An Investigation of Experimental Muscle Pain on Neuromuscular Fatigue and Endurance Performance

This thesis is presented for the degree of Doctor of Philosophy at the University of Kent

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Declaration

No part of this thesis has been submitted in support of an application for any degree or other qualification of the University of Kent, or any other University or Institution of learning.

Covid Mitigation Statement

During my second year as a PhD candidate, the Coronavirus global pandemic begun and caused a significant level of disruption to my studies. As the virus was initially spreading within the UK during early March 2020, emergency university procedures resulted in my PhD data collection being ceased with immediate effect. At this time, I was in the process of completing study two and three and I was performing pilot work for study four. Unfortunately, three participants were taking part in my studies and their data had to be discarded. With an initial correspondence from the government estimating twelve weeks to overcome the spread of the virus, we held off from implementing a mitigation strategy as we were ahead of schedule with data collection. Testing was only permitted to resume for myself on the 1st of August 2020. Subsequently, two lockdowns occurred from the 2nd of November to 2nd December 2020 and from 6th January 2021 to 8th March. Whilst testing was allowed in these latter two lockdowns, restrictions to travel, testing, and restrictions to external participants impeded the ability to test. As a result, study four suffered from a smaller sample size. The completion of an online or non-contact study as an alternative was not suitable due to the mechanistic and human based focus of the PhD.

Abstract

The determinants of exercise-induced fatigue are a contentious topic within exercise physiology and the limitations to endurance performance are still not fully understood. Exercise-induced pain is often present during high intensity exercise and typically occurs in parallel with exercise-induced fatigue. Therefore, it is possible that exercise-induced pain may be in part responsible for the development of neuromuscular fatigue and be a limitation to endurance performance. Therefore, the purpose of this thesis was to experimentally increase muscle pain and investigate the development of neuromuscular fatigue during endurance exercise. Experimental muscle pain was caused with an intramuscular injection of hypertonic saline into the vastus lateralis whereas an isotonic injection served as the non-painful control.

Four experimental studies were conducted for this thesis. The first study aimed to investigate the test-retest reliability of an isometric time to task failure (TTF) of the knee extensors and measures of neuromuscular fatigue. This was to determine if these measures were sufficiently reliable to investigate the effects of pain on fatigue and endurance performance. The second study was to determine the effect of localised muscle pain on the performance of an isometric TTF of the knee extensors and measures of neuromuscular fatigue. Study three was to explore if non-local pain could also affect isometric endurance performance and the development of neuromuscular fatigue. The final study sought to investigate if whole-body, self-paced cycling exercise was also impaired by elevated muscle pain.

The results of study one showed that an isometric knee extensor TTF displayed good reliability displayed by a coefficient of variation = 5.1% [95% CI: 2.9 - 7.3] and standard error of measurement = 21 s. Similarly, measures of neuromuscular fatigue displayed good reliability in the presence of exercise-induced fatigue (all coefficient of variation < 10%). Study two revealed that elevated muscle pain reduced isometric endurance time by 16% and this was due to a reduction in maximal strength and a reduction in voluntary activation (exacerbated central fatigue). Similarly, study three showed that non-local pain can reduce endurance time by 10% due to a decreased voluntary activation. Short duration self-paced cycling exercise remained largely unaffected in the presence of elevated muscle pain and the development neuromuscular fatigue was also unaltered.

In conclusion, muscle pain appears to exacerbate the development of neuromuscular fatigue and impose a significant limitation to single-limb time to exhaustion exercise. However, self-paced exercise appears to be less affected by elevated pain and requires further investigation.

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During my PhD, there has been several people who have been invaluable for their personal and/or a professional impact on myself. Without these people, this PhD would undoubtedly be nowhere near as smooth or as good without them.

I would firstly like to thank my primary supervisor Dr Lex Mauger for having the faith in me to do a PhD with him. Throughout the last three years he has given me the freedom and independence to allow me to develop into an independent researcher but has also been there whenever I have needed the help. And for that I am very grateful.

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Finally, I would like to thank all other members of staff and students within the Kent School of Sport and Exercise Sciences for being such a pleasant bunch and making the whole PhD experience much more enjoyable. With the isolation of the coronavirus pandemic, I have come to appreciate the positive environment our school fostered.

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Abbreviations

± = Plus or minus

< = Less than

>..= More than

% = Percentage

0.5RT = Half relaxation time

ADM = Adbductor digiti minimi

Ago = Agonist Muscle

Ag = Silver

Ag/Cl = Silver chloride

AMT = Active motor threshold

Ant = Antagonist muscle

ANOVA = Analysis of variance

ASICs = Acid sensing ion channels

AUC = Area under the curve

CAR = Central activation ratio

CM = Centimetres

CMEP = Cervicomedullary evoked potential

CT = Contraction time

CTRL = Control condition

CV = Coefficient of variation

d = Cohen's D effect size

DOMS = Delayed onset muscle soreness

EMG = Electromyography

EIP = Exercise-induced pain

ERT = Estimated resting twitch

H^+ = Hydrogen ions

HIIT = High intensity interval training

HYP = Hypertonic saline condition

ICC = Intraclass correlation coefficient

ICF = Intracortical facilitation

ISO = Isotonic saline condition

ITT = Interpolated twitch technique

K^+ = Potassium ions

Kg = Kilograms

La^- = Lactate

LICI = Long interval cortical inhibition

M1 = Motor cortex

MDC = Minimum detectable change

MEP = Motor evoked potential

MICT = Moderate intensity continuous exercise

M = Metres

Ms = milliseconds

mV = millivolt

M_{max} = Maximum M-wave amplitude

MVC = Maximum voluntary contraction

MVF = Maximum voluntary force

MVT = Maximum voluntary torque

N = Newtons

$N.s^{-1}$ = Newtons per second

NLMF = Non-local muscle fatigue

NMF = Neuromuscular fatigue/function

PANAS = Positive and negative affects schedule

$PETO_2$ = End-tidal partial pressure of oxygen

$PETCO_2$ = End-tidal partial pressure of carbon dioxide

Pi = Inorganic phosphate

PNS = Peripheral nerve stimulation

PPO = Peak power output

RF = Rectus femoris

RMS = Root mean square

RMT = Resting motor threshold

RPE = Rating of perceived effort

S = seconds

SEM = Standard error of measurement

SICI = Short interval cortical inhibition

SP = Silent period

Stb = Stabiliser muscle

Syn = Synergist muscle

TENS = Transcutaneous electrical nerve stimulations

TMS = Transcranial magnetic stimulation

TT = Time-trial

TTF = Time to task failure

VA = Voluntary activation

VAS = Visual analogue scale

$\dot{V}e$ = Minute ventilation

VL = Vastus lateralis

VM = Vastus medialis

$\dot{V}O_2$ = Volume of oxygen consumed

W = Watts

Publications and Presentations

Articles Published in Scientific Journals.

Norbury, R., Smith, S.A., Burnley, M., Judge, M. and Mauger, A.R., 2021. The effect of elevated muscle pain on neuromuscular fatigue during exercise. *European Journal of Applied Physiology*, 122(1) pp.113-126. https://doi.org/10.1007/s00421-021-04814-1

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Conference Presentations

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Chapter 1: Introduction and Literature Review

Section 1 – Fatigue

1.1.1 What is Fatigue?

The term 'fatigue' is broad and can be interpreted in a multitude of ways. Fatigue can be described as a disabling symptom which encompasses perceived fatigability (e.g., subjective measures) and performance fatigability (e.g., declines in muscle force/contractile function) (Enoka and Duchateau, 2016). As outlined by Enoka and Duchateau, pain may be one input into the development of fatigue by either increasing the perceived fatigability and/or performance fatigability through a decrease in voluntary activation or maximal strength (Thomas, Goodall and Howatson, 2018).

1.1.2 Aetiology of Fatigue

As previously mentioned, fatigue is a multi-faceted phenomenon and even when we study a specific type of fatigue (e.g., performance fatigability), the aetiology of force generating capacity can still originate from a multitude of mechanisms that are influenced by the type, duration, intensity, and environment. For the scope of this literature review, the aetiology of fatigue will primarily be focussed on short duration (< 10 minutes) exercise which is above the critical power/torque, classified as severe or extreme intensity (Burnley and Jones, 2018).

Fatigue can be dichotomised into two distinct origin, central and peripheral (Bigland Ritchie *et al.*, 1978; Kent-Braun, 1999). Central fatigue refers to the reduction in muscle force from changes to the central nervous system which causes a failure to adequately drive the motor neurones (Gandevia, 2001). Conversely, peripheral fatigue refers to changes within the periphery at or distal to the neuromuscular junction which causes a reduction in the force generating capacity of the muscle (Allen, Lamb and Westerblad, 2008). The relative contribution of each type of fatigue is depends on the exercise task performed. To summarise, peripheral fatigue tends to develop to a greater extent during short duration, high intensity bouts of exercise whereas central fatigue is usually greater during longer duration, lower intensity exercise (Thomas *et al.*, 2016). Nevertheless, there is often a combination of both central and peripheral fatigue present after exercise.

It is important to not view these two different types of fatigue in isolation, as there is likely an interaction between the development of central and peripheral fatigue, known as the 'afferent feedback' model of fatigue (Amann *et al.*, 2020). Intense muscular contractions cause the accumulation of metabolites including (but not limited to) Hydrogen Ions (H^+) , Potassium Ions

(K^+), lactate (La^-), and bradykinin. High intramuscular and interstitial concentrations of these metabolites have been shown to increase the discharge rate of type IV afferent fibres (Kumazawa and Mizumura, 1977; Graham *et al.*, 1986; Sinoway *et al.*, 1993). Type III afferent fibres are also stimulated by intense muscular work, however these fibres are preferentially stimulated by mechanical deformation and high intramuscular pressures (Kaufman and Rybicki, 1987). High concentrations of these biochemicals can sensitise type III afferents, reducing their stimulus threshold (Fock and Mense, 1976; Mense and Meyer, 1988). These fatigue-sensitive muscle afferents in exercising muscles relay neural information to the central nervous system on the physiological state of the muscle and therefore regulate the amount of central motor drive to the muscle to prevent a catastrophic disruption to muscle homeostasis. Support for this concept comes from a consistent level of end-exercise peripheral fatigue development across a variety of conditions during cycling exercise (Romer *et al.*, 2007; Amann and Dempsey, 2008; Johnson *et al.*, 2015). The idea of a 'critical threshold' concept is that once a certain (critical) level of peripheral fatigue is attained, central motor drive to that muscle will be constrained to prevent further development of peripheral fatigue. Experimental evidence for the critical threshold of peripheral fatigue was provided by Hureau and colleagues (Hureau *et al.*, 2014) who found that the development of peripheral fatigue after multiple repeated sprints was the same when prior peripheral fatigue was induced with electrically evoked contractions compared to no prior fatigue. Furthermore, partial blockade of afferent feedback results in an exacerbation of peripheral fatigue (Amann *et al.*, 2009; Hureau *et al.*, 2019). However, like all models of fatigue, this concept has been disputed (Marcora, 2010; Neyroud, Kayser and Place, 2016) and it seems that the consistent level of peripheral fatigue is more of an artifact of the mean data or is by-product of a critical loss of maximal force generating capacity which dictates task failure. Nevertheless, despite the existence of a critical threshold being debated, it is generally accepted that afferent feedback can constrain central motor drive.

Unfortunately, it is difficult to provide direct evidence of the cause-effect relationship for the afferent feedback model in-vivo because the methods required to measure the stimulation of group III/IV afferents requires unfeasibly invasive techniques. Much of the literature on muscle afferent firing has been conducted in anaesthetised animal models which makes the extrapolation to an intact human during exercise somewhat limited. Indirect experimental evidence for afferent feedback mediated fatigue pathway has been demonstrated using several novel experimental designs in the exercising human. Most notably, a study by Amann *et al.*

(2009) caused the pharmacological blockade of afferent feedback from the locomotor muscles during cycling exercise. In this study, participants received an intrathecal injection of fentanyl to block afferent feedback before completing a 5 km cycling time-trial. As a result of this, in the first 2.5 km participants had a greater power output compared to the control (intrathecal injection of saline). Consequently, the latter 2.5 km saw a reduction in mean power output and a greater magnitude of peripheral fatigue, while central motor drive (inferred by EMG) was 8% higher. This indicates that afferent feedback constrained central motor drive, thus a higher central motor drive was permitted. One major limitation of this study was that the cardio-pulmonary response to exercise was attenuated, resulting hypoventilation as the cardiovascular and ventilatory response to exercise is partially regulated by activity of specific afferent fibres (which were affected by the fentanyl). This may partly explain why performance was unaffected, as future research using the same experimental design but with a preservation of O_2 delivery resulted in improved endurance with afferent feedback blockade (Hureau *et al.*, 2019). On the other hand, increasing the amount of afferent feedback has been shown to exacerbate central fatigue (Khan *et al.*, 2011; Amann *et al.*, 2013; Kennedy *et al.*, 2013; Johnson *et al.*, 2015). Methods of increasing muscle afferent feedback typically involve performing a fatiguing task and subsequently occluding venous return with a sphygmomanometer. This prevents the clearance of metabolites and maintains firing of afferent feedback. When this is performed, voluntary activation does not recover (Gandevia *et al.*, 1996).

These findings are particularly interesting here because studies maintaining afferent feedback firing with muscle occlusion also cause high levels of muscle pain. Furthermore, experimental muscle pain such as hypertonic saline injections stimulates the type IV afferent nociceptors. The findings of the saline studies also find an impaired force generating capacity without changes to the peripheral apparatus (Graven-Nielsen *et al.* 2002; Khan *et al.* 2011; Park and Hopkins 2013).

It would be unjust to solely attribute the limiting factor of short duration, high intensity endurance exercise to the inhibition mediated by afferent feedback. There is of course the accumulation of metabolites impairing calcium ion release (Allen, Lamb and Westerblad, 2008), respiratory muscle fatigue mediated vasoconstriction (Romer and Polkey, 2008), muscle pain (Stevens *et al.*, 2018) and psychobiological components (Pageaux, 2014) which all play important roles in the aetiology of fatigue. One model which takes a more wholistic approach

and assimilates many of these factors is the sensory tolerance limit (Hureau, Romer and Amann, 2018). This model considers the inhibitory afferent feedback from the locally worked muscles as well as non-local muscles (e.g., respiratory muscles during cycling exercise or prior exercised muscles). Feedforward mechanisms are also considered, primarily the magnitude of efferent copies detected in the sensory cortex associated with central motor command required to generate forceful contractions. The sum of all of these components contribute to a global 'sensory tolerance limit' (Figure 1.1) and the proximity to this sensory tolerance limit could be measured by the rating of perceived exertion.

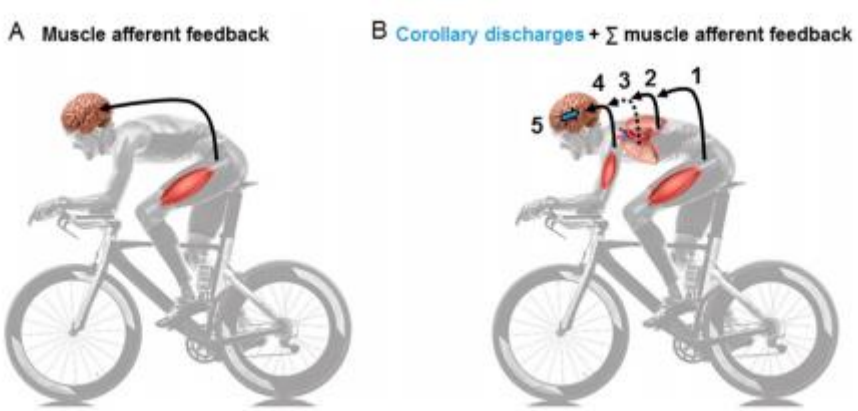

Figure 1.1. From Hureau, Romer and Amann (2018). The conceptual basis of the sensory tolerance limit. Corollary discharge and afferent feedback from skeletal muscle contributes to the limit of exercise tolerance.

Evidence for the support of a sensory tolerance limit was demonstrated in a simple yet intelligent study design by Rossman *et al.* (2014). They got participants to perform dynamic knee extensor exercise to the limit of exercise tolerance with either one leg (unilaterally) or simultaneously with both legs (bilaterally). When the knee extensor exercise was performed bilaterally there was a 19% reduction in time to task failure and 25% less peripheral fatigue compared to when performed unilaterally, suggesting that the inhibitory afferent feedback from the extra leg muscle mass and the additional corollary discharge required to drive two limbs as opposed to one caused an individual reach their sensory tolerance limit faster and thus less peripheral fatigue could develop. Similarly, upper body arm cranking exercise which preceded lower body cycling reduced the time to task failure by 38% which coincided with less of a decrement in maximum voluntary force and peripheral fatigue compared to no prior exercise (Johnson *et al.*, 2015). This data demonstrates that the amount of muscle mass active before/during exercise can play an important role in the magnitude of peripheral fatigue that is

generated. Small muscle mass exercise (e.g., single limb isometric knee extensor contractions) may allow for the attainment of greater levels of peripheral fatigue as the individual muscle is contributing majorly to the sensory tolerance limit (Thomas, Goodall and Howatson, 2018). This is important because the aetiology of fatigue from small muscle mass exercise (e.g., single-limb isometric) may be different to that of large muscle mass exercise (e.g., whole-body dynamic) and therefore the limitations to exercise may be different.

The different components of fatigue (e.g., central, and peripheral) have briefly been defined at the beginning of this section, however it is important to provide a detailed background on these measures and to evaluate their usefulness and limitations for the study of exercise induced fatigue research. The following sections will address the most common measures and interpretations of exercise-induced fatigue.

1.1.3. Global Fatigue

Global fatigue, or more commonly referred to as exercise-induced fatigue, reflects a reduction in the force generating capacity of the muscle or a reduction in exercise capacity. Therefore, there is no specific distinction for the site of fatigue, rather it is the summation of different mechanisms which effects the end-product of force generating capacity.

Measuring Global Fatigue

Global fatigue is non-specific, which means that it does not distinguish between the aetiology of the loss in force. Because of this, the measurement of global fatigue is relatively simple. Typically, global fatigue is quantified as any reduction in the outcome measurement of a task that requires a maximal effort. Outlined below are some of the common measures of global fatigue.

Maximum voluntary contractions (MVC) are commonly used to quantify global fatigue. Participants are seated in a dynamometer with their body fixed at specified joint angles. An individual then attempts to push or pull (depending on the joint action) as hard as possible against a non-compliant strap which is connected to a force transducer. This can be either isometric (static joint angle) or isokinetic (set joint range of motion and angular velocity). The maximum torque achieved during these efforts are recorded at baseline in a non-fatigued state and are subsequently repeated after exercise. Because of this, strong verbal encouragement is required to ensure participants exert maximally. Reductions in the maximum force that is produced relative to baseline is indicative of global fatigue. The advantage of this method is that it can be combined with other measures of neuromuscular fatigue such as peripheral nerve

stimulation and electromyography. It is also quick and simple to perform which allows for multiple measurements during and after a bout of exercise. The MVC is not without its limitations however, participants require a large degree of familiarisation and motivation to be able to exert their true maximal force. Additionally, a brief isometric contraction in a single muscle group is limited in interpretation to explain the aetiology of fatigue during dynamic, whole-body exercise where multiple muscle groups and muscle lengths are used. Furthermore, a reduction in MVIC does not necessarily reflect the aetiology of fatigue during submaximal exercise (Place and Millet, 2019). Nevertheless, when adopting a reductionist and more mechanistic methodology an MVC can be useful and is considered the 'gold standard' of strength measurement (Vøllestad 1997).

In a similar manner to MVCs, an assessment of PPO requires participants to maximally turn their legs on the pedals to maximise cycling cadence thus power. This exercise requires multiple muscle groups to exert force dynamically and is arguably more applicable to sporting performance. Unfortunately, it is difficult to combine a measure of PPO with other measures of neuromuscular fatigue which limits how informative this measure can be.

Measures of exercise capacity/exercise performance are often used to quantify the effect of fatigue on exercise performance. Any reduction in the performance of an exercise task can often be attributed to an exacerbation of fatigue. There are two distinct exercise tasks that can be performed which will be outlined. A time trial task requires participants to complete as much work as possible in a specific amount of time or to cover a specific distance as quickly as possible. This method of assessing performance involves an element of pacing thus variables which are manipulated which may be implicated in the conscious regulation of work would be more applicable to real world endurance performance (Hettinga *et al.*, 2017). Conversely, there is the constant load time to task failure (TTF) exercise task. This requires participants to complete a fixed intensity of exercise for as long as possible until they can no longer sustain the task. The inability to continue may relate to a cut-off point set by the investigator (e.g., fall in cadence or target force) or may be from a voluntary disengagement from the participant. A reduction in TTF or distance covered/completion time between conditions is indicative of greater fatigability. Constant load tasks are sometimes referred to as time to exhaustion, however this is a bit of a misnomer as true exhaustion is often not reached, in fact there is still a significant force generating capacity of the muscle, just not enough to sustain the task. The primary limitation of TTF tests is that it lacks validity to sporting performance which makes

the extrapolations of fatigue from this task somewhat limited as the end point is arbitrary. However, it could be argued that races are a pseudo-TTF for all but the race winner. This is because competitors who are not the fastest are attempting to keep pace with the fastest until it is no longer possible for them.

Despite its limitations, the TTF exercise modality benefits from a greater ability to hold multiple physiological variables constant (e.g., power/speed and cadence) and can provide useful insight into the mechanistic basis of an intervention.

Reliability

Before a discussion of the reliability of these measures can be made, it is imperative to clearly establish what reliability is and how it is quantified. Broadly speaking, reliability refers to how consistent a measure is. In the context of this thesis the term reliability will refer to the test-retest reliability of a measure which means how similar are values measured when recorded by the same investigator on the same participant across different testing sessions (days). Reliability can be categorised into two separate measures: absolute reliability and relative reliability. Absolute reliability refers to the degree to which measures are consistent across different days. Relative reliability refers to how consistent a measure stays within its position amongst a number of other measures. The coefficient of variation (CV%) measures absolute reliability which is calculated as:

$$CV\ (\%) = \frac{Standard\ Deviation}{Mean} * 100$$

A CV of < 10% is arbitrarily considered acceptable (Atkinson and Nevill, 1998). Relative reliability is calculated using the intraclass correlation coefficient (ICC) with a value being closer to 1 representing greater relative reliability. The interpretation of these values is not fully agreed on. Values of >0.9 are considered excellent with values 0.8 – 0.9 considered good, 0.75 – 0.5 is good and < 0.5 poor (Koo and Li, 2016). The type of error which manifests is also important to consider. There is random error which refers to variation that exhibits no pattern on repeated measurements. Systematic bias on the other hand is variation that has some systematic trend for a change in a particular direction (Hopkins, 2000). This can be determined with a paired samples t-test or repeated measures ANOVA (Weir, 2005).

Reliability of Global Fatigue

An isometric MVC exhibits excellent test-retest reliability as a measure when recorded in both a fresh and fatigued state (Rochette *et al.*, 2003; Clark, Cook and Ploutz-Snyder, 2007; Place

et al., 2007; Behrens *et al.*, 2017). Maximum voluntary contraction force typically does not suffer from a systematic bias (Nuzzo, Taylor and Gandevia, 2018), however if many repeated sessions are performed (> 3) then a training effect of an increase in force overtime becomes more likely, particularly for those who are unfamiliar to isometric contractions and are untrained.

Quantifying the reliability of exercise tasks is more ambiguous because there are vast differences in the contraction intensity, duty cycle, and determination of task failure. For this literature review, a focus will be placed on a continuous submaximal isometric contraction in healthy individuals. One study by Clark, Cook and Ploutz-Snyder (2007) has examined the reliability of a submaximal isometric plantarflexion contraction to task failure at 20% of maximum voluntary force and found a CV of 16.1% and an ICC of 0.64 which indicates poor absolute and relative reliability. There was also some systematic error (approximately 12 s). Conversely another study assessing the same protocol for the knee extensor muscles found no difference in endurance time across three repeated sessions (Rochette *et al.*, 2003). However, they only assessed the systematic bias (repeated measures ANOVA) and relative reliability (ICC = 0.68). The same 20% isometric contraction with the elbow flexors yielded a mean 60% (range: 25 – 115%) increase in endurance time from sessions 1 to 3 in a specific set of 'responders' (Hunter and Enoka, 2003). A study by Mathur, Eng and MacIntyre (2005) found that a 20% submaximal isometric contraction of the knee extensors exhibited an ICC of 0.96 with a standard error of measurement of 58 s.

The discrepancy in reliability likely arises from the different muscle groups and participants (e.g., men vs women, sedentary vs trained). Nevertheless, the reliability of an isometric contraction to task failure is still not clear and further exploration is needed.

1.1.4 Central Fatigue

Measuring Central Fatigue

Central fatigue refers to the reduction in force due to changes at spinal or supraspinal sites which consequently causes a failure to drive the motoneurons adequately (Gandevia, 2001). There are numerous methods to quantify central fatigue which will be discussed below.

Central Activation Ratio (CAR). This measure requires participants to perform a maximal voluntary isometric contraction. Once the participant reaches peak force, a single or train of electrical stimuli (13-26 pulses at 50-100Hz) of the nerve innervating the muscle group is delivered. If not all motoneurons are firing, then the electrical stimulation will recruit these

additional motoneurons and generate a brief superimposed twitch force. This is termed maximal evocable force. The ratio between the maximum voluntary force and maximal evocable force is quantified as the central activation ratio, with a ratio of 1 being indicative of complete activation. A baseline value of > 0.95 is typically seen in a fresh state for the quadriceps femoris as complete activation is not always possible for some individuals (Kent-Braun and Le Blanc, 1996). Any value of < 1 or a decrease in CAR value demonstrates differing degrees of central activation failure (Stackhouse *et al.*, 2000). The CAR assumes that the superimposed twitch in combination with an MVC will evoke an individual's true maximum force, however this is not always the case (Shield and Zhou, 2004). Therefore despite the CAR being a reliable measure (Park and Hopkins 2013) it is not considered an appropriate method to assess central fatigue.

Interpolated twitch technique (ITT). Expanding upon CAR is the ITT (Merton, 1954). Whilst similar in application, the ITT requires the delivery of a resting electrical stimulation which is in close proximity to the prior superimposed electrical stimuli. A comparison is then made between the superimposed twitch during the MVC in relation to the resting twitch. The advantage of this is that the size of the resting twitch considers the changes within the peripheral apparatus (i.e., peripheral fatigue) that might reduce the mechanical response to an evoked electrical stimulus. Because of this, the CAR is often greater than the ITT, suggesting an overestimation of voluntary activation (Krishnan and Williams, 2010; Zarkou *et al.*, 2017). The resting twitch which is usually delivered within 5 s of the MVC as it requires full potentiation, because the superimposed twitch during the MVC is fully potentiated, therefore a similar amount of potentiation is required to ensure a valid comparison is made between the two stimuli. In fact, it may take up to three maximal voluntary contractions to achieve full potentiation (Kufel, Pineda and Mador, 2002). On top of this, there are several methodological considerations with the ITT which need to be carefully selected to produce a valid and reliable measure of voluntary activation level. Firstly, the dynamometer that the participant is seated into needs to have minimal compliance with regards to the load cell attachment and the harness which connects the limb to the force transducer. If there is compliance within the system such as cushioning on the harness, then small superimposed twitches become absorbed and reduce the size of the twitch which has the subsequent effect overestimating voluntary activation (Loring and Hershenson, 1992). In addition to this, the sampling frequency of the force signal needs to be of a great enough resolution to be able to capture rapid changes in force caused by a twitch. A sampling frequency of 1000 Hz is recommended (Nuzzo, Taylor and Gandevia,

2018) to be able to safely capture frequent fluctuations in force. Another consideration is the sequence of the electrical stimuli. Typically, single pulses (twitches) or double pulses (doublets) are used. A twitch is just one stimulation whereas a doublet is two electrical stimulations delivered in proximity (1 ms pulse duration, 100 ms inter-stimulus duration) which is often referred to as a '100 Hz paired stimuli'. A superimposed doublet is considered to be more valid than a single twitch because it generates a larger superimposed twitch which provides a greater 'signal to noise ratio' (Shield and Zhou, 2004), however this has not been demonstrated (Behm, St-Pierre and Perez, 1996). Conversely, increasing the number of stimuli from one to four decreased the variability of the ITT (Suter and Herzog, 2001), however even just the method of using one single twitch provides a low amount of variation (standard deviation = 4.1%). The use of a single twitch for studying fatigue may not be appropriate as low frequency fatigue will cause single twitches to drop to a greater extent than doublets or triplets (Shield and Zhou, 2004). On the other hand, increasing the number of stimulations can drastically increase the level of discomfort of the protocol for the participant. A good balance between these two factors would be to select a doublet as this reduces the variability and increases the signal to noise ratio without dramatically increasing discomfort levels. Finally, the timing of the delivery of the stimulus is key. The superimposed stimulation needs to be delivered when the participant reaches peak force. Unfortunately, this is not always simple to achieve as the force rarely stays constant and, in some participants, there can be an inability to maintain a peak plateau in force particularly in the presence of fatigue. If the stimulation is delivered before or after peak force during the MVC then the superimposed twitch will be of a greater amplitude and voluntary activation will be underestimated. If this occurs, a repeat should be completed, however this is not always possible because some measures of voluntary activation are constrained to be collected within a certain time frame. In these instances, a correction can be applied (Strojnik and Komi, 1998) which accounts for the stimulus being delivered slightly before peak force. Nevertheless, the investigator should ensure that the participant is thoroughly familiarised with performing maximum voluntary contractions correctly (e.g., similar to the torque trace in figure 1.2.) when applying the ITT technique especially as the anticipation of a noxious stimuli from electrical stimulation may reduce force.

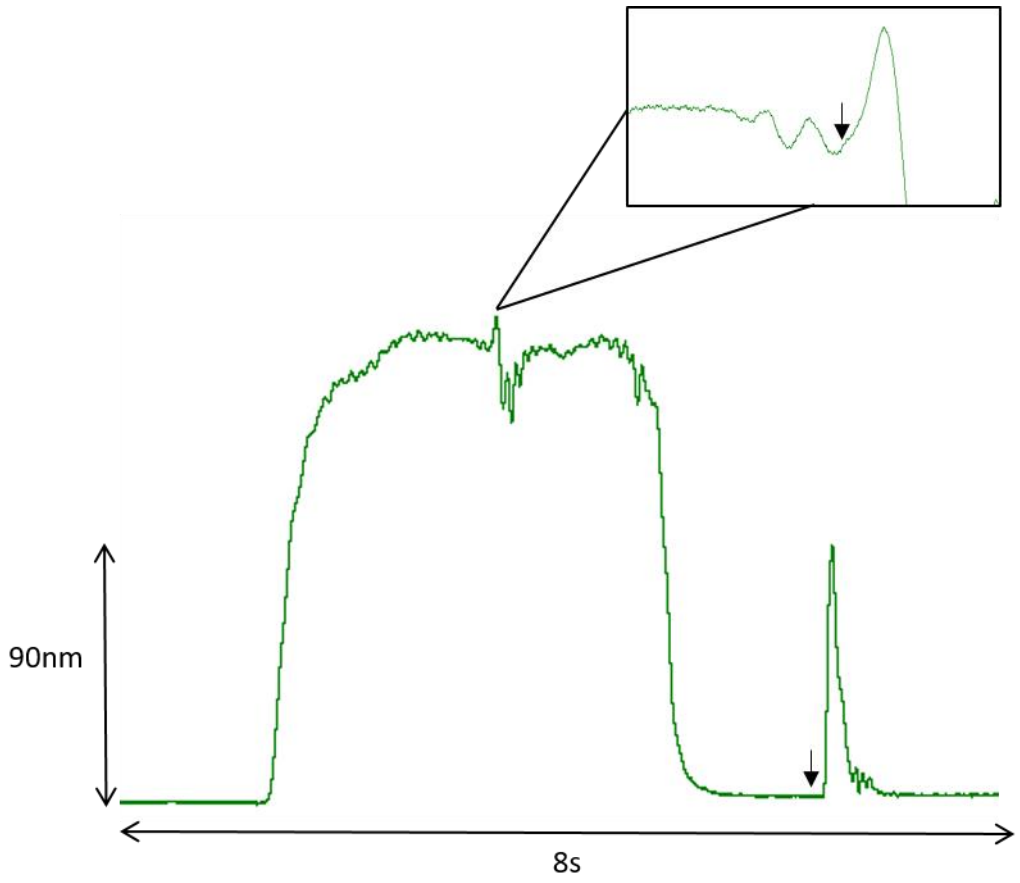

Figure 1.2. A representative torque trace of a maximum voluntary isometric contraction of the knee extensors with a superimposed and resting potentiated doublet. Downward arrows indicate the delivery time of the stimulus.

The reliability of the ITT when using doublet stimulations appear to be excellent with a CV < 10% (Clark, Cook and Ploutz-Snyder, 2007; Sidhu, Bentley and Carroll, 2009; Behrens *et al.*, 2017), even after fatigue (Place *et al.*, 2007). In summary, the ITT is a reliable and valid measure of central fatigue, although it is not without limitation (Dotan, Woods and Contessa, 2021). It is not possible to delineate the specific site where a suboptimal output is occurring (e.g., cortical, or spinal). Secondly it can only be used with maximal contractions and thus its applicability to determining central fatigue during submaximal contractions is limited.

Transcranial Magnetic Stimulation (TMS). Another method to investigate central fatigue is TMS. This involves directly stimulating the motor cortex, the area of the brain which is responsible for the execution of voluntary movement. A magnetic pulse released from a magnetic stimulator induces an electrical current which can pass through the scalp and excite underlying tissues (Goodall *et al.*, 2014). For TMS, a stimulating coil is placed over the motor

cortex (M1) contralateral to the intended side of the measured muscle. Different areas of the motor cortex are responsible for innervating specific regions of the body, it is therefore possible for specific muscles to be activated by placing the coil over the part of the M1 that innervates that particular body part (see figure 1.3). The peak-to-peak amplitude of the response evoked by the TMS can be measured with surface electromyography in the target muscle, termed motor evoked potential, when normalised to the peak-to-peak amplitude of the M-wave by peripheral nerve stimulation can act as a measure of corticospinal excitability.

The most common sites used for TMS are the first dorsal interosseous, biceps brachii and quadriceps femoris muscles. A muscle group particularly of interest with TMS is the knee extensors as this muscle group is commonly used in exercise settings (e.g., cycling, running, and jumping etc.) There are multiple measures of (or associated with) central fatigue for which TMS can be used to measure, the most common ones will be discussed.

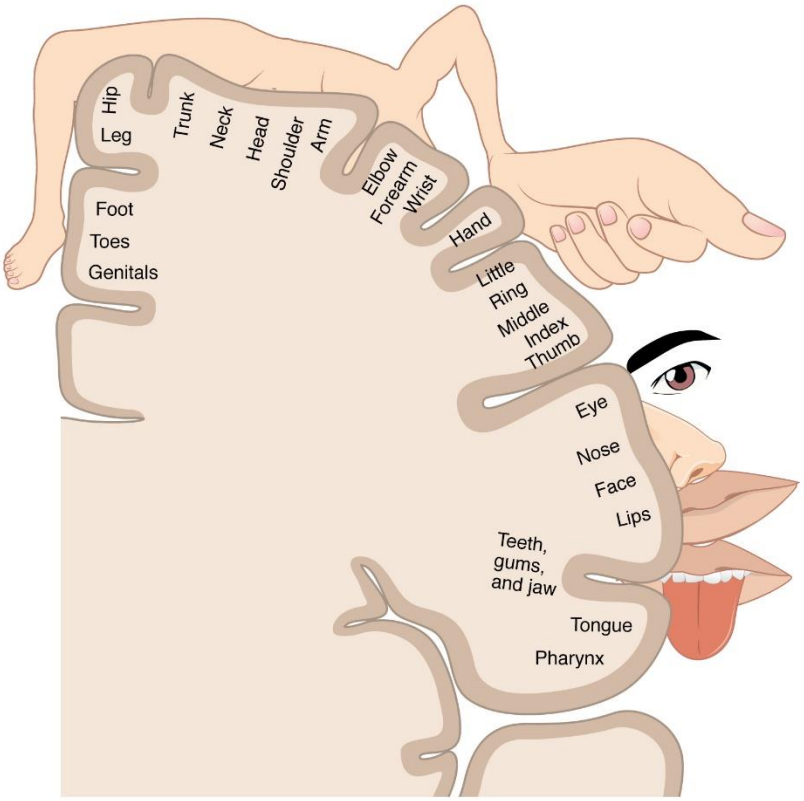

Figure 1.3. The motor homunculus of the M1.

Voluntary Activation With TMS. In a similar fashion to the CAR and ITT, measuring voluntary activation with TMS requires the superimposition of a single pulse (1 ms duration) during a maximum voluntary isometric contraction when the peak force has been reached (Goodall, Romer and Ross, 2009). Any additional increment in force evoked by the TMS pulse is

assumed to be due to a suboptimal recruitment of all motor neurones at a sufficient rate to maximise force development (Todd, Taylor and Gandevia, 2003). One of the differences of TMS compared to the ITT is that it identifies that central fatigue was not because the motoneurons were unresponsive to extra input (Todd, Taylor and Gandevia, 2003). This is because the brain is directly stimulated whereas the ITT only stimulates the motor nerve and therefore cannot differentiate the sites of limitation within the CNS (Goodall *et al.*, 2014). The size of the superimposed twitch needs to be compared to the size of the evoked resting twitch, however because corticospinal excitability is much lower at rest compared to an active contraction, simply delivering a TMS pulse during rest will not work to evoke a maximal twitch force response. Instead, the twitch needs to be estimated by extrapolating the negative linear relationship between contraction intensity and superimposed twitch magnitude. The y-intercept of this relationship is determined by the estimated resting twitch (ERT). To get enough data points to calculate a valid and reliable ERT, multiple submaximal contractions need to be performed. Typically protocols perform isometric contractions at 100, 75 and 50% of maximum voluntary force (Jubeau *et al.* 2014; Lee, Gandevia and Carroll 2009; Todd, Taylor and Gandevia 2003; Todd, Taylor and Gandevia 2004; see figure 1.4).

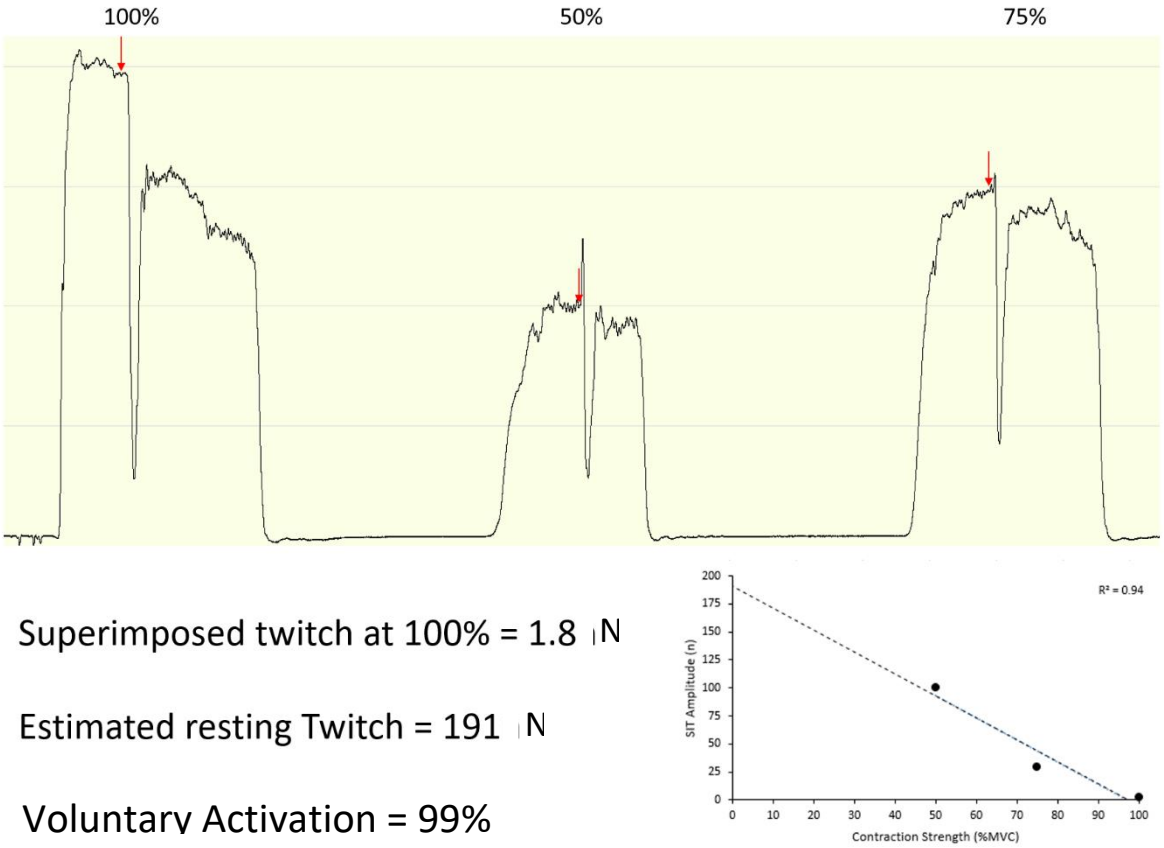

Figure 1.4. A typical force trace during the measurement of TMS-derived voluntary activation at in an unfatigued state. Red arrows represent single pulse TMS. (top). The figures derived from the voluntary activation (bottom left), The relationship between contraction intensity and superimposed twitch force (bottom right).

One limitation with using TMS to calculate voluntary activation is that obtaining the ERT can take several minutes and can induce neuromuscular fatigue itself, especially when multiple trials of the submaximal contractions are performed (e.g. 3 contractions with TMS at 50%, 75% and 100% of maximum voluntary force) which are sometimes used to obtain greater levels of reliability (Dekerle, Greenhouse-Tucknott, *et al.*, 2019). This is particularly an issue when measures of voluntary activation are needed immediately after an exercise task because significant recovery will occur by the time the latter contractions are performed and consequently underestimate the level of central fatigue (Dekerle, Ansdell, *et al.*, 2019). Additionally, as a strategy to maximise force, participants often produce a 'jolting' motion of the body when exerting a maximal effort which makes the ability to deliver a TMS pulse to the optimal stimulation site more challenging. Despite these challenges, voluntary activation derived from TMS has found to be reliable in the knee extensor muscles (Goodall, Romer and

Ross, 2009; Sidhu, Bentley and Carroll, 2009; Malcolm *et al.*, 2021) as well as the upper body (Todd, Taylor and Gandevia, 2004; Lee, Gandevia and Carroll, 2009) in a fresh state, but suffers substantially in a fatigued state with a smallest detectable change of 27.1% which meant that only a detectable change was seen in 2/20 measures (Dekerle, Ansdell, *et al.*, 2019). That does not even consider if the measurement is valid due to recovery when determining the ERT. Because the ITT is more temporally advantageous, does not require careful coil positioning and exhibits a slightly lower CV% (TMS = 3.1, ITT = 2.7%), for the investigation of the effect of muscle pain on neuromuscular fatigue it is perhaps a superior method for assessing voluntary activation after a fatiguing protocol (Todd, Taylor and Gandevia, 2016).

Motor Evoked Potential (MEP). Recording a MEP involves the delivery of a single TMS pulse at rest or superimposed over a submaximal isometric contraction at 10-50% of maximal voluntary force. The descending volley caused by the electromagnetic current results in an MEP which is measured as the electromyographic response in the target muscle (Figure 1.8). The peak to peak amplitude of the MEP reflects corticospinal excitability (Goodall *et al.*, 2014), therefore if corticospinal excitability is decreased the amplitude of the MEP would be expected to decrease and vice versa. Exercise-induced fatigue has not been consistently shown to cause decreases in MEPs (Kennedy *et al.*, 2016; Finn *et al.*, 2018), but this is most likely related to differences in the exercise task and TMS protocol.

The protocol used for the measurement of TMS related variables can considerably change the outcome of the MEP. Firstly, MEP amplitude increases as a function of stimulation intensity up to a certain point which is described as a sigmoidal curve (Carroll, Riek and Carson, 2001). A great enough stimulus intensity is needed to get a detectable MEP response and maximise the signal to noise ratio, however excessively high intensities can become uncomfortable for the participant and begin to recruit antagonist muscles. There are three common methods to determine the optimal stimulation intensity. The first method requires stimulation during a rested state or during muscle contraction and the stimulator intensity is increased from 30% upwards in increments of 5% until an MEP of at least 0.05 mV peak to peak amplitude seen in 50% of the trials. This is called the resting motor threshold (RMT) and active motor threshold (AMT), respectively. The intensity is then set to 120-130% of the this. The second method involves delivering a number of stimuli during a submaximal contraction and increasing the stimulator intensity up until a plateau is seen in the peak-to-peak MEP (< 5% increase) which is referred to as a stimulus-response curve (Temesi *et al.*, 2014). All methods work effectively,

however either AMT or stimulus-response curves are more suitable for investigating the corticospinal pathway of the knee extensor muscles.

The intensity of the background contraction can also influence the size of the MEP. The MEP amplitude tends to increase as contraction intensity increases up until 50% of maximum voluntary force (Goodall *et al.*, 2014). Intensities between 10-50% of maximum voluntary force are often used for the assessment of corticospinal excitability in the knee extensors. The use of contractions at ~20% of maximum voluntary force seem to be advantageous as similar stimulus response curves are obtained compared to 50% with a reduced the risk of inducing neuromuscular fatigue (Temesi *et al.*, 2014). Additional measures include the MEP latency which reflects the central motor conduction time defined as the time between stimulus delivery and onset of the MEP and the MEP area (figure 1.5, shaded red) which accounts for the MEP size and duration.

The reliability of motor evoked potentials is primarily determined by the number of measurements acquired. Delivering one single TMS pulse is inadequate to reliably assess corticospinal excitability. In fact to achieve high intra-session and intersession reliability, a minimum of ~20 pulses are needed (Goldsworthy, Hordacre and Ridding, 2016) with some data suggesting that 30 pulses are required for the most reliable estimated of corticospinal excitability (Cuypers, Thijs and Meesen, 2014). In a systematic review by Cavaleri, Schabrun and Chipchase (2017) ten stimuli were deemed the minimum number of trials to obtain between trials reliability (ICC = 0.83) while at least five are needed for excellent within session reliability (ICC = 0.92). For the knee extensors, ten measures produced excellent test-retest reliability (Temesi, Ly and Millet, 2017). Differences in the reliability are likely explained by the factors such as the coil type, stimulation intensity and area of the motor cortex stimulated. In neuromuscular fatigue research it is often unfeasible to deliver many consecutive TMS pulses because the effect of fatigue needs to be captured before significant recovery can occur. Therefore, a balance between using enough MEPs to reliably capture changes without allowing for too much recovery should be performed. Previous research has found significant alterations in corticospinal excitability between trials with as few as 4 MEPs (Angius *et al.*, 2018).

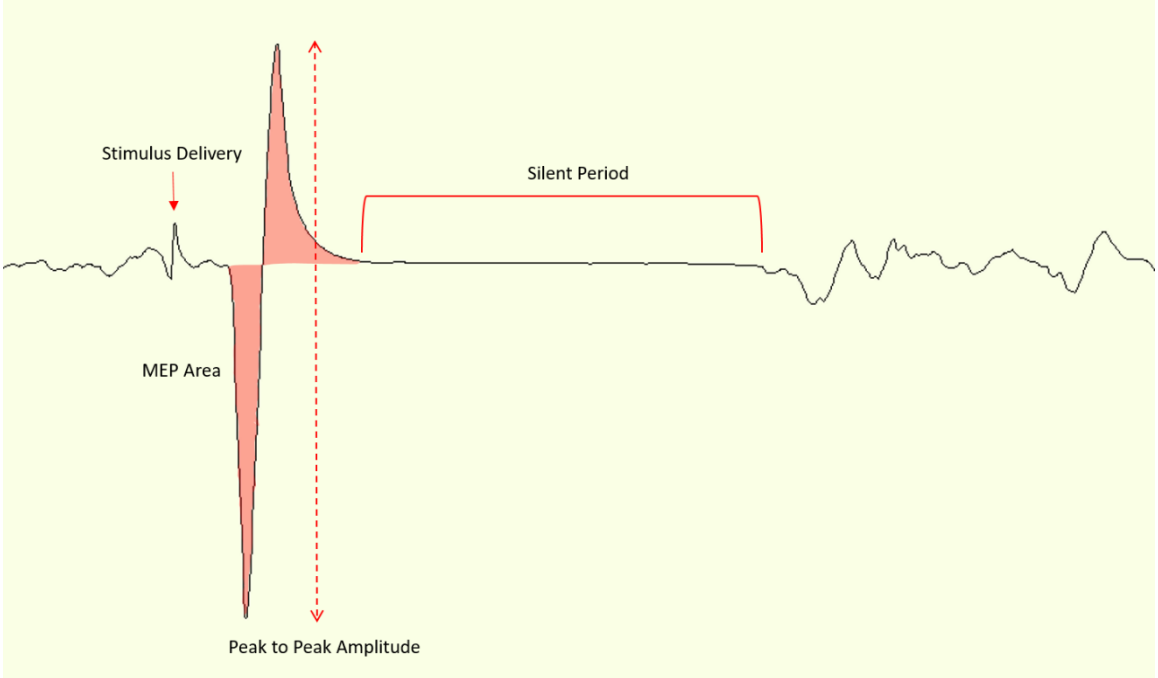

Figure 1.5. A typical motor evoked potential response in the vastus lateralis during a submaximal isometric contraction and the associated indices of corticospinal excitability and inhibition.

TMS Silent Period. The TMS silent period (SP) is the brief period of EMG 'silence' that follows a MEP before the return of voluntary muscle activity (see figure 1.5). The length of the SP (ms) represents the amount of corticospinal inhibition with a lengthening of the SP being indicative of greater levels of inhibition. The mechanisms of the induced silent period are not fully understood, but it is has previously been thought that the initial part of the silent period is spinal in origin (50-80ms) and the latter part being of cortical (Škarabot *et al.*, 2019), however this has been challenged and the spinal components may span for up to 150 ms (Yacyshyn *et al.*, 2016). The SP is thought to be mediated by the activation of receptor B of the gamma-aminobutyric acid neurotransmitter ($GABA_B$) as $GABA_B$ reuptake inhibitors can lengthen the SP (Werhahn *et al.*, 1999). In a similar fashion to MEP amplitude, SP increases as a function of stimulator intensity (Säisänen *et al.*, 2008) but not the background contraction intensity (Wu *et al.*, 2002).

Electromyography (EMG). The non-invasive and ease of use of bipolar surface EMG systems have made their application widespread in fatigue research. The measure of muscle activity from bipolar surface electrodes superficial to the muscle of interest have frequently been used to infer the level of neural drive to the muscle (Dideriksen, Enoka and Farina, 2011). Indeed,

the decrease in amplitude of the processed signal during a maximal effort has been attributed to central fatigue (Pageaux, Marcora and Lepers, 2013). In the presence of fatigue, the inability to fully recruit all motor units should result in a reduction in the amplitude of the EMG signal during maximal contractions (Burnley, 2009). On the other hand, fatiguing submaximal contractions will induce a progressive increase in EMG amplitude as an increase in the number of motor units and their firing rate increased to maintain force (Conwit *et al.*, 2000), known as the size principle (Henneman, Somjen and Carpenter, 1965). The EMG amplitude is not the sole product of neural drive or motor unit recruitment, as multiple central and peripheral factors can influence the EMG amplitude (De Luca *et al.*, 2010). Many of these can be accounted for by normalisation, however, to be able to solely infer central causes to the change in EMG amplitude is erroneous. Alterations in the periphery such as the muscle fibre propagation velocity and size of the intracellular action potentials can influence the signal (Vigotsky *et al.*, 2018). Additionally, comparison between activation levels of vasti muscles is inappropriate as the size of motor unit action potentials influences the signal amplitude more than the neural drive (Martinez-Valdes *et al.*, 2018). The root mean square (RMS) EMG amplitude of an MVC normalised to the peak-to-peak measurement of the compound muscle action potential (M-wave) evoked by a percutaneous electrical stimulation of the muscle nerve shortly after is thought to provide a more valid measure of central fatigue as the peripheral changes within the EMG signal are largely accounted for (Millet and Lepers, 2004). However this measure does not seem to change after exhaustive exercise (Pageaux *et al.*, 2015) despite robust reductions in voluntary activation. Furthermore, the EMG amplitudes of the quadriceps tends to exhibit considerable levels of variability (Place *et al.*, 2007; Buckthorpe *et al.*, 2012) when the MVC is normalised to the M-Wave (CV% 10-15%). However when submaximal isometric contractions are employed, the reliability is better (Kollmitzer, Ebenbichler and Kopf, 1999; Mathur, Eng and MacIntyre, 2005). Taken together, EMG amplitudes should be considered with caution and only used as a crude measure of neural drive.

Non-Local Muscle Fatigue

Within the discussion of central fatigue, the body of literature investigating the effects of non-local muscle fatigue (NLMF) on neuromuscular fatigue development of non-exercised muscles is warranted (Halperin, Chapman and Behm, 2015). Significant reductions in exercise tolerance (Bangsbo *et al.*, 1996; Amann *et al.*, 2013; Johnson *et al.*, 2015; Behm *et al.*, 2019), voluntary activation (Doix, Lefèvre and Colson, 2013; Kennedy *et al.*, 2013; Johnson *et al.*, 2015) and reductions in corticospinal excitability (Šambaher, Aboodarda and Behm, 2016; Aboodarda *et*

al., 2017) have been observed. Exacerbations in perceived effort (Amann *et al.*, 2013; Johnson *et al.*, 2015, 2018) have also been observed with NLMF, however this hasn't always been found (Elmer *et al.*, 2013). The heterogeneity with the fatigue protocol, neuromuscular assessment and muscle groups fatigued/tested are likely to explain the discrepancies in findings. Nevertheless, NLMF has been observed in conditions of fatiguing the knee extensors/elbow flexors and testing the contralateral limb, upper body fatigue on lower body fatigue (and vice versa) and even respiratory muscle fatigue on lower limb exercise. Interestingly, there isn't an exacerbation of peripheral fatigue with non-local muscle fatigue as evidenced by no difference (Aboodarda *et al.*, 2015) or less peripheral fatigue (Amann *et al.*, 2013; Rossman *et al.*, 2014; Johnson *et al.*, 2015), indicating a that central changes are responsible for the limitation to maximal force output and exercise tolerance.

1.1.5 Peripheral Fatigue

Measuring Peripheral Fatigue

Peripheral fatigue is defined as the reduction in muscle force due to changes at or distal to the neuromuscular junction. Peripheral fatigue is thought to be caused by changes within the metabolic milieu within the muscle. Specifically, the accumulation of metabolic by-products associated with intense muscular contractions are often implicated within the fatigue process such as Pi, K^+ and H^+ (Allen, Lamb and Westerblad, 2008). An accumulation of Pi can impair cross bridge cycling (Dahlstedt, Katz and Westerblad, 2001). An increase in H^+ causes a fall in muscle pH and subsequently can impair Ca^{2+} handling (Nelson, Debold and Fitts, 2014). The efflux of K^+ from intracellular to extracellular spaces to repeatedly produce action potentials is hypothesised to decrease the excitability of the sarcolemma causing a lower action potential in response to a given electrical impulse, or a greater electrical impulse needed to generate the same action potential. With a reduced excitability of the muscle fibre(s), there becomes a decrease in force producing capability (Balog and Fitts, 1996).

One way to measure the magnitude of peripheral fatigue is by directly stimulating the nerve or muscle with an electrical current (Rozand *et al.*, 2015), termed peripheral nerve stimulation (PNS). A short duration (1-2 ms) electrical pulse causes a momentary muscle twitch which can be measured for its force amplitude. Indeed, the force evoked by an electrical stimulation of the nerve is the most common method of directly assessing the magnitude of peripheral fatigue after exercise when it is compared to values acquired in a fresh state. Because the depolarisation and generation of action potentials are generated by an external stimuli (i.e. an electrical

stimulator system), no voluntary central motor command is required, thus when stimulated in a fully relaxed state, a decrease in the evoked isometric force response recorded from a force transducer connected to the stimulated limb is indicative of peripheral fatigue (Bigland Ritchie *et al.*, 1978). Not only is the amplitude informative about the development of peripheral fatigue but the temporal characteristics of the PNS can provide valuable information about the state of the contractile apparatus. The maximum rate of force development (MRFD) of the twitch may represent the $Ca2^{+}$ release from the sarcoplasmic reticulum and capacity of cross bridge cycling (Lepers *et al.*, 2002). The MRFD is commonly acquired from the first derivative of the force signal (Casartelli, Lepers and Maffiuletti, 2014; Maffiuletti *et al.*, 2016). Additionally, the maximal relaxation rate (MRR), also calculated with the first derivative or half relaxation time (i.e. time for twitch to go from peak force to 50% of that) may reflect the ability of myosin head detachment from actin filament, possibly due to elevations in fatigue inducing metabolites (Allen, Gandevia and McKenzie, 1995). Peripheral fatigue is reflected as a decrease in the MRFD, an increase in MRR and slowing of the half relaxation time (figure 1.6).

The EMG response of the electrical stimulation can be measured to supplement the mechanical measures of peripheral fatigue. The M-Wave is thought to reflect the level of sarcolemmal excitability of the stimulated muscle. However during fatigue, the M-Wave peak to peak amplitude does not seem to change (Pageaux *et al.*, 2015; Hureau, Ducrocq and Blain, 2016) whereas some have found a small (5%) decrease (Morgan *et al.*, 2019) indicating that excitability at the sarcolemma remains largely unaffected in the presence of fatigue. Recently, the notion that the peak to peak amplitude of the M-Wave decreasing reflects reduced sarcolemmal excitability has been challenged (Rodriguez-Falces and Place, 2018). Rather, the increase in the first phase of the M-wave is thought to reflect decreased sarcolemmal excitability as this has been observed after fatiguing maximal intermittent isometric contractions (Rodriguez-Falces and Place, 2019) and only in maximal isometric contractions where a significant level of fatigue is present (Rodriguez-Falces *et al.*, 2019). There are, a plethora of methodological factors which need to be considered which can impact the reliability, validity, type, and magnitude of peripheral fatigue. These will be outlined below.

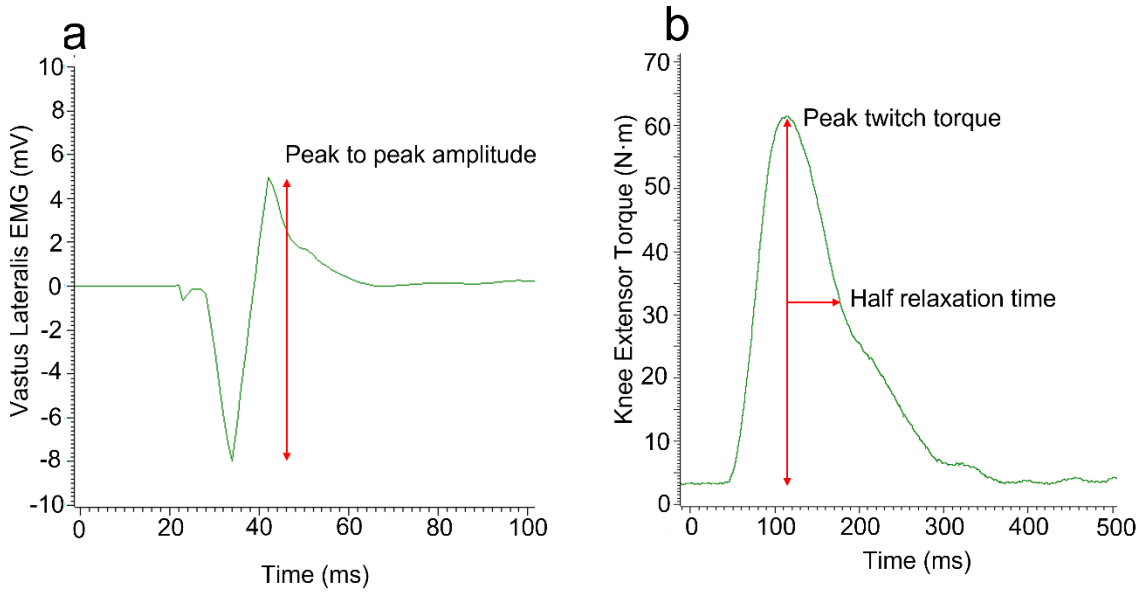

Figure 1.6. The electromyographic (a) and mechanical (b) response to peripheral nerve stimulation innervating the quadriceps femoris.

Firstly, the stimulation method can be delivered via numerous mediums. A common method employed for quadriceps electrical stimulation is via a motor point stimulator pen pressed into the femoral triangle, which is superficial to the femoral nerve, or similarly an electrode placed over the femoral nerve. Muscle stimulation is also used by placing several large electrodes over the muscle belly and has the advantage of being more comfortable for the participants, however it can take up considerable space of the stimulating muscle of interest therefore limiting the employment of other measurement techniques such as electromyography, near infrared spectroscopy or intramuscular injections. PNS delivered with a motor point pen may be more susceptible to error due to inconsistencies in pressure and location when compared to PNS via an electrode. Therefore, PNS with an electrode placed over the femoral nerve is the most favourable option for PNS in the context of this thesis.

The stimulation intensity is crucial for obtaining valid measures of peripheral fatigue. The intensity should start at approximately 50-100mA and increase in 10-20mA increments until a plateau in the evoked force and M-Wave is reached, this is termed the maximal stimulation amplitude. An additional 20-30% is added to this stimulation intensity (Neyroud *et al.*, 2014) to ensure supramaximal stimulation because the excitability of the motor axons become reduced with fatigue thus making a maximal stimulation intensity no longer able to activate all motor axons (Vagg *et al.*, 1998). In general, stimulation intensities should be minimised where

possible by accurately placing the cathode directly over the femoral nerve. A consequence of using unnecessarily higher stimulation intensities is that they can innervate the antagonists (e.g., biceps femoris for quadriceps stimulation) which may reduce the size of the evoked twitch force.

An additional important consideration for motor nerve stimulation is the use of a single (twitch), or multiple electrical stimuli (two = doublet, three = triplet). Similar to the ITT, a doublet (100Hz paired stimuli) may be more reliable (Shield and Zhou, 2004) but does cause greater discomfort to the participants.

2.5.2 Reliability of Peripheral Fatigue Measures

Measures of peripheral fatigue via the delivery of PNS appear to exhibit excellent reliability (Place *et al.*, 2007; Johnson *et al.*, 2015; Behrens *et al.*, 2017). As there is no voluntary effort required with these measurements, unlike with maximal voluntary force or the ITT, motivation and attentional focus are eliminated. Poorer reliability is only likely to become apparent when measures of peripheral fatigue are made post-exercise in the case where the measurements are taken at slightly different time points in relation to task failure as there is a rapid recovery in metabolic perturbation within the first 30 s (Bogdanis *et al.*, 1995). Additionally, muscle damage can reduce the size of the evoked twitch (Prasartwuth, Taylor and Gandevia, 2005) due to disruption of the actin-myosin filaments. Therefore, if PNS is delivered at the same time points during a fatiguing task, then they should exhibit excellent reliability.

Section 2 - Pain

1.2.1 What is Muscle Pain?

Pain can be defined as “an unpleasant emotional sensation associated with actual or potential tissue damage” (Treede, 2018). The overwhelming majority of individuals are well versed with the sensation of pain as its prevalence is common throughout many of life’s activities and experiences. The location, magnitude and quality of pain are all dependent on the context of the ‘actual or potential tissue damage’. Furthermore, as the definition of pain refers to it as an emotional sensation, this indicates that it manifests in a subjective and individualistic manner and importantly, the pain experience is not always synonymous with the magnitude of the nociceptive stimulus.

Nociception refers to the transmission of sensory signals that are derived from the stimulation of nociceptors in the periphery which are relayed along the spinal cord and to sensory

processing areas of the brain (Julius and Basbaum, 2001). The reason the magnitude of nociception does not directly relate to the magnitude of the pain experience is because there is physiological and psychological processing of the nociceptive signal (Garland, 2012), and the context of the specific painful situation can dictate how pain is processed and interpreted (Carlino, Frisaldi and Benedetti, 2014).

Because pain is an extremely complex and multifactorial process, it is inappropriate to assume all types of pain are the same, therefore this thesis will focus specifically on muscle pain with particular emphasis on exercise-induced pain (EIP) that is felt primarily due to chemical changes within the skeletal muscle tissue. The two foci of this will be from intense exercise and from the experimental induction of pain with an intramuscular injection of hypertonic saline injection. It is important to be clear that this is type of pain is different from other types of exercise related pain, such as muscle injury or delayed onset muscle soreness (DOMS), which this thesis will only briefly introduce in a later section. Therefore, the subsequent sections in this literature review will outline the aetiology of muscle pain, the processing of nociceptive signals and the different methods of measuring pain.

1.2.2 Aetiology of Exercise-Induced Pain.

The aetiology of pain involves a complex relay of peripheral, spinal, and cortical processing which is still not fully understood. For EIP, the threshold of pain appears to occur at approximately 50% of peak power output or the intensity corresponding to 50% of maximal oxygen uptake ($\dot{V}O_{2max}$) during cycling exercise (Cook *et al.*, 1997) although the variability in this threshold appeared to be large between individuals. Within the Cook paper, the authors stated that the variability of this leg pain was not purely a function of the muscle metabolic by-products but rather due to the error with the methods of measuring pain. However, this conclusion appears unsupported as only limited reference is made to lactate or Hydrogen ion accumulation being a linear function of relative exercise intensity whereas pain was not. An alternative view of exercise intensity such as the power duration relationship (Burnley and Jones, 2018) and the associated exercise intensity domains (specifically heavy/severe) may partially explain this variability. The critical power/speed represents the boundary between a steady metabolic environment (moderate and heavy domains) and a progressive increase in oxygen consumption and metabolite accumulation which are the severe and extreme domains (Poole *et al.*, 2016). Below the critical power, (within the heavy domain), pain may still occur but is unlikely to increase linearly as a function of intensity. The threshold for exercised

induced pain is more likely to occur at the lactate threshold where some noxious biochemicals which are responsible for causing muscle pain are present. The critical power likely reflects the threshold where EIP will start to increase inexorably if exercise is maintained above this intensity and the further above the critical power the exercise is, the more rapid the onset of EIP.

Importantly, the critical power and lactate threshold can occur at a wide range of relative exercise intensities between individuals. For example, Scharhag-Rosenberger *et al.* (2010) showed that blood lactate concentration at 60% of $\dot{V}O_{2max}$ ranged from 0.7-5.6 mmol.l^{-1} which may explain the vast differences in EIP threshold when assessed against a percentage of peak power output or $\dot{V}O_{2max}$. Nevertheless, EIP appears to scale as a function of exercise intensity when at least above the lactate threshold. EIP will also increase as a function of time at a given fixed severe-domain intensity (Smith *et al.*, 2020).

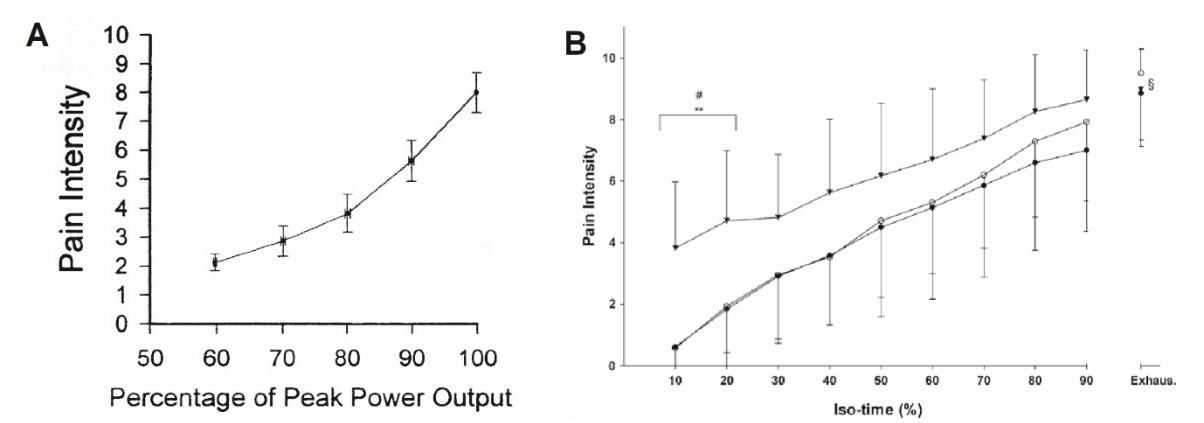

Figure 1.7 A. From Cook et al. (1997) displaying the increase in EIP in relation to exercise intensity. B. From Smith et al. (2020) displaying exercise induced pain at a fixed intensity above the critical torque.

With the exercise intensity required to cause EIP established, it is prudent to specify the primary cause of EIP. Because EIP is related to exercise intensity, the accumulation of certain biochemicals associated with high intensity exercise acts as a noxious stimulus and begins the cascade of the pain response. Indeed, algesic substances produced during high intensity exercise include Bradykinin (Mense and Schmidt, 1974; Mense and Meyer, 1988), K^+ (Fock and Mense, 1976), lactic acid/H^+ (Rotto *et al.*, 1990; Sinoway *et al.*, 1993), substance P (Herbert and Schmidt, 2001) and the fall in pH (Mense, 2008). The consequence of an elevated concentration of these substances is the stimulation of myelinated and unmyelinated afferent nociceptors (i.e., the muscle pain receptors). There are two general types of nociceptors for

pain. Type IV nociceptors respond to high levels of metabolites and algesic substances (e.g., those that accumulate during intense exercise). Secondly, there are type III mechanosensitive afferents which are primarily stimulated by mechanical pressure which are generated during forceful muscle contractions (Jankowski *et al.*, 2013). Furthermore, there is an interaction of these afferents whereby type IV metabosensitive afferents sensitise the type III afferents by reducing their threshold for excitation (Herbert and Schmidt, 2001). Acid sensing ion channels (ASICs) have also received recent attention as an important component in the development of EIP (Khataei *et al.*, 2020). ASICs appear to sense reductions in muscle pH of around 6.7 to 7.0 which are seen during intense exercise. In rats, knockout of ASIC3 resulted in a blunted pain sensitivity compared to wildtypes (Khataei *et al*., 2020).

These afferent nociceptors transmit impulses from the muscles in the periphery through to the dorsal horn within the spinal cord up to supraspinal areas via the spinothalamic tract. The destination includes several brain area such as the insula, anterior cingulate cortex and the somatosensory cortex region of the brain, which are all involved in generating the perception of the pain (Garland, 2012). The right somatosensory cortex perceives pain from the nociceptive stimuli on the left side of the body and vice versa. Furthermore, specific regions of the somatosensory cortex process pain from different body areas (e.g., arms, legs, and trunk, etc.) However, the spinal transmission of the nociceptive information does not make a simple transit from peripheral to central areas and back. There is a considerable level of sensory processing from the ascending and descending pathways (i.e., from the brain to the dorsal horn). Importantly, the descending pathway can modulate the magnitude of synapsing at the dorsal horn (presynaptic inhibition) between first and second order neurons of the ascending pathway through the release of serotonin/noradrenaline (Pertovaara, 2006) thus modulating the nociceptive signal of the ascending pathway and subsequently attenuating or even accentuating the perceived pain.

There are a number of psychological factors which can influence pain processing such as attentional focus; whereby increasing attention to pain increases the pain response (Arntz, Dreessen and Merckelbach, 1991). Cognitive appraisal also plays a role whereby positive appraisal may reduce the pain perception (Neufeld, 1970). For example, re-evaluating pain to be pleasurable such as viewing burning pain as ‘warm’. The emotional state of an individual may also be a factor. Negative emotions such as fear, anger or sadness associated with pain can amplify the perception via increased activation of the brain areas associated with emotion/pain

processing. Additionally, negative pain emotions may bias the attention of an individual to the perceived pain. Also, negative emotions can induce a physiological response with an increase in adrenaline and cortisol which may attenuate pain (Pertovaara, 2006), increase blood flow (Barcroft and Konzett, 1949) and increase muscle tension (Lundberg *et al.*, 1999). Expectation of the intensity of a noxious stimuli can modulate the perceived pain (Keltner *et al.*, 2006). There is also a complex array of psychological factors present during exercise which may also dictate how an individual responds to pain. These include the motivation to win a race or beat a personal best, excitement (or lack of) towards the exercise bout, and acknowledgment that EIP is necessary for the attainment of peak performance. Because of these factors, the relationship between exercise and pain becomes even more intricate and could be considered to be even more complex than pathological or injury related pain.

These observations about altering the pain experience based upon different circumstances are not exhaustive but illustrate the point that the magnitude of pain does not always scale with the nociceptive stimulus, thus it is imperative to attempt to account for (and measure) certain psychological factors when investigating the effect of experimental pain. Now that pain has been defined and with the aetiology outlined, it is important to quantify the pain response.

