THE IMPACT OF EXPERIMENTALLY-INDUCED MUSCLE PAIN ON THE PERFORMANCE OF SINGLE-LIMB AND WHOLE-BODY EXERCISE TASKS

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

by

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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

Abstract

Exercise-induced pain (EIP), which is often accompanied by fatigue, has been suggested to have a limiting or regulatory role during endurance performance, with the ability to tolerate or overcome pain a determinant of success. Despite this, the potential impact of EIP on endurance performance is not well understood, partly because prior research investigating this relationship has employed methods of pain induction that are inappropriate in representing the transmission and experience of EIP.

The focus of this thesis was to investigate the role of EIP on exercise performance through the experimental induction of muscle pain using a model that closely replicates the experience of naturally occurring EIP. There were two overarching main aims of this thesis which were addressed in four experimental studies. The first aim was to investigate and confirm the hypertonic saline model as a suitable experimental method of muscle pain induction to investigate the fatigue-pain relationship. The second aim was to apply the hypertonic saline model to evaluate the impact of EIP on exercise tasks relevant to endurance performance.

When combined with muscle contraction, hypertonic saline injected into the vastus lateralis induced a muscle pain that felt like naturally occurring EIP of a greater contraction intensity (Study 1). Applied both unilaterally and bilaterally at rest, is was found that this method is unlikely to directly elicit a confounding response that may influence exercise performance (i.e. exercise pressor reflex) (Study 3). When applied to exercise, the increased muscle pain from the hypertonic saline impaired both the accuracy of single-limb isometric torque reproduction (Study 2) and time to task failure performance (i.e. an accelerated progression of fatigue) in both single-limb (Study 1) and whole-body exercise tasks (Study 4).

In summary, the findings of this thesis provide evidence and advances understanding of the potential limiting impact of EIP on endurance performance tasks. This thesis also has practical application in providing a novel experimental model that can be applied in future investigations of experimental muscle pain and the fatigue-pain relationship.

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Symbols, Abbreviations and Definitions

$CI_{.95}$	Confidence interval
^{o}C	Degrees Celsius
Δ	Delta
>	Greater than
<	Less than
≤	Less than or equal to
µl	Microlitre
%	Percent
±	Plus-minus
ADP	Adenosine diphosphate
Ag	Silver
Ag/Cl	Silver chloride
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
BIL	Bilateral
$B[La^{-}]$	Blood lactate concentration
BF	Breathing frequency
bpm	Beats per minute
Ca^{2+}	Calcium
CGM	Central governor model
cm	Centimetres
CMD	Central motor drive
CNS	Central nervous system
CO	Cardiac output
CON	Control
DOM	Dominant
DOMS	Delayed onset muscle soreness
EC	Excitation-contraction
EEG	Electroencephalography
EIP	Exercise-induced pain
EMG	Electromyography

G	Gauge
GET	Gas exchange threshold
H^+	Hydrogen ion
h	Hours
HR	Heart rate
HYP/HS	Hypertonic saline
Hz	Hertz
IM	Intramuscular
ISO/IS	Isotonic saline
K^+	Potassium
Kg	Kilogram
L	litre
La^-	Lactic acid
LT	Lactate threshold
m	Metre
MEG	Magnetoencephalography
min	Minute
mL	Millilitre
mM	Millimolar
mm	Millimetre
mmHg	Millimetre of mercury
MPQ	McGill pain questionnaire
MRI	Magnetic resonance imaging
MVC	Maximum voluntary contraction
MVCs	Maximal voluntary contractions
MVT	Maximum voluntary torque
ND	Non-dominant
NRS	Numerical rating scale
P_i	Inorganic phosphate
PANAS	Positive and negative affect schedule
η_p^2	Partial eta squared
$PETCO_2$	End-tidal partial pressure of carbon dioxide
$PETO_2$	End-tidal partial pressure of oxygen

PRI(T)	Pain rating index (total)
PRS	Pain resilience scale
RER	Respiratory exchange ratio
RF	Rectus femoris
ROF	Rating of fatigue
RPE	Rating of perceived exertion
rpm	Revolutions per minute
s	Seconds
SD	Standard deviation
sEMG	Surface electromyography
SPCS	Situation-specific pain catastrophizing scale
SR	Sarcoplasmic reticulum
SRI	Subclass rating index
SSEIT	Schutte self-report emotional intelligence test
SV	Stroke volume
TENS	Transcutaneous electrical nerve stimulation
TMS	Transcranial magnetic stimulation
TTF	Time to task failure
V_E	Minute ventilation
V_E/VCO_2	Ratio of pulmonary ventilation to carbon dioxide output
VA	Voluntary activation
VAS	Visual analogue scale
VCO_2	Carbon dioxide production
VL	Vastus lateralis
VM	Vastus medialis
VO_2	Oxygen uptake
VO_{2MAX}	Maximal oxygen uptake
W	Watts

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Chapter 1 – Introduction & Literature Review

The experience of pain is commonplace in exercise and sport, with the performance of intense and prolonged contractions causing an acute and naturally occurring pain in the exercising muscle (exercise-induced pain; EIP), which is typically associated with the intensity and/or duration of exercise (Cook et al. 1997; O'Connor and Cook 1999). Arising from the sensitisation and activation of Group III and IV muscle afferents, EIP is often accompanied by fatigue (Pollak et al. 2014). Based on this, it has been suggested that the nociceptive processing and/or the psychological drive to escape the experience of EIP may indeed *contribute* to the development of fatigue during exercise (Mauger 2014). Therefore, the ability to tolerate or overcome pain is believed to be a key to success in exercise performance (Anshel and Russell 1994; Mauger 2013). Despite this, the potential fatiguing-impact of EIP on endurance exercise has received limited exploration or attention in contemporary models of fatigue and endurance performance and is therefore still poorly understood. In addition, previous investigations have demonstrated this to be challenging due to the complexity of both constructs, and in isolating the experience of pain (from exercise intensity). Therefore, the initial purpose of this literature review is to provide an overview and background to the relevant theory and research in the areas of "pain", "fatigue" and "endurance performance". This chapter then evaluates the prior experimental approaches used to manipulate EIP, with the fundamental aim to review our current understanding of role of EIP, and how this may potentially contribute to fatigue during endurance exercise tasks.

1.1 Pain

This section provides a comprehensive discussion of the literature regarding the experience of pain. A brief overview to pain and pain theory will be discussed, alongside an insight into the measurement and induction of pain. As a central element of the thesis and importance for all experiments performed, this will be followed by a detailed discussion on the hypertonic saline (HS) experimental pain model. This will include the physiological action of the solution, alongside the prior application of this model in research. Whilst it is acknowledged that the literature in this area is

extensive, this section will primarily focus on concepts of acute pain that are of more relevance to the thesis and EIP, a central theme of this thesis.

1.1.1. An overview of pain

Pain, as defined by the International Association for the Study of Pain, is an "unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (Loeser and Treede 2008). Classified based on factors such as duration (acute, chronic), anatomic location, aetiology and pathophysiology (nociceptive, neuropathic, inflammatory), the causal stimuli for each type of pain varies (e.g. tissue or neural damage, noxious stimuli) (Vadivelu et al. 2009), and may therefore be perceived and responded to differently (Astokorki and Mauger 2017a). As a universally recognised perception, the acute experience of pain in the human body is an important protective function (Sherrington 1906), providing a warning to initiate an adaptive response to minimise tissue damage and modify future behaviour (Vadivelu et al. 2009).

Prior to its contemporary understanding as a complex physiological and psychological construct, the experience of pain was primarily deliberated during ancient times and the Middle Ages from a philosophical context. The term "pain", was believed to be first documented in an ancient text of traditional Chinese medicine, describing pain to be resultant from an disparity between "yin" (negative) and "yang" (positive) (Chen 2011). Subsequently, philosophers begun to postulate on the nature of pain and its experience from an alternate perspective. In particular, the primary source of pain was deliberated, with Aristotle proposing that the heart was the "seat of sensations" with pain as a "passion of the soul" (Dallenbach 1939).

On the other hand, there was growing acknowledgement of the possibility that the location of pain may instead originate within the brain. However, the movement away from the Aristotelian perspective towards the role of the brain did not significantly emerge until the 17th century with work by Harvey and philosopher Descartes (Keller and Krames 2009). In particular, Descartes, influenced by a scientific principles, described the first major theory of pain as a perceptual experience that occurs from the transduction of sensory information (i.e. a noxious stimulus) traveling in a single

pain pathway via specific receptors and peripheral nerves through the spinal cord to the brain (Moayedi and Davis 2013a) (Figure 1). Descartes' pioneering concept of this somatosensory pain pathway in humans and progressive discoveries in the fields of anatomy and physiology of pain underpinned the first of four primary theories of acute pain.

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Fig 1. A sketch depicting Decartes' theory of pain (Decartes et al. 1664). From a sketch reproduced by Moayedi and Davis (2013).

Theories of pain

An influential theory in the field of pain (Bell and Shaw 1868), the Specificity theory proposes that each somatosensory modality has a singular dedicated pathway (from a dedicated receptor through to a particular area of the brain for that sensory modality eliciting one response) (Melzack and Wall 1965; Keller and Krames 2009; Moayedi and Davis 2013a). In the instance of pain, free nerve endings (considered to be pain receptors or "nocicpetors") are stimulated by a specific stimuli when above the

noxious threshold (Melzack and Wall 1965; Perl 2007). This will transmit the signal of pain, which is projected via an associated afferent fiber (a-delta and C-fibres) and the lateral spinothalamic tract in the spinal cord and received in the thalamus of the brain, with the pain perceived proportional to the intensity of the noxious stimuli (Melzack and Wall 1965; Coffey and Mahon 1982). A simplistic concept, there are evidently issues with the viewpoint that one type of receptor elicits a singular response (Coffey and Mahon 1982). In addition, the model is unable to explain certain pain conditions (e.g. low pain intensity, serious injury) (Keller and Krames 2009), and the assumption of pain being associated with peripheral injury, and stimulus severity dictating the degree of pain experienced is inadequate.

Contrary to the Specificity theory, the Intensity theory of pain does not account for the proposal of dedicated and distinct pathways in the body for each somatosensory modality (Moayedi and Davis 2013a). Based and developed upon Plato's initial principle of pain as an emotion that only occurs when an appropriate stimulus intensity is attained (Plato 1998), the Intensity theory describes the effect of afferent activity and postulates that the incidence of pain can arise in any system based on a supra-threshold or summative stimulus intensity (Dallenbach 1939). For example, a noxious stimulus (an intense stimulus) will generate a greater afferent activity than an innocuous stimulus (a weak stimulus), which would indicate a painful over a non-painful event (Moayedi and Davis 2013a). The credibility of this theory was however questioned upon the introduction of *specialised* nerve endings ("nociceptors") and nociception by Sherrington's framework (1906), which outlined that nociceptors specifically respond to the occurrence of tissue injury or damage (i.e. a "noxious" stimuli) (Sherrington 1906; Moayedi and Davis 2013a).

A further opponent to both the Specificity and Intensity theories is the Pattern theory of pain. This theory disregards specific fibers and nerve endings, and hypothesises that sensory organs react to an extensive range of stimulus intensity (innocuous to noxious) with varying levels of responsivity (Nafe 1929). As a result, the summation of activity from the particular firing pattern of individual afferent neurons (residing in a specific region of the body) encodes and signals the type and location of the applied stimulus (chemical, mechanical and thermal), producing the sensation of pain (Nafe 1929). Whilst progressive, these theories all have shortcomings in terms of viewing

the brain as a recipient of information and failing to consider any potential psychological involvement in pain processing (Melzack 1993; Keller and Krames 2009).

The Gate Control theory (Melzack and Wall 1965) incorporates key elements of prior theories (pain receptor specificity, pain transmission patterning) and highlights the importance of the central nervous system (CNS) and psychological factors in pain processing (Keller and Krames 2009; Moayedi and Davis 2013a). The model proposed a "gating mechanism" in the dorsal horn of the spine that is modulated by sensory afferent input to spinal cord transmission (T) cells, and descending impulses at a supraspinal level from the brain (Melzack and Wall 1965). At the periphery, the balance in relative activity of small-diameter nociceptive (C-fibres) and myelinated large-diameter (A-fibres) afferents partially control the "gate", with A-fibres considered to be inhibitory of pain and C-fibres facilitating pain (Melzack and Wall 1965). In summary, when the transmission of nociceptive signals surpass the critical level of inhibition from large fibre stimulation, the gate is "opened" and this activates the "action system" leading to the experience of pain (Melzack and Wall 1965; Melzack 1993). This theory is considered to be the most prominent theory within the literature and an influential framework in furthering the understanding of pain to date (Perl 2007; Moayedi and Davis 2013a). However, it has since been scrutinised for elements of inaccuracy and an oversimplification including the neuroanatomical structure of the spinal cord, the location and mechanism by which large-diameter afferent fibres exert an inhibitory action, and the spinal modulation of nociceptive information (Nathan 1976; Humphries et al. 1996; Moayedi and Davis 2013b). Figure 2 summarises the four aforementioned pain theories.

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Fig 2. Diagrams summarising the assumptions made by the theories of pain (Specificity theory, a; Intensity theory, b; Pattern theory, c; Gate control theory, d) regarding the relationship between noxious or innocuous stimuli and afferent signalling. From Perl (2007)

Pain pathway

In order to induce the experience of pain, four key neurological processes occur: transduction, transmission, modulation and perception (Vadivelu et al. 2009). The process of transduction refers to activation and sensitisation of nociceptors in primary

afferent neurons by mediating chemical substances (e.g. bradykinin, potassium, serotonin) released in response to a noxious stimulus, and the subsequent conversion of this into an action potential and a neural signal (Vadivelu et al. 2009). This pain impulse is then transmitted via Group III and IV afferents to the dorsal horn, ascending to the brain stem, and the thalamus and somatosensory cortex of the brain (transmission) (Vadivelu et al. 2009). Throughout this pathway, pain modulatory networks at the dorsal horn modify the nociceptive information, either inhibiting or facilitating the pain signal (modulation) (Loeser and Melzack 1999). When relayed to the brain, the signal is integrated and processed, and interpreted as the experience of pain (perception) (Vadivelu et al. 2009).

It is important to establish that pain and nociception are independent entities (Loeser and Treede 2008). The concept of nociception refers to the "objective" detection and neural processing of a noxious stimulus (i.e. any stimuli that causes actual or potential tissue damage) via nociceptive afferents to the CNS and higher brain centres that results in the conscious sensation of pain (Mense 1993; Almeida et al. 2004). The experience of pain is a conscious phenomenon that occurs resultant of neural activity in the brain (Mense 1993). As pain is highly sensitive to changes in sensory, affective, cognitive and motivational factors (Almeida et al. 2004), and is consequently likely to be processed differently between individuals, it can be considered to be a subjective experience.

Role of psychological and cognitive factors

As an unpleasant, complex and subjective experience with both sensory and affective components, the perception of pain is not necessarily proportional to the nociceptive input and can be strongly influenced by psychological and cognitive factors (Wiech and Tracey 2009). The Gate Control theory (see *theories of pain*) (Melzack and Wall 1965) first proposed that psychological processes contribute to modulation of the "gating mechanism" through descending pathways (Rhudy and Meagher 2001; Peerdeman et al. 2016). Therefore, in the experimental investigation of pain, it is important to acknowledge that the experience of pain can also be altered by the psychological state of the individual (Price 2000). This can encompass many variables, however the most prominent factors in pain modulation are expectations,

emotion/mood and attentional state (Villemure and Bushnell 2002; Linton and Shaw 2011; Atlas and Wager 2012).

It has been widely accepted that pain expectations (cognitions on the predicted probability about future events or outcomes based on prior information of a stimulus) can have a strong influence on the perception of a noxious stimuli (Atlas and Wager 2012). For example, research investigating the manipulation of pain expectations has shown that greater expectations of acute pain are associated with a greater pain experienced, and vice versa (even in the presence of innocuous stimulation) (Sawamoto et al. 2000; Koyama et al. 2005; Tracey 2010; Atlas and Wager 2012; Wiech et al. 2014; Peerdeman et al. 2016). Expectancies in this context are not solely limited to the effect of a stimulus but can also interact with the individual's perceived ability to cope with the expected pain (i.e. pain-specific self-efficacy), which may also influence or be predictive of pain tolerance to an aversive stimulus (Litt 1988; Peerdeman et al. 2016). It is however suggested that the effects of expectations on pain may be resultant from its influence on overriding processes such as emotion and attention (Atlas and Wager 2012)

Indeed, attentional state can moderate the experience of both the sensory (e.g. intensity) and affective (e.g. unpleasantness) components of pain (Villemure and Bushnell 2002; Villemure et al. 2003; Wiech et al. 2008). The experimental manipulation of attentional focus has demonstrated that the pain experienced is exacerbated when instructed to focus on the pain (Levine et al. 1982) whilst an attenuated perception of pain occurs when distracted and redirected away from the pain (Miron et al. 1989; Longe et al. 2001). Research investigating perceptual differences when required to attend to a painful stimulus are however inconsistent, with findings that focusing on the pain paradoxically reduces its perception (Villemure and Bushnell 2002). In addition, pain can also demand attention and serve as interruptive function (Eccleston and Crombez 1999), confounding the ability to ascertain the extent at which pain is susceptible to attentional modulation (Villemure and Bushnell 2002; Wiech et al. 2008). With a finite attentional capacity, the threatening experience of pain may result in a significant demand for these available resources (Eccleston 1995; Rainville et al. 2005; Wiech and Tracey 2013). When occurring concurrently with an additional sensory modality, cognitive processes or

exercise task, the experience of pain could cause a redirection of attention and potentially compromise task performance or result in an attenuation of pain perception (Wiech et al. 2008).

Mood, emotions, and the ability to process and manage emotional states (e.g. emotional intelligence) are also key factors that can provide an explanation for intra- and inter-individual differences in pain experience and tolerance (Price 2000; Keefe et al. 2001; Ruiz-Aranda et al. 2011), and are also closely associated with the previously mentioned variables (Keogh et al. 2001; Öhman et al. 2001; Rainville et al. 2005). Experimental manipulations to improve mood and emotion (e.g. "pleasant" stimuli) have generally demonstrated a reduced perception of pain and improved pain tolerance (Zelman et al. 1991; De Wied and Verbaten 2001; Meagher et al. 2001), whilst inducing a negative mood or increased negative affect (the experience of feeling emotion) has the opposite effect (Zelman et al. 1991; De Wied and Verbaten 2001; Meagher et al. 2001; Wiech and Tracey 2009). In addition, the subconscious or conscious ability to process affective information, and effectively manage and reduce negative affect may serve to diminish the intensity of perceived pain (Tracey 2010; Ruiz-Aranda et al. 2011). A key element of processing information, emotional intelligence (the ability to perceive, facilitate, appraise, and manage emotions within oneself and in others) has been associated with negative effect, with individuals possessing a greater emotional intelligence reporting a lower negative affect and subsequently perceiving less pain (Ruiz-Aranda et al. 2011). This suggests that emotional intelligence may therefore be an important additional factor in understanding the variability in acute pain perception between individuals. Due to the implications of the aforementioned variables on the modulation of pain and endurance performance (McCormick et al. 2015), the implementation of appropriate psychological scales is of great importance for the experimental investigation of pain during exercise.

1.1.2. Measurement and induction of acute pain

This section will provide a brief discussion on the standard methods of inducing pain (e.g. thermal, ischemic, electrical and chemical), with a specific focus on EIP, and the common methods used to evaluate the pain induced by these models. The current

understanding of pain (in terms of nociception and transmission) predominantly comes from brief and phasic models of pain induction (e.g. evaluating the brain electrical activity from a short-duration heat stimulus) (Carmon et al. 1976; Iannetti et al. 2003) however knowledge of tonic pain (a stimuli that can induce pain extending over several minutes up to less than one hour) (Treede 1995) is more limited. Experimental pain models applied to healthy individuals provide a controlled and standardised means to activate the nociceptive system, and measure perceptions of the evoked pain (Staahl and Drewes 2004). There are various models including mechanical, electrical, chemical, ischemic and exercise-induced that can be applied in different tissues of the body (skin, muscle viscera) and allow for the physiological response to the experience of pain to be measured (Staahl and Drewes 2004; Olesen et al. 2012). In experimental research, the pain model should be carefully selected to ensure that the processing and experience of pain elicited is of the greatest relevance to the desired type of pain (e.g. nociceptive, neuropathic, inflammatory) (Section 1.1.1) to be investigated. However at present, there are limited satisfactory methods in existence for the experimental induction of muscle pain similar to that experienced during exercise (a central element of this thesis).

The measurement of the pain induced by human experimental models is challenging due to the overall complexity of pain and the inability to directly record nociceptive activity. Despite this, an indicative measure of pain can be made through several accepted techniques. This can be achieved either objectively via neurophysiological assessment (e.g. electrical stimulation, measurement of brain activity) (Chen 2001), or subjectively, through observational reports (also this can be a biased and unreliable measure) or self-report unidimensional or multidimensional pain scales (Jensen and Karoly 2011; Katz and Melzack 2011). As a multidimensional construct encompassing intensity, location and quality, these measures attempt to define the pain experienced from either a singular or combined context.

