnel wounds)?

Do you know of any reason you should not exercise?

Are you recovering from an illness, injury or operation?

When you perform physical activity, do you feel a pain in your chest?

When not performing physical activity, have you recently suffered chest pain?

Do you ever lose consciousness or lose your balance due to dizziness?

Do you have bone or joint problems that may be made worse with physical activity?

Have you had to suspend your training in the last 2 weeks due to injury or illness?

Do you suffer from any muscle or joint injury that could affect your ability to exercise?

Do you have any history of stroke, epilepsy, head trauma or migrane?

Signature of Participant.....Date.....

Appendix 2 – Elite performance readiness questionnaire

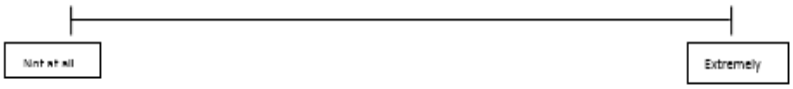
Participant code:

Elite Performance Readiness Questionnaire

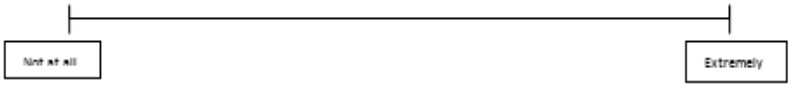
Below are 10 questions we would like you to answer concerning how you feel. These questions are answered by indicating your feelings on a continuous line that ranges from 'not at all' to 'extremely.' Please read each one carefully then put a vertical line along the line that best describes HOW YOU FEEL RIGHT NOW in relation to your sport. Make sure you answer every question.

E.g.

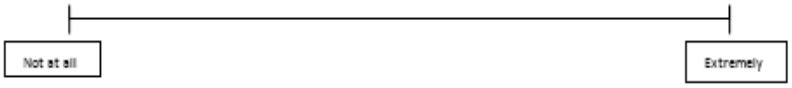
1. How angry do you feel?



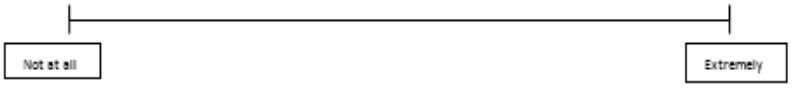
2. How confused do you feel?



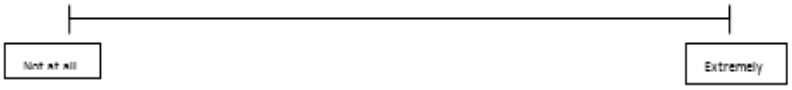
3. How depressed do you feel?



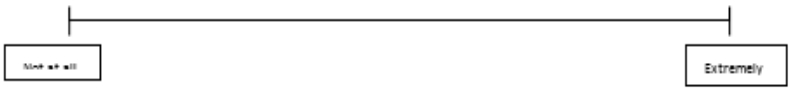
4. How fatigued do you feel?



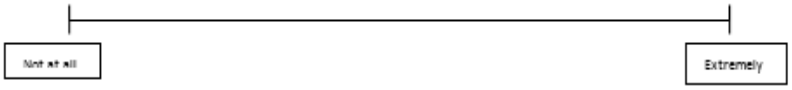
5. How tense do you feel?



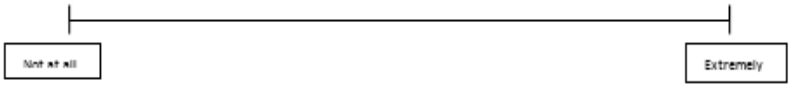
6. How alert do you feel?



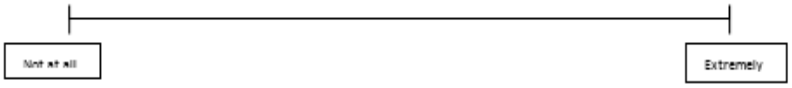
7. How confident do you feel?



8. How sore do your muscles feel?



9. How motivated to train do you feel?



10. How well do you feel you have slept?

Appendix 3 – Belief questionnaire

Belief questionnaire

(PRE – to be completed before the intervention)

Participant: _____ Garment? (PCM_{AMB}/PCM_{COLD}): _____

How effective *do you think* this garment *will be* for enhancing your recovery? Please circle your answer below.

1	2	3	4	5
Not effective at all	Minimally effective	Moderately effective	Very effective	Extremely effective

(POST – to be completed AFTER the intervention – 72 h post)

Participant: _____ Garment? (PCM_{AMB}/PCM_{COLD}): _____

How effective *did you think* this garment *was* for enhancing your recovery? Please circle your answer below.

1	2	3	4	5
Not effective at all	Minimally effective	Moderately effective	Very effective	Extremely effective

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