1.2.3 Measuring Pain

There are multiple factors that need to be considered when trying to measure the experience of pain. These are primarily the intensity, quality, location, and the affective dimensions of the pain. Additionally, it is possible to systematically quantify an individual's pain tolerance and pain threshold. These measures and their applicability to EIP will be outlined below.

Pain Quantity

Pain quantity, often referred to as pain intensity, is defined as the magnitude of pain experienced and is the most common measure of pain. The Cook pain scale (Cook *et al.*, 1998) is commonly used to measure pain intensity during exercise. This scale anchors 0 at 'no pain at all' and 10 at 'worst pain imaginable' (see figure 1.8.) there is also an additional point above 10 to rate pain which is subsequently more intense than a participants perceived 10, thus preventing the limitation of a 'ceiling effect'. Visual analogue scales exist for pain intensity which requires participants to indicate on a line the intensity of pain and can be scaled from 0-10 or 0-100. A 100 point scale has been credited for being more precise to changes in a given rating compared to a 10 point scale (Pageaux, 2016).

Not only should pain the measurement ratings be able to detect small changes, but they should also be sampled at a high frequency in order more accurately sample the temporal pain response and prevent aliasing. Typically, measures of pain are recorded in 30 to 60 second intervals, however this will not always provide enough information about the kinetics of pain if the intensity of pain is rapidly changing. This is particularly true when experimental pain models are applied such as intramuscular hypertonic saline injections where the onset of pain quickly increases shortly after the infusion (Graven-Nielsen, Svensson and Arendt-Nielsen, 1997; Ciubotariu, Arendt-Nielsen and Graven-Nielsen, 2004; Khan *et al.*, 2011). In this case, a sampling frequency of every 2-5 s would appear to be more appropriate. Unfortunately, published guidelines for the sampling frequency of pain has not been published, and therefore a pragmatic approach to sampling frequency is required based upon the duration and kinetics of the pain response. A linear potentiometer device can automatically record pain intensity as it changes and be retrospectively analysed in a desired frequency. Alternatively, when verbal ratings are asked for, a lower frequency of 30 s seems appropriate to not become overwhelming for the participant. The benefit of a high sampling frequency is that the area under the curve (AUC) of pain can be calculated to determine the volume of the pain experienced. In terms of its applicability to exercise induced pain, the scale has been used successfully in exercise settings (Angius *et al.* 2015; Cook *et al.* 1997; Astokorki and Mauger 2017). A further important consideration is how the ratings of pain are anchored. The rating of pain that is at the upper bounds of the Cook pain scale should not be anchored to the worst pain ever felt by the individual giving the rating (e.g., broken bones, burns or cuts etc.). Instead, the upper bound should be calibrated to the greatest EIP felt from during a previous bout of exercise.

Overall, pain intensity is a simple, quick, and valid measure to take which makes its utility for measuring EIP invaluable because individuals often experience continual changes which need to be relayed to the investigator while simultaneously maintaining attentional resources to perform exercise.

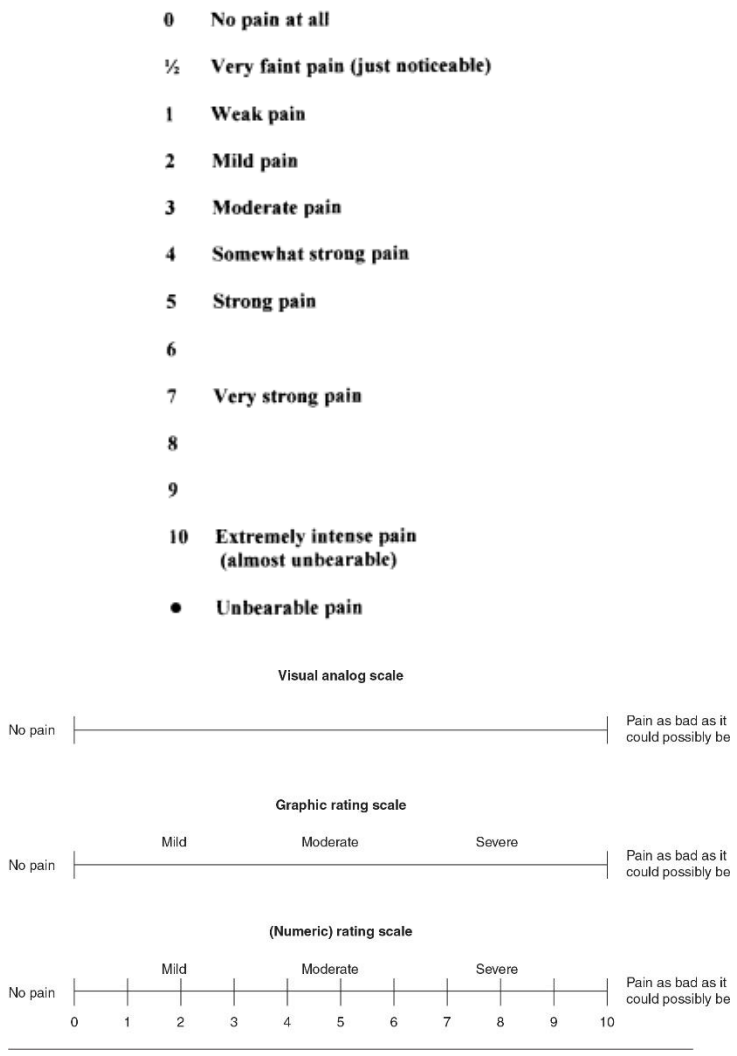

Figure 1.8. The Cook pain scale. From Cook et al. (1998) and three different types of visual analogue scales for pain.

Pain Quality

Pain quality refers to the descriptive sensation of the pain experience which is heavily dependent on the cause and location of the pain. For example, pain which originates from muscle tissue typically has a quality described as 'tearing' and 'cramping' while cutaneous pain is typically described as 'stabbing' and 'burning' (Mense, 2008). Pain which is evoked by different experimental methods can also generate different qualities of pain. Muscle pain induced by ischaemia produced higher ratings of 'stabbing' 'burning', 'heavy' and 'exhausting' while an intramuscular hypertonic saline injection caused more of a 'cramping' sensation (Graven-Nielsen *et al.*, 2003). Even intramuscular versus intradermal injections of the same algesic substance (capsaicin) can create differing qualities of pain (Witting *et al.*, 2000). These findings suggests that certain afferent fibres are more responsive to specific

noxious stimuli (Burgess and Perl, 1974). If EIP is to be studied, the method of inducing pain must stimulate nociceptors within the muscle and should at least share some common descriptive qualities of pain as EIP. This is because different populations of nociceptors are responsible for evoking differing qualities of pain. For example, the use of heat pain may be effective in providing a standardised pain response however, thermal specific nociceptors would be stimulated which might have different functional consequences to that of chemical or mechanoreceptors which are stimulated during painful exercise.

With some of the diversity of pain qualities identified, it is essential to determine the quality of pain that is experienced during the studies of this thesis. Exercise-Induced pain is derived from high intramuscular pressures, mechanical deformation and most significantly, the presence of high concentrations of noxious biochemicals which are described as "tiring, exhausting, heavy, aching, and hot/burning" (Cook *et al.*, 1997). These descriptors of pain are derived from the McGill long form pain questionnaire (Melzack, 1975). This questionnaire has been extensively used in the pain literature and is deemed to be valid and reliable (Hawker *et al.*, 2011) as well as sensitive to changes in pain (Jenkinson *et al.*, 1995). The McGill pain questionnaire has several descriptive words in a category that describes one specific quality of pain (e.g., dull, sore, hurting, aching and heavy). Each word is associated with the severity of that feeling (i.e., Sore (2) is a worse feeling than dull (1) and hurting (3) is a more severe feeling than sore). There are a total of 20 different groups of descriptors which are categorised into four different dimensions of pain: sensory, affective, evaluative, and miscellaneous. Sensory refers to the actual quality of pain in regards to the temporal, spatial, pressure and thermal aspects (Melzack, 1975). Affective refers to the psychological distress associated with the pain such as 'frightful' or 'exhausting'. Evaluative refers to the psychological evaluation of the pain experienced in terms of its overall intensity (Annoying, troublesome, miserable, intense, and unbearable). Finally, Miscellaneous involves words that don't fit any of the three preceding dimensions (e.g., nauseating). Whilst the McGill pain questionnaire is a useful tool in the measurement of pain it does have limitations. Firstly, many of the descriptors appear synonymous or have very subtle differences in their definition which can confuse to the participant about which to select. Therefore, a list of definitions for each word should be provided when completing the questionnaire. Secondly, it can take several minutes to administer. Therefore, the questionnaire could not be administered repeatedly to determine changes in pain quality over time. However, there is the short form McGill questionnaire which has only has 15 descriptors on a four point intensity scale of 0 = none, 1 = mild, 2 = moderate and 3 = severe (Melzack, 1987) which

could capture the quality of pain more rapidly. Measures of pain quality are important because it can provide insight about how the pain might affect behaviour. For example, a high affective score could be linked to the desire to avoid or escape the pain.

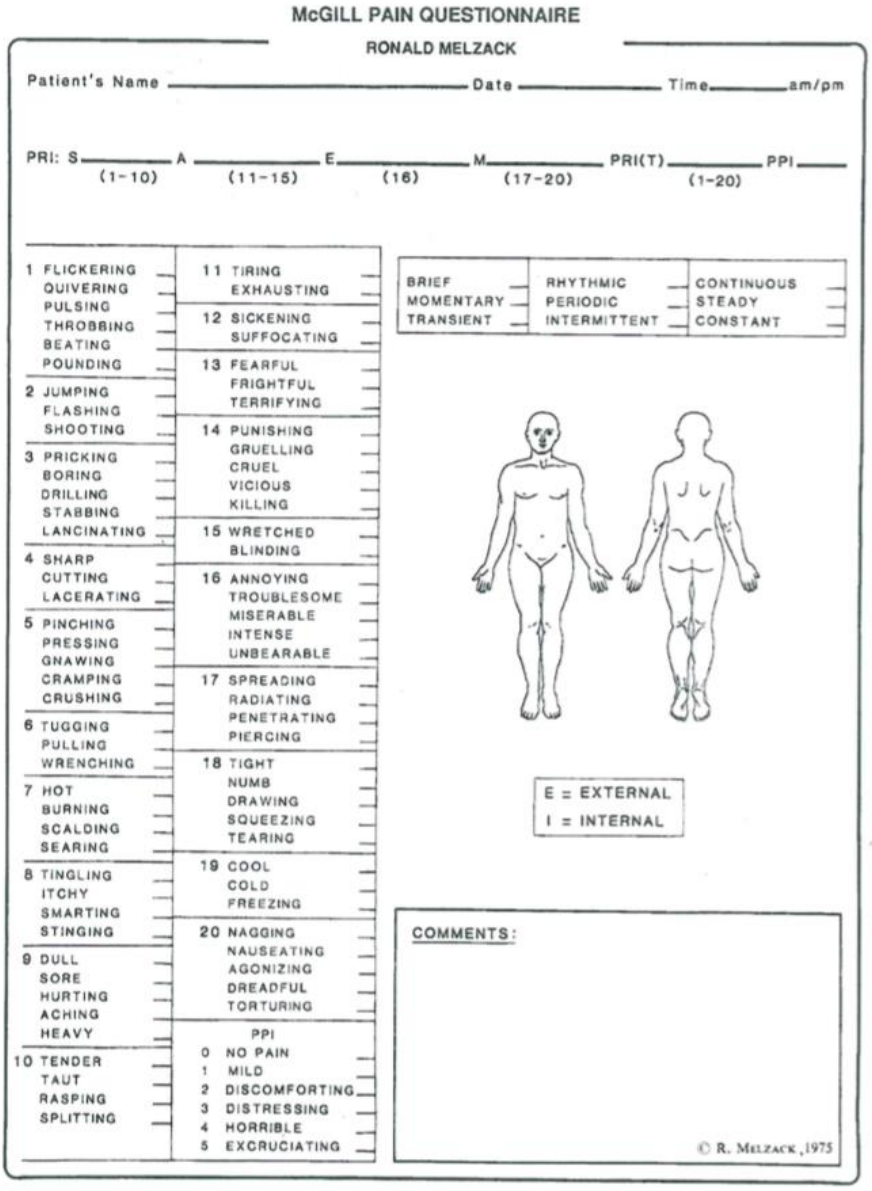

Figure 1.9. The long form McGill pain questionnaire to assess pain quality from Melzack (1983).

Pain Threshold

Pain threshold is another measure of pain that is often recorded, particularly in clinical settings. The measurement of pain threshold refers to the minimum stimulus required to evoke a painful response (O'Connor and Cook, 1999). This is done by systematically applying a noxious stimulus such as heat, cold, electricity or pressure in an ascending or descending intensity until

the participant identifies the onset/offset of pain. An average of several repeated trials is needed to determine a reliable pain threshold, which further highlights the plasticity of the pain pathways modulation of noxious stimuli. Several acute interventions can modulate the pain threshold. For example intramuscular injections of lidocaine reduces the pressure pain threshold by approximately 50% in healthy individuals (Staud *et al.*, 2009). This effect is not exclusive to pharmaceutical modification, even placebo analgesic injections have been shown to significantly reduce pressure pain threshold in those with fibromyalgia (Staud *et al.*, 2014) partly due expectation effects which implicates a psychological component to pain threshold. Indeed, other interventions such as the presence of a loved one (Tamam *et al.*, 2019) or laughter (Dunbar *et al.*, 2012). Gate control stimulation of a-fibres with transcutaneous electrical nerve stimulation can also increase pain threshold (Aarskog *et al.*, 2007). Therefore, a range of physiological, psycho-social, psychological, and pharmacological interventions can influence pain threshold.

Another influencer of pain threshold can be exercise. while intense exercise has been shown to cause naturally occurring muscle pain, there is also a well-documented hypoalgesic effect of aerobic (Koltyn *et al.*, 1996), dynamic resistance (Koltyn and Arbogast, 1998) and most notably isometric exercise (Naugle, Fillingim and Riley, 2012) with the effect extending to the whole body level (Naugle, Fillingim and Riley, 2012). This effect is only transient with a duration of up to 15-30 minutes (Kemppainen *et al.*, 1990; Koltyn *et al.*, 1996). The mechanisms which underpin this effect are numerous, but are primary related to the post-exercise release of endogenous opioids, hormones and endocannabinoids (Lesnak and Sluka, 2020) in combination with conditioned pain modulation (Lemley, Hunter and Bement, 2015).

One might expect that repeated bouts of aerobic exercise would enhance the pain threshold of an individual, however there is not sufficient evidence to support this as cross-sectional studies which have assessed the pain threshold between athletes and non-exercising controls have failed to reveal a consistent effect (Tesarz *et al.*, 2012). Six weeks of aerobic exercise failed to enhance pain threshold in untrained individuals (Jones *et al.*, 2014) and pain threshold did not predict time-trial performance (r = -0.016, P > 0.05) in healthy individuals (Astokorki and Mauger 2017) which casts uncertainty on the role of pain threshold in determining exercise performance, at least in normal conditions. An important consideration however is that methods of pain threshold testing typically involve algometry, contact heat, or electrical stimulation, which poorly reflect the type of pain elicited during exercise. It is plausible that

pain threshold adaptations may be specific to the aetiology of exercise induced pain i.e., the accumulation of a higher concentration of noxious biochemical are needed to evoke a pain response, however it would be methodologically challenging to experimentally increment noxious biochemical in a systematic order in a rested state.

Pain Tolerance

Pain tolerance is defined as the duration that an individual can voluntarily engage with a noxious stimulus for. There are a range of different methods to assess pain tolerance. One common method that is employed is the cold pressor test (Edens and Gil, 1995). This involves participants submerging their hand into cold water (usually 0-2ºC) for a period of time, causing pain. The goal is to keep their hand submerged for as long as possible, however due to ethical constraints, a limit is usually set on submersion time (Angius *et al.*, 2015) to prevent tissue damage, which introduces a ceiling effect on those with exceptional pain tolerance. Furthermore the ability to tightly control water temperature and provide circulation can significantly alter pain tolerance times (Mitchell, MacDonald and Brodie, 2004) making comparisons between studies difficult. Finally, the comparison of this particular sensation of pain to EIP is questionable (Angius *et al.* 2015; Astokorki and Mauger 2017).

Another method of assessing pain tolerance involves the occlusion of a limb with a tourniquet combined with low intensity isometric (5-20% of MVC) rhythmic contractions (Graven-Nielsen *et al.*, 2003). The occlusion of the muscle prevents venous return which prevents muscle metabolites being removed, consequently causing an accumulation, especially of substance P which stimulates and sensitises the muscle nociceptors (Herbert and Schmidt, 2001). The duration that the participant can/will perform these contractions is recorded and determined as pain tolerance. While ischaemic contractions are advantageous because they closely reflect EIP, it is confounded by the effect of muscle fatigue, particularly when higher contraction intensities and high duty cycles are employed (Iridiastadi and Nussbaum, 2006). Pain tolerance can also be even more malleable than pain threshold. For example, participants that thought they were receiving a strong analgesic but were getting a placebo improved their pain tolerance time with ischaemic contractions from 14.7 ± 2.5 minutes to 16.7 ± 2.5 minutes (Benedetti, Pollo and Colloca, 2007). In the context of exercise, pain tolerance appears to be much more plastic to the individual's circumstances within the context of the exercise bout. For example, during self-paced exercise an individual can freely moderate the level of EIP by varying power/speed. Changes in levels of motivation, prior experience and knowledge of the

exercise endpoint are all critical factors in determining pain tolerance (Mauger, 2014). With the right incentive (e.g., competition or financial gain), pain tolerance will improve (Bouaziz *et al.*, 2017) and previous painful experiences can alter self-efficacy of an ability to tolerate pain (Bandura *et al.*, 1987).

Given the psycho-social aspect of pain, it is unsurprising that pain tolerance can be 'trainable' (Anshel and Russell, 1994; Jones *et al.*, 2014). Indeed, a landmark study by O'Leary *et al.* (2017) compared the effects of a six week moderate intensity continuous training (MICT) program versus a volume matched, but more painful high intensity interval training (HIIT) program on a cycling ergometer. Despite similar improvements in maximal oxygen uptake, lactate threshold and peak power output, the HIIT group improved their performance on a time to task failure exercise test (intensity = 50%Δ between lactate threshold and $\dot{V}O_{2max}$) by 43 ± 56% whereas no improvement was seen in the MICT group. This performance increase was associated with the increase in pain tolerance determined by an ischaemic contraction test (r = 0.51, $p = 0.011$). While a constant load exercise task provides good control, it would be insightful to explore whether an increase in pain tolerance provides similar performance enhancements to self-paced exercise. Nevertheless, these findings provide evidence on role of pain tolerance on endurance performance success. While the mechanism(s) for this change are not fully understood, it does not appear to be exclusively peripheral adaptations as the tourniquet test was performed on the arm, suggesting an improved central processing of the nociceptive signal (O'Leary *et al.*, 2017). One peripheral mechanism may arise from the downregulation of ASIC protein expression which are required for EIP (Khataei *et al.*, 2020).

An increase in pain tolerance may make it possible for an individual to continue exercise at near maximal pain intensities and potentially 'access their physiological reserve' (Mauger, 2014). Additionally, an individual could produce a greater power/speed for a given pain intensity. The link between EIP and performance of endurance exercise has started to receive more attention. Pain which is present during maximal exercise may play an important role in endurance performance success and the development of fatigue, with the latter two being potentially interrelated. The next section will attempt to make sense of the link between exercise, muscle pain and the development of exercise-induced fatigue.

1.2.4 Exercise, Pain, and Fatigue Interaction

As previously discovered, EIP appears to scale linearly with exercise intensity above 50% of peak power output or most likely above critical power/speed (Cook *et al.*, 1997). Similarly, the

greater the perception of effort, the greater the perception of aches and pain in the leg (Borg, Ljunggren and Ceci, 1985). Interestingly, muscle fatigue also appears to increase as a function of exercise intensity when above the critical torque i.e., the greater the exercise intensity the greater the rate of the development of global, central and peripheral fatigue (Burnley, Vanhatalo and Jones, 2012). During constant load cycling or isometric knee extensor exercise in the severe intensity domain, pain progressively increases to (near) maximal levels at the point of task failure (Aboodarda *et al.*, 2020; Smith *et al.*, 2020). Interestingly, the development of central fatigue during the initial parts of the exercise is minimal but central fatigue appears to be present towards the latter part of the exercise (Decorte *et al.*, 2012). It is plausible that the progressive increase in pain intensity during exercise is causing fatigue that is centrally mediated. Unfortunately, this has not been tested in the context of EIP, and a limitation of many studies investigating the development of neuromuscular fatigue during exercise do not measure pain intensity which makes it difficult to directly assess how pain can cause fatigue.

Because EIP and exercise-induced fatigue appear to develop in tandem, it could be suggested that exercise induced fatigue is at least in part caused by pain. From a theoretical standpoint this seems possible because pain ultimately serves as a protective mechanism to prevent damage to the body. So, if a high amount of pain is present, muscle force might be downregulated in a feedback loop to prevent the development of further pain and consequently prevent the development of catastrophic levels of muscle fatigue. The decrease in muscle force (fatigue) may further act as a protective mechanism in response to pain as fatigue can reduce the force and ability to move the painful muscle which in itself can be a protective method to reduce the threat of damage.

From an evolutionary perspective, extreme levels of muscle fatigue are unfavourable as this would result in acute ambulatory issues which could be catastrophic in the presence of danger. Whilst speculative, this may also explain why a 'physiological reserve' exists as the necessity of survival supersedes the acute protection from fatigue. This also could explain why it is possible to endure higher levels of pain in exercise where the imminent disengagement from exercise is anticipated.

Because muscle pain is a conscious sensation and is generally regarded as unpleasant and aversive, there is a psychological drive to reduce exercise intensity or stop altogether for relief (Navratilova and Porreca, 2014). Also, there is some evidence that pain can act on a neurophysiological (i.e., involuntary basis) by causing a reduction in the excitability of the

corticospinal pathway (Farina *et al.*, 2001; Le Pera *et al.*, 2001; Svensson *et al.*, 2003). The painful signals processed by the brain may constrain central motor drive to the painful muscle. Essentially, muscle pain can impair muscle function, but it is unclear whether the mechanism is primarily due to a voluntary disengagement of activity or is it due to a physiological and involuntary down regulation of force generating capacity. Unfortunately, it is difficult to experimentally test how or whether EIP causes fatigue. This is because fatigue is a multifaceted process which introduces many confounding factors. To elaborate, as exercise intensity increases so does the accumulation of fatigue inducing metabolites such as inorganic phosphate (Pi) and H^+ (Vanhatalo *et al.*, 2010). Similarly, muscle damage can occur at higher exercise intensities (Peake *et al.*, 2005) which can independently induce long lasting decrements in force production and cause further increases in pain sensitivity (Pearcey *et al.*, 2015). Finally substrate depletion during more prolonged endurance exercise can compromise exercise tolerance (Bergstrom *et al.*, 1967). With all these confounding factors outlined, it is unsurprising that the role of pain on fatigue and exercise performance remains ambiguous. One way to understand the fatigue-pain relationship would be to decouple the intensity of pain from exercise intensity to either exacerbate or attenuate pain for a given exercise intensity, however some methods which have attempted to do this have limitations which are discussed later (see sections 3 and 4).

This section has outlined what pain is and how it is caused and how it might be involved with the development of fatigue. The next section will turn focus to exercise-induced fatigue by providing a background on the types and aetiology of fatigue.

Section 3 – Effects of Reduced Pain on Fatigue

1.3.1 The Purpose of Reducing Pain

To fully investigate the role of pain on endurance performance, we must explore how reducing pain can impact endurance performance and neuromuscular fatigue. Reducing pain involves minimising the perception of pain from a given nociceptive stimulus or directly reducing the magnitude of the nociceptive stimulus itself. There has been a significant amount of attention in clinical research in ways to reduce pain to help manage conditions which cause chronic muscle pain (e.g., fibromyalgia, lower back pain). However, there has been a growing interest in attenuating EIP to enhance exercise performance. As EIP is hypothesised to limit exercise tolerance and neuromuscular fatigue (see 'Exercise Pain and Fatigue Interaction), an individual

with lower pain levels may be able to produce a greater work rate for a given level of pain they are willing to tolerate or be able to attenuate the potentially fatiguing effects of pain. This could have significantly important implications for exercise performance. This next section will outline different models of reducing muscle pain that have been utilised in the research and their effect on endurance performance and fatigue.

1.3.2 Experimental Models of Reducing Pain

Paracetamol

Paracetamol (also known as acetaminophen) is a commonly used and widely available analgesic (Piletta, Porchet and Dayer, 1991). The mechanism to which paracetamol reduced pain is primarily through the serotonergic descending pain pathways (Anderson, 2008) and the inhibition of cyclooxygenase enzymes which stimulate nociceptors through prostaglandin synthesis. Paracetamol therefore could be used as a model of experimentally reducing muscle pain.

Several studies have investigated the effects of paracetamol ingestion on exercise performance. One of the first studies to test this was by Mauger, Jones and Williams (2010). They tested the effects of 1.5g of paracetamol on trained cyclists during a 10-mile time trial in comparison to a placebo. Time trial completion time was improved by a small but meaningful 2% without any perceptual changes in exertion or pain, suggesting that the participants could produce a greater power output for the same level of pain and exertion. Additionally, at the midpoint of the time trial blood lactate concentrations and heart rate were greater with paracetamol indicating that a greater physiological strain could be 'tolerated'. Further evidence for this ergogenic effect of analgesia was demonstrated in a study by (Foster *et al.*, 2014) which examined the effect 1.5g of paracetamol on 8 × 30 seconds cycling repeated sprints (2 minutes recovery). The mean power output across the latter three sprints was improved by 8-9% when exercise induced pain was the greatest. Furthermore, an identical protocol in women saw no differences in mean power but a greater peak power with paracetamol ingestion (Delextrat *et al.*, 2015) suggesting a preservation of maximal force production with analgesia. Why this did not translate to a greater mean power is not clear. Conversely, ingestion of 1.5g of paracetamol for the same intermittent sprint protocol as the two previous studies but for treadmill running did not influence performance despite marked reductions in pain perception during the latter bouts (Park *et al.*, 2016). Unfortunately, the precise mechanisms of how paracetamol might alter the development of neuromuscular fatigue cannot be inferred as these were not measured.

One study by Mauger and Hopker (2013) found that corticospinal excitability is improved with paracetamol ingestion, therefore it is uncertain if lowering pain levels was responsible for producing these minor improvements in performance or whether this was due to a greater excitability of the corticospinal pathway.

More recent work has attempted to investigate the mechanisms behind the potential ergogenic effect of paracetamol. One study by Morgan *et al.* (2018) investigated the neuromuscular fatigue responses of maximal intermittent isometric contractions after the ingestion of 1 g of paracetamol or placebo. With paracetamol, the mean torque across all contractions was 61 ± 11% of MVC with paracetamol and 58 ± 14% with placebo. This was accompanied by a much greater end exercise EMG amplitude (87 ± 28% versus 59 ± 17% respectively). This attenuated decrease in muscle activation may reflect the reduction in excitation of group III/IV afferent feedback caused by paracetamol ingestion thus exerting less inhibition of the CNS and the preservation of voluntary activation, however if this was the case, an exacerbation in peripheral fatigue would be expected, but this was not observed. Furthermore, this increase in muscle activity may have been mediated by the aforementioned improvement in corticospinal excitability. Unfortunately, pain was not recorded in this study making it difficult to discern if pain intensity was even altered during this exercise. These findings have also been extended to whole body exercise (Morgan *et al.*, 2019). The ingestion of 1 g of paracetamol improved critical and mean power determined by the 3 minute all out test. Subsequent T_{lim} calculations also predicted a 1.1 - 3% improvement of 100-1000kJ time trials. These findings were also accompanied by an attenuation in the fall of the EMG amplitude.

In summary, the paracetamol model of experimentally reducing muscle pain presents some evidence that pain may be a factor related to endurance performance success by allowing for a greater power output for a given amount of pain and potentially 'permitting' a greater muscle activation and physiological strain for a given exercise task, however the effect is only mild (~1-5% performance improvement). This may be due to the weak, non-localised effect paracetamol offers into reducing pain (Tiippana *et al.*, 2013). Further research is needed to investigate the role of paracetamol on constant load exercise with measures of neuromuscular fatigue and pain intensity to provide stronger evidence for the role of analgesia on the development of neuromuscular fatigue and exercise tolerance. This has been investigated in the heat (Mauger *et al.*, 2014) but paracetamol has an antipyretic effect which could improve exercise tolerance in the heat independent of analgesia. A model of reducing pain with a more

localised and more potent analgesic substance may provide more insight, such as intrathecal injections of fentanyl.

Intrathecal Fentanyl

Fentanyl is a potent opioid analgesic. When injected into the vertebral interspace of the lumbar spine (intrathecal injection), it blocks around 50% of the ascending afferent signalling (Hureau *et al.*, 2018) which includes pain (Hilty *et al.*, 2011) whilst keeping the descending central motor drive intact. Therefore, this is an experimental model which blocks pain and other forms of afferent feedback and has been used to assess the role of afferent feedback (and not pain per se) on exercise performance and neuromuscular fatigue.

The first study to pioneer this this model was by Amann *et al.* (2009). They performed a 5 km time trial after a fentanyl, placebo (saline) or no injection (control) trial combined with measures of central and peripheral fatigue pre and post exercise. Interestingly, there was no difference in the mean power output during the time trial between conditions. But upon closer inspection of the data, during the first half of the time trial participants had a greater mean power with fentanyl compared to the other two conditions but had a lower power output on the second half resulting in a similar mean power. It is likely that that the fentanyl caused participants to adopt a suboptimal pacing strategy whereby participants were too aggressive in the first half of the performance. When looking at the neuromuscular function data, peripheral fatigue was greater with fentanyl, which is interesting because the end exercise peripheral fatigue is remarkably similar across an array of conditions (Romer *et al.*, 2007; Burnley *et al.*, 2010; Vanhatalo *et al.*, 2010) which suggests an 'override' of the individuals critical peripheral fatigue threshold occurred. This was accompanied by a greater EMG amplitude with fentanyl, which the authors suggested infers greater central motor drive. Together these findings indicate that the attenuation of inhibitory afferent feedback permitted a greater level of central motor drive and power output but consequently caused a much greater amount of metabolic perturbation which impaired power output on the second half of the time trial when severe levels of peripheral fatigue developed. While these results provide important insight into the role of afferent feedback on fatigue, there are several methodological concerns with intrathecal fentanyl which make interpreting the findings challenging. Firstly, an additional consequence of the fentanyl is a blunted exercise pressor reflex. Indeed, a 3% lower tidal volume was observed with fentanyl which resulted in a much greater end tidal CO_2 and reduced SpO_2 due to hypoventilation. This alone may counteract the potential improvement in performance with

recued afferent feedback due to an impaired oxygen delivery. Secondly, measures of neuromuscular fatigue were taken three minutes after task failure which allows for considerable recovery, particularly of voluntary activation (Pageaux *et al.*, 2015) which may explain why there was no difference between conditions. To mitigate the potential effect of this altered pacing strategy with the fentanyl, the same lab published a study using a similar design except the exercise modality was a constant load cycling time to task failure (Amann *et al.*, 2011). Surprisingly, in this study, exercise tolerance was reduced by 21% with the fentanyl compared to placebo. Again, this was presumably due to the exacerbation of peripheral fatigue caused by the insufficient cardio-respiratory response with afferent feedback blockade. Peripheral fatigue was about one third greater with the fentanyl injection and central motor drive inferred by EMG was lower at task failure in the placebo condition suggesting a role of inhibitory pain/afferent feedback on central motor drive. More recent evidence has expanded on these findings (Blain *et al.*, 2016) and found similar performance and neuromuscular changes but with the addition of muscle biopsy measures. There was a greater metabolic perturbation as seen by greater increases in inorganic phosphate, ADP, pH, and La^-. Again, this suggests that the intramuscular perturbation is greater with afferent feedback blockade due to a lack of inhibition on central motor drive with reduced afferent feedback. Further mechanistic work also found that with the fentanyl model applied during cycling, the cortical silent period assessed by TMS was shorter compared to the control condition (Sidhu *et al.*, 2017), showing that corticospinal inhibition was attenuated with the blockade of afferent feedback whereas no difference in MEP amplitude which assesses corticospinal excitability was seen.

Whilst these studies provide novel insight into the role of afferent feedback/pain on exercise performance and the development of neuromuscular fatigue, they are confounded by the fentanyl-induced hypoventilation. Some studies have applied the same model to isometric small muscle mass exercise which has a much lower cardio-pulmonary demand and circumvent the issue of hypoventilation. Firstly Broxterman *et al.*, (2017) required participants to perform isometric knee extensor contractions above the critical torque (~58% MVF) with a 3 s contraction time interspersed with 2 s of rest. This protocol was performed within a magnetic resonance imaging (MRI) machine to capture the intramuscular metabolic perturbation at a high resolution. Similar to the previous studies, no difference in time to task failure was observed (Fentanyl = 239 ± 73 s, Control = 257 ± 79 s). However, oxidative ATP synthesis was not different suggesting no cardiopulmonary limitations. On the other hand, the rate (and magnitude) of perturbations in PCr, Pi and pH was much greater with fentanyl, indicating that

afferent feedback is in part responsible for maintaining efficient skeletal muscle bioenergetics. In the subsequent year the same authors repeated the experiment, but used the all-out intermittent critical torque test (Broxterman *et al.*, 2018). This test requires participants to perform 60 MVCs (3 s on, 2 s off) for a total of 5 minutes. The cumulative integrated force of the contractions over the first minute was ~8% greater with fentanyl compared to control, with no difference being observed between minutes 2-5. This shows that early on in exercise the blocking afferent feedback/pain can result in an enhancement of exercise performance.

Finally, a recent study attempted to overcome the limitations of the fentanyl by attempting to preserve the muscle oxygenation of the locomotor muscles when intrathecal fentanyl is administered (Hureau *et al.*, 2019). To do this, participants received 100% oxygen to inspire with and without the intrathecal fentanyl injection along with a trial with a normoxic condition and intact afferent feedback. A 5 km time trial was performed, and measures of neuromuscular function were recorded 30 s after exercise completion. The exercise time was 3.5% faster with hyperoxia and intact afferent feedback compared to normal conditions. However, the fentanyl trial with hyperoxia was 3.3% faster than the trial with hyperoxia and intact afferent feedback. This was accompanied by an average 8% greater EMG amplitude during the time trial. The decrease in MVC force was greater with fentanyl as was the decrease in potentiated quadriceps twitch torque (i.e., peripheral fatigue). No difference in voluntary activation was observed. Cardio-respiratory variables such as minute ventilation, heart rate, femoral blood flow or leg O_2 delivery were not different indicating that the hyperoxic inspiration preserved the cardio-pulmonary kinetics. Thus, to date, this study provides the strongest evidence that pain/afferent feedback constrains locomotor muscle fatigue and central motor drive to a critical/intolerable level.

To summarise, intrathecal fentanyl injections provide a novel insight into the role of pain and afferent feedback on exercise performance and neuromuscular fatigue. From the evidence presented, it appears that afferent feedback may limit the magnitude of the intramuscular metabolic perturbation to a maximum tolerable limit by constraining central motor drive to the active musculature. A limitation with this experimental model is that it is impossible to discern the role of muscle pain on constraining central motor drive because the intrathecal fentanyl blocked the transmission of both nociceptive and non-nociceptive group III/IV afferents. While the evidence suggests that afferent feedback may be a limiting factor in endurance performance, it also has an ergogenic effect in mediating an appropriate cardio-pulmonary

response to exercise, resulting in group III/IV afferents being a 'double edged sword'. Ultimately, nociceptors and metabosensitive afferent receptors play a role in protecting the body from a 'catastrophic' level of muscle fatigue, therefore blocking these protective mechanisms can result in levels of peripheral fatigue and pacing strategies that end up being detrimental to performance. While some may interpret this evidence as weak for the role of pain and afferent feedback on fatigue (Marcora, 2010) however, when considering the limitations of the research and framing the fentanyl model as a 'proof of concept', there is a strong mechanistic link between pain, afferent feedback and neuromuscular fatigue. To further strengthen this hypothesis, a series of studies using a more localised analgesic approach such as intramuscular injections of lidocaine can provide strong analgesia to a small portion of the locomotor muscles whilst leaving enough important sensory information intact.

Lidocaine Injection

Lidocaine is an anaesthetic which can be applied to a localised area to cause a temporary reduction in pain perception. One use for Lidocaine is to assess shoulder function in those who have impingement or rotator cuff tears to assess the strength of the rotator cuff muscles. These types of injuries cause high levels of pain and result in severe impairment of external rotation forces that are thought to be related to pain and not the contractile function. Research by Steenbrink *et al.* (2006) measured external rotation maximal force and pain before and after a subacromial injection of 10 mL of lidocaine. The resulting effect was a large reduction in pain intensity from 7.7 ± 1.2 down to 0.9 ± 1.6 which coincided with a 34% increase in strength. Further work by Park, Lee and Lee (2008) also used subacromial lidocaine injections and found only an 11% increase in external rotation strength which was only observed in those with a full thickness tear of a rotator cuff. With a clear ergogenic effect of plain blockade present in those with pre-existing pain and injury, it is unclear how this would affect healthy populations or those with naturally occurring EIP. Interestingly, a lidocaine injection into the subacromial space did not reduce external rotation strength in those who were healthy and free of pain (Farshad *et al.*, 2012). Therefore, with no apparent impairment to central drive and a strong analgesic effect, lidocaine injections could provide a potential method for studying the fatigue-pain relationship.

Transcutaneous Electrical Nerve Stimulation (TENS)

TENS is a method of inducing analgesia by delivering a weak electrical current over a muscle. The resulting effect is the stimulation of large diameter non-nociceptive afferents, which are

responsive to touch, pressure, and vibration. Thin diameter nociceptive afferents respond to noxious chemical stimuli, and it is proposed that these afferents project to the same areas at the spinal cord. The electrical current causes the non-nociceptive afferents to preferentially project to the spine and 'close the gate' thus inhibiting the transmission of nociceptive afferents to higher brain areas where pain is processed (Sluka and Walsh, 2003). This mechanism is referred to as gate control theory (Melzack and Wall, 1965). Therefore, the application of TENS over the painful muscle during exercise can be used as an experimental model to reduce pain (Aarskog *et al.*, 2007; Ferreira *et al.*, 2017).

One particularly interesting study by Astokorki and Mauger (2017) used TENS during the performance of a 20% of maximum voluntary force isometric elbow flexion to task failure. There was a 27% improvement in endurance time which was accompanied by a mean 12% reduction in pain perception in comparison to a placebo. Furthermore, a 33 s improvement in a 10-mile cycling time trial time was observed which extends these findings to whole body, self-paced exercise. Unfortunately, it is unclear whether this ergogenic effect of TENS was related to psychological factors of reducing the unpleasant sensation of pain or due to the attenuation of central fatigue mediated by group III/IV afferent nociceptors. Interestingly, no difference was observed in the end exercise maximum voluntary force between conditions which is unexpected considering that the TENS trial exercised for a much greater duration. If pain was reduced, an individual would be likely to reach closer to their true 'physiological limit' and induce greater levels of exercise induced fatigue. In contrast, the application of TENS during a 5 km time trial did not significantly alter pain perception or completion time (Hibbert *et al.*, 2017). However, TENS was only applied before the time trial (and not during like previously done) which may explain why the intervention was not effective.

In a novel study design, Son *et al.* (2016) applied TENS or a placebo sham treatment during experimental knee pain induced by a continuous hypertonic saline infusion into the infrapatellar fat pad. The maximum voluntary force of the knee extensors acutely decreased by 26-29% however the application of TENS reduced pain by about 50% from a pain rating of around 4 down to 2 which partially recovered maximal force so that there was only a 15% decrement from baseline. Similarly, pain reduced central activation ratio (a measure of central fatigue) by 10-11% but TENS recovered it to only be reduced by 4% while no recovery was seen with the sham condition. Therefore, it appears that acute analgesia can immediately attenuate the central fatigue induced by pain.

More recently, a study by Behm *et al.* (2019) extensively investigated the effect of TENS on endurance performance. Participants underwent either 20 minutes of TENS, a TENS sham, or a control. Subsequently, in three different protocols, participants completed an isometric knee extensor contraction at 30% of maximum force until fatigue in the TENS treated leg, in the contralateral non-treated leg (after TENS) and the contralateral non-treated leg after a fatiguing task of the TENS treated leg (2 × 100 s MVCs). There was an 11.7% improvement in time to task failure with the TENS treated leg compared to control but no difference between TENS and sham. No differences were observed at all within the contralateral trial between conditions. However, when the knee extensors were fatigued prior to the contralateral exercise, the addition of TENS improved the time to task failure indicating a non-local effect of pain and fatigue. Unfortunately, no measures of pain were recorded in this study which makes it uncertain as to whether a reduction of pain was responsible for improving exercise performance. As a non-local effect was also observed, it could be speculated that the nociception from one limb may project inhibitory feedback on the contralateral limb and the attenuation of this with TENS can mitigate this.

In conclusion, TENS appears to be an effective method at reducing localised pain and provides strong evidence that the attenuation of pain can preserve neuromuscular function and provide an ergogenic effect when applied proximal to the painful tissues during exercise. However, TENS may act under a secondary confounding mechanism of promoting vasodilation (Hallén *et al.*, 2010; Tomasi *et al.*, 2015) thus increasing oxygen delivery, metabolite clearance and improving performance. Nevertheless, the partial recovery of maximal strength in a rested painful state with the application of TENS would argue against this notion as improving blood flow or oxygen delivery is unlikely to further improve neuromuscular function at rest.

1.3.3 Summary of Reducing Pain and Fatigue

Taken together, there is a strong body of evidence to suggest that analgesia has an ergogenic effect on endurance performance and can attenuate the development of central fatigue. The use of multiple models of experimental hypoalgesia (e.g., paracetamol, TENS, lidocaine etc.) provide novel methods of testing the fatigue-pain relationship, however these methods are partially confounded by other factors which may also improve endurance performance independent of pain. It is also important to consider that while analgesia may be beneficial for exercise performance to some extent, completely blocking pain could end up reversing the ergogenic effects. This is because pain ultimately serves as a warning to actual or potential

tissue damage and 'overriding' this may cause levels of tissue damage or metabolic perturbation which can pose a significant risk to the health and function of an individual. Furthermore, pain serves as an important sensory feedback tool to optimise pacing strategy. For example in the Amann *et al.* (2009) paper, partial blockade of sensory feedback resulted in an extremely aggressive positive pacing strategy and participants had ambulatory problems after the time-trial. Such a positive pacing strategy may not be optimal for most endurance bouts. To rigorously test the fatigue and pain relationship, the other end of the spectrum should be explored. How does the experimentally increasing pain impact endurance performance and neuromuscular fatigue? Does it cause the opposite effects of reducing pain (i.e., reduced exercise performance and causes central fatigue? The next section will explore this concept.

Section 4 – The Effect of Elevated Pain on Fatigue

1.4.1 The Purpose of Elevating Pain

As previously mentioned, EIP increases linearly as a function of intensity (Cook *et al.*, 1997). Exercise induced fatigue also increases with exercise intensity (Burnley, Vanhatalo and Jones, 2012). Therefore, it could be hypothesised that the increase in pain could be causing this increase in exercised induced fatigue. However, other factors that are also related to fatigue development such as metabolite accumulation may confound this relationship in normal conditions of endurance exercise. To experimentally test this, there needs to be an increase in the perceived pain independent of the exercise intensity to disassociate the magnitude of pain from the exercise intensity. Alternatively, pain can be experimentally induced during a resting state. Currently, it is thought that an elevation of pain stimulates group III/IV nociceptors which relays inhibitory afferent feedback to the central nervous system and subsequently causes an inhibition of the motor cortex (Bank *et al.*, 2013). Therefore, an increase in pain during endurance exercise is likely to exacerbate the magnitude of central fatigue and consequently limit endurance performance. This next section will now outline several different common models of experimentally inducing pain and their effect on neuromuscular fatigue and endurance performance.

1.4.2 Experimental Models of Pain

<u>Ischemia</u>

Ischemia is defined as the inadequate blood supply to a tissue. This is typically achieved with blood flow restriction (BFR) which is the process of placing an inflatable cuff around the proximal site of the muscle of interest (e.g., quadriceps). Inflation of the cuff compresses the

vasculature and impedes venous return. When ischemia is added to low intensity resistance exercise there is an elevated pain response (Hollander *et al.*, 2010; Loenneke *et al.*, 2011) due to the stimulation of group III/IV afferent nociceptors which respond to the accumulating levels (and lack of clearance) of noxious biochemical substances induced by ischemic conditions (Kaufman and Rybicki, 1987). The pain of ischemic contractions is similar to that of EIP (Graven-Nielsen and Arendt-Nielsen, 2003; Arendt-Nielsen and Graven-Nielsen, 2008) which makes BFR a good model of exploring the effects of exercise-related pain on fatigue. Research has found that the increase in pain perception with BFR is accompanied by a reduced exercise tolerance (Loenneke *et al.*, 2012) and an exacerbation of central fatigue (Russ and Kent-Braun, 2003; Kennedy *et al.*, 2013, 2015). However, while BFR might effectively increase pain, a side-effect of causing ischemia is that the reduction in blood flow can cause peripheral fatigue (Russ and Kent-Braun, 2003; Karabulut *et al.*, 2010). One way to circumvent this issue is to cause ischemic muscle pain on non-exercising muscles. For example Kennedy *et al.* (2014) required participants to complete a 2 minute maximum voluntary isometric contraction of the adductor policis muscle (in the hand). This muscle was then occluded to maintain firing of nociceptors and then the neuromuscular function of the proximal, unfatigued elbow flexor was measured. They found that voluntary activation and torque of the elbow flexors was reduced in the presence of 'strong pain' (mean rating 5.3/10) caused by ischemia. In a subsequent study by Kennedy *et al.* (2015), knee extensor neuromuscular function was assessed after a 2 minute knee flexion MVC or a 2 minute contralateral knee extensor MVC. The subsequent voluntary force and voluntary activation was lower in the knee extensors after fatigue and maintained ischemia of the ipsilateral knee flexors, but no differences were observed in neuromuscular function after fatigue and maintained ischemia of the contralateral knee extensors, despite strong to very strong pain levels being recorded. The reason for the lack of effect is puzzling as previous work has clearly demonstrated a decrease in maximal force and voluntary activation of the unfatigued quadriceps (Martin and Rattey, 2007; Amann *et al.*, 2013; Doix, Lefèvre and Colson, 2013) after fatiguing contractions of the contralateral quadriceps even without subsequent ischemia. To elucidate the role of either fatigue or pain on neuromuscular fatigue, a study attempted to compare the non-local effects of either concurrent rising pain or prior contralateral quadriceps fatigue on performance and neuromuscular fatigue of the non-fatigued/non-painful quadriceps (Aboodarda *et al.*, 2020). Using a custom built cycling dynamometer which allows for the performance single-limb cycling exercise and isometric contractions (Doyle-Baker *et al.*, 2018), participants completed a time to task failure protocol

at 80% of peak power output which was either preceded by rest (CTRL), the same fatiguing exercise protocol in the contralateral leg (FAT), or simultaneous occlusion of the non-exercised, contralateral leg which progressively increased the perception of pain up to maximal levels (PAIN). Exercise capacity was reduced by in 43% FAT and by 21% in PAIN compared to CON, but the reduction was greater in FAT compared to PAIN. As a result, the end exercise decrease in maximum voluntary force was less in FAT and PAIN compared to CON while the potentiated twitch (i.e., peripheral fatigue) was only reduced to a lesser extent in FAT. Interestingly no differences were seen in voluntary activation measured by both TMS and PNS, although the same amount of central fatigue occurred in a shorter exercise time. There were also no differences in corticospinal excitability and inhibition between conditions except for a reduced silent period during an MVC in FAT compared to CON. Therefore, this study provides evidence that muscle pain that is non-local in origin can reduce exercise capacity, potentially due to a faster rate of central fatigue or attainment of intolerable pain levels.

To summarise, contractions during ischemia (and ischemia at rest) cause strong muscle pain which is similar in quality to EIP. This can promote central fatigue and reduce exercise tolerance, although this is confounded by the elevation of peripheral fatigue due to reduced blood flow, however a non-local effect of ischemic muscle pain has been observed, suggesting an additional centrally mediated effect.

Delayed onset of Muscle Soreness (DOMS)

DOMS is another model of experimental pain that has been utilised in pain research (Graven-Nielsen and Arendt-Nielsen, 2003). Typically, repeated eccentric muscle contractions are performed such as decline treadmill running or maximal eccentric elbow flexion exercise because lengthening muscles under high loads is more efficacious at promoting muscle soreness and pain compared to isometric or concentric muscle actions (Clarkson *et al.*, 1986; Newham, 1988). The cause of pain is likely related to the disruption to the integrity of the ultrastructure of the muscle tissue which stimulates the release of algesic and inflammatory substances such as prostaglandins, histamine and serotonin etc. (Newham, 1988; Tegeder *et al.*, 2002). The pain caused by this model is not particularly elevated in resting conditions (Tegeder *et al.*, 2002) however, hyperalgesia is present during muscle contractions or external mechanical pressure (Graven-Nielsen and Arendt-Nielsen, 2003) suggesting a sensitisation of nociceptors. Temporally, DOMS presents an advantage to other models of pain because it is long lasting. Pain can be elevated for as long as 2-4 days (Prasartwuth, Taylor and Gandevia,

2005; Behrens, Mau-Moeller and Bruhn, 2012) after the exercise bout which makes it possible to record a plethora of fatigue related measurements. Therefore, assessing central and peripheral alterations with PNS and TMS can be performed in the presence of DOMS to provide insight into the role of pain on neuromuscular function.

One study by Prasartwuth, Taylor and Gandevia (2005) employed this design by getting participants to complete eccentric contractions of the elbow flexors until maximum voluntary torque was reduced by 40%. Voluntary activation was assessed for multiple days after the exercise when muscle pain during the contractions was elevated. While voluntary activation was lowered in the presence of pain, the two variables were not significantly related, suggesting that the soreness present during the neuromuscular function assessment was not directly causing the deficit in voluntary activation. Nevertheless, increased soreness (and pain) appears to cause central fatigue. These findings have been replicated in subsequent studies (Racinais *et al.*, 2008; Behrens, Mau-Moeller and Bruhn, 2012). Furthermore, when DOMS is reduced through the repeated bout effect, there is less pain in conjunction with a lower decline in maximum voluntary strength after performance of the same exercise bout which further suggests a link between muscle pain/soreness and the degree of fatigue (Goodall *et al.*, 2017).

While DOMS is easy to induce and produces pain like EIP, it is confounded by the muscle damage it causes as Z-band streaming (i.e., direct damage to contractile properties) is likely to have a large effect on an individual's maximal force output. For example in Behrens, Mau-Moeller and Bruhn (2012), resting twitch amplitude was still reduced by 24.2% 24hrs after the exercise protocol. Because of this it is difficult to infer a cause-effect relationship between pain and reductions in endurance performance or maximum strength using the DOMS paradigm. Furthermore, the aetiology of pain from DOMS is related to mechanical damage and their inflammatory algesic counterparts which may stimulate different subgroups of nociceptors in comparison to acute EIP which originates from noxious stimuli such as high intramuscular pressures, muscle acidity and high concentrations of metabolites (e.g., La^-, K^+ and H^+).

To summarise, DOMS has been used to partially assess the fatigue-pain relationship, however it can only be used when the cause of fatigue can be differentiated i.e., central versus peripheral. DOMS has consistently been shown to cause central fatigue via a reduction in voluntary activation which is potentially due to inhibitory feedback from group III/IV nociceptors of the painful, damaged muscles.

Hypertonic Saline Injections

The final model of experimental pain that will be discussed in this section is the intramuscular injection of sterile saltwater (hypertonic saline). This model of experimental pain was first used in the late 1930's (Kellgren, 1938). The infusion of 0.5 – 1.5 mL of 5-6% NaCl concentration stimulates nociceptors, potentially through membrane depolarisation (Graven-Nielsen, McArdle, *et al.*, 1997; Mense, 2009) or indirectly through the release of the algesic substance glutamate (Tegeder *et al.*, 2002). A bolus of hypertonic saline can also increase intramuscular pressure (Graven-Nielsen, McArdle, *et al.*, 1997) which may cause pain via direct stimulation of group III nociceptors.

The hypertonic saline experimental model of muscle pain is particularly attractive for use in studying the fatigue-pain relationship because it is non-toxic (Svendsen *et al.*, 2005) and does not interfere with the electrophysiological properties of the muscle (Farina, Arendt-Nielsen and Graven-Nielsen, 2005). Because of this it has a distinct advantage over the previously described models of experimental pain (e.g., Ischemia or DOMS). Furthermore, the quality of pain is similar to that of clinical pain (Graven-Nielsen *et al.* 1997) and more recently a study compared the pain response from an intramuscular hypertonic saline injection to that EIP from isometric exercise (Smith *et al.*, 2020)**.** The quality of pain was different when comparing a resting hypertonic saline injection to the pain experienced during a 20%$_{MVF}$ submaximal isometric contraction to task failure. The 20%$_{MVF}$ produced more feelings of 'sharp' and 'exhausting' whereas resting hypertonic saline injections caused a 'shooting' feeling. Sensory and evaluative dimensions in the McGill pain questionnaire were similar between conditions. However, when the hypertonic saline pain quality was compared with the 20%$_{MVC}$ combined with a hypertonic saline injection, the quality of pain was similar. Therefore, by using a combination of light exercise and hypertonic saline injections, it is possible to replicate the quality of EIP.

To add to this, hypertonic saline injections are useful because the location, amount and time-course of the pain can be standardised. This is reflected by a good intra-individual reliability of the VAS area (r = 0.636; p = 0.048), VAS peak (r = 0.745; p = 0.013) and duration (r = 0.677; p = 0.032) of pain (Graven-Nielsen *et al.* 1997), however there is a large inter-individual pain response to a given volume, concentration or infusion rate (Graven-Nielsen *et al.* 1997) which makes it difficult to homogenise the pain response across a cohort of participants unless a constant infusion is performed where the rate of infusion is individualised to produce a desired pain response. Nevertheless, the injection protocols used in research have often

provided at minimum, a notable increase in pain intensity across most individuals. With the above points mentioned, the experimental induction of pain with hypertonic saline appears to be an appropriate choice for a model to uncouple the relationship between pain and exercise intensity which subsequently enables the experimental manipulation of pain and its role in neuromuscular fatigue and endurance performance.

Hypertonic saline injections are commonly injected into muscle tissue to simulate clinical muscle pain or into the infrapatellar fat pad of the knee to attempt to replicate knee pain. While the scope of this thesis is orientated around the role of EIP (i.e., deep muscle pain) the research on hypertonic saline induced knee pain will still be considered because although there could be differences between the effects of knee and muscle pain, there is a greater volume of research on knee pain which could aid in the interpretation of the research on muscle pain. Therefore, this section will synthesise the current knowledge on the role of experimental muscle (and knee) pain induced by hypertonic saline on neuromuscular fatigue and endurance performance.

1.4.3 Hypertonic Saline and Neuromuscular Function

A plethora of studies have investigated the effect of experimental pain using hypertonic saline on a variety of neuromuscular variables, particularly in the context of clinical pain. These include global fatigue, peripheral fatigue, central fatigue, EMG amplitude and TMS responses. All of these different components of neuromuscular fatigue have been somewhat investigated and each section will describe the current research on each specific area.

Endurance/Global Fatigue

One of the first studies using the hypertonic saline model of pain on neuromuscular function was that of by Graven-Nielsen, Svensson and Arendt-Nielsen (1997). They performed two experiments after injection 0.5 mL of 5% hypertonic or 0.9% isotonic saline injected into the tibialis anterior. Initially, they evaluated maximum dorsiflexion strength throughout the duration after isotonic or hypertonic saline. At the time of peak pain (approximately 40 s after infusion) peak torque was lower by 7.5% in hypertonic condition. Furthermore, the pain intensity was reported to be significantly correlated to the changes in maximum voluntary torque, although no correlation co-efficient is reported. In the subsequent experiment, participants received the same injections but in this time were required to perform an isometric contraction at 80% of maximum torque until it decreased to 30%. With the addition of hypertonic saline, endurance time was reduced by 19.9% (isotonic = 38.2 ± 20.1 s, hypertonic

= 30.6 ± 27.6 s). The authors speculated that these reductions in performance were due to a deficit in central activation (i.e., central fatigue) caused by elevated pain, however no measures were recorded to directly support this. While this study provided one of the first pieces of experimental evidence that pain can limit endurance performance and maximal strength, it is limited in its ability to explain the mechanisms as to why this occurred.

A subsequent study from the same research group (Graven-Nielsen *et al.*, 2002) sought to build upon this previous work. They injected 1.5 mL of hypertonic (5.8%) or isotonic saline (0.9%) in the rectus femoris (RF) muscle. With hypertonic saline, a 21 ± 7% reduction in maximum voluntary torque of the knee extensors was seen which recovered back to baseline after the cessation of pain. Unfortunately, no information was provided at which point after the saline injections the MVCs were performed or what the peak pain response was. Nevertheless, the inhibitory effects of pain appear to extend to the knee extensors. Two years later, a study by Ciubotariu, Arendt-Nielsen and Graven-Nielsen (2004) tested the extent to which experimental muscle pain reduced endurance performance. They required participants to complete isometric contractions at 50% and 80% of maximum voluntary torque with the tibialis anterior (dorsiflexion) and gastrocnemius (plantarflexion) after the injection of 1 mL (6%) hypertonic into either muscle or no injection. Endurance time, defined as the time before the participants were unable to keep the torque within 10% of the target for more than 3 s was reduced in all conditions by a range of 9.9 – 25.5% compared to the control condition (see table 1.1). Furthermore, the torque in the hypertonic saline trials was lower than during the control trial but was still within the 10% cut-off suggesting that participants exercised at a slightly lower intensity during the pain trial. Perhaps if the definition of task failure was more stringent (i.e., failure to maintain the exact target for 3 s) then the exercise intensities would have been similar and task failure would have occurred even earlier with hypertonic saline which means that the 25% reduction in endurance time may be an underestimation of the effect of elevated pain on endurance performance. In contrast, a study by Schulte *et al.* (2004) required participants to perform an isometric elbow flexion task at 40% of maximum voluntary force to fatigue (> 10% drop in target force for 3 s) when the pain response plateaued after the injection 1 mL of hypertonic (5.8%) or isotonic (0.9%) saline into the biceps brachii. No difference was observed in endurance time (hypertonic = 89.3 ± 22.6 s, isotonic = 102.3 ± 32.4 s). There are several explanations for this lack of change. Firstly, the experimental pain caused a peak pain of only 3.2/10 on the visual analogue scale where as it reached 4.4 and 6.4 - 6.5 in previous studies (Graven-Nielsen, Svensson and Arendt-Nielsen, 1997; Ciubotariu, Arendt-Nielsen and

Graven-Nielsen, 2004), therefore the magnitude of pain may have not been great enough to cause a change in endurance time. Furthermore, the fact that the intensity of the contraction was the lowest of all studies at 40% of maximum voluntary torque compared to 50% and 80% in other studies may have allowed participants to sustain the task after the pain from the hypertonic saline had mostly dissipated. All of the outlined studies were at least in part conducted by the same research group; therefore, these findings need to be replicated in a different lab. In 2011 a different research group (Khan *et al.*, 2011) employed the hypertonic saline model by injecting 1 mL of 5% hypertonic saline into the biceps brachii. The subsequent pain was approximately 5/10 which caused a small but significant 5% reduction in maximum voluntary torque. Interestingly despite strong pain being caused, the reduction in force was smaller than reported previous studies (7.5 – 20%). It may be that this effect is muscle specific, and the elbow flexors/upper body muscles are more resistant to pain induced reductions in neuromuscular fatigue. This would also explain why no difference was seen in endurance time of 40% MVC elbow flexion task within the paper by Schulte *et al.* (2004). However, experimental pain caused by hypertonic saline into the extensor carpi radialis brevis caused a ~11% reduction on maximum wrist extensor force, with the magnitude of force decrement being inversely related to peak pain (r = -0.38, P = 0.007). Table 1.1 provides a summary of the research on hypertonic saline induced experimental muscle pain and endurance performance.

In a more recent study by Smith and colleagues (Smith *et al.*, 2020) participants performed an isometric contraction of the knee extensors at 10% of maximum voluntary torque to task failure with either a 1 mL isotonic saline injection (0.9%) or hypertonic saline injection (5.85%) into the VL. They found a 26% decrease in time to task failure in the presence of pain. Interestingly, despite a large reduction in endurance time, the isometric MVC torque was not different between conditions, suggesting a potential acceleration in global fatigue.

Table 1.1. Summary of literature on the effects of experimental muscle pain induced by hypertonic saline on endurance performance. MVT = maximum voluntary torque. * Denotes significantly different from the respective control condition ($P < 0.05$).

Study	Hypertonic Saline Model	Exercise Task	Change in Endurance Time (%)	Effect Size (Cohen's D)
Graven-Nielsen, Svensson and Arendt-Nielsen (1997)	0.5 mL, 5% NaCl, Tibialis Anterior	80% MVT dorsiflexion isometric contraction	↓ 19.9*	0.31
Ciubotariu, Arendt-Nielsen and Graven-Nielsen (2004)	1 mL, 6% NaCl, Tibialis Anterior	50% MVT dorsiflexion isometric contraction	↓ 10*	0.68
	1 mL, 6% NaCl, Tibialis Anterior	80% MVT dorsiflexion isometric contraction	↓ 22.3*	1.12
	1 mL, 6% NaCl, Gastrocnemius	50% MVT plantarflexion isometric contraction	↓ 9.9*	0.81
	1 mL, 6% NaCl, Gastrocnemius	80% MVT plantarflexion isometric contraction	↓ 25.5*	1.49
Schulte *et al.* (2004)	1 mL, 5.8% NaCl, Biceps Brachii	40% MVT elbow flexion isometric contraction	↔	0.42
Smith *et al*. 2020	1 mL, 5.85% NaCl, Vastus Lateralis	10% MVT knee extension isometric contraction	↓ 26.0*	0.60

Research which has investigated the effect of knee pain induced by hypertonic saline on maximum voluntary torque has also been undertaken. One of the first studies to do this was by Henriksen *et al.* (2011)**.** They injected 1 mL of 5.8% hypertonic saline into the infrapatellar fat pad before completing isometric and isokinetic maximal contractions of the knee extensors and

flexors. After the hypertonic saline injection, all measures of knee extensor strength significantly decreased by 5-15% compared to the isotonic condition. The same was observed for the knee flexors except for the highest angular velocity ($180^{o}.s^{-1}$). Interestingly, even after cessation of pain, isometric torque remained depressed by 5.8%. These findings were replicated in a subsequent study by a different research group (Son *et al.*, 2016) except this study used a constant infusion of hypertonic saline to produce a stable increase in knee pain (30-40/100 VAS). A 26% decrease in maximum torque was seen with experimental knee pain which was correlated with the magnitude of perceived pain ($P < 0.001$, $r = 0.33$). Additional research has also found a 34% decrement of maximum voluntary torque in the presence of knee pain (Park and Hopkins 2013).

Performing a more detailed examination, one study used experimental knee pain and recorded an array of measures related to maximum force production (Rice *et al.*, 2019). They got participants to perform maximal isometric, concentric and eccentric contractions in the presence of knee pain caused by 1 mL of hypertonic saline injected into the infrapatellar fat pad. Peak torque was reduced by 9.7, 5.8 and 7.4% compared to baseline for the isometric, concentric and eccentric contractions, respectively. Furthermore, the rate of force development of the isometric contractions revealed a 12.5% reduction in the peak torque slope and at the early (0-100ms) slope of the isometric contraction, with no difference in the later phases (100-200ms). This is interesting because the initial rate of force development is thought to be primarily related to the level of neural drive (Folland, Buckthorpe and Hannah, 2014). Therefore, this study provided interesting evidence that not only is isometric torque compromised in the presence of pain but so is the ability to rapidly produce that force.

Finally a study by Oda *et al.* (2018) assessed the effects of experimental medial versus lateral knee pain on maximum voluntary torque of the painful knee extensors, non-painful contralateral knee extensors and the non-painful hand grip muscles. Medial knee pain caused by 0.5 $mEq.mL^{-1}$ of hypertonic saline caused a large amount of localised knee pain (median peak pain = 85.4/100 VAS) which resulted in a considerable 44% decrease in maximum voluntary force of the painful knee extensor. Moreover, the contralateral, non-painful knee extensor experienced a 20% drop in force and handgrip strength fell by 18% on the ipsilateral side and by 10% on the contralateral side. This study provides interesting evidence that intense knee pain can exert even non-local reductions in strength, an indication that acute intense pain may exert inhibitory effects at the central level.

Overall, the weight of the evidence strongly suggests that global fatigue is exacerbated by the experimental induction of pain as represented by a consistent decrement in both endurance time and maximal force generating capacity. Table 1.1 summarised the current literature available on the effect of hypertonic saline induced pain on endurance performance while table 1.2 summarises the effects of pain on maximal force generating capacity. It is important to note that there is a considerable amount of variation between studies and within studies. This may be explained by differing methodological variables with the hypertonic saline injection such as volume, concentration and infusion time (Graven-Nielsen *et al.* 1997) which can influence the magnitude of the pain intensity. Furthermore, the time when these measures of global fatigue were taken in relation to the injection can also influence the magnitude of effect. For example in Rice *et al.* (2019) some participants pain ratings had returned to zero before the assessment of maximum force had been completed.

Table 1.2**.** Summary of Literature on experimental pain induced by hypertonic saline injections on maximum voluntary torque/force (MVT/MVF) of the painful muscle. MVT = maximum voluntary torque. * = significantly different from no pain/control condition P <0 .05.

Study	Hypertonic Saline Model	Measurement	ΔMVT/MVF	Effect Size (Cohen's D)
Graven-Nielsen, Svensson and Arendt-Nielsen (1997)	0.5 mL, 5% NaCl, Tibialis Anterior	Isometric dorsiflexion MVT	↓ 7.5%*	0.29
Graven-Nielsen *et al.* (2002)	1.5 mL, 5.8% NaCl, Rectus Femoris	Isometric dorsiflexion MVT	↓ 21%*	1.12
Slater *et al.* (2003)	0.5 mL, 5.8% NaCl, extensor carpi radialis	Isometric wrist extension MVT	↓ 11%*	n/a
Khan *et al.* (2011)	1 mL, 5% NaCl, Biceps Brachii	Isometric elbow flexor MVT	↓5%*	n/a
Henriksen *et al.* (2011)	1 mL, 5.8%, Infrapatellar fat pad	Isometric Knee Extensor MVT	↓15%*	n/a
Henriksen *et al.* (2011)	1 mL, 5.8%, Infrapatellar fat pad	Isometric Knee Flexor MVT	↓7.5%*	n/a

Park and Hopkins (2013)	1 mL 5% NaCl, Infrapatellar fat pad	Isometric Knee Extensor MVT	↓34%*	n/a
Stackhouse *et al.* (2013)	1.5 mL 5% NaCl, Subacromial space	Isometric External Rotation MVT	↓32.8%*	n/a
Son *et al.* (2016)	0.154 mL.min^{-1} 5% NaCl Infrapatellar fat pad	Isometric Knee Extensor MVT	↓26%*	n/a
Salomoni *et al.* (2016)	1 mL 5% NaCl, Infrapatellar fat pad	Isometric Knee Extensor MVT	↓10%*	0.67
Oda *et al.* (2018)	0.5 mL, 1mEq.mL^{-1} NaCl, Tibial Insertion of Medial Collateral Ligament	Isometric Knee Extensor MVT	↓44%*	n/a
Rice *et al.* (2019)	1 mL, 5.8% NaCl, Infrapatellar fat pad	Isometric Knee Extensor MVT	↓9.7%*	0.96

Central Fatigue

As some of the research on the effect of pain on global fatigue were suggestive of a central effect being responsible for decreases in task performance, studies including a direct measure of central fatigue in response to pain need to be investigated. However, only a limited number of studies have used direct measurements of central fatigue during hypertonic saline induced experimental pain.

Firstly, a study by Khan *et al.* (2011) used both motor point stimulation and transcranial magnetic stimulation in two separate visits to assess voluntary activation for elbow flexion contractions during pain. Neither measure of voluntary activation decreased with pain, although surprisingly, voluntary activation with electrical stimulation was lower at the first measurement after the pain had dissipated compared to the isotonic saline condition. These findings are peculiar as it would be expected that if voluntary activation was reduced after pain had subsided then it should at least be reduced to a similar extent during pain. One explanation may be that the ITT is not sensitive enough to detect small changes in voluntary activation (Herbert and Gandevia, 1999), especially as the reduction in maximal voluntary force was only small (approximately 5%) and single twitches as opposed to doublets were used which are less sensitive to changes in voluntary activation (Folland and Williams, 2007). A further study by Park and Hopkins (2013) which assessed the effect of experimental knee pain on central

activation ratio found a 5% decrease with pain compared to the isotonic saline condition. In another study with a similar design, central activation of the quadriceps decreased from 0.99 to 0.89 (Son *et al.*, 2016). Unfortunately, the central activation ratio typically underestimates deficits in central activation failure due to the lack of normalisation to a resting stimulation. Another study in the same year by Salomoni *et al.* (2016) induced experimental knee pain and assessed voluntary activation with the ITT, however no difference in voluntary activation level was observed in comparison to the isotonic saline injection. Whilst the ITT was used, this study poses a different methodological issue because the reported mean baseline voluntary activation level was 76% (range 27-97%). This is well below the normative data provided in other studies of 85-95% (Becker and Awiszus, 2001; Newman, Jones and Newham, 2003; Lanza, Balshaw and Folland, 2017) which questions the validity of this voluntary activation data.

In conclusion, there is mixed evidence that pain can cause central fatigue. Unfortunately, due to limitations with the assessment of voluntary activation, there is a clear lack of methodologically robust studies to demonstrate muscle pain mediated reductions in voluntary activation. Another method to investigate central alterations to muscle pain is with TMS which can provide information about the integrity of the corticospinal pathway in response to pain.

One of the first studies to use the hypertonic saline model with TMS was by Le Pera *et al.* (2001). They injected 0.2 mL of 5% hypertonic saline into the right abductor digiti minimi (ADM) muscle. The contralateral motor cortex was then stimulated via single pulse TMS at the time of peak pain (5.8 ± 1.3 VAS). Remarkably, the amplitude of the motor evoked potential decreased by 40% compared to a non-painful injection of isotonic saline. In a second experiment, the authors injected the hypertonic saline into the left ADM and assessed the excitability of the right ADM. The MEP amplitude remained unchanged, suggesting that the pain induced decrease in corticospinal excitability does not affect the non-painful contralateral muscle. However, it should be considered that the muscles examined in the study were relatively small in comparison to the locomotor muscles and therefore the smaller absolute nociceptive stimulus may not be strong enough to exert a robust contralateral effect. Additionally, upper, and lower limb muscles are functionally different (i.e., dexterity for hands vs locomotion for quadriceps) making this study's extrapolation to quadriceps muscles limited. Subsequently, in a study by Martin *et al.* (2008), participants received a hypertonic saline injection into the biceps to maintain a pain rating of 3-5/10. Corticospinal excitability was subsequently assessed with TMS of the motor cortex with the addition of corticospinal tract

stimulation to obtain Cervicomedullary motor evoked potentials (CMEPs; i.e., spinal excitability). While the participants were relaxed, no difference in MEP was observed, but a 50% increase in CMEP amplitude was seen which consequently reduced the MEP/CMEP ratio. Similarly, when a contraction was held at 20% of peak EMG the findings were similar, however when a constant force contraction was performed, MEP amplitude was decreased with no changes in CMEP amplitude. These differences may be due to the varying total synaptic input between conditions. During a constant EMG contraction, synaptic input likely remained similar which means that afferent stimulation provided an excitatory input whereas descending excitatory input would remain similar to maintain the constant EMG output. Conversely, with constant force, the reduction in voluntary EMG in the presence of pain suggests less synaptic input and thus reduced MEPs. No difference in MEPs seen during rest but a decrease in CMEP and consequent a decrease in MEP/CMEP suggests inhibition of cortical output cells. Further mechanistic work by Schabrun and Hodges (2012) used a paired pulse TMS paradigm to measure short intracortical inhibition (SICI) and intracortical facilitation (ICF) of the first dorsal interosseous. During muscle pain, only intracortical facilitation decreased while intracortical inhibition was unchanged during pain but decreased after the pain had resolved, indicating that pain may be responsible for reducing excitation of cortical cells rather than being inhibitory in nature. Furthermore, a study by Rice *et al.* (2015) assessed corticospinal excitability and short intracortical inhibition in the lower limb muscles after experimentally inducing knee pain. Interestingly, in the presence of pain, the MEP amplitude of the VL and vastus medialis (VM) increased by 50%. No change in MEP amplitude was observed for the biceps femoris or tibialis anterior, suggesting only a local effect of pain on corticospinal excitability. Additionally, pain induced corticomotor suppression may also be muscle specific as evidenced by a reduced MEP amplitude in the first dorsal interosseous (hand muscle) but not in the extensor carpi radialis (forearm muscle) despite similar levels of pain being caused (Larsen *et al.*, 2018). The reason for this is not clear but solidifies the notion that pain does not exert a uniform inhibitory effect on corticospinal pathways and may even have an excitatory influence. One consideration with the paired pulse TMS measures such as SICI and ICF is that they have a much greater variability in the measured values (O'Leary *et al.*, 2015; Hermsen *et al.*, 2016; Biabani *et al.*, 2018) with ICCs of ~0.6-0.8 and CVs in excess of 15%. Therefore, no difference in ICF or SICI may be a product of high variability rather than a lack of an inhibitory effect. To further complicate matters, differences in pain location, muscles tested and background muscle activity (i.e., during relaxation or contraction) may cause some of these

differences in findings. More mechanistic work which quantifies intra-cortical facilitation, cortical silent period and CMEPs during exercise and pain may help elucidate the specific corticospinal mechanisms involved with pain.