Measurement of pain

Unidimensional self-report include the visual analogue scale (VAS), numerical rating scale (NRS) and verbal rating scale, faces pain scale, Iowa pain thermometer and category ratio scales (Cook et al. 1997) (Figure 3). These scales typically measure

pain intensity (the sensory dimension) on a scale of 0-10 or 0-100 and are commonly anchored by verbal descriptors ranging from “no pain” to “worst imaginable”, “severe” or “unbearable” pain (Williamson and Hoggart 2005; Ferreira-Valente et al. 2011; Hawker et al. 2011). These easily administrable and robust scales are considered to provide a reliable and valid measure of pain (Price et al. 1983; Jensen et al. 1986; Ferreira-Valente et al. 2011). In particular, the VAS (0-100) and NRS are more responsive and highly sensitive to changes during acute experimental pain (Rosier et al. 2002; Price et al. 2008; Ferreira-Valente et al. 2011).

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Fig 3. Common scales for the unidimensional assessment of pain (visual analogy scale, VAS; numerical rating scale, NRS; verbal rating scale). From Williamson and Hoggart (2005).

These scales are generally administered in verbal or written form, although scales such as the VAS are translatable to electrical devices to continuously record pain over time. This is beneficial if stimulus-response curves to experimental pain are required (Staahl and Drewes 2004). A continuous measure of pain intensity also permits the identification of an individual’s pain threshold (“the minimum stimulus intensity that

is initially perceived as painful") and tolerance ("the length of time an individual is willing to endure a noxious stimuli" or "the maximal level of perceived pain that one will endure) (O'Connor and Cook 1999). However the assumption of pain intensity as the focal measure of pain only provides a singular classification of the overall experience, and therefore additional adapted VAS scales would need to be implemented to define other dimensions (e.g. affect) (Rainville et al. 1992) or soreness (Svensson and Arendt-Nielsen 1995). This would therefore render the sole use of a unidimensional scale inefficient when attempting to elucidate the detailed overall experience of acute experimental pain or gain an understanding of underpinning mechanisms (Olesen et al. 2012).

Alternatively, with several elements (location, quality) and components (Melzack and Casey 1968; Katz and Melzack 2011), the use of tools that measure outside just the magnitudinal aspect of acute pain and consider it in a multidimensional context, are desirable. A "gold standard" tool that details the broad experience and qualities of acute experimental pain is the McGill Pain Questionnaire (MPQ) or Short-Form MPQ (SF-MPQ) (Melzack 1975, 1987) (Figure 4). The MPQ is an inventory of descriptors across 20 subcategories used to outline the "language of pain", defining the sensory, affective, evaluative and miscellaneous

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Fig 4. The McGill Pain Questionnaire (MPQ; top panel) and short-form MPQ (SF-MPQ; bottom panel). From Melzack (1975).

aspects of pain experienced. Responses from the MPQ also have a scale to summarise the overall pain intensity (PPI) and an anatomical map to indicate distribution (Melzack 1975). As such, the MPQ provides information on the intensity of pain alongside its emotional and physical components, as well as interpreting both quantitative (e.g. number of words chosen, pain rating scores) and qualitative (e.g. the descriptors selected) elements of the pain experience (Fernandez and Boyle 2002; Hawker et al. 2011). Evidence supports the construct validity of the MPQ for acute pain (Reading 1982), however due to its comprehensive nature, it could be perceived as overly complex (requiring an understanding of the 78 adjectives) and inefficient in a time-sensitive research setting (Hawker et al. 2011; Olesen et al. 2012). At the expense of evaluating pain in a broader context, the SF-MPQ (containing a reduced number of words describing only the sensory and affective classifications) is an established and valid measure that allows for a more rapid assessment and correlates with the overall pain score of the MPQ (Melzack 1987).

Neurophysiological methods after the administration of a noxious stimuli provide an alternate assessment of experimental pain. The most common methods are the measurement of brain activity (e.g. magnetic resonance imaging (MRI), electroencephalography (EEG) and the nociceptive withdrawal reflex (Staahl and Drewes 2004; Olesen et al. 2012). The nociceptive withdrawal reflex is elicited by the cutaneous application of a noxious stimulus (Plaghki et al. 1998; Andersen et al. 1999, 2006). The size of the reflex, as indicated by electromyography (sEMG), is well correlated with subjective pain intensity stimulus-response (Staahl and Drewes 2004), and therefore provides an objective measure of experimental pain induction (Gracely 1999). In addition, methods such as MRI and EEG which can indicate neuronal activity and brain-evoked potentials in response to noxious stimuli, have been applied to aid understanding of central pain processing (Chen 2001). There is however limited evidence for the application of these neurophysiological methods to tonic pain induction.

Induction of pain

In the study and assessment of pain, there are various techniques to stimulate acute pain in humans. Experimental models provide a specific and controlled means to

induce and mimic the experience of pain under varying conditions (Staahl and Drewes 2004). This allows for isolated psychophysiological, behavioural or neurophysiological measures and mechanisms in response to the administered pain to be recorded (Graven-Nielsen and Arendt-Nielsen 2003; Staahl and Drewes 2004). Each model is responded to, and processed differently (Olesen et al. 2012), and therefore careful consideration is required when selecting a technique. A majority of research investigating experimental models have been performed on cutaneous pain; a superficial "burning" and "sharp" pain experienced locally at the site of injury (Svensson et al. 1997a; Graven-Nielsen and Arendt-Nielsen 2003). However, as the premise of this thesis is to explore the impact of deep tissue pain experienced in the muscle during exercise, this subsection will focus on specifically muscle pain (the "cramping", "aching" localised experience of pain caused by the activation of Group III and IV nociceptive afferents (Mense 1993).

Endogenous methods

Ischemic pain is an extensively employed and reliable model as a general pain stimulus (Lewis 1932; Sternbach et al. 1977). This form of pain is induced by limb occlusion in combination with isometric or dynamic contractions, resulting in an accumulation of metabolites and mechanical pressure (Graven-Nielsen et al. 2003). Dependent on the intensity, frequency or duration of contraction, an unpleasant and deep sensation of moderate to strong pain (described as "burning", "heavy" and "exhausting") develops over time until the experience becomes intolerable and the tourniquet is released (Mills et al. 1982; Svensson and Arendt-Nielsen 1995; Graven-Nielsen et al. 2003). As indicated by the reported quality of pain, it should be noted that the pain from this model is not specific to the muscle or muscle group and also occurs in superficial tissues (Staahl and Drewes 2004; Graven-Nielsen 2006; Olesen et al. 2012).

Isometric or dynamic concentric muscular contractions or exercise (cycle ergometry) of a heavy intensity or with insufficient rest provides an alternative method that can isolate pain to a specific muscle group (e.g. quadriceps) (Cook et al. 1997; Staahl and Drewes 2004; Graven-Nielsen 2006). Pain during concentric muscle contractions are believed to be consequential of impaired blood flow, therefore potentially an

environment similar to ischaemic muscle pain (Graven-Nielsen and Arendt-Nielsen 2003). In addition, cycling exercise of a moderate to high intensity reliably produces a transient muscle pain that is proportional to the intensity of concomitant exercise (O'Connor and Cook 2001). Whilst this model has the benefit of specificity to muscle pain, the involvement of several large muscle groups has issues with control and is preventative of investigating pain in a singular muscle (Olesen et al. 2012). In contrast to the occurrence of pain during exercise, performing unfamiliar or eccentric muscle contractions may cause a delayed onset muscle soreness (DOMS). Dependent on duration and intensity of exercise performed, the experience of DOMS peaks approximately 24 to 48 hours after exercise completion and diminishes within 96 hours (Newham 1988; Cheung et al. 2003; Connolly et al. 2003). The underpinning cause of DOMS induced by micro-injuries is believed to be related to a combination of mechanisms including structural tissue damage, the release of algesic substances, muscle spasms and inflammation (Cheung et al. 2003). Uniquely, DOMS does not result in pain experienced under resting conditions, and instead requires movement or mechanical pressure of the affected muscle or muscles to evoke a painful sensation (Cheung et al. 2003; Olesen et al. 2012). However, as DOMS is resultant from muscle damage, it is difficult to discern between damage-induced or specifically pain-induced changes in task performance.

Exogenous methods

Electrical stimulation evoked via needle electrodes applied either intramuscularly or proximal to the sensory fascicle of the nerve supplying the muscle is an invasive yet tissue-specific, reliable and well controlled method to evoke and assess muscle pain (Laursen et al. 1997; Schulte et al. 2003). Dissimilar to the naturally occurring muscle pain experienced during exercise, the pain from this method is described as a "boring", "penetrating" local and referred sensation that is present during the stimulation with no long-lasting effects (Laursen et al. 1997). As such, this method can be applied in an "on" or "off" fashion (Laursen et al. 1997). When applied repeatedly or at an increased intensity, this method can induce temporal summation resulting in a proportional increase in spread of pain area and the experience of referred pain, demonstrating central changes (Arendt-Nielsen et al. 1997; Schulte et al. 2003).

Compared to the muscle pain during exercise (caused by the activation of Group III and IV nociceptive afferents), electrical stimulation is however a non-physiological method of pain induction that circumvents the nociceptors and directly facilitates both nociceptive and non-nociceptive afferent input (Graven-Nielsen and Mense 2001; Staahl and Drewes 2004; Olesen et al. 2012). In addition, dependent on electrode placement, electrical stimulation also can evoke a concurrent muscle twitch, confounding the produced sensations of pain and causing issues with reproducibility (Graven-Nielsen and Arendt-Nielsen 2003).

Manual or computer-controlled algometry is an alternate and common mechanical method of pain stimulation in a small volume of the muscle. Due to its extensive use, a standardised algometry technique has been devised, with various reference values for different muscles (Staahl and Drewes 2004). Manual application of this model is however associated with inconsistency in the pressure rate applied, with further methodological concerns regarding inter-experimenter variability (Antonaci et al. 1998). Techniques such as computer-controlled algometry (allowing the pressure to be automatically controlled) or cuff algometry (Graven-Nielsen et al. 1998b; Polianskis et al. 2002) address these issues and are able to assess the relationship between pressure and pain intensity (Staud et al. 2003). Regardless of technique, the ability to investigate muscle pain is confounded by the unavoidable stimulation of the cutaneous nociceptors and low-threshold non-nociceptors (Graven-Nielsen and Arendt-Nielsen 2003). Therefore, similar to electrical stimulation, algometry is an experimental pain model that is non-specific. As this model induces both cutaneous and muscle pain in a small portion of the muscle, and is problematic to apply during locomotive exercise, algometry is unlikely to be an appropriate method to mimic the naturally occurring EIP experienced during exercise.

A final exogenous model of experimental muscle pain is through the intramuscular injection of algesic substances (e.g. capsaicin, bradykinin, glutamate). When injected into the muscle these substances activate the nociceptors, sensitise muscle afferents and generally induce a significant mild to moderate pain intensity, with notable inter-individual differences in response (Mense 1993; Mørk et al. 2003). This is however is dependent on the experimental paradigm (e.g. volume, concentration, rate of infusion,

location) (Graven-Nielsen 2006). Unlike the electrical and mechanical stimulation methods, once administered, this method continuously evokes pain until the cessation of solution action (Laursen et al. 1999). The most extensively employed form of chemical stimulation in the study of muscle pain is the HS model, which will be discussed in the following section.

1.1.3. Intramuscular injections of hypertonic saline

Hypertonic saline (HS) as an experimental method of evoking muscle pain was first introduced in the 1930s (Lewis 1938; Kellgren 1938), but remained relatively unexplored until the 1990s. Since then, it has become a widely accepted method that induces a quality of pain replicative of muscle pain in a specific muscle with good intra-individual reliability, and the advantage of being placebo-controlled with an isotonic saline solution (IS) (Graven-Nielsen et al. 1997b). As this model does not cause muscle toxicity (at concentrations up to 6%), and is not related to tissue damage it is considered safe for human experimentation, with the occurrence of minimal side effects after numerous applications across many studies (Graven-Nielsen and Arendt-Nielsen 2003; Svendsen et al. 2005; Graven-Nielsen 2006).

From a mechanistic perspective, it was suggested that muscle pain from the HS may be resultant from local muscle spasms (Lewis and Kellgren 1939) or intramuscular pressure (Graven-Nielsen et al. 2000). However a study investigating changes in resting intramuscular and surface EMG activity in both the masseter and tibialis anterior muscles found no significant differences in muscle activity between the application of HS or IS (Svensson et al. 1998), implying that muscle pain from spasms is not the case (Graven-Nielsen 2006). In addition, another study demonstrated a significant temporary elevation in intramuscular pressure regardless of the concentration injected was isotonic and hypertonic, thus suggesting that the muscle pain by this model is not induced by changes in intramuscular pressure (Graven-Nielsen et al. 1997d). Instead, it is believed that the high sodium concentration is the effective painful stimulus (Mense 2009).

When injected, HS was found to predominantly excite Group IV nociceptors with a more minor contribution from Group III afferents (Laursen et al. 1999). Correlating

with the large proportion of group IV afferent activation (Svendsen et al. 2005), the HS produces a deep "moderate" to "somewhat strong" pain with a quality that is relevant to naturally occurring acute EIP (e.g. "aching", "throbbing", "cramping") (Kellgren 1938; Graven-Nielsen et al. 2003). Using an MPQ, acute stimulation is mainly described in the sensory pain classification, with affective descriptors principally selected when the experience is more prolonged (Stohler and Kowalski 1999). In addition, whilst an invasive procedure that punctures the skin to access the deep muscle, studies which have applied the model to anesthetized and normal skin have shown that cutaneous nociceptors are likely to have minimal to no contribution to the perceived pain (Arendt-Nielsen et al. 1996; Svensson et al. 1996). Combined, as HS has both a nociceptive pathway specific to the muscle and potential experience of pain that is not dissimilar from acute musculoskeletal pain, this method could therefore provide an experimental model to replicate EIP.

It should be noted that the HS model does have significant inter-individual variations in the experience of pain as measured by a VAS and MPQ (Graven-Nielsen et al. 1997b). However, a high level of reproducibility with good re-test reliability in pain intensity, quality and distribution is present for individuals receiving repeated injections either within the same session or as a series of singular administrations separated by one week (Graven-Nielsen et al. 1997a; Schulte et al. 2003; Graven-Nielsen 2006). Therefore, when applied in an experimental setting that requires repeated visits and more than one injection, this model can be used with confidence that participants will have a similar experience in each session.

1.1.4. Considerations for hypertonic saline experimental model

When applying any experimental model, a consideration of factors that may influence and underpin the experience of pain should be carefully considered to ensure a robust and reliable response. Prior research has ascertained that the experience of pain (intensity, quality and distribution) elicited by the HS experimental muscle pain model is dependent on the infusion paradigm (volume, concentration, rate and tissue injected) (Graven-Nielsen et al. 1997b). Commonly, the HS is applied unilaterally or bilaterally (in a singular bolus or in a repeated sequential manner), or as a larger volume continuously infused into the muscle over time (Graven-Nielsen et al. 1997a,

2003; Svensson et al. 1997b; Ge et al. 2006; Larsen et al. 2016). Earlier studies performed this technique manually; however, the development of computer-controlled syringe pumps improved the infusion standardisation (Graven-Nielsen and Arendt-Nielsen 2003).

Indeed, through cannulation and a computer controlled syringe pump, combined with the assistance of continuous feedback from a VAS (as a measure of pain intensity), studies have employed a controlled infusion of HS over a set period to maintain a desired steady-state pain intensity (Fazalbhoy et al. 2012a; Kobuch et al. 2015). On the other hand, some protocols have involved a constant infusion rate to ensure a stimulation of a longer duration (e.g. 15 to 20 minutes) (Graven-Nielsen et al. 1998a; Schabrun and Hodges 2012), although this is believed to decrease the overall experience of pain (Zhang et al. 1993; Graven-Nielsen et al. 1997c). Access to equipment and the suitability of this method to be applied during exercise (i.e. inability for the needle to remain within the muscle during contraction) are however limitations.

A single bolus injection generally induces pain for up to 5 minutes (Graven-Nielsen et al. 1997c), with a rapid onset of localised and referred pain that intensifies, reaches a maximal point and then declines back to the state of "no pain" (Lewis 1938; Kellgren 1938; Svensson and Arendt-Nielsen 1995; Graven-Nielsen 2006) (Figure 5). Greater pain intensity and distribution (local pain) has been observed after sequential injections (4 x 0.1 mL) with a 90 s inter-stimulus interval at spatially separated sites in the same muscle when compared to a single bolus infusion of the combined volume (0.4 mL) (Graven-Nielsen et al. 1997a). Bilateral injections of HS have also been associated with a greater pain intensity and spread in the trapezius and longissimus muscles (Ge et al. 2006; Larsen et al. 2016), demonstrating an effect of spatial summation (Graven-Nielsen et al. 1997a).

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Fig 5. Exemplar of pain intensity profiles (as recorded by a visual analogue scale; VAS) from 10 participants who received a single bolus injection of hypertonic saline (into the tibialis anterior). Note the inter-individual variation in response between participants. From Graven-Nielsen et al (1997b).

Whether injected as a singular bolus, administered sequentially or continuously infused, it has been reported that the pain experienced from the HS is dependent on the concentration and volume of solution, and the rate of infusion (Graven-Nielsen et al. 1997b). Most studies employing a bolus injection of HS administer a concentration of between 5.0 to 6.7%, with 0.9% IS used as a placebo. However, pain duration and quality was found to correlate with saline concentration, with progressive increases parallel with greater solution concentrations (up to 20% saline) (Graven-Nielsen et al. 1997b), whilst pain intensity also proportionally increased up to concentrations of between 10 to 11.5% saline upon where a plateau occurred (Graven-Nielsen et al. 1997b). Smaller volumes of HS (e.g. 0.1 mL) produce a reduced peak pain intensity, quality and VAS area than a larger volume of HS (e.g. 0.5 mL) with the same rate of infusion (e.g. 20 s) (Graven-Nielsen et al. 1997b). However, the rate of infusion does not influence these parameters when the same volume of solution is administered (Graven-Nielsen et al. 1997b). Instead, the "faster" infusion rate (e.g. 20 s) resulted in a quicker onset of pain intensity than a "slower" infusion rate (100 s), which was attributed to an earlier activation of the nociceptors (Graven-Nielsen et al. 1997b). In

general, most experimental work utilising the HS model appear to employ a 20 s infusion window (e.g. Graven-Nielsen et al. 1997b, d, 1998b).

From a perspective of both safety and differences in chemical sensitivity, the volume and infusion rate is also constrained by the tissue injected (Ogston-Tuck 2014). Within the literature, a single bolus of HS has been applied in differing volumes (ranging from 0.2 to 1.5 mL) in a diverse range of muscles. This includes the tibialis anterior (Graven-Nielsen et al. 1997e; Farina et al. 2005a), trapezius (Ge et al. 2006; Falla et al. 2009), longissimus (Arendt-Nielsen et al. 1996), biceps brachii (Ervilha et al. 2005; Khan et al. 2011), masseter (Svensson et al. 1996), gastrocnemius medialis, erector spinae (van den Hoorn et al. 2015) or first dorsal interosseus (Larsen et al. 2018). The notion that different muscle volumes may affect the pain intensity response suggests that the size and morphology of the selected muscle should be considered to ensure an appropriate volume, concentration and infusion rate (Kellgren 1938; Schmidt-Hansen et al. 2006; Henriksen et al. 2007). This however has not been consistently observed, with no difference in pain intensity or quality between the tibialis anterior and brachioradialis (Graven-Nielsen et al. 1997b) or lumbar erector spinae muscles (Loram et al. 2009) after 0.5 mL of HS at the same rate of infusion. In addition, the muscle in which the HS is applied has also been shown to dictate the distribution of pain (i.e. localised or referred) (Graven-Nielsen et al. 1997b). For example, local pain is typically observed in muscles such as the biceps brachii (Graven-Nielsen 2006) whilst a delayed experience of referred pain towards proximal joints has appeared after infusions in muscles such as the tibialis anterior (Graven-Nielsen et al. 1997c).

Not related to the experimental paradigm of HS intramuscular injections but of importance is the influence of sex. Females are commonly more sensitive to pain than males, regardless of the site or pain induction method used, although this is most consistently observed in experimental stimuli that evokes deep muscle pain (Fillingim and Maixner 1995; Loram et al. 2009). An elevated pain intensity (mean and peak pain) in females has been recorded in studies employing the HS model in the trapezius (Ge et al. 2004; Falla et al. 2008) and tibialis anterior (Lei and You 2012) muscles, although no differences were observed for pain quality or distribution. Intriguingly, this has however not been consistently observed, with a similar intensity

but different pain quality reported in males and females after HS was injected in the tibialis anterior and lumbar erector spinae (Loram et al. 2009). The reasons for these inconsistent findings are somewhat uncertain (Falla et al. 2008) and could be resultant of an array of factors (e.g. genetic, hormonal, psychological and social) (Lei and You 2012). Differences in muscle volume between the sexes are an unlikely reason due to similar distributions of pain reported (Falla et al. 2008; Lei and You 2012).

1.1.5. Previous applications of hypertonic saline experimental model

The induction of muscle pain by HS has been extensively employed to enable the investigation of pain mechanisms and responses. These are typically assessed through the use of single-limb isometric or dynamic tasks utilising small muscles or muscle groups at either the ankle or elbow joints (e.g. Graven-Nielsen et al. 1997e; Ciubotariu et al. 2004; Khan et al. 2011), with limited investigation into the muscles surrounding the knee. As pain can cause changes in movement, muscle coordination and contraction performance (Hodges and Tucker 2011), the most prominently researched area using this model is the study of the reflex and central mechanisms that underpin the alternation of motor control strategy during acute muscle pain (e.g. Svensson et al. 1996; Farina et al. 2005b; Madeleine et al. 2006), alongside the study of referred pain patterns from musculoskeletal structures (Ge et al. 2006; Schmidt-Hansen et al. 2006).

Of importance for appropriate motor control is the sense of proprioception, the awareness of limb position and movement, and perception of force produced by the muscle (Proske and Gandevia 2012; Bank et al. 2013). The activation of Group III and IV afferents and the associated presence of muscle pain is believed to cause a pronounced deterioration in the ability to estimate the force applied by a muscle (Weerakkody et al. 2003; Proske and Gandevia 2012), with evidence demonstrating an unimpaired position or movement sense at the ankle joint (Matre et al. 2002). Prior research investigating the impact of experimental muscle pain on the judgement of torque or force has predominantly applied the HS model in the upper limb in a small muscle group (biceps brachii) (Proske et al. 2003, 2004; Weerakkody et al. 2003). These studies support the notion that pain impedes accuracy in a torque matching and estimation task, with participants specifically overestimating the torque being

produced by the painful biceps brachii and therefore producing less torque than the outlined target (Proske et al. 2003, 2004; Weerakkody et al. 2003).

Dependent on the conditions (e.g. resting, static or dynamic contractions), short-term adaptations occur in the presence of HS-induced pain as a preventative mechanical response to reduce pain and minimise further damage (Lund et al. 1991; Hodges and Tucker 2011). The manner in which muscle activity is redistributed has been extensively debated, with several theories proposed to explain these changes (Johansson and Sojka 1991; Lund et al. 1991; Hodges and Tucker 2011) (Section 1.4.1). Adaptations in muscle recruitment pattern are mainly detected by changes in sEMG activity via electrodes placed on the relevant agonist and antagonist muscles for the movement performed (Graven-Nielsen et al. 2000) (Figure 6). Measures of resting sEMG levels have demonstrated no consistent evidence for the effects of pain on muscle activity (Graven-Nielsen et al. 1997e; Svensson et al. 1998), with it suggested that this may be due to differences between experimental pain model used and the muscle stimulated (Svensson et al. 2004; Graven-Nielsen and Arendt-Nielsen 2008).

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Fig 6. An example of pain induced by intramuscular injection of hypertonic saline into a muscle of the back with recordings of EMG to assess changes in muscle activity and co-ordination. From Graven-Nielsen et al (2000).

During both isometric and dynamic contractions of the upper and lower limb, a majority of studies investigating changes within or between muscles have shown that HS experimental muscle pain generally reduces motor unit firing rate (Sohn et al. 2000; Farina et al. 2005b) and the recruitment of the painful agonist muscle (Graven-Nielsen et al. 1997e; Ciubotariu et al. 2004; Henriksen et al. 2007, 2009). In addition, unilateral muscle pain induced in two knee extensor muscles (vastus lateralis and medialis) during a bilateral cycling task reduced sEMG activity during the extension phase in both the painful muscles and a 'non-painful' quadriceps muscle (rectus femoris) of the painful leg (Brøchner Nielsen et al. 2017).

Compensatory HS-induced alterations in motor control and muscle activity (of both the painful and surrounding muscles) have also been demonstrated during the performance of typical daily movements such as gait (Arendt-Nielsen et al. 1996; Graven-Nielsen et al. 1997e; Henriksen et al. 2007; van den Hoorn et al. 2015) or mastication (Svensson et al. 1996, 1997b). It should be noted that other studies, however, have not reported any change in sEMG activity (Hodges et al. 2008; Tucker and Hodges 2009). It has been postulated that these contrasting findings could be explained by differences in contraction intensity or level of muscle activity for the respective area tested (upper or lower limbs, shoulder-neck region) with the sEMG technique used also limitative (Bank et al. 2013).

Finally, an evaluation of task performance in the presence of experimental pain during isometric contractions provides a well-established indicator of the effects of pain on motor control characteristics (Bank et al. 2013). During sustained contractions of a single-limb, experimental muscle pain induced by HS may cause significant reductions in endurance time compared to non-painful conditions (Graven-Nielsen et al. 1997e; Ciubotariu et al. 2004). This has been evidenced in tibialis anterior and gastrocnemius lateralis at 50 and 80% maximal voluntary torque (MVT) (Graven-Nielsen et al. 1997e; Ciubotariu et al. 2004), but not in the biceps brachii at 40% MVT (Schulte et al. 2004). It has also been reliably reported that acute experimental muscle pain causes an inhibition of MVT (Graven-Nielsen et al. 2002; Henriksen et al. 2011). These changes may compromise the exercise task, which are likely to explain reductions in endurance time. To understand the mechanistic basis for this

reduction in sustained isometric performance, a knowledge of "fatigue" is however required.

1.2 Fatigue

Fatigue is a broad term that can encompass a range of definitions dependent on discipline. Originating from the Latin word "*fatigare"* ("to weary or tire out"), fatigue defines internal or subjective feelings of extreme tiredness from mental or physical exertion, or as a symptom of an illness or disease (Hawley and Wolfe 1997; Davis and Walsh 2004; Twomey et al. 2017). Generally, this represents a decline in physical performance, with an actual or perceived increase in task difficulty or effort to exert force (Enoka and Stuart 1992). In the context of the muscle, exercise and performance, fatigue was initially defined as the "inability to maintain the required force" (Edwards 1981). This limited definition considers fatigue to be associated with a sudden point of task failure, and does not consider fatigue as a complex or transient phenomenon that occurs via various physiological processes (Abbiss and Laursen 2005; Boyas and Guével 2011).

Since fatigue was acknowledged to be a multifactorial experience that gradually develops from the onset of contraction (Bigland-Ritchie and Woods 1984), a revised definition was proposed. Muscle fatigue has since been defined as "any exercise-induced reduction in the maximal voluntary force or power generated by a muscle or muscle group regardless of whether the exercise task can be sustained or not" (Bigland-Ritchie and Woods 1984; Gandevia 2001). This can occur in different forms of exercise ranging from sustained isometric, single-joint exercise utilising small muscles or muscle groups to dynamic, whole-body exercise (i.e. prolonged cycling or running) involving a larger muscle mass. The mechanisms which cause fatigue however vary, and are highly dependent on the type of exercise task performed (i.e. modality, duration and intensity) (Enoka and Stuart 1992).

An initial viewpoint was that the mechanisms underpinning the decline in force production from the onset of contraction originated within the contractile apparatus (Allen et al. 2008; Taylor et al. 2016). However, it has since been acknowledged that force production is dependent on the entirety of the neuromuscular system (from the

brain to the muscle cross-bridge) (Enoka and Stuart 1992; Taylor and Gandevia 2008; Boyas and Guével 2011). Any alteration in any part of this sequence of processes could therefore modify motor output and the capacity to voluntarily generate force (Boyas and Guével 2011; Taylor et al. 2016). Hence, exercise-induced reductions in force and therefore muscle fatigue has both central (central nervous system) and peripheral (skeletal muscle) neuromuscular origins (Figure 7) which act in combination, as opposed to independently (Gandevia 2001; Twomey et al. 2017).

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Fig 7. Schematic illustration of the different central and peripheral sites contributing to muscle fatigue. Muscle fatigue can occur from alterations in: (1) activation of the primary motor cortex; (2) propagation of the command from the central nervous system to the motoneurons; (3) activation of the motor units and muscle fibres; (4) neuromuscular propagation; (5) excitation-contraction coupling; (6) metabolic substrate availability; (7) state of intracellular medium; (8) contractile apparatus; (9) blood supply to the muscle. Modified from Bigland-Ritchie (1981) by Boyas and Guevel (2011).

1.2.1. Central and peripheral fatigue

Peripheral fatigue denotes processes that occur at or distal to the neuromuscular junction (Gandevia 2001). It therefore refers to changes within the muscle itself, alongside alterations in contractile mechanisms and the transmission of action potentials, which reduce the muscle fibres' contractile ability to produce force (Gandevia 2001). Central fatigue, which represents a limitation of the CNS, is defined as a "progressive exercise-induced decrease in voluntary activation (VA) (the level of neural "drive" to the muscle) of the motoneurons and muscle fibres" (Gandevia 2001; Taylor et al. 2006, 2016). This occurs at both a spinal and supraspinal level and fundamentally means that from the onset of exercise, there has been a steady decrease in both the quantity and discharge rate of the recruited motor units (Gandevia 2001; Taylor et al. 2006; Boyas and Guével 2011).

A common method to quantify fatigue is through the measurement of a maximal voluntary contraction (MVC) of the involved muscle group during a fatiguing task (e.g. a sustained isometric contraction until task failure) (Vøllestad 1997). Compared to a baseline MVC, the performance of an MVC immediately post-exercise or at periodic intervals during exercise provides an indication of the rate of fatigue development (Vøllestad 1997). Furthermore, several motor nerve and muscle stimulation techniques are able to ascertain and distinguish the extent at which specific sites in the motor pathway have contributed to the reduction in force production (Merton 1954; Taylor and Gandevia 2001). The most frequent method of assessing central or peripheral fatigue development is through the use of sEMG combined with electrical or magnetic supramaximal stimulation of the peripheral motor nerve innervating a muscle (circumventing the central nervous system) either at rest or during an isometric MVC, which evoke a muscle twitch (Cairns et al. 2005). This approach allows for changes in force production, neuromuscular excitability (e.g. muscular wave) and muscle contractile properties to be examined (Twomey et al. 2017). For example, when measured at rest prior to and immediately after exercise, any decreases in force produced by the muscle of the stimulated nerve can be used to quantify peripheral fatigue (Vøllestad 1997; Taylor and Gandevia 2008).

Impairments in VA and therefore central fatigue are demonstrated through the gold-standard "interpolated-twitch technique" which involves a comparison between a twitch superimposed on an MVC (superimposed twitch) with a twitch immediately post-MVC (Merton 1954; Vøllestad 1997; Gandevia 2001). The occurrence and increase of any superimposed twitch demonstrates an inability to recruit the entirety of the muscle (i.e. a reduction in VA) to produce the required force, resultant of a decline in motor unit firing or recruitment (Merton 1954; Taylor et al. 2006). From this approach, any decline in force is therefore attributed to upstream central processes (Taylor et al. 2006). Direct stimulation of the motor cortex through transcranial magnetic stimulation (TMS) during contraction, which provides an index of corticospinal excitability, is an additional method implemented to determine the role of supraspinal structures in central fatigue (Gandevia et al. 1996; Todd et al. 2003). In addition, alongside the previously described non-invasive methods to study fatigue, there are further techniques such as invasive muscle biopsies (Bergström et al. 1969), nuclear magnetic resonance imaging (Dawson et al. 1978) and blood samples that are used to explain reductions in force production capacity during prolonged or sustained exercise (Allen et al. 2008; Place et al. 2010). These approaches have provided an insight into mechanisms at several levels (e.g. neural, muscular, metabolic and cellular) that may correlate with impairments in muscle performance (Place et al. 2010).

Peripheral fatigue mechanisms

Substrate availability

An impairment in the resynthesis of adenosine triphosphate (ATP) to meet the energy demand of exercise due to a reduction in substrate availability is a factor that has been linked with declines in muscle function and peripheral fatigue (Ament and Verkerke 2009; de Lima et al. 2018). Alongside other fuel sources, glycogen stores in the skeletal muscle provides the predominant energy source during prolonged exercise (Allen et al. 2008), which allow for the slow or relatively rapid regeneration of ATP (Ament and Verkerke 2009). These stores transiently decline over time or with changes in exercise intensity, and combined with an increase in blood glucose consumption, results in low or exhausted levels of availability, which impede the

ability to produce force and continue exercise (Hermansen et al. 1967; Ament and Verkerke 2009).

This effect is not necessarily observed in all forms of exercise, and is dependent on exercise type and intensity (Enoka and Stuart 1992; Boyas and Guével 2011). However it is well established that there is a strong correlation between muscle glycogen reserves in the muscle fibres and time to task failure (TTF) in intense endurance exercise (Bergström et al. 1967; Hermansen et al. 1967; Allen et al. 2008). Consuming additional glucose appears to offset the reductions in force and allow for a greater exercise duration (Coyle et al 1986). How changes in muscle glycogen content leads to fatigue is still uncertain, however some evidence suggests that depleted glycogen stores may affect excitation-contraction (EC) coupling through a reduction in the release of calcium (Ca^{2+}) from the sarcoplasmic reticulum (Duhamel et al. 2006; Allen et al. 2008).

Blood supply to the muscle

A limitation in blood supply and subsequent impairment in oxygen delivery to the working muscle during intense and prolonged muscular contraction was one of the initial recognised limiting mechanisms contributing the development of peripheral fatigue (Allen et al. 2008; Boyas and Guével 2011). An increase in blood flow is key to the ability to meet the metabolic demand of exercise, remove metabolites and dissipate heat (Boyas and Guével 2011). However during intense contractions, muscle blood flow becomes either partially or completely occluded, reducing blood and oxygen supplied to the working muscle and potentially creating an ischaemic environment which can impair muscle fibre recovery (Boyas and Guével 2011). This can increase the reliance on anaerobic metabolism and result in an accentuated accumulation of metabolites (Boyas and Guével 2011). The extent at which this occurs appears to be dependent on the muscle group and the intensity of the contraction performed (Ament and Verkerke 2009). Isometric contractions greater than 15% MVC have been shown to increase intramuscular pressure which occludes muscle blood perfusion, with a transient decrease in blood supply as contraction intensity increases (Barcroft and Millen 1939; Lind and McNicol 1967; Sjøgaard et al. 1988). Whilst an occlusion of blood supply and the subsequent limitation of oxygen

delivered to the muscle is implicated in peripheral fatigue, as established previously, the accumulation of metabolites and increase mechanical pressure resultant from this environment is likely to also induce the perception of pain (Section 1.1.2).

Accumulation of metabolites

A predominant utilisation of anaerobic glycolysis in the resynthesis of ATP is established to result in an increase in concentration of metabolites (e.g. inorganic phosphate (P_i), adenosine triphosphate (ADP), hydrogen ions (H^+), potassium (K^+) and lactic acid (La^-)), which are believed to have a key deleterious role on muscle contractile capacity and the subsequent development of peripheral fatigue (Dawson et al. 1978). Greater levels of these metabolites in the muscle are also responsible for the stimulation of Group III and IV afferents (Rotto and Kaufman 1988), which during exercise results in the perception of EIP (Section 1.4). As such, these metabolites are implicated as causal of both peripheral fatigue and pain, and based on this have been suggested to (indirectly) contribute to the development of central fatigue (Sections 1.2.2 and 1.4.1). The rate of metabolite accumulation is dependent on the exercise task (e.g. duration and intensity) as well as the volume of active muscle mass (Ament and Verkerke 2009). An accentuated rate of accumulation may also occur in exercise conditions where blood flow to the working muscle is impaired, and therefore oxygen availability is insufficient to meet the metabolic demand of the exercise (Fitts 1994).

Conventionally, from the anaerobic metabolism of glycogen, the production of La^{-1} and its subsequent dissociation causing an increase in concentration of lactate and H^+ (causing acidosis or reduced pH) was believed to have a negative effect on cross-bridge activity and was associated with decreases in the ability to maximally generate force (Fitts 1994). The accumulation of La^{-1} and the decreased pH of the muscle tissue is also believed to be one of the predominant chemical stimuli causal of muscle pain during continuous contractions, an effect accentuated in the presence of ischemia (Mense 2009). In addition to the postulation that lactate does not have a direct role in force production decrements (Allen et al. 2008), the extent at which an increase in $[H^+]$ and consequent acidosis is a cause of peripheral fatigue has since been questioned (Westerblad et al. 1997). As such, there has been a greater emphasis on the

role of increased P_i, from the hydrolysis of phosphocreatine, on muscle function and peripheral fatigue (Westerblad et al. 1998).

It is believed that greater levels of P_i reduces cross-bridge functionality and has several proposed detrimental effects on Ca^{2+} handling (e.g. decreased sensitivity, availability or uptake), which could impair the EC coupling pathway (Westerblad et al. 1998, 2002). A contraction-induced efflux of K^+ from the intracellular space causing an extracellular accumulation of $[K^+]$ in the t-tubular system is also believed to be an additional contributing factor to peripheral fatigue (Sejersted and Sjogaard 2000; Cairns and Lindinger 2008). A rise in $[K^+]$ significantly above resting levels causes a depolarisation and a loss of excitability, which can have a negative effect on action potential transmission, muscle activation and therefore a decline in force produced (Cairns and Lindinger 2008; McKenna et al. 2008). There are several compensatory mechanisms in place (e.g. Na^+-K^+ pumps) (Sejersted and Sjogaard 2000), although whether these pumps are inactivated during intense and fatiguing contractions is debated (McKenna et al. 2008).

Excitation-contraction uncoupling

Excitation-contraction (EC) uncoupling has been identified as the predominant cause of peripheral fatigue (Bigland-Ritchie and Woods 1984; Boyas and Guével 2011). The process of EC coupling describes the steps resulting in the transformation of an action potential at the neuromuscular junction into the formation of a cross-bridge in the muscle (Melzer et al. 1995; Place et al. 2010). A key step in this is the release of Ca^{2+} from the sarcoplasmic reticulum (SR) to bind to troponin and facilitate the muscle contraction, and the subsequent reuptake into the SR when the muscle relaxes (Ashley et al. 1991). As fatigue develops, an accumulation of noxious metabolites (see *"accumulation of metabolites"*) and changes in the intracellular environment has been found to directly impede the contractile apparatus, or decreases the release and reuptake of Ca^{2+} in the SR (Bigland-Ritchie and Woods 1984; Westerblad and Allen 1991; Westerblad et al. 1993). As such, this may have a negative impact on EC coupling (Bigland-Ritchie and Woods 1984), with a potential reduction in cross-bridge cycling and therefore a decline in force or power produced by the muscle (Vøllestad 1997).

Central fatigue mechanisms

Suboptimal drive from the motor cortex

Supraspinal fatigue (a suboptimal descending motor cortical output to drive the exercising muscle maximally despite a maximal effort from the individual) is a component of a central decline in force production during, and briefly after, exercise (Gandevia et al. 1996; Taylor et al. 2000, 2016; Søgaard et al. 2006). It should be considered that this is not necessarily implicative of an absolute decrease in descending drive or excitability during fatiguing contractions, but perhaps a reduction in the efficacy of motor cortex output driving the motoneurons, which have progressively become less excitable (Taylor and Gandevia 2008; Taylor et al. 2016). Evidence suggests that the contribution of supraspinal fatigue is somewhat limited during brief, sustained MVCs, with a greater incremental contribution to the development of fatigue during sustained submaximal contractions of a longer duration (Taylor et al. 2016). At present, the mechanisms that underpin supraspinal fatigue are unclear, however there is the suggestion from some evidence that a decline in cerebral oxygen availability may be associated with a reduced exercise tolerance (Amann et al. 2007; Subudhi et al. 2008).

Alteration in brain neurotransmitters

One proposed mechanism linked to central fatigue is the exercise-induced reduction or accumulation of certain brain neurotransmitters and monoamines (e.g. serotonin, dopamine, noradrenaline) (Roelands and Meeusen 2010; Boyas and Guével 2011; de Lima et al. 2018). It is believed that central fatigue is not caused by these neurotransmitter systems acting individually, and instead is caused by a complex interaction between one another (Meeusen et al. 2006a; Roelands and Meeusen 2010). Changes in the synthesis and concentration of neurotransmitters has been suggested to have inhibitory effects on motor behaviour and psychological variables (e.g. motivation, arousal, attention) that can have an impact on voluntary motor drive (Meeusen and De Meirleir 1995; Gandevia 2001).

Initially, the most commonly investigated monoamine believed to have a key role in central fatigue was serotonin, where it was suggested that an increase in brain

serotonin concentration increases feelings of lethargy and loss of drive (decreasing motor unit recruitment) and has inhibitory effects on mood and arousal (Meeusen et al. 2006b). However, contrasting evidence questions whether the presence of experimentally increased serotonin either accelerates the development of fatigue (thereby impairing performance) (Wilson and Maughan 1992) or has no effect on performance (Roelands et al. 2009), with a lack of robust evidence for a role of this neurotransmitter (Meeusen et al. 2006b). Likely due to the complexity of the serotonin neurotransmitter system (Taylor et al. 2016), these findings question the extent at which this neurotransmitter has a significant inhibitory effect and influences central fatigue (Roelands and Meeusen 2010).

Other neurotransmitters and catecholamines (e.g. dopamine, noradrenaline) are suggested to have a greater effect on central fatigue (Roelands and Meeusen 2010). Manipulations of dopamine have presented contrasting evidence for an individual role on central fatigue (Roelands et al. 2008; Klass et al. 2012), whilst the minimal research on noradrenaline reuptake inhibition has demonstrated a potentially limiting effect on endurance performance (Klass et al. 2012, 2016). It has been suggested that as a singular neurotransmitter does not underpin brain function, it is possible that an interaction between two or more neurotransmitters may instead have a role in the onset of central fatigue (Meeusen et al. 2006b). Indeed, a revised hypothesis proposes that the ratio of serotonin to dopamine is key, with an increase in ratio associated with lethargy and tiredness (and an acceleration of fatigue) and a decrease in ratio promotes motivation and arousal, improving exercise performance (Davis and Bailey 1997).