Peripheral Fatigue

Only a few studies have investigated the effect of hypertonic saline induced pain on peripheral fatigue. Assessing peripheral fatigue in response to pain is important because it provides an extra level of confidence in concluding that centrally mediated mechanisms are responsible for the observed reductions in endurance time or maximum voluntary force. In the case of hypertonic saline injections, peripheral function measurements can confirm that the saline does not exert changes to muscle activity and consequently alter exercise performance.

The first study to assess peripheral muscle function in response to hypertonic saline pain was by Svensson *et al.* (1998). They infused hypertonic saline into the right masseter muscle which caused a pain of 60-70/100 on a VAS. In the presence of pain, electrical stimulations to evoke the compound muscle action potential were measured to determine changes in sarcolemmal excitability. The mean of the 20 stimulations between pre-pain and during pain were not different in peak-to-peak amplitude or response latency. However, the saline was injected into the contralateral masseter and therefore does not directly assess the peripheral properties of the painful muscle. To further investigate this, another study (Farina, Arendt-Nielsen and Graven-Nielsen, 2005) induced pain into the tibialis anterior by infusing incremental boli of hypertonic saline of 0.2, 0.5 and 0.9 mL. No difference in M-Wave amplitude, conduction velocity or spectral content was observed. Unfortunately, the twitch torque evoked could not be analysed due to the low frequency of stimulation (10Hz), so it remains elusive as to whether the force generating capacity remained intact with hypertonic saline. However, in a study by Graven-Nielsen *et al.* (2002) there was no difference between the superimposed twitch torque at various contraction levels (25-100%) with hypertonic saline compared to isotonic saline suggesting unaltered mechanical properties of the muscle. Furthermore, Salomoni *et al.* (2016) used the ITT which requires the delivery of a resting potentiated twitch after the injection of hypertonic or isotonic saline to cause experimental knee pain. The authors didn't report the amplitude of the twitch force but included all of the data as a supplement. Twitch force decreased by 2.3% after the injections, but there was no difference between isotonic or hypertonic injections and this decrease was likely a consequence of mild levels of peripheral fatigue from performing

repeated MVCs. Unfortunately, because this study induced knee pain and not muscle pain, it is difficult to extend these findings to when the hypertonic saline is injected intramuscularly.

Taken together, these findings indicate that experimental muscle pain induced via the injection of hypertonic saline is unlikely to have an effect on the electrical or peripheral apparatus of the muscle. While no change in M-Wave peak to peak amplitude may suggest this, some researchers have proposed that the peak to peak amplitude in the M-Wave does not necessarily indicate a change in sarcolemmal excitability (Rodriguez-Falces and Place, 2018). Rather, an increase in the first phase of the M-Wave is thought to reflect this, which has been observed after fatiguing isometric contractions (Rodriguez-Falces and Place, 2019; Rodriguez-Falces *et al.*, 2019). Nevertheless, a lack of difference in evoked force with electrical stimulation still indicated that the force generating capacity of the periphery is unaltered. Therefore, it is likely that any changes that are seen in global fatigue in response to hypertonic saline induced pain e.g., reduced maximum voluntary force or reduced endurance time are likely attributable to changes within the central nervous system. However, further research is needed to confirm these findings. In particular, peripheral fatigue needs to be measured during a fatiguing exercise task of the muscle which has received an intramuscular injection to see if pain can accelerate the development of peripheral fatigue via preferential recruitment of high threshold (more fatigable) motor units.

Electromyographic Responses (EMG)

Many studies have sought to investigate the effect of pain on muscle activity assessed via EMG. Using the hypertonic saline model of experimental pain, most experimental research has measured bipolar or high-density EMG activity in muscles relating to the painful site. Measurements of EMG during submaximal and maximal contraction intensities have been performed across isometric, isokinetic, and isotonic movements. Various measures of muscle activity can be derived from these signals such as amplitude, motor unit activity and area of activity.

A good starting point for investigating pain-related effects on muscle activity is to look at bipolar surface EMG activity of a painful muscle. In the tibialis anterior, there has been a reduction in the surface amplitude in response to saline-induced pain during a non-fatiguing 30% MVF contraction (Farina, Arendt-Nielsen and Graven-Nielsen, 2005). Similar findings were seen in a study by Ciubotariu, Arendt-Nielsen and Graven-Nielsen (2004). EMG amplitude of the agonist and the synergists of the dorsiflexors measured during high intensity

(50-80% of maximum) isometric contractions were significantly decreased at 0-80% of endurance time suggesting a uniform decrease in muscle activation among the prime movers during a constant isometric contraction. Additionally, during dynamic elbow flexion task, the EMG of the biceps brachii and brachioradialis are reduced, despite participants being able to complete the task just as effectively (Ervilha *et al.*, 2004). Interestingly, in another study, the amplitude of the trapezius muscle decreased during a constant isometric arm abduction contraction for 90 s at 15-20% of maximal voluntary force (Schulte *et al.*, 2004). Furthermore, in a study using a high density array of electrodes (HDEMG) to assess the spatial distribution of activity with pain (Madeleine *et al.*, 2006), hypertonic saline resulted in a shift that was more caudal, while with the isotonic saline a more cranial shift was seen. In other words, less activation was seen in the upper part of the trapezius which was proximal to the injection site. These findings have also been confirmed in another study on the trapezius during a repetitive dynamic task (Liew *et al.*, 2019). This indicates that a more localised change in muscle activity may take place in the presence of pain as opposed to inhibition of the whole muscle.

Whilst a number of studies have found a reduction in EMG amplitude in response to pain, there several studies which have failed to observe an effect. For example, in the study by Graven-Nielsen, Svensson and Arendt-Nielsen (1997) no difference was seen in surface EMG amplitude of the tibialis anterior during an 80% MVF isometric dorsiflexion until fatigue between isotonic and hypertonic saline conditions. However, only the tibialis anterior activity was measured whereas other muscles are responsible for dorsiflexion, so it is unclear if synergist muscle activity changed. Schulte *et al.* (2004) also saw no decrease in the biceps brachii EMG amplitude during a sustained 40% MVC isometric elbow flexion contraction but saw an increase in trapezius activity which could indicate that a compensatory mechanism of an increase in activity of the non-painful muscles may occur in some instances. During isometric knee extension exercise at 10% of MVF, there was no difference in VL, VM or RF EMG amplitude in hypertonic compared to isotonic saline. Therefore, it is currently unclear how muscle pain impacts EMG activity during submaximal contractions.

The reasons for the discrepancies in the findings for submaximal EMG amplitude are not immediately clear but could be that the level of pain induced caused by differing volumes and concentrations of hypertonic saline may also explain the difference. For example 0.5 mL of 5% was used in the study by Graven-Nielsen, Svensson and Arendt-Nielsen (1997) who saw no difference in activity but when the volume was doubled in a later study using the same exercise

protocol (Ciubotariu, Arendt-Nielsen and Graven-Nielsen, 2004), there was a significant decrease in agonist muscle activity. Similarly when 2 mL of hypertonic saline was injected into the VL a decrease in VL EMG was seen during cycling exercise (Canestri *et al.*, 2021). Therefore, high levels of pain may be required to cause a measurable change to muscle activity. Alternatively, the detection volume of the EMG electrodes in some studies may not be in close enough proximity to the saline-induced pain. For example, if the injection site is far away from the electrodes, or if the pain is deep within the muscle tissue, then the muscle fibres within the detection volume of the EMG electrodes could possibly be unaffected by the pain. Finally, there is the possibility that pain does not cause a uniform inhibition to the painful muscle and there is more likely a combination of excitatory and inhibitory inputs which result in no net change in EMG amplitude, and a more detailed analysis of muscle activity is warranted to determine more specific changes in muscle activity.

During maximal contractions, a more consistent change in EMG amplitude is observed. This is even the case when the decrease in force is accounted for (hypertonic = 2.96μV/Nm, isotonic = 3.60 μV/Nm; Graven-Nielsen, Svensson and Arendt-Nielsen 1997). Furthermore, these findings have been extended to maximal concentric and eccentric contractions during experimental knee pain (Rice *et al.*, 2019). However, no EMG amplitude in the quadriceps was seen after experimental knee pain in another study (Salomoni *et al.*, 2016), although the efficacy of the experimental pain is questionable as only 50% of participants had a force reduction >5% which may not be great enough to detect given that measures of EMG are more variable than force (Clark, Cook and Ploutz-Snyder, 2007). The surface EMG amplitude associated with a maximal voluntary contraction represents the maximum motor unit recruitment and firing rate possible by the individual in their current physiological state. As experimental pain often reduces strength, the reduction in EMG amplitude may partly be a product of a lower force generating capacity. Under resting conditions, the relationship between force and EMG amplitude is typically linear (Alkner, Tesch and Berg, 2000; Onishi *et al.*, 2000; Campy, Coelho and Pincivero, 2009). But, given the reductions in EMG amplitude even when force is accounted for (Graven-Nielsen, Svensson and Arendt-Nielsen, 1997) there could still be some other mechanism which is altering motor unit activity and causing a reduction in muscle activity.

Table 1.3. The effects of hypertonic saline induced pain on EMG amplitude during submaximal contractions. MVT = maximum voluntary torque. Ago = agonist muscle, Syn = synergist

muscle, Ant = antagonist muscle, Stb = stabiliser. Muscles examined: TA = tibialis anterior, ECU = extensor carpi ulnaris, ECR = extensor carpi radialis, FCR = flexor carpi radialis, EHL = extensor hallucis longus, GL = gastrocnemius lateralis, gastrocnemius medialis, SOL = soleus, VL = vastus lateralis, VM = vastus medialis, RF = rectus femoris, BF = biceps femoris.

Study	Hypertonic Saline Model	Measurement	EMG
Graven-Nielsen, Svensson and Arendt-Nielsen (1997)	0.5 mL, 5% NaCl, Tibialis Anterior	Dorsiflexion 80% MVT isometric contraction	↔ TA (Ago)
Birch *et al.* (2000)	0.3 mL, 5% NaCl Extensor carpi ulnaris	Wrist extension 10% MVT isometric contraction	↔ ECU (Ago) ↔ ECR (Syn) ↔ FCR (Ant)
Farina *et al.* (2004)	0.2, 0.5 and 0.9 mL, 5.8% NaCl, Tibialis anterior	Dorsiflexion 10% MVT isometric contraction	↔ TA (Ago)
Schulte *et al.* (2004)	1 mL, 5.8% NaCl, Biceps Brachii	Elbow flexion 40% MVT isometric contraction	↔ Biceps Brachii (Ago) ↔ Brachioradialis (Syn) ↑ Trapezius (Stb)
Ciubotariu, Arendt-Nielsen and Graven-Nielsen (2004)	1 mL, 6% NaCl, Tibialis Anterior	Dorsiflexion 50 & 80% MVT isometric contraction	↓ TA (Ago) ↓ EHL(Syn)
	1 mL, 6% NaCl, Gastrocnemius lateralis	Plantarflexion 50 & 80% MVT isometric contraction	↓ GL (Ago) ↓ GM (Syn) ↑ SOL (Syn) ↔ TA (Ant)
Farina, Arendt-Nielsen and Graven-Nielsen (2005)	0.2, 0.5 and 0.9 mL, 5.8% NaCl, Tibialis anterior	Dorsiflexion 30% MVT isometric contraction	↓ TA (Ago)
Smith et al. (2020)	1 mL, 5.85% NaCl, Vastus lateralis	Knee extension 20% MVT isometric contraction	↔ VL (Ago) ↔ VM (Ago) ↔ RF (Ago)
Canestri et al. (2021)	2 mL, 6% NaCl, Vastus Lateralis	Cycling TTF at 80% of peak power	↓ VL (Ago) ↑ BF (Ant)

As bipolar surface EMG has found equivocal evidence on the effect of muscle pain on submaximal muscle activity, the different aspects of motor units need to be investigated. This can be achieved with HD-EMG or intramuscular fine-wire EMG. One study (Farina *et al.*, 2004) used an array of 16 surface electrodes and intramuscular EMG on the tibialis anterior and performed isometric contractions at 10% of maximal force to determine motor unit conduction velocity and motor unit firing rates. In the presence of experimental pain induced by hypertonic saline, low threshold motor units decreased in their firing rate with no decrease in conduction velocity. Furthermore, this study incrementally applied boli of hypertonic saline during repeated non-fatiguing contractions to assess the relationship between the magnitude of pain and changes in EMG. There was a significant inverse correlation between pain and firing rates $(r = -0.45, P < .001)$ meaning that the greater the pain, the greater the reduction in the motor unit firing rate. No difference was seen in motor unit recruitment; however, it is important to consider that the detection volume is small with intramuscular EMG and activity at the localised area may not reflect changes across the entire tibialis anterior. Nevertheless, no adjustment to conduction velocity infers that the peripheral properties were kept intact and the changes in motor unit firing rates were centrally mediated. To further investigate this a subsequent study Hodges, Ervilha and Graven-Nielsen (2008) investigated the role of experimental muscle pain on motor unit activity of synergist muscles (medial gastrocnemius and soleus) of the painfully injected muscle (lateral gastrocnemius). Participants contracted at an intensity to allow for the recording of 1-4 motor units at baseline (no pain) or in the presence of low levels or high levels of pain. Motor unit firing rates were decreased by 12.3% for both muscles in high pain (4.9/10 VAS) and by 10.1% and 7.5% in low pain (3.3/10 VAS) for the medial gastrocnemius and soleus respectively but the magnitude of pain was not correlated with the change in motor unit firing rate. Interestingly, there was no difference in the surface EMG amplitude in either muscle. Therefore, experimental pain seems to cause a decrease in the firing rate of low threshold motor units and therefore higher threshold motor units were recruited to maintain force which subsequently caused no net change in the global surface amplitude. Additional research by Tucker *et al.* (2009) sought to confirm these findings and further elucidate the mechanisms as to how pain effects muscle activity. They experimentally induced pain within the infrapatellar fat pad to cause knee pain and measured motor unit activity in the VL and VM. Pain was also induced in a muscle which does not have any synergists (the flexor pollicis longus). The results found that motor unit discharge rate was reduced after the induction of pain in the synergists for the quadriceps and the prime mover for

the flexor pollicis longus. To compensate and maintain force, new motor units were recruited, however not all of the newly recruited motor units recruited in their predicted orderly fashion. In fact, 12 out of the 19 additional motor units that were recorded during pain were not the same motor units that are recruited during higher force contractions in a pain-free condition. This means that the normal increase in higher threshold motor units is not observed with pain but rather a redistribution of motor unit activity occurs instead. One hypothesis that has been proposed is that the direction of applied force is altered to minimise aggravation of the painful tissue. For example, during knee extension, more lateral and less anterior force may be applied. While this was not measured in the Tucker study, a previous study has found no differences in force direction with experimental knee pain (Salomoni *et al.*, 2016). It is therefore likely that the reduced motor unit firing rate caused by pain must be overcome by a greater central drive from the nervous system to produce the same amount of force resulting in the recruitment of additional, higher threshold motor units. To investigate if higher threshold motor units are affected by pain, recent work found that low threshold motor units decreased in firing rate during pain whereas high threshold motor units increased their firing rate and had a reduced recruitment threshold (Martinez-Valdes *et al.* 2020).

There have been several models to explain the response of muscle activity in response pain. Firstly, the vicious cycle theory hypothesised that muscle activity of the painful muscle increases during resting painful conditions (Roland, 1986) and this consequently exacerbates the symptoms of pain overtime. Contrary to this model, studies have found that pain induced by hypertonic saline does not cause long lasting changes in muscle activity nor is it related to pain intensity or duration (Graven-Nielsen, Svensson and Arendt-Nielsen, 1997; Svensson *et al.*, 1998). Hyperactivity would also suggest a greater EMG amplitude at low contraction intensities, which has not been consistently demonstrated. Therefore, the vicious cycle theory is unlikely to be occurring for acute muscle pain.

Alternatively, there is the pain adaptation theory (Lund *et al.*, 1991) which proposes that the muscle activity and range of motion of the painful muscle and its synergists is reduced to prevent further pain. Concomitantly, the antagonist to the painful muscle becomes hyperactive to constrain activity of the agonist. While this hypothesis has more empirical evidence to support it, several studies have challenged this hypothesis (Ervilha *et al.*, 2004; Hodges, Ervilha and Graven-Nielsen, 2008; Rice *et al.*, 2015) by finding excitatory responses in response to pain. Based upon these findings, a new model, ‘moving differently in pain’ was proposed

(Hodges and Tucker, 2011). This model proposes that there is not a uniform inhibition or excitability of the neuromuscular pathway in the presence of pain but rather a redistribution of muscle activity and several excitatory and inhibitory processes which compete at the central level. The weight of the evidence supports this theory as seen in increases in motor unit recruitment, corticospinal excitability, and changes in spatial activity patterns in response to pain. This is particularly likely for pain during submaximal exercise where potential sites of inhibition may need to be compensated for by greater excitatory processes in order to maintain a required task. However, the result of such competition could be less efficient and promote a more rapid attainment of fatigue (Martinez-Valdes *et al.* 2020).

1.4.4 Summary of Pain and Neuromuscular Fatigue

In summary, muscle pain from an intramuscular injection of hypertonic saline appears to reduce muscle strength and shorten endurance time. These functional changes appear to be underpinned by an exacerbation to central fatigue whereas peripheral fatigue remains unaffected. Centrally mediated effects appear to be primarily due to a decline in voluntary activation and an inhibition to the firing rates of low threshold motor units.

The majority of the research has been performed in resting or non-fatiguing conditions and a limited number of neuromuscular measurements have been recorded in each study. Given that discrepancies in findings are likely related to methodological differences, the performance of multiple neuromuscular measures during muscle pain in the same study or across similar methodological setups is warranted. Furthermore, the effect of pain on the development of neuromuscular fatigue during exercise is relatively unknown. The purpose of this thesis was to investigate the effect of muscle pain on endurance performance and neuromuscular fatigue during exercise. Therefore, the aims of the study were as follows:

1. To establish whether measures of neuromuscular fatigue are reliable.
2. To measure neuromuscular fatigue in response to pain during fatiguing exercise.
3. To assess endurance performance in the presence of elevated muscle pain.

In light of these aims, there are several hypotheses of this thesis which are based upon the previously outlined literature.

H_1 = Muscle pain from an intramuscular injection of hypertonic saline into the quadriceps would reduce endurance performance and maximal strength of the (contralateral) quadriceps

H_2 = A reduction in performance would be attributed to an exacerbation of central fatigue and peripheral fatigue would be unaffected.

H_3 = Pain would impair short duration, self-paced exercise performance

H_4 = Measures of neuromuscular fatigue would be reliable in fresh and fatigued conditions

Chapter 2: General Methods

2.1 Outline

Several common experimental measures, techniques and questionnaires were used across all four studies of this thesis. The aim of this section is to provide a description of these common methods employed within the thesis.

Ethical Approval. Prior to each study being conducted, ethical approval was obtained from the University of Kent's School of Sport and Exercise Sciences Research Ethics Advisory Group and all studies were conducted in accordance with the declaration of Helsinki. Prior to conducting any of the procedures below, participants read a participant information sheet and then provided written informed consent to participate.

2.2 General Procedures

Prior to each experimental study of this thesis, several pre-test procedures were completed, outlined below.

2.2.1 Health Questionnaires

Prior to participants undergoing any experimental procedures, a general health questionnaire was administered. This was to identify any known health conditions which would contraindicate a prospective participant to strenuous exercise. If participants answered yes to any of the questions in the initial sections and yes to any of the questions in the follow up section, then they were excluded from participation.

As this questionnaire is generalised to readiness for physical activity, there were some experimental techniques used in the studies which could pose risks to specific individuals. Therefore, after the general health questionnaire, a study-specific health questionnaire was administered to screen for any potential contraindications to the experimental techniques. This included specific questions regarding suitability for intramuscular injections (e.g., needle phobia, known allergies) and questions to determine contraindications for transcranial magnetic stimulation as per the guidelines from Rossi and colleagues (Rossi *et al.*, 2011).

2.2.2 Anthropometric Measures

Body mass was recorded with a set of scales and was recorded to the closes 0.1 kg while participants wore light clothing (i.e., shorts and t-shirt) with no shoes. Height was recorded with a stadiometer and participants stood straight against the device without shoes and with a neutral head position. Height was recorded to the closest 1 centimetre.

2.2.3 Familiarisation(s)

The first visit in each study always compromised of a familiarisation session of both the experimental measures and the designated exercise protocol. For the experimental procedures, this was to ensure that participants were comfortable with the measures (e.g., peripheral nerve stimulation and intramuscular injections) and to improve their reliability and validity. For the exercise protocols, a familiarisation was performed to determine the time to task failure of the exercise task (for studies 1-3) to ensure a task failure was achieved within four to six minutes. A second familiarisation was performed for studies one to three to ensure the time to task failure was of the appropriate duration and to further improve the reliability.

2.3 Experimental Procedures

2.3.1 Intramuscular Injections

A single bolus of 1 mL hypertonic saline (5.85% NaCl) was injected in the VL (the middle third of the muscle belly) of the leg to induce muscle pain. The site was cleaned with an alcohol swab and then the saline was manually infused using a 3 mL Luer-Lok syringe (BD, New Jersey, USA) connected to a 1.5 inch 25-gauge hypodermic needle (SurGuard2, Terumo, Japan) over a 20 s window. The needle was initially inserted then an aspiration was performed to ensure that that the needle tip was not in a blood vessel. If blood was drawn up on aspiration, then the injection procedure was restarted. After needle insertion (5 s), the infusion period commenced which involved manually infusing 1 mL of saline over the period of 10 s. The needle remained in situ for a further 5 s before being removed. An identical injection protocol was performed with the isotonic saline for the CTRL condition in studies two and three and ISO in study four.

Figure 2.1 General set up of the lab for the studies within this thesis.

2.3.2 Dynamometry

Participants were seated in an isokinetic dynamometer (Cybex NORM isokinetic dynamometer, CMSi, Computer 267 Sports Medicine Inc, Stoughton USA) for study 1 and a custom-built isometric chair for studies 2-4 with a fixed hip and knee angle of 90˚ (0˚ being full extension) to complete isometric contractions of the right knee extensors. Participants were strapped around the torso and at the ankle ~3 cm superior to the malleoli. Torque/Force was acquired via a cable from the dynamometer into data acquisition modules (CED Micro 1401-3, Cambridge Electronic Design, Cambridge, UK and MP150, Biopac Systems Inc., California, USA) which was sampled torque at a frequency of 2 KHz. To determine maximum voluntary torque (MVT), participants completed isometric contractions of the knee extensor muscles whilst torque was displayed instantaneously on a screen visible to the participants.

2.3.3 Peripheral Nerve Stimulation

An electrical stimulator (DS7a/DS7R, Digitimer, Hertfordshire, UK) with a maximum voltage = 400 V capable of delivering a single square wave pulse was used to deliver electrical stimulations to the femoral nerve to innervate the right quadriceps femoris. An anode (100 mm × 50 mm; Phoenix Healthcare Products Ltd, Nottingham, UK) was secured to the right gluteal fold. The cathode was initially a motor point pen (Compex; DJO Global, Guildford, UK). The pen was placed within the femoral triangle and single square wave pulses (200 μs duration) were delivered at 100 mA to determine the specific site at which the greatest twitch torque and compound muscle action potential amplitude was obtained. An Ag/AgCl electrode (32 × 32 mm, Nessler Medizintechnik, Innsbruck, Austria) was subsequently placed over the optimal stimulation site to be used as the cathode for the rest of the stimulations. Twitches were delivered in stepwise increments of 20 mA beginning from 100 mA to determine the stimulation intensity where a plateau in both the M-Wave and twitch torque was observed. To ensure supramaximal stimulation, an additional 30% stimulation intensity (Millet *et al.*, 2011) was added. Supramaximal stimulations were delivered as paired stimuli (1 ms duration, 10 ms inter interval pulse) and as single stimuli (2 ms pulse duration) where appropriate. See physiological measures below.

2.3.4 Transcranial Magnetic Stimulation

Single pulse TMS was delivered with a magnetic stimulation (Magstim 200^2, The Magstim Company Ltd, Carmarthenshire, UK) via a double cone coil (110 mm diameter) delivering a posterior-anterior current which was placed over the motor cortex to assess corticospinal

excitability and inhibition. Initially the participants' vertex was marked as the midpoint between the nasal-inion and the tragus. The coil was initially placed 2 centimetres to the left of this position to evoke a response in the right quadriceps. Stimulations were superimposed during a submaximal isometric contraction of the knee extensors equivalent to ~20% of the baseline maximum voluntary force. The stimulator intensity was set at 50% and location adjusted to determine the hotspot which evoked the greatest MEP peak to peak amplitude of the VL. Once this site was identified, four to five stimulations were delivered at 45% and increased in stepwise increments of 5% until a plateau in MEP amplitude was observed (< 5% increase). This position was marked on a tight-fitting swimming cap for subsequent stimulations.

2.3.5 Electromyography

Muscle activity of the lower limb muscles was recorded with Ag/AgCl electrodes. The muscles of interest were the VL, VM, RF and BF. Electrode sites were selected as the muscle belly most proximal to the knee for the VL as these sites would not interfere with the intramuscular injection sites and procedure. The RF was palpated for, and the electrodes were placed in the centre of the muscle belly. For the biceps femoris, the location at the midpoint between the knee joint centre and iliospinal tract was used. Once the site was selected, it was shaved, abraded, and cleaned with an isopropyl swab to reduce impedance. EMG data was band pass filtered (10 – 500 Hz) and sampled at 2KHz for wireless and 1.25KHz for wired. The wired system was used for the VL signal due it is having a ± 10 mV range which allows for recording evoked responses (e.g., M waves) without the issue of clipping whereas the wireless system was confined to ± 5 mV and was used to record voluntary muscle activity.

2.4 Physiological Measures

2.4.1 Global fatigue

The amalgamation of central and peripheral factors is what can be characterised as global fatigue and therefore the measure of the decrease in baseline maximum voluntary force/torque are considered the primary measure of global fatigue. The peak instantaneous measure of isometric force of the knee extensors is the value derived for this measure. Secondary to this is a reduction in endurance performance in relation to the same performance in a control condition is also an indicator of global fatigue.

2.4.2 Central Fatigue

The measurement of voluntary activation is used as the measure of central fatigue. Voluntary activation is calculated using the ITT method (Shield and Zhou, 2004). During a maximum voluntary contraction, a peripheral nerve stimulation doublet is delivered once peak force is reached, followed by another doublet stimulation during rest within 5 s of completion of the MVC. Because it is not always possible to deliver the doublet at peak a correction proposed by Strojnik and Komi (Strojnik and Komi, 1998) is applied where the peak force and force when the superimposed doublet was delivered are included in the calculation of voluntary activation. The calculation for voluntary activation is as follows:

$$100 - \text{SI Doublet} * \frac{(\text{force before SI doublet/peakforce})}{\text{resting potentiated doublet}} * 100$$

Any decrease in the value derived from a baseline measure is indicative of central fatigue.

2.4.3 Peripheral Fatigue

Changes to peripheral characteristic were primarily determined by the change in the peak instantaneous force of an evoked doublet from peripheral nerve stimulation. Within the evoked doublet, secondary measures of peripheral fatigue were taken such as the maximal rate of twitch development and maximum relaxation rate which are defined as the highest and lowest values of the first derivative of the force signal. These reflect the ability of the muscle's ability to contract and relax, respectively. Half relaxation time also reflects the ability of the muscle to relax which is defined as the time taken from peak force to 50% of that. Contraction time reflects the ability of the muscle to rapidly contract.

2.4.4 Corticospinal excitability

The excitability of the corticospinal pathway was quantified as the average of the peak-to-peak amplitude of the motor evoked potential normalised to the peak-to-peak amplitude of the M wave. This M wave was delivered after the series of MEPs to acquire the $MEP \cdot M_{max}^{-1}$ ratio. This accounts for peripheral changes which might influence the amplitude of the MEP. The decrease in this $MEP \cdot M_{max}^{-1}$ ratio from baseline is reflective of a decrease in corticospinal excitability.

2.4.5 Corticospinal Inhibition

To reflect inhibition within the corticospinal pathway, the TMS silent period was quantified as the point of stimulation, which was displayed in the data acquisition software until the

resumption of voluntary EMG activity which was visually determined by the same investigator. Any increase in the duration of this period from the baseline period was indicative of corticospinal inhibition.

2.5 Perceptual Measures

2.5.1 Pain Intensity

Perceived pain intensity was recorded on a custom built pain device (linear potentiometer) with the Cook pain scale (Cook *et al.*, 1997). The lower bound was 0 (no pain at all) and upper bound was 100 (extremely intense pain, almost unbearable). Anchors were also set at 10, 20, 30, 40, 50 and 70 which corresponded to weak pain, mild pain, moderate pain, strong pain, and very strong pain, respectively. Pain ratings were specifically instructed to be selected based upon the pain from exercise and/or the injected solutions and the upper limit was calibrated to the worst EIP ever perceived. Participants were explicitly informed not anchor pain experienced from other sources (e.g., bone fracture) or from a theoretical imagined maximal pain (e.g., severe burns).

Figure 2.2. Custom built pain recording device (linear potentiometer) to record rating of pain every 2s.

2.5.2 Rating of Perceived Effort

Measures of perception of effort were recorded with the 6-20 Borg scale (Borg 1982). The definition of RPE given to participants was “the effort to drive the limb” and participants were specifically instructed not to incorporate feelings of pain or fatigue within their rating. The lower bound, six, was anchored to “no effort at all” (i.e., during rest) and the upper bound at twenty was anchored at maximal effort and was compared to the level of effort required for a maximal voluntary contraction. Ratings at 7, 9, 11, 13, 15, 17 and 19 were anchored with the

words extremely light, light, somewhat hard, hard (heavy), very hard and extremely hard, respectively.

2.6 Questionnaires

2.6.1 Pain Expectation and Coping Confidence

At the beginning of each experimental visit, ratings of pain expectation and pain coping confidence were asked for on 0-10 scales. For pain expectation participants were instructed to rate "how much pain do you expect to experience?" with zero being "no pain at all" and ten being "the worst possible pain". For pain coping confidence, the instruction was to rate "How confident are you that you will be able to cope with the pain experienced?" with zero at "Not confident at all" and ten at "Completely confident".

2.6.2 Positive and Negative Affect

A measure of affect was also administered at the beginning of each experimental by using the positive and negative affect schedule (Watson, Clark and Tellegen, 1988). The schedule has 20 words for positive and negative feelings and emotions (10 words each) where participants would have to rate 1 (very little or not at all) to 5 (extremely) on how they felt at the present moment.

2.6.3 McGill Long Form Pain Questionnaire

To quantify the quality of the pain experienced in the experimental visits, participants completed the McGill Long Form Pain Questionnaire (Melzack, 1975). This form has a series of boxes containing multiple words describing a specific quality of pain (e.g., cool, cold, freezing). Participants completed this form post-exercise and were instructed to mark words which reflect the quality of pain they experienced. Each set of words in the box are in descending order of intensity. For example, box nineteen with cool, cold, and freezing, the word freezing implies a more intense feeling of cold pain than cool. The analysis of the McGill questionnaire can be separated into distinct components of the pain including sensory (boxes 1-10), affective (boxes 11-15), emotional (box 16) and miscellaneous (boxes 17-20). Furthermore, words which are selected more than one third of the time across participants can be determined as commonly selected words.

2.6.4 Pain Catastrophising Scale

The pain catastrophising scale (Edwards *et al.*, 2006). This questionnaire contains a series of statements about the thoughts and feelings experienced during the hypertonic saline injection and exercise combined. The scale runs from 0 (not at all) to 4 (all the time) for each statement.

Chapter 3: The Test-Retest Reliability of Measures of Neuromuscular Fatigue after a Fatiguing Submaximal Isometric contraction

3.1 Abstract

Introduction. The reliability of neuromuscular fatigue (NMF) has been assessed in fresh conditions, but limited evidence exists as to how reliable these measures are in the presence of exercise induced fatigue. Additionally, isometric task intensity is often set at a percentage of maximum force. It is unknown if homogenising the time to task failure (TTF) duration may improve reliability. *Methods.* Twelve healthy participants (mean ± SD age: 26.2 ± 3.8 yrs, height: 1.76 ± 0.09 M, body mass: 72.0 ± 12.5 kg) volunteered to participate in the study. After an initial familiarisation visit of procedures, participants completed six visits where they were required to perform an isometric contraction of the knee extensors at either 20% of maximum voluntary force ($TTF_{20\%}$) or at an adjusted intensity which caused a TTF between 4 to 6 minutes ($TTF_{4\text{-}6min}$). Measures of neuromuscular fatigue were recorded pre and post exercise. Data from three repetitions of each condition were analysed for reliability with the coefficient of variation (CV), ICC, standard error of measurement (SEM) and minimum detectable change (MDC). *Results.* $TTF_{20\%}$ and $TTF_{4\text{-}6min}$ both displayed good reliability CV = 7.3% [95% CI: 4.7 – 9.9%] and CV = 5.1% [95% CI: 2.9 – 7.3%] respectively. Maximum voluntary force (MVF), doublet amplitude and voluntary activation (VA) exhibited good reliability post-exercise (CV < 10% for all). MVF MDC was 127 N after fatigue in $TTF_{20\%}$ whereas it was 90 N in $TTF_{4\text{-}6min}$. Doublet MDC was 39 N in $TTF_{20\%}$ and 36 N in $TTF_{4\text{-}6min}$. VAL MDC was 10.8% in $TTF_{20\%}$ and 11.7% in $TTF_{4\text{-}6min}$. EMG amplitude CV during the TTF exercise was ~15% in $TTF_{20\%}$ and ~10% in $TTF_{4\text{-}6min}$. **Conclusion.** The $TTF_{4\text{-}6min}$ protocol exhibits good reliability in TTF and NMF variables post-exercise. Overall, $TTF_{4\text{-}6min}$ is an advantageous method of prescribing exercise intensity during a submaximal isometric TTF.

3.2 Introduction

Exercise-induced fatigue is defined as a transient reduction in the ability to produce force which is reversible by rest (Gandevia, 2001). The aetiology of exercise-induced fatigue can be broadly dichotomised as either central or peripheral in origin. Central fatigue refers to any impairments in force that are proximal from the neuromuscular junction (i.e., spinal and supraspinal areas) whereas peripheral fatigue refers to changes at or distal to the neuromuscular junction (i.e., actin-myosin filaments, sarcolemma). The quantification of both types of fatigue is commonly referred as neuromuscular fatigue (NMF). NMF can occur in varying degrees during isometric (Burnley, Vanhatalo and Jones, 2012) as well as whole body dynamic exercise (Thomas *et al.*, 2014) and these processes may interact and influence the development of each other (Hureau, Romer and Amann, 2018; Amann *et al.*, 2020). Nevertheless, exercise-induced fatigue, regardless of origin is likely to limit endurance capacity.

Measures of NMF are frequently recorded in studies which aim to explore the mechanisms of fatigue during endurance performance in response to an intervention. Typically, measures of NMF are taken at baseline, in an unfatigued state and are compared to measures which are recorded during or after endurance exercise (e.g. see Angius *et al.* 2016). This allows the magnitude/progression of neuromuscular fatigue to be quantified. Additionally, the values of a particular NMF variable at a given time point can be compared to values of the same measure on a separate occasion with/without an intervention. For example, maximum voluntary force, a measure of global fatigue can be compared over time when pain has been induced via the injection of hypertonic saline versus an experimental, non-painful control (Khan *et al.*, 2011; Smith *et al.*, 2020). Because measures of NMF are used to infer the mechanisms of the aetiology of exercise induced fatigue, it is important that NMF variables are reliable in their measurement values.

Reliability refers to how repeatable/consistent a value derived from a particular variable is (Atkinson and Nevill, 1998; Hopkins, 2000). Good reliability is reflected by consistent values across multiple repeated measures. There are two types of reliability, absolute and relative. Absolute reliability refers to the consistency of a measurement of an individual variable which is repeated multiple times and is commonly quantified as the coefficient of variation (CV) or standard error of measurement (SEM), whereas relative reliability refers to how an individual variable maintains their rank in a sample across repeated measures which is quantified with the ICC (Atkinson and Nevill, 1998). Furthermore, the error in a measurement can be random or systematic. Random error is the change that can increase or decrease and is usually a function

of biological variation or measurement error of the equipment. Conversely, systematic error is the change of a measure in one direction. For example, measurements of muscle strength in a series of lab visits may induce a learning or training effect and consequently the values increase over the course of the sessions.

Previous research has studied the aetiology of fatigue using a single limb isometric time to task failure (TTF) protocol. This entails the participant performing an isometric contraction with a limb (usually knee extensors or elbow flexors) which is fixed into a specific joint angle and connected to a force transducer. Participants perform a maximal voluntary contraction (MVC) and subsequently have to maintain an isometric contraction at a given percentage of this maximum for as long as possible (Crenshaw *et al.*, 1997; Ciubotariu, Arendt-Nielsen and Graven-Nielsen, 2004; Angius *et al.*, 2016). NMF variables recorded in conjunction with the TTF typically include maximum voluntary force (MVF) with peripheral nerve stimulation (PNS) during and shortly after an MVC to obtain measures of voluntary activation (central fatigue) and twitch force (peripheral fatigue) using the ITT (Shield and Zhou, 2004; Rozand *et al.*, 2015). Also, the associated electromyography response of the muscle(s) of interest is continuously recorded. In order for studies to identify the mechanisms of a particular intervention (e.g. the role of pain on endurance performance), these measurements must display adequate reliability as some NMF measures are considered to be insensitive to changes caused by exercise-induced fatigue (De Haan, Gerrits and De Ruiter, 2009) which may result in erroneous conclusions being made.

Previous work has sought to determine the reliability of NMF measures (Clark, Cook and Ploutz-Snyder, 2007; Place *et al.*, 2007; Behrens *et al.*, 2017) and has found that in an unfatigued state, measurements of MVF, VA and twitch force exhibit good to excellent reliability (< 10% CV, > 0.8 ICC). While this is promising, it is unclear if the reliability of a NMF variable at baseline can be translated to when a participant is fatigued. There is limited research on the reliability of NMF variables after a fatiguing exercise task. Research by Place *et al.* (2007) assessed NMF after a 2-minute sustained maximum voluntary contraction to determine if the presence of exercise induced fatigue impacted the reliability of NMF measures. For maximum voluntary force, M-wave amplitudes and electrically evoked contractions, the typical error of the measurement was similar in fresh and fatigued states, however coefficients of variation were consistently greater after fatigue. This indicates some reduction in reliability with neuromuscular fatigue present, although the reliability could still be considered good (CV

< 10%). Similarly, a CV of 7% was found in the post-exercise MVC after a 4 minute MVC (Kent-Braun and Le Blanc, 1996). More recently, research by Goodall *et al.* (2017) investigated the reliability of NMF measures after two repeats of simulated soccer play. Baseline measures of NMF reliability were good (CV < 10%, ICC > 0.9) but as fatigue developed at half-time, full time and after extra time, this resulted in a decrease in reliability with CV values exceeding 10% at full time for MVC, twitch torque and voluntary activation.

It is currently unknown if the same level of reliability is present after a submaximal isometric TTF protocol. Sustained MVCs have a standardised duration and level of effort whereas submaximal isometric contractions to task failure have variable durations and the aetiology of fatigue is different to those of their maximal counterparts (Taylor and Gandevia, 2008), therefore it is unknown if submaximal isometric exercise tasks exhibit similar levels of reliability after fatigue. This is important because many studies have used submaximal isometric contractions as an exercise task to assess the effect of an intervention because these more closely reflect the demands of endurance exercise (e.g. see Angius *et al.* 2016; Ciubotariu, Arendt-Nielsen and Graven-Nielsen 2004; Plaskett and Cafarelli 2001). Submaximal isometric tasks are performed at a fixed intensity relative to maximum voluntary force and are performed to task failure which often results in disparate inter-individual endurance times due to differences in endurance capacity and maximum muscle strength. It has been found that at differing intensities, the profile of neuromuscular fatigue varies (Eichelberger and Bilodeau, 2007; Burnley, Vanhatalo and Jones, 2012). Therefore, individuals exercising at a fixed percentage of maximum force are likely to exhibit a greater inter-individual variability in NMF. This may be problematic because an individual with an exceptional level of strength and poor endurance will get a shorter TTF at a given intensity whereas an individual with the opposite characteristics would never reasonably reach TTF. Subsequent data analysis between conditions of an intervention which compare measures at absolute time points (e.g., minute 3 between two experimental conditions) as opposed to relative time points (e.g., 75% of TTF) will be limited to analysing up to the shortest TTF in that sample. Furthermore, a greater inter-individual variability in NMF may mean that some individuals may not respond to a given intervention due to a lack of change in the NMF variable itself. As an example, if an individual does not experience central fatigue from an exercise protocol due to it being insufficient in duration, an intervention that is hypothesised to exacerbate central fatigue during exercise (e.g., elevated muscle pain) will not be detected.

One pragmatic way to prescribe exercise intensity is to manually adjust it based upon the response of an individual to a fixed percentage of MVC. Manually adjusting the intensity to fall within a specific TTF range such as 4 to 6 minutes can reduce the inter-individual variation in TTF, but it is unknown how the reliability of this method could compare to a fixed intensity prescription. Therefore, the purpose of this study was two-fold. 1.) to assess and compare the reliability of NMF variables before versus after fatigue. 2.) To determine whether a fixed intensity at 20% of MVC ($TTF_{20\%}$) or a manually adjusted target designed to achieve a TTF within 4-6 minutes ($TTF_{4\text{-}6min}$) has similar levels of reliability. The hypothesis is two-fold. 1.) The variability of NMF variables will be good at baseline and more variable after fatigue but still acceptable. 2.) that the $TTF_{4\text{-}6min}$ will demonstrate reduced inter-individual variability in all variables and have similar intra-individual variability to $TTF_{20\%}$.

3.3 Methods

3.3.1 Participants

Twelve healthy participants (3 female; mean ± SD age: 26.2 ± 3.8 yrs, height: 1.76 ± 0.09 M, mass: 72 ± 12.5 kg) volunteered to take part in the study and provided written informed consent before any testing commenced. Exclusion criteria included: those who had a lower limb injury in the past three months, those who were taking long term medication for pain relief and anyone with pacemakers or other cardiac related issues. This was determined before testing with a health screening questionnaire. The study was approved by the university of Kent research ethics advisory group (prop 34_2018_19). See appendix 1 for ethics documentation.

3.3.2 Experimental Design

Participants initially completed a familiarisation session to become accustomed measurements of neuromuscular fatigue and the isometric time to task failure of the right knee extensors. Then in a randomised order, participants completed six main visits of the study. Three visits comprised of baseline measures of neuromuscular function (maximum voluntary contractions with peripheral nerve stimulation and electromyography) before an exercise task which was a continuous submaximal isometric contraction of the right knee extensor muscles at an intensity of 20% of maximum voluntary torque until task failure followed by post exercise measures of neuromuscular function. The other three visits comprised of the same exercise protocol except the target intensity was adjusted to cause task failure within 4-6 minutes. A schematic of the experimental visits can be seen in figure 3.1.

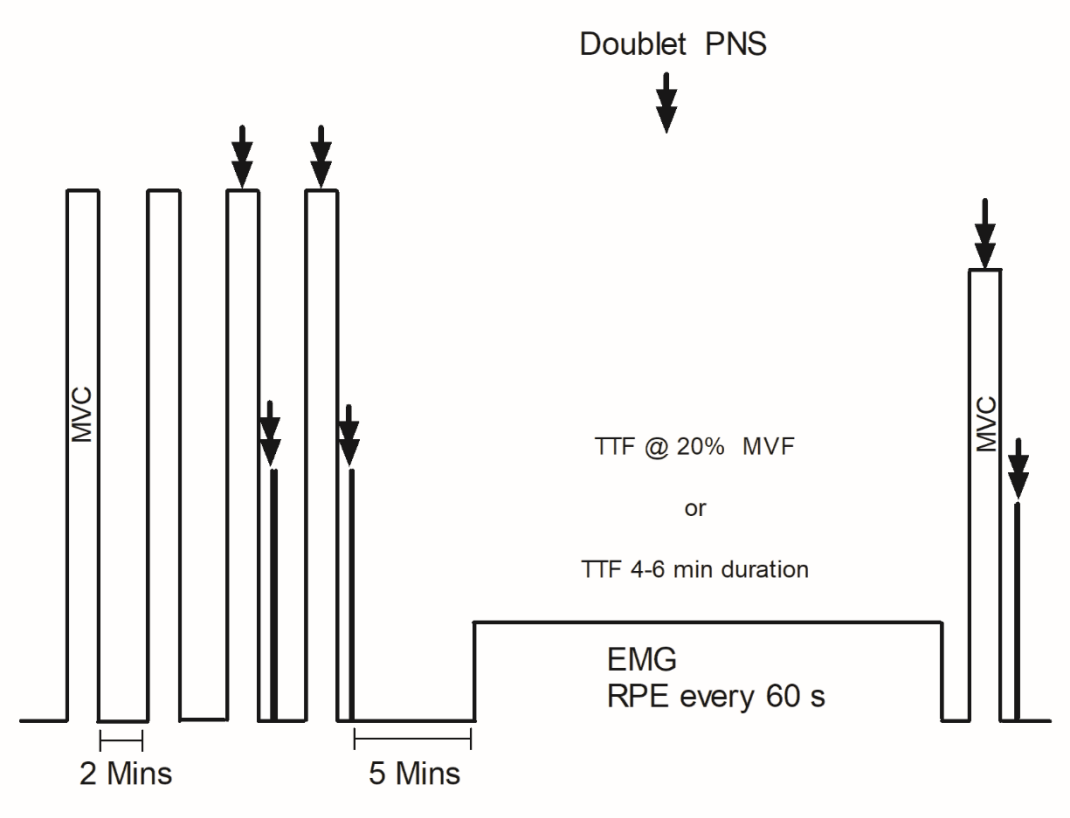

Figure 3.1. A visual schematic of the experimental visits. Each TTF was performed three times for a total of six experimental visits. EMG of the VL was recorded continuously and RPE was recorded at the end of each minute and at task failure.

3.3.3 Equipment and Measures

Dynamometry. Isometric force was recorded in this study with the method described in the general methods section 2.3.2.

Peripheral Nerve Stimulation (PNS). An electrical stimulator (DS7a, Digitimer, Hertfordshire, UK; Maximum voltage = 400V) was used to deliver PNS as described in the general methods section 2.3.3. The mean stimulation intensity was 316mA (range: 182 – 540mA).

Electromyography (EMG). Bipolar surface EMG of the VL was acquired by placing two Ag/AgCl electrodes (Nessler Medizintechnik, Innsbruck, Austria) parallel with the fibres on the muscle belly proximal to the knee. The skin of the site was shaved, abraded, and cleaned to reduce impedance. The electrode location was marked with indelible ink so that the electrodes could be placed in the same location for all trials. Muscle activity was recorded continuously at a sampling frequency of 2KHz and amplified at a gain of 1000 (EMG100c,

Biopac systems, California, USA) which was band passed (10-500Hz) before being analysed offline in compatible software (Spike2 v7, Cambridge Electronic Design, UK).

Rating of perceived effort (RPE). The rating of perceived effort was recorded using the Borg 6-20 scale (Borg 1982) with 6 being anchored at 'no exertion' and 20 anchored at 'maximal exertion'. Participants were instructed to provide a number on the scale that reflects the conscious effort to drive the limb to produce the target force (Pageaux, 2016).

3.3.4 Procedures

Main Visits. Participants visited the lab at a similar time of day (± 2hrs) after having abstained from vigorous physical activity 48hrs, caffeine 4hrs and any analgesics 6hrs before testing commenced. Initially participants had their skin prepared for surface EMG and their optimal stimulation site for PNS was found followed by the determination of their supramaximal stimulation intensity. Participants then completed a standardised warm-up consisting of 10 contractions at 50% of their perceived maximum effort for 3 seconds interspersed with 3 seconds of recovery. Participants then (after 2 minutes of rest) completed baseline measures of neuromuscular function. This entailed four MVCs with two minutes of rest in between each attempt. On MVC three and four, a peripheral nerve stimulation (doublet) was superimposed onto the MVC once peak torque was attained and plateaued. Another potentiated doublet was delivered at rest within 5 s of completion of the MVC. Stimulations were only performed during the latter two MVCs as it typically takes 3 MVCs to achieve full potentiation (Kufel, Pineda and Mador, 2002). Five minutes of rest was given before the start of the isometric exercise task which was a continuous submaximal isometric contraction of the right knee extensor muscles. Two separate 'arms' of the study were conducted. One 'arm' aimed to assess the reliability of a fixed submaximal contraction intensity that corresponded to 20% of the peak torque achieved from four MVCs of the first trial from that 'arm'. The other 'arm' was a manually determined target that would cause task failure within a 4–6-minute time range, thus the relative percentage of peak torque was different for each participant. This target was adjusted based upon the TTF at 20% of maximum voluntary torque that was performed in the familiarisation. An additional familiarisation was performed if the TTF was still out of the 4-6 minutes after the first manual adjustment. The participants had to attempt to push against the non-compliant strap of the dynamometer to this target for as long as possible. Task failure was defined as an inability to maintain the target for three consecutive seconds despite strong verbal encouragement. RPE was recorded every minute and at task failure. Instantaneous feedback on

torque was visible to the participant and they were also provided with verbal feedback from the experimenter as to when they were below the target torque and approaching task failure. Once task failure had been reached, participants completed one MVC with a superimposed and resting potentiated doublet to acquire post-exercise measures of neuromuscular function. Each 20% trial and 4–6-minute trials were repeated three times to assess the reliability of these exercise tests and their associated fatigue parameters.

3.3.5 Data Analysis

Maximum voluntary torque was compared from pre to post exercise and was indicative of global fatigue (i.e., the summation of central and peripheral fatigue). The reduction in peak torque acquired from the potentiated doublet was indicative of peripheral fatigue. Superimposed and resting potentiated doublets were used for the calculation of VA using the ITT (Shield and Zhou, 2004) which was calculated as:

$$VA\ (\%) = 1 - \left(\frac{Superimposed\ doublet\ torque\ (nm)}{Resting\ potentiated\ doublet\ torque\ (nm)}\right) * 100$$

Where the superimposed doublet torque is the increment in torque achieved from a superimposed doublet and the resting potentiated doublet torque is the peak torque achieved from the subsequent potentiated doublet. Occasionally the superimposed doublet was delivered slightly before or after peak torque was achieved, therefore a correction formula was implemented to correct for this (Strojnik and Komi, 1998). Any reduction in VAL was indicative of central fatigue.

Electromyography amplitude was calculated with the root mean square (RMS) function in software Spike2 using a 100 ms time constant. For maximal voluntary contractions, the mean EMG signal of a 500 ms epoch (250 ms each side of peak torque) was taken. This was determined manually due to the stimulus artefact from the superimposed doublets occasionally interfering with this criterion. If this was the case, the greatest torque which did not include the stimulus artefact was used. The EMG amplitude was normalised against the RMS of the most proximal M-Wave from a potentiated doublet to account for peripheral/impedance changes that may influence the EMG signal, termed MVC/Mmax. Any fall in the MVC/Mmax was indicative of central fatigue. During the exercise task the EMG_{RMS} was averaged over each minute and at the 30 s prior to task failure. This data was normalised relative to the EMG amplitude obtained from the baseline MVCs of that trial.

3.3.6 Statistical Analysis

All data was analysed in SPSS (SPSS Statistics 25; IBM; Chicago, IL) and JAMOVI. Data was initially checked for normality with the Shapiro-wilk test and Q-Q plots. Measures of neuromuscular function from pre to post exercise were analysed with a paired samples t-test and Cohen's D effect size (Cohen, 1992) where 0.2 = small effect, 0.5 medium effect, 0.8 = large effect. Statistical significance was set at $P < 0.05$. To detect systematic bias, a one-way repeated measures ANOVA was performed on baseline measures across visits for each variable. The data from all six trials were used, whereas for post-exercise measures, only the three trials from each condition were checked for systematic bias. A Holm-Bonferroni correction was applied to subsequent post-hoc tests (Holm, 1979).

Inter individual and intra individual reliability on all measures of neuromuscular function and TTF were calculated with the coefficient of variation (CV) which was calculated as:

$$CV\ (\%) = \frac{Standard\ deviation}{Mean} * 100$$

Relative reliability of measures were calculated with the ICC using an ICC 3,1 (i.e. fixed effects) as this study was to determine the reliability for subsequent studies using similar measurements (Weir, 2005). ICC scores < 0.5, 0.5 – 0.75, >0.75 – 0.9 and > 0.9 were considered as poor, moderate, good, and excellent relative reliability respectively.

The typical error was also calculated for each dependent variable. This was calculated as:

$$Standard\ Error\ of\ Measurment = \sqrt{MS_E}$$

Where MS_E is the mean square error term from the repeated measures ANOVA table (Weir, 2005). Minimum detectable change (MDC) was subsequently calculated from the SEM as:

$$MDC = SEM * 1.96 * \sqrt{2}$$

Where 1.96 is the Z score for a 95% confidence interval. This reflects the minimum change required in a measurement to be 95% confident that the change was greater than the amount of measurement error.

3.4 Results

3.4.1 Time to Task Failure

The mean ± SD TTF for $TTF_{20\%}$ was 224 ± 64 s, 225 ± 72 s, and 231 ± 72 s respectively for each session. As expected, For $TTF_{4\text{-}6min}$, the TTF was 307 ± 28 s, 312 ± 37 s, and 316 ± 25 s respectively. The mean ± SD intensity for $TTF_{4\text{-}6min}$ was 17.0 ± 3.4% (range = 11.1 to 22.2%) of maximum voluntary force.

No systematic bias was present for $TTF_{20\%}$ ($F_{2,22} = 0.482$, $P = 0.624$) or $TTF_{4\text{-}6min}$ ($F_{2,22} = 0.542$, $P = 0.589$). The mean intra-individual coefficient of variation for $TTF_{20\%}$ was 7.3 [4.7 - 9.9]% whereas it was 5.1 [2.9 - 7.3]% for $TTF_{4\text{-}6min}$. For the inter-individual CV of the first visit, $TTF_{20\%}$ had a CV which was 28.8% whereas it was 9.2% in $TTF_{4\text{-}6min}$. The $ICC_{3,1}$ for $TTF_{20\%}$ was 0.916 [0.796 – 0.973] whereas it was 0.543 [0.195 – 0.819] for $TTF_{4\text{-}6min}$. The SEM was 20 and 21 seconds for $TTF_{20\%}$ and $TTF_{4\text{-}6min}$ respectively. Subsequently, the minimum detectable change for TTF was 55 s for $TTF_{20\%}$ and 58 s for $TTF_{4\text{-}6min}$.

3.4.2 Maximum Voluntary Force (MVF)

Reliability statistics for MVF can be seen in table 3.3 and 3.4. Across the baseline MVT for all six trials, a one-way repeated measures ANOVA revealed that no systematic bias was present. ($F_{5,55} = 1.845$, $P = 0.119$). The mean time from task failure to commencement of the post-exercise MVC was 11 ± 5 s. There was no systematic bias for $TTF_{20\%}$ ($F_{1.12,12.33} = 0.372$, $P = 0.577$). or $TTF_{4\text{-}6min}$ ($F_{2,22} = 0.858$, $P = 0.438$).

3.4.3 Voluntary Activation

At baseline there was no systematic bias present for VAL ($F_{5,55} = 0.763$, $P = 0.580$). During post-exercise, no systematic bias was seen for $TTF_{20\%}$ ($F_{2,22} = 0.341$, $P = 0.715$) or $TTF_{4\text{-}6min}$ ($F_{2,22} = 0.332$, $P = 0.721$). Baseline and post-exercise reliability statistics can be seen in table 3.3 and 3.4.

3.4.4 Doublet Amplitude

There was no systematic bias for doublet amplitude at baseline ($F_{5,55} = 1.135$, $P = 0.252$) or at task failure for $TTF_{20\%}$ ($F_{2,22} = 0.042$, $P = 0.959$) and $TTF_{4\text{-}6min}$ ($F_{2,22} = 1.195$, $P = 0.322$). Baseline and post-exercise reliability statistics can be seen in table 3.3 and 3.4.

3.4.5 Electromyography

Baseline M_{max} displayed no systematic bias ($F_{1.996,19.960} = 1.617$, $P = 0.224$). There was no systematic bias at post-exercise for $TTF_{20\%}$ ($F_{2,20} = 0.085$, $P = 0.919$) or $TTF_{4\text{-}6min}$ ($F_{2,20} = 0.366$,

P = 0.698). Similarly, there was no systematic bias for EMG amplitude at the post-exercise time-point $F_{2,22}$ = 0.845, P = 0.443 and $F_{2,22}$ = 0.272, P = 0.764 for $TTF_{20\%}$ and $TTF_{4\text{-}6min}$ respectively. During exercise, EMG amplitude displayed no systematic bias at any time point (all P > 0.05). Baseline and post-exercise reliability statistics can be seen in table 3.3 and 3.4.

Table 3.1. Raw values for neuromuscular function variables measured at baseline. Data presented as mean ± SD.

	Session Number					
	1	2	3	4	5	6
MVF (N)	529 ± 97	558 ± 128	533 ± 122	547 ± 109	562 ± 127	579 ± 127
VA (%)	94.4 ± 3.5	95.1 ± 3.0	94.2 ± 3.7	94.0 ± 3.1	94.1 ± 3.2	94.9 ± 3.1
Doublet (N)	212 ± 53	212 ± 58	211 ± 58	212 ± 57	220 ± 62	219 ± 60
M_{max} (mV)	14.6 ± 2.2	14.1 ± 2.2	14.5 ± 2.2	14.9 ± 2.4	14.8 ± 3.1	15.4 ± 3.0

Table 3.2. Neuromuscular function raw values measured post-exercise. Data presented as mean ± standard deviation.

	$TTF_{20\%}$			$TTF_{4\text{-}6min}$		
	1	2	3	1	2	3
MVF (N)	335 ± 117#	347 ± 122#	344 ± 127#	354 ± 128#	337 ± 136#	351 ± 130#
%Δ	39.0	38.0	38.5	36.0	40.9	40.0
VA (%)	90.5 ± 6.0*	89.2 ± 6.7*	89.8 ± 5.4*	88.7 ± 6.4*	88.4 ± 5.2*	87.4 ± 6.0*
%Δ	4.7	5.4	4.3	5.7	6.8	7.4
Doublet (N)	151 ± 70#	151 ± 75#	150 ± 76#	152 ± 69#	151 ± 76#	159 ± 79#
%Δ	32.1	33.1	31.5	29.9	31.1	29.8
EMG_{RMS} (%max)	81.1 ± 23.5*	87.0 ± 27.2	85.6 ± 25.8	89.1 ± 21.5	86.3 ± 25.0	87.7 ± 36.6

M_{max} (mV)	14.5 ± 1.7	14.4 ± 2.3	14.7 ± 3.4	14.0 ± 2.2	14.3 ± 2.7	14.3 ± 3.3
%Δ	0.6	0.0	3.3	1.5	4.0	5.1

MVF = maximum voluntary force, VAL = voluntary activation level, EMG_{RMS} = electromyography root mean square, Mmax = maximum M-wave amplitude. * = significantly different from baseline ($P < 0.05$). # = significantly different from baseline ($P < 0.001$)

Table 3.3. Reliability of neuromuscular function parameters measured at baseline. Data presented as mean and 95% confidence interval in brackets.

	CV (%)	ICC (3,1)	SEM	MDC
MVF	5.5 [4.2 – 6.9]	0.931 [0.855 – 0.976]	31 N	87 N
VAL	1.8 [1.2 – 2.4]	0.641 [0.414 – 0.851]	2.0%	5.4%
Doublet	5.7 [4.7 – 6.8]	0.948 [0.890 – 0.982]	13 N	36 N
M_{max}	7.9 [4.2 – 11.5]	0.767 [0.570 - 0.917]	1.9 mV	5.2 mV

CV = coefficient of variation; ICC = intraclass correlation coefficient, SEM = standard error of measurement; MDC = minimum detectable change; MVT = maximum voluntary torque; VAL = voluntary activation level; EMG_{RMS} = electromyography root mean square; M_{max} = m-wave peak to peak amplitude.

Table 3.4. Reliability of neuromuscular function parameters measured post-exercise. Data presented as mean and 95% confidence interval in brackets.

		CV (%)	ICC (3,1)	SEM	MDC
MVF	$TTF_{20\%}$	6.8 [3.6 – 9.9]	0.921 [0.808 – 0.974]	46 N	127 N
	$TTF_{4\text{-}6min}$	8.2 [4.1 – 12.3]	0.939 [0.848 – 0.980]	33 N	90 N
VA	$TTF_{20\%}$	3.7 [2.1 – 5.4]	0.584 [0.244 – 0.839]	3.9%	10.8%
	$TTF_{4\text{-}6min}$	4.1 [2.5 – 5.6]	0.491 [0.135 – 0.792]	4.2%	11.7%
Doublet	$TTF_{20\%}$	8.8 [5.0 – 12.5]	0.963 [0.906 – 0.988]	14 N	39 N
	$TTF_{4\text{-}6min}$	7.4 [4.0 – 10.9]	0.969 [0.921 – 0.990]	13 N	36 N
EMG_{RMS}	$TTF_{20\%}$	13.2 [10.6 – 17.3]	0.793 [0.554 – 0.929]	11.6%	32.2%
	$TTF_{4\text{-}6min}$	13.7 [9.5 – 18.0]	0.802 [0.569 – 0.932]	4.0%	11.1%
M_{max}	$TTF_{20\%}$	9.7 [4.2 – 15.3]	0.575 [0.215 – 0.844]	1.7 mV	4.6 mV
	$TTF_{4\text{-}6min}$	7.1 [3.8 – 10.4]	0.855 [0.657 – 0.955]	1.1 mV	2.9 mV

CV = coefficient of variation; SEM = standard error of measurement; MDC = minimum detectable change; ICC = intraclass correlation coefficient; MVT = maximum voluntary torque; VAL = voluntary activation level; EMG_{RMS} = electromyography root mean square; M_{max} = m-wave peak to peak amplitude.

EMG during exercise. Due to differing durations of endurance time in $TTF_{20\%}$ between individuals, only minute 1, 2 and task failure could be analysed for reliability with n = 12. For $TTF_{4\text{-}6min}$ analysis could be performed on minutes 1 to 4 as well as task failure but only data on minute 1, 2 and TF was analysed to match $TTF_{20\%}$. No systematic bias was seen at any

timepoint for $TTF_{20\%}$ or $TTF_{4\text{-}6min}$ (all $P > 0.05$). Raw data for normalised EMG amplitude and reliability statistics can be seen in table 3.5.

Table 3.5. Reliability statistics of normalised surface EMG amplitude during each minute of exercise and at task failure for $TTF_{20\%}$ and $TTF_{4\text{-}6min}$.

$TTF_{20\%}$	Session 1 (%)	Session 2 (%)	Session 3 (%)	CV (%)	ICC (3,1)	SEM (%)	MDC (%)
Minute 1	18.9 ± 4.6	19.1 ± 4.8	19.3 ± 6.8	15.2 [8.7 – 21.7]	0.361 [0.006 – 0.718]	5.7	15.9
Minute 2	21.3 ± 4.8	22.0 ± 7.9	21.6 ± 8.8	15.9 [10.4 – 21.4]	0.499 [0.145 – 0.797]	6.6	18.3
Task Failure	43.4 ± 18.1*	47.4 ± 33.1*	46.1 ± 24.8*	13.3 [8.8 – 17.8]	0.809 [0.583 – 0.935]	11.4	31.5
$TTF_{4\text{-}6min}$	Session 1 (%)	Session 2 (%)	Session 3 (%)	CV (%)	ICC (3,1)	SEM (%)	MDC (%)
Minute 1	16.9 ± 9.5	15.2 ± 5.1	15.1 ± 5.1	10.7 [6.0 – 15.3]	0.878 [0.715 – 0.960]	1.9	5.2
Minute 2	17.5 ± 5.8	15.5 ± 5.0	15.9 ± 5.2	10.0 [4.2 – 15.8]	0.857 [0.673 – 0.952]	2.5	6.8
Task Failure	42.3 ± 21.3*	45.5 ± 30.2*	43.6 ± 29.5*	15.6 [11.1 – 20.2]	0.886 [0.732 – 0.962]	9.2	25.6

* Denotes significantly different from minute 1 and minute 2 ($P < 0.05$).

3.5 Discussion

The aim of the present study was two-fold. Firstly, it was to compare the reliability of two different types of exercise intensity prescriptions, a fixed percentage of maximum force (i.e., $TTF_{20\%}$) and an individually prescribed intensity that causes a TTF within 4-6 minutes ($TTF_{4\text{-}6min}$). Secondly, it was to identify the reliability of key NMF variables in a fresh and fatigued state. The main findings are that $TTF_{4\text{-}6min}$ displays similar reliability to a conventionally prescribed intensity (e.g., $TTF_{20\%}$) and the intra-individual reliability is similar for NMF variables post-exercise. Secondly, NMF variables display acceptable reliability in fresh and fatigued stated except for normalised EMG amplitude.

3.5.1 Time to Task Failure

There was excellent reliability in $TTF_{20\%}$ (ICC = 0.916), however in $TTF_{4\text{-}6min}$ it was moderate (0.543). Conversely, the CV for each type of protocol was deemed as acceptable (< 10%) with both protocols also displaying similar SEMs and MDCs. The poor ICC in $TTF_{4\text{-}6min}$ can be

explained by the homogeneity of the time to task failure values as the ICC determines the relative position within a sample an individual moves (Weir, 2005). There was no systematic bias for either protocol which means that there is no learning or training effect present, presuming that at least one familiarisation trial is performed. Taken together, it appears that the two exercise protocols provide similar levels of intra-individual reliability, however, the inter-individual CV for TTF was 9.7% for $TTF_{4\text{-}6min}$ whereas it was 30.7% for $TTF_{20\%}$. This greater homogeneity in $TTF_{4\text{-}6min}$ is advantageous because subsequent analysis of data can be performed on a series absolute time points (e.g., minute 1 of trial A versus minute 1 of trial B) which allows for an isolated comparison of the effect of a given intervention (i.e., the effect hypertonic saline vs isotonic saline on isometric TTF). If one were to employ this method of data analysis with $TTF_{20\%}$ then the absolute time analysis would be limited to the longest common timepoint reached across the sample. In $TTF_{20\%}$ this would be limited to 2 minutes whereas in $TTF_{4\text{-}6min}$ this would be 4 minutes. This issue would become amplified when the addition of hypertonic saline during the $TTF_{20\%}$ is performed which would further reduce endurance time by 20-25% (Graven-Nielsen, Svensson and Arendt-Nielsen, 1997; Ciubotariu, Arendt-Nielsen and Graven-Nielsen, 2004; Smith *et al.*, 2020) and limit the number of absolute time comparisons that could be made.

3.5.2 Measures of Reliability at Baseline

All measures of NMF displayed excellent absolute reliability in a fresh state at baseline (CV < 10%; see table 3.3), however the relative reliability of VAL and M_{max} were moderate and good respectively whereas all other variables displayed excellent reliability (ICC > 0.9). Similar to $TTF_{4\text{-}6min}$, baseline VAL measures are homogenous between individuals as a ceiling effect occurs (i.e. VAL cannot exceed 100%) and most healthy individuals are able to achieve high levels (85-95%) in a fresh state (Shield and Zhou, 2004). An explanation for the lower ICC and CV scores with M_{max} may be because the raw EMG peak to peak amplitude is used. The raw EMG signal is influenced by several non-physiological variables such as electrode positioning and skin preparation. While there was an effort to repeat the location of the EMG signal by using indelible ink, small deviations from the initial site may occur between sessions and thus alter the size of the M-Wave. This is particularly the case as the baseline NMF analysis was pooled between $TTF_{20\%}$ and $TTF_{4\text{-}6min}$ which means that six sessions were analysed with measures that were taken over the time course of 2 to 6 weeks per participant. Nevertheless, the ICC was still rated as good and CV < 10%. Furthermore, the M_{max} is often used to normalise EMG values during voluntary contractions or transcranial magnetic stimulation and therefore

the within-session reliability would be much more important for M_{max}. Unfortunately it is beyond the scope of this study to quantify within-session reliability, however excellent within session reliability has been found for VL m-wave peak to peak amplitude (Place *et al.*, 2007). No systematic bias was seen for any of the baseline NMF variables which indicates that no learning effect or training effect was present, even after six repeated sessions. This was expected as the volume and frequency of isometric contractions was unlikely to cause a measurable increase in MVF or doublet amplitude and these tasks are relatively simple thus one familiarisation session is all that is needed to maximum competency.

3.5.3 Measures of Reliability Post-Exercise

No systematic bias was observed for any NMF variable post-exercise which means that the same exercise protocol appears induce a similar level of neuromuscular fatigue when repeated. Furthermore, each protocol induced a robust decrement in MVF, VA, Doublet amplitude, indicating that significant levels of central and peripheral fatigue occurred. This is important in the context of reliability because if the protocol did not induce neuromuscular fatigue, then assessing reliability in a fresh versus fatigued state would be invalid. In terms of reliability, for all NMF variables, absolute reliability remained acceptable as seen by a CV of < 10% for MVF, doublet, VAL and M_{max}. However, EMG_{RMS} amplitude was 13.2% and 13.7% for $TTF_{20\%}$ and $TTF_{4\text{-}6min}$ respectively. Unfortunately, this value cannot be compared to a 'fresh' value as the EMG amplitude is normalised to baseline MVC amplitude. Relative reliability remained excellent in MVF and Doublet after exercise but was moderate and poor in $TTF_{20\%}$ and $TTF_{4\text{-}6min}$ respectively. Again, this may be because even after fatigue, VAL is still somewhat homogenous between individuals. EMG measures had moderate to good relative reliability post-exercise.

The SEMs and MDCs appear to favour the $TTF_{4\text{-}6min}$ as being more sensitive to measures of NMF after a fatiguing bout of exercise. Generally, a lower SEM and MDC indicate stronger reliability. Interestingly, the SEMs (and subsequently the MDCs) did not largely increase after fatigue with the $TTF_{4\text{-}6min}$ protocol. In fact, the MVF SEM only increased by 2 N and the doublet amplitude remained the same whereas M_{max} decreased. The SEM for VA increased and the reason for the increase in VA is unclear (Oskouei *et al.*, 2003). However, in this case it may be because the participants are able to reach a similar peak force after fatigue which would explain why MVF reliability didn't change, but the ability to maintain this peak may have been compromised, thus small variations at what force the superimposed twitch was delivered at

may have contributed to differences in voluntary activation calculation. Furthermore, the measure of VAL at baseline was taken as the mean of two measurements, whereas the post-exercise measurement was only one. The variability of the superimposed twitch may be accounted for by performing multiple trials as has been previously cited as necessary (Suter and Herzog, 2001). Unfortunately, only one measurement is possible post-exercise as recovery of neuromuscular fatigue occurs rapidly (Froyd, Millet and Noakes, 2013). Place *et al.* (2007) found that the typical error (similar to SEM) was 6.7% which is greater than the SEM of VAL in this study (3.9 and 4.2%). This may be due to differences in the fatigue protocol employed i.e., maximal versus submaximal contractions and open versus closed loop exercise.

3.5.4 EMG During Exercise.

EMG amplitude increased only at task failure, indicating a stable EMG amplitude during the first two minutes of exercise and a progressive increase in amplitude towards task failure as higher threshold motor units are recruited to maintain target force. Interestingly, $TTF_{4\text{-}6min}$ displayed better intra-individual reliability than $TTF_{20\%}$ (see table 3.5). This was true for all measures of reliability at all timepoints, although the difference in variability was less at task failure. The reason for these differences is most likely due to better neuromuscular control in $TTF_{4\text{-}6min}$. This is because the mean intensity for $TTF_{4\text{-}6min}$ was lower (17%) whereas it was 20% in $TTF_{20\%}$ and this may result in less fatigue at minute 1 and 2 and therefore better force steadiness. Indeed, this has been seen by both da Silva *et al.* (2016) and Mathur, Eng and MacIntyre (2005) who found improved reliability in lower intensity contractions.