Motoneuronal pool excitability

Changes in the excitability of the motoneuron pool contributing to a decline in motor unit firing rate during prolonged contractions is suggested to contribute to central fatigue at a spinal level (Gandevia 2001). Motoneuronal slowing has been proposed to be caused by changes in inhibitory and excitatory input (synaptic input from both descending drive and the ‘bottom up’ processing of sensory feedback such as pain), and an alteration in the intrinsic properties of the motoneurons, consequently decreasing their responsiveness (Taylor et al. 2006, 2016; Taylor and Gandevia

2008). As a compensatory response to maintain motoneuronal output, an increase in excitatory input is also likely to occur, causing a potential increase in the perceived effort required for the same motor output (Carroll et al. 2016) (see *'Psychobiological Model'* - Section 1.3.4).

Role of muscle afferent feedback

During sustained and prolonged exercise, motoneuron activity decreases (Taylor et al. 2016). One factor which influences and modulates the excitability of the motoneuron pool is the input from muscle afferents to the brain and spinal cord (Gandevia 2001; Taylor and Gandevia 2008). These afferents are classified as Group I to IV based on their differing function as well as diameter and conduction velocity (dependent on level of myelination) (Lloyd 1943). For example, Group I afferents, innervating the muscle spindles and Golgi tendon organs (Group Ib) are thickly myelinated fibres that conduct rapid impulses and provide the CNS with information muscle length and intramuscular tension (Proske and Gregory 2002). The existing knowledge on the role Group Ib afferents on motoneuron drive is somewhat limited due to difficulty in experimentally isolating the afferents, although it is believe that these afferents have a minimal role (Gandevia 2001). Conversely there is a well-established hypothesis for changes in neuromuscular spindle (Group Ia and II afferents) facilitation during fatiguing exercise (Gandevia 2001; Boyas and Guével 2011).

The muscle spindles typically have an excitatory input on the motoneurons, however during sustained isometric contractions, the discharge rate of the Group Ia afferents is believed to progressively decline in addition to an enhanced presynaptic inhibition (i.e. a fatigue-impaired efficacy of Group Ia input to facilitate the motoneuron pool) (Macefield et al. 1991; Rossi et al. 1999; Taylor et al. 2016). During the sustained and fatiguing contraction, it has been suggested that the phasal decrease (an initial rapid followed by slow decline) in spindle activity will lead to a gradual disfacilitation of α-motoneurons, which have a key contribution to reductions in motor unit recruitment (Macefield et al. 1991; Gandevia 2001). The extent at which the neuromuscular spindles exert an inhibitory effect is however still unclear particularly at the spinal level where their influence can be diminished (Boyas and Guével 2011).

Alternatively, during sustained contractions, an alteration in feedback from mechanically and chemically sensitive Group III and IV afferents innervating the working muscle is considered a determinant of central fatigue (Gandevia 2001; Boyas and Guével 2011; Taylor et al. 2016). Group III afferents are thinly myelinated and respond to mechanical stimuli (e.g. pressure, stretch and contraction), whilst the unmyelinated Group IV afferents (and some Group III) are chemically sensitive to the accumulation of noxious intramuscular metabolites and changes in the metabolic milieu of the muscle during contraction (Kaufman et al. 1983; Rotto and Kaufman 1988). Activation of these receptors in response to mechanical and chemical stimuli during fatiguing exercise, results in an increased discharge of the Group III and IV afferents that project centrally via the lumbar dorsal horn of the spinal cord (Mense and Craig 1988). Increased activity of these afferents can consequently lead to declines in motoneuron firing and VA (Bigland-Ritchie et al. 1986; Garland and McComas 1990; Taylor et al. 2016).

The specific action of Group III and IV afferents are uncertain, with the inhibitory effects are believed to occur at several points throughout the motor pathway (Gandevia 2001; Taylor and Gandevia 2008). At a spinal level, this may have a direct or indirect inhibition on α-motoneuronal output for some muscle groups, although could be facilitatory for other groups (Martin et al. 2006; Taylor et al. 2016). Understanding of the direct action at a supraspinal and cortical level is limited, however evidence of a decline in post-exercise VA whilst ischaemic conditions of the working muscle are maintained is evidential of a supraspinal contribution to central fatigue (Gandevia et al. 1996; Kennedy et al. 2013). Overall, whilst it is apparent that afferent feedback is an important determinant of central fatigue, it should be highlighted that the perception of pain is ultimately resultant from a form of afferent feedback (i.e. nociception), which could provide one explanation for why pain may be fatiguing (Section 1.4.1).

1.2.2. Relationship between peripheral and central fatigue

A recent postulation is that peripheral and central factors interact to regulate exercise performance and prevent the development of peripheral fatigue beyond the "critical threshold" (Amann et al. 2006; Amann and Dempsey 2008; Amann 2011). These

theories were based on an array of evidence (Amann and Dempsey 2008; Amann et al. 2009, 2011a, 2013) detailing the correspondence of exercise termination with a specific and severe extent of peripheral fatigue that could not be voluntarily surpassed (the critical threshold) (Amann 2011). As such, during exercise, peripheral fatigue and the concomitant sensory feedback can only develop up to an individual and task dependent threshold (Amann 2011). Specifically, the hypothetical "critical threshold of peripheral fatigue" and "sensory tolerance limit" models suggest that sensory feedback from metabosensitive Group III and IV afferents within the muscles directly or indirectly involved in exercise are a contributory factor in tightly regulating peripheral muscle fatigue during exercise (Amann and Dempsey 2008; Hureau et al. 2018a) (Figure 8). A substantial volume of evidence exists for the essential role of these afferents on cardiorespiratory control during exercise (Coote et al. 1969; McCloskey and Mitchell 1972; Kaufman et al. 1983; Amann et al. 2010), however their role in the interaction between peripheral and central fatigue is less documented (Hureau et al. 2018a)

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Fig 8. Schematic illustration summarising the hypothetical "critical threshold of peripheral fatigue" (A) and the "sensory tolerance limit" (B). From Hureau et al (2018).

These models propose that the Group III and IV afferents transmit information on exercise-induced peripheral fatigue to the CNS where this neural information is then processed and the magnitude of central motor drive (CMD) is adjusted (Amann 2011). From the commencement of exercise, contraction-induced mechanical and

chemical stimuli activate receptors located at the terminus of these afferents and subsequently increase their discharge (Mense and Craig 1988). As stated previously, the noxious environment from muscular contraction can also sensitise or stimulate nociceptors, resulting in the perception of pain alongside other sensations/perceptions (Cook et al. 1997; O'Connor and Cook 1999), suggesting that these may therefore have a role in these models.

Fundamentally, in this negative feedback loop, once the critical threshold of peripheral fatigue and sensory information is attained, exercise is either voluntarily terminated (open-loop exercise – see section 1.3) or the CNS limits CMD (i.e. the development of central fatigue) (closed-loop exercise) as a means to protect the individual from abnormal threats to homeostasis, overexertion and intolerable sensations of pain (Amann et al. 2006, 2009; Amann 2011; Hureau et al. 2018a). Plainly according to this perspective, peripheral fatigue via the process of afferent feedback contributes to the development of central fatigue during prolonged and sustained contraction, thereby influencing exercise performance (Gandevia 2001; Amann et al. 2006; Amann 2011). Hypothetically, an increase in inhibitory afferent feedback and therefore pain could consequently have a role in promoting the earlier attainment of this critical limit (Aboodarda et al. 2020).

An initial approach to test this hypothesis was to either manipulate oxygen availability, or to perform "pre-fatiguing" exercise at varying intensities prior to a cycling time-trial, and subsequently compare measures of performance, neural drive and locomotor muscle fatigue (Amann et al. 2006; Amann and Dempsey 2008). With greater pre-existing fatigue, power output was reduced and performance time increased (Amann and Dempsey 2008), whilst a similar response was observed from hypoxic to hyperoxic arterial oxygen content (Amann et al. 2006). However, an important finding was an identical extent of peripheral fatigue post-exercise (as measured by resting quadriceps twitch force) despite the differences in performance and pre-exercise interventions. Whilst this finding is supportive of a peripheral fatigue contribution to central fatigue, prior exercise can have key confounding effects on central fatigue such as changes in neurotransmitter levels, glycogen content and temperature (see section 1.2.1) (Amann and Dempsey 2008). To circumvent this limitation, follow-up studies (Gagnon et al. 2009; Hureau et al. 2014) employed a

neuromuscular electrical stimulation technique to induce specifically peripheral fatigue, and subsequently corroborated these findings.

However, none of the aforementioned interventions are able to markedly isolate the central effects of the Group III and IV afferents and associated sensations (e.g. pain), which arguably are the fundamental component of the critical threshold concept. One approach is to infuse a metabolite "soup" (equivalent to a physiological level of metabolites produced by the contracting muscle) into the muscle, which at varying concentrations activate populations of Group III and IV afferents and cause the perceptions of fatigue and pain (Pollak et al. 2014). An alternate method to manipulate feedback from Group III and IV afferents and prevent their central inhibitory projection (therefore attempting to bypass the "critical threshold" and allowing a greater peripheral fatigue), is through the partial pharmacological attenuation of afferent feedback from the locomotive limb. An epidural administration of Lidocaine or Bupivacaine into the intervertebral space of the lumbar spine was initially used to block afferent feedback (Smith et al. 2003; Amann et al. 2008b). However these local anaesthetics also have an efferent action which significantly reduce locomotor muscle strength (inevitably requiring an increase in CMD to maintain force), reducing feedback during constant-load exercise and thus are inadequate to evaluate the role of Group III and IV afferents (Amann et al. 2008b, 2010; Amann 2011).

As a solution to this issue, the administration of a lumbar intrathecal injection of fentanyl has been demonstrated to inhibit approximately 60% of the ascending input from nociceptive, metabosensitive and mechanosensitive Group III and IV afferents without affecting neuromuscular function and the capacity of the locomotor muscle to produce maximal force (Amann et al. 2009, 2010; Amann 2011; Blain et al. 2016; Sidhu et al. 2018). Fentanyl, a μ-opioid receptor (as found in the dorsal horn and afferent fibres) agonist, increases pain tolerance and has been demonstrated to reduce the muscle pain intensity reported from both electrical and HS pain models (Eichenberger et al. 2003; Amann et al. 2009). The fentanyl model therefore provides a more appropriate method to investigate the effect of Group III and IV afferent feedback on limiting the development of peripheral fatigue and performance during endurance exercise (Amann et al. 2009).

In a formative series of studies, it was found that blocking the central projection of Group III and IV afferents through fentanyl during intense whole-body endurance exercise significantly increases the magnitude of motoneuronal output (as indicated by sEMG) (Amann et al. 2009, 2011a; Sidhu et al. 2014; Hureau et al. 2019), which is typically decreased during high-intensity exercise with intact feedback (Martin et al. 2008). In terms of exercise performance the greater CMD substantially increased power output resulting in a quicker first half of a 5 km cycling time-trial (Amann et al. 2009). This change exacerbated metabolic and respiratory acidosis, accelerating the development of peripheral fatigue, and impairing the second half of the trial, resulting in an overall cycling time-trial completion not dissimilar to the placebo condition (Figure 9). This change in pacing response suggests that afferent feedback (and potentially pain and discomfort), rather than effort alone (see Section 1.3.4), could be an important element to gauge appropriate work-rate regulation. Performance of a high-intensity constant load cycling trial also reported a significantly reduced time to exhaustion performance (Amann et al. 2011a).

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Fig 9. The effect of partial blockade of afferent feedback (via intrathecal fentanyl) on EMG (A) and power output (B) during a 5 km cycling time-trial compared to placebo and control conditions. From Amann et al (2009).

Post-exercise, a significantly larger pre to post-exercise reduction in quadriceps potentiated twitch in the fentanyl condition (i.e. greater peripheral fatigue) suggests that participants were able to endure a greater development exercise-induced peripheral fatigue beyond the "critical threshold" that was observed with intact afferent feedback after the same exercise (Amann et al. 2009, 2011a; Hureau et al. 2019). It is interesting to note that participants also anecdotally indicated severe issues with muscle soreness and ambulation post-exercise (Amann et al. 2009). It has been

argued that the ambulatory problems may have been due to the migration of fentanyl to the brain centres, causing feelings of sickness, confusion and an impairment in movement (Taylor 2010), a view which is supported by the participants not suffering any lasting harm post-exercise. Surpassing the "critical threshold" and not having any lasting impairments could indeed question the purpose of such a threshold, which from an evolutionary perspective could be interpreted as counterintuitive (Taylor 2010). Nonetheless, the authors concluded that these findings emphasise the critical role of Group III and IV afferents on inhibiting spinal motoneuronal output and therefore the prevention of over-exertion (Hureau et al. 2019), but also demonstrate a potential key effect on work rate regulation (Amann 2011).

Further support of this is evidence of a shortened or unchanged cortical silent period evoked by TMS during the performance of both an exhaustive single-limb isometric task of the knee extensors and locomotor exercise in the presence of the fentanyl blockade when compared to a placebo (Hilty et al. 2011; Sidhu et al. 2017, 2018). During fatiguing exercise, it has been established that the cortical silent period progressively increased in duration both during and post-exercise, which is believed to be indicative of an increase in intracortical inhibition (McNeil et al. 2009). As such, these findings reinforce the postulation that Group III and IV afferents may facilitate intracortical inhibition, which is considered a key contributing factor to the development of central fatigue (Hilty et al. 2011; Sidhu et al. 2017, 2018).

It should however be noted that Group III and IV afferents also project to sites within the CNS responsible for cardiovascular and ventilatory regulation (e.g. ventral lateral medulla), which during endurance exercise, contributes to minimising the development of peripheral fatigue (Amann et al. 2009, 2010; Hureau et al. 2019). As such, these afferents have both a facilitatory (e.g. circulatory and respiratory regulation) and inhibitory role on endurance performance (Amann et al. 2011a). During exercise, the partial blockade of Group III and IV afferent feedback by fentanyl administration minimises the facilitatory effects, causing hypoventilation, and impairing the exercise pressor response, including a notably attenuated cardiorespiratory response, a decline in blood flow to the locomotor muscle and a reduced contractile efficiency (Amann et al. 2010, 2011b, 2015; Broxterman et al. 2018; Hureau et al. 2018b). This physiological interference subsequently limits

arterial oxygenation and the delivery of oxygen to the exercising muscle (Amann et al. 2011a), which as an important mechanism of fatigue development (section 1.2.1), accelerates peripheral fatigue and could consequently compromise performance (Amann et al. 2011a; Sidhu et al. 2017).

This is a key limitation, with the exaggeration of peripheral fatigue from the Group III and IV afferent blockade confounding previous findings on the impact of these afferents on specifically central fatigue (Amann et al. 2009, 2011a; Sidhu et al. 2014). Only recently has this been addressed (Hureau et al. 2019), through the provision hyperoxic inspirate (allowing for the careful control of oxygen delivery to the exercising muscle) during the performance of the same cycling time-trial distance used in the previous fentanyl studies (Amann et al. 2009). Here, motoneuronal output was significantly increased (i.e. decreased central fatigue) permitting a higher power output and a significantly faster completion time. Again, as with previous studies, the peripheral fatigue recorded post-exercise was greater in the fentanyl condition opposed compared to the slower placebo and control conditions. It is therefore clear that with the inclusion of carefully controlled oxygen delivery, Group III and IV afferents have a prominent limiting effect on both peripheral fatigue development and endurance exercise performance via a centrally-mediated inhibition on motoneuronal activation of the exercising muscle (Hureau et al. 2019).

There is however some refutation on the concept of an explicit individual and task-specific critical threshold or sensory tolerance limit, with another standpoint considers the role of afferent feedback on CMD and endurance performance to be insignificant. This alternate view suggests that motor unit recruitment is solely regulated by the conscious brain (for the 'Psychobiological Model' see section 1.3.4.) (Marcora 2008, 2010a; Pageaux 2014). This model proposes that tolerance of an exercise task is based on maintaining effort to a "tolerable" level that an individual is willing to exert, which is mediated centrally by the corollary discharge (efferent processes of CMD) (Marcora 2008, 2009; Marcora et al. 2008; Wright 2008). Interestingly, the Psychobiological viewpoint accepts muscle pain (resultant from the stimulation of Group III and IV afferents at the periphery), which is commonly associated with fatigue, does have a role on work-rate regulation, although as a motivational function (Marcora 2010a). Evidently a highly debated area (Taylor 2010), the complex process

of fatigue and the influence on endurance exercise performance is more likely to be explained in terms of a combination of both afferent and efferent systems (Taylor 2010).

1.3 Endurance performance

Sections 1.1 and 1.2 provided a discussion of pain and the interaction of central and peripheral processes involved in the development of fatigue. Based on the evidence examined previously, there is a probable relationship between the experience of pain during exercise and the process of fatigue. However, as most methods of experimental pain-induction are different in their processing and response compared with naturally occurring EIP, the ability to examine this relationship has been limited. As identified previously, the HS experimental pain model could provide a method that produces representative EIP. Consequently, to understand the potential fatiguing impact of EIP on performance, it is important to recognise the exercise context in which these factors are most prominent and relevant. As EIP is commonly reported during intense and prolonged muscle contractions (i.e. endurance exercise), this section will focus on endurance performance and its proposed determinants, with an emphasis on the models that attempt to explain the process of fatigue and performance during this form of exercise.

Endurance exercise is characterised by the prolonged (> 75 s) maintenance of constant or self-regulated exercise at a range of intensities (i.e. from low to maximal) involving different forms of muscle contraction (i.e. isometric or dynamic) (Gastin 2001; Burnley and Jones 2007; McCormick et al. 2015). Dependent on characteristics of exercise (e.g. mode, duration, intensity), the development of fatigue and endurance performance is limited by the complex interaction of numerous mechanisms (e.g. physiological, psychological, biomechanical and environmental) (Abbiss and Laursen 2005). Due to the integrative nature of factors underpinning performance, there have been numerous models proposed that attempt to explain how performance is regulated, tolerated and limited (Section 1.3.4), with a singular model yet to be agreed upon.

"Performance" of endurance events in response to an experimental manipulation can be assessed in the laboratory or field by an "open-loop" or "closed-loop" task (Coyle 1999). Open-loop tasks, known as time to exhaustion/task failure protocols, are measured by the length of time at which set submaximal constant-intensity exercise (e.g. power/velocity or torque/force) or incremental intensity over time can be maintained (i.e. a longer time represents greater endurance) until task failure (Laursen et al. 2007; Currell and Jeukendrup 2008). Closed-loop tasks, known as time-trial protocols, are evaluated by either the time taken to complete a known fixed distance or amount of work, or the completion of as much work as possible within a set time (Laursen et al. 2007; Currell and Jeukendrup 2008). As participants are typically made aware of the end point of the time-trial, these closed-loop tasks therefore allow for the self-regulation of work rate in order to successfully pace the effort towards the known distance or time (Laursen et al. 2007). As such, the mechanisms which underpin performance in these two protocols are likely to be dissimilar.

The protocol selected when exploring the effect of experimental intervention (e.g. HS experimental pain) on endurance performance should be based primarily on the research question (Currell and Jeukendrup 2008). Both types of protocol are considered to be reliable and sensitive to factors that may influence performance, and have evident advantages and limitations (Laursen et al. 2007; Amann et al. 2008a). Time-trials provide an applied context to assess changes in pacing and a simulation of actual performance, whereas time to exhaustion tests allow for a greater control in the investigation of physiological, psychological and perceptual changes which can affect performance (Amann et al. 2008a; Currell and Jeukendrup 2008; Hettinga et al. 2017b). As an aim of the thesis is to examine the influence of elevated EIP on endurance performance and the effect this may have on physiological and perceptual measures, the use of a time-trial would be inappropriate, as participants would be able to continuously adjust their work rate (Amann et al. 2008a) to moderate the perception of pain to a tolerable level, which could potentially mask any changes in measures. This thesis therefore has a predominant focus on open-loop exercise (Chapters 3 and 6), although as EIP is suggested to have a role in the regulation of work-rate (Mauger 2014), there is some acknowledgement and discussion on the role and importance of pacing during closed-loop exercise (Chapter 4).

1.3.1. Single-limb and whole-body endurance

The mechanisms associated with the development of fatigue and endurance performance likely differ for different exercise modalities (i.e. whole-body exercise, single-limb isometric or dynamic exercise tasks). At present, mechanisms of fatigue (both central and peripheral) have been widely explored in both isometric and dynamic single-limb exercise (Gandevia et al. 1996; Gandevia 2001; Taylor and Gandevia 2008). Whole-body locomotor exercise presents more of a challenge due to the need to transfer the participant between an ergometer and a dynamometer before and after exercise, which causes a notable delay in the assessment of changes in neuromuscular function (Pageaux et al. 2016), and can underestimate the magnitude of fatigue (Cairns et al. 2005). Evaluating fatigue and endurance performance in whole-body locomotor exercise has superior ecological validity for "real" sporting competition scenarios ranging from short-duration sprints to long-duration ultra-endurance events. This form of exercise requires a greater muscular and cardiorespiratory demand than exercise isolated to a single limb which provides a more controlled method of investigating central and peripheral mechanisms of fatigue in an specific muscle group (Sidhu et al. 2013; Hettinga et al. 2017b).

There is a simple divergence in the muscle mass active during single-limb tasks, which are typically isolated to a singular muscle or muscle group, and whole-body locomotive exercise, which utilises a significantly larger muscle mass. An evaluation of open-loop exercise tasks consisting of either constant-load dynamic knee extensor exercise (small muscle mass) or cycling (large muscle mass) to exhaustion revealed a significantly greater peripheral fatigue (as measured by quadriceps potentiated twitch) after the exercise task with smaller active muscle mass (Rossman et al. 2012). This finding is however confounded by the differing and task-specific cardiorespiratory and neural responses of the two exercise modalities (Rossman et al. 2012, 2014). A follow-up study investigating differences in quadriceps fatigue between single-limb and double-limb knee extension (thus minimising the aforementioned limitations) supported these findings (Rossman et al. 2014). A greater post-exercise peripheral fatigue in exercise utilising a singular compared with both quadriceps muscle groups was found (Rossman et al. 2014), potentially due to greater central fatigue when more muscle mass was engaged in the double-limb exercise task.

There is therefore is a likely difference in the Group III and IV afferent signalling from the exercising muscle or muscles to the CNS between exercise modes (Rossman et al. 2012; Sidhu et al. 2013). As opposed to single-limb exercise where feedback is constrained to an isolated muscle mass, during whole-body exercise afferent feedback will arise from an "ensemble" of feedback from the active muscle in addition to other sources such (e.g. cardiorespiratory system and non-exercising muscles) (Rossman et al. 2012, 2014; Weavil and Amann 2019). For example, strenuous whole-body exercise has a significant pulmonary demand, causing a decrease arterial oxygen saturation (arterial hypoxaemia), and requiring a significant degree of respiratory muscle activation (causing the gradual accumulation of noxious metabolites which activate Group IV afferents and subsequently restricts locomotor blood flow) (Dempsey et al. 2002; Amann 2012a). These responses can therefore compromise the adequate delivery of oxygen to the working muscle relative to the exercise demand, which is likely to accelerate the development of peripheral fatigue (Amann 2012a).

The differences in end-exercise peripheral fatigue between exercise modes can be explained from the perspective of the sensory tolerance limit, where the sum of all afferent feedback (and associated sensations in addition to feedforward signals) contributes to the regulation of peripheral fatigue (Gandevia 2001; Hureau et al. 2018a). Based on this theory, to reach the same individual threshold, a greater afferent signal would be required from the smaller muscle mass to match the cumulative signal from both the exercising and non-exercising muscles in whole-body exercise (Hureau et al. 2018a). As such, the CNS therefore "permits" a greater extent of homeostatic disturbance in the reduced volume of active muscle mass compared to exercise employing a greater muscle mass, which will have an overall reduced severity of metabolic disturbance (Rossman et al. 2012, 2014). The findings of these studies are suggestive of a link between the size of muscle mass employed and afferent feedback (Freund et al. 1978; Hureau et al. 2018a) and reinforce the role of afferent feedback in the regulation of peripheral fatigue. With likely variances in afferent signalling between single-limb and whole-body exercise, which utilise differing volumes of muscle mass, it would be of interest whether there is a disparity in the associated perception of pain reported between the two forms of exercise.

Differences in central alterations (at a cortical and spinal level) are also believed to be observed between strenuous whole-body exercise and single-limb exercise tasks to failure (Sidhu et al. 2013). Whilst the increases intracortical inhibitory processes are similar between single-limb and whole-body exercise, there is a disparity in corticospinal excitability, which has been demonstrated to be reduced during sustained whole-body compared to single-limb exercise (requiring a greater CMD to maintain muscle activation) (Sidhu et al. 2012, 2013). In addition, as a form of exercise that is typically associated with large muscle mass, and a greater demand cardiorespiratory demand, whole-body exercise can cause additional homeostatic perturbations (e.g. hyperthermia, respiratory muscle work, a decline in cerebral oxygen availability, and alterations in brain neurotransmitters) compared to single-limb exercise that may influence the responsiveness of the corticospinal cells (Sidhu et al. 2013) and have been associated with reductions in descending drive (see Section 1.2.1.) (Nybo and Nielsen 2001; Nybo and Rasmussen 2007). Overall, whilst single-limb exercise provides a tightly controlled model to investigate and indicate the mechanisms associated with pain and fatigue in whole-body exercise, the differences in physiological response and causes of fatigue between these modes of exercise should be taken into account when attempting to extrapolate findings. As such, the mechanistic findings from single-limb exercise can be used as indicative but not necessarily informative of locomotor exercise, and should therefore be interpreted with caution (Sidhu et al. 2013).

1.3.2. Determinants of endurance performance

It is well established that success in whole-body endurance exercise is primarily determined by the ability to optimally produce power or velocity whilst limiting the development of central or peripheral fatigue (Mauger 2013). A predominant emphasis in the literature has been on the traditional variables of aerobic fitness that dictate the capacity and tolerance of endurance exercise. These parameters are also used to demarcate the four exercise intensity domains (moderate, heavy, severe and extreme) which produce uniform physiological responses and mechanisms of fatigue that can be sustained for an approximate period of time (Burnley and Jones 2007). This includes critical power/velocity, maximal oxygen uptake (VO_{2MAX}), the fractional utilisation of VO_{2MAX} and the energetic cost of exercise (economy/efficiency) (Bassett

and Howley 2000; Burnley and Jones 2007; Joyner and Coyle 2008). The extent of blood lactate accumulation during exercise is commonly associated with the fractional utilisation of VO_{2MAX}, which is also known as either the lactate threshold (LT), gas exchange threshold (GET) or ventilatory threshold (Joyner and Coyle 2008). The relative extent at which these, and other factors determine endurance performance is however dependent on the exercise characteristics.

From exclusively a physiological perspective, the model outlined by Joyner and Coyle (2008) is a widely accepted explanation of the factors which underpin this endurance performance velocity or power (Figure 10). According to this model, aerobic components of fitness such as VO_{2MAX}, the LT, anaerobic capacity and economy/efficiency interact to predict the maximally sustainable power output or velocity during performance (Joyner and Coyle 2008). Fundamentally, it is believed that VO_{2MAX} represents the "upper limit" of aerobic metabolism and the LT is related to the greatest fraction of VO_{2MAX} that can be utilised and sustained for a period of minutes to hours, without the accumulation of lactate (Costill 1970; Joyner and Coyle 2008). Both VO_{2MAX} and the LT operate to determine performance oxygen uptake (VO_2) (Joyner and Coyle 2008). This "performance VO_2" is then related to efficiency or economy, which is associated with the actual velocity or power that can be generated at the consumption of that given VO_2 (i.e. the ability to move economically) (Bassett and Howley 2000; Joyner and Coyle 2008). The concept of VO_{2MAX} and its fractional utilisation are well understood, however efficiency/economy has received growing recognition (Coyle 1999), and is believed to be a differentiating factor in performance between athletes with a similar VO_{2MAX} (Bassett and Howley 2000).

[REDACTED]

Fig 10. Schematic overview of the conceptual framework outlining the physiological determinants of performance velocity or power output. From Joyner and Coyle (2008).

Fundamentally however, several elements of endurance performance cannot be accounted for by solely physiological variables (Noakes 2011). Firstly, deviations in performance within or between individuals with unchanged aerobic parameters are unable to be explained solely by physiological variables (Noakes 2011, 2012). Secondly, the model outlined by Joyner and Coyle (2008) does not account for the differing causes of fatigue in single-limb and whole-body exercise (section 1.3.1). Finally, these postulations assume that the individual is exercising at a constant-intensity and is willing to or are sufficiently motivated to consistently exert an optimal effort and level of performance without any variations in work-rate (e.g. the "end-spurt" during self-paced exercise) (Noakes 2011, 2012). Essentially, these models neglect the role of the brain in the regulation of exercise and development of fatigue during endurance exercise (Noakes 2011). This viewpoint was actually acknowledged by Joyner and Coyle (2008) to be equally as important as the development of fatigue in muscular, cardiovascular and neuromuscular factors in the regulation and tolerance of endurance exercise. In particular, contemporary models of fatigue and endurance

performance place an increased value on the role of the brain, with some emphasis on psychological and perceptual factors (Section 1.3.4).

Role of perceptions

Perceptions, which refer to the conscious experience of sensation, occur from the central processing and response to neural impulses resultant from a physical stimulus and its concomitant sensory signals (Gardner and Martin 2000). These highly subjective and variable constructs can be measured by self-report psychophysiological tools during exercise (Abbiss et al. 2015). A recent debate within exercise science has been around the possibility that the rating of effort perception (which may be involved in pacing, fatigue and exhaustion) is the key determinant (as opposed to predictor) of endurance performance (Marcora 2010b, a; Marcora and Staiano 2010)(see section 1.3.4). During endurance exercise, the degree of this perceived sensation is typically recorded by the Borg 6 ("no exertion") to 20 ("maximal exertion") rating of perceived exertion (RPE) scale (Borg 1982) . The category-ratio scale is also employed as a means to rate exertion, and can also be used as a measure of alternate perceptions (e.g. pain) (Borg 1982).

There has however been some prior misunderstanding on what the RPE scale is measuring, with the argument for a discrepancy between perceptions of "effort" and "exertion" (Abbiss et al. 2015). Effort has received numerous definitions including "the amount of mental or physical energy being given to a task" (Abbiss et al. 2015), or "the conscious sensation of how hard, heavy, and strenuous a physical task is" (Marcora and Staiano 2010). Exertion on the other hand is defined as "the subjective intensity of effort, strain, discomfort and/or fatigue that is experienced during physical exercise" (Noble and Robertson 1996), which therefore includes additional perceptions. Particularly, the term "discomfort" unequivocally refers to the perception of pain, and as such any changes in perceived pain are likely to causally result in a changed perceived exertion (Abbiss et al. 2015). However, evidence indicates that that the perception of pain should be treated as an isolated construct. Indeed, the experimental manipulation of pain has directly resulted in changes in pain tolerance (after aerobic exercise training), work-rate regulation and performance (Section

1.4.2), suggesting that the experience of pain during exercise is a key contributing factor in endurance exercise (Mauger 2014).

The same issue is also evident with the perception of fatigue, defined as "the awareness of a decreased capacity for physical and/or mental activity due to an imbalance in the availability, utilisation, and/or restoration of resources needed to perform activity" (Aaronson et al. 1999). Not only is perceived fatigue argued to be different from perceived exertion, but fatigue itself has been linked and synonymous with other feelings such as sleepiness, exhaustion and illness (Micklewright et al. 2017b). This has led to the recent development of the 11-point numerical 'rating-of-fatigue' (ROF) scale (with accompanying descriptions and diagrams) ranging from 0 ("not fatigued at all") to 10 ("total fatigue and exhaustion – nothing left"), providing a scale that discriminates from other perceptions (e.g. exertion) (Micklewright et al. 2017b). The subjective ratings of fatigue from this scale have been shown to have a linear relationship with objective measures of fatigue, therefore providing a straightforward means to approximate the exercise-induced reduction in force generating capacity of the muscle (Whittaker et al. 2019).

Clearly, these differences in definition, the consideration of supplementary perceptions, and possible variations in use between laboratories could limit comparisons between the literature and potentially confound any interpretations on the singular role of these perceptions on endurance performance (Smirmaul 2012; Abbiss et al. 2015; Micklewright et al. 2017b). Indeed, at present, findings supporting their contribution during exercise and on overall endurance performance are inconclusive (Smirmaul 2012). Nonetheless, these perceptions are evidently resultant from separate processes and therefore the possibility to differentiate between perceptions has since been demonstrated (O'Connor and Cook 2001; Pageaux et al. 2015a; Astokorki and Mauger 2017a). As such, when employed during experimental studies, these perceptions should be dissociated in definition and recorded as different entities with independent scales to avoid inaccurate perceptual measures.

1.3.3. Role of pacing in endurance performance

In closed-loop endurance exercise tasks, where successful performance is evaluated by the fastest time to completion or the completion of maximal work possible in a fixed period of time, the pacing behaviour employed is recognised as a determinant of performance (Edwards and Polman 2013). Pacing has been defined as the "goal directed distribution and management of effort across the duration of an exercise bout" (Edwards and Polman 2012). As such, pacing is fundamentally a conscious and informed decision-making process on the effective use of available energetic resources to achieve optimal performance (Edwards and Polman 2013). In the context of time-trial performance, "management of effort" refers to the careful regulation and distribution of energy expenditure to complete the task in the quickest time possible/perform the maximal amount of work (i.e. finish the task with minimal energy stores in reserve), whilst maintaining metabolic capacity and homeostasis to prevent early task failure (Foster et al. 2003; St Clair Gibson et al. 2006; Edwards and Polman 2013; Roelands et al. 2013; Smits et al. 2014). For example, an overly aggressive pace at the start of a time-trial would risk premature fatigue (requiring the adjustment of exercise intensity to continue) whilst a conservative start could result in sub-optimal performance.

The pacing employed during an endurance event is determined by interactions between circumstantial factors, consisting of internal (physiological, biomechanical, psychological, perceptual) and external (event, environment: e.g. opposition, terrain, climate, altitude and course) components (St Clair Gibson et al. 2006; Smits et al. 2014; Konings and Hettinga 2018). Numerous theoretical models have been proposed in an attempt to explain how these factors interact to determine work rate regulation and exercise tolerance during endurance performance (see Section 1.3.4.).

One proposed framework, which has received experimental support (Konings et al. 2016, 2018; Konings and Hettinga 2020), takes an ecological-psychological perspective and is centred around the affordance-competition hypothesis (Smits et al. 2014; Hettinga et al. 2017a; Konings and Hettinga 2018). This perspective outlines how the direct coupling between perception and action as well as the interaction between the athlete's action capabilities and environment combine to form "affordances" (relevant opportunities produced by the environment that may or may

not be actioned upon) (Hettinga et al. 2017a). These affordances are presented simultaneously and continuously, changing over time and competing with one another (i.e., some affordances are actualised whilst others may be ignored or resisted) (Hettinga et al. 2017a). Based on the affordances available and the competition between them, the individual is presented with a continuous decision to make on the appropriate exercise behaviour, whether it is persisting with a behaviour (maintain the current pace) or modifying behaviour and therefore implementing an alternative one (i.e., speed up or slow down) (Smits et al. 2014; Hettinga et al. 2017a). Motivation, athlete experience and internal factors or sensory information, such as pain or fatigue, will however have a mediating influence on the opportunities that can be actioned (Hettinga et al. 2017a; Konings and Hettinga 2018). This perspective is believed to provide an understanding of the more complex and nuanced tactical decisions and pacing situations (e.g., the "end-spurt") particularly in response to the actions of opponents in a competitive situation (Konings et al. 2016, 2018; Konings and Hettinga 2020).

Despite some similarities between each of the proposed frameworks, the principal underpinning mechanisms are still debated. A consistent theme in these models has however placed an increased emphasis on the role of the brain and the conscious experience of sensations (i.e. perceptions) during exercise (e.g. effort, fatigue and pain) (see Section 1.3.2.). In particular, due to the linear relationship between pain intensity and work-rate (Cook et al. 1997), EIP has been postulated to indicate the relative strain of the working muscle and therefore a be contributing factor in the pacing strategy and work-rate regulation (Mauger 2014) (see Section 1.4.2.).

1.3.4. Models of fatigue and endurance performance

Central Governor Model

The 'Central Governor Model' (CGM) based on, the "teleoanticiaption" model (Ulmer 1996), suggests that, in response to afferent feedback from numerous physiological systems and within the context of additional variables (e.g. the environment and exerciser experience), a "governor" in the CNS (likely to be located in the brain) tightly regulates the recruitment of skeletal muscle in the exercising limb and continuously changes work-rate (Noakes et al. 2005; Noakes 2012). The proposed

aim of this model would be to maintain homeostasis and protect from catastrophic physiological failure (Noakes et al. 2005; Noakes 2012). Therefore, the CGM suggests that all exercise is submaximal and is performed with a reserve of available motor units (further motor unit recruitment would compromise homeostasis) (Noakes 2012) (Figure 11).

The CGM suggests an alternate definition of fatigue as an individual sensation or emotion based on the interpretation of subconscious CNS processes as opposed to a physical event (St Clair Gibson et al. 2003; Noakes et al. 2005). According to this model, the sensation of fatigue is the key factor in ensuring that work-rate remains within the physiological capacity of the individual (St Clair Gibson et al. 2003; Noakes 2012). It should be noted that the CGM does acknowledge the important function of metabolites (which also result in the sensation of pain and can influence muscle contractile ability) and sensory afferent feedback as a contributing factor to the determination and "oscillation" of the pacing strategy (Noakes et al. 2004, 2005). Overall, the model outlines that the multifaceted integration of several physiological and environmental factors, as well as afferents from the peripheral organs results in the brain-generated and conscious sensation of fatigue (Noakes 2012).

Specifically, the sensation of fatigue is fundamentally a calculation which considers the prior and current knowledge of exercise task, the perceived capacity of the body to complete the exercise task at the existing work rate (i.e. current metabolic state) and potential future threats to the preservation of homeostasis (i.e. the environment) (St Clair Gibson et al. 2003; Noakes et al. 2004). Any changes in these factors (i.e. an increased or decreased difficulty in preserving homeostasis) and therefore a conscious awareness of an adjusted RPE (which regulates the sensation of fatigue) will be interpreted as a change in the sensation of fatigue, which could result in a changed pacing strategy (Noakes et al. 2005; Noakes 2011). It is suggested that RPE is pre-determined at the commencement of exercise, with this measure changing as a linear function based on the duration of exercise completed or remaining (Noakes et al. 2004; Noakes 2011). Consequently, the performance of exercise requiring a maximal effort should reach a maximal RPE value upon task completion. It should be noted that the definition of RPE used in this model is akin to "exertion" opposed to "effort" (which includes feelings of pain and discomfort) (Noakes 2012) (see Section 1.3.2),

therefore according to this model the perception of pain could form an important marker of feedback.

However, it should be noted that the exercise modality employed (i.e. closed-loop and open-loop) will influence the "calculations" made by the central governor, and as such is likely to influence the strategy employed (St. Clair Gibson and Noakes 2004). The CGM outlines that exercise in open-loop tasks, where the duration or distance is unknown, exercise is terminated by the CNS prior to this critical incidence of fatigue that may result in bodily harm (St. Clair Gibson and Noakes 2004; Noakes 2012). In the context of closed-loop exercise, where the endpoint is known, the CGM is initially anticipatory (Noakes et al. 2005; Tucker 2009; Noakes 2011, 2012). The central governor is proposed to calculate and predict the initial-work rate that can be sustained for the expected duration of exercise (i.e. a feed-forward control of skeletal muscle recruitment) until the point of exercise completion based on the integration of numerous physiological, psychological and environmental factors (Noakes 2011, 2012). Upon the commencement of exercise, cyclical evaluations between feedforward and feedback control subsequently informs a continuous (sub)conscious alteration of work-rate to ensure exercise is performed within the individual physiological limit (St. Clair Gibson and Noakes 2004; Noakes et al. 2005). In both forms of exercise, the CGM therefore proposes that individuals able to control the symptom of fatigue and minimise its impact on performance are more likely to be successful (Noakes 2012).

[REDACTED]

Fig 11. Diagrammatic representation of the most recent version of the central governor model. From Noakes (2012).

Evidently, opposed to the primarily physiological determinants of endurance performance (see Section 1.3.2.), the CGM could provide a credible psychophysiological explanation. Indeed, its conception, and the emphasis on the role of the brain, arguably prompted a shift in endurance performance investigations (Marcora 2008; Konings and Hettinga 2018). However, despite its merits in places, the CGM has been widely criticised (Weir et al. 2006; Marcora 2008; Shephard 2009; Inzlicht and Marcora 2016). The "black box" nature of the CGM (which has been regularly amended to include additional variables) means any outcome can be explained by the model, which therefore make it difficult to test or falsify (Noakes 2012; Inzlicht and Marcora 2016). Indeed, direct support for the CGM is limited, with most evidence used for the CGM is focused on caveats of other models. In addition, the prospect of conscious-subconscious control has been widely disputed, questioning the theoretical basis for the existence of a subconscious regulator in the brain

(Marcora 2008; Inzlicht and Marcora 2016; Konings and Hettinga 2018; Venhorst et al. 2018). In some respects, it is questionable whether this model has significantly advanced understanding of work-rate regulation and exercise tolerance during performance (Micklewright et al. 2017a; Venhorst et al. 2018).

A further contention with the CGM is the proposed function of the model to preserve "homeostasis". Firstly, homeostasis, by definition, can be compromised during fatiguing exercise (e.g. the occurrence of physiological catastrophe), with changes in factors at the periphery (e.g. the rapid accumulation of metabolites) evidently not constituting a relatively constant environment (Weir et al. 2006; Smits et al. 2014). Secondly, motivational interventions have been suggested to override this function (Inzlicht and Marcora 2016). Finally, scenarios where an individual continues to exercise despite the presence of warning signals (e.g. EIP) to modify work-rate is contradictory to the postulation of homeostatic control (St Clair Gibson et al. 2018). These points therefore question the use of the term "homeostasis" (if the processes of the CGM are ineffective or can be surpassed) or whether alternate mechanisms are present that permit the continuation of exercise despite the potential or actual occurrence of tissue damage (Weir et al. 2006; St Clair Gibson et al. 2018).

Based on these highlighted criticisms and issues, themes of the CGM has since been refined, with the recent development of the Integrative Governor theory (St Clair Gibson et al. 2018) The Integrative Governor theory is founded on set of "rules", with the relative weighting between antagonistic psychological (to increase work-rate) and physiological (to decrease work rate) homeostatic drives (underpinned by negative feedback loops) central to determining pacing and the development of fatigue during endurance exercise (St Clair Gibson et al. 2018). The presence of competing drives would explain why individuals are able to surpass homeostatic control mechanisms and continue to exercise despite the threat or incidence of damage, however whether the Integrated Governor meaningfully progresses understanding of endurance performance is questionable as, similar to the CGM, the theory could be difficult to falsify.