Comparison of $TTF_{20\%}$ versus $TTF_{4\text{-}6min}$. There was limited difference between the two protocols for the coefficient of variation or ICC, however the SEMs and MDCs appear to be smaller for MVF, doublet and EMG responses in $TTF_{4\text{-}6min}$. This stronger intra-individual variation with $TTF_{4\text{-}6min}$ in comparison with $TTF_{20\%}$ shows that employing the $TTF_{4\text{-}6min}$ protocol may be more beneficial for detecting changes in global and peripheral fatigue as well as EMG responses to an intervention during fatiguing exercise. There was not a difference in inter-individual variation for NMF variables at post-exercise which was expected. This is because the magnitude of neuromuscular fatigue appears to be similar at post-exercise, irrespective of intensity above an individual's critical force (Burnley *et al.*, 2010; Burnley, Vanhatalo and Jones, 2012). However, the rate of change of neuromuscular fatigue differs. This may explain why EMG stronger better inter-individual reliability as this was recorded during exercise when the development of neuromuscular fatigue will have been more

homogenous in $TTF_{4\text{-}6min}$. Furthermore, recent evidence has shown that individuals exhibit unique profiles of neuromuscular fatigue (Chartogne *et al.*, 2020) which are correlated between muscles. In other words, one individual may be more prone to developing peripheral fatigue whereas another may display large reductions in voluntary activation at task failure. Therefore, attempting to homogenise the neuromuscular response to endurance exercise may be more complex than just manipulating exercise intensity.

3.5.5 Methodological Considerations

In $TTF_{20\%}$, 4 out of 12 participants achieved a TTF of within 4-6 minutes, meaning that the exercise intensity between the two conditions for these individuals was not notably different. Consequently, these participants were exercising at a similar point of their force-time curve and would not be expected to differ in their reliability. Additionally, no participant in this sample exceeded a TTF of 6 minutes so it is plausible that reliability could be different if some participants were able to exercise for much longer than six minutes, something which may have been observed with a larger sample size.

The current study was conducted on a Cybex isokinetic dynamometer with a knee and hip angle of 90°. Therefore, these reliability statistics may not reflect neuromuscular function testing on different dynamometers/isometric chairs e.g. due to differences is compliance (Loring and Hershenson, 1992) or at different muscle lengths. Particularly with a dynamometer, the small amount of cushioning may result in the absorption of twitch forces.

Another limitation may be with ICC on the VAL and $TTF_{4\text{-}6min}$. The variable displayed low ICC's which was 0.543 for time to task failure and 0.496 – 0.584 for measures of VAL. This may be due to the ceiling effect of the VA measurements (i.e., cannot exceed 100%) and the lack of heterogeneity of the data, thus, this data must be interpreted with caution. Others have chosen to exclude VA from an ICC analysis (Place *et al.*, 2007).

3.3.6 Conclusions and Practical Applications

In conclusion, an exercise intensity that is prescribed based upon the time to task failure it yields (e.g., 4-6 minutes) appears to be just as reliable as the traditionally prescribed percentage of maximum force. However, $TTF_{4\text{-}6min}$ will produce a more homogenous TTF across participants which will allow for improved data analysis when comparing absolute time points between participants. Reliability of key neuromuscular fatigue variables should be considered in the presence of fatigue as this may reduce the reliability. In this case, MVF, doublet and VAL exhibit acceptable reliability in the presence of fatigue, however, EMG variables such as

RMS amplitude of MVCs or submaximal contractions should be interpreted with caution as these variables displayed poor-moderate reliability. With the reliability of key endurance tasks and neuromuscular measures quantified, it is possible to confidently use these measures to assess the effect of muscle pain on endurance performance and the development of neuromuscular fatigue.

Chapter 4: The Effect of Experimental Muscle Pain on Endurance Performance and Neuromuscular Fatigue

4.1 Abstract

Purpose: Muscle pain can impair exercise performance but the mechanisms for this are unknown. This study examined the effects of muscle pain on neuromuscular fatigue during an endurance task. *Methods:* On separate visits, twelve participants completed an isometric time to task failure (TTF) exercise of the right knee extensors at ~20% of maximum force following an intramuscular injection of isotonic saline (CTRL) or hypertonic saline (HYP) into the VL. Measures of neuromuscular fatigue were taken before, during and after the TTF using transcranial magnetic stimulation (TMS) and peripheral nerve stimulation. *Results:* The mean pain intensity was 57 ± 10 in HYP compared to 38 ± 18 in CTRL ($P < 0.001$). TTF was reduced in HYP (4.36 ± 0.88 min) compared to CTRL (5.20 ± 0.39 min; $P = 0.003$). Maximum voluntary force was 12% lower at minute 1 ($P = 0.003$) and 11% lower at minute 2 in HYP ($P = 0.013$) compared to CTRL. Voluntary activation was 4% lower at minute 1 in HYP compared to CTRL ($P = 0.006$) but not at any other time point (all $P > 0.05$). The TMS silent period was 9% longer at 100 s during the TTF in HYP compared to CTRL ($P = 0.026$). *Conclusion:* Muscle pain reduces exercise performance through the exacerbation of neuromuscular fatigue that is central in origin. This appears to be from inhibitory feedback from group III/IV nociceptors which acts to reduce central motor output.

4.2 Introduction

Exercise requires repeated or sustained muscular contractions and can cause a progressive decline in the force generating capacity of a muscle, known as exercise-induced fatigue (Gandevia, 2001). The aetiology of exercise-induced fatigue can be central (changes at the spinal or supraspinal level) and/or peripheral (changes at or distal to the neuromuscular junction) in origin (Bigland Ritchie *et al.*, 1978; Kent-Braun, 1999) but most exercise appears to encompass both types of fatigue in a feedback-feedforward system to regulate exercise tolerance (Hureau, Romer and Amann, 2018).

Strenuous exercise is usually accompanied by EIP. Pain can be defined as an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage (Raja *et al.*, 2020). The naturally occurring and non-damaging exertional pain accompanying strenuous exercise (EIP) can be described as "aching" or "cramping" and increases as a function of time/exercise intensity (Cook *et al.*, 1997; Smith *et al.*, 2020). The feeling of EIP arises from the accumulation of noxious biochemicals, reduced muscle pH and increases in intramuscular pressure which consequently stimulates group III/IV nociceptive afferents (O'Connor and Cook, 1999; Mense, 2008). Since EIP and exercise intensity (and consequently the development of fatigue) are associated, it may be possible that EIP contributes to the fatigue process, however this is not known.

Previous work has found that in combination with traditional physiological parameters (e.g. lactate threshold), pain tolerance (i.e. the maximum level of perceived pain someone can tolerate) can partially predict cycling time-trial performance (Astokorki and Mauger, 2017) and that reducing muscle pain through the ingestion of acetaminophen results in an improvement in endurance performance (Astokorki and Mauger 2017; Foster *et al.* 2014; Mauger, Jones and Williams 2010; Morgan *et al.* 2019). Conversely, elevating muscle pain through the intramuscular injection of hypertonic saline has been shown to reduce isometric TTF performance (Graven-Nielsen, Svensson and Arendt-Nielsen, 1997; Ciubotariu, Arendt-Nielsen and Graven-Nielsen, 2004; Smith *et al.*, 2020) and maximum muscle strength (Graven-Nielsen *et al.*, 2002; Slater *et al.*, 2003; Khan *et al.*, 2011). The mechanisms which underpin these changes are suggested to be centrally mediated (Le Pera *et al.*, 2001; Schabrun and Hodges, 2012) but the fatiguing effect of pain during exercise is unclear. Additionally, the experience of muscle pain may reduce endurance performance by acting as an aversive stimulus which causes a voluntary disengagement from exercise or reduction in exercise intensity. On the other hand, muscle pain may independently cause fatigue by altering motor

unit recruitment thresholds/firing rates or reducing central motor drive and act on a physiological, unconscious basis (i.e., the nociceptive component).

Recently, Smith and colleagues (2020) induced muscle pain using an intramuscular injection of hypertonic saline during submaximal isometric knee extensor exercise. They found that this produced a similar pain quality to EIP and allowed the authors to decouple the pain-intensity relationship during knee extensor exercise. The increased muscle pain caused a mean decrease of 26% in endurance time, despite a similar end exercise maximum voluntary torque, which suggests that fatigue occurred more rapidly when pain was exacerbated.

The use of peripheral nerve stimulation allows for the measurement of peripheral changes in muscle function (e.g., resting twitch amplitude) as well as central changes in voluntary activation (via the ITT). Transcranial magnetic stimulation (TMS) allows for the non-invasive quantification corticospinal excitability and inhibition during exercise and in combination would provide novel information on the development of neuromuscular fatigue in response to elevated muscle pain. Consequently, these methods allow us to further understand the mechanisms of how muscle pain may act to limit endurance performance as opposed to isolated measures of motor function that have previously been explored (e.g. Le Pera *et al.*, 2001; Khan *et al.*, 2011).

Therefore, the purpose of this study was to perform an isometric TTF of the knee extensors with elevated muscle pain from an intramuscular injection of hypertonic saline while simultaneously recording measures of neuromuscular fatigue to identify the mechanisms behind how muscle pain limits endurance performance. It was hypothesised that the intramuscular injection of hypertonic saline would decrease isometric TTF through an exacerbation of central fatigue (i.e., decreased voluntary activation).

4.3 Methods

4.3.1 Participants

Twelve healthy and recreationally active individuals (two female) with a mean ± SD age 26.6 ± 3.9 years, height: 175 ± 8.2 cm, body mass: 72.2 ± 11.7 kg volunteered to take part in the study. All participants had no lower limb injury within the past three months, were not taking medication for the treatment of pain or have any pain related conditions. Participants were also screened for any contraindications to TMS. All participants provided written informed consent

before testing. The study was approved by the university of Kent SSES Research Ethics Advisory Group (Prop 30_2018_2019) and was conducted in alignment with the declaration of Helsinki. Documentation for ethics can be found in appendix 2.

4.3.2 Experimental Protocol

Participants visited the laboratory on four occasions separated by a minimum of 48 h between visits 1 and 2 and at least 7 days between visits 3 and 4. Participants performed the experiment at a similar time of day (± 1.5 h) and avoided strenuous physical activity 48 h, caffeine 4 h, alcohol 24 h and analgesics 6 h prior to testing. In visit one, participants were familiarised with measures of neuromuscular function (see neuromuscular function testing), questionnaires, perceptual measures, the isometric TTF exercise and the intramuscular injection of hypertonic saline if they had not received one before. Visit two comprised of a second familiarisation of the isometric exercise task where the intensity (%MVC) was adjusted from the first visit if the TTF was not within four to six minutes. This was to ensure that the isometric time to task failure coincided with the typical pain duration from the intramuscular injection of hypertonic saline into the VL (Smith *et al.*, 2020). Visits three and four were experimental visits (Figure 4.1) completed in a randomised order. Participants arrived at the laboratory and completed the positive and negative affect schedule (PANAS) and pain expectation/pain coping confidence. They then underwent baseline measures of neuromuscular function involving peripheral nerve stimulation and single pulse TMS during isometric contractions of the right knee extensors. Participants then waited ten minutes before receiving an intramuscular injection of 1 mL of isotonic saline (0.9%) or hypertonic saline (5.85%) in the muscle belly of the VL. The isotonic saline condition served as a non-painful injection matched control (CTRL) while the hypertonic saline caused acute muscle pain (HYP). Immediately after the injection, participants began the submaximal isometric TTF protocol with intermittent measures of peripheral nerve stimulation and TMS while providing measures of pain and RPE until task failure, where post-exercise measures of neuromuscular fatigue were performed along with the pain catastrophizing scale (Edwards *et al.*, 2006) and McGill long form pain questionnaire (Melzack, 1975) .

4.3.3 Equipment and Procedures

Experimental Muscle Pain. Hypertonic saline injections were used to cause muscle pain as described in the general methods section 2.3.1. An identical injection protocol was performed with the isotonic saline (CTRL condition).

Exercise Protocol. The exercise protocol was a semi-constant submaximal isometric TTF of the right knee extensors at an individualised intensity to cause TTF within 4-6 mins in CTRL. The mean intensity for the participants was 20% of maximum voluntary force (MVF), but this ranged from 13 to 25% of MVF. Three seconds before the end of each minute in the TTF participants were instructed to relax and prepare to perform an MVC with superimposed doublet and subsequently relax for 3 s while a resting doublet was delivered. Four TMS pulses and a single peripheral nerve stimulation was delivered during the submaximal contraction phase of the TTF task at 10 s and 100 s. Participants were encouraged to go for as long as possible until they were unable to maintain the target for three consecutive seconds or voluntarily withdrew from the task. Participants also continuously rated their pain and provided RPE every minute and at task failure. A schematic of the experimental protocol can be seen in figure 4.1.

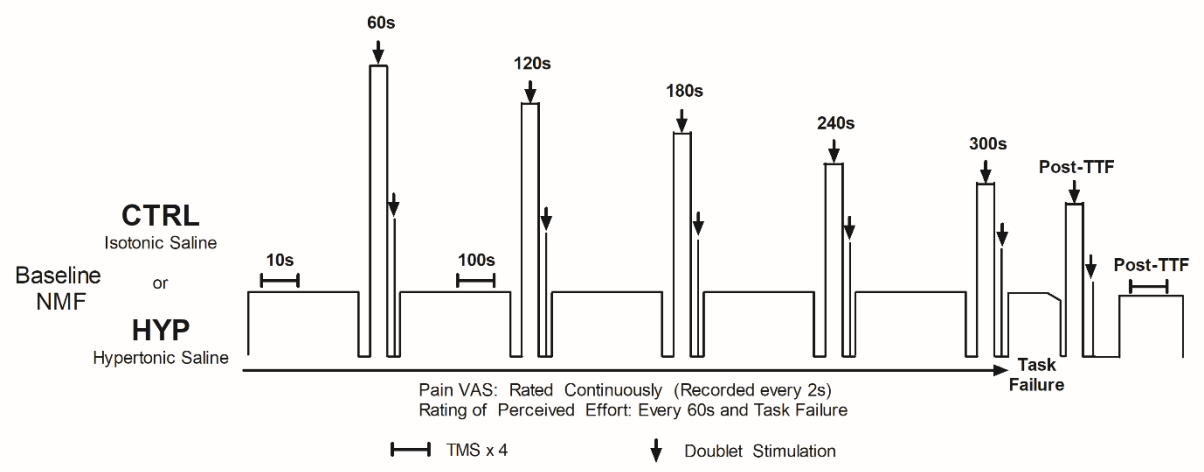

Figure 4.1. A schematic of the procedures for the experimental visits (CTRL and HYP).

Mechanical Recordings. Participants were strapped into a custom-built isometric chair with a hip and knee angle of 90° (0° being full extension). Force of the knee extensors was recorded as described in the general methods section.

Electromyography (EMG). Bipolar surface electromyography was used to record activity of the VL, VM and BF with 37.5 mm × 37.5 mm Ag/AgCl electrodes (Whitesensor 4831Q, Ambu Ltd, Denmark) at an inter electrode distance of 37.5 mm. The VL electrodes recorded evoked responses from TMS and peripheral nerve stimulation and voluntary muscle activity whereas the VM was used to assess changes in synergist activity in response to muscle pain. The BF measures were used to check and minimise antagonist motor evoked potential amplitudes.

The electrode location was on the muscle belly proximal to the knee and parallel to the fibres of the muscle for the VL and VM while the BF was placed on the muscle belly 50% of the distance between the ischial tuberosity and the lateral epicondyle of the tibia. Each site was shaved, abraded, and cleaned to reduce impedance and the electrode locations were marked for replication in subsequent visits. All EMG data was recorded continuously at a frequency of 2.5 KHz and amplified (gain 1000 for VL, 2000 for VM and BF) with a signal amplifier (EMG2-R, Biopac Systems, California, USA and EMG100c, Biopac Systems, California, USA) before being band pass filtered (10-500 Hz) and recorded onto compatible software (Acqknowledge v5.0, Biopac Systems, California, USA).

Peripheral Nerve Stimulation. An electrical stimulator (DS7r, Digitimer, Hertfordshire, UK; maximum voltage = 400 v) capable of delivering a single square wave pulse was used for peripheral nerve stimulation. The method for electrical stimulation can be found in the general methods section 2.3.3.

Transcranial Magnetic Stimulation. Delivery of TMS was performed as described in the general methods section 2.3.4. The mean stimulator intensity was 67 ± 5% of maximum stimulator output in CTRL and 66 ± 7% in HYP.

Perceptual Measures. Pain intensity was recorded onto a linear potentiometer every 2 s (see general methods) and recorded the data on an SD card. Rating of perceived effort (RPE) was recorded on the 6-20 point scale (Borg 1982) every 60 s and at task failure.

Questionnaires. Before each experimental visit the PANAS (Watson, Clark and Tellegen, 1988) was administered to confirm participants arrived the lab in a similar psychological state (see general methods 2.6.2. Additionally, pain expectation and perceived pain coping ability were recorded as described in general methods 2.6.1. The situation-specific pain catastrophizing scale (Edwards *et al.*, 2006) and the long form McGill pain questionnaire (Melzack, 1975) was administered immediately post-exercise.

Neuromuscular Function Testing. For baseline measures of neuromuscular function, participants initially performed a warmup consisting of ten contractions at 50% of perceived maximum effort (3 s contracting, 3 s relaxing). This was followed by four maximum voluntary contractions of 4 s in duration separated by 2 min of rest. On the third and fourth MVC a superimposed doublet was delivered once peak force was reached and a resting potentiated doublet was delivered within 5 s of the end of the MVC. Twelve TMS stimulus were delivered

during twelve submaximal contractions (3 sets of 4 contractions) at the target force of the subsequent exercise. One single peripheral nerve stimulation was delivered on a contraction after. Post-exercise (within 10 s), a single MVC with peripheral nerve stimulation was delivered followed by four submaximal contractions superimposed with TMS and one contraction superimposed with single peripheral nerve stimulation to measure corticospinal excitability and inhibition.

4.3.4 Data Analysis

The baseline neuromuscular variables were calculated as the mean raw value and the raw value was taken for each measure during every minute. MVF and doublet amplitude was recorded as the peak instantaneous force achieved. Voluntary activation, a measure of central fatigue, was calculated using the ITT described in general methods section 2.4.2

The TMS MEP was analysed for corticospinal excitability and inhibition as per the description in general methods 2.4.4 and 2.4.5. The root mean square (RMS) of the EMG waveform was calculated offline in software (Acqknowledge V5.0; Biopac systems Inc, California, USA) using a 100 ms time constant. The mean 500 ms of the RMS (250 ms either side of peak force) was analysed for MVCs and the mean 20 s of data was analysed at the beginning of each minute and before task failure of the exercise task and was normalised to MVC EMG amplitude. The ΔMVF, ΔVAL, ΔDoublet, ΔSP/ΔTime were calculated as the change in value from pre- to post-exercise divided by the TTF as an indicator of the rate of fatigue development. Pain data was taken as the VAS recorded at every 20 s and at task failure.

4.3.5 Statistical Analysis

All data are presented as mean ± SD or a mean and interquartile range when not normally distributed. Data was analysed in JAMOVI 1.0.7.0. (The Jamovi Project, 2020). Data was initially checked for normality with the Shapiro-Wilk test and sphericity with the Mauchly test. If these assumptions were violated, data was analysed with a non-parametric test or Greenhouse-Geiser corrected, respectively. A paired samples t-test was used to compare TTF between CTRL and HYP. A 2 × 4 repeated measures ANOVA (condition × time) was used to analyse neuromuscular variables at baseline, minute one, two (or 10 s and 100 s for TMS data) and task failure. A 2 × 8 repeated measures ANOVA was used to analyse pain VAS data. Follow-up paired samples t-tests were used to determine differences between conditions at different time points and were Bonferroni-Holm corrected where appropriate (Holm, 1979). Paired samples t-tests were used for differences in TTF and the ΔMVF, ΔVAL, ΔDoublet,

ΔSP/ΔTime which were Bonferroni corrected. ICC (2,1) were calculated and presented as point estimate and 95% confidence interval for doublet amplitude between CTRL and HYP at minute one, two and task failure for confirmation of similarity.

Confidence intervals (95%), Cohen's d effect sizes (Cohen, 1992) and partial eta squared (η_p^2) were reported where appropriate. A Pearson correlations matrix was used to examine the relationship between changes in pain at minute 1 between conditions against change in neuromuscular variables between conditions at minute 1, and was Bonferroni corrected.

4.4 Results

4.4.1 Time to Task Failure.

There was a 16.2% shorter TTF in HYP (4.36 ± 0.88 minutes) compared to CTRL (5.20 ± 0.39 minutes) (mean difference = 0.84 minutes, 95% CI [0.34, 1.33 minutes], $t_{11} = 3.728$, $P = 0.003$, $dz = 1.08$; figure 4.2a).

4.4.2 Pain Intensity and Pain Quality

Prior to each experimental visit, there was no difference in pain expectation ($P = 0.602$) or pain coping confidence (Wilcoxon $P = 1.000$). The mean pain intensity, matched for exercise time was greater in HYP (57 ± 10) compared to CTRL (38 ± 18) (mean difference = 19, 95% CI [11, 28], $t_{11} = 5.18$, $P < 0.001$, $dz = 1.50$). When also matched for exercise time peak pain was greater in HYP (94.5 [75.8 – 99.3]) compared to CTRL (85.5 [55.8 – 99.0]) (Wilcoxon $P = 0.047$). For pain intensity throughout the TTF there was a condition × time interaction ($F_{3.42, 37.59} = 10.7$, $P < 0.001$, $\eta_p^2 = 0.493$; figure 4.2a). Pain intensity was elevated from 20 s to 140 s (all $P < 0.001$) in HYP compared to CTRL but was not different between conditions at 0 s ($P = 0.142$) or at task failure ($P = 1.000$; figure 4.2b). For pain quality assessed by the McGill long form questionnaire, Cramping (50%), Aching (58%), Tiring (58%) and Intense (50%) were the most common words selected in CTRL whereas in HYP, Cramping (50%), Aching (42%), Gruelling (42%), Intense (67%) were most selected. No difference was seen in the total pain rating ($P = 0.466$) or the sensory ($P = 0.686$), affective ($P = 0.515$), evaluative (Wilcoxon $P = 0.269$) or miscellaneous ($P = 0.160$) dimensions of pain.

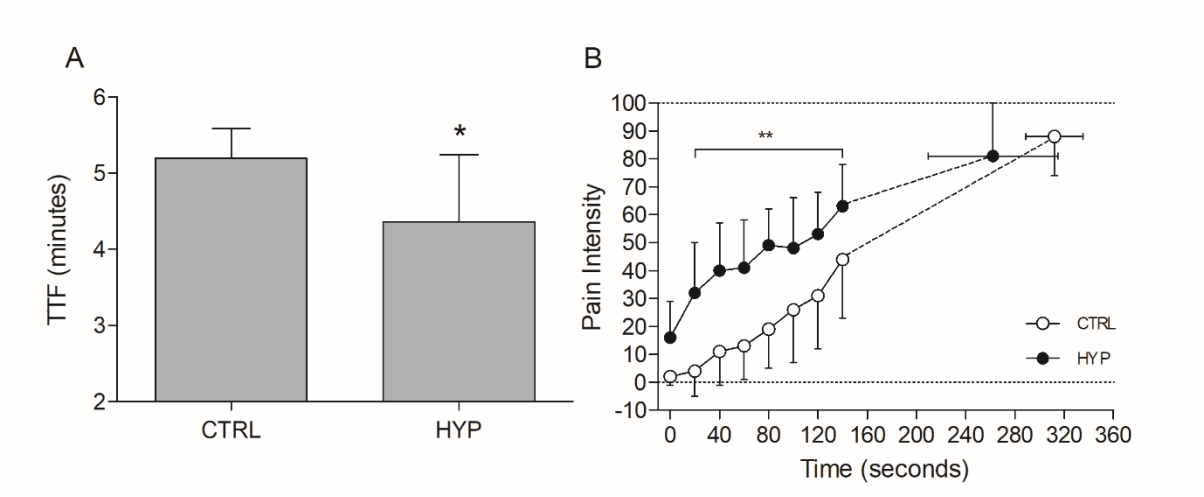

Figure 4.2 A. TTF of the isometric endurance task. Data presented as mean ± SD. * denoted significantly different from CTRL ($P < 0.05$). B. Pain VAS data through the isometric TTF. Data presented as mean ± SD. ** denotes significantly different from CTRL ($P < 0.001$).

4.4.3 Maximum Voluntary Force (MVF)

For MVF there was a condition × time interaction ($F_{1.77, 19.43} = 6.81$, $P = 0.007$, $\eta_p^2 = 0.382$). Subsequent post-hoc tests revealed that MVF decreased by 43% (mean difference = 278 N, 95% CI [218, 338 N], $t_{11} = 14.09$, $P < 0.001$, $dz = 2.96$) and 45% (mean difference = 293 N, 95% CI [244, 342 N], $t_{11} = 14.85$, $P < 0.001$, $dz = 3.78$) in CTRL and HYP respectively, with no difference between conditions ($P > 0.999$). However, during the exercise task, MVF was lower at minute 1 in HYP (509 ± 139 N) compared to CTRL (577 ± 155 N) (mean difference = 68 N, 95% CI [26, 109 N], $t_{11} = 4.001$, $P = 0.003$, $dz = 1.02$). Similarly, MVF at minute 2 was lower in HYP (470 ± 124 N) compared to CTRL (527 ± 141 N) (mean difference = 56 N, 95% CI [10, 102 N], $t_{11} = 3.334$, $P = 0.013$, $dz = 0.78$) (figure 4.3A.). The change in ΔMVF/ΔTime was greater in HYP than in CTRL (Wilcoxon $P = 0.015$) (CTRL = 52 [43 – 63] $N.min^{-1}$, HYP = 67 [56 – 81] $N.min^{-1}$).

4.4.4 Voluntary Activation (VA)

No interaction effect was observed for VA ($F_{1.56, 17.18} = 1.34$, $P = 0.282$, $\eta_p^2 = 0.108$). However, there was a main effect of condition ($F_{1.11,} = 7.60$, $P = 0.019$, $\eta_p^2 = 0.409$). Post-hoc tests revealed that VA was lower in HYP (92.2 ± 5.1%) than CTRL (96.4 ± 2.5%) at minute 1 (mean difference = 4.2%, 95% CI [1.44, 6.9%], $t_{11} = 3.36$, $P = 0.006$, $dz = 0.97$) but was not different at minute 2 (mean difference = 4.3%, 95% CI [-0.2%, 8.8%], $t_{11} = 2.08$, $P = 0.061$, $dz = 0.60$) or at task failure (mean difference = 6.5%, 95% CI [-0.5%, 13.5%], $t_{11} = 2.04$, $P = 0.066$, $dz =$

0.59). There was also a main effect of time for VA ($F_{1.45, 15.91}$ = 17.31, P < 0.001, η_p^2 = 0.611). VA decreased from 95.8% to 85.9% in CTRL (mean difference = 9.9%, 95% CI [6.1, 13.8%], t_{11} = 4.047, P = 0.003, dz = 1.64) and from 96.1% to 79.4% in HYP (mean difference = 16.69%, 95% CI [7.8, 25.5%], t_{11} = 6.807, P < 0.001, dz = 1.20) (figure 4.3B). There was a greater ΔVAL/ΔTime in HYP (3.9 ± 3.0%.min^{-1}) compared to CTRL (1.9 ± 1.2%.min^{-1}) (P = 0.036).

4.4.5 Doublet Amplitude and M_{max}

For doublet amplitude there was no condition × time interaction ($F_{1.48, 16.24}$ = 0.346, P = 0.649, η_p^2 = 0.030) or main effect of condition ($F_{1, 11}$ = 1.578, P = 0.235, η_p^2 = 0.125). However, there was a main effect of time ($F_{1.07, 11.80}$ = 22.136, P < 0.001, η_p^2 = 0.668). Doublet amplitude decreased by 34% in CTRL (mean difference = 106 N, 95% CI [64, 148 N], t_{11} = 7.725, P < 0.001, dz = 1.60) and by 33% in HYP (mean difference = 103 N, 95% CI [57, 149 N], t_{11} = 7.510, P < 0.001, dz = 1.43; figure 4.3C). There was no difference in the ΔDoublet/ΔTime (P = 0.218). Intraclass correlation coefficients for doublet amplitude were 0.935 (0.795 – 0.981), 0.948 (0.836 – 0.985) and 0.944 (0.819 – 0.984). For M_{max}, there was no condition × time interaction ($F_{3, 33}$ = 1.360, P = 0.272, η_p^2 = 0.110) or main effect of condition ($F_{1, 11}$ = 0.074, P = 0.790, η_p^2 = 0.007) and time ($F_{1.90, 20.91}$ = 3.26, P = 0.061, η_p^2 = 0.229).

4.4.6 MEP·M_{max}^{-1}

For MEP·M_{max}^{-1}, no condition × time interaction was observed ($F_{1.69, 18.54}$ = 0.370, P = 0.660, η_p^2 = 0.033) or main effect of condition $F_{1, 11}$ = 2.411, P = 0.149, η_p^2 = 0.180). There was a main effect of time ($F_{3, 33}$ = 3.942, P = 0.017, η_p^2 = 0.264) for an increase in MEP·M_{max}^{-1} but subsequent post-hoc tests with a holm-Bonferroni correction revealed no significant differences.

Table 4.1. Data for MEP·M_{max}^{-1} ratio over time and between conditions. Data presented as mean ± SD.

	Baseline	10 s	100 s	Post-Exercise
CTRL	0.44 ± 0.19	0.50 ± 0.24	0.44 ± 0.21	0.51 ± 0.25
HYP	0.45 ± 0.21	0.53 ± 0.27	0.47 ± 0.25	0.52 ± 0.27

4.4.7 TMS Silent Period

There was no condition × time interaction for the TMS silent period ($F_{2.08, 22.85}$ = 1.84, P = 0.181, η_p^2 = 0.143). However, there was a main effect of time ($F_{1.24, 13.66}$ = 10.56, P = 0.004, η_p^2 = 0.490) and condition ($F_{1, 11}$ = 6.47, P = 0.027, η_p^2 = 0.370). Silent period increased by 28% in CTRL (mean difference = 40 ms, 95% CI [9, 72 ms], t_{11} = 3.368, P = 0.031, dz = 0.81) and by 36% in HYP (mean difference = 51 ms, 95% CI [15, 87 ms], t_{11} = 4.304, P = 0.003, dz = 0.91) but was not different between conditions (mean difference = 13 ms, 95% CI [-5, 31 ms], t_{11} = 1.60, P = 0.138, dz = 0.46). A longer silent period was observed at the 100 s time point (mean difference = 17 ms, 95% CI [2, 31 ms], t_{11} = 2.57, P = 0.026, dz = 0.74), but not at 10 s (mean difference = 5 ms, 95% CI [-3, 14 ms], t_{11} = 1.42, P = 0.183, dz = 0.41; figure 4.3D).

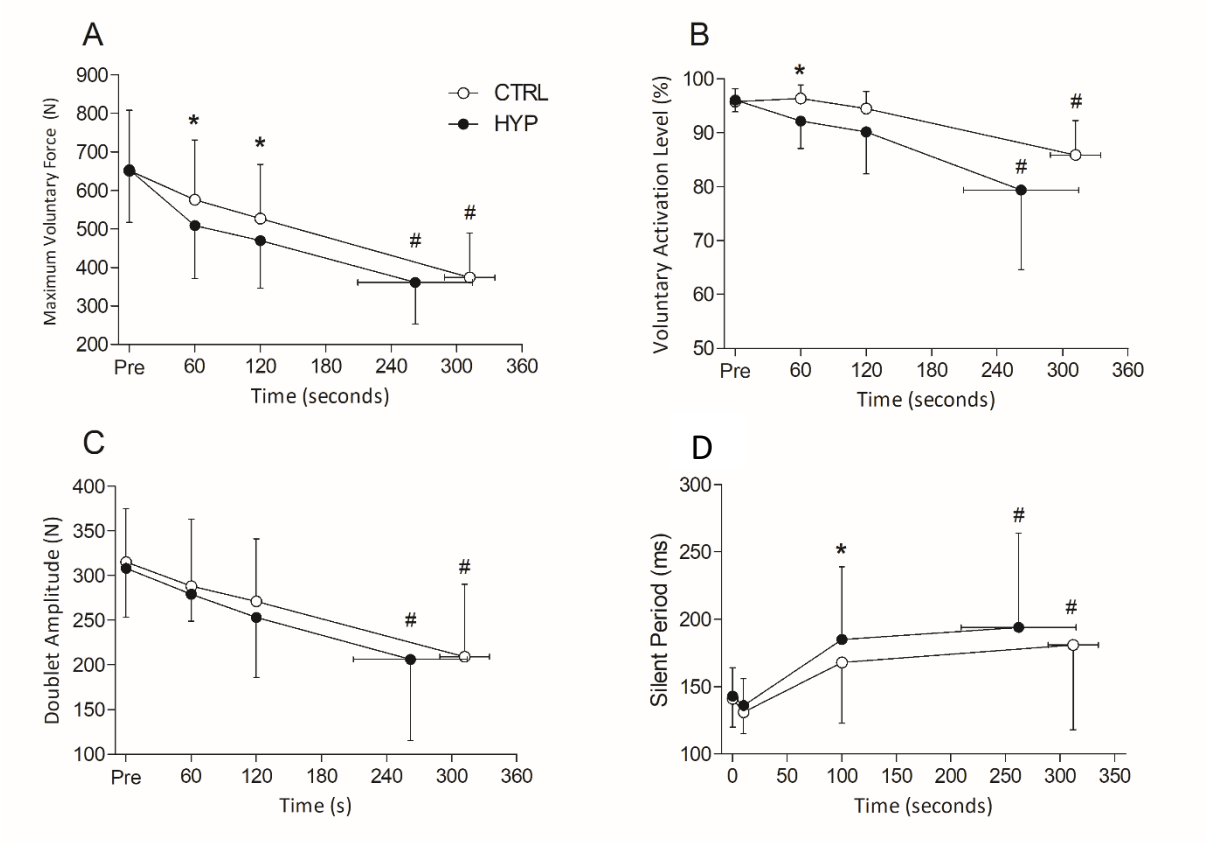

Figure 4.3. Neuromuscular fatigue variables at each minute of the isometric TTF. A. Maximum voluntary force. B. Voluntary activation level. C. Doublet amplitude. D. TMS silent period. * Denotes significantly different from CTRL (P < 0.05). # Denotes significantly different from baseline (P < 0.05).

4.4.8 Electromyography

Vastus Lateralis. For EMG_{RMS} amplitude of the VL during MVCs there was a condition × time interaction ($F_{2, 22}$ = 4.74, P = 0.019, η_p^2 = 0.301). EMG_{RMS} was lower at minute 1 (mean difference = 24.8%, 95% CI [12.6, 37.1%], t_{11} = 4.978, P < 0.001, dz = 1.29) and minute 2 (mean difference = 15.1%, 95% CI [4.0, 26.1%], t_{11} = 3.024, P = 0.044, dz = 0.87) in HYP compared to CTRL. No difference was seen at task failure (mean difference = 4.4%, 95% CI [-5.1, 13.8%], t_{11} = 0.877, P = 1.000, dz = 0.29). EMG_{RMS} decreased in CTRL from minute 1 to task failure (mean difference = 31.9%, 95% CI [14.4, 49.4%], t_{11} = 5.180, P < 0.001, dz = 1.16) but not it HYP (P = 0.500; figure 4.4A). For EMG amplitude during the submaximal TTF, there was a condition × time interaction ($F_{1, 11}$ = 5.018, P = 0.047, η_p^2 = 0.313). EMG_{RMS} was not different at minute 1 (mean difference = 1.1%, 95% CI [-1, 3.1%], t_{11} = 0.743, P = 0.465, dz = 0.33). EMG_{RMS} increased in amplitude at task failure for both conditions, however EMG_{RMS} was lower in HYP compared to CTRL (mean difference = 5.2%, 95% CI [1.3, 9.2%], t_{11} = 3.795, P = 0.011, dz = 0.84; figure 4.4C)

Vastus Medialis. For EMG_{RMS} amplitude of the VM during MVCs there was no condition × time interaction ($F_{2, 22}$ = 3.20, P = 0.060, η_p^2 = 0.225). However, there was a main effect of condition ($F_{1, 11}$ = 8.58, P = 0.014, η_p^2 = 0.225). MVC EMG_{RMS} amplitude was lower at minute 1 (mean difference = 20.7%, 95% CI [5.7, 35.7%], t_{11} = 3.04, P = 0.033, dz = 0.88) and minute 2 (mean difference = 21.2%, 95% CI [3.8, 38.8%], t_{11} = 2.68, P = 0.042, dz = 0.77) but not different at the task failure MVC (mean difference = 8.0%, 95% CI [-2.5, 18.5%], t_{11} = 1.67, P = 0.123, dz = 0.48). There was also a main effect of time ($F_{1.253, 13.779}$ = 15.49, P < 0.001, η_p^2 = 0.585). MVC EMG_{RMS} decreased from minute 1 to task failure in CTRL (mean difference = 29.4%, 95% CI [13.5, 45.4%], t_{11} = 5.17, P < 0.001, dz = 1.17) and in HYP (mean difference = 16.7%, 95% CI [7.8, 25.6%], t_{11} = 3.69, P = 0.004, dz = 1.20; figure 4.4B). For EMG_{RMS} amplitude during the submaximal TTF there was no condition × time interaction ($F_{1, 11}$ = 3.401, P = 0.092, η_p^2 = 0.236) or main effect of condition ($F_{1, 11}$ = 2.355, P = 0.153, η_p^2 = 0.176). There was a main effect of time ($F_{1, 11}$ = 8.705, P = 0.013, η_p^2 = 0.442). EMG_{RMS} increased from minute 1 to task failure in CTRL (mean difference = 21.0%, 95% CI [5.6, 36.3%], t_{11} = 3.005, P = 0.012, dz = 0.87) and in HYP (mean difference = 16.8%, 95% CI [3.6, 30.0%], t_{11} = 2.807, P = 0.017, dz = 0.81; figure 4.4D).

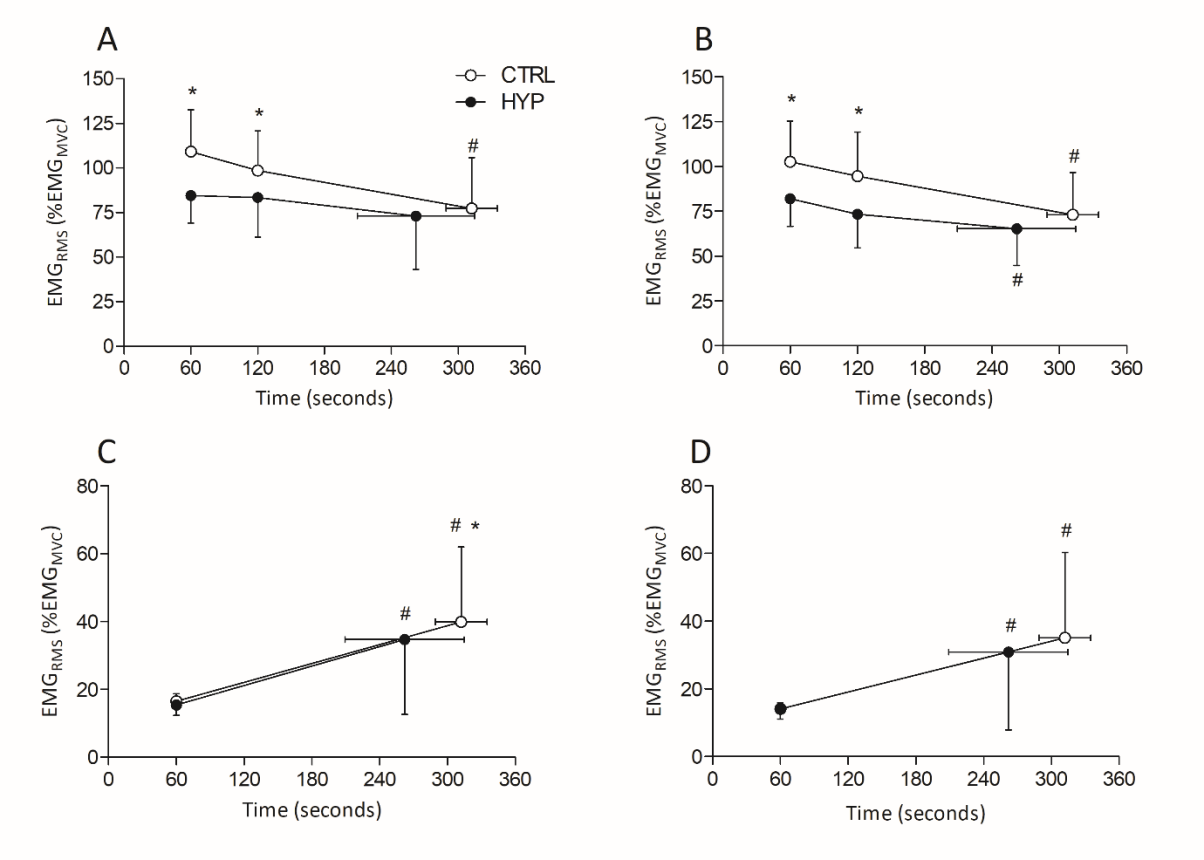

Figure 4.4. Root mean square electromyographic recordings during MVCs and the submaximal isometric TTF. A. Vastus Lateralis MVC EMG amplitude. B. Vastus Medialis MVC EMG amplitude. C. Vastus Lateralis isometric TTF EMG amplitude. D. Vastus Medialis isometric TTF EMG amplitude. * Denotes significantly different from CTRL ($P < 0.05$). # Denotes significantly different from Minute 1 ($P < 0.05$).

Correlations

A Pearson correlation matrix with a Bonferroni correction revealed a significant negative relationship between the change in mean pain VAS from CTRL to HYP of minute 1, against the change in MVF (r = -0.859, P = 0.001) and VAL (r = -0.773, P = 0.013) but not between doublet amplitude (r = -0.174, P = 1.000) or MVC EMG amplitude (r = -0.344, P = 1.000) between conditions (figure 4.5).

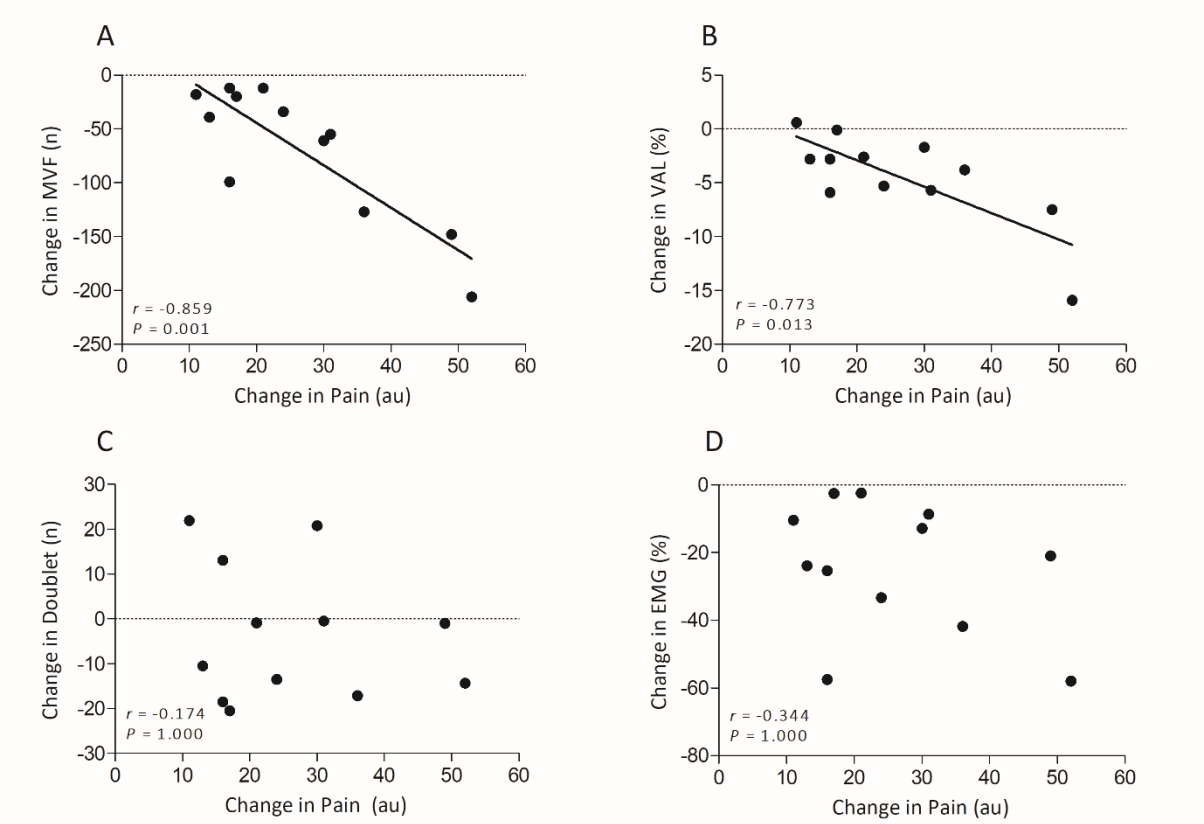

Figure 4.5. Pearson correlations between the change in pain between conditions for the first minute of the isometric TTF against the difference in the change in neuromuscular function variables at minute 1. A. Maximum Voluntary Force. B. Voluntary activation level. C. Doublet amplitude. D. EMG MVC amplitude of the VL.

Rating of Perceived Effort

For RPE there was a condition × time interaction ($F_{2, 22}$ = 12.6, P < 0.001, η_p^2 = 0.553). RPE increased over time but was greater at minute 1 in HYP (13 [12.4 – 14.0]) compared to CTRL (12 [11 – 12]) (Wilcoxon P = 0.008) and minute 2 (HYP = 15 [15 – 15.3], CTRL = 14 [13.4 – 14]) (Wilcoxon P = 0.024). No difference was seen at task failure (CTRL = 20 [20 – 20]), HYP = 20 [19.8 – 20]) (Wilcoxon P = 1.000).

Pain Catastrophising and PANAS

There was no difference in the sum of pain catastrophising score (mean difference = 1.1, 95% CI [-3.4, 1.1], t_{11} = 1.13, P = 0.281, dz = 0.33). No difference in positive affect (P = 0.396) or negative effect (P = 0.766) was observed between conditions.

4.5 Discussion

The novel findings of the study are twofold: i) Increased muscle pain reduces endurance performance and maximal strength, ii), these reductions in performance can be attributed to the exacerbation of central fatigue as seen by greater decreases in voluntary activation and a longer silent period in HYP compared to CTRL. Furthermore, similar decreases in evoked responses were achieved in a shorter time.

4.5.1 Pain on Isometric TTF

The intramuscular injection of hypertonic saline prior to a submaximal isometric TTF elevated leg muscle pain by 36% when conditions were matched for exercise time, and pain was particularly exacerbated within the first two minutes of exercise in HYP compared to CTRL (figure 4.2B). This increase in leg muscle pain, which was similar in quality to that of EIP (i.e., no difference in McGill questionnaire ratings) resulted in a mean 50 s, 16.2% (dz = 1.08) decrease in isometric TTF. This is beyond the standard error of measurement observed for TTF in chapter three (21 s) but not quite of the minimum detectable change (58 s).

These findings are similar to other studies which have investigated endurance performance in response to pain such as Graven-Nielse*n et al* (1997) who saw a ~20% reduction in TTF during an isometric dorsiflexion at 80% of maximum torque when hypertonic saline was injected into the tibialis anterior and Smith *et al.* (2020; d = 0.6) who injected hypertonic saline into the VL and performed an isometric TTF at 10% of maximum torque for the knee extensors. Conversely, no difference in TTF at 40% of maximum torque was observed when the biceps brachii were injected with hypertonic saline (Schulte *et al.*, 2004). Variations in the TTF reducing effect is likely a product of the different muscle groups tested, chosen exercise intensity and volume/concentration of hypertonic saline used. A fixed volume of hypertonic saline (1 mL, 5.85%) is also likely to cause a varying pain response among individuals (Graven-Nielsen, Arendt-Nielsen, *et al.*, 1997) as there are likely differences in pain processing among participants (Fillingim, 2017). This appeared to be the case in the present study as VAS ratings varied greatly (see figure 4.2B). This may explain some of the variability in changes in TTF in this study as some participants' mean pain was only slightly greater in HYP compared to CTRL. Nevertheless, this study demonstrates a notable decrease in endurance performance when muscle pain is increased in a locomotor muscle which is functionally important for common endurance tasks (e.g., running or cycling).

4.5.2 Pain and Neuromuscular Performance

Elevated muscle pain in HYP resulted in a decrease in maximum voluntary force at minute 1 and 2 during the TTF compared to CTRL, both of which exceeded the standard error of measurement in chapter three. This decrease not only demonstrates the ability of pain to reduce maximal strength which has been observed by others (Graven-Nielsen, Svensson and Arendt-Nielsen, 1997; Graven-Nielsen *et al.*, 2002; Slater *et al.*, 2003; Khan *et al.*, 2011) but also represents the accentuation in the development of fatigue in HYP compared to CTRL. No difference in end exercise MVF was observed, similar to Smith *et al.* (2020), despite a marked reduction in exercise time. This is reflected by the significantly greater ΔMVF/ΔTime in HYP compared to CTRL. It is likely that the force generating capacity is reduced to a level which is associated with an inability of the participant to maintain sufficient neural drive to maintain target force, in line with the theory of the sensory tolerance limit (Hureau, Romer and Amann, 2018). During the earlier parts of the TTF, the reduction in MVF likely reflects a net inhibition of the motor unit pool which are used to generate knee extensor forces. Consequently, participants were exercising at the same absolute intensity, but a greater relative exercise intensity in HYP compared to CTRL.

In combination with measures of MVF was the delivery of peripheral nerve stimulation during and after each MVC which allowed for the quantification of central and peripheral fatigue during exercise. Voluntary activation, a measure of central fatigue, was significantly lower within in HYP compared to CTRL at minute 1 which demonstrates the centrally mediated reduction in maximum force (figure 4.3B). Furthermore, this change was greater than the standard error of measurement observed in chapter three. No difference was observed at minute 2 which is unexpected because the maximum force was reduced at this time point. It is plausible that the ITT which was used to calculate voluntary activation may be insensitive to detect changes near maximal contraction intensities (Herbert and Gandevia, 1999). As pain from the saline would have started to decrease in some individuals in tandem with an increase in naturally occurring EIP, the inhibitory effect of pain may have been more difficult to capture with the ITT at minute 2 compared to minute 1. Furthermore, peripheral fatigue was not responsible for the change in MVF as the amplitude of the potentiated doublet remained unchanged between conditions (figure 4.3C), suggesting that the hypertonic saline had no impact on excitation-contraction coupling processes.

An interesting and novel finding within the present study is that the difference in the mean pain over the first minute of exercise between CTRL and HYP had a strong negative correlation with the difference in the change in MVF from baseline to minute 1 (r = -0.859, P = 0.001; figure 4.5A). This was also the case for voluntary activation (r = -0.773; P = 0.013; Figure 4.5B), therefore providing strong evidence that central fatigue is mediated by the magnitude of pain perception and that pain may act in a 'dose response' effect to cause central inhibition. In the present study, it was not possible to discern whether this effect is originating from the magnitude of the nociceptive signal or whether it is the conscious perception of the pain mediating this response, but future work could investigate this phenomenon.

4.5.3 Electromyographic Responses

There was a reduction in EMG amplitude at minute 1 and minute 2 in HYP compared to CTRL for both vasti muscles which was above the standard error of measurement (Figure 4.4A-B). This is in agreement with previous experimentally induced pain research (Graven-Nielsen, Svensson and Arendt-Nielsen, 1997; Rice *et al.*, 2019). A reduction in EMG amplitude may reflect a reduction in maximal central motor output to the quadriceps, that is likely centrally mediated. However, EMG amplitude may only provide a crude measure of neural drive (Farina *et al.*, 2010) and it is likely that the reduced amplitude is an artefact of a reduction in force, as these two variables tend to scale linearly (Alkner, Tesch and Berg, 2000; Campy, Coelho and Pincivero, 2009). Nevertheless, a reduction in force/EMG without a change in doublet or M-Wave amplitude strengthens the notion that the reduction in force is centrally mediated. The bipolar EMG setup preclude the ability to identify which specific neural mechanisms are responsible for this, although previous work using fine-wire intramuscular EMG or high-density surface electromyography (HDEMG) during muscle pain may provide useful insight (Farina *et al.* 2004; Tucker *et al.* 2009; Martinez-Valdes *et al.* 2020). A reduction in motor unit firing frequency has previously been observed (Farina *et al.*, 2004; Tucker *et al.*, 2009) along with a de-recruitment of low threshold motor units (Martinez-Valdes *et al.* 2020). Therefore, the reduction in force and EMG amplitude seen in this study may be due to a centrally mediated inhibition of motor units and/or a decrease in firing frequency in some of the motor units across the motor-neuron pool.

Interestingly no difference was observed between conditions for submaximal EMG amplitude at minute 1 (when hypertonic saline pain was likely evoking the peak pain response; figure 4.4C). It could have been expected that the earlier recruitment of higher threshold motor units

to compensate for the pain mediated central inhibition and consequent acceleration of fatigue would have led to a greater increase in the EMG amplitude with HYP. However as previously seen, changes to motor unit firings rates and recruitment thresholds have been found without a concomitant change to the surface EMG amplitude (Martinez-Valdes *et al.* 2020). It is likely that a combination of excitatory and inhibitory processes occur in response to pain (Hodges and Tucker, 2011) and during exercise the task, the demands can be maintained but at the cost of accelerated fatigability. Therefore, complex adjustments to motor control may not be detectable by a bipolar surface configuration. Furthermore, evidence suggests that muscle pain may result in a shift in the centre of gravity of activation (Liew *et al.*, 2019) meaning that regional variations in muscle activity may occur, potentially outside of the detection volume of the bipolar configuration. However, it is not known if a similar change occurs with more widespread naturally occurring EIP as opposed to the more localised muscle pain with hypertonic saline. At the point of task failure, there was a lower EMG amplitude in the VL but not the VM in HYP compared to CTRL. The reduced EMG amplitude is likely a reflection of the shorter TTF and an inability for the individual to recruit as many high threshold motor units as possible in HYP which was necessary to prolong exercise time.

4.5.4 TMS Responses

TMS was delivered during the TTF to determine corticospinal excitability and inhibition in the presence of elevated muscle pain. Firstly, corticospinal excitability was not different between conditions at any time point and did not change over time. Discrepancies in motor cortex excitability in response to acute pain have been observed, with both a decrease (Le Pera *et al.*, 2001) and increase (Rice *et al.*, 2015) in excitability, whereas fatigue from a 2 minute MVC also increased MEP area but was unchanged with the maintenance of group III/IV afferent firing (Kennedy *et al.*, 2016). Differences in motor cortical excitability may be related to the level of muscle activity present during TMS delivery. MEPs evoked at rest appear to show a reduction in corticospinal excitability but not when delivered during an active contraction (Burns, Chipchase and Schabrun, 2016). Nevertheless, it appears that a reduction in corticospinal excitability was not responsible for the impaired endurance performance with elevated pain. On the other hand, corticospinal inhibition assessed with the TMS silent period increased over time and was greater at 100 s in HYP compared to CTRL, but not 10 s or at task failure. The lack of difference between conditions at 10 s is likely due to the lack of time for the saline to reach a level of pain which would cause a measurable lengthening of the silent

period as pain VAS within the beginning of exercise was not different between CTRL and HYP (figure 4.2C).

The silent period is thought to reflect activity of the gamma-aminobutyric acid b neurotransmitter which may be acting to inhibit the motor cortical activity, thus potentially impacting motor control and descending drive of the quadriceps during the TTF. Additionally, lengthening of the TMS silent period can be caused by changes at the spinal level which could be elucidated by corticomedullary evoked potentials. EIP or fatigue may therefore also act to inhibit spinal motoneurons (Goodall, Howatson and Thomas, 2018; Škarabot *et al.*, 2019). Consistent with these findings, Hilty *et al.* (2011) found that partial blockade of group III/IV afferents (including nociceptors) attenuated the lengthening of the silent period during exercise. In combination, these findings suggest that pain or an increased nociceptive firing acts to inhibit the corticospinal pathway and inhibit descending central drive to the quadriceps.

4.5.5 Task Disengagement versus Fatigue

One potential mechanism of how pain may have reduced endurance performance relates to the aversiveness of pain due to the enhanced negative affective-motivational component associated with the hypertonic saline injection combined with the intense exercise. This potentially contributes to an increased avoidance drive to escape the pain from the endurance task (Navratilova and Porreca, 2014; Stevens *et al.*, 2018), and in this study, participants ended the exercise at a similar, potentially intolerable level of EIP. However, end exercise MVF and doublet amplitude was similar and a premature withdrawal from exercise would have likely resulted in less end-exercise fatigue. Furthermore, no difference in pain catastrophizing was seen between conditions which is associated with exercise performance and task disengagement (Nijs *et al.*, 2008). It is plausible that a voluntary task disengagement did occur, but this effect was 'masked' by the exacerbation of neuromuscular fatigue. Furthermore this 'voluntary disengagement' effect may be more prevalent in whole-body, longer duration exercise, or in non-exercised muscle groups, however this warrants further investigation. Collectively, whilst there is not sufficient evidence to rule out task disengagement under the present experimental conditions, the differences in neuromuscular measures suggest that in this form of exercise their impact is greater. We therefore contend that an amplification of central fatigue best explains the reduction in TTF in HYP.

4.5.6 Methodological Considerations

Two females took part in the study, however we did not control for what phase they were in of the menstrual cycle which may have altered their response to experimental pain (Sherman and LeResche, 2006) potentially via 'luteal analgesia' (Vincent *et al.*, 2018) and exercise performance/neuromuscular fatigue (Ansdell *et al.*, 2019; McNulty *et al.*, 2020). Future work should attempt to control for this factor.

The TMS stimulus intensity for the MEPs was determined by delivering stimulus during contractions at around 20% of MVF to generate a stimulus response curve. The lowest intensity to evoke a maximal increase in the VL whilst minimising BF MEP was selected (Temesi *et al.*, 2014). We acknowledge that by maximising the MEP amplitude there may be a potential for a 'ceiling effect' with MEP amplitude. In the present study, there was a main effect for time for $MEP \cdot M_{max}^{-1}$ but subsequent post-hoc tests revealed no differences between time points. It was possible that pain may have reduced the MEP, but fatigue increased it, thus resulting in no net difference. Indeed, several other studies using the same stimulus intensity method have observed an increase in the MEP with fatigue (Pageaux *et al.*, 2015; Kennedy *et al.*, 2016; Aboodarda *et al.*, 2020). Nevertheless, the utilisation of a stimulus intensity at 120% of active motor threshold may have provided greater sensitivity for increases in corticospinal excitability.

The hypertonic saline injected was 1 mL of 5.85% NaCl solution. Possibly due to differences in pain threshold and pain tolerance, there was a variable response in pain VAS to the hypertonic saline injection. One important consideration is those participants who did not report a significant increase in pain following the injection of hypertonic saline. For example, the difference in mean pain VAS scores when normalised for the same exercise time was on average 19 points greater in HYP compared to CTRL whereas in three participants it was as low as -4, 3 and 6 units, respectively. While this heterogeneous pain response may have allowed for a robust correlation analysis, some of the data in changes of neuromuscular parameters between CTRL and HYP may have become 'diluted' with these low responding participants. Indeed, work by Graven-Nielsen and colleagues (Graven-Nielsen, Arendt-Nielsen, *et al.*, 1997) demonstrated some individuals only rated peak pain as 1 cm on a pain VAS whereas others were around 5-6 cm (out of 10 cm). Future work may want to take an individualised approach with injection volume to evoke a consistent pain response equal to 'strong' (~5/10 pain VAS).

4.5.7 Conclusion

In summary, elevated muscle pain reduces strength and endurance performance due to centrally mediated mechanisms. It is likely that feedback from group III/IV afferent nociceptors are responsible for constraining motor output to the painful muscle group. A redistribution/reorganisation of motor control may also be acting to maintain the demands of the isometric TTF but in a manner that causes fatigue to occur more rapidly. Given that these effects are centrally mediated, it is plausible that this pain could impact the function of contralateral muscles or proximal muscles within common cortical networks. Therefore, the next experimental chapter will focus on the effect of muscle pain on the fatigue and endurance of the contralateral quadriceps.

Chapter 5: The Effect of Hypertonic Saline Evoked Muscle Pain on Neurophysiological responses and Exercise Performance in the Contralateral Limb.

5.1 Abstract

Background: Non-local muscle pain may impair endurance performance through neurophysiological mechanisms, but these are relatively unknown. This study examined the effects of muscle pain on neuromuscular and neurophysiological responses in the contralateral limb. *Methods:* On separate visits, nine participants completed an isometric time to task failure (TTF) using the right knee extensors after intramuscular injection of isotonic saline (CTRL) or hypertonic saline (HYP) into the left vastus lateralis. Measures of neuromuscular fatigue were taken before, during and after the TTF using transcranial magnetic stimulation (TMS) and peripheral nerve stimulation. *Results:* Mean pain intensity was greater in the left leg in HYP (3.3 ± 1.9) compared to CTRL (0.4 ± 0.7; $P < 0.001$) which was combined with a TTF by 9.8% in HYP (4.54 ± 0.56 min) compared to CTRL (5.07 ± 0.77 min; $P = 0.005$). Maximum voluntary force was not different between conditions (all $P > 0.05$). Voluntary activation was lower in HYP compared to CTRL ($P = 0.022$). No difference was identified between conditions for doublet amplitude ($P > 0.05$). Furthermore, no difference in $MEP \cdot M_{max}^{-1}$ or the TMS silent period between conditions was observed (all $P > 0.05$). *Conclusions:* Non-local pain impairs endurance performance of the contralateral limb. This impairment in performance is likely due to the faster attainment of the sensory tolerance limit from a greater amount of sensory feedback originating from the non-exercising, but painful, left leg.

5.2 Introduction

Pain is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage (Raja *et al.*, 2020). Muscle pain during exercise is caused by an accumulation of noxious biochemicals (Mense, 2009) along with an increase in intramuscular pressure (O'Connor and Cook, 1999). This sensation is referred to as exercise-induced pain, which increases as a function of exercise intensity (Cook *et al.*, 1997) and time (Smith *et al.*, 2020).

Exercise-induced fatigue can develop during exercise, which can be defined as a transient reduction in the maximal force generating capacity of the muscle that is reversible by rest (Gandevia, 2001). Exercise-induced pain is often accompanied by exercise-induced fatigue (Pollak *et al.*, 2014). Therefore, the two may be interrelated and consequently exercise-induced pain may at least in part be responsible for the development of fatigue (Mauger, 2013) and be detrimental to endurance performance (Mauger, Jones and Williams, 2010; Astokorki and Mauger, 2017; Stevens *et al.*, 2018). Support for this notion comes from previous work which has found that muscle pain reduces the maximal force generating capacity of the painful muscle (Graven-Nielsen et al. 1997, 2002; Khan et al. 2011; Norbury et al. 2022) and impairs endurance performance (Ciubotariu, Arendt-Nielsen and Graven-Nielsen, 2004; Smith *et al.*, 2020). This effect appears to be driven by neurophysiological changes (Le Pera et al. 2001; Graven-Nielsen et al. 2002; Khan et al. 2011; Norbury et al. 2022) such as reductions in voluntary activation, corticospinal excitability and an increase in corticospinal inhibition. Because the fatigue related effects of muscle pain appear to be centrally mediated, it is possible that non-local muscle pain may also influence the development of neuromuscular fatigue in a non-local exercising limb, and subsequently reduce endurance performance (Hureau, Romer and Amann, 2018).

The effect of non-local pain on fatigue and endurance performance has recently been explored by Aboodarda and colleagues (2020). When ischaemia was induced on the left leg to gradually increase muscle pain, a 21% reduction in single limb cycling time to task failure (TTF) was seen in the right leg, which was coupled with lesser end-exercise reduction in maximum force and potentiated twitch force compared to no prior fatigue. Additionally, reductions in voluntary activation of non-local muscles have been found following a fatiguing protocol and subsequent maintenance of group III/IV afferent firing (and pain) through ischemia (Kennedy *et al.*, 2013, 2014, 2015; Finn *et al.*, 2020). Therefore, it is unclear how muscle pain may act at a non-local level to impact neuromuscular fatigue and endurance performance.

Intramuscular injections of hypertonic saline have previously been used to cause acute muscle pain (Graven-Nielsen *et al.*, 2002; Khan *et al.*, 2011; Smith *et al.*, 2020, 2021). To explore the relationship between pain and fatigue, hypertonic saline may be advantageous to ischaemia as ischaemia traps blood in the occluded limb and lowers O_2 availability. Furthermore, hypertonic saline induced pain can provide a different time course of pain intensity in comparison to ischemia, whereby saline produces a rapid increase, then slow decrease in pain intensity. Because of this, it is possible to determine the neurophysiological effects of non-local pain when exercise-induced pain and fatigue is low in the contralateral leg. Peripheral nerve stimulation and transcranial magnetic stimulation (TMS) are measurement techniques that can be used to investigate the neuromuscular function (NMF) of an individual in response to non-local muscle pain.

Therefore, the purpose of this study was to induce muscle pain in the left quadriceps and simultaneously assess endurance performance, neuromuscular fatigue, and corticospinal responses in the contralateral quadriceps. It was hypothesised that acute muscle pain would reduce endurance performance, and this would be accompanied by a decrement in the maximal force generating capacity as well as reductions in voluntary activation. Furthermore, we expect corticospinal excitability to be reduced and corticospinal inhibition to increase in response to muscle pain.

5.3 Methods

5.3.1 Participants

Twelve healthy individuals (3 female) with a mean ± SD age of 26 ± 5 y, height: 176 ± 9 cm, and body mass: 74.1 ± 13.0 kg participated in the study. A sample size calculation was performed to determine the number of participants required to detect a statistically significant effect in TTF with pain. Based on an effect size of dz =1.09 from Aboodarda and colleagues (2020) of the change in TTF between the pain and control conditions. An estimated n = 9 was needed to detect an effect. All participants were free of lower limb injuries from the past three months, were not taking medication for the treatment of pain and had no pain related conditions. Participants were also screened for any contraindications to TMS (Rossi *et al.*, 2011). Before testing commenced, all participants provided written informed consent and the study was approved by the University of Kent SSES research ethics advisory group (Prop 19_2019_20) and conducted in accordance with the declaration of Helsinki without registering the study (see appendix 3 for ethics documentation).

5.3.2 Experimental Protocol

Participants visited the laboratory on four occasions separated by at least 72 h and at a similar time of day (± 2 h). Prior to visits, participants avoided strenuous lower body activity for 48 h, caffeine for 4 h, alcohol for 24 h and analgesics for 6 h. In the first visit, participants were familiarised with measures of neuromuscular function, questionnaires, perceptual measures, the isometric TTF exercise and the intramuscular injection of hypertonic saline (if they had not received one before, $n = 6$). Visit two comprised of a second familiarisation of the isometric exercise task where the intensity (% of maximum voluntary contraction) was adjusted from the first visit if the TTF was lower than four minutes or greater than six minutes. This was to ensure that task failure coincided with the typical pain duration from the intramuscular injection of hypertonic saline (Smith *et al.*, 2020). Visits 3 and 4 comprised of the two experimental visits which were completed in a randomised order (see figure 5.1). Participants initially underwent baseline measures of NMF involving peripheral nerve stimulation and TMS during isometric contractions of the right knee extensors. Participants then waited ten minutes before receiving an intramuscular injection of 1 mL of isotonic saline (0.9%) or hypertonic saline (5.85%) in the left VL. The isotonic saline condition served as a non-painful, injection matched control (CTRL) while the hypertonic saline caused acute muscle pain (HYP). Immediately after the injection, participants began the submaximal isometric TTF protocol with the right leg whilst measures of peripheral nerve stimulation and TMS were performed. Measures of pain intensity and rating of perceived effort (RPE) were recorded until task failure, where post-exercise measures of NMF were performed along with the Situation Specific Pain Catastrophizing Scale.

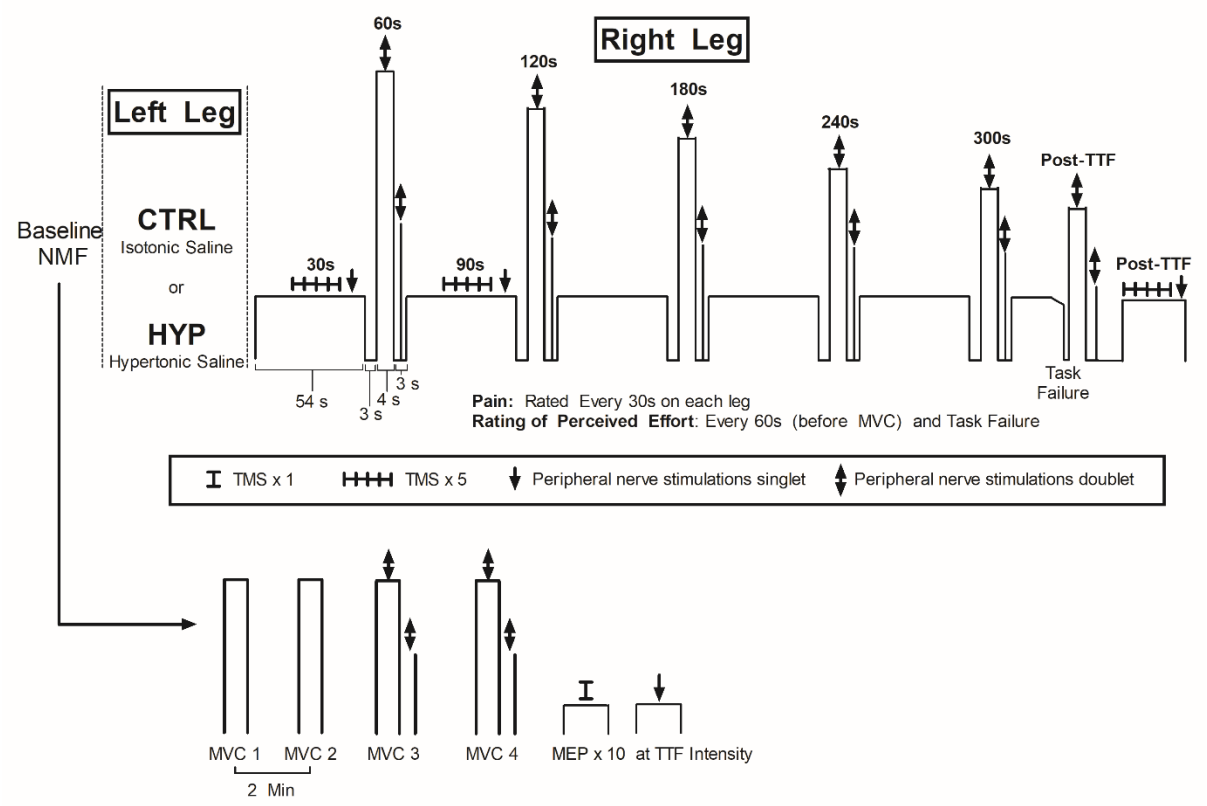

Figure 5.1 Schematic of the experimental protocol. NMF = neuromuscular function, TMS = transcranial magnetic stimulation. TMS was delivered at 90 s to allow for five stimulations instead four stimulations.

5.3.3 Equipment and Procedures

Hypertonic Saline Injection. A bolus of 1 mL hypertonic saline (5.85% NaCl) was injected into the middle third of the muscle belly of the left VL to induce muscle pain. This method can be found in section 2.3.1. An identical injection protocol was performed with the isotonic saline (CTRL condition).

Isometric Endurance Task. The endurance task was a single limb isometric contraction of the right knee extensors until task failure, which was defined as the inability to maintain the target force for three consecutive seconds despite verbal encouragement to return to the target. The intensity of the endurance task was individually prescribed to attain task failure in 4-6 minutes in CTRL (mean = 19% maximum voluntary force (MVF), range 14 – 25% MVF). At the end of each minute of the endurance task, participants were instructed to relax and to prepare to perform a 4 s maximum voluntary contraction (MVC) with a superimposed doublet. They were instructed to relax for a further 3 s after the MVC while a resting potentiated doublet was delivered. At 30 s and 90 s five TMS pulses were delivered (~3 s between stimulations).

Neuromuscular Function Testing. Baseline measures of NMF were completed after a warmup of ten contractions at 50% of the participants' perceived maximum effort (3 son, 3 s off). Four MVCs were then performed (3-5 s duration, 2 min rest between attempts). The latter two MVCs had a superimposed and resting potentiated doublet delivered during and after the MVC. TMS pulses were delivered in a batch of 10 submaximal contractions at the same target force of the subsequent endurance task. One additional contraction was performed at the end of this batch with a single superimposed electrical stimulation. Within 10 s of cessation of the endurance task, another MVC with a superimposed doublet was performed along with 5 submaximal contractions with TMS and one contraction with a single electrical stimulation.