Anticipatory-RPE Feedback Model

Extending upon the CGM (although without reference to the CGM), the Anticipatory-RPE Feedback Model proposes that the conscious rating of perceived exertion (RPE) is the principal factor in the regulation of exercise performance (Tucker 2009). This is with the aim to ensure optimal performance whilst protecting the individual from a catastrophic homeostatic disturbance (Tucker 2009; Tucker and Noakes 2009). Anticipatory/feedforward forecasting, based on prior knowledge and experience of the exercise (e.g. duration or distance), integrated with physiological input (i.e. afferent feedback) are fundamental tenets outlined by this model (Tucker 2009; Tucker and Noakes 2009). A body of work investigating changes in work-rate regulation in conditions of environmental (e.g. heat, hyperoxia) (Nybo and Nielsen 2001; Tucker et al. 2004, 2007) and metabolic (e.g. substrate depletion) (Baldwin et al. 2003) extremity/stress as well as after experimental manipulation (e.g. exercise misinformation) (Albertus et al. 2005) were key in the formation of this model.

The Anticipatory-RPE Feedback Model can explain "performance" in terms of both open- and closed-loop exercise (Figures 12 and 13). According to the model, during fixed-work rate protocols, volitional termination of exercise occurs upon the attainment of the subjective "maximal tolerable RPE" (immediately prior to the incidence of bodily harm), and therefore TTF is determined by the rate at which the RPE increases to this maximal level. Again, similar to the CGM, this model employs a definition of RPE consistent with "exertion", and therefore includes the perception of pain (see Section 1.3.2) (Tucker 2009). In this form of exercise, the anticipated safe duration of exercise is predicted by the brain which sets the initial rate of increase in RPE. During exercise, the brain utilises afferent feedback from a range of physiological systems to incessantly adjust the rate at which RPE increases until the maximal tolerable RPE is reached.

[REDACTED]

Fig. 12 Schematic diagram detailing the anticipatory-RPE feedback model of endurance performance during open-loop exercise tasks (e.g. time to task failure). From Tucker (2009).

In time-trials, the exerciser is free to adjust work-rate in response to an RPE which is perceived to be excessive or unmaintainable. The anticipatory component of the model consists of what is termed the "template" RPE and an initially selected exercise work-rate. These are set to allow for the optimal completion of the task (based on pre-exercise expectations, knowledge of exercise distance/duration, afferent feedback and psychological factors) where exercise is concluded at the point of maximal tolerable RPE (and not before). During exercise, the integration of afferent feedback from changes in numerous physiological systems (influenced by exercise intensity and environment) and knowledge of remaining exercise distance/duration forms a "conscious" RPE (a verbalised rating).

The conscious RPE is then continuously compared to the template RPE, with any mismatch between the two constructs resulting in an alteration in work-rate via changes in muscle recruitment to prevent premature exhaustion and ensure that the exerciser completes the task at the point of maximal tolerable RPE (Tucker 2009; de Koning et al. 2011; Roelands et al. 2013). The perceived strictness of a subconscious RPE template has however been criticised for a potential inability to explain applied

competition scenarios which require an element of flexibility in terms of decision-making and response to competitor behaviour (Konings and Hettinga 2018). The model has also been criticised for not specifying whether the decision to adjust work-rate is conscious or subconscious (Swart et al. 2012), however as highlighted previously, this dichotomy is highly debated. Since its conception, this model has received minimal attention in subsequent research, and incidentally resulted in the amendment of the CGM, which when first proposed did not include RPE (Marcora 2008). This has led in some uncertainty on exactly how these models differ, but also raise the question of whether RPE is a redundant factor in the CGM (Marcora 2008)

[REDACTED]

Fig 13. Schematic diagram detailing the anticipatory-RPE feedback model of endurance performance during closed-loop exercise (e.g. time trial). From Tucker (2009).

Psychobiological Model

The Physiobiological Model is another model centred around the role of perception of effort (not exertion) as the key determinant of endurance performance (Marcora 2008, 2010a; Marcora et al. 2008). This model defines perception of effort as the "conscious sensation of how hard, heavy or strenuous a physical task is" (Marcora 2010a). Here, perception of effort is believed to be independent of sensory feedback from the periphery (contrary to the previously mentioned models and the inhibitory feedback model) and resultant from the central integration of corollary discharge (efferent neural processes) associated with the central motor command (Marcora 2009) (Figure 14). This model has received support from a several experimental studies which have employed physiological (e.g. muscle fatigue) and psychological (e.g. mental fatigue and subliminal priming) manipulations and have demonstrated changes endurance performance associated with alterations in perception of effort (e.g. Marcora et al. 2008; Blanchfield et al. 2014; Pageaux et al. 2015b).

[REDACTED]

Fig 14. Schematic diagram summarising the two models (Afferent feedback, A; Corollary discharge, B) proposing the mechanisms underlying the generation of perceived exertion during exercise. From Marcora (2009)**.**

Based on motivational intensity theory (Brehm 1989; Wright 2008) and incorporating the construct of "potential motivation" (the maximum effort an individual is prepared to expend to achieve a task or goal), the Psychobiological Model stipulates than an individual will participate in exercise until the attainment of the maximal effort they are willing to expend (Marcora 2008; Marcora et al. 2008; Wright 2008). This model is supported by the finding that individuals (with high levels of motivation) typically complete exercise with very high ratings of perceived effort (despite no evident physiological failure), and terminating with similar end-task values (Marcora and

Staiano 2010; Smirmaul et al. 2013). Compared to the aforementioned models, the Psychobiological Model places a greater emphasis on cognition and describes the singular importance of psychological and perceptual factors in both the conscious regulation of work-rate and exercise tolerance, disregarding the role for anticipatory or subconscious processes (Micklewright et al. 2017a). The model does acknowledge the function of environmental factors as well as traditional physiological determinants of endurance performance, but instead argues that these influence perception of effort or potential motivation opposed to a direct effect on performance itself (Marcora 2010b, a; Smirmaul et al. 2013). Importantly, the Psychobiological Model argues against the role of afferent feedback and EIP as an endurance performance determinant, and instead suggests that it serves as a motivational stimulus to terminate exercise (Marcora 2010a).

Like previously described models, the Psychobiological Model is proposed to explain "performance" in terms of both open- and closed-loop exercise tasks. During exercise performed at a fixed work rate, perception of effort increases proportionally with time (Marcora and Staiano 2010). From the Psychobiological perspective, this gradual increase in perception of effort is representative of a "moment-by-moment" increase in CMD (to both the working and respiratory muscle) as a compensatory response to maintain motoneuronal output for the same fixed work rate (see Section 1.2.1.) (Marcora 2008). As the perception of effort continues to rise towards maximal levels, the conscious decision of task disengagement is made, where the individual "gives up" (Marcora and Staiano 2010). Task disengagement can occur under two different conditions: the effort required by the task increases to the level set by potential motivation or the individual believes maximal effort has been expended and continuation of exercise is believed to be impossible based on individual physical ability (Marcora 2008, 2010a; Marcora et al. 2008; Marcora and Staiano 2010).

During closed-loop exercise tasks, the regulation of work-rate is suggested to be consciously determined, primarily by potential motivation and perception of effort in addition to a further three factors: 1) knowledge of the distance/time to cover, 2) knowledge of distance/time remaining and 3) previous experience (all of which are suggested to be sensitive to environmental or physiological factors) (Pageaux 2014). The psychobiological model postulates that individuals adjust their work-rate on a

moment-to-moment basis, primarily based on perception of effort (e.g. a low perception of effort at a specific distance/time of a time-trial is likely to result in an increase in work-rate), to ensure that the task is successfully completed (ideally at the subjective maximal perception of effort) (Marcora 2010a; Pageaux 2014). Any change in perception of effort or motivation will change work-rate and therefore overall performance (Pageaux 2014). Argued to be contrary to the CGM and the inhibitory feedback model, the Psychobiological model is also able to provide an explanation for pacing behaviour such as the "end-spurt" (e.g. a more reliable conscious perception of effort at a given-work rate combined with knowledge of limited time/distance remaining resulting in a significant increase in work-rate without compromising performance prior to task end-point) or a more cautious work-rate in the initial stages of the task (Marcora 2010a; Smirmaul et al. 2013; Pageaux 2014).

According to the Psychobiological Model, a change in performance in either form of exercise through physiological or psychological experimental intervention (e.g. mental fatigue, pre-fatigued muscles, muscle damage, motivational incentives, pharmacological) or training-induced adaptations (Marcora 2008; Marcora and Staiano 2010; Smirmaul et al. 2013) occur as a result of the respective factors manipulating perception of effort (Blanchfield et al. 2014), positioning perception of effort as an all-encompassing factor. Whilst it could be suggested that RPE is a simple means to explain exercise tolerance and regulation of work rate, the reliance on a singular scale also be considered to be an *oversimplified* construct that provides a limited explanation of what is clearly multifaceted and integrative exercise behaviour (also a limitation of the CGM and Anticipatory-RPE Feedback Model) (Renfree et al. 2014; Venhorst et al. 2018). Between these models, RPE has been defined differently (see Section 1.3.2.), thereby confounding the interpretation of prior research through each respective model.

This is also somewhat contradictory to the statement that the Physiobiological Model is a "simpler" and therefore more valid model compared to those formerly outlined (Marcora 2008). For example, the mechanisms of fatigue and afferent feedback outlined in sections 1.2.1. and 1.2.2. can affect RPE and influence performance. A further challenge with the Psychobiological Model is that it is outlined and discussed

over a collection of separate papers (e.g. predominantly comment pieces) (Marcora 2008, 2010a; Pageaux 2014) and, is yet to be centralised into a singular published paper. As a result, the ability to test the model is limited, which could explain the lack of empirical evidence disproving or challenging the model.

Inhibitory Feedback Model

The Inhibitory Feedback Model is a feedback loop with the primary aim to restrict the development of locomotor muscle fatigue below an individual critical threshold/sensory tolerance limit (which is never surpassed), and therefore preserve the muscle reserve capacity (Amann and Dempsey 2009, 2016). The model outlines that the development of fatigue at the periphery is associated with an increase in inhibitory feedback from metabosensitive Group III and IV afferents (Amann and Dempsey 2016) in response to the production of metabolites in the muscle milieu. These afferents project to a cortical level which (whether consciously and/or subconsciously) regulates the magnitude of descending motor drive to the locomotor muscle (influencing power output) (Amann and Dempsey 2009). Any alteration in power output will result in a change in the metabolic milieu of the locomotor muscle, which consequently informs the extent of the afferent feedback (continuing the loop) (Amann and Dempsey 2009) (Figure 15).

In the context of open-loop exercise, exercise is voluntarily terminated upon the attainment of the critical threshold of peripheral fatigue (Amann 2011). In closed-loop exercise as this threshold is approached, work-rate is reduced through a CNS-mediated reduction in CMD (i.e. the development of central fatigue), allowing for the continuation of exercise (Amann 2011). As such, this feedback loop and the contribution of peripheral fatigue to the development of central fatigue is therefore suggested to serve as a protective function to avoid the occurrence of a serious impairment in muscular function, intolerable sensations of pain and abnormal threats to homeostasis (Amann and Dempsey 2008, 2009, 2016). This proposed relationship is in direct contrast to the Psychobiological Model, which deem central fatigue to be irrelevant during endurance exercise (Marcora 2010a; Taylor 2010).

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Fig 15. Schematic illustration of the inhibitory feedback model. The solid black line represents efferent nerve activity (central motor drive), the dashed line represents afferent nerve activity. From Amann and Dempsey (2016).

Unlike the previously outlined models, which are either problematic to falsify or have received minimal experimental challenge, the Inhibitory Feedback Model has received support from a series of studies which were able to experimentally manipulate Group III and IV afferent feedback (Amann et al. 2009, 2010; Amann 2011; Blain et al. 2016; Sidhu et al. 2018). (see Section 1.2.2.). The most prominent intervention was the lumbar intrathecal injection of fentanyl during both constant work-rate and self-paced cycling exercise (Amann et al. 2009, 2011a). Experimentally isolating the Group III and IV afferents is challenging, and this method did initially receive criticism for potentially causing an impaired exercise pressor response (accelerating peripheral fatigue) and confounding the ability investigate the central effect of these afferents (Amann et al. 2009, 2011a; Sidhu et al. 2014). The careful control of oxygen delivery to the working muscle during time-trial exercise has since provided a solution to this issue (Hureau et al. 2019).

In the studies employing lumbar intrathecal injection of fentanyl, the blockade of afferent-feedback (and complete removal of EIP) reduced performance during the constant work-rate trial, and significantly altered the ability to regulate work-rate during a time-trial, resulting in a change to an unsustainable aggressive positive pacing strategy (Amann et al. 2009, 2011a). This latter finding is suggestive that

afferent feedback during exercise (and potentially EIP), rather than just effort (as outlined by prior models) may be important factor in the determination and gauging of an appropriate pacing strategy (Amann et al. 2009). The ability of the Inhibitory Feedback Model to explain pacing strategies and behaviour during endurance events is however contested. In particular, this model is unable to explain phenomenon such as "end-spurt", a significant increase in work-rate toward the end of exercise (i.e. an increased CMD) despite the presence of an intolerable and fatiguing metabolite concentration (Marcora 2010a). This criticism applies to all models predominantly or completely based on physiological concepts, which are able to explain a maintainable average work-rate but are perceived to be unable to provide a rationale for tactical pacing decisions (Taylor 2010). Evidence of decrements in power output coupled with an increase in integrated EMG (of the vastus lateralis and biceps femoris) toward the end of a middle-distance cycling time trial is also suggestive of a peripheral limitation of performance opposed to CNS-mediated decline in CMD (Hettinga et al. 2006), questioning the ability of this model to explain performance in specific exercise contexts.

It is however difficult to dispute that afferent feedback (and accompanying sensations) has an important *role* in work-rate regulation (Hettinga 2010) based on prior evidence of altered CMD and a change in pacing in the absence of afferent feedback (Amann et al. 2009). Furthermore, it is also acknowledged that the determination of CMD magnitude during endurance performance is a complex process, and that psychological and cognitive factors (e.g. motivation, previous experience, emotions) are likely to be important contributory factors (Hettinga 2010; Amann and Secher 2010). Whilst the psychological factors are not incorporated into the model, it has been suggested that the inhibitory afferent feedback can be bypassed by conscious self-regulation to determine work-rate (Marcora 2010a; Taylor 2010). As such, it is suggested that a singular model is likely to be insufficient in the explanation of the multifaceted nature of work-rate regulation and endurance performance, and an integrative approach, is required (Hettinga 2010). A summary of the four models previously outlined can be seen in Table 1.

Table 1. An overview of the models of fatigue and endurance performance outlined in this thesis

Model	**Proposed aim**	**Overview**	**Performance**	**Key Points**
Central Governor Model (CGM)	Maintain homeostasis and protect from catastrophic physiological failure	• A "governor" in the CNS regulates muscle recruitment and changes in work-rate • All exercise is submaximal and is performed with a motor unit reserve • Fatigue is a sensation/emotion based on the interpretation of subconscious CNS processes (opposed to a physical event) which is regulated by perceived exertion, and is the key factor in ensuring that work-rate remains within the individual physiological capacity • Fatigue considers: - Prior and current knowledge of exercise task	• Open-loop tasks - Exercise is terminated by the CNS prior to the critical incidence of fatigue that may result in bodily harm • Closed-loop exercise, - Anticipatory: an initial prediction of the initial-work rate that can be sustained for the expected duration of exercise - During exercise: cyclical evaluations between feedforward and feedback control informing a continuous alteration of work-rate • The ability to control the symptom of fatigue and minimise its impact	• The "black box" nature of the CGM make it an "all-knowing" entity that is difficult to test or falsify • Communicated over a series of papers where the model has been regularly amended to include additional variables (impaired clarity and understanding) • The use of the term "homeostasis" (which by definition can be compromised during exercise) • Lack of direct supporting evidence, with the focus predominantly on the caveats of the other models

		- Perceived capacity of the body to complete the exercise task at the existing work rate - Potential future threats to the preservation of homeostasis	on performance is associated with success	
Anticipatory-RPE Model	Ensure optimal performance whilst protecting the individual from a catastrophic homeostatic disturbance	• Conscious rating of perceived exertion is the key factor in the regulation of exercise performance • Anticipatory/feedforward forecasting, based on prior knowledge and experience of the exercise, integrated with physiological input (i.e., afferent feedback) are fundamental tenets	• Open-loop tasks - Anticipatory: the brain predicts the safe duration of exercise, setting the initial rate of increase in RPE - During exercise: afferent feedback continually adjusts the rate at which RPE increases until the attainment of maximal tolerable RPE (immediately prior to the incidence of bodily harm) • Closed-loop tasks - Anticipatory: "template" RPE and an initially selected exercise	• A subconscious RPE template (which is strict) is unable to explain scenarios which require flexibility in terms of decision-making and response to competitor behaviour • Does not specify whether the decision to adjust work-rate is conscious or subconscious • Has received minimal attention in the literature • Uncertainty on how this model differs from the CGM

			work-rate (to allow for the optimal completion of the task where exercise is completed at the maximal tolerable RPE) - During exercise: afferent feedback and knowledge of remaining exercise distance/duration forms a "conscious" RPE (a verbalised rating), which is regularly compared to the template RPE. Any mismatch results in a change in work-rate	
Psychobiological Model	Participate in exercise until the attainment of the maximal effort willing to expend	• Perception of effort (not exertion) is the key determinant of endurance performance • Perception of effort is independent of sensory feedback and instead, resultant from the central integration	• Open-loop tasks - Perception of effort increases proportionally with time (representative of a "moment-by-moment" increase in central motor drive as a compensatory response	• Able to provide an explanation for pacing strategies and behaviour (e.g., the "end-spurt" or an initially cautious work-rate) • Positions RPE as an all-encompassing factor

		of corollary discharge associated with the central motor command • Incorporates the construct of "potential motivation" • Describes the singular importance of psychological and perceptual factors in work-rate regulation and exercise tolerance • Disregards the role for anticipatory or subconscious processes • Environmental and physiological factors influence perception of effort or potential motivation opposed to a direct effect on performance itself	to maintain motoneuronal output for the same fixed work rate) - As the perception of effort continues to rise towards maximal levels, the conscious decision of task disengagement is made • Closed-loop tasks - Work-rate regulation is consciously determined by potential motivation and perception of effort in addition to knowledge of the distance/time to cover, knowledge of distance/time remaining and previous experience - Work-rate is regulated on a moment-to-moment basis based on perception of effort (with any change in perception of effort or motivation changing work-rate) to	• Sole reliance on RPE is an oversimplified and limited explanation of what is clearly multifaceted and integrative exercise behaviour • The viewpoint that simplicity makes this explanation more valid is questionable • Outlined and discussed over a collection of separate papers and yet to be centralised into a singular published paper. • Limited ability to test the model • Lack of empirical evidence disproving or challenging the model

			ensure that the task is completed at the subjective maximal perception of effort	
Inhibitory Feedback Model	Restrict the development of locomotor muscle fatigue below an individual critical threshold/sensory tolerance limit	• A feedback loop • Development of peripheral fatigue is associated with an increase in inhibitory feedback from Group III and IV afferents in response to the production of metabolites in the muscle milieu • These afferents project to a cortical level which regulates the magnitude of descending motor drive to the locomotor muscle (influencing power output) • Alterations in power output will change the metabolic milieu of the locomotor muscle, which informs	• Open-loop exercise - Exercise is voluntarily terminated upon the attainment of the critical threshold of peripheral fatigue • Closed-loop exercise - Work-rate is reduced through a CNS-mediated reduction in central motor drive as the critical threshold is approached (allowing for the continuation of exercise)	• Received support from a series of studies • Unable to explain certain pacing strategies and behaviour during endurance events (such as the "end-spurt") • Does not acknowledge the role of psychological and cognitive factors

		the extent of the afferent feedback (continuing the loop)		

1.4 Exercise-induced pain

The experience of naturally occurring pain in the knee extensor muscles during moderate to severe-intensity exercise is relatively commonplace and well-recognised in healthy individuals participating in exercise (Cook et al. 1997, 1998; O'Connor and Cook 2001; Mauger 2014; Astokorki and Mauger 2017a). Considering the widely agreed definition of pain, this EIP is a subjective and emotional experience that involves the integration of both central and peripheral physiological mechanisms (see Sections 1.1.1.) as well as psychological factors (Price 2000; Ray and Carter 2007). Arising from the sensitisation and activation of ascending group III and IV afferents in response to nociceptive stimuli (see Sections 1.1.1. and 1.4.1.), EIP is often accompanied by fatigue (Pollak et al. 2014) suggesting that EIP may be a factor partially responsible for the development of fatigue (Mauger 2014).

EIP typically arises from exercise involving intense, continuous and prolonged muscle contractions (e.g. endurance exercise) which produces a reproducible pain threshold equal to approximately 50% of peak power output, peak VO_2 or peak RPE (Cook et al. 1997). From this point, EIP intensity increases proportionally with exercise intensity (open-loop exercise), or distance complete or time elapsed (closed-loop exercise) until maximal levels are attained at the end of exercise (Cook et al. 1997, 1998; Mauger et al. 2010). Described as a "cramping", "burning", "aching", "exhausting" and "intense" sensation (i.e. predominantly sensory and affective MPQ descriptors), the subjective experience of EIP is initially localised in the working muscle which subsequently spreads to additional locations over time (Mense 1993; Cook et al. 1997; Motl et al. 2007; Stevens et al. 2018).

Based on the observed linear relationship between pain intensity and exercise work-rate or duration, it has been suggested that EIP (fundamentally a protective mechanism), can provide helpful sensory feedback regarding the relative state of the working muscle (Mauger 2014). This has led to the notion that pain may have a prominent limiting or regulatory role during endurance performance (Mauger 2013, 2014). For example, sensations of EIP may contribute to the conscious and informed regulation of work-rate across an exercise bout (i.e. pacing) to ensure optimal performance (see Sections 1.3.3. and 1.4.2.) (O'Connor and Cook 2001; Mauger

2013, 2014). An increase in inhibitory feedback from Group III and IV afferents and the concomitant experience of EIP is also suggested to limit CMD and therefore contribute to the development of central fatigue (see Sections 1.2.1, 1.2.2 and 1.4.1). In addition, as EIP is a subjective sensory and emotional experience it is proposed that the ability or willingness to tolerate and moderate sensations of pain is a key differentiating factor in successful performance between athletes with a similar physiological capacity (Anshel and Russell 1994; Cook et al. 1997; Mauger 2014; Astokorki and Mauger 2017a; O'Leary et al. 2017) (see Section 1.4.2.).

Despite being a common experience, and the belief of its importance (Mauger 2014), the impact of EIP on endurance exercise has received limited attention and is therefore still inadequately understood. Afferent feedback is a central component in the models of fatigue and endurance performance outlined previously (Section 1.3.4), yet there is scarce reference to an isolated role of EIP. The Psychobiological model does acknowledge EIP, however as discussed, cites this only having a motivational function. This section will provide an outline of the mechanisms of EIP, determine its potential impact on endurance performance, and discuss prior literature that has attempted to manipulate sensations of EIP during both open- and closed-loop exercise tasks. Finally, based on the evidence presented and discussed, an experimental method of evoking muscle pain that feels like naturally occurring EIP will be proposed, which will form the basis of this thesis.

1.4.1. Exercise-induced pain (EIP)

Processing of EIP

Resistance and aerobic exercise of different modes, intensities and duration can elicit the experience of EIP (Dannecker and Koltyn 2014). During exercise, the stimulation of free nerve endings (unmyelinated axon terminals) supplied by Group III and IV afferents innervating the skeletal muscle (muscle nociceptors) are responsible for mediating the neural processing of noxious (i.e. tissue threatening) stimuli and eliciting the subsequent perception of muscle pain (Mense 1993; Graven-Nielsen and Mense 2001; Graven-Nielsen 2006). A bulk of the muscle nociceptors are formed from the Group IV afferents, which solely terminate in free nerve endings, whereas not all Group III afferents terminate in free nerve endings (Stacey 1969; Mense 2009). Free nerve endings of Group III and IV afferents are densely populated in the muscle, located in the connective tissue and the wall of blood vessels, and are able to distinguish between noxious and innocuous stimuli (Mense 1993) (Figure 16). In particular, they are sensitive to the algesic chemical by-products produced by the damaged or contracting muscle (likely due to their location in blood vessels) (Mense 1993; O'Connor and Cook 1999).

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Fig 16. A sketch depicting the location and pathway of nociceptive (Group III and IV) and non-nociceptive (Group Ia and Ib) afferents involved the processing of sensory information. From O'Connor and Cook (1999).

Nociceptors respond to the application of chemical (e.g. exogenous and endogenous algesics), mechanical (e.g. pressure) and thermal stimuli (see Section 1.1.2. for examples of common pain induction methods). Group III nociceptive afferents are predominantly activated by pressure, whereas Group IV nociceptive afferents respond to chemical stimuli, which when activated results in the sensation of "dull", "aching" and "cramping" muscle pain (Marchettini et al. 1996). However, with some being activated by innocuous mechanical stimuli, it should be noted that not all free nerve endings supplied by Group IV afferents are nociceptive, and instead are believed to have an alternate non-nociceptive function (e.g. sensations of pressure in the muscle or moderating cardiovascular response) (Graven-Nielsen et al. 2004; Hoheisel et al. 2005; Laurin et al. 2015).

Weak everyday stimuli (i.e. low-intensity pressure, stretch or muscle contractions) will not activate the nociceptors (Mense 1993, 2009). Instead, a tissue-threatening (noxious) intensity of mechanical stimuli (i.e. muscle squeeze or pinch) or a range of algesic chemicals (past a certain level of concentration) applied exogenously (via intramuscular injection) are required to activate what are known as the "high-threshold mechanosensitive" polymodal receptors, for the perception of pain to occur (Mense 1993, 2009; Graven-Nielsen and Mense 2001; Pollak et al. 2014). Nociceptors can also be sensitized by a noxious stimulus (typically algesics from tissue damage or inflammation), which is characterised by a decreased nociceptive activation threshold or an increased responsiveness of dorsal horn neurons (O'Connor and Cook 1999; Graven-Nielsen and Mense 2001; Julius and Basbaum 2001). A sensitized nociceptor terminal will respond and be activated by a lower mechanical input (e.g. innocuous muscle tissue deformation or activity) yet also demonstrate an exaggerated reaction to typical noxious stimuli (Graven-Nielsen and Mense 2001). As such, this will likely facilitate greater afferent activity and an exacerbated experience of muscle pain (i.e. hyperalgesia) (Olesen et al. 2012).

When applied to the nociceptor, a stimuli is converted into an electrical signal (Olesen et al. 2012). Dependent on the magnitude of the stimulus and therefore whether the electrical signal exceeds the activation threshold value, the generation of an action potential and release of neurotransmitters occurs (Graven-Nielsen and Mense 2001). Transmitted through the nociceptive afferents, the impulse enters the dorsal root

ganglia and the dorsal horn of the spinal cord (the afferents predominantly synapse superficially in laminae I and II, with some also terminating in the deeper laminae V) (Mense 1993; Graven-Nielsen 2006; Olesen et al. 2012) (Figure 16). The nociceptive signal is then projected to areas within the brain, via the ascending tracts (primarily the spinothalamic) (Graven-Nielsen 2006).

As an inherently complex subjective experience modulated by numerous factors (e.g. sensory, affective, cognitive and social factors), the perception of pain involves a multitude of brain regions. Areas of the brain that are activated or stimulated by the muscle pain include the thalamus, the primary and secondary somatosensory cortex, prefrontal cortexes (Svensson et al. 1997c; Coghill et al. 1999; Jensen et al. 2016), with the insular cortex and anterior cingulate cortex believed to be of central importance (Peyron et al. 2000; Almeida et al. 2004; Jensen et al. 2016). Combined, these regions of the brain are of believed to have numerous roles, including coding for components of the sensory, affective and cognitive experience of muscle pain (Peyron et al. 2000). Nociceptive information is also transmitted to areas such as the amygdala, hypothalamus, orbitofrontal cortex, which alongside the rostral region of the anterior cingulate cortex, are believed to be involved in the emotional-motivational aspect of pain, potentially explaining the key relationship between pain and emotion (Rainville 2002; Almeida et al. 2004).

Aetiology of EIP

Whilst the specific aetiology of EIP during exercise is yet to be fully established, it is hypothesised to occur from three factors (which act either individually or in combination). A bout of intense and prolonged muscle contractions in the production of force places the working muscle under notable strain (increasing intramuscular pressure and deformation of tissue), and creates an environment of high mechanical pressure and an increase in concentration of these noxious metabolites (e.g. bradykinin, serotonin, K^+, H^+, histamine, substance P, prostaglandins and adenosine) (Mense 1993, 2009; O'Connor and Cook 1999; Mauger 2014).

EIP has been demonstrated to typically arise at an exercise intensity equivalent to approximately 50% of peak power output, peak VO_2 or peak RPE (i.e. a moderate

exercise intensity) (Cook et al. 1997). Further increases in work-rate, particularly above the LT, will result in a build-up of noxious metabolites, stimulating and/or sensitising the afferents and subsequently increasing the sensations of muscle pain (Cook et al. 1997; O'Connor and Cook 1999). Research has provided strong evidence in favour of the relationship between metabolite concentrations (equivalent to varying exercise intensities) and the sensation of pain (Pollak et al. 2014). When infusing a combination of metabolites into the muscle, it was found that concentrations at rest or equivalent to low-intensity contractions did not elicit pain. An increase in concentration of metabolites corresponding to moderate-high intensity contractions induced sensations of pain, which increased linearly proportional to concentration. An accumulation of chemical substances is not however isolated to supra-threshold exercise tasks and can instead be observed at a local muscle level in exercise at sub-threshold, low-force intensities utilising prolonged and repetitive muscle contractions (e.g. single-limb isometric contractions) (Mense 2009), which creates an ischemic environment and thus an eventual increase in metabolite concentration reducing pH (Mense 2009).

Based on its association with muscle pain across different exercise tasks, the accumulation of noxious metabolites is likely a contributing factor underpinning the occurrence of EIP. Nonetheless, there are also exercise conditions (e.g. short-duration, high-power) where muscle pain is also reported, yet noxious chemicals have had an insufficient period of time to significantly accumulate, and as such alternate/additional mechanisms may be present (Cook et al. 1997). For example, during an 8 second bout of cycling at 250 W, a "weak" intensity of muscle pain was reported (Cook et al. 1997). In this form of exercise, it is plausible that contractions producing a high level of force will increase intramuscular pressure and tissue deformation, providing a sufficient mechanical stimulus that will predominantly stimulate the nociceptive afferents (Cook et al. 1997; O'Connor and Cook 1999).

Fatiguing impact of EIP

Nociceptive processing and the subsequent experience of EIP is often accompanied by fatigue. With this thesis outlining the mechanisms causal of both constructs (see Section 1.2.1 for mechanisms of fatigue), an association is perhaps unsurprising.

Primarily, the activation and sensitisation of Group III and IV afferents by metabolites which accumulate during muscle contraction induce the sensation of muscle pain but are also implicated in (peripheral) fatigue and the description of its perception (e.g. "tired" or "exhausted") (Light et al. 2008; Jankowski et al. 2013; Pollak et al. 2014). This has led to the notion that processing and experience of EIP may contribute to the development of fatigue during exercise (Mauger 2014). Indeed, there are several mechanisms (outlined below) which could provide explain how EIP may exacerbate or contribute to the development of fatigue, and therefore limit endurance performance. Due to the complexity of both EIP and fatigue and the difficulty in isolating the experience of pain, investigating the fatigue-pain relationship is challenging and has received limited attention. In addition, with evidence of fatigue occurring without the sensation of pain and vice versa, a simple causal relationship should be approached with caution (Amann et al. 2009; Flood et al. 2017).

Role of metabolites in pain and fatigue

An elevated intramuscular pressure (occluding the supply of blood and oxygen to the working muscle) or an increase in exercise intensity (and a greater reliance on anaerobic metabolism) all result in the gradual accumulation of the aforementioned metabolites (Section 1.2.1) (Boyas and Guével 2011), which impede the function of the contractile apparatus and therefore result in a reduced ability to produce force or power (i.e. peripheral fatigue) (Bigland-Ritchie and Woods 1984; Westerblad and Allen 1991; Westerblad et al. 1993; Fitts 1994; Vøllestad 1997). During exercise, at a noxious concentration, these metabolites also stimulate or sensitise Group III and IV nociceptive afferents, increasing afferent feedback and resulting in the perception of EIP (Section 1.4). As these same metabolites have also been shown to cause peripheral/central fatigue, this presents a unique challenge where it difficult to experimentally discern whether EIP in itself is fatiguing. A method which allows for exacerbation of EIP without affecting the fatigue-causing metabolites (Section 1.4.3) would potentially allow this to be tested.

Afferent feedback

An increase in feedback from Group III and IV afferents is suggested to inhibit CMD (and the ability to recruit motor units) and promote the development of central fatigue

(Blain et al. 2016; Hureau et al. 2019; Aboodarda et al. 2020). This has been evidenced in studies where increased afferent activity resulted in a decline in VA (Kennedy et al. 2013), and a reduced capacity to maximally produce force (Graven-Nielsen et al. 2002; Henriksen et al. 2011). An increase in nociceptive afferent feedback could therefore have a negative impact (i.e. a greater relative task difficulty) on performance of exercise where maintaining a set work-rate is required for successful tasks completion. In response to this, to maintain the same motor output, a compensatory increase in descending motor drive and potentially a greater effort would be required (Graven-Nielsen and Arendt-Nielsen 2008). These changes could accelerate the development of supraspinal fatigue and subsequently impair endurance performance (Gandevia 2001; Graven-Nielsen and Arendt-Nielsen 2008). In the context of an open-loop exercise task, an increase in feedback from Group III and IV afferents (and the associated sensation of pain) could also contribute to the earlier attainment of the "Sensory Tolerance Limit" (Aboodarda et al. 2020).

Ascertaining whether nociceptive activity (a specific type of afferent feedback) facilities central fatigue is compounded by the non-nociceptive role of some Group IV afferents (Hoheisel et al. 2005), and the additional sources of feedback also transmitted by the Group III and IV afferents (Laurin et al. 2015). Prior research employing the experimental manipulation of afferent feedback during exercise performance (see Section 1.2.2) are confounded by an intervention-induced inhibition of the exercise pressor response (exacerbating peripheral fatigue) (Amann et al. 2009, 2011a; Sidhu et al. 2014, 2017), which limits the ability to understand the isolated role of EIP. Even when this was addressed (Hureau et al. 2019), these studies have seldom reported participant perceptual data on the pain experienced and therefore provided little insight into the notion of EIP influencing fatigue and exercise performance. Evidently these factors present a shortcoming of the prior experimental approach, with future work requiring a more specific method to both manipulate EIP and measure its subjective perception.

Influence on motor system

It is commonly accepted that the experience of pain is often associated with changes in motor behaviour with the fundamental purpose to protect the body from further

tissue damage/injury (i.e. alleviate the load on the painful tissue) and reduce the perception of pain (Hodges and Tucker 2011; Bank et al. 2013). Whilst such alterations could be immediately beneficial, these adjustments can also cause movement abnormalities and various acute debilitating effects (Hodges and Tucker 2011). There are three major theories that attempt to explain the mechanisms for the pain-induced changes in movement; the Vicious Cycle (Roland 1986; Johansson and Sojka 1991), the Pain Adaptation Model (Lund et al. 1991) and the Moving differently in pain theory (Hodges and Tucker 2011).

The Vicious Cycle theory suggests that an initiating factor such as muscle stiffness (from the facilitation of muscle spindles via Group III and IV nociceptive input) results in "muscle hyperactivity" which leads to ischaemia and the subsequent accumulation of pain-producing metabolites, thereby continuing the cycle (Roland 1986; Johansson and Sojka 1991). Simply, the experimental induction of pain in a specific muscle will subsequently result in an increase in activity of the same muscle, which would result in further pain and likely accelerate the development of fatigue (Schulte et al. 2004).

Alternatively, based on experimental observations, the Pain Adaptation Model argues that the excitation of Group III and IV afferents and the presence of muscle pain during movement results in a consistent uniform inhibitory/facilitatory effect on agonist (i.e. the painful muscle) and antagonist muscle activity (Lund et al. 1991). It is proposed that the purpose of such adaptation is to reduce movement amplitude and velocity (i.e. smaller and slower movements) and the force produced by the painful muscle as a protective mechanism from any further damage (Lund et al. 1991; Peck et al. 2008).

Both of these theories provide relatively simplistic and general explanations of pain-induced changes in movement that are unable to account for variable or unpredictable changes (Hodges and Tucker 2011). Particularly, the Vicious Cycle theory has little evidence of support (Graven-Nielsen et al. 2000; Peck et al. 2008; Hodges and Tucker 2011), whilst the Pain Adaptation Model is able to provide a consistent explanation for some experimental observations (Arendt-Nielsen et al. 1996; Graven-Nielsen et al. 1997e; Madeleine et al. 2006), but not for several others (e.g. decreased motoneuron

discharge rate or no change in agonist sEMG activity) (Birch et al. 2000; Schulte et al. 2004; Farina et al. 2005a; Hodges et al. 2008; Tucker et al. 2009; Salomoni and Graven-Nielsen 2012). However, it has since been recognised that pain does not produce uniform inhibition and excitation effects across the motor neurone pool (Hodges and Tucker 2011)

The "moving differently in pain" theory is able to explain the variation between individuals and tasks (e.g. different contraction intensities) and postulates that muscle pain (or the threat of pain) initiates changes across the motoneuron pool, causing a redistribution of activity between and within muscles and a change in mechanical behaviour (e.g. the direction of force) (Hodges and Tucker 2011; Bank et al. 2013) (Figure 17). The immediate benefit is to protect from further pain, prevent additional injury to the painful area or both. However, this change in strategy also has consequences that may affect task performance. For example, based on the assumption that the most optimal and efficient movement strategy is selected in conditions of no pain, any change may be considered "sub-optimal" and consequently impair exercise efficiency, influencing the rate of fatigue progression (Tucker and Hodges 2010; Hodges and Tucker 2011).

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Fig 17. Schematic diagram summarising the motor adaptations to pain proposed in the "Moving differently in pain" theory. From Hodges and Tucker (2011).

At a motor unit level, modifications in recruitment order and firing frequency observed in response to muscle pain could also influence the rate of fatigue and exercise performance. Typically, during prolonged muscle contraction it is assumed that motor units are recruited in a systematic approach from low-threshold to high-threshold (Henneman et al. 1965; Milner-Brown et al. 1973). However, during isometric contractions, a selective decrease in the discharge rate of low-threshold motor units is observed in response to muscle pain (and inhibitory input from nociceptive afferents) (Farina et al. 2004; Tucker et al. 2009). As a compensatory mechanism to maintain force or torque, an increase in firing of low-threshold motor units in the synergist or antagonists was originally suggested (Falla et al. 2007; Farina et al. 2008). However, this has been refuted and instead the recruitment of additional motor units (unaffected by the noxious stimuli) was proposed as the compensatory response to allow for task continuation (Hodges et al. 2008; Tucker and Hodges 2009;

Tucker et al. 2009; Hodges and Tucker 2011). Opposed to conventional sEMG (which describes the global muscle activation), the use of fine-wire electrodes and high-density sEMG techniques allow for the concurrent measurement of motor unit activity and therefore have advanced understanding of this (Madeleine et al. 2006; Falla and Gallina 2020).

During both low-intensity and high-intensity muscle contractions in the presence of muscle pain, it has been demonstrated that an increased voluntary drive and the preferential activation of the larger high-threshold motor units compensate for the inhibition of low-threshold motor units and allow for the contraction to be maintained (Martinez-Valdes et al. 2020). It was also demonstrated that the threshold of recruitment and de-recruitment of these high-threshold motor units was lowered, therefore prolonging the duration of their activation (Martinez-Valdes et al. 2020). Whilst advantageous for the immediate development of force and the maintenance of the contraction (Hodges and Tucker 2011), an adaptation towards a more metabolically inefficient motor unit strategy (high-threshold motor units are more susceptible to fatigue) is likely to accelerate metabolite accumulation and exacerbate muscle fatigue (Martinez-Valdes et al. 2020) which could impair performance (Edwards, 1981).

Psychological drive

The mechanisms previously discussed attempt to explain the potential fatiguing role of EIP from solely a physiological perspective. It should however be reiterated that pain could also have psychological impact on exercise performance. Pain can be described by two unique components: sensory-discriminative and affective-motivational (Treede et al. 1999; Price 2000). Typically measured by unidimensional numerical scales (see Section 1.1.2), the sensory-discriminative dimension localises a stimulus and provides information on its modality and intensity (i.e. nociception or sensory pain) (Boggio et al. 2009; Horn et al. 2012). Assessed by multidimensional or qualitive scales, the affective-motivational dimension refers to the emotional response (e.g. fear, sadness, anxiety, helplessness) in anticipation of or in response to a an aversive and painful stimulus (i.e. pain unpleasantness) (Boggio et al. 2009; Horn et al. 2012). Whilst distinct components of pain, the affective-motivation component of

pain is in part informed by the processing of input from sensory-discriminative information and therefore requires supplementary information to contextualise the nociceptive stimulus (Price 2000; Moseley and Arntz 2007).

As highlighted previously, the acute experience of muscle pain is ultimately providing a physiological warning signal of actual or potential threat to the body (Eccleston and Crombez 1999; Auvray et al. 2010; Horn et al. 2012). This subsequently motivates the defensive thoughts or behaviour of the individual to avoid, escape, overcome the source of pain (Fields 1999; Auvray et al. 2010). Therefore, as an important protective function, intolerable EIP during endurance exercise could provide a powerful psychological drive to modify exercise behaviour in order to avoid the aversive sensations of pain (Fields 1999). With the established linear relationship between EIP and work-rate, the exerciser can either reduce the work-rate or disengage from the exercise task to reduce EIP (Mauger 2014). Therefore the ability to overcome this drive or use it to carefully inform adjustments in work-rate could be a key factor in endurance performance (Mauger 2014). Evidence of this potential effect during exercise is however limited, with the affective-motivational component generally overlooked (Eccleston and Crombez 1999). Despite a given perceived intensity, pain unpleasantness is influenced by experimental pain stimulus (Rainville et al. 1992) and can be moderated by contextual factors (Price 2000; Moseley and Arntz 2007), and therefore investigations evaluating the impact of EIP during exercise should therefore separate the two distinct measures of pain intensity and pain unpleasantness.