Mechanical Recordings. Participants were strapped into a custom-built isometric chair with a hip and knee angle of 90° (0° being full extension) as described in section 2.3.2.

Electromyography (EMG). Bipolar surface EMG was used to record muscle activity of the VL as described in section 2.3.5

Peripheral Nerve Stimulation. Electrical stimuli were delivered as per the methods described in section 2.3.3.

Transcranial Magnetic Stimulation. Single pulse TMS was delivered with a magnetic stimulator (Magstim 200^2, The Magstim Company Ltd, Carmarthenshire, UK) via a double cone coil (110 mm diameter). The method employed for TMS is described in section 2.3.4. The mean stimulation intensity was 64 ± 8% maximum stimulator output. Each batch of TMS pulses (10 at baseline, 5 during exercise) were accompanied by the delivery of a single peripheral nerve stimulation to acquire $MEP \cdot M_{max}^{-1}$ ratio.

Perceptual Measures. To assess pain intensity, the pain perception scale was used (Cook *et al.*, 1997) and participants rated their muscle pain for each leg every 30 s. Details about pain recordings can be found in section 2.5.1. Rating of perceived effort (RPE) was recorded on the 6-20 point scale (Borg 1982) which is described in section 2.5.2.

Questionnaires. Post exercise, the Situation Specific Pain Catastrophizing Scale (Edwards *et al.*, 2006) was administered (see section 2.6.4).

5.3.4 Data Analysis

Baseline NMF was calculated as the mean of the raw value. MVF and doublet amplitude were calculated as the peak instantaneous force. Voluntary activation was calculated as described in

section 2.4.2. TMS measures of corticospinal excitability and inhibition was analysed as described in section 2.4.4 and 2.4.5. The root mean square (RMS) of the electromyogram was calculated offline using a 100 ms time constant. MVC RMS amplitude was calculated as the 250 ms either side of peak force, whereas for the submaximal contraction amplitude the mean amplitude of 20 s at the start of each minute and 20 s before task failure was used. Both EMG variables (i.e., MVC and endurance task amplitude) were normalised to the baseline value and expressed as a percentage of max.

5.3.5 Statistical Analysis

All data are presented as mean ± SD or as mean and interquartile range if not normally distributed. Data was analysed in JAMOVI 1.6.7. Data was initially checked for assumptions with the Shapiro-Wilk test and the Mauchly test. If these assumptions were violated, data was analysed with a non-parametric test (Wilcoxon's signed-rank test) or Greenhouse-Geiser corrected, respectively. A 2 × 5 repeated measures ANOVA (condition × time) was used to analyse neuromuscular variables at baseline, minutes one, two, three, and task failure. A 2 × 4 repeated measures ANOVA (condition × time) was used to analyse TMS data. A 2 × 8 repeated measures ANOVA (condition × time) was used to analyse pain data. If an interaction effect was observed, followed-up post-hoc tests were performed to determine differences between conditions at different time points and were Bonferroni-Holm corrected (Holm, 1979). Paired samples t-tests were used to test for differences in TTF. 95% confidence intervals, Cohen's d effect sizes (Cohen, 1992) where 0.2, 0.5 and 0.8 represent small, medium and large effect sizes respectively and partial eta squared (h_p^2) were reported where appropriate and 0.01, 0.06 and 0.14 reflect small, medium and large effect sizes respectively.

5.4 Results

5.4.1 Time to Task Failure

TTF was 9.3% shorter in HYP (4.62 ± 0.54 min) compared to CTRL (5.09 ± 0.68 min) (mean difference = 0.47 min, 95% CI [0.18, 0.76 min], $t_{11} = 3.60$, $P = 0.004$, $dz = 1.04$; figure 5.2a).

5.4.2 Pain Intensity

Left Leg (Saline-Injected Leg). There was a condition × time interaction for left leg pain ($F_{2.03, 22.32} = 8.53$, $P < 0.001$, $h_p^2 = 0.437$). Left leg pain did not change over time in CTRL (all $P > 0.05$), whereas in HYP, pain intensity was increased and greater than CTRL at all timepoints (all $P < 0.01$) except at 210 s ($P = 0.088$) and at task failure ($P = 0.592$; see figure 2b). Peak

pain was also greater in HYP (4.6 ± 2.2) than CTRL (1 ± 1.1) (mean difference = 3.6, 95% CI [2.2, 4.9], $t_{11} = 5.80$, $P < 0.001$, $dz = 1.67$).

Right Leg (Exercising Leg). No condition × time interaction was observed for right leg pain ($F_{2.90, 31.94} = 0.965$, $P = 0.419$, $h_p^2 = 0.081$) or main effect of condition ($F_{1, 11} = 1.053$, $P = 0.327$, $h_p^2 = 0.087$). There was a main effect of time ($F_{2.48, 27.31} = 85.145$, $P < 0.001$, $h_p^2 = 0.886$). Pain intensity increased at every time point from 30 s to task failure (all $P < 0.009$; figure 2b). There was also no difference in peak pain (CTRL = 9.0 [1.9], HYP = 9.0 [1.4] (Wilcoxon $P = 0.371$). There was no difference in the sum of the Situation Specific Pain Catastrophizing Scale post-TTF ($P = 0.733$).

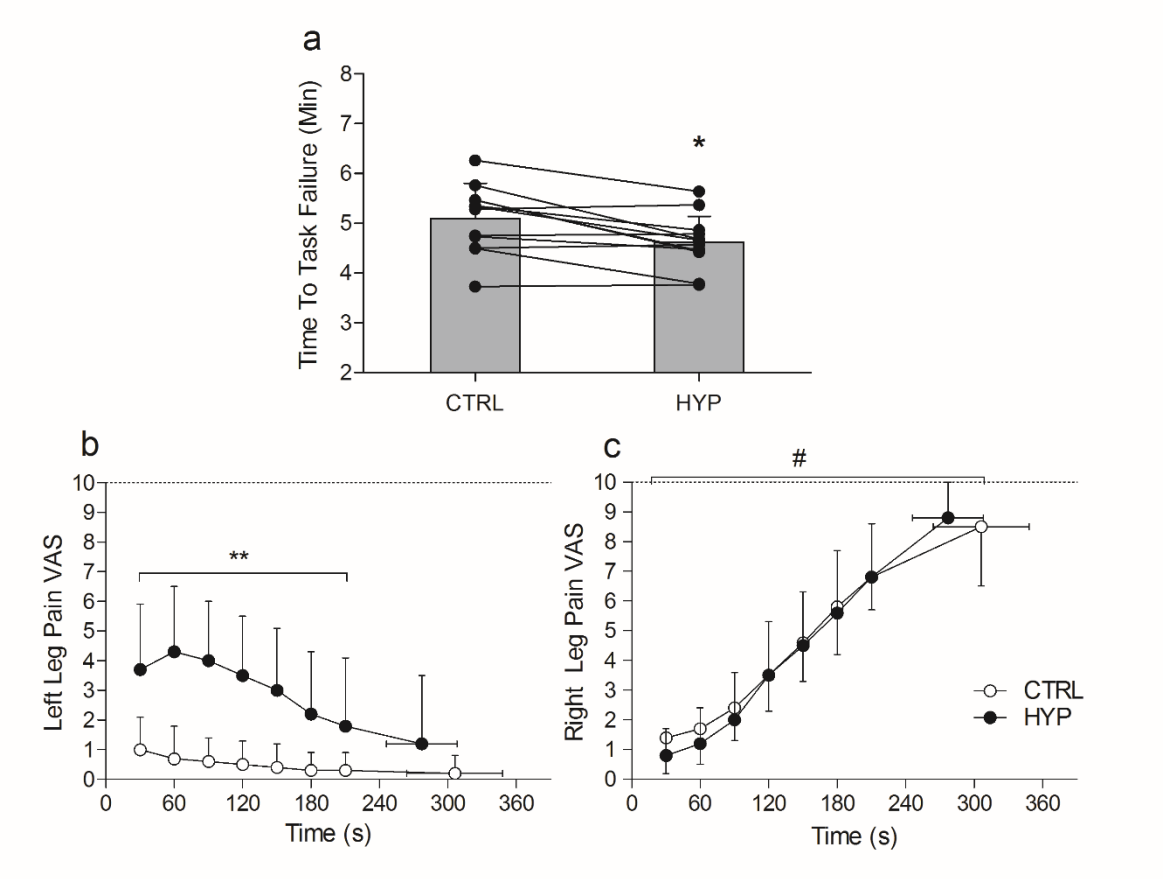

Figure 5.2. a. TTF of the endurance task. * Denotes significantly different from CTRL ($P = 0.005$). Data presented as mean and individual data. b. Left leg pain during the endurance task. ** denotes significantly different from CTRL (Interaction effect; $P < 0.001$). c. Right leg pain during the endurance task. # Denotes significant main effect of time ($P < 0.05$).

5.4.3 Maximal Voluntary Force

For MVF there was no condition × time interaction ($F_{1.32\ 14.53}$ = 1.90, P = 0.190, h_p^2 = 0.147) or main effect of condition ($F_{1,\ 11}$ = 0.002, P = 0.958, h_p^2 = 0.001). However, there was a main effect of time ($F_{1.85,\ 20.40}$ = 28.45, P < 0.001, h_p^2 = 0.721). MVF decreased at each timepoint from baseline (692 ± 168 N) until task failure (446 ± 141 N) (all P < 0.05) (figure 5.3a).

5.4.4 Voluntary Activation

No condition × time interaction was observed for voluntary activation ($F_{1.57,\ 17.29}$ = 0.312, P = 0.684, h_p^2 = 0.162). However, there was a main effect of condition ($F_{1,\ 11}$ = 6.029, P = 0.032, h_p^2 = 0.354) and main effect of time ($F_{2.53,\ 27.87}$ = 9.640, P < 0.001, h_p^2 = 0.467). VA was lower in HYP compared to CTRL. Over time, VA decreased from baseline (96.6 ± 2.3%) to minute 3 (90.7 ± 5.4%) (P = 0.007) but did not decrease any further at task failure (89.4 ± 8.3%) (P = 0.334) (figure 5.3b).

5.4.5 Potentiated Doublet

For potentiated doublet there was no condition × time interaction ($F_{2.13,\ 23.42}$ = 2.638, P = 0.090, h_p^2 = 0.193) or main effect of condition ($F_{1,\ 11}$ = 0.159, P = 0.698, h_p^2 = 0.014), but there was a main effect of time ($F_{1.38,\ 15.19}$ = 28.923, P < 0.001, h_p^2 = 0.724). Doublet amplitude decreased at every timepoint from baseline (338 ± 78 N) to task failure (245 ± 88 N) (all P < 0.05) (figure 5.3c).

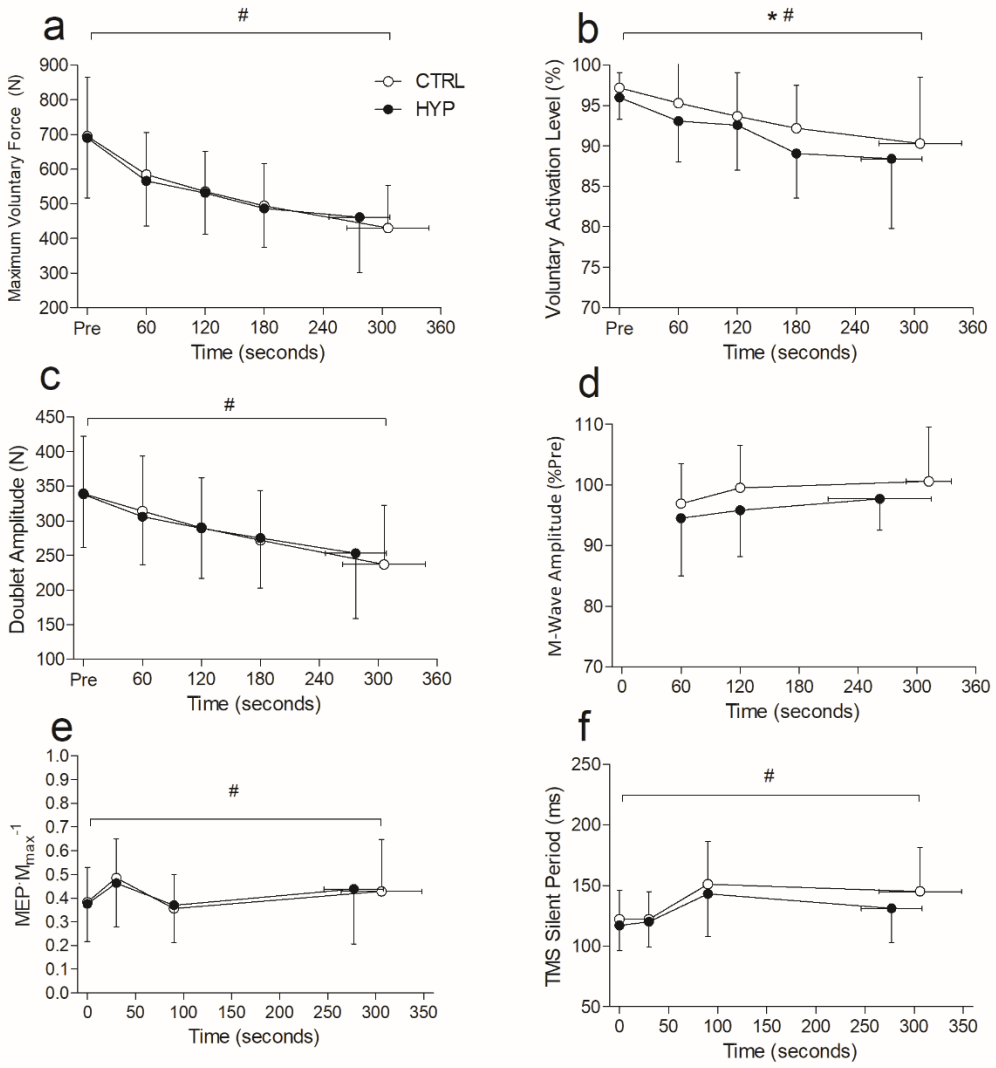

Figure 5.3. Neuromuscular variables during the isometric TTF. a. Maximum voluntary force. b. Voluntary Activation Level. c. Doublet amplitude. d. Change in M_{max}. e. Corticospinal excitability as $MEP \cdot M_{max}^{-1}$. f. Corticospinal inhibition as the TMS silent period. Data presented as mean ± SD. * denotes main effect of condition ($P < 0.05$). # Denotes main effect of time ($P < 0.05$).

5.4.6 M_{max}

No condition × time interaction was observed for M_{max} ($F_{1.32, 14.48} = 1.880$, $P = 0.193$, $h_p^2 = 0.146$). There was also no main effect of condition ($F_{1, 11} = 3.250$, $P = 0.099$, $h_p^2 = 0.228$) or main effect of time ($F_{1.41, 15.51} = 1.59$, $P = 0.233$, $h_p^2 = 0.126$).

5.4.7 MEP·M_{max}^{-1}

There was no condition × time interaction for MEP·M_{max}^{-1} ($F_{3, 33}$ = 1.147, P = 0.345, h_p^2 = 0.094) or main effect of condition ($F_{1, 11}$ = 0.006, P = 0.936, h_p^2 = 0.001), but there was a main effect of time ($F_{3, 33}$ = 7.866, P < 0.001, h_p^2 = 0.417). MEP·M_{max}^{-1} increased from baseline (0.38 ± 0.15) to 30 s (0.48 ± 0.17) (P = 0.001) and subsequently decreased from 30 s to 90 s (0.36 ± 0.15) (P < 0.001) and then remained unchanged at task failure (0.43 ± 0.22) (P = 0.178). Representative traces of MEPs and M-Waves can be seen in figure 5.4

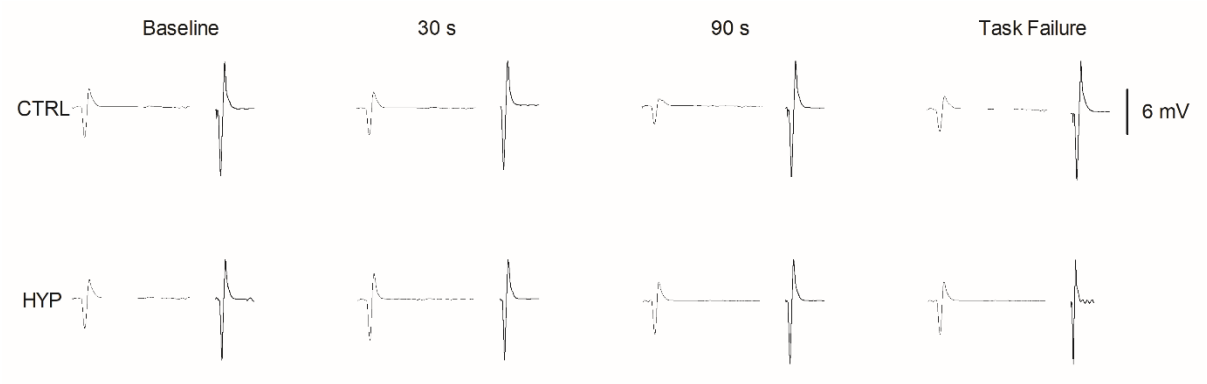

Figure 5.4 Representative traces of motor evoked potentials and M-Waves for each experimental condition at each time point. First trace is average of the MEPs and the second trace is the M-Wave.

5.4.8 TMS Silent Period

No condition × time interaction was seen for the TMS silent period ($F_{1.82, 20.01}$ = 1.92, P = 0.176, h_p^2 = 0.148) or main effect of condition ($F_{1, 11}$ = 3.39, P = 0.093, h_p^2 = 0.235). However, there was a main effect of time ($F_{1.46, 16.07}$ = 7.92, P = 0.007, h_p^2 = 0.419). The TMS silent period did not increase from baseline (120 ± 22 ms) to 30 s (121 ± 21 ms) (P = 0.836) but increased from 30 to 90 s (147 ± 34 ms) (P = 0.019) but did not change any further at task failure (138 ± 33 ms) (P = 0.105; figure 5.3 D).

5.4.9 Electromyography Amplitude

MVCs. No condition × time interaction was observed for MVC EMG_{RMS} amplitude ($F_{1.73, 19.01}$ = 3.077, P = 0.076, h_p^2 = 0.219). There was also no main effect of condition ($F_{1, 11}$ = 0.920, P = 0.358, h_p^2 = 0.077) but there was a main effect of time ($F_{1, 11}$ = 10.592, P < 0.001, h_p^2 = 0.491). MVC EMG_{RMS} amplitude decreased from minute 1 to minute 2 (P = 0.028,) but did not decrease further at minute 3 or at task failure (both P > 0.05; figure 4).

Time to Task Failure. For EMG_{RMS} amplitude of the isometric TTF there was no condition × time interaction ($F_{2.19, 24.04} = 0.847$, $P = 0.450$, $h_p^2 = 0.071$). However, there was a main effect of condition ($F_{1, 11} = 11.983$, $P = 0.005$, $h_p^2 = 0.521$) whereby EMG_{RMS} was greater in HYP compared to CTRL. There was also a main effect of time ($F_{1.04, 11.48} = 7.384$, $P = 0.019$, $h_p^2 = 0.402$). EMG_{RMS} increased from minute 1 (16.4 ± 5.4%) to minute 3 (18.8 ± 7.1%) ($P = 0.047$) and from minute 3 to minute 4 (21.7 ± 8.8%) ($P = 0.036$) but did not further increase at the point of task failure (34.4 ± 23.4%) ($P = 0.083$).

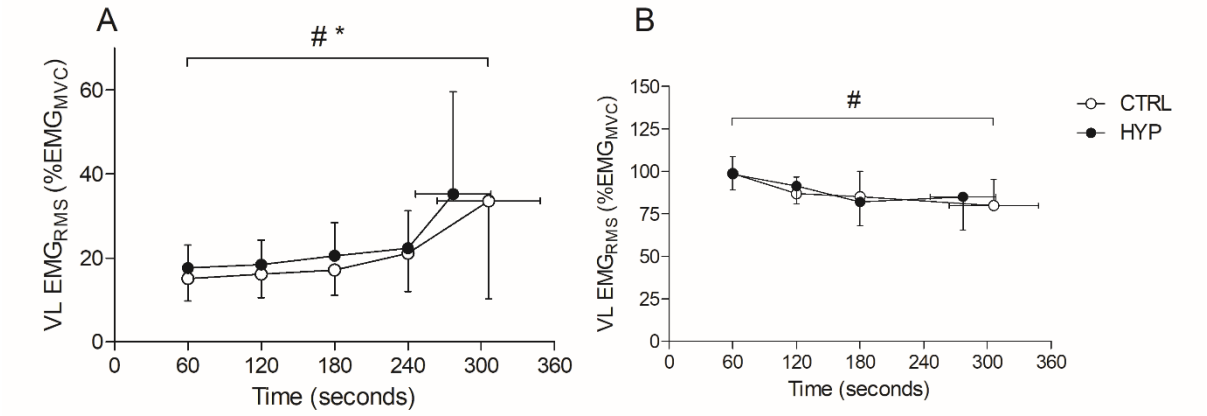

Figure 5.5. Root mean square electromyography Amplitude during the isometric TTF. A. Submaximal amplitude of the vastus lateralis muscle. B. MVC EMG amplitude of the vastus lateralis * Denotes significant main effect of condition ($P < 0.05$). # Denotes significant main effect of time ($P < 0.05$).

5.4.10 Rating of Perceived Effort (RPE)

For RPE, no condition × time interaction effect was observed ($F_{3, 33} = 0.791$, $P = 0.507$, $h_p^2 = 0.067$) and there was no main effect of condition ($F_{1, 11} = 3.561$, $P = 0.086$, $h_p^2 = 0.245$). There was a main effect of time ($F_{1.83, 20.10} = 148.689$, $P < 0.001$, $h_p^2 = 0.930$) whereby RPE increased at every timepoint from minute 1 (11 ± 2) to task failure (19 ± 1) (all $P < 0.001$).

5.5 Discussion

The primary finding of this study is that acute muscle pain reduces endurance performance in the contralateral limb. The mean change in endurance time (28 s) also exceeded the standard error of measurement of this TTF protocol from chapter three (21 s). This reduction in TTF appears to be due an exacerbation of perceptual responses (i.e., left leg pain) along with a decrement to voluntary activation.

5.5.1 Pain and TTF

The hypertonic saline injection caused a rapid increase in pain intensity of the left leg which peaked by 60 s at ~4.5/10 (somewhat strong pain), and then slowly decreased over time due to the gradual dissipation of the intramuscular saline (figure 5.2b). By the end of the TTF, the pain in the left leg was not significantly different from CTRL. As expected, in the exercising limb there was a gradual increase in pain intensity during the TTF, which reached near maximal levels. However, there was no difference in pain intensity of the right leg between HYP and CTRL, meaning that the pain in the exercising leg was unaffected by concurrent pain in the contralateral leg.

Only one other study has investigated the impact of exclusively non-local muscle pain on endurance performance (Aboodarda *et al.*, 2020). They found a 21% reduction in TTF with concurrent rising pain compared to the control, almost two-fold greater than in the current study. This may be explained by the gradual increase of muscle pain in the contralateral limb due to the ischemic environment induced, whereas with a hypertonic saline injection, muscle pain rapidly increases then slowly decreases (i.e., figure 5.2b.). As a result, there was likely a greater summation of afferent feedback in the latter parts of the exercise in the Aboodarda study which was exerting a greater inhibitory effect on endurance performance.

5.5.2 Neuromuscular Fatigue

Despite a significant pain response in the left leg, the impact of pain on neuromuscular fatigue during the endurance task was limited. No difference was observed in maximum voluntary force between conditions (figure 5.3a). This is in contrast to work by others (Deschamps *et al.*, 2014) who found a reduction in maximal hopping performance after a hypertonic saline injection into the contralateral VL. However, it is difficult to draw direct comparisons between MVCs and maximal hopping efforts as there are differences in muscle activation and stability between tasks. On the other hand, when hypertonic saline was injected into iliotibial tract (targeting non-muscle nociceptors), a decrement in the force generating capacity of the contralateral knee extensors and ipsilateral hand grip muscles was observed (Oda *et al.*, 2018). This was however observed with a greater pain intensity than achieved in this study (peak pain of 8.5) Whilst it cannot be ruled out that pain may reduce contralateral muscle strength, the findings of the present study do not suggest that a reduction in maximal force generating capacity was responsible for the shorter TTF. This is in contrast with localised muscle pain,

which does appear to reduce maximal force generating capacity (Graven-Nielsen, Svensson and Arendt-Nielsen, 1997; Smith *et al.*, 2020).

There was a main effect of condition for voluntary activation whereby it was lower in HYP compared to CTRL (figure 5.3b). Interestingly, this did not result in a decrease in the maximal force generating capacity of the knee extensors. It is not clear why this occurred, as peripheral fatigue also did not differ between condition. The reduction in VA likely reflects a combination of neural inhibitory feedback from both limbs acting to constrain voluntary drive to the right knee extensor. In the left leg, the pain-related feedback from the nociceptors stimulated by the hypertonic saline. In the right leg, there was stimulation of fatigue-sensitive and nociceptive afferents. In combination, the additional afferent feedback in HYP caused a greater reduction in central motor output. The reason for this reduction in central motor drive is likely to prevent the attainment of an intolerable level of voluntary activity (Gandevia, 2001; Hureau, Romer and Amann, 2018).

Post-exercise neuromuscular fatigue was not different between CTRL and HYP which is in contrast with several studies which have induced contralateral pain or fatigue and then performed a subsequent TTF (Amann *et al.*, 2013; Johnson *et al.*, 2015; Aboodarda *et al.*, 2020). Neural inhibitory feedback which can 'spill over' from the non-local area is thought to cause an individual to reach their sensory tolerance limit at an accelerated rate (Hureau, Romer and Amann, 2018). A shortened TTF would result in less end-exercise neuromuscular fatigue, which is typically seen with an attenuated reduction in maximum voluntary force and peripheral fatigue. It is unclear in the current study because despite a reduction in TTF, there was no difference in end-exercise neuromuscular fatigue. Perhaps only a modest reduction in TTF observed in this study (~10%) was insufficient to cause a significant attenuation of end-exercise neuromuscular fatigue, whereas in prior studies reductions in TTF have been much greater (21 – 50% reduction; Amann et al. 2013; Johnson et al. 2015; Aboodarda et al. 2020).

5.5.3 TMS Responses

The $MEP \cdot M_{max}^{-1}$ ratio increased at 30 s then decreased at 90 s during the exercise task reflecting an increase in excitability early on in the exercise, before exercise-induced fatigue likely decreased the excitability of the corticospinal pathway (Finn *et al.*, 2018). However, there was no observable difference in $MEP \cdot M_{max}^{-1}$ between conditions. Therefore, the excitability of the corticospinal pathway was unaffected by non-local muscle pain. These findings are in agreement with Le Pera and colleagues (Le Pera *et al.*, 2001) who observed no effect of muscle

pain on motor evoked potential amplitude of the contralateral hand. The TMS silent period, which is thought to reflect inhibition of the corticospinal pathway (Goodall, Howatson and Thomas, 2018; Škarabot *et al.*, 2019), increased 90 s into the endurance task but was not further increased with the addition of non-local muscle pain. This is also in alignment with previous work (Aboodarda *et al.*, 2020) where no increase of the TMS silent period with non-local pain and a shortening with non-local fatigue was observed. Therefore, when pain is non-local, it appears to have no influence on the corticospinal pathway during fatiguing exercise. However, this may not be the case for localised muscle pain where corticospinal adjustments may be responsible for changes in motor function (Schabrun and Hodges, 2012).

5.5.4 Electromyographic Responses

An interesting finding in the present study was that the VL EMG amplitude during the TTF was greater in HYP than in CTRL (figure 5.5a). The EMG recorded during submaximal tasks are thought to provide a crude measure of the neural drive to the muscle (Farina *et al.*, 2010). In this study, pain in the left VL could have subsequently required an increased neural drive to the right VL during the endurance task due to the centrally mediated inhibition caused by muscle pain (Farina *et al.* 2004; Liew *et al.* 2019; Martinez-Valdes *et al.* 2020) which could necessitate a greater need for central drive to ensure the maintenance of force. As a result, the earlier recruitment of more fatigable, higher threshold motor units could lead to the earlier development of fatigue and a shortened TTF.

5.5.5 Sensory Tolerance Limit

The sensory tolerance limit (Hureau, Romer and Amann, 2018) postulates that sensory feedback from muscles not directly involved in the exercise and corollary discharge summates until an intolerable level is achieved which causes a decrease in voluntary activation and termination of exercise. Within this study, it appears that the additional sensory neural feedback in the non-exercised (but painful) left leg combined with the rising exercise-induced fatigue and pain in the right leg caused the individual to reach their sensory tolerance limit sooner and cause a premature task failure through a constrained voluntary activation. Indeed, a reduced TTF for quadriceps exercise has occurred with additional sensory neural feedback from respiratory muscles (Wüthrich, Notter and Spengler, 2013), contralateral quadriceps (Amann *et al.*, 2013; Aboodarda *et al.*, 2020) and upper body muscles (Johnson *et al.*, 2015).

5.5.6 Methodological Considerations

The acute nature of the hypertonic saline injection resulted in a decreasing pain intensity within the left leg at the latter stages of the endurance task. It should be acknowledged that the neuromuscular and endurance performance reducing effects of non-local pain may have been more pronounced if a consistent or increasing level of pain was induced. Therefore, the effects in the present study are likely an underestimation of the role of non-local muscle pain on endurance performance.

Three females participated in this study which may introduce sex differences which could influence the results observed; particularly as we did not control for phases of the menstrual cycle or hormonal contraceptives. Firstly, in terms of the TTF intensity, it is likely that females performed the isometric TTF under different levels of ischemia compared to males due to (on average) a lower absolute strength (Males means absolute target = 145 N, Females = 118 N). This may influence the aetiology of pain and fatigue between the sexes and is acknowledged as a limitation within the present study. Furthermore, neurophysiological measures such as voluntary activation and short intracortical inhibition can change during different phases of the menstrual cycle, possibly due to differing levels of circulating oestrogen and progesterone (Ansdell *et al.,* 2019). In regard to sex differences in pain perception, it appears males and females experience a similar intensity of pain from hypertonic saline injections (Loram, Horwitz and Bentley, 2009; Yekkalam *et al.,* 2019). However, we acknowledge that differing phases of the menstrual cycle and the use of oral contraceptives can also be important as the response to experimental pain has been shown to be influenced by the menstrual cycle phase (Sherman and LeResche, 2006). Specifically, analgesia in the luteal phase (Vincent *et al.*, 2018) may have caused differing levels of pain in response to exercise. However, in the exercising leg, pain was similar. Therefore, any influence of the menstrual cycle would have likely been minimal. Fortunately, only two experimental visits were performed which were no longer than 7 days apart for females which would minimise the chance of the experimental visit being completed at different phases of the menstrual cycle.

5.5.7 Conclusion

In summary, muscle pain/nociceptive activity in a contralateral limb causes a significant reduction in endurance performance. This effect is centrally mediated and likely arises from a faster attainment of the sensory tolerance limit due to elevated levels of sensory neural feedback relayed from nociceptors in the painful left leg. The prior chapters have been

constrained to single limb isometric exercise in the TTF modality. To translate these findings to ‘real world’ endurance, the effect of muscle pain on self-paced, whole-body exercise is needed.

Chapter 6: The Effects of Bilateral Pain on Self-Paced Cycling Performance and Neuromuscular Fatigue

6.1 Abstract

Intro: Muscle pain may be a limiting factor in endurance performance, however the effect of elevated muscle pain on self-paced exercise performance is unknown. *Methods:* Eight healthy participants completed three experimental visits after a familiarisation session. After baseline measures of maximal voluntary force, voluntary activation, potentiated twitch force, and corticospinal excitability and inhibition, participants either received no injection (CTRL), a bilateral isotonic saline injection (1 mL, 0.9% NaCl; ISO) or a bilateral hypertonic saline injection (5.85% NaCl) into the VL to cause quadriceps muscle pain (HYP). Participants then completed a 5-minute cycling time-trial and performed the same pre-exercise neuromuscular assessments post-exercise. *Results:* Time-trial performance in HYP (3.12 ± 0.38 km) was not different from CTRL (3.21 ± 0.24 km) and ISO (3.20 ± 0.26 km) ($P = 0.171$, $\eta_p^2 = 0.223$). Pain intensity was similar between CTRL and ISO at all timepoints (all $P > 0.05$) but was greater in HYP compared to CTRL and ISO from 30 s to 150 s (all $P < 0.05$). There was a significant correlation between the mean pain intensity within minute 1 in HYP and the change in mean power between HYP and ISO ($r = 0.717$; $P = 0.045$). There was a similar decline in maximum voluntary force (-27%; $P = 0.413$), and potentiated doublet amplitude (-29%; $P = 0.560$) across conditions whereas voluntary activation remained unchanged from baseline ($P = 0.071$). No differences in MEP responses were seen over time or between conditions (all $P > 0.05$). *Conclusion:* Elevated muscle pain within the quadriceps appears to have limited impact on short duration, self-paced endurance performance and does not alter the magnitude of neuromuscular fatigue. Therefore, pain may have a distinct neurophysiological effect during self-paced exercise performance.

6.2 Introduction

Self-paced endurance exercise requires an individual to complete as much distance as possible in a fixed time, or to complete a fixed distance as quickly possible (MacInnis, Thomas and Phillips, 2019). Within self-paced exercise, an individual can alter their work rate (i.e., power or speed) ad libitum. Successful endurance performances are primarily characterised by an ability to sustain the greatest average speed/power over the course of the event, and this is achieved by managing exercise induced fatigue and the conscious perceptions associated with strenuous exercise. Exercise-induced fatigue is defined as a reduction in force generating capacity of the muscle which is reversible by rest (Bigland-Ritchie and Woods, 1984). Conscious perceptions may include (but are not limited to) effort, fatigue, and pain. In other words, self-paced exercise tasks are characterised by managing exercise-induced fatigue and the associated perceptions to produce the greatest distance or shortest time for the given task.

The decision to change work rate may be influenced by several psycho-physiological variables. These include prior experience, motivation, knowledge of exercise endpoint (Mauger, 2014) and perceptions of fatigue, effort, dyspnoea and pain (Wüthrich, Notter and Spengler, 2013; Staiano *et al.*, 2018; Stevens *et al.*, 2018). EIP is of particular interest because it is a prominent sensation that is often experienced during exercise; however, it has received little attention on how it may mediate self-paced exercise performance. Pain can defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage (Treede, 2018). During exercise, EIP originates from the excitation of group III/IV afferent nociceptors which relay the nociceptive signal to sensory areas of the brain which are processed as pain. EIP appears to increase linearly as a function of exercise intensity and time (Cook *et al.*, 1997; Smith *et al.*, 2020).

The role of EIP on the performance of self-paced exercise is not well understood but may limit exercise performance in several ways. Primarily, pain can cause and facilitate the development of neuromuscular fatigue as stimulation of nociceptive afferents is relayed from the painful exercising muscle to the brain. Consequentially, central motor drive to the working muscles is reduced to prevent further pain or 'physical disruption' which is caused by deleterious concentrations of noxious biochemicals (Hureau, Romer and Amann, 2018). This has been reflected as a reduction in maximal voluntary activation and/or maximal force (Graven-Nielsen *et al.*, 2002; Slater *et al.*, 2003; Khan *et al.*, 2011) and decreases in corticospinal excitability (Burns, Chipchase and Schabrun, 2016). Furthermore, muscle recruitment strategies change in

response to pain in order to sustain the demands of the exercise task such as the increased recruitment of higher-threshold motor units at the expense of increased fatigability (Martinez-Valdes *et al.* 2020). Alternatively, or in addition to altered neuromuscular responses, pain may provide important sensory feedback for the regulation of exercise intensity. The perception of EIP during exercise may serve to indicate whether the current exercise intensity is too great to be sustained and if continued, may result in intolerable levels or an unfavourable magnitude of muscle fatigue.

Previous research has used several experimental interventions to reduce muscle pain during exercise. Most notably, exercise performance after the oral ingestion of paracetamol has produced equivocal results, with a recent meta-analysis only finding ergogenic effects of reducing pain during time to task failure exercise and not during self-paced bouts (Grgic and Mikulic, 2021). Similarly, the ingestion of tramadol has improved 20 minute time-trial cycling performance (Holgado *et al.*, 2018) and had no effect in comparison to placebo (Bejder *et al.* 2020). The use of non-pharmacological interventions such as transcutaneous electrical nerve stimulation (Astokorki and Mauger 2017) or viewing pleasant versus painful images (Astokorki, Flood and Mauger, 2021) have reduced EIP and improved self-paced cycling performance. Taken together, there is limited evidence to suggest pain may be a limiting factor for self-paced exercise performance.

Reducing pain is only 'one side of the coin' to determining if EIP affects exercise performance. To fully explore this phenomenon there is a need to elevate pain and assess time-trial performance, however this paradigm has received much less attention. The intramuscular injections of hypertonic saline has successfully been used as a model to cause acute muscle pain (Smith *et al.* 2021; Smith *et al.* 2020; Martinez-Valdes *et al.* 2020; Martinez-Valdes *et al.* 2021) without altering peripheral function (Farina, Arendt-Nielsen and Graven-Nielsen, 2005). Therefore, by using the hypertonic saline model we can isolate the effect of elevated muscle pain on the performance of self-paced exercise. In addition, the use of peripheral nerve stimulation and transcranial magnetic stimulation pre to post exercise will elucidate the mechanisms of pain on the development of neuromuscular fatigue and corticospinal responses.

No study has yet investigated the effect of experimental muscle pain on self-paced cycling exercise performance and the associated neuromuscular fatigue response. Therefore, the aim of the present study was to bilaterally induce experimental muscle pain of the quadriceps and

assess short duration cycling time-trial performance and the associated neuromuscular fatigue parameters. Based upon previous data of analgesia and self-paced exercise, it was expected that experimental pain would reduce power output, particularly during the earlier part of the time-trial (when saline pain is strongest) and this would result in less end-exercise peripheral fatigue and an exacerbation of central fatigue.

6.3 Methods

6.3.1 Participants

Eight participants (2 females; mean ± SD) age: 27 ± 5 years, height: 176.5 ± 7.1 cm, body mass: 75.6 ± 10.5 kg volunteered to take part in the study. All participants were healthy and had not sustained a lower limb injury within the past three months, were not taking any pain related medication and did not have any contraindications to transcranial magnetic stimulation (TMS). The study was approved by the university ethics committee (see appendix 4 for ethics documents).

6.3.2 Study Design

Participants attended the lab on four to five separate occasions at a similar time of day (± 2h). Each visit was separated by at least 72 h and participants were instructed to avoid vigorous physical activity 48h, alcohol 24h, analgesics 6h and caffeine 4h prior to each visit.

Visit 1 was a familiarisation session which included familiarising participants with measures of neuromuscular function (NMF) in the isometric chair (see procedures). Participants then underwent a familiarisation of the 5-minute cycling time-trial (TT) which included practising dismounting the cycle ergometer and moving to the isometric chair for post-exercise measures of NMF within 30 s. A second familiarisation to further accustom the participants to the TT and post-exercise NMF assessment transition was performed if needed (n = 4). Then within a randomised order, participants completed three experimental visits where they performed baseline measures of NMF and then received either a bilateral injection of hypertonic saline (HYP) to cause experimental muscle pain, isotonic saline to serve as an injection matched control (ISO) or no injection (CTRL).

6.3.3 Equipment and Measures

Experimental Muscle Pain. A bilateral intramuscular injection of 1 mL of hypertonic saline (5.85%) was used to induce muscle pain in the left and right VL. The injection protocol was identical to the described method in section 2.3.1 for each leg.

Cycle Ergometry. An electronically braked cycle ergometer (Cyclus 2, Avantronic, Leipzig, Germany) with an attachable cycle frame was used to assess self-paced exercise performance. This set up allows for participants to freely adjust cadence and gear ratio to alter power output power output. Feedback of time elapsed was provided but power, cadence and heart rate were blinded.

Cardiorespiratory Variables. Measures of gas exchange were recorded breath by breath with a metabolic cart (Cortex Metalyser 3b, Leipzig, Germany). Initially, the device was calibrated before participants wore a facemask (7450 V2, Hans Rudolph, Birmingham, UK) with an attached flow sensor and sample line which acquired measures of minute ventilation ($\dot{V}_E$), breathing frequency (f_R), oxygen uptake ($\dot{V}O_2$) and partial pressure of end tidal O_2 and CO_2 ($PETO_2$ and $PETCO_2$ respectively). Heart rate was continuously recorded with a heart rate monitor (Garmin 010-10997-07; Garmin ltd, Hampshire, UK) during the TT.

Mechanical Recordings. Participants were seated into a custom-built isometric chair as described in section 2.3.2.

Peripheral Nerve Stimulation. For the assessment of central and peripheral fatigue, peripheral nerve stimulation was performed as described in section 2.3.4

Electromyography. Bipolar surface electromyography (EMG) was used to record the myoelectric activity of the right VL, VM, RF and BF using methods described in section 2.3.5.

Transcranial Magnetic Stimulation (TMS). Single pulse TMS were delivered in line with the methods described in section 2.3.4.

Perceptual Measures. The intensity of pain experienced was continuously measured with a linear potentiometer attached to the stem of the cycle and pain was recorded using the methods outlined in section 2.5.1. Pain quality was measured using the McGill long form questionnaire

described in section 2.6.3. RPE was recorded at the end of every minute during the TT as described in section 2.5.2.

Psychological Questionnaires. Before each experimental session, participants reported states of affect with the PANAS (see section 2.6.2). Additionally, pain expectation and pain coping confidence were recorded (see section 2.6.1). Post-exercise, the pain catastrophising scale was administered as described in section 2.6.4.

6.3.4 Procedures

Visit 1. Familiarisation. Participants arrived at the lab and had measures of their height and body mass recorded. Participants were then familiarised with the pain expectation/coping confidence and PANAS. Participants were then seated in the isometric chair where their settings were recorded for future visits. A familiarisation of PNS, maximum voluntary isometric contractions and TMS was performed in an identical manner to how they were performed during the experimental visits (see visits 2,3 and 4 below). Participants then completed a warmup (5 minutes, self-selected pace) and performed their familiarisation of the 5-minute time trial where they were instructed to cover as much distance as possible in that time. A familiarisation of dismounting the cycle ergometer and moving to the isometric chair for post-exercise measure of NMF was then performed. If participants had not received a bilateral hypertonic saline injection in a prior study, they were familiarised with this technique after they had recovered from the TT (n = *3)*. Some participants also completed another familiarisation of only the 5-minute TT and post-exercise MVC if needed (n = 4).

Visits 2, 3 and 4. Experimental Trials. Participants initially completed the PANAS and pain expectation/coping confidence questionnaires. Subsequently, EMG was prepared, and the participants were seated in the isometric chair where their optimal stimulation site and intensity were found for PNS. A warmup was then completed which consisted of 10 contractions at 50% of the participants perceived maximum effort (3 s on, 3 s off). Two minutes after, participants completed four MVCs interspersed with 2 mins of recovery between each effort. The latter two MVCs had a PNS doublet superimposed once peak force was achieved and plateaued, followed shortly (within 5 s) by a resting PNS doublet. Participants were instructed to contract as hard an as fast as possible and to reach peak force as soon as possible. After the final PNS doublet, three single twitches were immediately delivered to assess contractile function. Subsequently, TMS was performed by finding the optimal stimulation site and intensity. Then 10 stimuli were

delivered during intermittent isometric contractions equivalent to 20% of the previous best MVC force. Following this, participants had 5 minutes of rest where the injection sites were marked on the VL. A 5-minute self-selected warmup was then performed before participants rested for another 3 minutes. During these 3 minutes participants were seated on the cycle ergometer with stools supporting the feet to create a knee joint angle of 90°. At 2 minutes 35 seconds of the 3 minutes the injection procedure commenced (HYP and ISO), or participants remained seated (CON). The TT commenced shortly after the needles were withdrawn (within 15 s). After completion of the TT, participants dismounted the cycle ergometer and were seated in the isometric chair where they completed one MVC with a superimposed PNS doublet and resting potentiated PNS doublet followed by three single PNS twitches. Then five TMS pulses were delivered at the same contraction intensity as the baseline measures. Finally, the McGill long form and pain catastrophising questionnaire were administered. A schematic of the experimental trials can be seen in figure 6.1.

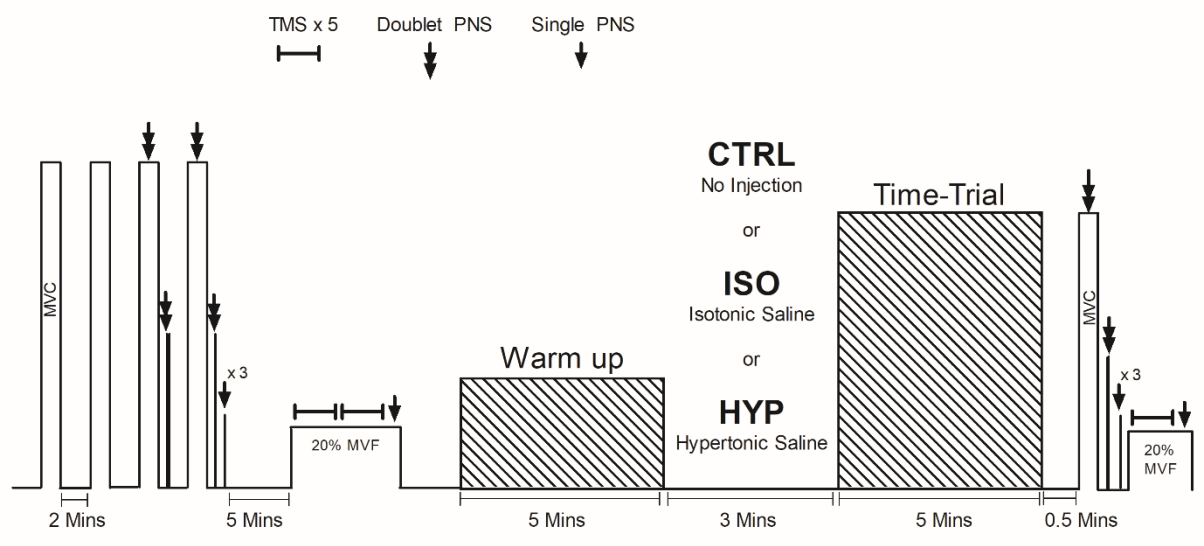

Figure 6.1. A schematic of the protocol during the experimental visits.

6.3.5 Data Analysis.

The raw value of all evoked NMF responses measures was taken and the maximum value for pre and post exercise measures was used for analysis. MVF was recorded as the peak instantaneous force achieved. Half relaxation time (0.5_{RT}) was calculated as the time from peak force to half of the relaxation force, contraction time (CT) was calculated as the onset of twitch force until peak force, maximum rate of force development (MRFD) and maximum relation rate (MRR) were calculated as the maximum and minimum values from the first derivative of

the force signal. Voluntary activation was calculated as described in the general methods section 3.4.3

Measures of corticospinal excitability and inhibition from MEPs was analysed using the methods described in section 2.4.4 and 2.4.5.
The root mean square (RMS) of the EMG waveform was calculated offline in software (Acqknowledge V5.0; Biopac systems Inc, California, USA) using a 100 ms time constant. The mean 500 ms (250 ms either side of peak force) was analysed for MVCs.

6.3.6 Statistical Analysis.
Data was initially checked to be normally distributed with a Shapiro-wilk test. A one-way repeated measures ANOVA was used to compare TT performance and questionnaire responses across conditions. A 3 × 2 repeated measures ANOVA with condition (CTRL, HYP and ISO) and time (PRE and POST) as factors was used to analyse all NMF variables. For variables analysed during the TT (e.g., heart rate, RPE and breath by breath measures) a 3 × 3 repeated measures ANOVA for condition (CTRL, ISO and HYP), time (minutes 1, 3 and 5) and a condition × time interaction was used. Pairwise comparisons with a Holm-Bonferroni correction were performed when a main effect or interaction effect was observed (Holm, 1979). A Pearson correlation was used to determine the change in pain between ISO and HYP during minute 1 and the change in mean power between ISO and HYP at minute 1. The alpha level was set at 0.05. Effect sizes were calculated from the partial eta squared (η2p). All data is presented as mean ± SD except for non-parametric data which is presented as median ± interquartile range.

6.4 Results

All participants arrived in a similar psychological state as indicated by similar positive (P = 0.191) and negative (P = 0.593) affect scores on the PANAS.

6.4.1 Time-Trial Performance
Distance completed for the TT was 3.21 ± 0.24 km in CTRL, 3.20 ± 0.26 km in ISO, and 3.12 ± 0.38 km in HYP which was not different between conditions ($F_{2, 14}$ = 2.01, P = 0.171, η_p^2 = 0.223; Figure 6.2A). Similarly, there was no difference in mean power ($F_{2, 14}$ = 2.31, P = 0.136, η_p^2 = 0.248) or peak power ($F_{2, 14}$ = 2.47, P = 0.120, η_p^2 = 0.261; figure 6.2B). Upon inspection

of the early, mid, and late phases of the TT (i.e., Minute 1, minute 3 and minute 5) there was no condition × time interaction for mean power ($F_{1.63, 11.44} = 1.09$, $P = 0.355$, $\eta_p^2 = 0.135$) or main effect of condition ($F_{2, 14} = 2.83$, $P = 0.093$, $\eta_p^2 = 0.288$). A time-effect was observed ($F_{2, 14} = 4.11$, $P = 0.035$, $\eta_p^2 = 0.370$) whereby mean power increased only from minute 3 to minute 5 (mean difference = 34 W, t = 3.758, $P = 0.021$; Figure 6.2C). A Pearson correlation found a significant relationship between pain intensity in the first minute of exercise and change in power output between HYP and ISO in the first minute ($r = 0.717$, $P = 0.045$). In other words, the greater the pain intensity, the lower the power output.

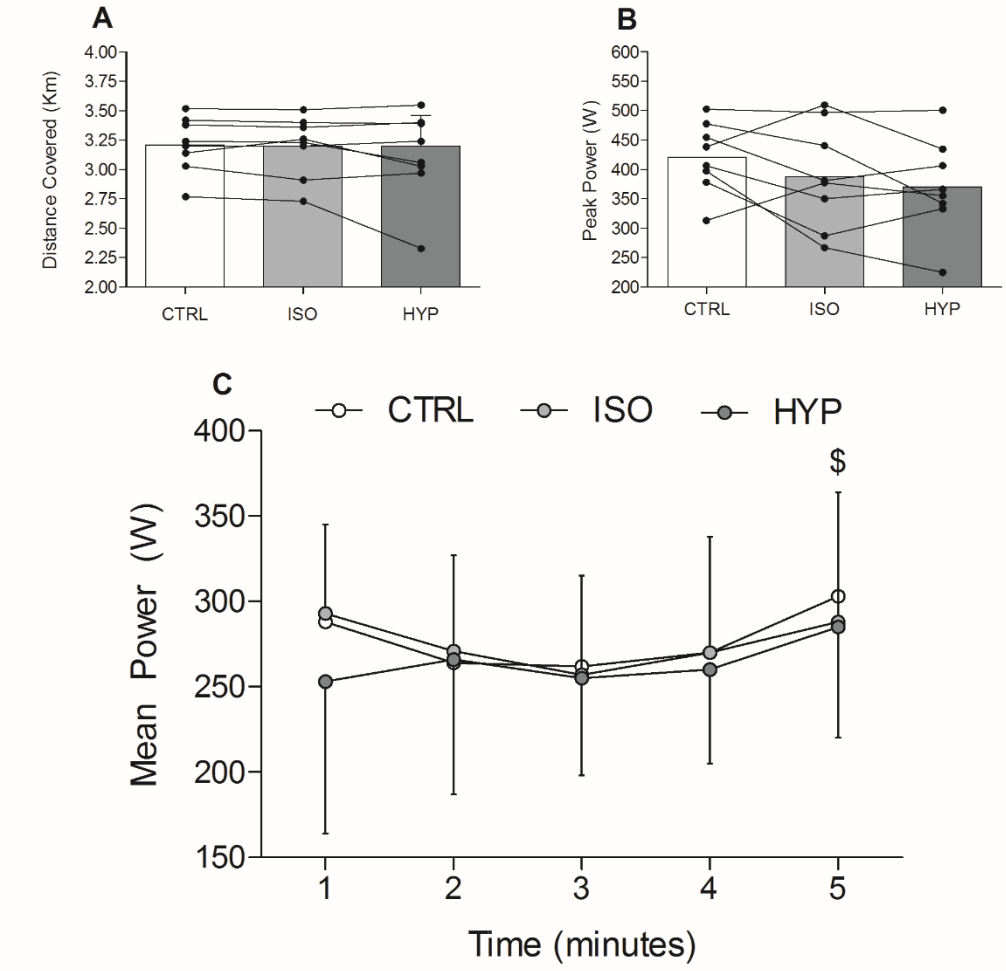

Figure 6.2. Performance on the 5-minute time-trial. A. Total distance covered. Data presented as mean and individual. Four individuals had a lower distance covered in HYP compared to the average of CTRL and ISO, one was identical and three slightly improved. B. Peak power during the time-trial. Data presented as mean and individual. C. Mean power during each minute of the time trial. Data presented as mean and individual response in a and b and mean ± SD in c. $ denotes significantly different from minute 3 (main effect of time).

6.4.2 Pain Recordings and Perceptual Measures

Prior to each experimental visit, there was no difference in pain expectation ($F_{1.11, 7.77} = 3.21$, $P = 0.111$, $\eta_p^2 = 0.314$) or pain coping confidence ($F_{2, 14} = 1.04$, $P = 0.378$, $\eta_p^2 = 0.130$). For pain intensity during exercise, there was a condition × time interaction ($F_{20, 140} = 14.1$, $P < 0.001$, $\eta_p^2 = 0.668$). Follow up post-hoc tests revealed that pain was not different at any timepoint between CTRL and ISO (all $P > 0.05$) however pain was greater in HYP compared to CTRL from 0 s to 150 s (all $P < 0.05$) but was not different between conditions from 180 s to 300 s (all $P > 0.05$). Pain intensity was also greater in HYP compared to ISO from 30 s to 150 s (all $P < 0.05$*)* but was not different between conditions at 0 s ($P = 0.084$) and from 180 s to 300 s (all $P > 0.05$). For peak pain intensity there was no difference between conditions ($F_{2, 14} = 0.268$, $P = 0.768$, $\eta_p^2 = 0.037$) (CTRL = 84 ± 14, ISO = 81 ± 17, HYP = 84 ± 16).

Pain Catastrophising was different between conditions ($F_{1.22, 8.55} = 5.470$, $P = 0.041$, $\eta_p^2 = 0.439$) whereby a greater sum of scores was seen in HYP compared to CTRL (mean difference = 4, t = 2.995, $P = 0.029$) and ISO (mean difference = 3.6, t = 2.714, $P = 0.034$) but was not different between CTRL and ISO ($P = 0.783$). Within the McGill pain questionnaire, the most selected words can be seen in table 6.1. There was a difference in the sensory component of the pain ($F_{2, 14} = 9.54$, $P = 0.002$, $\eta_p^2 = 0.577$) whereby there was a greater sensory sum of scores in HYP compared to CTRL ($P = 0.041$) and ISO ($P = 0.041$), but CTRL and ISO were not different ($P = 0.231$). For affective ($P = 0.219$), evaluative ($P = 0.742$) or miscellaneous ($P = 0.135$) scores there was no difference between conditions.

Table 6.1. Most selected words to describe the quality of pain experienced during the TT across all three experimental Conditions.

	CTRL	ISO	HYP
Sensory	Cramping (63%) Hot (38%) Aching (63%)	Cramping (50%) Hot (38%) Aching (38%) Heavy (38%)	Throbbing (38%) Shooting (50%) Stabbing (38%) Sharp (50%) Cramping (75%) Pulling (38%) Stinging (38%) Aching (63%)
Affective	Tiring (50%)	Tiring (50%)	Tiring (38%) Exhausting (38%) Gruelling (38%)
Evaluative	Intense (38%)	Intense (50%)	Intense (63%)

Miscellaneous	Spreading (38%) Tight (38%)	Spreading (38%) Radiating (38%)	Spreading (50%)

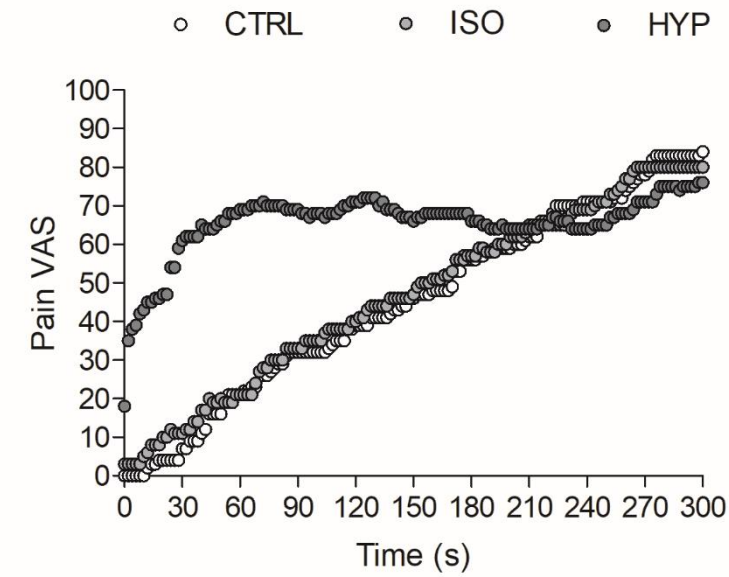

Figure 6.3. Pain intensity during the 5-minute time-trial. Data presented as mean for each 2 s timepoint. Standard deviation omitted to improve clarity.

6.4.3 Rating of Perceived Effort (RPE)

For RPE there was no condition × time interaction ($F_{4, 28}$ = 2.444, P = 0.070, η_p^2 = 0.259) or main effect of condition ($F_{2, 14}$ = 0.637, P = 0.638, η_p^2 = 0.062). There was a main effect of time ($F_{1.10, 7.70}$ = 211.245, P < 0.001, η_p^2 = 0.968) whereby RPE at each time point (all P < 0.05; see table 6.2).

Table 6.2. Rating of Perceived effort across the TT. All data presented but only statistical analysis performed on minutes one, three and five.

	1	2	3	4	5
CTRL	12.9 ± 1.9	14.8 ± 1.5	16.5 ± 1.3$^{\#}$	17.9 ± 1.1	19.4 ± 0.7$^{\#}$
ISO	13.0 ± 1.3	14.9 ± 0.7	16.1 ± 0.8$^{\#}$	17.8 ± 0.5	19.5 ± 0.5$^{\#}$
HYP	13.9 ± 1.0	15.7 ± 1.2	16.6 ± 0.9$^{\#}$	17.8 ± 1.2	18.9 ± 0.9$^{\#}$

= significantly different from previous analysed timepoint (P < 0.05).

6.4.4 Maximum Voluntary Force

No condition × time interaction ($F_{2, 14}$ = 0.711, P = 0.508, η_p^2 = 0.092) or main effect of condition ($F_{1, 7}$ = 0.944, P = 0.413, η_p^2 = 0.119) was observed, however there was a main effect

of time ($F_{1, 7}$ = 38.207, P < 0.001, η_p^2 = 0.845). MVF decreased from PRE (673 ± 116 N) to POST (488 ± 121 N) exercise by 27 ± 12% (see figure 6.4A).

6.4.5 Maximum Rate of Force Development

There was no interaction effect for the maximum rate of force development ($F_{2, 14}$ = 0.361, P = 0.703, η_p^2 = 0.049) or main effect of condition ($F_{2, 14}$ = 0.469, P = 0.635, η_p^2 = 0.063). There was a main effect of time ($F_{1, 7}$ = 11.186, P = 0.012, η_p^2 = 0.615) whereby the rate of force development decreased by 26 ± 32% from 6039 ± 3179 $N.s^{-1}$ at baseline to 4467 ± 3341 $N.s^{-1}$ post-exercise (see figure 6.4B).

6.4.6 Voluntary Activation

At baseline VAL was 95.1 ± 4.1% in CTRL, 96.1 ± 3.3% in ISO and 96.1 ± 2.9% in HYP and after the time-trial was 93.6 ± 5.9% in CTRL, 93.7 ± 4.2% in ISO and 93 ± 6.2% in HYP. No condition × time interaction was observed for voluntary activation ($F_{2, 14}$ = 0.902, P = 0.428, η_p^2 = 0.114). Additionally, there was neither a main effect of condition ($F_{1, 7}$ = 0.369, P = 0.698, η_p^2 = 0.050) or time ($F_{2, 14}$ = 4.507, P = 0.071, η_p^2 = 0.392; see figure 6.4D).

6.4.7 Doublet Amplitude

For Doublet amplitude, there was no condition × time interaction ($F_{2, 14}$ = 2.084, P = 0.161, η_p^2 = 0.229) or main effect of condition ($F_{2, 14}$ = 0.605, P = 0.560, η_p^2 = 0.080) but there was a main effect of time ($F_{1, 7}$ = 12.430, P = 0.010, η_p^2 = 0.640). Doublet amplitude decreased by 29 ± 17% from PRE (328 ± 65 N) to POST (233 ± 39) exercise (figure 6.4C). All other measures of peripheral function can be seen in table 6.2.

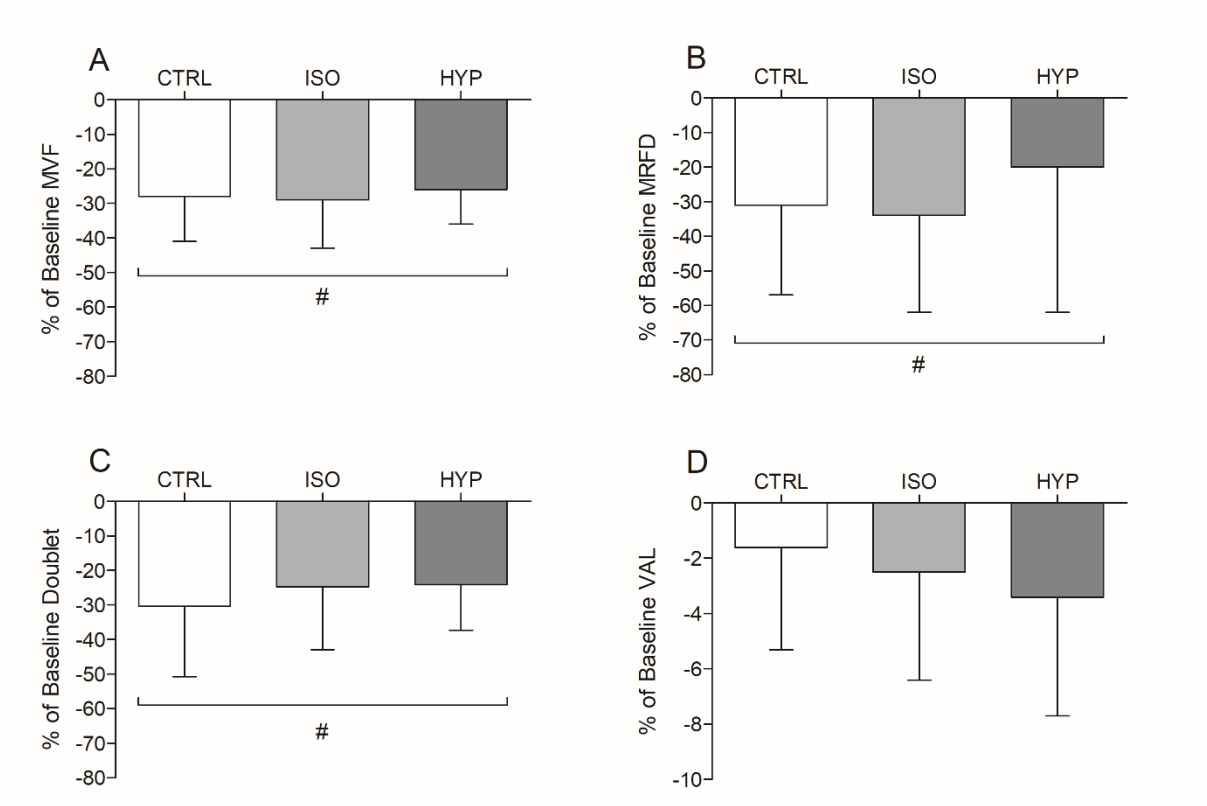

Figure 6.4. Magnitude of neuromuscular fatigue after the 5-minute time trial. A. Maximum Voluntary Force. B. Maximum Rate of Force Development. C. Potentiated Doublet. D. Voluntary activation level. # = significantly reduced from baseline (main effect of time).

Table 6.2. Indices of peripheral function in response to peripheral nerve stimulation at baseline and after the 5-minute time trial. Qtw = quadriceps potentiated twitch force, MRTD = maximum rate of twitch development, MRR = maximum relaxation rate, 0.5RT = half relaxation time, CT = contraction time, Mmax = muscle compound action potential peak to peak amplitude.

					Two Way ANOVA		
		CTRL	ISO	HYP	Condition	Time	Interaction
Qtw (N)	Pre	227 ± 45	228 ± 50	221 ± 45	$F_{2,14} = 0.620$	$F_{1,7} = 19.815$	$F_{1.22,8.52} = 2.133$
					$P = 0.758$	$P = 0.003$	$P = 0.181$
	Post	119 ± 35	129 ± 23	134 ± 20	ES = 0.039	ES = 0.739	ES = 0.234
MRTD ($N.s^{-1}$)	Pre	3672 ± 847	3656 ± 937	3539 ± 790	$F_{2,14} = 0.230$	$F_{1,7} = 17.855$	$F_{1.10,7.71} = 2.293$
					$P = 0.797$	$P = 0.004$	$P = 0.170$
	Post	1649 ± 706	1819 ± 507	1947 ± 267	ES = 0.032	ES = 0.718	ES = 0.247
MRR ($N.s^{-1}$)	Pre	2701 ± 1067	2606 ± 971	2495 ± 929	$F_{2,14} = 0.258$	$F_{1,7} = 14.567$	$F_{2,14} = 1.484$
					$P = 0.776$	$P = 0.007$	$P = 0.260$
	Post	1032 ± 34	1141 ± 396	1100 ± 226	ES = 0.035	ES = 0.675	ES = 0.175
0.5 RT (ms)	Pre	68 ± 17	66 ± 19	68 ± 16	$F_{2,14} = 0.532$	$F_{1,7} = 5.748$	$F_{2,14} = 0.440$
					$P = 0.599$	$P = 0.048$	$P = 0.653$
	Post	95 ± 24	83 ± 40	94 ± 38	ES = 0.071	ES = 0.451	ES = 0.059

CT (ms)	Pre	83 ± 4	82 ± 4	83 ± 3	$F_{2,14}$ = 1.880 P = 0.190 ES = 0.233	$F_{1,7}$ = 5.030 P = 0.060 ES = 0.418	$F_{1.18,8.25}$ = 2.130 P = 0.183 ES = 0.233
	Post	94 ± 15	90 ± 13	85 ± 5			
Mmax (mV)	Pre	12.0 ± 3.3	10.8 ± 3.9	11.8 ± 3.8	$F_{2,14}$ = 0.524 P = 0.630 ES = 0.070	$F_{1,7}$ = 5.800 P = 0.047 ES = 0.453	$F_{2,14}$ = 0.338 P = 0.719 ES = 0.046
	Post	12.8 ± 3.8	11.9 ± 3.5	12.5 ± 3.5			

6.4.8 MEP·M_{max}^{-1}

No condition × time interaction effect was found for MEP·M_{max}^{-1} ($F_{2, 14}$ = 0.0167, P = 0.983, η_p^2 = 0.002). Similarly, there was no main effect of condition ($F_{2, 14}$ = 1.126, P = 0.352, η_p^2 = 0.139) or main effect of time ($F_{1, 7}$ = 2.249, P = 0.177, η_p^2 = 0.243).

6.4.9 TMS Silent Period

There was no condition × time interaction for the TMS silent period ($F_{2, 14}$ = 3.304, P = 0.067, η_p^2 = 0.321). Similarly, no condition ($F_{2, 14}$ = 1.093, P = 0.362, η_p^2 = 0.135) or time effect was observed ($F_{1, 7}$ = 0.339, P = 0.578, η_p^2 = 0.046).

6.4.10 Electromyography

MVC EMG Amplitude. A one-way repeated measure ANOVA for the normalized amplitude of the post-exercise MVC revealed no main effect of condition for the VL ($F_{2, 14}$ = 1.41, P = 0.276, η_p^2 = 0.168), VM ($F_{2, 14}$ = 1.47, P = 0.264, η_p^2 = 0.173) or RF ($F_{2, 14}$ = 0.508, P = 0.613, η_p^2 = 0.068).

6.4.11 Cardiorespiratory Measures. Oxygen uptake ($\dot{V}O_2$)

No condition × time interaction was found for $\dot{V}O_2$ ($F_{8, 56}$ = 0.791, P = 0.613, η_p^2 = 0.102) or main effect of condition ($F_{2, 14}$ = 1.093, P = 0.362, η_p^2 = 0.135). There was a main effect of time ($F_{4, 28}$ = 115.623, P < 0.001, η_p^2 = 0.943). Post-hoc tests revealed that $\dot{V}O_2$ increased from minute 1 to 2 (mean difference = 1.31 L.min^{-1}, t = 13.52, P < 0.001) and from minute 2 to minute 3 (mean difference = 0.25 L.min^{-1}, t = 2.58, P = 0.046), and then further increased from minute 3 to minute 5 (mean difference = 0.28 L.min^{-1}, t = 2.84, P = 0.033; figure 6.5A).

Minute Ventilation ($\dot{V}e$). There was no interaction effect observed for $\dot{V}e$ ($F_{8, 56}$ = 0.362, P = 0.936, η_p^2 = 0.049) or main effect of condition ($F_{2, 14}$ = 1.176, P = 0.337, η_p^2 = 0.144) but there was a time effect ($F_{4, 28}$ = 132.655, P < 0.001, η_p^2 = 0.950). $\dot{V}e$ increased during every minute during the exercise from the previous minute (all P < 0.05; figure 6.5B).

Tidal Volume (vT). There was no condition × time interaction ($F_{8, 56}$ = 0.811, P = 0.596, η_p^2 = 0.104) or a main effect of condition ($F_{2, 14}$ = 2.081, P = 0.162, η_p^2 = 0.229). There was a main effect of time ($F_{4, 28}$ = 34.312, P < 0.001, η_p^2 = 0.831). Follow up post-hoc tests revealed that vT only increased from minute 1 to minute 2 (mean difference = 0.79 L, t = 7.81, P < 0.001) but did not change any further throughout the rest of the exercise (all P > 0.05; figure 6.5C).

Breathing Frequency (f_R). No interaction effect was observed for f_R ($F_{8, 56}$ = 0.794, P = 0.610, η_p^2 = 0.102) or main effect of condition ($F_{2, 14}$ = 1.257, P = 0.315, η_p^2 = 0.152). A time effect was observed whereby f_R increased at each timepoint throughout the exercise (all P < 0.05; figure 6.5D).

$PETCO_2$. A condition × time interaction was observed for $PETCO_2$ ($F_{8, 56}$ = 2.328, P = 0.031, η_p^2 = 0.250). Subsequent post-hoc tests revealed no differences between conditions at any timepoint (all P > 0.05) however in CTRL and ISO, $PETCO_2$ increased from minute 1 to 2 and then decreased at each time-point thereafter (all P < 0.05). In HYP, PETCO2 remained unchanged throughout exercise (all P > 0.05; figure 6.5E).

Heart Rate (HR). Due to signal loss of one participant, only n = 7 could be used for analysis. There was no condition × time interaction for HR ($F_{8, 56}$ = 0.925, P = 0.504, η_p^2 = 0.134), however there was a main effect of condition ($F_{2, 14}$ = 8.943, P = 0.004, η_p^2 = 0.598) and a main effect of time ($F_{4, 28}$ = 52.195, P < 0.001, η_p^2 = 0.897). Heart rate was greater in CTRL (mean difference = 4 $B.Min^{-1}$, t = 2.86, P = 0.029) and ISO (mean difference = 6 $B.Min^{-1}$, t = 4.13, P = 0.004) compared to HYP but was not different between CTRL and ISO (P = 0.230; see figure 6.5F).

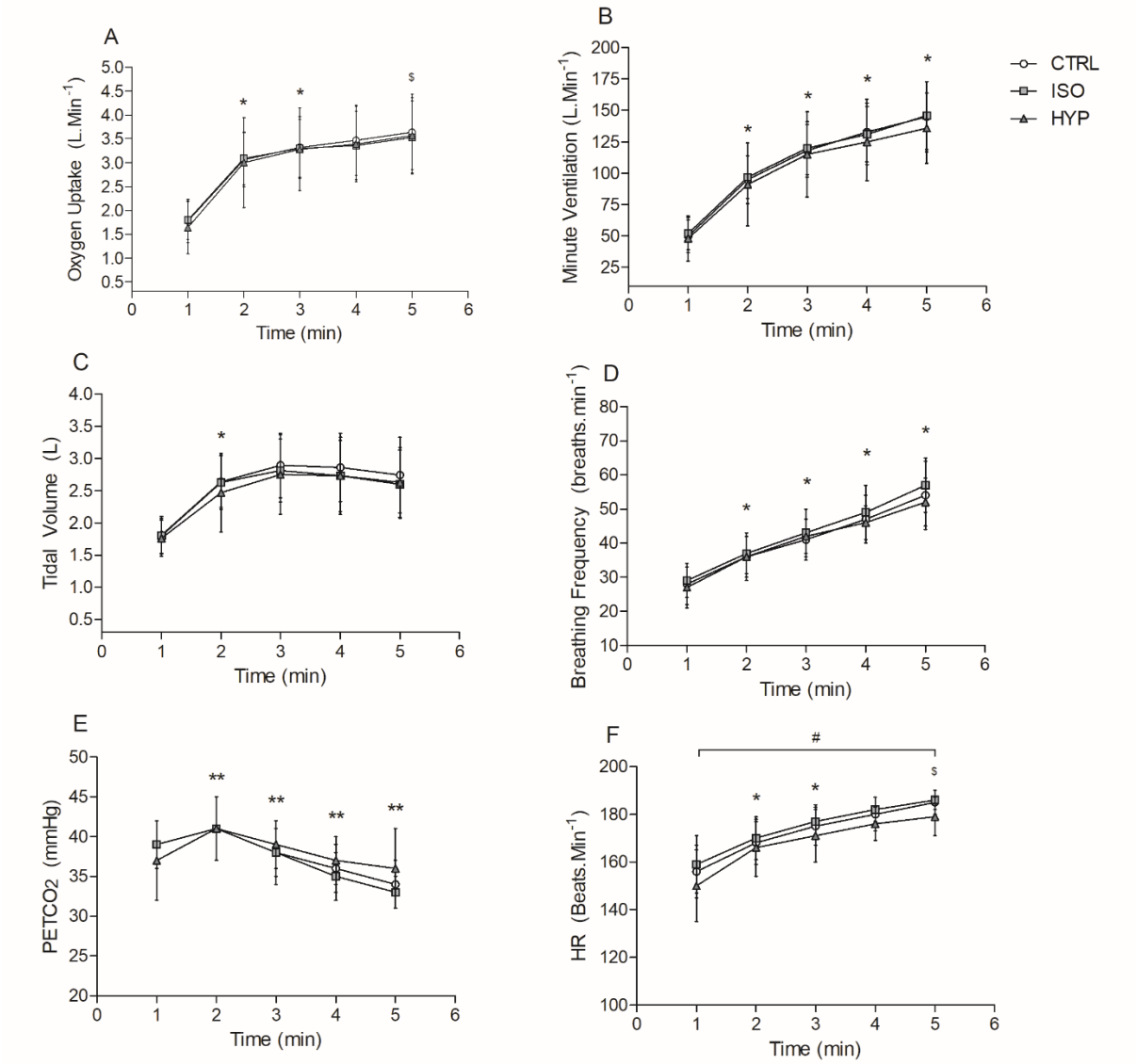

Figure 6.5. Cardiorespiratory variables for each condition during the 5-minute time-trial. A. Oxygen uptake absolute values. B. Minute ventilation. C. Tidal Volume. D. Breathing frequency. E. Partial pressure of end tidal CO_2. E. Heart Rate. * Denotes significantly different from previous time point ($P < 0.05$). $ denotes significantly different from minute 3 ($P < 0.05$). ** denotes significantly different from previous timepoint for CTRL and ISO (interaction effect). # Denotes main effect of condition.

6.5 Discussion

The main findings of the present study were that acute muscle pain did not significantly impair short duration cycling TT performance, however the pacing strategy shifted from a typical parabolic profile in CTRL/ISO to a more even profile in HYP (figure 6.2C). Additionally, elevated muscle pain did not alter the developments of neuromuscular fatigue or corticospinal responses. However, muscle pain, appeared to increase the amount of pain catastrophising.

Only one previous study has investigated the effects of increased pain on TT performance. Astokorki and colleagues (Astokorki, Flood and Mauger, 2021) found that 10 mile cycling time-TT was impaired by 3.8% when EIP was increased from viewing images depicting pain (via compassional hyperalgesia) when compared to neutral images. While these findings contrast with this study, the exercise protocol of the Astokorki study is of longer duration where elevated pain may become increasingly significant for exercise performance. Furthermore, the exacerbation of pain was not experimentally induced which may have affected the psychological interpretation of the pain in comparison to the hypertonic saline injections in this study. Conversely, when pain is experimentally reduced with paracetamol, a meta-analysis by Grgic and colleagues (Grgic and Mikulic, 2021) found no difference in time-trial performance which is in agreement with this study, supporting the notion that altered pain perception may not be a major factor for the successful performance of self-paced, high intensity exercise.

Whilst the weight of the evidence seems to suggest that pain may not impact self-paced exercise performance, there is limited agreement in the findings, perhaps an indication that the negative effect of pain on endurance performance is circumstantial. Indeed, multiple studies have displayed evidence that reducing pain improves self-paced exercise performance (Mauger, Jones and Williams 2010; Astokorki and Mauger 2017; Holgado *et al.* 2018) and elevating pain reduces time to task failure (Graven-Nielsen, Svensson and Arendt-Nielsen, 1997; Ciubotariu, Arendt-Nielsen and Graven-Nielsen, 2004; Smith *et al.*, 2020). Divergent effects of pain may in-part be (but not limited) to differences in the demands of the individual exercise task such self-paced versus fixed power (Grgic and Mikulic, 2021), high versus low intensity and duration (Martinez-Valdes *et al.* 2020) and isolated, isometric versus dynamic, whole-body exercise. Future work is required to investigate a wider array of exercise tasks which differ in these aforementioned characteristics to better understand the extent pain can limit endurance performance.

6.5.1 Neuromuscular Effects of Pain on Self-Paced Exercise

Emerging evidence has shown that pain does not exert a uniform inhibitory effect on muscle function, but instead both excitatory and inhibitory processes occur simultaneously within the neuromuscular system to allow for the maintenance of the desired task whilst minimising pain (Hodges and Tucker, 2011). Experimental muscle pain has been shown to reduce the firing frequency of low threshold motor units at low forces but also decrease the recruitment threshold of higher threshold motor units at higher force contractions (Martinez-Valdes *et al.* 2020).

Similarly, discharge rates during low velocity contractions were reduced in response to pain but were maintained during higher velocity contractions (Martinez-Valdes *et al.*, 2021). These findings may partially explain why TT performance was unchanged in response to pain in our study. The participants in this study were able to maintain the required muscle forces (i.e., power output) in HYP to achieve similar TT performance to that in CTRL and ISO. However, a caveat of the altered motor unit properties in response to pain is that there could be an increase in fatigability of the painful muscle due to the higher proportion of fatigable, higher threshold muscle fibres (Stephens and Usherwood, 1977)**.** While this was not observed in the present study, as the same amount of neuromuscular fatigue developed between conditions (see figure 6.4), it might be that once pain from the saline dissipated near the end of the TT, the function of lower threshold motor units was restored to their previous non-painful function. Whether these findings would remain if the pain was kept consistent during the TT remains unknown, but we hypothesise that a noticeable impairment to TT performance would be observed if that was the case.

Altered synergist (i.e., VM and RF) and agonist (i.e., hamstrings, gastrocnemius) muscle activation patterns may be able to compensate for the pain mediated inhibition in the VL to maintain performance. However, no differences in the change in EMG amplitude of the post-exercise MVC for the individual quadricep muscles does not support this notion. Furthermore, experimental work by Hodges and colleagues (Hodges, Ervilha and Graven-Nielsen, 2008) did not find adjustments to firing rates of plantarflexion synergists during experimental pain of the gastrocnemius.

6.5.2 TMS Responses

There was no difference between conditions in $MEP \cdot M_{max}^{-1}$ or the TMS silent period. Muscle pain has been shown to either cause a decrease or have no effect on MEP amplitude (Le Pera *et al.*, 2001; Martin *et al.*, 2008; Schabrun, Burns and Hodges, 2015; Burns, Chipchase and Schabrun, 2016; Sanderson *et al.*, 2021). However, the findings of these studies were measured during rest with the presence of experimental pain. Our measure of corticospinal excitability was measured post-exercise to quantify corticospinal changes in response to painful and fatiguing exercise. As pain intensity and fatigue at the end of exercise were similar between all conditions, it is perhaps unsurprising that there were no differences in $MEP \cdot M_{max}^{-1}$ between conditions. However, MEP amplitude has shown differing changes after fatiguing protocols (Weavil and Amann, 2018) due to differences in TMS methodology. Nevertheless, a

combination of excitatory and inhibitory factors seem to result in no net change in the MEP amplitude (Weavil and Amann, 2018). Similarly, there was no differences in the TMS silent period between conditions and from pre to post exercise which again could be explained by the previous points discussed. It seems that if changes in the corticospinal pathway are present, they are likely only detectable in the presence of strong muscle pain, as was seen in chapter four.

6.5.3 Psycho-Physiological Effects of Pain

One key consideration about the experimental pain is that all participants were previously familiarised with a bilateral injection of hypertonic saline and therefore were aware of the typical intensity, quality, and duration of the experimental pain. Because of this, participants knew that the pain sensation in the legs (from the saline) would only be transient and was not a part of the naturally occurring EIP which is used as sensory feedback to inform the individual of the physiological state of the exercising muscle (Mauger, 2014). Because of this, participants may have disregarded the experimental pain as a form of sensory feedback to inform pacing and this might partly explain why endurance performance was unaltered. Nevertheless, the inhibitory nociceptive activity in HYP should theoretically have contributed to a more rapid attainment of a sensory tolerance limit and exercise intensity, regardless of the origin of pain. One potential explanation to this would be that the pain from the both the exercise *and* saline needs to reach intensities that are high enough to be perceived as intolerable. In this case then participants would be unable to adapt to the pain and see a reduction in exercise intensity This is somewhat supported within the data as the correlation analysis showed a relationship between the change in power within the first minute of exercise between ISO and HYP and average pain in the first minute of exercise in HYP ($r = 0.719$). Those participants who tended to report very strong pain (e.g., > 70/100) in the early stages of exercise from the saline appeared to also have reduced power outputs in the initial parts of the time trial. Those who had moderate to strong pain intensities appeared to be able to tolerate pain and adapt to the pain to maintain performance. In the scenario of TTF exercise where elevated pain has been shown to reduce endurance, the work rate cannot be adapted with pain, thus a reduction in TTF occurs. It should be acknowledged that while a correlation revealed a significant relationship, this phenomenon is probably on a threshold basis. That is, an individual will have a specific (yet dynamic) pain tolerance which is modified by factors such as end-point proximity, motivation, previous experience etc.

Whilst for some participants their performance was not affected by pain, recent research has shown that muscle pain can impair accurate estimate of force production (Smith *et al.*, 2021). This could theoretically impair self-paced exercise performance as the adopted pacing strategy in HYP could be altered from the participant's template of their 'optimal' strategy by causing an over or under shoot in force production. The shift in overall pacing strategy (figure 6.2C) showed that in CTRL and ISO participants exhibited a typical parabolic pacing profile. Whereas in HYP, the pacing profile became more even throughout the TT. This more balanced approach in HYP could be also related to the inability to accurately reproduce desired forces and therefore a flatter profile remained more viable for a successful endurance performance in HYP. While this is plausible, the findings by Smith and colleagues were in an isometric model of exercise and do not necessarily reflect the ability to select power outputs during whole-body dynamic exercise. Exercise-induced pain is one of multiple sensory feedback tools that can be used to regulate pace (Mauger, 2014; Whitehead *et al.*, 2017) and participants in this study could potentially gauge their exercise intensity on other perceptions such as effort, fatigue and respiratory exertion. Indeed, RPE increased over time during exercise but was not different between conditions. Values for effort increased linearly and reached near maximum levels by the end of the exercise (18 - 20) for all participants. This demonstrates that participants were able to accurately gauge their effort to drive the limb and supports our previous point that this could have been used as feedback to regulate pace.

6.5.4 Cardiorespiratory responses

There was no difference between conditions for any respiratory variable (see figure 6.5.) and because power output is closely related to oxygen consumption (Abrantes *et al.*, 2012), this was expected as there was no significant difference in power output between conditions. Group III/IV afferents are important for the cardiovascular and ventilatory control of exercise (Amann *et al.*, 2010; Bruce and White, 2012), but whilst hypertonic saline injections stimulate group III/IV afferent nociceptors, they did not appear to contribute to an elevated cardiovascular and respiratory response (and therefore change in exercise performance) in the current study. Interestingly, there was a main effect of condition for heart rate whereby heart rate was lower in HYP compared to CTRL and ISO (figure 6.5). It is unclear as to why this occurred as similar power outputs were observed between conditions. It would have been expected that the heart rate would have been greater in HYP given the possibility of additional stimulation of group III/IV afferent feedback from the saline or due to anxiety to the initial pain. Nevertheless, this

further supports the notion that additional nociceptive afferent feedback did not alter the cardiovascular response to exercise and did not confound exercise performance.

6.5.5 Methodological Considerations

One major consideration of the study design was that the time-trial was only five minutes in duration. This was to ensure that there was an elevated pain response in HYP compared to CTRL and ISO for the majority of the exercise duration because pain from 1 mL of hypertonic saline (5.85%) has been shown to last for a mean ± SD duration of 4.68 ± 1.4 minutes (Smith *et al.*, 2020). Indeed, we saw elevated pain in HYP compared to CTRL and ISO for most of the exercise, but this may limit the translation of the findings to longer duration exercise or results may have differed if a consistent level of pain was induced for the whole TT.

Neuromuscular function assessments were conducted rapidly post exercise with the mean ± SD time to the performance of the post-exercise MVC being 33 ± 5 s. However, even this small delay may mean that the magnitude of the post-exercise neuromuscular fatigue may be an underestimation. This is particularly the case for TMS measures which were conducted after the MVC and may be in part as to why no condition or time effect were observed. Furthermore, these changes in post-exercise measures of fatigue between conditions were well within the standard error of measurements observed in chapter three, indicating no change in the magnitude of fatigue between conditions. Nevertheless, the time for assessment of post-exercise fatigue was consistent between conditions (CTRL = 35 ± 7 s, ISO = 34 ± 3 s, HYP = 32 ± 3 s) and could only have been conducted faster with specialist equipment (see Doyle-Baker et al. 2018).

Finally, a more homogenous group of participants may have altered findings of the present study as trained and untrained individuals may respond differently to pain during exercise. Those who are well trained in endurance exercise typically have better pain tolerance (Tesarz *et al.*, 2012; O'Leary *et al.*, 2017) and better coping strategies to combat EIP (Kress and Statler, 2007; Buman *et al.*, 2008). Unfortunately, recruiting such a group of participants was not possible given the logistical and contextual constraints at the time of data collection.

6.5.6 Conclusion

In summary, elevated muscle pain from the bilateral injection of hypertonic saline appears to have little impact on 5-minute cycling TT performance. Participants appear to be able to maintain power output even in the presence of strong (but tolerable) muscle pain. This is accompanied with no discernible changes in neuromuscular fatigue between conditions. Future

research should explore the impact of muscle pain on longer duration TTs and investigate measures of neuromuscular fatigue during the exercise bout.

Chapter 7: General Discussion

7.1 Summary

The purpose of this thesis was to investigate the effect of muscle pain on endurance performance and the development neuromuscular fatigue as well as corticospinal responses. Specifically, the aim was to explore whether there is a link between muscle pain and the development of neuromuscular fatigue, whereby muscle pain could directly cause and/or facilitate fatigue. Therefore, the aims of the study were as follows:

1. To establish whether measures of neuromuscular fatigue are reliable.
2. To measure neurophysiological responses to muscle pain.
3. To assess endurance performance in the presence of elevated muscle pain.

The first experimental study of this thesis (chapter three) identified whether commonly used indices of neuromuscular fatigue and isometric endurance performance were sufficiently reliable on a session-to-session basis. The findings were that maximum voluntary force (global fatigue), voluntary activation (central fatigue) and peripheral nerve stimulation doublet amplitude (peripheral fatigue) were all sufficiently reliable (i.e., < 10% coefficient of variation) in both fresh and fatigued conditions. Additionally, an isometric endurance task which was set at an intensity to cause task failure within 4 to 6 minutes also displayed good reliability. Taken together, the use of an isometric endurance task with measures of neuromuscular fatigue were appropriate for the investigation of the pain- fatigue relationship.

The second study (chapter five) aimed to elucidate the mechanisms of how muscle pain can impact endurance performance. Whilst numerous papers have demonstrated a performance-reducing effect of muscle pain (Graven-Nielsen, Svensson and Arendt-Nielsen, 1997; Ciubotariu, Arendt-Nielsen and Graven-Nielsen, 2004; Smith *et al.*, 2020), there was a lack of studies utilising neurophysiological measures to explain why performance was reduced. We found that isometric time-to-task failure of the knee extensors was reduced by 16.2% in the presence of elevated muscle pain which was induced by the intramuscular injection of hypertonic saline into the VL. This was accompanied by an exacerbation of central fatigue (i.e., reduced voluntary activation) and decline in maximal voluntary force whilst peripheral fatigue was similar to the control condition (non-painful injection). Furthermore, during the exercise, measures of corticospinal inhibition measured from single pulse transcranial magnetic

stimulation were increased in the presence of pain, further providing evidence of a centrally mediated effect. Therefore, this was the first study to directly show that pain reduces voluntary activation and increases corticospinal inhibition during exercise.

Because a central effect of pain was observed in chapter four, we hypothesised that pain which was present in a remote, non-exercising muscle could also reduce performance of an exercising muscle (chapter five). Therefore, to investigate this we adopted a similar study design to the previous study but injected hypertonic saline into the left VL and required participants to perform the isometric knee extensor time to task failure in the right leg. Interestingly, there was a 9.8% reduction in time to task failure in the presence of non-local pain. This was accompanied by an increase in VL EMG amplitude and a decrement in voluntary activation. However, no differences were seen between conditions in TMS responses or maximal voluntary force. Nevertheless, this study provided compelling evidence that non-local pain can also limit endurance performance.

Whilst a clear detrimental effect of pain on endurance performance has been observed in isometric time to task failure tests, the effect of increasing pain on whole body self-paced exercise performance was unknown. The final study of this thesis (chapter six) investigated the effect of a bilateral hypertonic saline injection on short duration cycling-time trial performance. Elevated pain resulted in no consistent reduction in 5-minute cycling time-trial performance. However, due to a variable pain response to the saline, it is possible that those who experienced greater levels of pain from the saline reached an intolerable experience of pain early in the exercise which resulted them having to reduce their power output to reduce perceived pain levels. It also appeared that the presence of greater pain caused participants to adopt a different pacing strategy. Neuromuscular fatigue was unaffected by the pain with whole body exercise. Taken together, these four studies have significantly increased the understanding of how pain may impact endurance performance.

7.2 Reliability of Measures of Neuromuscular Fatigue

To be able to draw conclusions about the mechanistic underpinning of the fatigue-pain relationship, this thesis used a variety of techniques including isometric dynamometry, surface EMG, peripheral nerve stimulation and transcranial magnetic stimulation. These measurement techniques need to display sufficient reliability to allow for the correct interpretation of the data collected within the experimental chapters. If a measurement technique shows inadequate reliability through large test-retest variations within a participant, then the effect of pain on

these various neuromuscular measurements become undetectable which can result in type two errors.

Determining the between-session reliability was important because the studies conducted in chapters four to six in this included more than one experimental visit on separate days. Furthermore, whilst the reliability of measures of neuromuscular fatigue have been extensively investigated in fresh/rested conditions (Allen, Gandevia and McKenzie, 1995; Clark, Cook and Ploutz-Snyder, 2007; Place *et al.*, 2007; Behrens *et al.*, 2017), the data on their reliability in the presence of exercise-induced fatigue was scant. This is an important consideration because neuromuscular measures are frequently measured from pre to post exercise and whilst the measures at baseline may be reliable, it is unclear how changes to the muscle, nervous system, and participant (e.g., motivation, attention, and arousal) can affect reliability. Furthermore, small day-to-day variations in muscle strength and/or endurance capacity may cause downstream variations in the associated magnitude of exercise-induced fatigue, and it is well established that day to day variations in endurance performance can be attributed to a multitude of factors such as sleep, menstrual cycle, diet and temperature (Nuzzo, Taylor and Gandevia, 2018).

Another primary aim of this study was to investigate the test-retest reliability of a modified intensity isometric TTF whereby instead of prescribing exercise as a fixed percentage of maximum isometric force, the exercise intensity was set to yield a time to task failure within the range of 4 to 6 minutes. This method of setting exercise intensity was necessary because the hypertonic saline injections planned for the subsequent series of studies typically only elevated pain for approximately five minutes. If participants were able to reach a time to task failure which exceeded the duration of the experimental pain, then the participant may be able to 'outlast' the potential negative effects of pain. Indeed, this was observed by work of Smith and colleagues (Smith *et al.*, 2020) who had six participants exceed a time to task failure of twenty minutes during an isometric contraction at 10% of maximum voluntary torque. It was found that a TTF with an intensity to cause task failure in 4 to 6 minutes had a similar intra-individual reliability (7.3% and 5.1% for TTF 4-6 minutes and TTF 20%, respectively). Unsurprisingly, inter-individual reliability was much better in TTF 4-6 mins (CV = 9.2%) compared to TTF 20% (CV = 28.8%). By improving the inter-individual reliability of the time to task failure between individuals and causing similar time to task failures across participants, each participant should be within a similar part of their torque-duration relationship (Burnley,

Vanhatalo and Jones, 2012). However, this did not result in noticeably more reliable measures of neuromuscular fatigue in comparison to TTF 20%. Nevertheless, most measures of fatigue displayed sufficient reliability post-exercise (CV < 10%). Only measures of EMG (i.e., RMS amplitude and M_{max}) displayed CV values > 10% at the post-exercise time point. Therefore, the use of EMG data to infer changes in fatigue development during exercise should be interpreted with caution.

Overall, typical measures of neuromuscular fatigue displayed sufficient reliability in the presence of exercise-induced fatigue. In the studies within chapters four and five, measures of neuromuscular fatigue were measured at each minute, but we did not quantify the reliability at these time points in chapter 3. However, we speculate that reliability would be no worse than that observed at the post-exercise time-point as less exercise-induced fatigue would have occurred. This reliability study provided methodological rigour for the subsequent studies in this thesis which used these measurement techniques to investigate the fatigue-pain relationship.

7.3 The Effect of Elevated Pain on The Development of Neuromuscular Fatigue

Chapters four to six in this thesis explored how pain influences (or causes) the development of fatigue. Previous research in this area has used measures of neuromuscular fatigue in response to muscle pain to better understand the mechanisms of how muscle pain can cause fatigue. However, there are few studies which assess the effect of muscle pain on a key locomotor muscle (e.g., the quadriceps). This is especially true during exercise, as multiple studies only investigated measures of fatigue in resting painful conditions with a greater focus on pain in clinical contexts. It is not understood how pain can interact with exercise-induced fatigue to influence its development. This is important because in both healthy individuals and clinical populations, naturally occurring muscle pain from exercise is a significant part of exercise (Valkeinen *et al.*, 2006).

7.3.1 Global fatigue

Global fatigue which was measured as a decrease in maximum voluntary force and/or a decrease in the rate of force development can be considered as one the primary outcomes of interest as this is of the more tangible consequences of muscle pain.

In chapter four, there was considerable evidence of a decrease in the maximal force generating capacity of the knee extensors in response to pain. When muscle pain in the VL was induced by hypertonic saline injections, there was a significant reduction in maximum voluntary force

at minutes 1 and 2 during exercise compared to when isotonic saline was injected. This is in agreement with other studies which has consistently found a decrease in maximal strength in response to experimental muscle pain (Graven-Nielsen, Arendt-Nielsen, *et al.*, 1997; Graven-Nielsen *et al.*, 2002; Slater *et al.*, 2003; Khan *et al.*, 2011). This also occurs even when pain is induced in other areas such as the infrapatellar fat pad, (Henriksen *et al.*, 2011; Rice *et al.*, 2019), subacromial space (Stackhouse *et al.*, 2013) and iliotibial tract (Oda *et al.*, 2018). Stark differences in the magnitude of force decrease are apparent in the literature with small to large decreases observed (5-44%). We found a strong correlation in the difference between MVF between CTRL and HYP at minute 1 and the change in mean pain intensity at minute 1 between CTRL and HYP. In other words, the greater the pain intensity an individual perceived within that first minute of exercise, the greater the decrease in their maximum voluntary force. Therefore, the magnitude of perceived pain can at least partially explain some of the differing magnitudes in force decrement. Interestingly, a similar correlation was seen by Farina and colleagues (Farina *et al.*, 2004) with pain intensity and impairment to motor unit firing rate (a key determinant of force production), which further supports a dose response effect of pain on global fatigue and provides insight into the potential mechanisms for this reduction in force. It is likely that the reduction in the ability to produce maximum forces are partly caused by inhibition to firing rates of low threshold motor units, as HD-EMG work has demonstrated this (Martinez-Valdes *et al.* 2020). To observe a correlation in *both* maximum voluntary force and motor unit firing rates strengthens the notion that the magnitude of pain influences the magnitude of force decrement. It is difficult to ascertain whether it is the conscious perception of pain or the associated nociceptive activity that is responsible for these changes. Unfortunately, it is currently not possible to directly measure afferent feedback in vivo and pain intensity is not always associated with the magnitude of nociception which further complicates the matter. One way to determine this could be by experimentally reducing the perception of pain (through a psychological intervention) whilst keeping the magnitude of the nociception intact, but this is yet to be performed.

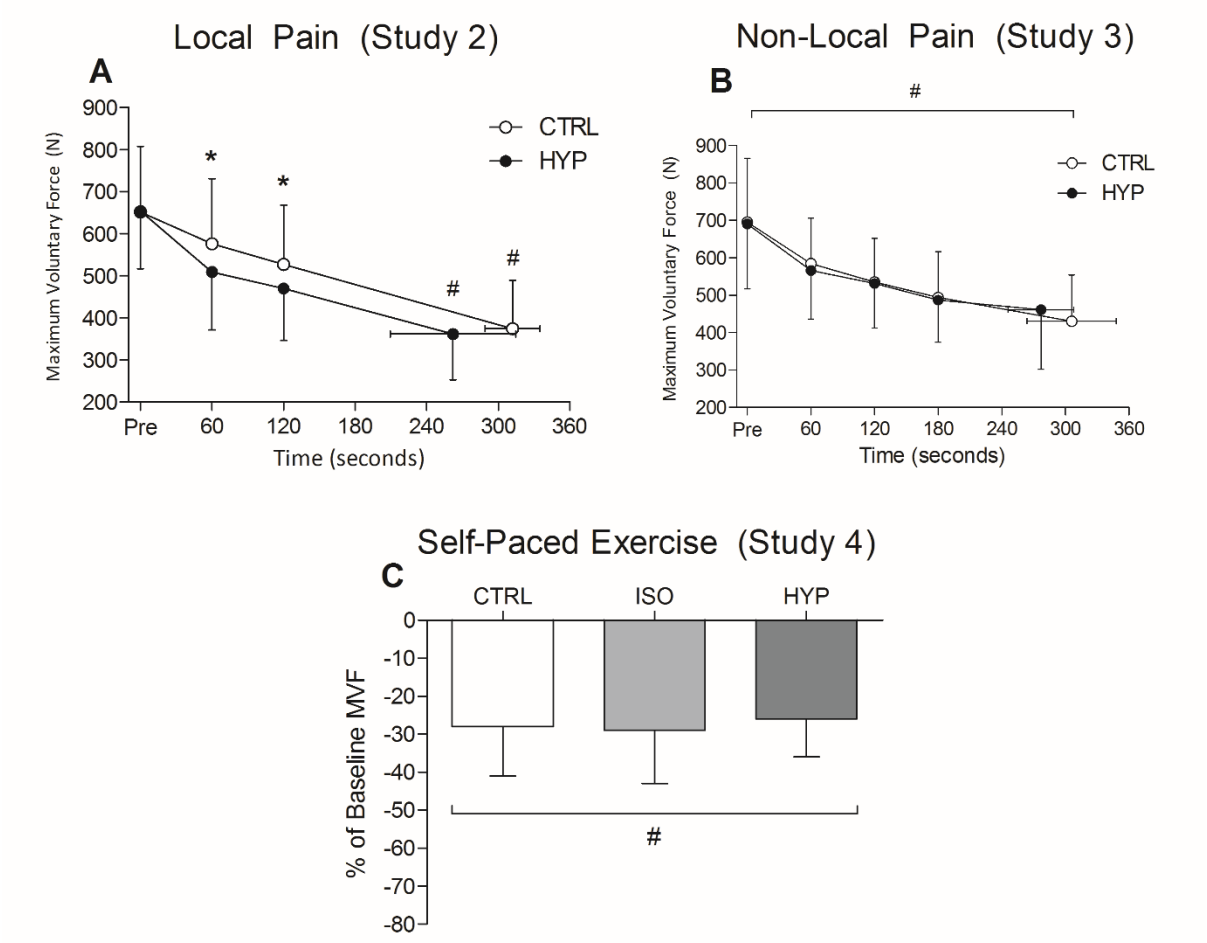

Figure 7.1. Global fatigue in each study measured as the changes in maximum voluntary force between conditions for experimental studies two, three and four. * Denotes condition effect. # Denotes times effect.

7.3.2 Central Fatigue

Central fatigue was quantified as the decline in voluntary activation measured with the ITT (Shield and Zhou, 2004). When participants performed maximal voluntary contractions, the superimposition of PNS at peak force briefly stimulates any of the remaining, inactive motor units, resulting in an additional increment of force. The amplitude of this superimposed twitch force relative to the resting stimulation delivered shortly after the MVC provided valuable insight into the development of central fatigue.

In chapters four and five, central fatigue development was tracked during the isometric TTF by performing the ITT during baseline, every minute of exercise and at task failure. This way, the kinetics of central fatigue development could be assessed during exercise in the presence of increased pain which up until present, had not been investigated. In chapter four, during localised muscle pain, there was significant evidence of an exacerbation of central fatigue,

particularly within the earlier parts of the exercise when muscle pain was much greater in HYP compared to CTRL. Furthermore, the intensity of muscle pain was correlated with the decrease in voluntary activation within the first minute of exercise, indicating that pain can act in a dose-response relationship to cause central fatigue. This is the first study to show that experimental muscle pain can impair voluntary activation as the only other research has which has investigated this has found that hypertonic saline impaired voluntary activation after pain had returned to baseline levels, but not during pain (Khan *et al.*, 2011). Similar work investigating knee pain caused by hypertonic saline, has found evidence of exacerbated central fatigue (Park and Hopkins 2013; Salomoni *et al.* 2016). Therefore, it seems that pain from multiple tissues (i.e., muscle, joints, and connective tissue) can impair voluntary activation. Whether these reductions occur through a similar physiological pathway to muscle pain is unclear, but evidence suggests different receptors converge into common ascending pathways (Schaible, Schmidt and Willis, 1987). Within muscle tissue, type III/IV nociceptors are stimulated in the presence of hypertonic saline which relays inhibitory feedback to the central nervous system to prevent or reduce further pain and/or damage to the affected tissues. As a result, there is an inhibition to the recruitment and firing rate of some motor units. Consequently, an individual in pain is no longer fully able to activate the muscle.

Muscle pain can also impair the voluntary activation of muscles which were not the source of inhibitory afferent feedback (Halperin, Copithorne and Behm, 2014; Johnson *et al.*, 2015; Šambaher, Aboodarda and Behm, 2016; Aboodarda *et al.*, 2017; Laginestra *et al.*, 2021). In chapter five, we wanted to extend the findings of chapter four and investigate if non-local pain could exacerbate central fatigue of contralateral the knee extensors. Interestingly, there was a main effect of condition on voluntary activation observed, whereby voluntary activation was lower in HYP than in CTRL. Therefore, muscle pain can exacerbate central fatigue of muscle beyond the source of the experimental pain. The extent of this effect warrants further investigation because central fatigue was only measured in the contralateral, homologous muscles. Whether this effect extends to non-homologous muscles (e.g., quadriceps pain impairing elbow flexor activation) remains unknown. Previous work has showed that fatigue and subsequent occlusion of a distal or remote muscle can impair the voluntary activation of a resting, unfatigued muscle (Kennedy *et al.*, 2014, 2015; Finn *et al.*, 2020). The post-exercise occlusion maintained high levels of pain but did not impede blood flow to the tested muscles and therefore provides further evidence that fatigue-sensitive and nociceptive afferent feedback can exacerbate central fatigue of non-fatigued and non-painful muscles.

In chapter six, voluntary activation was assessed from pre to post exercise after a 5-minute time-trial in the presence of pain (HYP) versus an injection matched control (ISO) and no injection (CTRL). There were no differences in voluntary activation between conditions or even from pre to post exercise. No time effect is surprising as a 4 km Time-trial has been shown to induce significant decreases in voluntary activation even when measures were recorded 2 minutes post-exercise (Thomas *et al.*, 2014). In study four, measurements were recorded approximately 30 s after exercise. Perhaps an $n = 8$ within this study meant that it was difficult to detect an effect of voluntary activation for this exercise as the effect size for time effect was $\eta_p^2 = 0.392$. On top of this, shorter duration high intensity exercise is typically characterised by higher levels of peripheral fatigue whereas longer duration exercise is likely to result in more central fatigue (Thomas *et al.*, 2016). The lack of condition effect is likely explained by two factors. Firstly, the lack of difference in TT performance and secondly, the post-exercise measure of voluntary activation was recorded when muscle pain was similar between conditions. It is possible that voluntary activation was impaired during the TT but testing for this was not possible without causing significant disruption to the exercise protocol. It was expected that with elevated pain, time-trial performance would be compromised (i.e., less total distance covered) and this would have been due to an exacerbation of central fatigue. Therefore, the magnitude of peripheral fatigue would be less or equivalent in HYP, but greater levels of central fatigue would be present.

The disparate findings between TTF and self-paced exercise is intriguing but perhaps relates to the difference the ability to modulate fatigue between exercise modalities. In TTF exercise, EIP is inexorable, particularly within HYP and this may result in significant levels of central fatigue occurring. During the self-paced exercise, EIP and fatigue can be moderated by adjusting power output, cadence, or technique/muscle recruitment pattern to prevent deleterious levels of pain and fatigue occurring. Therefore, the ability to adapt to increasing pain levels within self-paced exercise may be one explanation why central fatigue was unaffected with self-paced exercise.

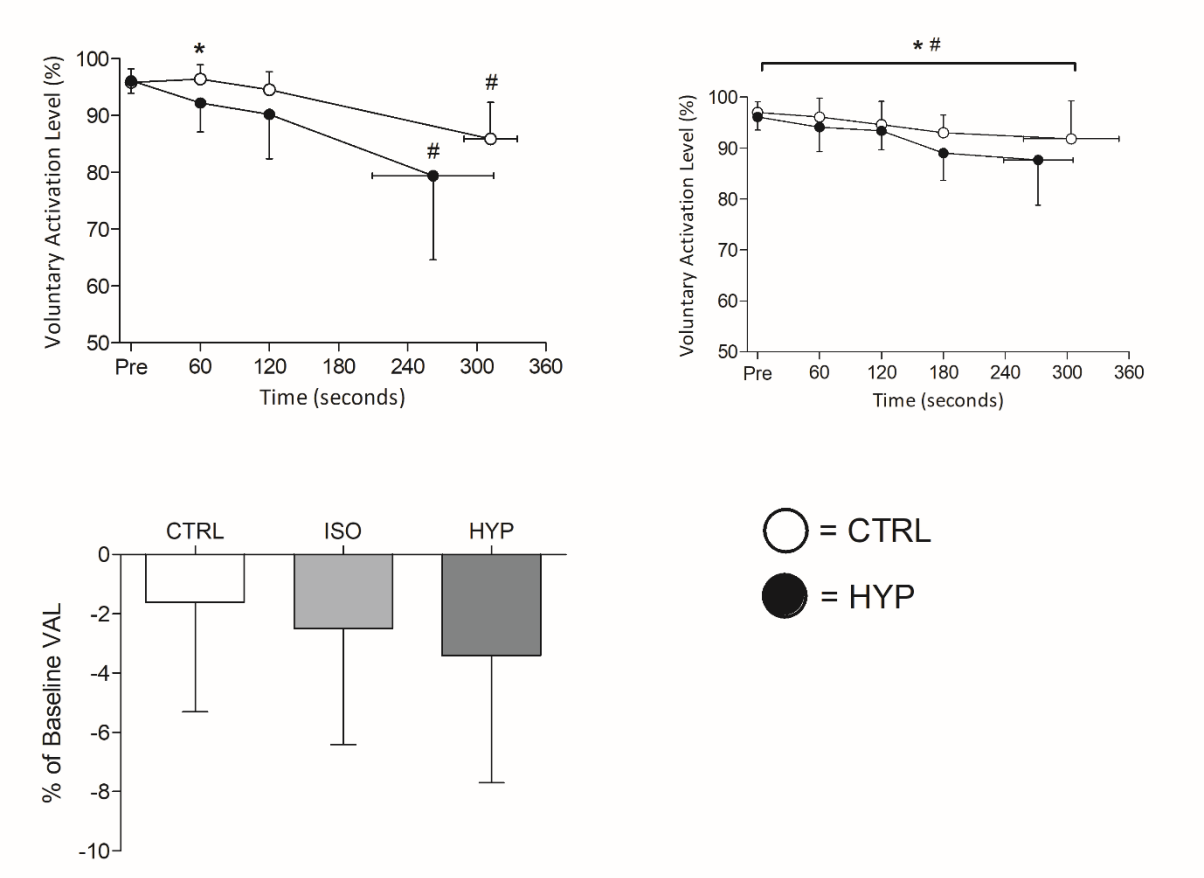

Figure 7.2. Central fatigue measures as the changes in voluntary activation between conditions for experimental studies two, three and four. * Denotes condition effect. # Denotes times effect.

Overall, the weight of the evidence presented suggests that muscle pain can cause central fatigue at least in TTF exercise as measured by the decrease in voluntary activation. Voluntary activation is not only reduced in painful muscles but also non-local muscles which share common neural pathways. This effect appears to be one of the primary mechanisms in which muscle pain reduces endurance performance. The decline in voluntary activation is most likely the cause of the reduced MVF observed in chapter four and reflects the net inhibitory effect of pain on the central nervous system. Unfortunately, the voluntary activation measure can only inform about the presence of central fatigue and cannot tell us at what specific part(s) within the central nervous system is becoming impaired (e.g., spinal vs supraspinal). Nevertheless, these findings provide the first steps in demonstrating a centrally mediated limitation to endurance performance in response to elevated muscle pain.

7.3.3 Peripheral fatigue

Peripheral fatigue refers to changes at or distal to the neuromuscular junction and was assessed by evoking twitches of the quadriceps muscle with peripheral nerve stimulation. With this

method, muscle contraction occurs without any input from the central nervous system and any changes to the properties of the evoked twitch can be attributed to altered peripheral function. Within this thesis, peripheral fatigue was primarily quantified as the reduction in the doublet twitch force amplitude from baseline.

Within chapters three to six we found significant evidence of peripheral fatigue during exercise (~25% decrease in doublet amplitude). However, there was no evidence of elevated pain impacting the development of peripheral fatigue. This was somewhat expected as there was previous but limited evidence that showed the peripheral characteristics of the muscle are preserved in response to hypertonic saline pain which included no change in conduction velocity, mean frequency or amplitude of the M-Wave (Farina, Arendt-Nielsen and Graven-Nielsen, 2005), and no change to resting twitch torque (Graven-Nielsen *et al.*, 2002). These findings were in resting conditions and means limited conclusions can be drawn about the development of peripheral fatigue during painful, fatiguing exercise. In chapter four, measurements of peripheral fatigue were recorded every minute during the isometric TTF. There were no differences in doublet amplitude at minutes one or two when the pain from the saline was at or approaching its peak which further supports the notion that the contractile function of the quadriceps was unaffected in the presence of muscle pain.

At the post-exercise time point, there was no difference in doublet amplitude between conditions in chapter four, five and six. This was somewhat unexpected for chapter four and five because a significant reduction in time to task failure is usually accompanied by a lesser end-exercise magnitude of peripheral fatigue (Amann *et al.*, 2013; Johnson *et al.*, 2015; Aboodarda *et al.*, 2020). This is because above the critical torque, the intramuscular metabolic environment becomes progressively perturbated (e.g., increase in inorganic phosphate, decrease in PCr) until task failure (Burnley *et al.*, 2010; Burnley and Jones, 2018) which is a likely contributor to peripheral fatigue (Allen, Lamb and Westerblad, 2008).

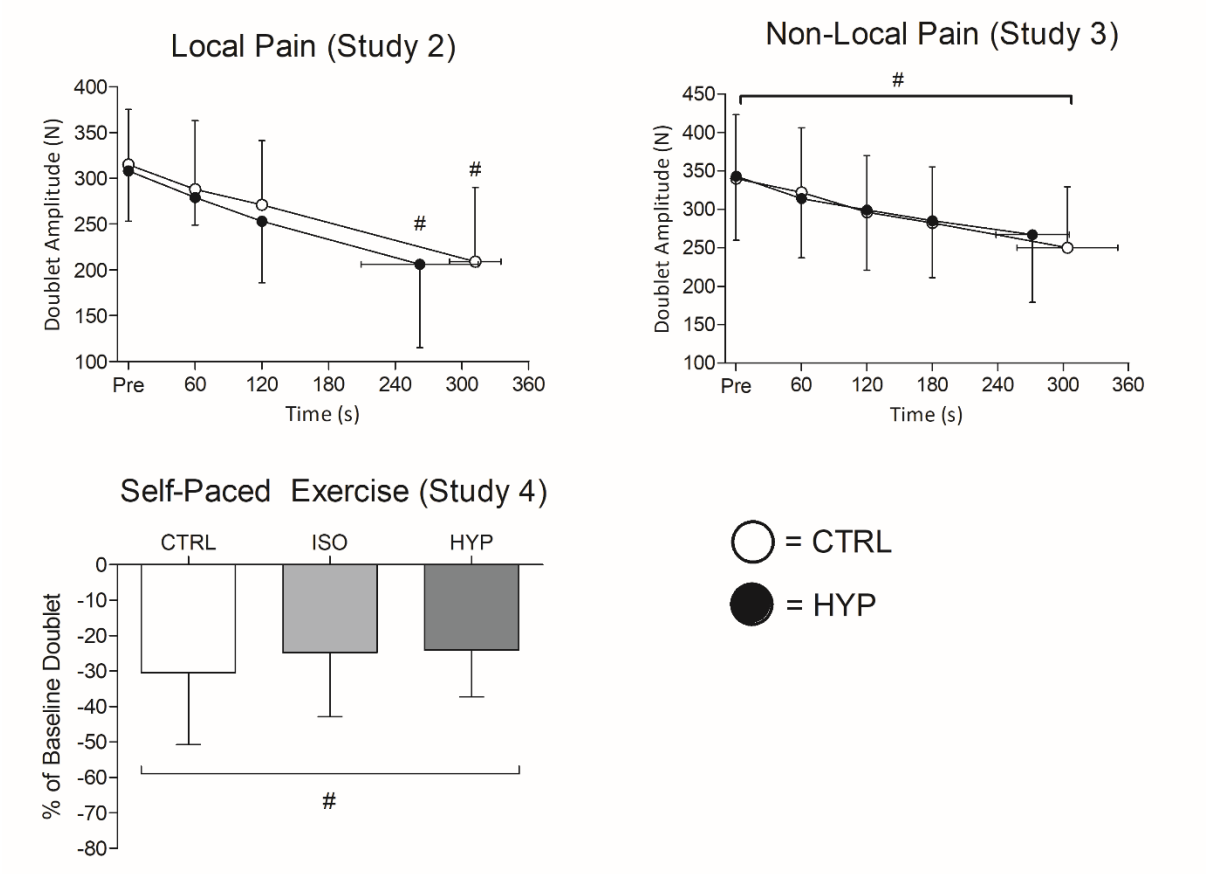

Figure 7.3. Measures of evoked doublet amplitude in experimental chapters four to six.

There are several explanations as to why we observed no differences in peripheral fatigue between conditions after exercise. Firstly, it appears that the kinetics of peripheral fatigue are not completely linear, instead, peripheral fatigue develops rapidly in the initial stages of exercise and then progressively declines until a 'critical threshold' is reached. Indeed, within chapter five, we only observed a significant decline in doublet amplitude from baseline to minute 1 and from minute 1 to minute 3. From minute 3 until task failure, there was no further decline in doublet amplitude (i.e., peripheral fatigue). Within chapter four, doublet amplitude increased at each minute but only included minutes 1, 2 and task failure with a smaller mean difference from minute 1 to 2 (17 N) than baseline to minute 1 (28 N). Furthermore, previous work has found that peripheral fatigue occurs to a greater extent during the first half of a fixed load exercise bout compared to the latter half (Decorte *et al.*, 2012). Therefore, it is plausible that large decreases in exercise duration are needed to observe a reduction in the magnitude of end exercise peripheral fatigue. Indeed, reductions in peripheral fatigue magnitude has been observed in studies where interventions incur large (> 30%) decreases in time to task failure (Amann *et al.*, 2013; Johnson *et al.*, 2015; Aboodarda *et al.*, 2020). Importantly, within the

work comparing the effect of prior fatigue versus concurrent pain by Aboodarda and colleagues (Aboodarda *et al.*, 2020), less end-exercise peripheral fatigue occurred with prior non-local fatigue (~40% reduction in TTF) but not with contralateral pain (~20% reduction in TTF).

It should not be completely discounted that the development of peripheral fatigue could be greater in the presence of pain. When pain was localised to the exercising muscle (i.e., in chapter four), the same amount of peripheral fatigue occurred in a shorter amount of time. This could be interpretated as peripheral fatigue occurring faster. Visual inspection of the kinetics of peripheral fatigue within figure 7.3 partially supports this. Mechanistically, pain causes an inhibition to the firing frequency of low threshold motor units and consequently, the recruitment of higher threshold units are required to maintain force (Tucker *et al.* 2009; Martinez-Valdes *et al.* 2020; Farina *et al.* 2004). During exercise, the deviation from the orderly recruitment of motor units (i.e., low to high threshold) and the preferential recruitment of higher threshold motor units could be responsible for increased fatigability during painful exercise (Stephens and Usherwood, 1977). However, the lack of difference in the ΔDoublet/ΔTime (CTRL = 20 ± 12 N.Min^{-1}, HYP = 23 ± 14 N.Min-1; P = 0.218) indicate that the rate of peripheral fatigue between conditions was not different. Within chapter five where the experimental pain was non-local, there was no evidence of peripheral fatigue which was expected as the hypertonic saline was non-local. Self-paced exercise within chapter six also showed no differences in any measure of peripheral function from pre to post exercise but this was unsurprising given the lack of change in distance covered between conditions.

Taken together, there is little evidence that muscle pain impacts the development of peripheral fatigue. It is unlikely that the peripheral characteristics of the muscle are impaired in resting painful conditions, but it is plausible that increased pain may cause changes to motor unit recruitment patterns which may exacerbate the development of peripheral fatigue during fatiguing exercise. Therefore, changes in exercise performance in response to elevated muscle pain are more likely to be attributed to changes to the central nervous system (i.e., mentioned in the previous section).

7.3.4 TMS Responses

The employment of transcranial magnetic stimulation within the experimental studies of this thesis allowed for the measurement of corticospinal excitability and corticospinal inhibition. The former was measured as the peak-to-peak amplitude of the motor evoked potential whilst the latter was measured as the duration of the silent period of the motor evoked potential. These

measures allowed for the elucidation of the mechanistic underpinning of pain on the central nervous system. Importantly, TMS evokes involuntary responses, thus any changes in TMS responses are likely attributable to physiological rather than psychological changes. TMS measures were recorded during the time to task failure exercise and at the post-exercise time points.

Throughout the studies within this thesis, there was no effect of pain on corticospinal excitability. Within the literature, the effect of pain on corticospinal excitability has been studied extensively but no study had examined how corticospinal excitability was altered during exercise within the painful quadriceps muscle. The findings within the literature are conflicting. This is due to the various methods employed for the assessment of corticospinal excitability including but not limited to: the individual muscle tested, type of pain, stimulator intensity and contraction intensity. Within the studies presented in this thesis, the VL muscle was investigated, as the quadriceps are a key locomotor muscle that individuals commonly experience EIP in. Because of this, TMS was performed during a submaximal contraction to facilitate excitability of the corticospinal pathway in order to evoke substantial MEPs. Additionally, TMS during isometric contractions allowed for the assessment of the silent period. It appears that the MEP amplitude remains unchanged in the presence of pain during an active muscle contraction but can decrease when tested in a resting muscle (Burns, Chipchase and Schabrun, 2016; Sanderson *et al.*, 2021). However, MEP amplitude increased within the rested knee extensors in the presence of knee pain (Rice *et al.*, 2015). During chapters four and five, corticospinal excitability was measured early on in exercise (10 s and 30 s, respectively) and later on (100 s and 90 s, respectively). TMS early in the exercise minimised the effect of exercise-induced fatigue on corticospinal excitability so the effect of pain could be studied in more isolation. There were differences in motor evoked potential amplitude which agrees with most of the literature and may suggest that the integrity of the corticospinal pathway is preserved to maintain the force target during exercise. Within chapter five, there was an increase from baseline to 30 s and then a decrease from 30 s to 90 s in MEP amplitude. This reflected a facilitation of corticospinal pathway early in the exercise due to a small increase in voluntary descending drive. Later on in the exercise, as voluntary descending drive increases substantially to maintain force requirements of the TTF, a plateau or decrease in the evoked response is typically observed (Weavil *et al.*, 2015). This is in combination with reductions in motoneuronal excitability from repetitive motor cortex activation (i.e., late spike frequency adaptation; Powers *et al.* 1999).

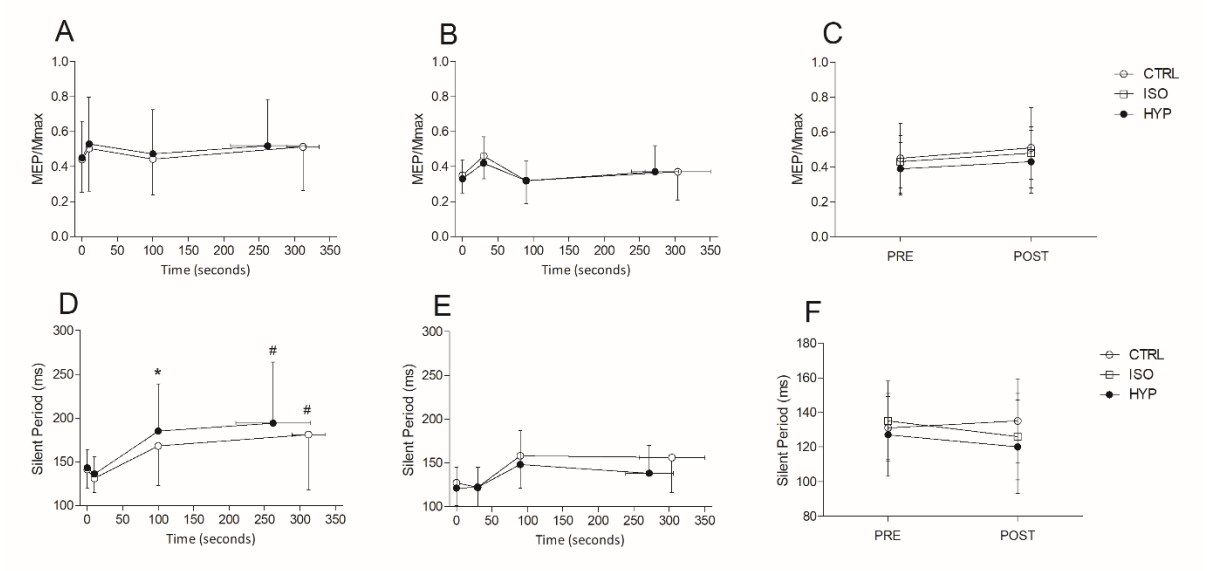

Figure 7.4. A-C. MEP amplitude in experimental chapters four to six. D-F. The TMS silent period in experimental chapters four to six. * Denotes significantly different between conditions.

Even if MEP amplitude changed in the presence of elevated muscle pain, it is unclear what functional implications this would have as recent work has found that increasing corticospinal excitability with transcranial direct current stimulation did not improve exercise performance (Kristiansen *et al.*, 2021).

Whilst corticospinal excitability does not explain the pain mediated changes in exercise performance, the amount of corticospinal inhibition was a mechanism of interest. The TMS silent period is thought to reflect corticospinal inhibition (Goodall *et al.*, 2014), with the early part attributed to spinal mechanisms and the latter part attributed to cortical mechanisms (Goodall *et al.*, 2014; Yacyshyn *et al.*, 2016; Škarabot *et al.*, 2019). In chapter four, there was a significantly longer TMS silent period in HYP compared to CTRL at only the 100 s time point. No differences between conditions were observed within chapter five, which suggests that elevated inhibition of the corticospinal pathway may only be exclusive to the painful muscle and provides novel evidence that non-local pain may have divergent neurophysiological consequences in comparison to localised muscle pain. In both chapters four and five, the TMS silent period increased during exercise which represents exercise-induced increases in corticospinal inhibition. The cause of an increase in the TMS silent period appears to be the increase in the neurotransmitter gamma-aminobutyric acid ($GABA_b$) which plays a role in reducing neuronal excitability. Whether muscle pain directly causes an increase in this neurotransmitter remains unknown. Functionally, we speculate that elevated inhibition within

the central nervous could be responsible for the inability to fully activate the muscle (e.g., reduced voluntary activation and force) through inhibition of firing rates of lower threshold motor units. Therefore, greater amounts of central motor command are needed to overcome this inhibition. Future work should investigate the effect of muscle pain on resting measures of the TMS silent period to eliminate the potential confound of exercise-induced fatigue and to better align the TMS measures with the peak pain response.

7.3.5 EMG responses

Bipolar surface EMG was recorded in all studies of this thesis to facilitate other neurophysiological measures (e.g., TMS and peripheral nerve stimulation) but also to elucidate the effect of pain on muscle activation. Indeed, surface EMG allows for the non-invasive and non-disruptive quantification of muscle activity of individual muscles during exercise. The primary focus of the EMG recordings in this thesis was to gain insight into changes in motor control strategies within the accessible quadriceps muscles VL, VM and RF. However, as observed in chapter three, reliability of the normalised EMG amplitude displayed reliability levels of CV > 10%. Therefore, the EMG findings and their interpretations outlined below should be met with caution.

EMG Amplitude During Exercise. In chapter four, there was no differences between VL or VM EMG amplitude between conditions during the first minute of the TTF when pain was approaching its peak. Unfortunately, due to measures of TMS being recorded in the latter portion of the second minute, it was not possible to extract an EMG signal for analysis at this timepoint. Nevertheless, a lack of change in the first minute of exercise between CTRL and HYP makes it unlikely that any difference was present in the second minute or beyond as many of the other neuromuscular variables were affected by pain in the first minute. However, in the VL at the point of task failure, EMG amplitude was lower in HYP compared to CTRL. The most likely explanation for this finding is because time to task failure was shorter in HYP, and participants were unable to reach as high levels of muscle recruitment than in CTRL. Indeed, visual inspection of the EMG data (figure 4.5C) reveals a strikingly similar trajectory of EMG amplitude, meaning that changes in EMG amplitude are mostly driven by changes in endurance time rather than a pain mediated alteration to muscle recruitment. It appears that the pain mediated and exercise-induced fatigue reductions to central motor drive make these higher levels of muscle activity unobtainable in HYP compared to CTRL.

In chapter five, there was a main effect of condition for submaximal EMG amplitude of the VL where the EMG amplitude was greater within HYP compared to CTRL. However, no difference between conditions was seen for the VM. The greater EMG amplitude at the same absolute time point reflects a greater muscle activation required to sustain the exercise task. This may come in the form of a higher firing frequency or the recruitment of more (and new) motor units (Christie *et al.*, 2009). Pain from the contralateral limb may have exerted inhibitory neural feedback and consequently constrained central motor drive to both the painful and knee extensors. Therefore, the recruitment of higher threshold motor units to overcome the pain mediated inhibition resulted in a larger EMG amplitude.

Multiple studies have recorded EMG of the agonist, synergist, and antagonist muscles during submaximal contractions in response to experimental muscle pain and the findings of those studies generally matched the findings within this thesis. During localised muscle pain, bipolar surface EMG has been shown to be unaffected in the presence of pain (Graven-Nielsen, Svensson and Arendt-Nielsen 1997; Birch *et al.* 2000; Farina *et al.* 2004; Smith *et al.* 2020; Martinez-Valdes *et al.* 2020). This is because pain does not have a uniform inhibitory effect on motor unit firing rates or recruitment patterns. Whilst lower threshold motor units become inhibited in response to pain, high threshold motor units or muscle fibres unaffected by the pain become recruited to compensate and maintain force. Therefore, the net balance of inhibition and excitation of various motor units result in no discernible change in the bipolar EMG amplitude. However, when pain is in the contralateral leg, there is only an increased in motor unit recruitment to overcome the pain mediated inhibition to central motor drive.

EMG Amplitude During Maximal Contractions. Within the studies of this thesis, there was conflicting findings regarding the EMG amplitude during the maximum voluntary contractions. In chapter four there was a noticeable decrease in maximal EMG amplitude during the exercise, whilst in chapters five and six, no difference between conditions was observed. The EMG amplitude during a muscle contraction appears to be closely related to the levels of force produced (Campy, Coelho and Pincivero, 2009) and therefore decreases in maximum force generating capacity are likely to be responsible for the change in EMG amplitude. With contralateral pain there appeared to be no difference in MVC EMG amplitude between conditions and this further supports the previous point as there was no difference in maximum voluntary force between conditions. Similarly, after the cycling TT in chapter six,

there was no difference between conditions for the decrease in MVC EMG amplitude which was paralleled by no difference in the decrease in MVC force.

Therefore, the electromyographic changes during elevated muscle pain are not uniform in response. It appears that EMG activity during submaximal contractions of localised muscle pain appears unchanged whereas it may increase when non-local pain is present. However, this is not consistent for all muscles. Unfortunately, bipolar surface EMG is limited in its ability to infer changes in neural control strategies. The work with experimental pain and HD-EMG during the time of this thesis by others has assisted in highlighting the divergent motor control strategies cause by muscle pain (Martinez-Valdes *et al.* 2020; Martinez-Valdes *et al.* 2021). Work employing HD-EMG techniques to further investigate the complex neural adjustments to muscle pain are the next step to elucidating the mechanisms of pain on neuromuscular fatigue.

7.4 The Effect of Pain on Endurance Performance

Another primary aspect of this thesis was to investigate how muscle pain can impact endurance performance. By implementing neurophysiological measures during and after exercise, it was possible to determine the mechanisms which would underpin any change in endurance performance. Endurance performance was assessed in exercises which primarily used the quadriceps, as this muscle group is primarily used in common endurance tasks such as running and cycling. Previous research has often tested muscles which are not heavily used for endurance tasks (e.g., tibialis anterior or hand muscles) and may contain different functional (e.g., posture, locomotion, fine motor control) and anatomical profiles (e.g., number of synergists/agonists, insertions, fibre type) which could be differently affected by pain.

7.4.1 Single limb TTF

Chapter four investigated how muscle pain impacted the performance of a single limb isometric TTF exercise of the knee extensors. The exercise intensity was set at approximately 20% but was adjusted to obtain task failure in 4-6 minutes. There was a modest reduction (16.1%) reduction in time to task failure within the HYP condition compared to CTRL.

These findings are consistent with the majority of the previous literature which has induced experimental muscle pain and assessed isometric time to task failure performance (Graven-Nielsen, Svensson and Arendt-Nielsen, 1997; Ciubotariu, Arendt-Nielsen and Graven-Nielsen, 2004; Smith *et al.*, 2020). One study by Schulte and colleagues (Schulte *et al.*, 2004) observed no difference in time to task failure but this may be explained by the investigation of a different

muscle (i.e. the biceps brachii). Activity from the trapezius muscle may have been able to compensate for any impaired function within the biceps. Furthermore, only a relatively mild intensity of pain was reported by participants (i.e., 3/10) which may not have been high enough to impair performance. Additionally, due to the transient nature of the saline, the pain could have subsided before task failure was achieved and therefore participants could have outlasted the pain.

There are a few potential mechanisms which may explain why pain causes a reduction in time to task failure. Firstly, a salient observation from the neuromuscular data is a reduction in maximum voluntary force. Consequently, participants exercising in the presence of pain are exercising at a higher relative intensity in comparison to the isotonic saline condition and given that higher exercise intensities above the critical power, this would result in shorter exercise times (Burnley, Vanhatalo and Jones, 2012). The effort ratings support this conclusion, as RPE (defined as 'effort to drive the limb') was greater in the first two minutes of exercise in HYP compared to CTRL. The concomitant decrease in voluntary activation is likely responsible for the decrease in maximal force observed during exercise. Stimulation of type III/IV nociceptors from the hypertonic saline act negatively on the central nervous system to inhibit central motor drive to the working muscle to prevent further symptoms of pain and mitigate any further perturbations from homeostasis.

In terms of the affected central motor drive to the muscle, the pain mediated-inhibition appears to primarily affect low threshold motor units by reducing their firing rates (Martinez-Valdes *et al.* 2020). The firing frequency of the higher threshold motor units appears to remain intact in the presence of pain, but they are recruited earlier. This can allow for the maintenance of high levels of force within the presence of pain, but not maximal forces as maximal force generation would require all motor units to be active and firing at their greatest frequency. As a result, not only are participants working at a greater relative intensity but the recruitment of motor units is not in its regular ordinal pattern (Tucker *et al.*, 2009). The earlier recruitment of higher threshold motor units which are more fatigable can result in the acceleration of fatigue. Therefore, pain may act through multiples mechanisms on the neuromuscular system to impair endurance performance.

The elimination of psychological factors contributing to an impaired exercise performance in the presence of elevated pain was not completely possible. Because intense pain acts as an aversive stimulus, a greater level of pain in HYP may have caused the participants to stop the

exercise task to escape the pain. Whilst this is a possibility given that task failure coincided with near maximal pain levels in both conditions, there was no difference in pain catastrophising.

Taken together, there is strong evidence that pain can limit isometric time to task failure performance. This does not definitively show that pain is a limiter to endurance exercise as time to task failure tests have often been criticised for not reflecting the demands of typical endurance events such as self-paced exercise (Marino, 2012). Furthermore, constant force, single-limb isometric exercise may have a differing aetiology of fatigue to that of whole body exercise (Place and Millet, 2019).

7.4.2 Whole body TTF

Given that isometric time-to task failure performance is reduced in the presence of elevated muscle pain, fewer studies have focused on the effects of pain on whole body time to task failure exercise. In fact, only one study has assessed the effect of elevating pain on whole body time to task failure (Canestri *et al.*, 2021). They found that 2 mL of hypertonic saline reduced cycling TTF at 80% of peak power output by 16.9%, similar in magnitude to the findings of chapter four. Therefore, it appears that the findings from this thesis are likely applicable to whole body, dynamic exercise performance.

7.4.3 Non- local Pain.

Chapter five investigated how muscle pain can impact endurance performance when the experimental pain was in the contralateral quadriceps muscles. Even when the pain was not within the exercising muscle there was still a 10% decrease in TTF. The magnitude of effect with non-local pain was less than that observed in chapter four with localised pain (16%). The neuromuscular measures indicate that the mechanisms that impair exercise performance may not be identical to those when pain is within the localised muscle. Firstly, there was no reduction in maximum voluntary force which suggests that participants were working at the same relative intensity, however voluntary activation was impaired which suggests that central fatigue may have still played a role in limiting the exercise performance. Furthermore, there was a greater EMG amplitude during exercise in the VL during pain. A greater activation in this muscle could result from the need for the recruitment of higher threshold motor units to overcome the centrally mediated inhibition caused by the contralateral pain. A physiological change is most likely to explain the reduction in endurance performance of the contralateral limb because, like localised pain, there was no difference in pain catastrophising. Furthermore,

pain in the exercising limb followed the same trajectory in both conditions whereas pain in the contralateral limb (injected leg) rapidly increased and then decreased until it was no longer different from the control condition after three minutes. If pain was causing a faster psychological disengagement from exercise during HYP then this should be caused by a greater pain intensity near the point of task failure, which was not observed for either leg.

An alternative model of pain (ischaemia) was used to investigate the effect of non-local quadriceps pain on endurance performance and there was a significant decrease in single limb cycling time to task failure compared to control (Aboodarda *et al.*, 2020). Similarly, to chapter five, there was evidence of greater amounts of central fatigue (similar decrease in voluntary activation in a shorter time) but exercise was terminated at the attainment of maximal pain levels in the contralateral leg. Therefore, differences in the kinetics of pain between chapter five and the work of Aboodarda may result in differing mechanisms of fatigue. Nevertheless, there is convincing evidence that muscle pain does not have to be exclusive to the exercising muscle for it to exacerbate neuromuscular fatigue and impair endurance performance.

7.4.4 Self-paced Exercise.

Whilst the majority of this thesis focussed on single limb isometric exercise, it was important to determine the effect of pain on the performance of whole body, self-paced exercise. No study had yet investigated the impact of elevated pain on cycling time-trial performance. We found that overall, bilateral muscle pain of the quadriceps did not reduce performance of a 5-minute cycling time-trial. Because there was no impact on the total distance covered, there was no differences in the development of neuromuscular fatigue from pre to post exercise. Furthermore, the only difference within cardio-respiratory variables was heart rate which was lower in HYP compared to CTRL and ISO.

There are several reasons why performance was unaffected by elevated muscle pain. The most likely reason is that the pain experienced by most individuals was not sufficient to cause a participant to reduce their power output. The correlation between pain in the first minute of the TT and the change in power between HYP and ISO showed that those with higher pain had a reduced power output in that first minute. Due to the variable pain response from the hypertonic saline model, some participants perceive very high levels of pain (> 70/100) which, in combination with naturally occurring EIP from the cycling exercise can cause intolerable levels of pain. In order to escape the intolerable levels of pain the only course of action possible is to reduce power until pain levels are at their maximum tolerable amount. Indeed, the profile of

pain the HYP condition within study four shows a rapid increase and then maintained intensity of pain at around 70/100 (very strong pain) until the end of exercise. In CTRL and ISO, it increased linearly until it reached similar levels at the end of exercise. Therefore, it seems that psychological factors may be more prevalent in affecting endurance performance. Indeed, pain catastrophising was also greater in HYP compared to CTRL and ISO which indicates that more thought was given to the negative aspects of the pain during the TT.

Upon examination of other research to answer this research question, much of the literature has investigated the effects of reducing pain (as opposed to increasing it) on self-paced cycling exercise performance. For example Mauger and colleagues (Mauger, Jones and Williams, 2010) found that paracetamol ingestion improved 10-mile cycling time-trial performance but the pain intensity remained similar, indicating that participants conform to an exercise intensity based upon the level of perceived pain. An important difference with reducing naturally occurring EIP (i.e. with paracetamol) and elevating pain with hypertonic saline is that the participants are who receive a saline injection are aware that the pain is non-threatening and transient (Ford *et al.*, 2021). Therefore, saline-induced pain may not have the same degree of perceived threat in comparison to naturally occurring EIP. The perceived threat in this context is the development of muscle fatigue that would compromise a successful endurance performance. It is just that if pain levels become high enough, an individual will try to escape the pain regardless of their knowledge or anticipation of the saline-induced pain.

A method which potentiates the naturally occurring EIP could better reflect the limitations EIP has on self-paced exercise performance as changes in power could effectively increase or decrease the perceived pain intensity. In addition, the assessment of endurance performance within study four could only be limited to short-duration exercise as the hypertonic saline only causes pain for about 5 minutes. Longer duration exercise may be differently affected by pain as individuals would have to tolerate pain for a prolonged period, although this remains to be investigated. These are just some of the limitations which need to be considered. The next section will focus on the methodological considerations of the thesis to help provide some perspective on the experimental findings.

7.5 Methodological Considerations

Whilst this thesis has furthered the understanding of how muscle pain affects endurance performance and the development of neuromuscular fatigue, there are several considerations with the methods employed which require consideration before final conclusions can be made.

Firstly, an integral part of this thesis was the use of the hypertonic saline injections to cause muscle pain. This model of pain was attractive to investigate the fatigue-pain relationship because the pain could be standardised, is similar to EIP when combined with light exercise (Smith *et al.*, 2020) and does not affect the peripheral characteristics of the injected muscle (Farina, Arendt-Nielsen and Graven-Nielsen, 2005). However, the limitations of the hypertonic saline model of experimental muscle pain warrant discussion. The biggest limitation with the hypertonic saline injection was the transient and dynamic pain intensity it caused. Shortly after infusion, there is a rapid increase in perceived pain intensity which usually peaks at 75 ± 31 s (Smith *et al.*, 2020) and then slowly declines until pain is no longer present, usually after 5 minutes. This time critical aspect of the hypertonic saline makes it challenging to investigate a wide number of neurophysiological changes. For example, we only observed significant differences in voluntary activation at minutes one and at 100 s for the TMS silent period chapter in four because the pain was only 'strong' at these time points. Assessment of endurance performance was also limited to 5 minutes of duration because longer durations would have resulted in participants being able to endure the initial pain 'wave'. Even with shorter duration tasks, the saline induced pain was often not significantly different from isotonic saline at task failure. If pain intensity could be sustained at a moderate to high level, then it would be more likely that endurance performance would be compromised to a greater extent.

Not only is the saline transient in nature, but also heterogenous in its pain response. Differences in pain threshold and pain tolerance, due to anatomical and psychological factors result in the same 1 mL of hypertonic saline evoking different intensities of pain within each participant (see figure 7.5). Whilst a variable response was advantageous to investigate relationships between pain intensity and neuromuscular fatigue, certain low-responders could 'dilute' the data to hide potentially important neurophysiological changes.

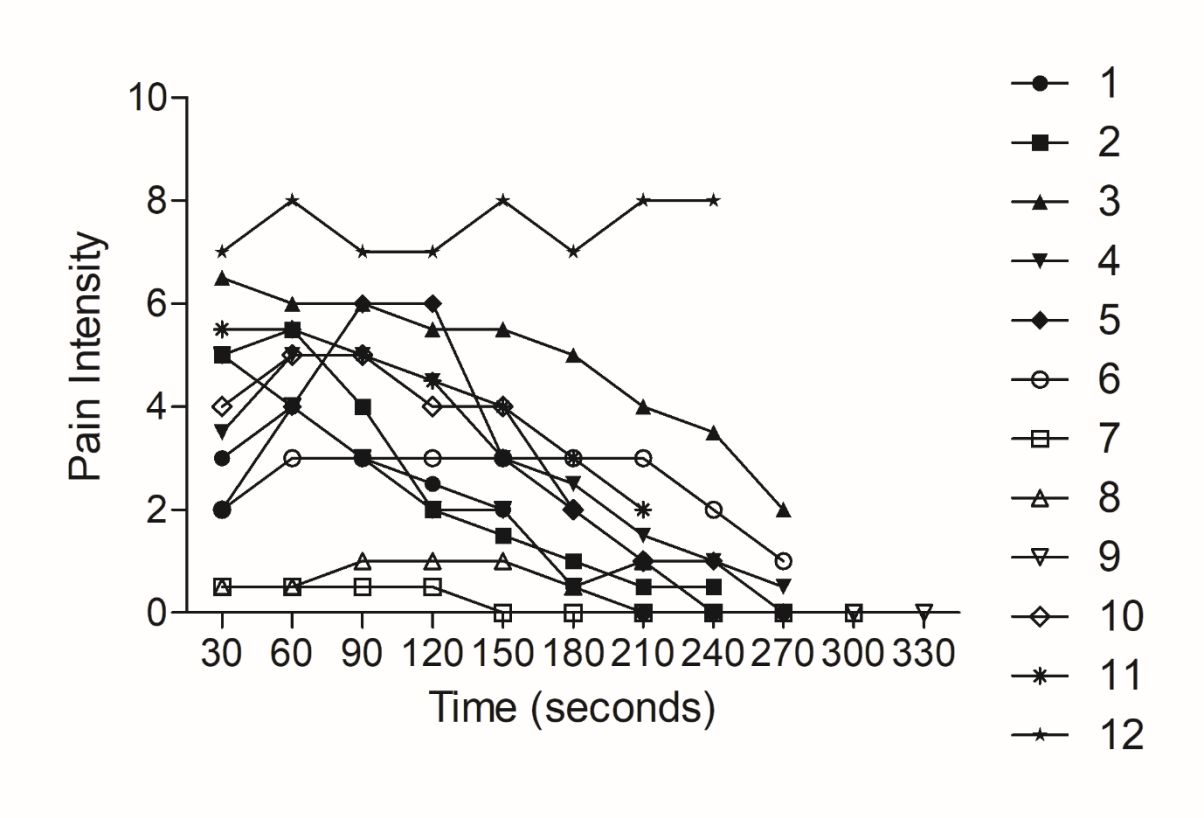

Figure 7.5. Individual pain responses of hypertonic saline in the resting leg from chapter five.

Another methodological consideration relates to the use of TMS throughout chapters four to six. The role of the TMS was to investigate corticospinal responses to pain during and after exercise. Unfortunately, single pulse transcranial magnetic stimulation has a large variability for measures of motor evoked potential amplitude (Goldsworthy, Hordacre and Ridding, 2016; Hermsen *et al.*, 2016; Biabani *et al.*, 2018). Therefore, multiple stimuli are required to obtain a reliable measure. This becomes challenging with the transient nature of the hypertonic saline and the rapid recovery from exercise-induced fatigue which provide only a short window for the assessment of corticospinal excitability/inhibition. During exercise, it was only possible to obtain four to five measures of corticospinal excitability before either another measure was required (i.e., MVC and PNS). Post-exercise, the rapid recovery of exercise-induced fatigue would have resulted in variable responses between the initial and latter part of the post-exercise measures. Furthermore, the use (or lack of) a submaximal contraction during the delivery of stimuli can also influence the TMS measures. In this thesis, the stimuli were delivered during a submaximal isometric contraction that was equivalent to the isometric time to task failure target (chapter four and five) or at 20% of baseline strength (chapter six). This method of contraction was selected because measures of corticospinal excitability were obtained during

the isometric TTF to investigate the level of excitability which was reflective of the exercise demands. Alternative methods could have included using a percentage of maximal EMG amplitude (to control for changes in descending drive), a percentage of the most recent MVC (to account for fatigue) or stimulations during rest (to eliminate differences in neuronal drive). Due to the need to rapidly conduct these measurements, the first two alternatives would not be possible, and the resting stimulations often yield low MEP responses due to a lack of facilitation of the corticospinal pathway. The development of exercise-induced fatigue necessitates a larger central motor drive to generate the same absolute target force, therefore at differing timepoints in these studies, MEPs were likely delivered during differing levels of voluntary descending drive which potentially confounded the measurements of corticospinal excitability. This is important considering the potential for the interaction between pain and fatigue to cause differing changes to voluntary descending drive during exercise between conditions. In an attempt to mitigate this confounding factor, TMS was conducted early on in exercise when exercise-induced fatigue would have been minimal and pain would be high, therefore reducing differences in voluntary descending drive. Furthermore, the selection of the stimulation intensity was to evoke maximal MEPs, this could be problematic because the ceiling effect could occur whereby saturation of the MEP makes the measurement unable to increase. However, this was not as much as much concern as it was expected that corticospinal excitability would decrease in the presence of pain. Moreover, there was still a significant increase in MEP amplitude in chapter five.

Finally, both males and females were recruited for the studies in this thesis. There was no control for what stage females were at in their menstrual cycle, or if they were using oral contraceptives. The use of contraceptive medication or stage of the menstrual cycle could of influenced the findings because different levels of circulating sex hormones can influence pain intensity and unpleasantness (Vincent *et al.*, 2018), exercise performance (McNulty *et al.*, 2020) and measures of voluntary activation (Ansdell *et al.*, 2019). This issue is somewhat lessened by the within-subject design that was employed in all four studies, however females transitioning through different stages of the menstrual cycle between the main experimental visits of the studies may have impacted the findings. However, it is unlikely that this impacted the outcome of the studies because only a small percentage of the sample (25%, 25%, 22% and 12.5% in studies one to four, respectively) were females. Ideally, the recruitment of male or female only participants would have been advantageous, but on balance given the difficulty in recruitment for these unpleasant and challenging studies, it was considered to be more

important to raise the sample size than being homogenous. Future work should at attempt to control for or at least state what phase of the menstrual cycle phase/contraceptive use and conduct sex specific studies to understand how pain effects fatigue in males and females independently.

7.6 Applications and Future Directions

The experimental studies conducted in this thesis have demonstrated the potential use of an acute experimental pain technique (hypertonic saline injection) in combination with various measures of neuromuscular function. The hypertonic saline method could be further utilised to explore the fatigue-pain relationship to extend the knowledge developed from this thesis. There is still much to understand on the role of muscle pain on the neuromuscular system.

The hypertonic saline model could be used to investigate the effects of differing volumes, concentrations, and infusion rates to manipulate the pain experience and provide further insight into how the magnitude or duration of muscle pain influences endurance performance and/or the development of fatigue. In terms of neurophysiological responses to muscle pain, this thesis used single pulse TMS and bipolar surface EMG. Given the observed decreases to voluntary activation, the increased TMS silent period and in some cases muscle activity in response to pain, more specialised research techniques are required to gain a deeper insight into the mechanisms of pain. This would include the employment of paired pulse TMS to investigate SICI, LICI and ICF. HD-EMG has emerged as a promising technique to investigate motor unit properties in response to painful isometric contractions. Persistent inward currents are also of interest (Mesquita, Škarabot and Pearcey, 2020) which can be estimated with HD-EMG but are yet to be investigated in the context of the research questions posed in this thesis. Specifically PICs are thought to contribute to the excitability of low threshold motor units and given this population of motor units seem to be primarily affected by pain (Martinez-Valdes *et al.* 2020), there could be a link between the two. PICs could be the next step to understanding the effect of pain on motor control. The rapid advancement of HD-EMG decomposition methods could allow for studies on motor unit activity in response to muscle pain of the quadriceps during dynamic contractions.

Whilst the effects of elevated muscle pain have been investigated within this thesis, these studies are only one side of fully understanding the fatigue-pain relationship. It would be informative to investigate if reducing pain has the opposite effects on endurance performance and the development of neuromuscular fatigue. Specifically, would partial blockade of pain

prolong endurance performance in both open and closed loop exercise and would this increase in performance be accompanied with the attenuation of central fatigue and the greater development of end-exercise peripheral fatigue? A number of studies have attempted to investigate the effect of reduced pain on exercise performance with mixed findings for paracetamol (Grgic and Mikulic, 2021) and tramadol (Holgado *et al.* 2018; Bejder *et al.* 2020). Intrathecal injections of fentanyl which reduced pain by about 50% has also failed to demonstrate a beneficial effect to endurance performance except when an adequate O_2 delivery was maintained (Hureau *et al.*, 2019). No study has investigated the effect of reducing muscle pain on the development of neuromuscular fatigue. Therefore, future work should utilise a model of reducing pain and investigate isometric endurance performance with measures of fatigue and TMS responses. One proposed model which has not been utilised is the intramuscular injection of lidocaine hydrochloride. Lidocaine, a potent analgesic blocked muscle pain by 50% in healthy controls when injected into the muscle (Staud *et al.*, 2009). Assuming this does not impair force generating capacity as with intrathecal lidocaine (Amann *et al.*, 2008), this model could become a valuable experimental method to further the understanding of the effect of pain on the development of fatigue.

By understanding the effects of increasing and reducing pain during exercise, it is then possible to explore ways to safely reduce the pain intensity and aversiveness for those with pain-related conditions (e.g., fibromyalgia) who might benefit from exercise programmes. This could be using psychological, nutritional, or physiological interventions. Alternatively, the purposeful and controlled implementation of muscle pain could be used to augment adaptation to exercise regimes through the development of improved pain tolerance or even develop resistance to neuromuscular impairments in the presence of high levels of EIP.

7.7 Conclusion

To summarise, this thesis aimed to investigate the effects of muscle pain on endurance performance and the development of neuromuscular fatigue. There is compelling evidence that muscle pain from the intramuscular injection of hypertonic saline can impair time to task failure exercise which is caused by an exacerbation of central fatigue. Self-paced exercise performance seems to be less affected. There is significant scope for future research to use the saline model of pain to fully understand the effect of pain and neuromuscular fatigue and endurance performance. It is hoped that the findings from this thesis will generate more research interest with the hypertonic saline model of pain and/or use of various neuromuscular measurement techniques. Furthermore, this research should stimulate interest in multiple professions relating

to exercise physiology (coaches, researchers, athletes etc) to consider muscle pain as a potential limiter to exercise performance, particularly when the level of EIP is high.

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Appendix

Appendix 1: Ethics Form for Chapter Three (Study One)

University of Kent

School of Sport & Exercise Sciences
Research Ethics and Advisory Group (REAG)
University of Kent at Medway
Chatham Maritime
Kent
ME4 4AG

Ethics Reference:

Prop 34_2018_19

Date: 9th January 2019

Dear Ryan Norbury,

Re: The Test-Retest reliability of a Submaximal Isometric Contraction to Fatigue

I am delighted to confirm that SSES REAG has approved your research study (REF No. Prop 34_2018_19) and you are now permitted to recruit participants and commence your research.

If you need to amend any aspect of your research, please ensure you inform SSES REAG by completing a request for amendment form and submitting all revised paperwork (e.g. participant information sheet, questionnaires).

If there should happen to be any adverse event during your study, please also ensure SSES REAG is kept informed.

I hope your study is successful.

With kind regards,

Louis Passfield

(Chair SSES REAG)

If any of the questions in Section IV B is answered 'yes', a full ethics application must be made to the REAG. This also applies for studies not defined as 'research' in the narrow sense, i.e. evaluations/audits, etc. Complete this form and send it to the Faculties Support Office along with supporting documentation: a copy of the full research proposal; any participant information sheets and consent forms; any surveys, interview schedules; any advertising material or proposed website wording. **It is important to note that you must not commence any research with human participants until full approval has been given by the Research Ethics Advisory Group – you will be notified via email when this has been granted.**

Overview
Name of Applicant(s)
Ryan Norbury
Contact Details (Please include your UoK address, email and telephone number)
Mobile: 07943524135 Email: rn247@kent.ac.uk School of Sport and Exercise Sciences Medway Building M0-27 Chatham Maritime Kent ME4 4AG
Title of Project
The Test-Retest reliability of a Submaximal Isometric Contraction to Fatigue
Lay Summary (Please provide a brief summary of the study)
Research within the sports and exercise science field which aims to investigate the effect of a specific intervention often uses the performance of exercise as a dependent variable. While exercise performance may be prone to change from a specific intervention, exercise performance between days may change due to factors that are not related to the independent variable. Usually, other variables which may influence exercise performance are controlled for to minimise this effect, however there can still be a small but perhaps significant level of variation. These variations in the performance measure may stem from the investigator, the participant or the measurement equipment. The consequence of this variation provides 'noise to the signal' and consequently mask a true meaningful effect of an intervention. Conversely this 'noise' may be interpreted as an effect of an intervention. Therefore it is of importance to be able to quantify the amount of variation in the performance of an exercise task with regards to the performance and physiological variables measured in order to ensure that the correct conclusions can be made from the sampled data. A Particularly common method of measuring exercise performance in a study of fatigue within sports and exercise science is the use of isometric contractions performed in a dynamometer. A dynamometer is advantageous because it allows for the standardisation of joint angles and body movements while certain muscle groups can perform exercise in isolation at a specified workload. However there are still small variations in performance which are currently unknown. An isometric exercise protocol that has often been employed in research (particularly within the university of Kent) is a constant submaximal contraction at 20% of an individual's maximum strength until task failure (an inability to maintain the force for 3 consecutive seconds). It is often found that a fixed intensity provides high levels of inter individual variability but it is unknown to the level of intra-individual variability between the test. The variability of a submaximal contraction that is aimed at achieving task failure in 4-6 minutes is unknown. Additionally, measurements of fatigue such as maximal voluntary contraction force (a measure of global fatigue), quadriceps potentiated twitch force (a measure of peripheral fatigue) and voluntary activation (a measure of central fatigue) and their variability need to be quantified as these fatigue measures are not very sensitive in detecting differences an intervention may cause. Therefore the aim of the present study is to determine the variability of two similar submaximal isometric contraction exercise tests. One with a fixed workload of 20% MVC and another with an adjusted MVC (~20% max) which achieves task failure in 4-6 minutes. The physiological measures mentioned above will also be tested for their repeatability at baseline and after the exhaustive exercise. This research add to the methodological rigour of previous and future studies that use this exercise protocol and these physiological measures of fatigue.

Name of Supervisor(s) (If applicable)
Dr Lex Mauger Dr Mark Burnley
Application Reference Number (For office use only)

Risks and ethical issues
Please list the principal inclusion and exclusion criteria
The study will look to recruit healthy, young, male and female participants (18 to 45 years old). Prior to testing, potential participants will be required to complete a general health questionnaire and a risk assessment questionnaire to ascertain their ability to participate in maximum and submaximal contractions. Exclusion: • Participants with a lower limb injury that occurred in the past three months • Participants with pre-existing neurological disorders • Participants with long-term medication use
How long will each research participant be in the study in total, from when they give informed consent until their last contact with the research team?
Approximately seven weeks, which will include a maximum of 4 hours laboratory time.
What are the potential risks and burdens for research participants and how will you minimise them? (Describe any risks and burdens that could occur as a result of participation in the research, such as pain, discomfort, distress, intrusion, inconvenience or changes to lifestyle. Describe what steps would be taken to minimise risks and burdens as far as possible)
Isometric Exercise. Participants may experience the usual risks associated with the performance of multiple voluntary contractions (MVC) such as joint sprains and muscle strains, however isometric MVCs have been performed in hundreds of different studies without causing injury and is generally accepted as a low-risk activity. Nonetheless, a thorough warm-up consisting of graded submaximal contractions will be performed to reduce the risk of muscle injury. The exercise protocol is a ~20% submaximal isometric contraction which is well within the capacity of the neuromuscular system and will carry a very-low risk of injury as well as cause minimal levels of muscle damage. Post-exercise muscle fatigue and soreness (up 48 hours post-exercise) is still a potential burden for participants, however the experience of muscle fatigue will dissipate rapidly (within 10 minutes) and soreness should be considerably low in healthy and recreationally active participants as isometric exercise causes much less muscle damage than traditional isotonic exercise. Additionally, the repeated bout effect will attenuate exercise induced muscle damage after the first familiarisation session of the exercise protocol. The risk assessment for isometric exercise on a dynamometer can found here under 'SSES Routine risk assessment, SSESRA34'. *Peripheral Electrical Stimulation.* The use of electrical stimulation (ES) of the femoral nerve will innervate the quadriceps muscle. ES are delivered in short duration pulses (~200μs) which evoke brief twitches of the innervated muscle mass. The safety of the technique is well documented and is unlikely to cause complications or injury. However, it may cause some discomfort for participants at higher stimulation intensities. Because of the short duration of the stimulations, the discomfort is very short lived resulting in no longer than a few seconds of discomfort over the course of an experimental trial. As a result, ES tends to be well tolerated among most individuals. Each investigator is trained in ES to minimise the discomfort received during the measurements. The risk assessment for peripheral electrical nerve stimulation can found here under 'SSES Routine risk assessment, SSESRA22'.
Please describe what measures you have in place in the event of any unexpected outcomes or adverse effects to participants arising from involvement in the project

The laboratory contains a defibrillator which can be operated by a trained individual. For all testing sessions, there will be two people present in the laboratory, with a minimum of one individual who is first aid trained.
Will interviews/questionnaires or group discussions include topics that might be sensitive, embarrassing or upsetting, or is it possible that criminal or other disclosures requiring action could occur during the study?
No
If yes, please describe the procedures in place to deal with these issues
N/a
What is the potential benefit to research participants?
Through performing maximal voluntary contractions, participants will gain an accurate measure of quadriceps muscle strength. Additionally, participants will be contributing to further our knowledge of the reliability of common measures which are undertaken to assess fatigue.
What are the potential risks to the researchers themselves?
No risks are posed to the researchers involved in data collection.
Will there be any risks to the University? (Consider issues such as reputational risk; research that may give rise to contentious or controversial findings; could the funder be considered controversial or have the potential to cause reputational risk to the University?)
No
Will any intervention or procedure, which would normally be considered a part of routine care, be withheld from the research participants? (If yes, give details and justification). For example, the disturbance of a school child's day or access to their normal educational entitlement and curriculum).
No

Recruitment and informed consent
How and by whom will potential participants, records or samples be identified?
The lead researcher will recruit participants through posters placed around the School of Sport and Exercise Sciences (Medway Building and Medway Park) and on social media, and through an e-mails distributed to all students in the school. Additionally, adverts may be placed on participant recruitment websites on the internet. Word of mouth from individuals involved in the study to students/staff members of the university.
Will this involve reviewing or screening identifiable personal information of potential participants or any other person? (If 'yes', give details)
Yes. Participants will complete a general health questionnaire. These will be kept in a locked cabinet by the researcher.
Has prior consent been obtained or will it be obtained for access to identifiable personal information?
Yes. Informed consent will be taken by the researcher.
Will you obtain informed consent from or on behalf of research participants? (If 'yes' please give details. If you are not planning to gain consent, please explain why not).
Yes. All participants will receive a 'Participant Information Sheet', which provides a full written explanation of the study, protocols and procedures. This information sheet also contains an appendix discussing the background and risk assessment of intramuscular injections. Should participants require further information, or have any additional questions, explanations will be provided verbally. Upon the completion of this, if the participants are content to participate, they will provide full written consent through the completion of an 'Informed Consent Form'.
Will you record informed consent in writing? (If 'no', how will it be recorded?)
Yes

How long will you allow potential participants to decide whether or not to take part?
Participants will be provided with a minimum of 48 hours after receiving the Participant Information Sheet, to decide whether to participate.
What arrangements have been made for persons who might not adequately understand verbal explanations or written information given in English, or have special communication needs? (eg, translation, use of interpreters?)
All participants will be strong and capable in speaking and understanding the English language. They will receive both written and verbal explanations of all relevant details in the study. No arrangements will therefore be made
If no arrangements will be made, explain the reasons (eg, resource constraints)
Financial limitations

Confidentiality
In this section personal data means any data relating to a participant who could potentially be identified. It includes pseudonymised data capable of being linked to a participant through a unique code number.
If you will be undertaking any of the following activities at any stage (including in the identification of potential participants) please give details and explain the safeguarding measures you will employ • Electronic transfer by magnetic or optical media, email or computer networks • Sharing of personal data outside the European Economic Area • Use of personal addresses, postcodes, faxes, emails or telephone numbers • Publication of direct quotations from respondents • Publication of data that might allow identification of individuals, either directly or indirectly • Use of audio/visual recording devices • Storage of personal data on any of the following: – Manual files – University computers – Home or other personal computers – Private company computers – Laptop computers
All hard files or written data will be stored in a secure locked cabinet. All electronic data will be stored in anonymised format and kept in a password-protected folder on a password-protected laptop computer belonging to the researcher.
How will you ensure the confidentiality of personal data? (eg, anonymisation or pseudonymisation of data)
All data will be anonymised through the use of participant number coding. A master code will be kept in a secure locked cabinet by the researchers.
Who will have access to participants' personal data during the study?
Ryan Norbury Samuel Smith Adam Hunt Dr Lex Mauger
How long will personal data be stored or accessed after the study has ended? (If longer than 12 months, please justify)
The data will be stored for up to four years. This is due to the data being collected as part of a PhD thesis, and thus may be required at a later date for initial analysis.
Please note: as best practice, and as a requirement of many funders, where practical, researchers must develop a data management and sharing plan to enable the data to be made available for re-use, eg, for secondary research,

and so sufficient metadata must be conserved to enable this while maintaining confidentiality commitments and the security of data.

Incentives and payments
Will research participants receive any payments, reimbursement of expenses or any other benefits or incentives for taking part in this research? (If 'yes', please give details)
No
Will individual researchers receive any personal payment over and above normal salary, or any other benefits or incentives, for taking part in this research? (If 'yes', please give details)
No
Does the Chief Investigator or any other investigator/collaborator have any direct personal involvement (e.g. financial, share holding, personal relationship, etc) in the organisations sponsoring or funding the research that may give rise to a possible conflict of interest? (If 'yes', please give details)
No

Publication and dissemination
How do you intend to report and disseminate the results of the study? If you do not plan to report or disseminate the results please give your justification
The results of the study will be an anonymised cohort results. These results will subsequently be analysed and written up in the form of one or more conference/peer-reviewed papers, which will form the basis of an experimental chapter in the PhD thesis
Will you inform participants of the results? (Please give details of how you will inform participants or justify if not doing so)
Yes. All participants will be able to request a summary of results from testing.

Management of the research		
Other key investigators/collaborators. (Please include all grant co-applicants, protocol authors and other key members of the Chief Investigator's team, including non-doctoral student researchers)		
Dr Lex Mauger Dr Mark Burnley Ryan Norbury Samuel Smith Adam Hunt		
Has this or a similar application been previously rejected by a research Ethics Committee in the UK or another country? (If yes, please give details of rejected application and explain in the summary of main issues how the reasons for the unfavourable opinion have been addressed in this application)		
No		
How long do you expect the study to last?		
• Planned start date: 15/01/19	• Planned end date: 15/01/2020	• Total duration: 24 months
Where will the research take place?		
The University of Kent physiology laboratory at Medway Park		

Insurance/indemnity
Does UoK's insurer need to be notified about your project before insurance cover can be provided? *The majority of research carried out at UoK is covered automatically by existing policies, however, if your project entails more than usual risk or involves an overseas country in the developing world or where there is or has recently been conflict, please check with the Insurance Office that cover can be provided. Please give details below.*
No

Children
Do you plan to include any participants who are children under 16? (If no, go to next section)
No
Please specify the potential age range of children under 16 who will be included and give reasons for carrying out the research with this age group
N/a
Please describe the arrangements for seeking informed consent from a person with parental responsibility and/or from children able to give consent for themselves
N/a
If you intend to provide children under 16 with information about the research and seek their consent or agreement, please outline how this process will vary according to their age and level of understanding
N/a

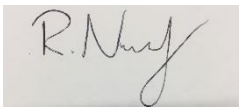
Participants unable to consent for themselves	
Do you plan to include any participants who are adults unable to consent for themselves through physical or mental incapacity? (If yes, the research must be reviewed by an NHS REC or SCREC)	
No	
Is the research related to the 'impairing condition' that causes the lack of capacity, or to the treatment of those with that condition?	
☐ Yes	If 'yes' proceed to next question
☒ No	If 'no' the study should proceed without involving those who do not have the capacity to consent to participation
Could the research be undertaken as effectively with people who do have the capacity to consent to participate?	
☒ Yes	If 'yes' then the study should exclude those without the capacity to consent to participation
☐ No	If 'no' then the inclusion of people without capacity in the study can be justified
Is it possible that the capacity of participants could fluctuate during the research? (If yes, the research must be reviewed by an NHS REC or SCREC)	
No	
Who inside or outside the research team will decide whether or not the participants have the capacity to give consent? What training/experience will they have to enable them to reach this decision?	
Dr Lex Mauger. He has been active researcher for 10 years, including a successful project ethics submission with the NHS, and the completion of NHS Good Clinical Practice Training.	
What will be the criteria for withdrawal of participants?	

A participant who has given consent and subsequently loses the capacity to provide consent will be withdrawn from the study. Participants will be informed both verbally and in written form that they can withdraw from the study at any time, without any disadvantage to themselves.

Declaration

To be signed by the Chief Investigator

- I agree to comply, and will ensure that all researchers involved with the study comply with all relevant legislation, accepted ethical practice, University of Kent policies and appropriate professional ethical guidelines during the conduct of this research project
- If any significant changes are made to the design of the research I will notify the Faculty of Sciences Research Ethics and Advisory Group (REAG) and understand that further review may be required before I can proceed to implement the change(s)
- I agree that I will notify the Faculty of Sciences Research Ethics Advisory Group of any unexpected adverse events that may occur during my research
- I agree to notify the Faculty of Sciences Research Ethics Advisory Group of any complaints I receive in connection with this research project

Signed:  Name: Mr Ryan Norbury	Date: 22/10/18

What to do next

Send your completed form, along with all supporting documentation, to the Faculties Support Office, at fso@kent.ac.uk.

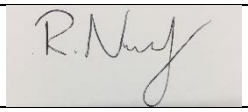
Checklist	
Please ensure you have included the following with your application (*where relevant):	
• Full research proposal (current project)	☒
• Participant information sheet	☒
• Consent form	☒
• *Covering letter	☒
• *Any questionnaires/interview schedules/topic guides to be used	☐
• *Any approved instruments/measures to be used	☐
• *Any advertising material to be used to recruit participants	☒
• *Confirmation that project is covered by UoK insurance policies (if necessary)	☐

A checklist should be completed for every research project in order to identify whether a full application for ethics approval needs to be submitted. The principal investigator or, where the principal investigator is a student, the supervisor, is responsible for exercising appropriate professional judgement in this review.

This checklist must be completed before potential participants are approached to take part in any research. All forms must be signed by the School's Research Ethics Advisory Group representative.

Section I: Project details			
Project title:	The Test-Retest reliability of a Submaximal Isometric Contraction to Fatigue		
Planned start date: 15/01/2019		Planned end date:15/01/2020	
Funder:	School of sports and exercise Science, University of Kent		

Section II: Applicant details			
Applicant name:	Ryan Norbury		
School/Department:	School of Sports and Exercise Science		
Email: Rn247@kent.ac.uk		Telephone number:	07943524135
Contact address:	School of Sport and Exercise Sciences Medway Building M0-27 Chatham Maritime Kent ME4 4AG		
Undergraduate ☐	Taught Postgraduate ☐	Research Postgraduate ☒	Staff ☐

Section III: Declaration and signatures			
Please note that it is your responsibility to follow, and to ensure that, all researchers involved with your project follow accepted ethical practice and appropriate professional ethical guidelines in the conduct of your study. You must take all reasonable steps to protect the dignity, rights, safety and well-being of participants. This includes providing participants with appropriate information sheets, ensuring informed consent and ensuring confidentiality in the storage and use of data.			
Applicant signature		**Date**	22/10/2018

Supervisor name	Dr. Lex Mauger	**Date**	15/11/2018
Supervisor signature			

School REAG rep signature (required for both staff and students)		**Date**	

If any question in Section IV(A) are answered 'yes':

1. Contact Nicole Palmer (University Research Ethics & Governance Officer) for advice
2. Send a copy of ethical approval to the Faculties Support Office, once received

If any questions in Sections IV(B) and/or IV(C) and/or IV(D) are answered 'yes':

1. Complete full application form together with supporting documentation
2. Send to the Faculties Support Office for review by the Research Ethics Advisory Group (REAG)

If all questions in Sections IV(A), IV(B), IV(C) and IV(D) are answered 'no':

1. Send the completed and signed form to the Faculties Support Office at fsoethics@kent.ac.uk.

Section IV: Research Checklist

Please answer all questions by ticking the appropriate box:

A) Research that may need to be reviewed by an NHS Research Ethics Committee, the Social Care Research Ethics Committee (SCREC) or other external ethics committee (if *yes*, please give brief details as an annex)	**YES**	**NO**
Will the study involve recruitment of patients through the NHS or the use of NHS patient data or samples?	☐	☒
Will the study involve the collection of tissue samples (including blood, saliva, urine, etc.) or other biological samples from participants, or the use of existing samples?	☐	☒
Will the study involve participants, or their data, from adult social care, including home care, or residents from a residential or nursing care home?	☐	☒
Will the study involve research participants identified because of their status as relatives or carers of past or present users of these services?	☐	☒
Does the study involve participants aged 16 or over who are unable to give informed consent (e.g. people with learning disabilities or dementia)?	☐	☒
Is the research a social care study funded by the Department of Health?	☐	☒
Is the research a health-related study involving prisoners?	☐	☒
Is the research a clinical investigation of a non-CE Marked medical device, or a medical device which has been modified or is being used outside its CE Mark intended purpose, conducted by or with the support of the manufacturer or another commercial company to provide data for CE marking purposes? (a CE mark signifies compliance with European safety standards)	☐	☒
Is the research a clinical trial of an investigational medicinal product or a medical device?	☐	☒

B) Research that may need full review by the Sciences REAG	**YES**	**NO**
Does the research involve other vulnerable groups: eg, children; those with cognitive impairment?	☐	☒

Is the research to be conducted in such a way that the relationship between participant and researcher is unequal (eg, a subject may feel under pressure to participate in order to avoid damaging a relationship with the researcher)?	☐	☒
Does the project involve the collection of material that could be considered of a sensitive, personal, biographical, medical, psychological, social or physiological nature.	☐	☒
Will the study require the cooperation of a gatekeeper for initial access to the groups or individuals to be recruited (eg, headmaster at a School; group leader of a self-help group)?	☐	☒
Will it be necessary for participants to take part in the study without their knowledge and consent at the time? (eg, covert observation of people in non-public places?)	☐	☒
Will the study involve discussion of sensitive topics (eg, sexual activity; drug use; criminal activity)?	☐	☒
Are drugs, placebos or other substances (eg, food substances, vitamins) to be administered to the study participants or will the study involve invasive, intrusive or potentially harmful procedures of any kind?	☐	☒
Is pain or more than mild discomfort likely to result from the study?	☒	☐
Could the study induce psychological stress or anxiety or cause harm or negative consequences beyond the risks encountered in normal life?	☐	☒
Will the study involve prolonged or repetitive testing?	☒	☐
Will the research involve administrative or secure data that requires permission from the appropriate authorities before use?	☐	☒
Is there a possibility that the safety of the researcher may be in question (eg, international research; locally employed research assistants)?	☐	☒
Does the research involve participants carrying out any of the research activities themselves (i.e. acting as researchers as opposed to just being participants)?	☐	☒
Will the research take place outside the UK? You may find the find the Proportionate Risk Assessment document useful.	☐	☒
Will the outcome of the research allow respondents to be identified either directly or indirectly (eg, through aggregating separate data sources gathered from the internet)?	☐	☒
Will research involve the sharing of data or confidential information beyond the initial consent given?	☐	☒
Will financial inducements (other than reasonable expenses and compensation for time) be offered to participants?	☐	☒
Are there any conflicts of interest with the proposed research/research findings? (eg, is the researcher working for the organisation under research or might the research or research findings cause a risk of harm to the participants(s) or the researcher(s) or the institution?)	☐	☒
Will the study involve the publication, sharing or potentially insecure electronic storage and/or transfer of data that might allow identification of individuals, either directly or indirectly? (e.g. publication of verbatim quotations from an online forum; sharing of audio/visual recordings; insecure transfer of personal data such as addresses, telephone numbers etc.; collecting identifiable personal data on unprotected** internet sites.) [**Please note that Qualtrics and Sona Systems provide adequate data security and comply with the requirements of the EU-US Privacy Shield.]	☐	☒

C) Security Sensitive Material	**YES**	**NO**
Does your research involve access to or use of material covered by the Terrorism Act? (The Terrorism Act (2006) outlaws the dissemination of records, statements and other documents that can be interpreted as promoting and endorsing terrorist acts. By answering 'yes' you are registering your legitimate use of this material with the Research Ethics	☐	☒

Advisory Group. In the event of a police investigation, this registration will help you to demonstrate that your use of this material is legitimate and lawful).		

D) Prevent Agenda	**YES**	**NO**
Does the research have the potential to radicalise people who are vulnerable to supporting terrorism or becoming terrorists themselves?	☐	☒

If the answer to any questions in Sections IV(B) and/or IV(C) and/or IV(D) is 'yes', please complete the full application form and send to the Faculties Support Office at fsoethics@kent.ac.uk together with required supporting documentation.

Appendix 2: Ethics Forms for Chapter Four (Study Two)

University of Kent

School of Sport & Exercise Sciences
Research Ethics and Advisory Group (REAG)
University of Kent at Medway
Chatham Maritime
Kent
ME4 4AG

Ethics Reference:

Prop 30_2018_19

Date: 16th January 2019

Dear Ryan Norbury,

Re: The Effect of Experimental Muscle Pain on Neuromuscular Function and Isometric Exercise Performance

I am delighted to confirm that SSES REAG has approved your research study (REF No. Prop 30_2018_19) and you are now permitted to recruit participants and commence your research.

If you need to amend any aspect of your research, please ensure you inform SSES REAG by completing a request for amendment form and submitting all revised paperwork (e.g. participant information sheet, questionnaires).

If there should happen to be any adverse event during your study, please also ensure SSES REAG is kept informed.

I hope your study is successful.

With kind regards,

Louis Passfield

(Chair SSES REAG)

If any of the questions in Section IV B is answered 'yes', a full ethics application must be made to the REAG. This also applies for studies not defined as 'research' in the narrow sense, i.e. evaluations/audits, etc. Complete this form and send it to the Faculties Support Office along with supporting documentation: a copy of the full research proposal; any participant information sheets and consent forms; any surveys, interview schedules; any advertising material or proposed website wording. **It is important to note that you must not commence any research with human participants until full approval has been given by the Research Ethics Advisory Group – you will be notified via email when this has been granted.**

Overview
Name of Applicant(s)
Ryan Norbury
Contact Details (Please include your UoK address, email and telephone number)
Mobile: 07943524135 Email: rn247@kent.ac.uk School of Sport and Exercise Sciences Medway Building M0-27 Chatham Maritime Kent ME4 4AG
Title of Project
The Effect of Experimental Muscle Pain on Neuromuscular Function and Isometric Exercise Performance
Lay Summary (Please provide a brief summary of the study)
During intense exercise, there is often a feeling of pain which increases as the exercise becomes more physically challenging. This feeling of pain is a very conscious and prominent sensation which may be implicated in the fatigue process and thus, may modulate exercise performance. When the muscle experiences significant metabolic perturbation (i.e. metabolite accumulation) and mechanical deformation that occurs during exercise, there is a stimulation of afferent fibres within the active muscle which play a role in nociception (I.e. causes pain) as well as providing inhibitory feedback to the central nervous system which can promote central fatigue. It is currently unknown whether the sensation of pain acts on a more psychological level, whereby an individual voluntarily disengages in the exercise task to reduce the levels of pain felt when an intolerable level of pain is felt, or whether pain acts on a more physiological/subconscious level by reducing the capacity of neuromuscular system in order to prevent further damage/pain that the brain may associate with a catastrophic level of muscle tissue disturbance. Therefore the mechanisms of pain and their implication to exercise performance are currently not well understood and need to be elucidated. One method of investigating this involves the intramuscular injection of a small amount (~1ml) of hypertonic saline (i.e. saltwater). This infusion of hypertonic saline stimulates the afferent fibres and causes intense feelings of pain within the injected site. Therefore it is possible to cause muscle pain without any of the other confounding factors that are implicated in the fatigue process such as muscle damage, metabolite accumulation or mental fatigue. This allows for a direct investigation of how muscle pain modulates neuromuscular function and exercise performance. Therefore the aim of this study is to examine how muscle pain of the quadriceps muscles effects neuromuscular function and isometric, isolated exercise performance.
Name of Supervisor(s) (If applicable)
Dr Lex Mauger Dr Mark Burnley
Application Reference Number (For office use only)

Risks and ethical issues

Please list the principal inclusion and exclusion criteria

The study will look to recruit 10 healthy, young, male and female participants (18 to 45 years old). Prior to testing, potential participants will be required to complete a general health questionnaire and an intramuscular injection risk assessment questionnaire to ascertain their ability to participate in maximum and submaximal contractions and receive intramuscular injections respectively

Exclusion:

- Participants with a lower limb injury that occurred in the past three months
- Participants with pre-existing medical conditions (neurological disorders and blood borne viruses (i.e. HIV, Hepatitis B/C))
- Participants with long-term medication use
- Participants with any allergy (e.g. nuts, fish, milk, egg, wheat and soya)
- Participants with needle phobia
- Those who have metallic hardware in close proximity to the head (such as cochlear implants, or an Internal Pulse Generator or medication pumps)

How long will each research participant be in the study in total, from when they give informed consent until their last contact with the research team?

Approximately three weeks, which will include a maximum of 4 hours laboratory time.

What are the potential risks and burdens for research participants and how will you minimise them? (Describe any risks and burdens that could occur as a result of participation in the research, such as pain, discomfort, distress, intrusion, inconvenience or changes to lifestyle. Describe what steps would be taken to minimise risks and burdens as far as possible)

Exercise. Participants may experience the usual risks associated with the performance of multiple voluntary contractions (MVC) such as joint sprains and muscle strains, however isometric MVCs have been performed in hundereds of different studies without causing injury and is generally accepted as a low-risk activity. Nonetheless, a thorough warm-up consisting of graded submaximal contractions will be performed to reduce the risk of muscle injury. The exercise protocol is a ~20% submaximal isometric contraction which is well within the capacity of the neuromuscular system and will carry a very-low risk of injury as well as cause minimal levels of muscle damage. Post-exercise muscle fatigue and soreness (up 48 hours post-exercise) is still a potential burden for participants, however the experience of muscle fatigue will dissipate rapidly (within 10 minutes) and soreness should be considerably low in healthy and recreationally active participants as isometric exercise causes much less muscle damage than traditional isotonic exercise. Additionally, the repeated bout effect will attenuate exercise induced muscle damage after the first familiarisation session of the exercise protocol. The risk assessment for exercise on an isokinetic dynamometer can be found here under SSESRA34.

Electrical Stimulation. The use of electrical stimulation (ES) of the femoral nerve will innervate the quadriceps muscle. ES are delivered in short duration pulses (~200μs) at an amplitude which does not exceed 400mA. This evokes brief twitches of the innervated muscle mass. The safety of the technique is well documented and is unlikely to cause complications or injury. However, it may cause some discomfort for participants at higher stimulation intensities. Because of the short duration of the stimulations, the discomfort is very short lived resulting in no longer than a few seconds of discomfort over the course of an experimental trial. As a result, ES tends to be well tolerated among most individuals. Each investigator is trained in ES to minimise the discomfort received during the measurements and complies with the risk assessment guideline. The risk assessment for transcutaneous peripheral electrical nerve stimulation can be found here under SSESRA68.

Transcranial Magnetic Stimulation. Single pulse transcranial magnetic stimulation (TMS) of the motor cortex is generally regarded as safe for the participants and is a measurement technique that has been safely used in thousands of published papers. 1ms pulses are delivered a stimulator intensity which is around 60-70% of maximum stimulator output. One of the primary risks with TMS is the induction of a seizure. However, since 1998, there have only been four reported cases of seizures (date of report: Dec. 2008). Three out of four of these cases were caused in participants that were taking pro-epileptogenic medication (Rossi et al. 2009). Syncope is also a cited concern with TMS, however this appears to be an epiphenomenon to TMS and is again unlikely to occur. In the rare instance syncope, participants will be lay down supine with the legs elevated until participants regain consciousness. Similar to ES, TMS may induce some discomfort at higher

stimulation intensities. The use of TMS is limited to minimise the amount of discomfort received. TMS is usually well tolerated by most individuals. Each investigator will have training on how to competently perform TMS to minimise the discomfort which may occur. The risk assessment for transcranial magnetic stimulation can be found here under SSESRA69.

Intramuscular Injection. Intramuscular injection of hypertonic saline as an experimental model of exercise-induced pain has previously been implemented within the present institution and has been approved for previous studies (**ethics references: Prop 139_2017_17, Prop 84_2016_17, Prop 140_2016_17, Prop 1_2018_19**). However, it is recognised that this method may still cause ethical concern. As stated, this model has been applied in an earlier research study conducted by the supervisor and one of the investigators of the proposed study. Combined, they have administered over 40 safe and successful injections. Each investigator of the study has received formal training from a registered medical practitioner and has been signed off as competent. All previous intramuscular injections have been administered and documented in line with NHS best practice and the School of Sport and Exercise Science IM injection risk assessment. This proposed study will build upon an extensive range of literature from various institutions located in Australia, Denmark, France, Germany, Sweden and USA where this method has been safely and successfully employed (Capra and Ro, 2000; Deschamps, Hug, Hodges, Tucker, 2014; Graven-Nielsen, Svensson and Arendt-Nielsen, 1997b; Khan et al., 2011; Schilder et al., 2014; Schulte et al., 2003), all of which have safely employed this method without any reported issues. Nonetheless, a detailed background and risk assessment of this method is presented below or can be found here under SSESRA20.:

1.1. Preface

As a fundamental protective function and an inhibiting factor that may contribute to fatigue, exercise-induced pain can be considered a key determinant of success in exercise adherence and endurance performance (Mauger, 2013). However, at present, investigations into the role of exercise-induced pain are somewhat limited due to the typical pain-inducing methods (e.g. heat, cold, pressure) being an inadequate replication of pain, without influencing other physiological factors (Mauger, 2013). However, the injection of a small volume of 5.8% hypertonic saline, (a strong solution of salt water) into muscle tissue, which causes sensations that are 'cramp-like' and 'aching', provides a method which closely represents exercise-induced pain (Graven-Nielsen and Mense, 2001). This method has been used extensively studies ranging from 1938 to the present day with no adverse events reported.

Therefore, the aim of this proposed programme of non-clinical research studies, conducted at the University of Kent, is to apply this method as a model of exercise-induced pain, and to investigate the mechanisms by which exercise-induced pain contributes to exercise intensity regulation and fatigue.

Currently, hypertonic saline (we plan to use Braun's sterile 5,85 % Sodium Chloride (PZN: 3158635)) is licenced for infusion, but not for intramuscular injection. This is because hypertonic saline is used to medically treat conditions such as hyponatremia (electrolyte imbalance in the blood), but has no medical use for intramuscular injection. Therefore, the aim of this document is to provide evidence and assurance that intramuscular injection of 5.85% saline is a common technique used in research, bears no risk above that of a standard intramuscular injection and that this technique is required to complete our planned programme of research. Research by Henriksen et al. (2007) and Danneskiold-Samsøe et al. (2007) has used the same volume and concentration into the vastus medialis while Ciubotariu, Arendt-Nielsen and Graven-Nielsen (2004) has used 6%NaCl into the tibialis anterior without issue. Therefore this volume and concentration has been shown to be safe. We will inject it into the vastus lateralis as this site poses less risk than the previously used vastus medialis. This exact protocol has been used in several studies internally (ethics references: Prop 139_2017_17, Prop 84_2016_17, Prop 140_2016_17, Prop 1_2018_19). A 5.8% concentration is used as this is what is commercially available and creates an appropriate amount of muscle pain when 1ml is injected.

1.2. Background

IM injections are a common alternative method to administer medications, drugs and vaccines with the substance, via the use of a syringe and needle, directly injected deep into the muscle tissue, below the muscle fascia and under the fatty subcutaneous layer (Boyd et al. 2013). This technique is a relatively simple process, is not defined as a medical procedure and therefore requires a clean procedure rather than aseptic. As the skeletal muscle is suggested to have scarcer nociceptors than subcutaneous tissue, IM injections involve decreased discomfort, and dependent on the site of injection

(i.e. deltoid, vastus lateralis, rectus femoris, ventrogluteal or dorsogluteal), this method enables comparatively large volumes of a substance to be quickly absorbed by the body, with a relatively prolonged action (Rodger and King, 2000).

When implementing IM injections, several considerations and decisions need to take place:

- Injection site (dependent on age, physical status and volume of the injectate)
- Substance to be injected
- Technique
- Equipment
- Environment in which injection is administered

It is proposed that the experimental pain model implemented in the proposed non-clinical studies is the IM injection of 1ml hypertonic saline (5.8% concentration). Hypertonic saline is a strong, sterile solution of salt water, with a concentration of sodium chloride greater than 0.9% (Mortimer and Jancik, 2006). It primarily functions by an osmotic effect, but can also be utilised for other clinical effects (hemodynamic, vasoregulatory, immunomodulatory, neurochemical and hypernatremic) (Doyle, Davis and Hoyt, 2001).

Additionally, after first being implemented by Kellgren in the late 1930s (Kellgren, 1938), hypertonic saline has since been extensively been used as an experimental model that characterises both the sensory and motor effects involved in muscle pain (Burton, Fazalbhoy and Macefield, 2016). This is predominantly due to the quality of the pain induced by IM injections of hypertonic saline effectively mimicking acute muscle pain, with the pain produced displaying localized and referred characteristics (Graven-Nielsen and Mense, 2001). Dependent on injection site and volume of solution, a bolus IM injection of hypertonic saline temporarily induces pain that can last up to ten minutes before disappearing, with a mean pain intensity rating of ~5.5 on the Cook 0-10 scale (Cook, O'Connor, Eubanks, Smith and Lee, 1997). This therefore allows the acute effects of pain to be studied (Burton et al. 2016).

The medical application of hypertonic saline is through intravenous infusion, for which the solution is licensed. At present, 5.8% hypertonic saline is not licensed for intramuscular injection because there is no medical basis for this method of application. However, as demonstrated by previous animal studies, this method does not cause any muscle toxicity (Svendsen, Edwards and Rasmussen, 2001), and the pain caused is unlikely to be related to tissue damage (Svendsen, Edwards, Lauritzen and Rasmussen, 2005). Thus this method can be deemed to be safe and acceptable for use in human experimentation (Graven-Nielsen, Lund, Arendt-Nielsen, Danneskiold-Samsøe and Bliddal, 2002).

Indeed, IM injections of hypertonic saline have been applied in over 35 studies ranging from 1938 to the present day, with no adverse effects reported (for example; Arendt-Nielsen, Graven-Nielsen, Svarrer and Svensson, 1996; Feinstein, Langton, Jameson and Schiller, 1954; Kennedy, McNeil, Gandevia and Taylor, 2016; Kellgren, 1938; Henriksen, Alkjær, Simonsen and Bliddal, 2009; Hodges, Moseley, Gabrielsson and Gandevia, 2003; Veerasarn and Stohler, 1992; Yavuz, Negro, Falla and Farina, 2015). Furthermore, we have contacted Professor Thomas Graven-Nielsen (http://vbn.aau.dk/en/persons/thomas-gravennielsen(474a635b-4e83-4b02-a90b-80d417d37e52)/cv.html?id=60483613), a leading professor in pain neuroscience who has extensively used this method (for example; Graven-Nielsen, Arendt-Nielsen, Svensson and Jensen, 1997a, Graven-Nielsen et al., 1997b; Graven-Nielsen, Babenko, Svensson and Arendt-Nielsen, 1998a, Graven-Nielsen et al., 1998b; Graven-Nielsen et al., 2002; Graven-Nielsen et al., 2003) for advice and guidance on the use of hypertonic saline. Prof. Graven-Nielsen stated that he has performed over 6000 injections, with no adverse effects having occurred and is therefore very confident in the safety of the technique.

When injected into the muscle, hypertonic saline predominantly causes the depolarisation of unmyelinated group IV muscle afferent units which directly stimulates the release of neuropeptides such as substance P at the periphery (Laursen, Graven-Nielsen, Jensen and Arendt-Nielsen, 1999). This means that a particular tissue and type of pain can be specifically targeted (Graven-Nielsen and Mense, 2001). However, at present, the mechanisms of muscle nociceptive processing excited by the intramuscular injection of hypertonic saline are uncertain (Mense, 2013). Several proposals have nonetheless been made, including: activation by augmented tonicity in the interstitial space, activation by ionic alterations and indirect activation by further algesic substances released from the muscle tissue or the nociceptive ending (Kress and Reeh, 1996).

In the present study, the hypertonic saline solution is planned to be injected into the vastus lateralis (middle third of the lateral aspect of the thigh between the greater trochanter and the lateral femoral condyle of the femur). This site provides a large muscle mass, is easy to access, has a reduced likelihood of injury and is not associated with any major blood vessels or significant nerve structures, minimising risks of damage (Dougherty and Lister, 2011). Additionally, the volume of fluid that is proposed to be injected into this site (1ml) is below the maximal recommended volume of 5ml for any substance that is injected into a large muscle mass (Roger and King, 2000). This volume is consistent with previous literature (Deschamps, Hug, Hodges and Tucker, 2014; Henriksen et al., 2007; Henriksen et al., 2009; Khan, McNeil, Gandevia and Taylor, 2011) and also below that utilised in other studies, where 1.5ml has been formerly applied (Ervilha, Farina, Arendt-Nielsen and Graven-Nielsen, 2005; Graven-Nielsen et al., 2002; Hodges et al., 2003).

1.3. Associated Risks and Side-effects

As a routine technique for drug administration for over one hundred and fifty years, IM injections are considered to be a method that is simple and safe, with the rare occurrence of complications or side-effects (Svendsen et al., 2005). In addition, the IM injection of hypertonic saline carries no further risk beyond which would occur from the IM injection of any substance (i.e. allergic or anaphylactic reaction), with this solution not causing muscle toxicity or tissue damage (Svendsen et al., 2001; Svendsen et al., 2005). Nonetheless, an IM injection can solely still result in several potential complications (Small, 2004) which could arise as a result of unsafe injections and poor technique. Although mainly preventable with trained and safe IM practice, it is important that these potential risks are taken into consideration. Potential complications of an IM injection include:

- Pain or mild discomfort for a short period after an injection is common
- Some bruising at the injection site may occur
- Muscle fibrosis can occur with repeated use of the same injection site
- An increased risk of injecting the substance intravenously if the needle is too deep
- Needle stick injury to the experimenter
- In more serious cases, nerve injury resulting in potential paralysis, atrophy, haematoma, bone injury, cellulitis, and sterile abscesses can occur
- Accidental femoral nerve damage due to incorrect needle placement and muscle atrophy from IM injection overuse are the predominant risks associated with the vastus lateralis site

Most of the complications highlighted previously may occur at any site of injection, and could potentially due to an inappropriate depth or rate of injection, or injecting into an incorrect site (Malkin, 2008).

1.4. Interventions in Place to Reduce Risk

A safe injection is defined as 'one that does not harm the recipient, does not expose the provider to any avoidable risk, and does not result in waste that is dangerous to other people' (Hutin et al., 2003: 492). In order to achieve a safe injection, and reduce the risks associated with IM injections the researcher will implement several precautions. These are detailed below:

- The lead researcher has received appropriate NHS training and guidelines, followed by a subsequent competency assessment of supervised practice to ensure safe, competent and consistent best practice when administering IM injections
  - Thorough understanding of injection site to reduce the likelihood of nerve injury and accidental intravenous injection
  - Performance of pre-injection checks (check drug name and ampule, check drug unit quantity or %, check expiry date, inspect drug for cloudiness and particles)
  - The use of appropriate syringe (Luer Lok) and needle size to ensure the solution is delivered to the muscle
  - The use of the Z track technique to minimise the pain experienced and reduce the likelihood of complications
  - Aspiration prior to delivery to ensure that the needle is not in a blood vessel. If blood appears, remove the needle and restart the process.

- Quick insertion of needle at 90^{o} followed by a slow injection and then quick withdrawal of the needle 10 seconds after the injection has occurred
- The documentation of solution administration, injection sites and even rotation of the site to prevent myopathy, muscle fibrosis and sterile abscesses
- The site of the injection will be marked on the participant's skin, and they will be instructed to maintain this for the duration of the study

- The lead researcher will implement best infection control practices for IM injections (Hutin et al., 2003)
  - The use of sterile injection equipment, and contamination prevention of injection equipment and solutions
  - Pre-checks of sterile equipment, with contaminated items replaced immediately
  - Wearing gloves and use aseptic non-touch technique when preparing
  - The safe handling of injection equipment, and prevention of needle-stick injuries to the provider
  - Cleaning of the injection site with alcohol wipe prior to administration
  - Prevention of access to needles and safe management and disposal of sharps waste to prevent potential needle stick injury
  - Practice of good hygiene in terms of provider (hand and skin integrity) and participant (site selection and skin preparation)
- A full risk assessment of intramuscular injections and hypertonic saline has been completed and documented
- Participants between the age of 18-45 will only be recruited for this study
- Participants will be screened prior to testing through completing a general health questionnaire. Participants with pre-existing medical conditions such as neurological disorders, blood borne viruses (i.e. HIV, Hepatitis B/C), lower limb injury, sore deep tissues, allergies to protein and long-term medication use will be excluded from participating in the study
  - Should any participants not disclose/be unaware of allergens, the lead researcher is aware of signs and symptoms of anaphylaxis and the appropriate first aid response
- Prior to injection, the injection site will be inspected to ensure it is free from redness, swelling, pain, tenderness infections, abrasions or necrosis.
- Documentation of factors such as solution, product batch number, site, date, time and adverse effects will be made after each IM injection
  - Should any adverse reactions or incidences occur, the completion of the Medical & Healthcare products Regulatory Agency (MHRA) yellow card form will occur, with all equipment used kept and safely stored
- The participants will be instructed to monitor the site two to four hours post-injection to ensure no adverse reactions have occurred (Mallet and Bailey, 1996). Any complications present will be documented.
- Previous contact with a Lead Professor in Pain Neuroscience at Aalborg University (Professor Thomas Graven-Nielsen) who has extensively used IM injection of saline. From over 6000 injections performed, there have been no serious side-effects reported.

Please describe what measures you have in place in the event of any unexpected outcomes or adverse effects to participants arising from involvement in the project

The laboratory contains a defibrillator which can be operated by a trained individual. For all testing sessions, there will be two people present in the laboratory, with a minimum of one individual who is first aid trained.

Will interviews/questionnaires or group discussions include topics that might be sensitive, embarrassing or upsetting, or is it possible that criminal or other disclosures requiring action could occur during the study?

No

If yes, please describe the procedures in place to deal with these issues

N/a

What is the potential benefit to research participants?

Through performing maximal voluntary contractions, participants will gain an accurate measure of quadriceps muscle strength. Additionally, participants will be contributing to further our knowledge, understanding and development of an experimental pain model (intramuscular injection of hypertonic saline), and the role of exercise-induced pain in neuromuscular function.

What are the potential risks to the researchers themselves?
There is the potential of a needle stick injury to occur to the researchers. This risk will however be minimalised through the researchers being appropriately trained and competent to ensure safe practice whilst handling the syringe and needles. During the research study, all intramuscular injections will be administered by Ryan Norbury, Samuel Smith, Adam Hunt or Dr Lex Mauger, all of whom have either declared themselves to be knowingly free of blood-borne viruses and have up to date immunisation for hepatitis B, or are undergoing the course of vaccinations.
Will there be any risks to the University? (Consider issues such as reputational risk; research that may give rise to contentious or controversial findings; could the funder be considered controversial or have the potential to cause reputational risk to the University?)
No
Will any intervention or procedure, which would normally be considered a part of routine care, be withheld from the research participants? (If yes, give details and justification). For example, the disturbance of a school child's day or access to their normal educational entitlement and curriculum).
No

Recruitment and informed consent
How and by whom will potential participants, records or samples be identified?
The lead researcher will recruit participants through posters placed around the School of Sport and Exercise Sciences (Medway Building and Medway Park) and on social media, and through an e-mails distributed to all students in the school. Additionally, adverts may be placed on participant recruitment websites on the internet. Word of mouth from individuals involved in the study to students/staff members of the university.
Will this involve reviewing or screening identifiable personal information of potential participants or any other person? (If 'yes', give details)
Yes. Participants will complete a general health questionnaire and an intramuscular injection risk assessment questionnaire. These will be kept in a locked cabinet by the researcher.
Has prior consent been obtained or will it be obtained for access to identifiable personal information?
Yes. Informed consent will be taken by the researcher.
Will you obtain informed consent from or on behalf of research participants? (If 'yes' please give details. If you are not planning to gain consent, please explain why not).
Yes. All participants will receive a 'Participant Information Sheet', which provides a full written explanation of the study, protocols and procedures. This information sheet also contains an appendix discussing the background and risk assessment of intramuscular injections. Should participants require further information, or have any additional questions, explanations will be provided verbally. Upon the completion of this, if the participants are content to participate, they will provide full written consent through the completion of an 'Informed Consent Form'.
Will you record informed consent in writing? (If 'no', how will it be recorded?)
Yes
How long will you allow potential participants to decide whether or not to take part?
Participants will be provided with a minimum of 24 hours after receiving the Participant Information Sheet, to decide whether to participate.
What arrangements have been made for persons who might not adequately understand verbal explanations or written information given in English, or have special communication needs? (eg, translation, use of interpreters?)

All participants will be strong and capable in speaking and understanding the English language. They will receive both written and verbal explanations of all relevant details in the study. No arrangements will therefore be made

If no arrangements will be made, explain the reasons (eg, resource constraints)

Financial limitations

Confidentiality

In this section personal data means any data relating to a participant who could potentially be identified. It includes pseudonymised data capable of being linked to a participant through a unique code number.

If you will be undertaking any of the following activities at any stage (including in the identification of potential participants) please give details and explain the safeguarding measures you will employ

- Electronic transfer by magnetic or optical media, email or computer networks
- Sharing of personal data outside the European Economic Area
- Use of personal addresses, postcodes, faxes, emails or telephone numbers
- Publication of direct quotations from respondents
- Publication of data that might allow identification of individuals, either directly or indirectly
- Use of audio/visual recording devices
- Storage of personal data on any of the following:
  - Manual files
  - University computers
  - Home or other personal computers
  - Private company computers
  - Laptop computers

All hard files or written data will be stored in a secure locked cabinet. All electronic data will be stored in anonymised format and kept in a password-protected folder on a password-protected laptop computer belonging to the researcher.

How will you ensure the confidentiality of personal data? (eg, anonymisation or pseudonymisation of data)

All data will be anonymised through the use of participant number coding. A master code will be kept in a secure locked cabinet by the researchers.

Who will have access to participants' personal data during the study?

Ryan Norbury
Samuel Smith
Adam Hunt
Dr Lex Mauger

How long will personal data be stored or accessed after the study has ended? (If longer than 12 months, please justify)

The data will be stored for up to four years. This is due to the data being collected as part of a PhD thesis, and thus may be required at a later date for initial analysis.

Please note: as best practice, and as a requirement of many funders, where practical, researchers must develop a data management and sharing plan to enable the data to be made available for re-use, eg, for secondary research, and so sufficient metadata must be conserved to enable this while maintaining confidentiality commitments and the security of data.

Incentives and payments

Will research participants receive any payments, reimbursement of expenses or any other benefits or incentives for taking part in this research? (If 'yes', please give details)

Yes. Participants will receive a total of up to £30 which will be provided pro-rata across the three testing sessions. This payment will account for the travel requirements and time in which the participant is providing for their participation in the laboratory testing.
Will individual researchers receive any personal payment over and above normal salary, or any other benefits or incentives, for taking part in this research? (If 'yes', please give details)
No
Does the Chief Investigator or any other investigator/collaborator have any direct personal involvement (e.g. financial, share holding, personal relationship, etc) in the organisations sponsoring or funding the research that may give rise to a possible conflict of interest? (If 'yes', please give details)
No

Publication and dissemination
How do you intend to report and disseminate the results of the study? If you do not plan to report or disseminate the results please give your justification
The results of the study will be an anonymised cohort results. These results will subsequently be analysed and written up in the form of one or more conference/peer-reviewed papers, which will form the basis of an experimental chapter in the PhD thesis
Will you inform participants of the results? (Please give details of how you will inform participants or justify if not doing so)
Yes. All participants will be able to request a summary of results from testing.

Management of the research		
Other key investigators/collaborators. (Please include all grant co-applicants, protocol authors and other key members of the Chief Investigator's team, including non-doctoral student researchers)		
Dr Lex Mauger Dr Mark Burnley Ryan Norbury Samuel Smith Adam Hunt		
Has this or a similar application been previously rejected by a research Ethics Committee in the UK or another country? (If yes, please give details of rejected application and explain in the summary of main issues how the reasons for the unfavourable opinion have been addressed in this application)		
No		
How long do you expect the study to last?		
• Planned start date: 15/01/19	• Planned end date: 31/12/2019	• Total duration: 11.5 months
Where will the research take place?		
The University of Kent physiology laboratory at Medway Park		

Insurance/indemnity
Does UoK's insurer need to be notified about your project before insurance cover can be provided?

The majority of research carried out at UoK is covered automatically by existing policies, however, if your project entails more than usual risk or involves an overseas country in the developing world or where there is or has recently been conflict, please check with the Insurance Office that cover can be provided. Please give details below.
No. Confirmation has been received that this activity (intramuscular injection of saline solutions) falls within the existing University insurance policy providing the risk assessment in place is strictly adhered to for the duration of the research activity (*see included email correspondence between Nicole Palmer and Dr Lex Mauger).*

Children
Do you plan to include any participants who are children under 16? (If no, go to next section)
No
Please specify the potential age range of children under 16 who will be included and give reasons for carrying out the research with this age group
N/a
Please describe the arrangements for seeking informed consent from a person with parental responsibility and/or from children able to give consent for themselves
N/a
If you intend to provide children under 16 with information about the research and seek their consent or agreement, please outline how this process will vary according to their age and level of understanding
N/a

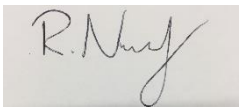
Participants unable to consent for themselves	
Do you plan to include any participants who are adults unable to consent for themselves through physical or mental incapacity? (If yes, the research must be reviewed by an NHS REC or SCREC)	
No	
Is the research related to the 'impairing condition' that causes the lack of capacity, or to the treatment of those with that condition?	
☐ Yes	If 'yes' proceed to next question
☒ No	If 'no' the study should proceed without involving those who do not have the capacity to consent to participation
Could the research be undertaken as effectively with people who do have the capacity to consent to participate?	
☒ Yes	If 'yes' then the study should exclude those without the capacity to consent to participation
☐ No	If 'no' then the inclusion of people without capacity in the study can be justified
Is it possible that the capacity of participants could fluctuate during the research? (If yes, the research must be reviewed by an NHS REC or SCREC)	
No	
Who inside or outside the research team will decide whether or not the participants have the capacity to give consent? What training/experience will they have to enable them to reach this decision?	
Dr Lex Mauger. He has been active researcher for 10 years, including a successful project ethics submission with the NHS, and the completion of NHS Good Clinical Practice Training.	
What will be the criteria for withdrawal of participants?	

A participant who has given consent and subsequently loses the capacity to provide consent will be withdrawn from the study. Participants will be informed both verbally and in written form that they can withdraw from the study at any time, without any disadvantage to themselves.

Declaration

To be signed by the Chief Investigator

- I agree to comply, and will ensure that all researchers involved with the study comply with all relevant legislation, accepted ethical practice, University of Kent policies and appropriate professional ethical guidelines during the conduct of this research project
- If any significant changes are made to the design of the research I will notify the Faculty of Sciences Research Ethics and Advisory Group (REAG) and understand that further review may be required before I can proceed to implement the change(s)
- I agree that I will notify the Faculty of Sciences Research Ethics Advisory Group of any unexpected adverse events that may occur during my research
- I agree to notify the Faculty of Sciences Research Ethics Advisory Group of any complaints I receive in connection with this research project

Signed:  Name: Mr Ryan Norbury	Date: 22/10/18

What to do next

Send your completed form, along with all supporting documentation, to the Faculties Support Office, at fso@kent.ac.uk.

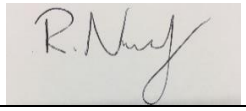
Checklist	
Please ensure you have included the following with your application (*where relevant):	
• Full research proposal (current project)	☒
• Participant information sheet	☒
• Consent form	☒
• *Covering letter	☒
• *Any questionnaires/interview schedules/topic guides to be used	☐
• *Any approved instruments/measures to be used	☐
• *Any advertising material to be used to recruit participants	☒
• *Confirmation that project is covered by UoK insurance policies (if necessary)	☒

3A checklist should be completed for every research project in order to identify whether a full application for ethics approval needs to be submitted. The principal investigator or, where the principal investigator is a student, the supervisor, is responsible for exercising appropriate professional judgement in this review.

This checklist must be completed before potential participants are approached to take part in any research. All forms must be signed by the School's Research Ethics Advisory Group representative.

Section I: Project details			
Project title:	The Effect of Experimental Muscle Pain on Neuromuscular Function and Isometric Exercise Performance		
Planned start date: 15/01/2019		Planned end date:31/12/2019	
Funder:	School of sports and exercise Science, University of Kent		

Section II: Applicant details			
Applicant name:	Ryan Norbury		
School/Department:	School of Sports and Exercise Science		
Email: Rn247@kent.ac.uk		Telephone number:	07943524135
Contact address:	School of Sport and Exercise Sciences Medway Building M0-27 Chatham Maritime Kent ME4 4AG		
Undergraduate ☐	Taught Postgraduate ☐	Research Postgraduate ☒	Staff ☐

Section III: Declaration and signatures			
Please note that it is your responsibility to follow, and to ensure that, all researchers involved with your project follow accepted ethical practice and appropriate professional ethical guidelines in the conduct of your study. You must take all reasonable steps to protect the dignity, rights, safety and well-being of participants. This includes providing participants with appropriate information sheets, ensuring informed consent and ensuring confidentiality in the storage and use of data.			
Applicant signature		**Date**	22/10/2018

Supervisor name	Dr. Lex Mauger		
Supervisor signature		**Date**	14/11/2018

School REAG rep signature (required for both staff and students)		**Date**	

If any question in Section IV(A) are answered 'yes':

3. Contact Nicole Palmer (University Research Ethics & Governance Officer) for advice
4. Send a copy of ethical approval to the Faculties Support Office, once received

If any questions in Sections IV(B) and/or IV(C) and/or IV(D) are answered 'yes':

3. Complete full application form together with supporting documentation
4. Send to the Faculties Support Office for review by the Research Ethics Advisory Group (REAG)

If all questions in Sections IV(A), IV(B), IV(C) and IV(D) are answered 'no':

2. Send the completed and signed form to the Faculties Support Office at fsoethics@kent.ac.uk.

Section IV: Research Checklist

Please answer all questions by ticking the appropriate box:

E) Research that may need to be reviewed by an NHS Research Ethics Committee, the Social Care Research Ethics Committee (SCREC) or other external ethics committee (if *yes*, please give brief details as an annex)	**YES**	**NO**
Will the study involve recruitment of patients through the NHS or the use of NHS patient data or samples?	☐	☒
Will the study involve the collection of tissue samples (including blood, saliva, urine, etc.) or other biological samples from participants, or the use of existing samples?	☐	☒
Will the study involve participants, or their data, from adult social care, including home care, or residents from a residential or nursing care home?	☐	☒
Will the study involve research participants identified because of their status as relatives or carers of past or present users of these services?	☐	☒
Does the study involve participants aged 16 or over who are unable to give informed consent (e.g. people with learning disabilities or dementia)?	☐	☒
Is the research a social care study funded by the Department of Health?	☐	☒
Is the research a health-related study involving prisoners?	☐	☒
Is the research a clinical investigation of a non-CE Marked medical device, or a medical device which has been modified or is being used outside its CE Mark intended purpose, conducted by or with the support of the manufacturer or another commercial company to provide data for CE marking purposes? (a CE mark signifies compliance with European safety standards)	☐	☒
Is the research a clinical trial of an investigational medicinal product or a medical device?	☐	☒

F) Research that may need full review by the Sciences REAG	**YES**	**NO**
Does the research involve other vulnerable groups: eg, children; those with cognitive impairment?	☐	☒

Is the research to be conducted in such a way that the relationship between participant and researcher is unequal (eg, a subject may feel under pressure to participate in order to avoid damaging a relationship with the researcher)?	☐	☒
Does the project involve the collection of material that could be considered of a sensitive, personal, biographical, medical, psychological, social or physiological nature.	☐	☒
Will the study require the cooperation of a gatekeeper for initial access to the groups or individuals to be recruited (eg, headmaster at a School; group leader of a self-help group)?	☐	☒
Will it be necessary for participants to take part in the study without their knowledge and consent at the time? (eg, covert observation of people in non-public places?)	☐	☒
Will the study involve discussion of sensitive topics (eg, sexual activity; drug use; criminal activity)?	☐	☒
Are drugs, placebos or other substances (eg, food substances, vitamins) to be administered to the study participants or will the study involve invasive, intrusive or potentially harmful procedures of any kind?	☒	☐
Is pain or more than mild discomfort likely to result from the study?	☒	☐
Could the study induce psychological stress or anxiety or cause harm or negative consequences beyond the risks encountered in normal life?	☐	☒
Will the study involve prolonged or repetitive testing?	☒	☐
Will the research involve administrative or secure data that requires permission from the appropriate authorities before use?	☐	☒
Is there a possibility that the safety of the researcher may be in question (eg, international research; locally employed research assistants)?	☐	☒
Does the research involve participants carrying out any of the research activities themselves (i.e. acting as researchers as opposed to just being participants)?	☐	☒
Will the research take place outside the UK? You may find the find the Proportionate Risk Assessment document useful.	☐	☒
Will the outcome of the research allow respondents to be identified either directly or indirectly (eg, through aggregating separate data sources gathered from the internet)?	☐	☒
Will research involve the sharing of data or confidential information beyond the initial consent given?	☐	☒
Will financial inducements (other than reasonable expenses and compensation for time) be offered to participants?	☐	☒
Are there any conflicts of interest with the proposed research/research findings? (eg, is the researcher working for the organisation under research or might the research or research findings cause a risk of harm to the participants(s) or the researcher(s) or the institution?)	☐	☒
Will the study involve the publication, sharing or potentially insecure electronic storage and/or transfer of data that might allow identification of individuals, either directly or indirectly? (e.g. publication of verbatim quotations from an online forum; sharing of audio/visual recordings; insecure transfer of personal data such as addresses, telephone numbers etc.; collecting identifiable personal data on unprotected** internet sites.) [**Please note that Qualtrics and Sona Systems provide adequate data security and comply with the requirements of the EU-US Privacy Shield.]	☐	☒

G) Security Sensitive Material	**YES**	**NO**
Does your research involve access to or use of material covered by the Terrorism Act? (The Terrorism Act (2006) outlaws the dissemination of records, statements and other documents that can be interpreted as promoting and endorsing terrorist acts. By answering 'yes' you are registering your legitimate use of this material with the Research Ethics	☐	☒

Advisory Group. In the event of a police investigation, this registration will help you to demonstrate that your use of this material is legitimate and lawful).		

H) Prevent Agenda	**YES**	**NO**
Does the research have the potential to radicalise people who are vulnerable to supporting terrorism or becoming terrorists themselves?	☐	☒

If the answer to any questions in Sections IV(B) and/or IV(C) and/or IV(D) is 'yes', please complete the full application form and send to the Faculties Support Office at fsoethics@kent.ac.uk together with required supporting documentation.

Appendix 3: Ethics Forms for Chapter Five (Study Three)

School of Sport & Exercise Sciences
Research Ethics and Advisory Group (REAG)
University of Kent at Medway
Chatham Maritime
Kent
ME4 4AG

Ethics Reference: Prop 19_2019_20

Date: 16.01.20

Dear Ryan,

Re: The Effect of Experimental Muscle Pain on the Fatigue of a Contralateral Non-Painful Muscle

I am delighted to confirm that SSES REAG has approved your research study (Prop 19_2019_20) and you are now permitted to recruit participants and commence your research.

If you need to amend any aspect of your research, please ensure you inform SSES REAG by completing a request for amendment form and submitting all revised paperwork (e.g. participant information sheet, questionnaires).

If there should happen to be any adverse event during your study, please also ensure SSES REAG is kept informed.

I hope your study is successful.

With kind regards,

K. Hambly

Karen Hambly

If any of the questions in Sections IV(B) and/or IV(C) and/or IV(D) is answered 'yes', a full ethics application must be made to the REAG. This also applies for studies not defined as 'research' in the narrow sense, i.e. evaluations/audits, etc. Complete this form and send it to the Faculties Support Office along with supporting documentation: a copy of the full research proposal; any participant information sheets and consent forms; any surveys, interview schedules; any advertising material or proposed website wording. **It is important to note that you must not commence any research with human participants until full approval has been given by the SSES Research Ethics Advisory Group – you will be notified via email when this has been granted.**

During term time we aim to process a research ethics application within two weeks, however during vacation periods and busy times (e.g. exams and marking period) it can take up to four weeks. It is the applicant's responsibility to ensure that their application is submitted in good time.
Overview
Name of Applicant(s)
Mr Ryan Norbury
Contact Details (Please include your UoK address, email and telephone number)
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Title of Project
The Effect of Experimental Muscle Pain on the Fatigue of a Contralateral Non-Painful Muscle
Lay Summary (Please provide a brief summary of the study)
Strong muscle pain which is experimentally induced by the intramuscular injection of hypertonic saline has previously been found to cause a reduction in maximal muscle strength, muscle activity and endurance performance in the injected painful muscle. Previous work has investigated the mechanisms which underpin these changes. It has been found that these reductions are likely mediated by changes at the central nervous system (i.e. inhibitory alterations to the brain and spine) while the periphery (i.e. muscle) remains largely unaffected. Therefore if pain induced changes are at the central level, it is possible that muscle pain could have a non-local effect and therefore reduce the muscle strength, activity and endurance of a non-painful muscle. To experimentally test this hypothesis, an intramuscular injection of 1ml of hypertonic saline to cause pain in the left quadriceps could be performed. Then a subsequent assessment of the endurance and neuromuscular function of the contralateral right quadriceps could be performed. On a different visit, another injection could be performed using isotonic saline which does not cause pain could be used to compare differences in fatigue between painful and non-painful conditions. The hypertonic saline method of inducing muscle pain is useful because it allows for the induction of pain without causing other undesired effects that might influence the performance of the contralateral limb.
Name of Supervisor(s) (If applicable)
Dr Lex Mauger Dr Mark Burnley

Risks and ethical issues

Please list the principal inclusion and exclusion criteria

The participants (n = 10-15) will be healthy male or female participants aged 18 to 45 years old. Prior to testing, potential participants will be required to complete a general health questionnaire, a screening form for transcranial magnetic stimulation and an intramuscular injection risk assessment questionnaire to ascertain their ability to participate in maximum and submaximal contractions, receive transcranial magnetic stimulation and receive intramuscular injections.

The exclusion criteria is as follows:

- Participants with a lower limb injury that occurred in the past three months
- Participants with pre-existing medical conditions (neurological disorders, pain disorders and blood borne viruses (i.e. HIV, Hepatitis B/C))
- Participants with long-term medication use for the treatment of pain
- Participants with any allergy (e.g. nuts, fish, milk, egg, wheat and soya)
- Those who have metallic hardware in close proximity to the head (such as cochlear implants, or an Internal Pulse Generator or medication pumps)

How long will each research participant be in the study in total, from when they give informed consent until their last contact with the research team?

Three to four weeks which will last a total duration of approximately 4.5-5 hours.

What are the potential risks and burdens for research participants and how will you minimise them? (Describe any risks and burdens that could occur as a result of participation in the research, such as pain, discomfort, distress, intrusion, inconvenience or changes to lifestyle. Describe what steps would be taken to minimise risks and burdens as far as possible)

Exercise. Participants may experience the usual risks associated with the performance of multiple voluntary contractions (MVC) such as joint sprains and muscle strains, however isometric MVCs have been performed in hundereds of different studies without causing injury and is generally accepted as a low-risk activity. Nonetheless, a thorough warm-up consisting of graded submaximal contractions will be performed to reduce the risk of muscle injury. The exercise protocol is a ~20% submaximal isometric contraction which is well within the capacity of the neuromuscular system and will carry a very-low risk of injury as well as cause minimal levels of muscle damage. Post-exercise muscle fatigue and soreness (up 48 hours post-exercise) is still a potential burden for participants, however the experience of muscle fatigue will dissipate rapidly (within 10 minutes) and soreness should be considerably low in healthy and recreationally active participants as isometric exercise causes much less muscle damage than traditional isotonic exercise. Additionally, the repeated bout effect will attenuate exercise induced muscle damage after the first familiarisation session of the exercise protocol. The risk assessment for exercise on an isokinetic dynamometer can be found here under SSESRA34.

Electrical Stimulation. The use of electrical stimulation (ES) of the femoral nerve will innervate the quadriceps muscle. ES are delivered in short duration pulses (~200μs) at an amplitude which does not exceed 400mA. This evokes brief twitches of the innervated muscle mass. The safety of the technique is well documented and is unlikely to cause complications or injury. However, it may cause some discomfort for participants at higher stimulation intensities. Because of the short duration of the stimulations, the discomfort is very short lived resulting in no longer than a few seconds of discomfort over the course of an experimental trial. As a result, ES tends to be well tolerated among most individuals. Each investigator is trained in ES to minimise the discomfort received during the measurements and complies with the risk assessment guidelines. The risk assessment for transcutaneous peripheral electrical nerve stimulation can be found here under SSESRA68.

Transcranial Magnetic Stimulation. Single pulse transcranial magnetic stimulation (TMS) of the motor cortex is generally regarded as safe for the participants (Rossi et al., 2009) and is a measurement technique that has been safely used in thousands of published papers. 1ms pulses are delivered a stimulator intensity which is around 60-70% of maximum stimulator output. One of the primary risks with TMS is the induction of a seizure. However, since 1998, there have only been four reported cases of seizures (date of report: Dec. 2008). Three out of four of these cases were caused in participants

that were taking pro-epileptogenic medication (Rossi et al. 2009). Syncope is also a cited concern with TMS, however this appears to be an epiphenomenon to TMS and is again unlikely to occur. In the rare instance syncope, participants will be lay down supine with the legs elevated until participants regain consciousness. Similar to ES, TMS may induce some discomfort at higher stimulation intensities. The use of TMS is limited to minimise the amount of discomfort received. TMS is usually well tolerated by most individuals. Each investigator will have training on how to competently perform TMS to minimise the discomfort which may occur. The risk assessment for transcranial magnetic stimulation can be found here under SSESRA69.

Intramuscular Injection. Intramuscular injection of hypertonic saline as an experimental model of exercise-induced pain has previously been implemented within the present institution and has been approved for previous on multiple occasions. An identical protocol to these studies will be used in this studies. See ethics refs:

Prop 139_2017_17
Prop 84_2016_17
Prop 140_2016_17
Prop 1_2018_19
Prop 30_2018_19

However, it is recognised that this method may still cause ethical concern. As stated, this model has been applied in multiple studies conducted by the lead investigator, supervisor and one of the investigators of the proposed study. Combined, we have administered over 150 safe and successful injections with zero complications related to the injection. Each investigator of the study has received formal training from a registered medical practitioner and has been signed off as competent. All previous intramuscular injections have been administered and documented in line with NHS best practice and the School of Sport and Exercise Science IM injection risk assessment. The proposed study will build upon an extensive range of literature from various institutions located in Australia, Denmark, France, Germany, Sweden and USA where this method has been safely and successfully employed (Capra and Ro, 2000; Deschamps, Hug, Hodges, Tucker, 2014; Graven-Nielsen, Svensson and Arendt-Nielsen, 1997b; Khan et al., 2011; Schilder et al., 2014; Schulte et al., 2003), all of which have safely employed this method without any reported issues. A detailed background and risk assessment of this method is presented below or can be found here under SSESRA20. An extensive background of the safety and applicability of hypertonic saline will now be outlined:

1.1. Preface

Currently, hypertonic saline (we plan to use Braun's sterile 5,85 % Sodium Chloride (PZN: 3158635)) is licenced for infusion, but not for intramuscular injection. This is because hypertonic saline is used to medically treat conditions such as hyponatremia (electrolyte imbalance in the blood), but has no medical use for intramuscular injection. Therefore, the aim of this section is to provide evidence and assurance that intramuscular injection of 5.85% saline is a common technique used in research, bears no risk above that of a standard intramuscular injection and that this technique is required to complete our planned programme of research. Research by Henriksen et al. (2007) and Danneskiold-Samsøe et al. (2007) has used the same volume and concentration into the vastus medialis while Ciubotariu, Arendt-Nielsen and Graven-Nielsen (2004) has used 1ml of 6%NaCl into the tibialis anterior without issue. Therefore greater volumes and concentrations into smaller areas has been shown to be safe. We will inject it into the vastus lateralis as this site poses less risk than the previously used vastus medialis. This exact protocol has been used in several studies internally (ethics references: Prop 139_2017_17, Prop 84_2016_17, Prop 140_2016_17, Prop 1_2018_19, Prop 30_2018_2019). A 5.8% concentration is used as this is what is commercially available and creates an appropriate amount of muscle pain when 1ml is injected.

1.2. Background

Intramuscular injections are a common method to administer medications, drugs and vaccines, via the use of a syringe and needle, directly injected deep into the muscle tissue, below the muscle fascia and under the fatty subcutaneous layer (Boyd et al. 2013). This technique is a relatively simple process, is not defined as a medical procedure and therefore

requires a clean procedure rather than aseptic. As the skeletal muscle is suggested to have scarcer nociceptors than subcutaneous tissue, IM injections involve decreased discomfort, and dependent on the site of injection (i.e. deltoid, vastus lateralis, rectus femoris, ventrogluteal or dorsogluteal), this method enables comparatively large volumes of a substance to be quickly absorbed by the body, with a relatively prolonged action (Rodger and King, 2000).

When implementing IM injections, several considerations and decisions need to take place:

- Injection site (dependent on age, physical status and volume of the injectate)
- Substance to be injected
- Technique
- Equipment
- Environment in which injection is administered

It is proposed that the experimental pain model implemented in the proposed non-clinical studies is the intramuscular injection of 1ml hypertonic saline (5.8% concentration). Hypertonic saline is a strong, sterile solution of salt water, with a concentration of sodium chloride greater than 0.9% (Mortimer and Jancik, 2006). It primarily functions by an osmotic effect, but can also be utilised for other clinical effects (hemodynamic, vasoregulatory, immunomodulatory, neurochemical and hypernatremic) (Doyle, Davis and Hoyt, 2001).

Additionally, after first being implemented by Kellgren in the late 1930s (Kellgren, 1938), hypertonic saline has since been extensively been used as an experimental model that characterises both the sensory and motor effects involved in muscle pain (Burton, Fazalbhoy and Macefield, 2016). This is predominantly due to the quality of the pain induced by IM injections of hypertonic saline effectively mimicking acute muscle pain, with the pain produced displaying localized and referred characteristics (Graven-Nielsen and Mense, 2001). Dependent on injection site and volume of solution, a bolus IM injection of hypertonic saline temporarily induces pain that can lasts ~5.4 minutes, with a mean pain intensity rating of ~5 (strong pain) on the Cook 0-10 scale (Cook, O'Connor, Eubanks, Smith and Lee, 1997). This therefore allows the acute effects of pain to be studied (Burton et al. 2016).

The medical application of hypertonic saline is through intravenous infusion, for which the solution is licensed. At present, 5.8% hypertonic saline is not licensed for intramuscular injection because there is no medical basis for this method of application. However, as demonstrated by previous animal studies, this method does not cause any muscle toxicity (Svendsen, Edwards and Rasmussen, 2001), and the pain caused is unlikely to be related to tissue damage (Svendsen, Edwards, Lauritzen and Rasmussen, 2005). Thus this method can be deemed to be safe and acceptable for use in human experimentation (Graven-Nielsen, Lund, Arendt-Nielsen, Danneskiold-Samsøe and Bliddal, 2002).

Indeed, IM injections of hypertonic saline have been applied in over 35 studies ranging from 1938 to the present day, with no adverse effects reported (for example; Arendt-Nielsen, Graven-Nielsen, Svarrer and Svensson, 1996; Feinstein, Langton, Jameson and Schiller, 1954; Kennedy, McNeil, Gandevia and Taylor, 2016; Kellgren, 1938; Henriksen, Alkjær, Simonsen and Bliddal, 2009; Hodges, Moseley, Gabrielsson and Gandevia, 2003; Veerasarn and Stohler, 1992; Yavuz, Negro, Falla and Farina, 2015). Furthermore, we have contacted Professor Thomas Graven-Nielsen (http://vbn.aau.dk/en/persons/thomas-gravennielsen(474a635b-4e83-4b02-a90b-80d417d37e52)/cv.html?id=60483613), a leading professor in pain neuroscience who has extensively used this method (for example; Graven-Nielsen, Arendt-Nielsen, Svensson and Jensen, 1997a, Graven-Nielsen et al., 1997b; Graven-Nielsen, Babenko, Svensson and Arendt-Nielsen, 1998a, Graven-Nielsen et al., 1998b; Graven-Nielsen et al., 2002; Graven-Nielsen et al., 2003) for advice and guidance on the use of hypertonic saline. Prof. Graven-Nielsen stated that he has performed over 6000 injections, with no adverse effects having occurred and is therefore very confident in the safety of the technique.

When injected into the muscle, hypertonic saline predominantly causes the depolarisation of unmyelinated group IV muscle afferent units which directly stimulates the release of neuropeptides such as substance P at the periphery (Laursen, Graven-Nielsen, Jensen and Arendt-Nielsen, 1999). This means that a particular tissue and type of pain can be specifically targeted (Graven-Nielsen and Mense, 2001). However, at present, the mechanisms of muscle nociceptive processing excited by the intramuscular injection of hypertonic saline are uncertain (Mense, 2013). Several proposals have nonetheless been made, including: activation by augmented tonicity in the interstitial space, activation by ionic

alterations and indirect activation by further algesic substances released from the muscle tissue or the nociceptive ending (Kress and Reeh, 1996).

In the present study, the hypertonic saline solution is planned to be injected into the vastus lateralis (middle third of the lateral aspect of the thigh between the greater trochanter and the lateral femoral condyle of the femur). This site provides a large muscle mass, is easy to access, has a reduced likelihood of injury and is not associated with any major blood vessels or significant nerve structures, minimising risks of damage (Dougherty and Lister, 2011). Additionally, the volume of fluid that is proposed to be injected into this site (1ml) is well below the maximal recommended volume of 5ml for any substance that is injected into a large muscle mass (Roger and King, 2000). This volume is consistent with previous literature (Deschamps, Hug, Hodges and Tucker, 2014; Henriksen et al., 2007; Henriksen et al., 2009; Khan, McNeil, Gandevia and Taylor, 2011) and also below that utilised in other studies, where 1.5ml has been formerly applied (Ervilha, Farina, Arendt-Nielsen and Graven-Nielsen, 2005; Graven-Nielsen et al., 2002; Hodges et al., 2003).

1.3. Associated Risks and Side-effects

As a routine technique for drug administration for over one hundred and fifty years, IM injections are considered to be a method that is simple and safe, with the rare occurrence of complications or side-effects (Svendsen et al., 2005). In addition, the IM injection of hypertonic saline carries no further risk beyond which would occur from the IM injection of any substance (i.e. allergic or anaphylactic reaction), with this solution not causing muscle toxicity or tissue damage (Svendsen et al., 2001; Svendsen et al., 2005). Nonetheless, an IM injection can solely still result in several potential complications (Small, 2004) which could arise as a result of unsafe injections and poor technique. Although mainly preventable with trained and safe IM practice, it is important that these potential risks are taken into consideration. Potential complications of an IM injection include:

- Pain or mild discomfort for a short period after an injection is common
- Some bruising at the injection site may occur
- Muscle fibrosis can occur with repeated use of the same injection site
- An increased risk of injecting the substance intravenously if the needle is too deep
- Needle stick injury to the experimenter
- In more serious cases, nerve injury resulting in potential paralysis, atrophy, haematoma, bone injury, cellulitis, and sterile abscesses can occur
- Accidental femoral nerve damage due to incorrect needle placement and muscle atrophy from IM injection overuse are the predominant risks associated with the vastus lateralis site

Most of the complications highlighted previously may occur at any site of injection, and could potentially due to an inappropriate depth or rate of injection, or injecting into an incorrect site (Malkin, 2008).

1.4. Interventions in Place to Reduce Risk

A safe injection is defined as 'one that does not harm the recipient, does not expose the provider to any avoidable risk, and does not result in waste that is dangerous to other people' (Hutin et al., 2003: 492). In order to achieve a safe injection, and reduce the risks associated with intramuscular injections the researcher will implement several precautions. These are detailed below:

- The lead researcher has received appropriate NHS training and guidelines, followed by a subsequent competency assessment of supervised practice to ensure safe, competent and consistent best practice when administering IM injections
    - Thorough understanding of injection site to reduce the likelihood of nerve injury and accidental intravenous injection
    - Performance of pre-injection checks (check drug name and ampule, check drug unit quantity or %, check expiry date, inspect drug for cloudiness and particles)
    - The use of appropriate syringe (Luer Lok) and needle size to ensure the solution is delivered to the muscle
    - The use of the Z track technique to minimise the pain experienced and reduce the likelihood of complications

- Aspiration prior to delivery to ensure that the needle is not in a blood vessel. If blood appears, remove the needle and restart the process.
- Quick insertion of needle at 90^{o} followed by a slow injection and then quick withdrawal of the needle 10 seconds after the injection has occurred
- The documentation of solution administration, injection sites and even rotation of the site to prevent myopathy, muscle fibrosis and sterile abscesses
- The site of the injection will be marked on the participant's skin, and they will be instructed to maintain this for the duration of the study

- The lead researcher will implement best infection control practices for IM injections (Hutin et al., 2003)
  - The use of sterile injection equipment, and contamination prevention of injection equipment and solutions
  - Pre-checks of sterile equipment, with contaminated items replaced immediately
  - Wearing gloves and use aseptic non-touch technique when preparing
  - The safe handling of injection equipment, and prevention of needle-stick injuries to the provider
  - Cleaning of the injection site with alcohol wipe prior to administration
  - Prevention of access to needles and safe management and disposal of sharps waste to prevent potential needle stick injury
  - Practice of good hygiene in terms of provider (hand and skin integrity) and participant (site selection and skin preparation)
- A full risk assessment of intramuscular injections and hypertonic saline has been completed and documented
- Participants between the age of 18-45 will only be recruited for this study
- Participants will be screened prior to testing through completing a general health questionnaire. Participants with pre-existing medical conditions such as neurological disorders, blood borne viruses (i.e. HIV, Hepatitis B/C), lower limb injury, sore deep tissues, allergies to protein and long-term medication use will be excluded from participating in the study
  - Should any participants not disclose/be unaware of allergens, the lead researcher is aware of signs and symptoms of anaphylaxis and the appropriate first aid response
- Prior to injection, the injection site will be inspected to ensure it is free from redness, swelling, pain, tenderness, infections, abrasions or necrosis.
- Documentation of factors such as solution, product batch number, site, date, time and adverse effects will be made after each IM injection
  - Should any adverse reactions or incidences occur, the completion of the Medical & Healthcare products Regulatory Agency (MHRA) yellow card form will occur, with all equipment used kept and safely stored
- The participants will be instructed to monitor the site two to four hours post-injection to ensure no adverse reactions have occurred (Mallet and Bailey, 1996). Any complications present will be documented.
- Previous contact with a Lead Professor in Pain Neuroscience at Aalborg University (Professor Thomas Graven-Nielsen) who has extensively used IM injection of saline. From over 6000 injections performed, there have been no serious side-effects reported.

Please describe what measures you have in place in the event of any unexpected outcomes or adverse effects to participants arising from involvement in the project

The Medway building contains a defibrillator which can be operated by a trained individual. For all experimental sessions using TMS, there will be two investigators present in the laboratory, with a minimum of one these individuals being first aid trained. There will also be a first aid kit present in the lab. Therefore immediate care can be provided when needed and the procedures for subsequent treatment are in place.

Will interviews/questionnaires or group discussions include topics that might be sensitive, embarrassing or upsetting, or is it possible that criminal or other disclosures requiring action could occur during the study?

No

If yes, please describe the procedures in place to deal with these issues

n/a

What is the potential benefit to research participants?
Through performing maximal voluntary contractions, participants will gain an accurate measure of their quadriceps muscle strength. Additionally, participants will be contributing to further our knowledge, understanding and development of an experimental pain model (intramuscular injection of hypertonic saline), and the role of exercise-induced pain in neuromuscular function.
What are the potential risks to the researchers themselves?
There is the potential of a needle stick injury to occur to the researchers. This risk will however be minimalised through the researchers being appropriately trained and competent to ensure safe practice whilst handling the syringe and needles. During the research study, all intramuscular injections will be administered by Ryan Norbury, Samuel Smith or Dr Lex Mauger, all of whom have either declared themselves to be knowingly free of blood-borne viruses and have up to date immunisation for hepatitis B.
Will there be any risks to the University? (Consider issues such as reputational risk; research that may give rise to contentious or controversial findings; could the funder be considered controversial or have the potential to cause reputational risk to the University?)
No
Will any intervention or procedure, which would normally be considered a part of routine care, be withheld from the research participants? (If yes, give details and justification). For example, the disturbance of a school child's day or access to their normal educational entitlement and curriculum).
No

Recruitment and informed consent
How and by whom will potential participants, records or samples be identified?
The lead researcher will recruit participants through posters placed around the School of Sport and Exercise Sciences (Medway Building and Medway Park) and on social media, and through an e-mails distributed to all students in the school. Additionally, adverts may be placed on participant recruitment websites on the internet. Word of mouth from individuals involved in the study to students/staff members of the university.
Will this involve reviewing or screening identifiable personal information of potential participants or any other person? (If 'yes', give details)
Yes. Participants will complete a general health questionnaire and an intramuscular injection risk assessment questionnaire. These will be kept in a locked cabinet by the researcher.
Has prior consent been obtained or will it be obtained for access to identifiable personal information?
Yes. Informed consent will be taken by the researcher.
Will you obtain informed consent from or on behalf of research participants? (If 'yes' please give details. If you are not planning to gain consent, please explain why not).
Yes. All participants will receive a 'Participant Information Sheet', which provides a full written explanation of the study, protocols, risks and benefits. This information sheet also contains an appendix discussing the background and risk assessment of intramuscular injections. Should participants require further information, or have any additional questions, explanations will be provided verbally. Upon the completion of this, if the participants are content to participate, they will provide full written consent through the completion of an 'Informed Consent Form'
Will you record informed consent in writing? (If 'no', how will it be recorded?)
Yes
How long will you allow potential participants to decide whether or not to take part?

Participants will be provided with a minimum of 24 hours after receiving the Participant Information Sheet, to decide whether to participate.

What arrangements have been made for persons who might not adequately understand verbal explanations or written information given in English, or have special communication needs? (eg, translation, use of interpreters?)

None

If no arrangements will be made, explain the reasons (eg, resource constraints)

Financial limitations

Confidentiality

In this section personal data means any data relating to a participant who could potentially be identified. It includes pseudonymised data capable of being linked to a participant through a unique code number.

If you will be undertaking any of the following activities at any stage (including in the identification of potential participants) please give details and explain the safeguarding measures you will employ

- Electronic transfer by magnetic or optical media, email or computer networks
- Sharing of personal data outside the European Economic Area
- Use of personal addresses, postcodes, faxes, emails or telephone numbers
- Publication of direct quotations from respondents
- Publication of data that might allow identification of individuals, either directly or indirectly
- Use of audio/visual recording devices
- Storage of personal data on any of the following:
  - Manual files
  - University computers
  - Home or other personal computers
  - Private company computers
  - Laptop computers

All hard files or written data will be stored in a secure locked cabinet. All electronic data will be stored in anonymised format and kept in a password-protected folder on a password-protected laptop computer belonging to the researcher.

How will you ensure the confidentiality of personal data? (eg, anonymisation or pseudonymisation of data)

All data will be anonymised through the use of participant number coding. A master code will be kept in a secure locked cabinet by the researchers.

Who will have access to participants' personal data during the study?

Ryan Norbury
Samuel Smith
Dr Lex Mauger
Dr Mark Burnley

How long will personal data be stored or accessed after the study has ended? (If longer than 12 months, please justify)

The data will be stored for up to four years. This is due to the data being collected as part of a PhD thesis, and thus may be required at a later date for initial analysis.

Please note: as best practice, and as a requirement of many funders, where practical, researchers must develop a data management and sharing plan to enable the data to be made available for re-use, eg, for secondary research,

and so sufficient metadata must be conserved to enable this while maintaining confidentiality commitments and the security of data.

Incentives and payments
Will research participants receive any payments, reimbursement of expenses or any other benefits or incentives for taking part in this research? (If 'yes', please give details)
Yes. Participants will receive up to £50 which will be provided pro-rata across the four testing sessions. This payment will account for the travel requirements and time in which the participant is providing for their participation in the laboratory testing.
Will individual researchers receive any personal payment over and above normal salary, or any other benefits or incentives, for taking part in this research? (If 'yes', please give details)
No
Does the Chief Investigator or any other investigator/collaborator have any direct personal involvement (e.g. financial, share holding, personal relationship, etc) in the organisations sponsoring or funding the research that may give rise to a possible conflict of interest? (If 'yes', please give details)
No

Publication and dissemination
How do you intend to report and disseminate the results of the study? If you do not plan to report or disseminate the results please give your justification
The results of the study will be an anonymised cohort results. These results will subsequently be analysed and written up in the form of one or more conference/peer-reviewed papers, which will form the basis of an experimental chapter in the PhD thesis
Will you inform participants of the results? (Please give details of how you will inform participants or justify if not doing so)
Yes. All participants will be able to request a summary of results from testing.

Management of the research
Other key investigators/collaborators. (Please include all grant co-applicants, protocol authors and other key members of the Chief Investigator's team, including non-doctoral student researchers)
Dr Lex Mauger (Primary Supervisor) Dr Mark Burnley (Secondary Supervisor) Ryan Norbury (Lead Investigator) Samuel Smith (Investigator) Megan Judge (Investigator)
Has this or a similar application been previously rejected by a research Ethics Committee in the UK or another country? (If yes, please give details of rejected application and explain in the summary of main issues how the reasons for the unfavourable opinion have been addressed in this application)
No
How long do you expect the study to last?

• Planned start date: 01/01/20	• Planned end date: 31/12/20	• Total duration: 12 Months
Where will the research take place?		
The psychobiological laboratory in the Medway Building (M0-02) at the University of Kent Medway Campus		

Insurance/indemnity
Does UoK's insurer need to be notified about your project before insurance cover can be provided? *The majority of research carried out at UoK is covered automatically by existing policies, however, if your project entails more than usual risk or involves an overseas country in the developing world or where there is or has recently been conflict, please check with the Insurance Office that cover can be provided. Please give details below.*
No. Confirmation has been received that this activity (intramuscular injection of saline solutions) falls within the existing University insurance policy providing the risk assessment in place is strictly adhered to for the duration of the research activity (*see included email correspondence between Nicole Palmer and Dr Lex Mauger).*

Children
Do you plan to include any participants who are children under 16? (If no, go to next section)
No
Please specify the potential age range of children under 16 who will be included and give reasons for carrying out the research with this age group
n/a
Please describe the arrangements for seeking informed consent from a person with parental responsibility and/or from children able to give consent for themselves
n/a
If you intend to provide children under 16 with information about the research and seek their consent or agreement, please outline how this process will vary according to their age and level of understanding
n/a

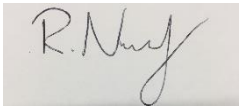
Participants unable to consent for themselves	
Do you plan to include any participants who are adults unable to consent for themselves through physical or mental incapacity? (If yes, the research must be reviewed by an NHS REC or SCREC)	
No	
Is the research related to the 'impairing condition' that causes the lack of capacity, or to the treatment of those with that condition?	
☐ Yes	If 'yes' proceed to next question
☒ No	If 'no' the study should proceed without involving those who do not have the capacity to consent to participation
Could the research be undertaken as effectively with people who do have the capacity to consent to participate?	
☒ Yes	If 'yes' then the study should exclude those without the capacity to consent to participation
☐ No	If 'no' then the inclusion of people without capacity in the study can be justified
Is it possible that the capacity of participants could fluctuate during the research? (If yes, the research must be reviewed by an NHS REC or SCREC)	

No
Who inside or outside the research team will decide whether or not the participants have the capacity to give consent? What training/experience will they have to enable them to reach this decision?
Dr Lex Mauger. He has been active researcher for >10 years, including a successful project ethics submission with the NHS, and the completion of NHS Good Clinical Practice Training.
What will be the criteria for withdrawal of participants?
A participant who has given consent and subsequently loses the capacity to provide consent will be withdrawn from the study. Participants will be informed both verbally and in written form that they can withdraw from the study at any time, without any disadvantage to themselves.

Declaration

To be signed by the Chief Investigator

- I agree to comply, and will ensure that all researchers involved with the study comply with all relevant legislation, accepted ethical practice, University of Kent policies and appropriate professional ethical guidelines during the conduct of this research project
- If any significant changes are made to the design of the research I will notify the SSES Research Ethics and Advisory Group (REAG) and understand that further review may be required before I can proceed to implement the change(s)
- I agree that I will notify the SSES Research Ethics Advisory Group of any unexpected adverse events that may occur during my research
- I agree to notify the SSES Research Ethics Advisory Group of any complaints I receive in connection with this research project

Signed: 	Date: 24/10/19
Name: Ryan Norbury	

What to do next

Send your completed form, along with all supporting documentation, to the SSES REAG, at N.Khan-360@kent.ac.uk.

Checklist	
Please ensure you have included the following with your application (*where relevant):	
• Full research proposal (current project)	☒
• Participant information sheet	☒
• Consent form	☒
• *Covering letter	☒
• *Any questionnaires/interview schedules/topic guides to be used	☐

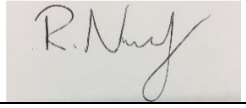
• *Any approved instruments/measures to be used • *Any advertising material to be used to recruit participants • *Confirmation that project is covered by UoK insurance policies (if necessary)	☐ ☒ ☒

A checklist should be completed for every research project in order to identify whether a full application for ethics approval needs to be submitted. The principal investigator or, where the principal investigator is a student, the supervisor, is responsible for exercising appropriate professional judgement in this review.

This checklist must be completed before potential participants are approached to take part in any research. All forms must be signed by the School's Research Ethics Advisory Group representative.

Section I: Project details			
Project title:	The Effect of Experimental Muscle Pain on the Fatigue of a Contralateral Non-Painful Muscle		
Planned start date: 01/01/20		Planned end date: 31/12/20	
Funder:	School of Sports and Exercise Science, University of Kent		

Section II: Applicant details			
Applicant name:	Ryan Norbury		
School/Department:	School of Sports and Exercise Science		
Email: Rn247@kent.ac.uk		Telephone number:	01634 888806
Contact address:	School of Sport and Exercise Sciences Medway Building M2-30 Chatham Maritime Kent ME4 4AG		
Undergraduate ☐	Taught Postgraduate ☐	Research Postgraduate ☒	Staff ☐

Section III: Declaration and signatures			
Please note that it is your responsibility to follow, and to ensure that, all researchers involved with your project follow accepted ethical practice and appropriate professional ethical guidelines in the conduct of your study. You must take all reasonable steps to protect the dignity, rights, safety and well-being of participants. This includes providing participants with appropriate information sheets, ensuring informed consent and ensuring confidentiality in the storage and use of data.			
Applicant signature		**Date**	24/10/2019

Supervisor name	Dr Lex Mauger	**Date**	01/11/2019
Supervisor signature			

School REAG rep signature (required for both staff and students)		**Date**	

If any question in Section IV(A) are answered 'yes':

5. Contact Nicole Palmer (University Research Ethics & Governance Officer) for advice
6. Send a copy of ethical approval to the Faculties Support Office, once received

If any questions in Sections IV(B) and/or IV(C) and/or IV(D) are answered 'yes':

5. Complete full application form together with supporting documentation
6. Send to the N.Khan-360@kent.ac.uk for review by SSES Research Ethics Advisory Group (REAG)

If all questions in Sections IV(A), IV(B), IV(C) and IV(D) are answered 'no':

3. Send the completed and signed form to the SSES REAG at N.Khan-360@kent.ac.uk

Section IV: Research Checklist

Please answer all questions by ticking the appropriate box:

I) Research that may need to be reviewed by an NHS Research Ethics Committee, the Social Care Research Ethics Committee (SCREC) or other external ethics committee (if *yes*, please give brief details as an annex)	**YES**	**NO**
Will the study involve recruitment of patients through the NHS or the use of NHS patient data or samples?	☐	☒
Will the study involve the collection of tissue samples (including blood, saliva, urine, etc.) or other biological samples from participants, or the use of existing samples?	☐	☒
Will the study involve participants, or their data, from adult social care, including home care, or residents from a residential or nursing care home?	☐	☒
Will the study involve research participants identified because of their status as relatives or carers of past or present users of these services?	☐	☒
Does the study involve participants aged 16 or over who are unable to give informed consent (e.g. people with learning disabilities or dementia)?	☐	☒
Is the research a social care study funded by the Department of Health?	☐	☒
Is the research a health-related study involving prisoners?	☐	☒
Is the research a clinical investigation of a non-CE Marked medical device, or a medical device which has been modified or is being used outside its CE Mark intended purpose, conducted by or with the support of the manufacturer or another commercial company to provide data for CE marking purposes? (a CE mark signifies compliance with European safety standards)	☐	☒

Is the research a clinical trial of an investigational medicinal product or a medical device?	☐	☒

J) Research that may need full review by the Sciences REAG	**YES**	**NO**
Does the research involve other vulnerable groups: eg, children; those with cognitive impairment?	☐	☒
Is the research to be conducted in such a way that the relationship between participant and researcher is unequal (eg, a subject may feel under pressure to participate in order to avoid damaging a relationship with the researcher)?	☐	☒
Does the project involve the collection of material that could be considered of a sensitive, personal, biographical, medical, psychological, social or physiological nature.	☐	☒
Will the study require the cooperation of a gatekeeper for initial access to the groups or individuals to be recruited (eg, headmaster at a School; group leader of a self-help group)?	☐	☒
Will it be necessary for participants to take part in the study without their knowledge and consent at the time? (eg, covert observation of people in non-public places?)	☐	☒
Will the study involve discussion of sensitive topics (eg, sexual activity; drug use; criminal activity)?	☐	☒
Are drugs, placebos or other substances (eg, food substances, vitamins) to be administered to the study participants or will the study involve invasive, intrusive or potentially harmful procedures of any kind?	☒	☐
Is pain or more than mild discomfort likely to result from the study?	☒	☐
Could the study induce psychological stress or anxiety or cause harm or negative consequences beyond the risks encountered in normal life?	☐	☒
Will the study involve prolonged or repetitive testing?	☒	☐
Will the research involve administrative or secure data that requires permission from the appropriate authorities before use?	☐	☒
Is there a possibility that the safety of the researcher may be in question (eg, international research; locally employed research assistants)?	☐	☒
Does the research involve participants carrying out any of the research activities themselves (i.e. acting as researchers as opposed to just being participants)?	☐	☒
Will the research take place outside the UK? You may find the find the Proportionate Risk Assessment document useful.	☐	☒
Will the outcome of the research allow respondents to be identified either directly or indirectly (eg, through aggregating separate data sources gathered from the internet)?	☐	☒
Will research involve the sharing of data or confidential information beyond the initial consent given?	☐	☒
Will financial inducements (other than reasonable expenses and compensation for time) be offered to participants?	☐	☒
Are there any conflicts of interest with the proposed research/research findings? (eg, is the researcher working for the organisation under research or might the research or research findings cause a risk of harm to the participants(s) or the researcher(s) or the institution?)	☐	☒
Will the study involve the publication, sharing or potentially insecure electronic storage and/or transfer of data that might allow identification of individuals, either directly or indirectly? (e.g. publication of verbatim quotations from an online forum; sharing of audio/visual recordings; insecure transfer of personal data such as addresses, telephone numbers etc.; collecting identifiable personal data on unprotected** internet sites.) [**Please note that Qualtrics and Sona Systems provide adequate data security and comply with the requirements of the EU-US Privacy Shield.]	☐	☒

K) Security Sensitive Material	YES	NO
Does your research involve access to or use of material covered by the Terrorism Act? (The Terrorism Act (2006) outlaws the dissemination of records, statements and other documents that can be interpreted as promoting and endorsing terrorist acts. By answering 'yes' you are registering your legitimate use of this material with the Research Ethics Advisory Group. In the event of a police investigation, this registration will help you to demonstrate that your use of this material is legitimate and lawful).	☐	☒

L) Prevent Agenda	YES	NO
Does the research have the potential to radicalise people who are vulnerable to supporting terrorism or becoming terrorists themselves?	☐	☒

If the answer to any questions in Sections IV(B) and/or IV(C) and/or IV(D) is 'yes', please complete the full application form and send to SSES REAG at N.Khan-360@kent.ac.uk together with required supporting documentation.

Appendix 4: Ethics Forms For Chapter Six (Study Four)

School of Sport & Exercise Sciences
Research Ethics and Advisory Group (REAG)
University of Kent at Medway
Chatham Maritime
Kent
ME4 4AG

Ethics Reference:

Date: 09.12.20

Dear Ryan,

Re: The Effect of Experimental Muscle Pain on Cycling Time Trial Performance and Neuromuscular Fatigue

I am delighted to confirm that SSES REAG has approved your research study (REF No.) and you are now permitted to recruit participants and commence your research.

If you need to amend any aspect of your research, please ensure you inform SSES REAG by completing a request for amendment form and submitting all revised paperwork (e.g. participant information sheet, questionnaires).

If there should happen to be any adverse event during your study, please also ensure SSES REAG is kept informed.

I hope your study is successful.

With kind regards,

K. Hambly

Karen Hambly

(Chair SSES REAG)

If any of the questions in Sections IV(B) and/or IV(C) and/or IV(D) is answered 'yes', a full ethics application must be made to the REAG. This also applies for studies not defined as 'research' in the narrow sense, i.e. evaluations/audits, etc. Complete this form and send it to the Faculties Support Office along with supporting documentation: a copy of the full research proposal; any participant information sheets and consent forms; any surveys, interview schedules; any advertising material or proposed website wording. **It is important to note that you must not commence any research with human participants until full approval has been given by the SSES Research Ethics Advisory Group – you will be notified via email when this has been granted.**

During term time we aim to process a research ethics application within two weeks, however during vacation periods and busy times (e.g. exams and marking period) it can take up to four weeks. It is the applicant's responsibility to ensure that their application is submitted in good time.
Overview
Name of Applicant(s)
Mr Ryan Norbury
Contact Details (Please include your UoK address, email and telephone number)
M2-30 The Medway Building University of Kent, Medway Campus, Central Avenue ME4 4AG Email: Rn247@kent.ac.uk Tel: 01634 888806
Title of Project
The Effect of Experimental Muscle Pain on Cycling Time Trial Performance and Neuromuscular Fatigue
Lay Summary (Please provide a brief summary of the study)
An elevated level of muscle pain has previously been shown to reduce endurance capacity during tasks which require participants to maintain a constant workload for as long as possible. However, sport and exercise performance often allows for the active individual to vary their workload as they desire, known as self-paced exercise. It is unknown if these previously found reductions in endurance capacity from constant workload tasks can extend to self-paced exercise. Cycling is a common form of endurance exercise which causes significant amounts of naturally occurring muscle pain which may play a role in mediating the workload selected or the level of fatigue experienced. In order to determine if muscle pain can effect self-paced cycling performance, the intramuscular injection of hypertonic saline will be injected into both quadriceps prior completing a 5 minute time trial (i.e. cycle as far as possible in 5 minutes). Hypertonic saline injections are a safe and effective method of acutely inducing muscle pain that is similar to exercise induced pain. Therefore participants will complete a 5 minute time trial with no injections, one with an injection of isotonic saline (does not cause pain but replicates the procedures of a painful injection) and one with a hypertonic saline injection to experimentally elevate the level of pain a participant feels during the exercise. Performance of the time trial will be compared between conditions and the level of fatigue will be assessed before and after exercise by performing electrical and magnetic stimulations on the participants as they contract their quadriceps against equipment that measures force production.
Name of Supervisor(s) (If applicable)
Dr Lex Mauger Dr Mark Burnley

Risks and ethical issues

Please list the principal inclusion and exclusion criteria

The participants (*n* = 10-15) will be healthy male or female participants aged 18 to 45 years old. Prior to testing, potential participants will be required to complete a general health questionnaire, a screening form for transcranial magnetic stimulation and an intramuscular injection risk assessment questionnaire to ascertain their ability to participate in maximum and submaximal contractions, receive transcranial magnetic stimulation and receive intramuscular injections.

The exclusion criteria are as follows:

- Participants with a lower limb injury that occurred in the past three months
- Participants with pre-existing medical conditions (neurological disorders, cardiovascular disorders, pain disorders and blood borne viruses (i.e. HIV, Hepatitis B/C)
- Participants with long-term medication use for the treatment of pain
- Participants with protein-based allergies (e.g. fish, milk, egg,)
- Those who have metallic hardware in close proximity to the head (such as cochlear implants, or an Internal Pulse Generator or medication pumps)
- Those with a phobia of needles or injections

How long will each research participant be in the study in total, from when they give informed consent until their last contact with the research team?

The participant will be involved in the research for approximately 6 hours in total, spread for 5 visits over the course of 3-5 weeks.

What are the potential risks and burdens for research participants and how will you minimise them? (Describe any risks and burdens that could occur as a result of participation in the research, such as pain, discomfort, distress, intrusion, inconvenience or changes to lifestyle. Describe what steps would be taken to minimise risks and burdens as far as possible)

Exercise. Participants may experience the usual risks associated with the performance of multiple voluntary contractions (MVC) such as joint sprains and muscle strains, however isometric MVCs have been performed in hundreds of different studies without causing injury and is generally accepted as a low-risk activity. Nonetheless, a thorough warm-up consisting of graded submaximal contractions will be performed to reduce the risk of muscle injury. The exercise protocol is a 5-minute cycling time trial on a cyclus 2 cycling ergometer. The time-trial will be preceded by a 5-minute self-selected warmup and will be followed by a cool down to minimise incidences of injury and post-exercise syncope. The cycling exercise is likely to cause a significant amount of exercise induced pain during the task, however the participants are free to adjust workload to reduce the level of pain if it becomes intolerable. There will also likely be post-exercise fatigue and soreness for up to 48hrs, however the short duration (5 minutes) will mean that this is much less severe in comparison to more traditional exercise protocols (e.g. 10km or 20km time trials). The risk assessment will be adhered to for cycling exercise which can be found here and for maximum contractions on an isometric chair can be found here.

Electrical Stimulation. The use of electrical stimulation (ES) of the femoral nerve will innervate the quadriceps muscle. ES are delivered in short duration pulses (~200μs) at an amplitude which does not exceed 400mA. This evokes brief twitches of the innervated muscle mass. The safety of the technique is well documented and is unlikely to cause complications or injury. However, it may cause some discomfort for participants at higher stimulation intensities. Because of the short duration of the stimulations, the discomfort is very short lived resulting in no longer than a few seconds of discomfort over the course of an experimental trial. As a result, ES tends to be well tolerated among most individuals. Each investigator is trained in ES to minimise the discomfort received during the measurements and complies with the risk assessment guidelines. The risk assessment for transcutaneous peripheral electrical nerve stimulation can be found here.

Transcranial Magnetic Stimulation. Single pulse transcranial magnetic stimulation (TMS) of the motor cortex is generally regarded as safe for the participants (Rossi et al., 2009) and is a measurement technique that has been safely used in thousands of published papers. 1ms pulses are delivered a stimulator intensity which is around 60-70% of maximum stimulator output. One of the primary risks with TMS is the induction of a seizure. However, since 1998, there have only been four reported cases of seizures (date of report: Dec. 2008). Three out of four of these cases were caused in participants that were taking pro-epileptogenic medication (Rossi et al. 2009). Syncope is also a cited concern with TMS, however this appears to be an epiphenomenon to TMS and is again unlikely to occur. In the rare instance syncope, participants will be lay down supine with the legs elevated until participants regain consciousness. Similar to ES, TMS may induce some discomfort at higher stimulation intensities. The use of TMS is limited to minimise the amount of discomfort received. TMS is usually well tolerated by most individuals. Each investigator will have training on how to competently perform TMS to minimise the discomfort which may occur. The risk assessment for transcranial magnetic stimulation can be found here.

Intramuscular Injection. Intramuscular injections of hypertonic saline as an experimental model of exercise-induced pain has previously been implemented within the present institution and has been ethically approved on multiple occasions. A protocol identical to these previously approves studies will be used in this study. See ethics refs:

Prop 1_2018_19
Prop 9_2018_19
Prop 55_2018_19

However, it is recognised that this method may still cause ethical concern. As stated, this model has been applied in multiple studies conducted by the lead investigator, supervisor and one of the investigators of the proposed study. Combined, we have administered over 150 safe and successful injections with zero complications related to the injection. Each investigator of the study has received formal training from a registered medical practitioner and has been signed off as competent. All previous intramuscular injections have been administered and documented in line with NHS best practice and the School of Sport and Exercise Science IM injection risk assessment. The proposed study will build upon an extensive range of literature from various institutions located in Australia, Denmark, France, Germany, Sweden and USA where this method has been safely and successfully employed (Capra and Ro, 2000; Deschamps, Hug, Hodges, Tucker, 2014; Graven-Nielsen, Svensson and Arendt-Nielsen, 1997b; Khan et al., 2011; Schilder et al., 2014; Schulte et al., 2003), all of which have safely employed this method without any reported issues. A detailed background and risk assessment of this method is presented below or can be found here. An extensive background of the safety and applicability of hypertonic saline will now be outlined:

1.1. Preface

Currently, hypertonic saline (we use Braun's sterile 5,85 % Sodium Chloride (PZN: 3158635)) is licenced for infusion, but not for intramuscular injection. This is because hypertonic saline is used to medically treat conditions such as hyponatremia (electrolyte imbalance in the blood), but has no medical use for intramuscular injection. Therefore, the aim of this section is to provide evidence and assurance that intramuscular injection of 5.85% saline is a common technique used in research, bears no risk above that of a standard intramuscular injection and that this technique is required to complete our planned programme of research. Research by Henriksen et al. (2007) and Danneskiold-Samsøe et al. (2007) has used the same volume and concentration into the vastus medialis while Ciubotariu, Arendt-Nielsen and Graven-Nielsen (2004) has used 1ml of 6%NaCl into the tibialis anterior without issue. Therefore greater volumes and concentrations into smaller areas has been shown to be safe. We will inject it into the vastus lateralis as this site poses less risk than the previously used vastus medialis. This exact protocol has been used in several studies internally (ethics references: Prop 139_2017_17, Prop 84_2016_17, Prop 140_2016_17, Prop 1_2018_19, Prop 30_2018_2019). A 5.8% concentration is used as this is what is commercially available and creates an appropriate amount of muscle pain when 1ml is injected.

1.2. Background

Intramuscular injections are a common method to administer medications, drugs and vaccines, via the use of a syringe and needle, directly injected deep into the muscle tissue, below the muscle fascia and under the fatty subcutaneous layer (Boyd et al. 2013). This technique is a relatively simple process, is not defined as a medical procedure and therefore requires a clean procedure rather than aseptic. As the skeletal muscle is suggested to have scarcer nociceptors than subcutaneous tissue, IM injections involve decreased discomfort, and dependent on the site of injection (i.e. deltoid, vastus lateralis, rectus femoris, ventrogluteal or dorsogluteal), this method enables comparatively large volumes of a substance to be quickly absorbed by the body, with a relatively prolonged action (Rodger and King, 2000).

When implementing IM injections, several considerations and decisions need to take place:

- Injection site (dependent on age, physical status and volume of the injectate)
- Substance to be injected
- Technique
- Equipment
- Environment in which injection is administered

It is proposed that the experimental pain model implemented in the proposed non-clinical studies is the intramuscular injection of 1ml hypertonic saline (5.8% concentration). Hypertonic saline is a strong, sterile solution of salt water, with a concentration of sodium chloride greater than 0.9% (Mortimer and Jancik, 2006). It primarily functions by an osmotic effect, but can also be utilised for other clinical effects (hemodynamic, vasoregulatory, immunomodulatory, neurochemical and hypernatremic) (Doyle, Davis and Hoyt, 2001).

Additionally, after first being implemented by Kellgren in the late 1930s (Kellgren, 1938), hypertonic saline has since been extensively been used as an experimental model that characterises both the sensory and motor effects involved in muscle pain (Burton, Fazalbhoy and Macefield, 2016). This is predominantly due to the quality of the pain induced by IM injections of hypertonic saline effectively mimicking acute muscle pain, with the pain produced displaying localized and referred characteristics (Graven-Nielsen and Mense, 2001). Dependent on injection site and volume of solution, a bolus IM injection of hypertonic saline temporarily induces pain that can lasts ~5.4 minutes, with a mean pain intensity rating of ~5 (strong pain) on the Cook 0-10 scale (Cook, O'Connor, Eubanks, Smith and Lee, 1997). This therefore allows the acute effects of pain to be studied (Burton et al. 2016).

The medical application of hypertonic saline is through intravenous infusion, for which the solution is licensed. At present, 5.8% hypertonic saline is not licensed for intramuscular injection because there is no medical basis for this method of application. However, as demonstrated by previous animal studies, this method does not cause any muscle toxicity (Svendsen, Edwards and Rasmussen, 2001), and the pain caused is unlikely to be related to tissue damage (Svendsen, Edwards, Lauritzen and Rasmussen, 2005). Thus this method can be deemed to be safe and acceptable for use in human experimentation (Graven-Nielsen, Lund, Arendt-Nielsen, Danneskiold-Samsøe and Bliddal, 2002).

Indeed, IM injections of hypertonic saline have been applied in over 35 studies ranging from 1938 to the present day, with no adverse effects reported (for example; Arendt-Nielsen, Graven-Nielsen, Svarrer and Svensson, 1996; Feinstein, Langton, Jameson and Schiller, 1954; Kennedy, McNeil, Gandevia and Taylor, 2016; Kellgren, 1938; Henriksen, Alkjær, Simonsen and Bliddal, 2009; Hodges, Moseley, Gabrielsson and Gandevia, 2003; Veerasarn and Stohler, 1992; Yavuz, Negro, Falla and Farina, 2015). Furthermore, we have contacted Professor Thomas Graven-Nielsen (http://vbn.aau.dk/en/persons/thomas-gravennielsen(474a635b-4e83-4b02-a90b-80d417d37e52)/cv.html?id=60483613), a leading professor in pain neuroscience who has extensively used this method (for example; Graven-Nielsen, Arendt-Nielsen, Svensson and Jensen, 1997a, Graven-Nielsen et al., 1997b; Graven-Nielsen, Babenko, Svensson and Arendt-Nielsen, 1998a, Graven-Nielsen et al., 1998b; Graven-Nielsen et al., 2002; Graven-Nielsen et al., 2003) for advice and guidance on the use of hypertonic saline. Prof. Graven-Nielsen stated that he has performed over 6000 injections, with no adverse effects having occurred and is therefore very confident in the safety of the technique.

When injected into the muscle, hypertonic saline predominantly causes the depolarisation of unmyelinated group IV muscle afferent units which directly stimulates the release of neuropeptides such as substance P at the periphery (Laursen, Graven-Nielsen, Jensen and Arendt-Nielsen, 1999). This means that a particular tissue and type of pain can be

specifically targeted (Graven-Nielsen and Mense, 2001). However, at present, the mechanisms of muscle nociceptive processing excited by the intramuscular injection of hypertonic saline are uncertain (Mense, 2013). Several proposals have nonetheless been made, including: activation by augmented tonicity in the interstitial space, activation by ionic alterations and indirect activation by further algesic substances released from the muscle tissue or the nociceptive ending (Kress and Reeh, 1996).

In the present study, the hypertonic saline solution is planned to be injected into the vastus lateralis (middle third of the lateral aspect of the thigh between the greater trochanter and the lateral femoral condyle of the femur). This site provides a large muscle mass, is easy to access, has a reduced likelihood of injury and is not associated with any major blood vessels or significant nerve structures, minimising risks of damage (Dougherty and Lister, 2011). Additionally, the volume of fluid that is proposed to be injected into this site (1ml) is well below the maximal recommended volume of 5ml for any substance that is injected into a large muscle mass (Roger and King, 2000). This volume is consistent with previous literature (Deschamps, Hug, Hodges and Tucker, 2014; Henriksen et al., 2007; Henriksen et al., 2009; Khan, McNeil, Gandevia and Taylor, 2011) and also below that utilised in other studies, where 1.5ml has been formerly applied (Ervilha, Farina, Arendt-Nielsen and Graven-Nielsen, 2005; Graven-Nielsen et al., 2002; Hodges et al., 2003).

1.3. Associated Risks and Side-effects

As a routine technique for drug administration for over one hundred and fifty years, IM injections are considered to be a method that is simple and safe, with the rare occurrence of complications or side-effects (Svendsen et al., 2005). In addition, the IM injection of hypertonic saline carries no further risk beyond which would occur from the IM injection of any substance (i.e. allergic or anaphylactic reaction), with this solution not causing muscle toxicity or tissue damage (Svendsen et al., 2001; Svendsen et al., 2005). Nonetheless, an IM injection can solely still result in several potential complications (Small, 2004) which could arise as a result of unsafe injections and poor technique. Although mainly preventable with trained and safe IM practice, it is important that these potential risks are taken into consideration. Potential complications of an IM injection include:

- Pain or mild discomfort for a short period after an injection is common
- Some bruising at the injection site may occur
- Muscle fibrosis can occur with repeated use of the same injection site
- An increased risk of injecting the substance intravenously if the needle is too deep
- Needle stick injury to the experimenter
- In more serious cases, nerve injury resulting in potential paralysis, atrophy, haematoma, bone injury, cellulitis, and sterile abscesses can occur
- Accidental femoral nerve damage due to incorrect needle placement and muscle atrophy from IM injection overuse are the predominant risks associated with the vastus lateralis site

Most of the complications highlighted previously may occur at any site of injection, and could potentially due to an inappropriate depth or rate of injection, or injecting into an incorrect site (Malkin, 2008).

1.4. Interventions in Place to Reduce Risk

A safe injection is defined as 'one that does not harm the recipient, does not expose the provider to any avoidable risk, and does not result in waste that is dangerous to other people' (Hutin et al., 2003: 492). In order to achieve a safe injection, and reduce the risks associated with intramuscular injections the researcher will implement several precautions. These are detailed below:

- The lead researcher has received appropriate NHS training and guidelines, followed by a subsequent competency assessment of supervised practice to ensure safe, competent and consistent best practice when administering IM injections
    - Thorough understanding of injection site to reduce the likelihood of nerve injury and accidental intravenous injection

- Performance of pre-injection checks (check drug name and ampule, check drug unit quantity or %, check expiry date, inspect drug for cloudiness and particles)
- The use of appropriate syringe (Luer Lok) and needle size to ensure the solution is delivered to the muscle
- The use of the Z track technique to minimise the pain experienced and reduce the likelihood of complications
- Aspiration prior to delivery to ensure that the needle is not in a blood vessel. If blood appears, remove the needle and restart the process.
- Quick insertion of needle at 90^{o} followed by a slow injection and then quick withdrawal of the needle 10 seconds after the injection has occurred
- The documentation of solution administration, injection sites and even rotation of the site to prevent myopathy, muscle fibrosis and sterile abscesses
- The site of the injection will be marked on the participant's skin, and they will be instructed to maintain this for the duration of the study

- The lead researcher will implement best infection control practices for IM injections (Hutin et al., 2003)
  - The use of sterile injection equipment, and contamination prevention of injection equipment and solutions
  - Pre-checks of sterile equipment, with contaminated items replaced immediately
  - Wearing gloves and use aseptic non-touch technique when preparing
  - The safe handling of injection equipment, and prevention of needle-stick injuries to the provider
  - Cleaning of the injection site with alcohol wipe prior to administration
  - Prevention of access to needles and safe management and disposal of sharps waste to prevent potential needle stick injury
  - Practice of good hygiene in terms of provider (hand and skin integrity) and participant (site selection and skin preparation)
- A full risk assessment of intramuscular injections and hypertonic saline has been completed and documented
- Participants between the age of 18-45 will only be recruited for this study
- Participants will be screened prior to testing through completing a general health questionnaire. Participants with pre-existing medical conditions such as neurological disorders, blood borne viruses (i.e. HIV, Hepatitis B/C), lower limb injury, sore deep tissues, allergies to protein and long-term medication use will be excluded from participating in the study
  - Should any participants not disclose/be unaware of allergens, the lead researcher is aware of signs and symptoms of anaphylaxis and the appropriate first aid response
- Prior to injection, the injection site will be inspected to ensure it is free from redness, swelling, pain, tenderness, infections, abrasions or necrosis.
- Documentation of factors such as solution, product batch number, site, date, time and adverse effects will be made after each IM injection
  - Should any adverse reactions or incidences occur, the completion of the Medical & Healthcare products Regulatory Agency (MHRA) yellow card form will occur, with all equipment used kept and safely stored
- The participants will be instructed to monitor the site two to four hours post-injection to ensure no adverse reactions have occurred (Mallet and Bailey, 1996). Any complications present will be documented.
- Previous contact with a Lead Professor in Pain Neuroscience at Aalborg University (Professor Thomas Graven-Nielsen) who has extensively used IM injection of saline. From over 6000 injections performed, there have been no serious side-effects reported.

Please describe what measures you have in place in the event of any unexpected outcomes or adverse effects to participants arising from involvement in the project

The Medway building contains a defibrillator which can be operated by a trained individual. For all experimental sessions using TMS, there will be two investigators present in the laboratory, with an additional first aider nearby. There will also be a first aid kit present in the lab. Therefore immediate care can be provided when needed and the procedures for subsequent treatment are in place.

Will interviews/questionnaires or group discussions include topics that might be sensitive, embarrassing or upsetting, or is it possible that criminal or other disclosures requiring action could occur during the study?

No

If yes, please describe the procedures in place to deal with these issues
n/a
What is the potential benefit to research participants?
Through performing maximal voluntary contractions, participants will gain an accurate measure of their quadriceps muscle strength. Additionally, participants will be contributing to further our knowledge, understanding and development of an experimental pain model (intramuscular injection of hypertonic saline), and the role of exercise-induced pain in neuromuscular function.
What are the potential risks to the researchers themselves?
There is the potential of a needle stick injury to occur to the researchers. This risk will however be minimised through the researchers being appropriately trained and competent to ensure safe practice whilst handling the syringe and needles. During the research study, all intramuscular injections will be administered by Ryan Norbury, Samuel Smith or Dr Lex Mauger, all of whom have either declared themselves to be knowingly free of blood-borne viruses and have an up to date immunisation for hepatitis B.
Will there be any risks to the University? (Consider issues such as reputational risk; research that may give rise to contentious or controversial findings; could the funder be considered controversial or have the potential to cause reputational risk to the University?)
No
Will any intervention or procedure, which would normally be considered a part of routine care, be withheld from the research participants? (If yes, give details and justification). For example, the disturbance of a school child's day or access to their normal educational entitlement and curriculum).
No

Recruitment and informed consent
How and by whom will potential participants, records or samples be identified?
The lead researcher will recruit participants through word of mouth. Potential participants will be able to ask the lead researcher any questions relating to ensure they are fully clear on the requirements of participation.
Will this involve reviewing or screening identifiable personal information of potential participants or any other person? (If 'yes', give details)
Yes. Participants will complete a general health questionnaire and an intramuscular injection risk assessment questionnaire. These will behind two physical/digital locks.
Has prior consent been obtained or will it be obtained for access to identifiable personal information?
Yes. Informed consent will be taken by the researcher.
Will you obtain informed consent from or on behalf of research participants? (If 'yes' please give details. If you are not planning to gain consent, please explain why not).
Yes. All participants will receive a 'Participant Information Sheet', which provides a full written explanation of the study, protocols, risks and benefits. This information sheet also contains an appendix discussing the background and risk assessment of intramuscular injections. Should participants require further information, or have any additional questions, explanations will be provided verbally. Upon the completion of this, if the participants are still happy to participate and

they meet the inclusion criteria, they will provide full written consent through the completion of an 'Informed Consent Form'

Will you record informed consent in writing? (If 'no', how will it be recorded?)

Yes

How long will you allow potential participants to decide whether or not to take part?

Participants will be provided with a minimum of 24 hours after receiving the Participant Information Sheet, to decide whether to participate.

What arrangements have been made for persons who might not adequately understand verbal explanations or written information given in English, or have special communication needs? (eg, translation, use of interpreters?)

None, these individuals will not be recruited to participate

If no arrangements will be made, explain the reasons (eg, resource constraints)

Financial limitations make this unfeasible.

Confidentiality

In this section personal data means any data relating to a participant who could potentially be identified. It includes pseudonymised data capable of being linked to a participant through a unique code number.

If you will be undertaking any of the following activities at any stage (including in the identification of potential participants) please give details and explain the safeguarding measures you will employ

- Electronic transfer by magnetic or optical media, email or computer networks
- Sharing of personal data outside the European Economic Area
- Use of personal addresses, postcodes, faxes, emails or telephone numbers
- Publication of direct quotations from respondents
- Publication of data that might allow identification of individuals, either directly or indirectly
- Use of audio/visual recording devices
- Storage of personal data on any of the following:
  - Manual files
  - University computers
  - Home or other personal computers
  - Private company computers
  - Laptop computers

All hard files or written data will be stored in a secure locked cabinet. All electronic data will be stored in anonymised format and kept in a password-protected folder on a password-protected laptop computer belonging to the researcher.

How will you ensure the confidentiality of personal data? (eg, anonymisation or pseudonymisation of data)

All data will be anonymised through the use of participant number coding. A master code will be kept in a secure locked cabinet by the researchers.

Who will have access to participants' personal data during the study?

Ryan Norbury
Dr Lex Mauger
Dr Mark Burnley
Sam Smith

How long will personal data be stored or accessed after the study has ended? (If longer than 12 months, please justify)
The data will be stored for up to three years. This is due to the data being collected as part of a PhD thesis, and thus may be required at a later date for subsequent analysis.
Please note: as best practice, and as a requirement of many funders, where practical, researchers must develop a data management and sharing plan to enable the data to be made available for re-use, eg, for secondary research, and so sufficient metadata must be conserved to enable this while maintaining confidentiality commitments and the security of data.

Incentives and payments
Will research participants receive any payments, reimbursement of expenses or any other benefits or incentives for taking part in this research? (If 'yes', please give details)
Yes. Participants will receive up to £50 which will be provided pro-rata across the four testing sessions. This payment will account for the travel requirements and time in which the participant is providing for their participation in the laboratory testing.
Will individual researchers receive any personal payment over and above normal salary, or any other benefits or incentives, for taking part in this research? (If 'yes', please give details)
No
Does the Chief Investigator or any other investigator/collaborator have any direct personal involvement (e.g. financial, share holding, personal relationship, etc) in the organisations sponsoring or funding the research that may give rise to a possible conflict of interest? (If 'yes', please give details)
No

Publication and dissemination
How do you intend to report and disseminate the results of the study? If you do not plan to report or disseminate the results please give your justification
The results of the study will be an anonymised cohort results. These results will subsequently be analysed and written up in the form of one or more conference/peer-reviewed papers, which will form the basis of an experimental chapter in the PhD thesis
Will you inform participants of the results? (Please give details of how you will inform participants or justify if not doing so)
Yes. All participants will be able to request a written and verbal summary of their results

Management of the research
Other key investigators/collaborators. (Please include all grant co-applicants, protocol authors and other key members of the Chief Investigator's team, including non-doctoral student researchers)
Dr Lex Mauger (Primary Supervisor) Dr Mark Burnley (Secondary Supervisor) Ryan Norbury (Lead Investigator)

Samuel Smith (Investigator) Megan Judge (Investigator)
Has this or a similar application been previously rejected by a research Ethics Committee in the UK or another country? (If yes, please give details of rejected application and explain in the summary of main issues how the reasons for the unfavourable opinion have been addressed in this application)
No
How long do you expect the study to last?

• Planned start date: 1/5/2020	• Planned end date: 1/5/2021	• Total duration: 12 Months

Where will the research take place?
In the Pain Research Lab (M0-02) of the Medway building on the University of Kent Medway Campus.

Insurance/indemnity
Does UoK's insurer need to be notified about your project before insurance cover can be provided? *The majority of research carried out at UoK is covered automatically by existing policies, however, if your project entails more than usual risk or involves an overseas country in the developing world or where there is or has recently been conflict, please check with the Insurance Office that cover can be provided. Please give details below.*
No. Confirmation has been received that this activity (intramuscular injection of saline solutions) falls within the existing University insurance policy providing the risk assessment in place is strictly adhered to for the duration of the research activity (*see included email correspondence between Nicole Palmer and Dr Lex Mauger).*

Children
Do you plan to include any participants who are children under 16? (If no, go to next section)
No
Please specify the potential age range of children under 16 who will be included and give reasons for carrying out the research with this age group
Please describe the arrangements for seeking informed consent from a person with parental responsibility and/or from children able to give consent for themselves
If you intend to provide children under 16 with information about the research and seek their consent or agreement, please outline how this process will vary according to their age and level of understanding

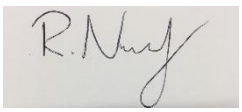
Participants unable to consent for themselves	
Do you plan to include any participants who are adults unable to consent for themselves through physical or mental incapacity? (If yes, the research must be reviewed by an NHS REC or SCREC)	
No	
Is the research related to the 'impairing condition' that causes the lack of capacity, or to the treatment of those with that condition?	
☐ Yes	If 'yes' proceed to next question

☒ No	If 'no' the study should proceed without involving those who do not have the capacity to consent to participation
Could the research be undertaken as effectively with people who do have the capacity to consent to participate?	
☒ Yes	If 'yes' then the study should exclude those without the capacity to consent to participation
☐ No	If 'no' then the inclusion of people without capacity in the study can be justified
Is it possible that the capacity of participants could fluctuate during the research? (If yes, the research must be reviewed by an NHS REC or SCREC)	
No	
Who inside or outside the research team will decide whether or not the participants have the capacity to give consent? What training/experience will they have to enable them to reach this decision?	
Dr Lex Mauger. He has been active researcher for >10 years, including a successful project ethics submission with the NHS, and the completion of NHS Good Clinical Practice Training.	
What will be the criteria for withdrawal of participants?	
A participant who has given consent and subsequently loses the capacity to provide consent will be withdrawn from the study. If participants become injured or subsequently display any evidence of the exclusion criteria during testing. Participants will also be informed both verbally and in written form that they can withdraw from the study at any time, without any disadvantage to themselves.	

Declaration

To be signed by the Chief Investigator

- I agree to comply, and will ensure that all researchers involved with the study comply with all relevant legislation, accepted ethical practice, University of Kent policies and appropriate professional ethical guidelines during the conduct of this research project
- If any significant changes are made to the design of the research I will notify the SSES Research Ethics and Advisory Group (REAG) and understand that further review may be required before I can proceed to implement the change(s)
- I agree that I will notify the SSES Research Ethics Advisory Group of any unexpected adverse events that may occur during my research
- I agree to notify the SSES Research Ethics Advisory Group of any complaints I receive in connection with this research project

Signed: 	Date: 3/3/2020
Name: Ryan Norbury	

What to do next

Send your completed form, along with all supporting documentation, to the SSES REAG, at N.Khan-360@kent.ac.uk.

Checklist	
Please ensure you have included the following with your application (*where relevant):	
• Full research proposal (current project)	☒
• Participant information sheet	☒
• Consent form	☒
• *Covering letter	☒
• *Any questionnaires/interview schedules/topic guides to be used	☐
• *Any approved instruments/measures to be used	☐
• *Any advertising material to be used to recruit participants	☐
• *Confirmation that project is covered by UoK insurance policies (if necessary)	☒

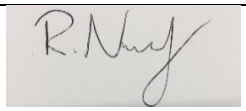
A checklist should be completed for every research project in order to identify whether a full application for ethics approval needs to be submitted. The principal investigator or, where the principal investigator is a student, the supervisor, is responsible for exercising appropriate professional judgement in this review.

This checklist must be completed before potential participants are approached to take part in any research. All forms must be signed by the School's Research Ethics Advisory Group representative.

Section I: Project details		
Project title:	The Effect of Experimental Muscle Pain on Cycling Time Trial Performance and Neuromuscular Fatigue	
Planned start date: 1/5/2020		Planned end date: 1/5/2021
Funder:	School of Sports and Exercise Science	

Section II: Applicant details			
Applicant name:	Ryan Norbury		
School/Department:	School of Sports and Exercise Science		
Email:	Rn247@kent.ac.uk	Telephone number:	01634 888806
Contact address:	M2-30 Medway Building, The University of Kent Medway Campus, Central Avenue, Kent, ME4 4AG		
Undergraduate ☐	Taught Postgraduate ☐	Research Postgraduate ☒	Staff ☐

Section III: Declaration and signatures
Please note that it is your responsibility to follow, and to ensure that, all researchers involved with your project follow accepted ethical practice and appropriate professional ethical guidelines in the conduct of your study. You must take all reasonable steps to protect the dignity, rights, safety and well-being of participants. This

includes providing participants with appropriate information sheets, ensuring informed consent and ensuring confidentiality in the storage and use of data.			
Applicant signature		**Date**	2/3/20

Supervisor name	Dr Lex Mauger	**Date**	17/3/20
Supervisor signature			

School REAG rep signature (required for both staff and students)		**Date**	

If the question *"Will the study involve the collection of tissue samples (including blood, saliva, urine, etc.) or other biological samples from participants, all the use of existing samples?"* in Section IV (A) is answered 'yes', if the human tissue samples taken are tested as soon as possible (within hours or days) and immediately disposed of, or rendered acellular you should reference the SSES Ethical Clearance for Standard Laboratory and Field Procedures document (https://www.kent.ac.uk/stms/research-ethics/sses-ethical-clearance-2015.docx) section 5 on research with human samples that sets out the methods by which the samples are rendered acellular or immediately disposed of in the full ethics application. This will confirm that these procedures (i.e. immediate analysis before disposable or rending acellular before storage) are allowed under the Human Tissue Act and do not require a licence.

7. Complete full application form together with supporting documentation
8. Send to the N.Khan-360@kent.ac.uk for review by SSES (REAG)

If any other question in Section IV(A) is answered 'yes' or human tissue samples taken are not tested as soon as possible (within hours or days) and immediately disposed of, or rendered acellular tissue:

7. Contact Nicole Palmer (University Research Ethics & Governance Officer) for advice
8. Send a copy of ethical approval to the Faculties Support Office, once received

If any questions in Sections IV(B) and/or IV(C) and/or IV(D) are answered 'yes':

9. Complete full application form together with supporting documentation
10. Send to the N.Khan-360@kent.ac.uk for review by SSES (REAG)

If all questions in Sections IV(A), IV(B), IV(C) and IV(D) are answered 'no':

4. Send the completed and signed form to the SSES REAG at N.Khan-360@kent.ac.uk

Section IV: Research Checklist

Please answer all questions by ticking the appropriate box:

M) Research that may need to be reviewed by an NHS Research Ethics Committee, the Social Care Research Ethics Committee (SCREC) or other external ethics committee (if *yes*, please give brief details as an annex)	YES	NO
Will the study involve recruitment of patients through the NHS or the use of NHS patient data or samples?	☐	☒
Will the study involve the collection of tissue samples (including blood, saliva, urine, etc.) or other biological samples from participants, or the use of existing samples?	☐	☒
Will the study involve participants, or their data, from adult social care, including home care, or residents from a residential or nursing care home?	☐	☒
Will the study involve research participants identified because of their status as relatives or carers of past or present users of these services?	☐	☒
Does the study involve participants aged 16 or over who are unable to give informed consent (e.g. people with learning disabilities or dementia)?	☐	☒
Is the research a social care study funded by the Department of Health?	☐	☒
Is the research a health-related study involving prisoners?	☐	☒
Is the research a clinical investigation of a non-CE Marked medical device, or a medical device which has been modified or is being used outside its CE Mark intended purpose, conducted by or with the support of the manufacturer or another commercial company to provide data for CE marking purposes? (a CE mark signifies compliance with European safety standards)	☐	☒
Is the research a clinical trial of an investigational medicinal product or a medical device?	☐	☒

N) Research that may need full review by the Sciences REAG	YES	NO
Does the research involve other vulnerable groups: eg, children; those with cognitive impairment?	☐	☒
Is the research to be conducted in such a way that the relationship between participant and researcher is unequal (eg, a subject may feel under pressure to participate in order to avoid damaging a relationship with the researcher)?	☐	☒
Does the project involve the collection of material that could be considered of a sensitive, personal, biographical, medical, psychological, social or physiological nature.	☐	☒
Will the study require the cooperation of a gatekeeper for initial access to the groups or individuals to be recruited (eg, headmaster at a School; group leader of a self-help group)?	☐	☒
Will it be necessary for participants to take part in the study without their knowledge and consent at the time? (eg, covert observation of people in non-public places?)	☐	☒
Will the study involve discussion of sensitive topics (eg, sexual activity; drug use; criminal activity)?	☐	☒
Are drugs, placebos or other substances (eg, food substances, vitamins) to be administered to the study participants or will the study involve invasive, intrusive or potentially harmful procedures of any kind?	☒	☐
Is pain or more than mild discomfort likely to result from the study?	☒	☐
Could the study induce psychological stress or anxiety or cause harm or negative consequences beyond the risks encountered in normal life?	☐	☒
Will the study involve prolonged or repetitive testing?	☐	☒
Will the research involve administrative or secure data that requires permission from the appropriate authorities before use?	☐	☒

Is there a possibility that the safety of the researcher may be in question (eg, international research; locally employed research assistants)?	☐	☒
Does the research involve participants carrying out any of the research activities themselves (i.e. acting as researchers as opposed to just being participants)?	☐	☒
Will the research take place outside the UK? You may find the find the Proportionate Risk Assessment document useful.	☐	☒
Will the outcome of the research allow respondents to be identified either directly or indirectly (eg, through aggregating separate data sources gathered from the internet)?	☐	☒
Will research involve the sharing of data or confidential information beyond the initial consent given?	☐	☒
Will financial inducements (other than reasonable expenses and compensation for time) be offered to participants?	☐	☒
Are there any conflicts of interest with the proposed research/research findings? (eg, is the researcher working for the organisation under research or might the research or research findings cause a risk of harm to the participants(s) or the researcher(s) or the institution?)	☐	☒
Will the study involve the publication, sharing or potentially insecure electronic storage and/or transfer of data that might allow identification of individuals, either directly or indirectly? (e.g. publication of verbatim quotations from an online forum; sharing of audio/visual recordings; insecure transfer of personal data such as addresses, telephone numbers etc.; collecting identifiable personal data on unprotected** internet sites.) [**Please note that Qualtrics and Sona Systems provide adequate data security and comply with the requirements of the EU-US Privacy Shield.]	☐	☒

O) Security Sensitive Material	**YES**	**NO**
Does your research involve access to or use of material covered by the Terrorism Act? (The Terrorism Act (2006) outlaws the dissemination of records, statements and other documents that can be interpreted as promoting and endorsing terrorist acts. By answering 'yes' you are registering your legitimate use of this material with the Research Ethics Advisory Group. In the event of a police investigation, this registration will help you to demonstrate that your use of this material is legitimate and lawful).	☐	☒

P) Prevent Agenda	**YES**	**NO**
Does the research have the potential to radicalise people who are vulnerable to supporting terrorism or becoming terrorists themselves?	☐	☒

If the answer to any questions in Sections IV(B) and/or IV(C) and/or IV(D) is 'yes', please complete the full application form and send to SSES REAG at N.Khan-360@kent.ac.uk together with required supporting documentation.