1.4.2. EIP and endurance performance

Role of EIP tolerance in endurance performance

With a clear association between acute muscle pain and work-rate or duration of exercise (Cook et al. 1997), it has long been postulated that the ability to tolerate or overcome pain is an inherent pre-requisite of exercise and potentially a differentiating factor in successful performance (Anshel and Russell 1994; Mauger 2013, 2014; Stevens et al. 2018). In other words, a greater ability to tolerate muscle pain for the duration of exercise could allow athletes to perform closer to their individual physiological capacity and surpass competitors with a lower pain tolerance

(O'Connor and Cook 1999). Generally supported by cross-sectional evidence, it is recognised that competitive athletes who participate in regular, vigorous and painful training are likely to be more "stoical" and have different perceptions or sensitivity to pain compared to non-competitive athletes (Ryan and Kovacic 1966; Scott and Gijsbers 1981; Ord and Gijsbers 2003; Tesarz et al. 2012, 2013). Interestingly, a majority of the studies comparing differences in pain perception between the competitive and non-competitive athletes reported a greater pain tolerance in the competitive athletes, with no difference in pain threshold (Tesarz et al. 2012), suggesting that pain tolerance opposed to pain threshold appears is the important distinguishing factor in performance.

This finding also alludes to the possibility that pain tolerance can potentially be improved through training. At present, a limited number of experimental training studies have demonstrated that in healthy yet previously untrained individuals, regular participation in chronic (6-12 weeks) aerobic training (cycling) elevated pain tolerance (measured by mechanical pressure and ischemic noxious stimuli) (Anshel and Russell 1994; Jones et al. 2014), but no difference in pain threshold (Jones et al. 2014). The same effect was not demonstrated in resistance training, with no change in pain tolerance whether this form of training was employed alone or in combination with the aerobic exercise over a 12 week period (Anshel and Russell 1994).

Both studies attributed the improvements in pain tolerance to psychological rather than physiological (i.e. reduced nociceptive processing or signalling) adaptations (Anshel and Russell 1994; Tesarz et al. 2012; Jones et al. 2014). For example, an increased exposure to prolonged training close to physiological capacity that elicits intense, unpleasant and painful sensations could necessitate the development of coping skills to increase pain control (Anshel and Russell 1994; Kress and Statler 2007; Tesarz et al. 2012; Jones et al. 2014). This could include an enhancement in pain-specific self-efficacy, resilience or attitudes, which are associated with pain tolerance (Ord and Gijsbers 2003; Rokke et al. 2004; Motl et al. 2007; Schmitz et al. 2013; Slepian et al. 2016). However, only one study recorded a psychological measure (mood) (Anshel and Russell 1994), and as neither study recorded changes in psychological measures, this is merely a hypothesis, and the physiological adaptations should not be discounted.

Although beneficial for understanding the relationship between pain tolerance and aerobic training, these studies do not provide a performance measure. This has since been addressed by two studies which evaluated the predictive value of pain tolerance on cycling time-trial performance (Astokorki and Mauger 2017a) and how an increased pain tolerance through aerobic interval training can improve cycling time to exhaustion (O'Leary et al. 2017). Combined with traditional physiological parameters of endurance performance ($VO_{2MAX,}$ GET and peak power output), pain tolerance (RPE clamp) was able to account for 7.5% of variance and therefore a significant predictor of endurance performance (Astokorki and Mauger 2017a).

This was supported by findings of a significantly increased ischaemic muscle pain tolerance after 6 weeks of interval training compared to a work-matched continuous aerobic training regimen, which was positively correlated with time to exhaustion performance (O'Leary et al. 2017). Incidentally, the interval training group had a much greater improvement in time to exhaustion compared to the continuous training group, despite both forms of training demonstrating similar improvements in aerobic markers of fitness ($VO_{2MAX,}$ LT, lactate turn-point and peak power output). Both studies provide further support for the notion that participation in aerobic training can improve pain tolerance, and, independent of aerobic fitness, these improvements are able to enhance endurance performance.

Experimental manipulation of EIP during endurance performance

The experimental manipulation of EIP during an exercise task provides a controlled means to evaluate the direct role of EIP on endurance performance. A common ergogenic intervention is caffeine (Doherty and Smith 2004; Ganio et al. 2009). The ingestion of caffeine is also believed to have a hypoalgesic effect, with several studies demonstrating a reduction in perceptions of EIP intensity during fixed work-rate submaximal (between 60-80% maximal aerobic capacity) cycling exercise (Motl et al. 2003, 2006; O'Connor et al. 2004; Gliottoni and Motl 2008; Gliottoni et al. 2009). In closed-loop exercise tasks significant improvements in performance have been shown despite no change in ratings of EIP intensity in the caffeine compared to a placebo condition (Jenkins et al. 2008; Astorino et al. 2012; Gonglach et al. 2016; Tomazini et

al. 2020). This suggests that either caffeine does not have a hypoalgesic effect beyond a threshold level of EIP, or that participants utilised perceptions of EIP as a factor in the regulation of work-rate. As such the ergogenic effect of the caffeine allowed greater work-rate to be maintained for a given level of perceived EIP (Gonglach et al. 2016). It should however be highlighted that there is notable interindividual variation in response to caffeine, which can be influenced by factors such as dose, timing, training status and polymorphisms (Pickering and Kiely 2018). In addition, caffeine elicits alternate physiological (e.g. motor unit recruitment), psychological and perceptual (e.g. perceived exertion) responses (Keisler and Armsey II 2006), which reduce the ability to elucidate whether the improvements in performance were solely resultant from the experimental manipulation of EIP.

Another method that has been applied to decrease EIP during exercise are analgesic drugs (i.e. "pain killers"). Initially, aspirin (Roi et al. 1994; Cook et al. 1997) and codeine (Cook et al. 2000; Ray and Carter 2007) were the pharmacological methods consumed, however these studies have produced equivocal results and, like caffeine, these drugs have additional actions (e.g. anticoagulation, fat oxidation) which make it difficult to attribute any ergogenic effect on performance solely to drug-induced analgesia (Mauger et al. 2010). Acetaminophen (i.e. paracetamol) (Mauger and Hopker 2012; Foster et al. 2014) was proposed as a suitable alternative to the previously used drugs in the investigation of EIP on endurance performance (Mauger et al. 2010).

A foundational study by Mauger and colleagues (Mauger et al. 2010) demonstrated that the consumption of 1.5 g of this centrally-acting analgesic significantly improved 16.1 km (10 mile) cycling time-trial performance by 2% in trained cyclists compared to a placebo condition. The quicker time-trial was attributed to the acetaminophen "permitting" the cyclists to maintain a greater work-rate (power output) during the middle section of the time-trial (Figure 18), with the quicker performance accompanied by an elevated heart rate and blood lactate concentration (i.e. greater physiological strain). An increased mean power output/torque has also been demonstrated across repeated sprint exercise (Foster et al. 2014) and maximal protocols (Morgan et al. 2018, 2019) after consumption of the same dose of acetaminophen. The latter studies did not report pain, however during the time-trial

and repeated sprint exercise, participants reported the same intensity of muscle pain, despite maintaining the greater power output. Again, it was proposed that the cyclists were willing to tolerate a certain level of EIP, and therefore in conditions of analgesia (with relatively less EIP) were able to produce a greater-work rate.

[REDACTED]

Figure 18. Mean power output profiles during 16.1 km time-trial across acetaminophen and placebo conditions. Acetaminophen (dark line) reduced the magnitude reduced the magnitude at which power output declined toward the middle section of the time-trial. From Mauger et al (2010).

These findings provide evidence for the association between exercise performance and pain tolerance, as well as a consideration that the regulation of work-rate during exercise and decision-making on pacing strategies could be partially based on EIP perceptions (i.e. work-rate may be regulated based on the set level of pain an individual is willing or able to tolerate). Transcutaneous electrical nerve stimulation (TENS) has also been used to induce analgesia prior to the performance of cycling time-trial exercise (Astokorki and Mauger 2017b; Hibbert et al. 2017) and a single-limb isometric TTF protocol of the knee extensors (Behm et al. 2019) and elbow flexors (Astokorki and Mauger 2017b). With the exception of study by Hibbert and colleagues (Hibbert et al. 2017) (potentially due to a difference in stimulation

parameters/procedures), the application of TENS prior to and during exercise has been shown to improve performance (Astokorki and Mauger 2017b; Behm et al. 2019). The study by Astokorki and Mauger (2017) also reported a reduction in sensations of EIP which were associated with an improvement in performance of both exercise tasks.

It should be noted that at rest, acetaminophen has been shown to potentially increase excitability and responsiveness of the corticospinal tract (Mauger and Hopker 2013), and alongside TENS, is therefore believed to attenuate the nociceptive signal at a spinal level (Astokorki and Mauger 2017b). As a decrease in corticospinal excitability has been associated with the development of fatigue (Ross et al. 2010), any enhancements in performance or exercise tolerance could instead be attributed to this action (Foster et al. 2014; Mauger et al. 2014). Resultantly, the action at a spinal level could confound the ability to attribute the improvements in performance to changes in CMD from the activation of Group III and IV afferents.

Emphasis in the literature has been placed on reducing EIP using various ergogenic interventions with analgesic properties, with a limited attention on experimental methods to *increase* EIP during exercise. The opioid antagonist naloxone, which increases pain, has been shown to significantly reduce time to exhaustion during incremental cycling (Sgherza et al. 2002) and submaximal treadmill running (Surbey et al. 1984). However in these studies the participant perceptions of pain were either not quantified (Sgherza et al. 2002) or limited (Surbey et al. 1984), inhibiting the understanding of whether the increase in pain was a factor in the impairment in performance. Other studies have employed methods of experimentally inducing pain (e.g. thermal, mechanical or electrical pain) during exercise (see Section 1.1.2). However, these induction methods are inappropriate for the investigation of EIP during exercise due to differences in their processing and response compared with the transmission and experience of EIP, in addition to the confounding actions that prevent the isolated investigation of EIP.

1.4.3. Hypertonic saline as a potential experimental model of EIP

Previous research has demonstrated the challenges involved in the experimental manipulation of pain independently of additional physiological, psychological and perceptual factors that may affect exercise performance. In order to examine and understand the sole effect of EIP on endurance performance and allow for its potential fatiguing impact to be investigated, an alternate pain induction model that can be safely applied during exercise and replicates the experience of EIP as closely as possible is required. Prerequisites of the desired method are that it 1) induces muscle pain that *feels like* naturally occurring EIP, 2) uncouples the relationship between EIP intensity and work-rate, and 3) does not elicit additional responses that may influence exercise performance.

As identified in sections 1.1.3 and 1.1.4, the intramuscular of HS could provide a method which may fulfil the aforementioned requirements. A safe and well-established method to experimentally induce a standardised experience of pain in an isolated muscle (with minimal contribution from cutaneous nociceptors), this model has a similar nociceptive pathway and has been demonstrated to evoke sensations equivalent to naturally occurring EIP (Kellgren 1938; Mense 1993; Laursen et al. 1999; Graven-Nielsen and Mense 2001; Graven-Nielsen et al. 2003; Graven-Nielsen 2006). This model allows for a good degree of experimental control through the benefit of a placebo-control in the form of IS (which produces a negligible or no pain response) and a good intra-individual reliability (i.e. participants are likely to have a similar experience across repeat experimental visits) (Graven-Nielsen et al. 1997b). It should however be noted that, at present, due to the dual role of Group III and IV afferents in both nociceptive processing and as a sensory moderator of the exercise pressor reflex (Coote et al. 1969; McCloskey and Mitchell 1972; Kaufman et al. 1983; Amann et al. 2010) it is not known whether the muscle pain induced by this method evokes a confounding cardiorespiratory response.

However, while HS injection is a recognised method for inducing muscle pain, the pain experience has yet to be directly compared with that of EIP, and there has been little attempt to use it to explore the fatigue-pain relationship in exercise conditions where the potential fatiguing-impact of EIP would be most prominent (e.g. endurance exercise). Research that has employed this model has demonstrated pain induced in

the tibialis anterior and gastrocnemius reduces performance of high intensity (50-80% MVC), short duration exercise (Graven-Nielsen et al. 1997e; Ciubotariu et al. 2004) and inhibits MVT (applied to the rectus femoris) (Graven-Nielsen et al. 2002). Whilst insightful, the characteristics of the exercise tasks (i.e. intensity, duration and muscle tested) evaluated in the presence of augmented muscle pain in prior research have limited translation to endurance performance. Resultantly, a concerted effort should be made to conduct experimental work which provides a more applied link to whole-body, locomotive exercise (e.g. investigating the effect of pain at contraction intensity and in a large muscle/muscle group relevant to exercise performance). This thesis will therefore apply the HS model of muscle pain within this context to ascertain its suitability as an experimental method to replicate the EIP experience and to explore its potential fatiguing impact on a series of exercise tasks relevant to endurance exercise.

1.4.4. Conclusion

This literature review has demonstrated that development of fatigue and endurance performance are highly complex and are underpinned by the interaction of several components (e.g. physiological, psychological, environmental). Numerous models have been proposed in an attempt to explain how performance is regulated, tolerated and limited. Whilst these models have been influential in progressing understanding and the approach towards endurance performance investigations, an overall consensus is yet to be reached. With the exception of the psychobiological model, most models of fatigue and endurance performance have acknowledged the role of afferent feedback and the accompanying sensations (e.g. EIP). However, the potential impact of specifically EIP on endurance exercise is still relatively unknown. Indeed, there are several proposed mechanisms which could explain how EIP may exacerbate or contribute to the development of fatigue, and therefore limit endurance performance, but these require further exploration.

The experimental manipulation of EIP during an exercise task provides a controlled means to evaluate this notion, however previous attempts to manipulate EIP have demonstrated this to be challenging and are limited by alternate confounding variables or responses. From reviewing the literature, it is apparent that the intramuscular

injection of HS may provide an appropriate model to induce an experience of muscle pain akin to naturally occurring EIP, however at present this has yet to be evaluated and therefore requires investigation.

1.5 Aims and hypotheses of the thesis

The overall aim of this thesis is two-fold: 1) to apply and confirm the HS model as a means for experimentally replicating the experience of EIP, and if successful, 2) to use this model as a method to evaluate the potential fatiguing role of EIP during exercise tasks relevant to endurance performance. Through four experimental studies, this thesis will employ the following aims and hypothesis to explore the aforementioned gaps in the literature

Chapter 3 – Study 1

Muscle pain induced by hypertonic saline in the knee extensors decreases single-limb isometric time to task failure

Aims: This study aimed to:

- Compare the qualitative experience (based on the total and subclass scores from the McGill Pain Questionnaire) of naturally occurring EIP to the pain elicited from an intramuscular injection of HS into a locomotor muscle
- Identify the effects of the experimental EIP elicited by this method on the performance time of an endurance exercise task performed in a muscle group, and at an intensity, that is relevant to locomotive exercise.

Hypothesis: It was hypothesised that the intramuscular injection of 5.8% HS into the vastus lateralis:

- Replicates the naturally occurring EIP (in terms of intensity and quality) from the performance of a higher intensity exercise, uncoupling the EIP and exercise intensity relationship;
- In addition to low intensity exercise results in a shorter exercise performance

Chapter 4 – Study 2

Muscle pain from an intramuscular injection of hypertonic saline increases variability in knee extensor torque reproduction

Aims: This study aimed to ascertain whether experimentally induced muscle pain in the vastus lateralis using an intramuscular injection of HS interferes with the ability to accurately reproduce the torque produced by the knee extensor muscles in a single-limb isometric torque reproduction task

Hypothesis: It was hypothesised that experimental muscle pain in the vastus lateralis reduces torque reproduction accuracy (as quantified by the variance in mismatch between target and actual torque) of low intensity isometric contractions when compared to a control (no pain) condition

Chapter 5 – Study 3

Cardiorespiratory and perceptual response to acute unilateral and bilateral muscle pain induced by hypertonic saline

Aims: This study aimed to:

- Investigate the cardiorespiratory response to acute unilateral and bilateral experimental muscle pain induced through the intramuscular injection of either IS (placebo) or HS (pain) in the right and left vastus lateralis at rest
- Evaluate and compare the perceptual response to acute bilateral muscle pain in contrast with unilateral muscle pain at rest, and determine the influence of limb dominancy on pain perception in the lower limb

Hypothesis: it was hypothesised that bilateral and simultaneous intramuscular injection of 5.8% HS into the vastus lateralis:

- Directly elicits a reflex cardiorespiratory response
- Results in a significant increase of muscle pain experienced (intensity and quality) compared to the unilateral muscle pain

Chapter 6 – Study 4

Acute bilateral muscle pain induced by hypertonic saline decreases cycling time to exhaustion

Aims: This study aimed to determine the effect of acute bilateral muscle pain administered in a knee extensor muscle (vastus lateralis), on short duration, heavy-intensity cycling endurance performance

Hypothesis: It was hypothesised that the addition of 5.8% HS into the VL of both the right and left leg during heavy intensity exercise to exhaustion would result in an elevated experience of muscle pain, and a reduced exercise performance compared to control and placebo conditions.

Chapter 2 – General Methods

2.1 Introduction

The purpose of this chapter is to outline and describe the primary methods (procedures, protocols and measurements) used across the experimental research conducted in Chapters 3-6. Procedures and protocols specific to an experimental chapter are also detailed in the methods section of the respective chapter. Data collection for each experimental chapter was conducted in the research laboratories of the School of Sport and Exercise Sciences, University of Kent.

2.2 Pre-test procedures

2.2.1 Ethical approval

Prior to commencement, the design and procedures of all experimental studies had received full ethical approval from the School of Sport and Exercise Sciences ethics committee at the University of Kent, with all procedures performed in line with the Declaration of Helsinki. In all studies, before confirmation of involvement, participants were provided with a written information sheet outlining the study and experimental protocols, as well as the expectations of the participant during the course of the study (see Appendix A). The information sheet also contained a detailed overview of the intramuscular (IM) injection procedure, the risks involved, and the measures in place to minimise the potential occurrence of these risks (see Appendix B). This information was subsequently verbally reinforced and confirmed with the participants.

Participants were then requested to complete a general health questionnaire and an IM injection risk assessment questionnaire (see Appendix A). These questionnaires were completed to ensure safe participation in the exercise and to confirm individual suitability to safely receive an IM injection. Participants were not permitted to take part if the questionnaires were not satisfactorily completed or if the administration of an IM injection was unsuitable or unsafe for the respective participant. Upon acceptable completion of the questionnaires, participants signed an informed consent form (see Appendix A). All participants were informed that their involvement in the study was voluntary, and that they were free to withdraw their consent at any time without reason.

For all studies, participants were instructed to arrive to the laboratory in a rested state (refrained from undertaking vigorous exercise in the past 24 h) and have abstained from the consumption of alcohol (48 h), caffeine (8 h) and analgesics (6 h) prior to each visit. Compliance to these pre-requisites was verbally confirmed with the participants at the start of each visit.

2.2.2 Participant familiarisation

For each of the four experiments, in the first visit to the laboratory, participants were familiarised with the experimental procedures and measures. The purpose of the familiarisation was primarily to reduce any potential learning effect and additionally to ensure participant comfort with the intramuscular injection procedure. Prior to commencement of experimental procedures, participants were instructed to read a set of written instructions for the scales implemented in each chapter (see Appendix C) and were also familiarised with the self-report psychological measurements implemented in each chapter (see Section 2.5 and Appendix D). This includes the 10-point Cook scale for pain intensity (Cook et al. 1997) (Chapters 3-6), the 15-point Borg (6-20) scale for rating of perceived exertion (Borg, 1998) (used in Chapters 3, 4 and 6), and the 11-point rating of fatigue scale (Micklewright et al. 2017b) (Chapters 3 and 6). Familiarisation of these scales are detailed in Section 2.4.2. All information on the perceptual measures and report instruments was subsequently verbally reinforced and confirmed with the participants. In addition, participants without prior experience of the hypertonic saline intramuscular injection procedure were also familiarised before commencing any experimental visit. Familiarisation procedures specific to each experiment are detailed in the methods section of the respective experimental chapter.

2.2.3 Anthropometric measures

Before the commencement of each experimental study, anthropometric and descriptive measures of age, height, body mass, and hours of physical activity engaged in per week were recorded. Height, recorded to the nearest 0.01 m, was measured using a Stadiometer, whilst body mass was measured to the nearest 0.1 kg using calibrated scales.

2.3 Experimental procedures

2.3.1 Intramuscular injections

All IM injections were performed either in the right vastus lateralis (VL) (Chapters 3 and 4), or simultaneously in the right and left vastus lateralis (Chapters 5 and 6). The vastus lateralis IM injection site was identified as the middle third of the lateral aspect of the thigh between the greater trochanter and the lateral femoral condyle of the femur. This provides a large and easily accessible muscle mass and is not associated with any major blood vessels or significant structures, minimising the risks of damage or likelihood of injury.

For all administrations of IM injections, thorough care and consideration was taken to implement best infection control practices and the safe handling of the injection equipment. All equipment was checked prior to use to ensure that the items were sterile and non-contaminated, and then safely disposed of after use. Before receiving an IM injection, the site was always inspected and palpated to ensure that it was free from contraindications, and then subsequently marked to ensure standardisation of location. Prior to the administration of a bilateral injection (Chapters 5 and 6), the injection site for the right and left leg was visually inspected to confirm similar location selection for both legs. The site was always prepared and cleansed with an alcohol swab before each injection.

The solutions administered for all studies were either a single bolus of 1.0 mL 5.8% hypertonic saline (B Braun Medical Industries), used to induce acute muscle pain, or a single bolus of 1.0 mL 0.9% isotonic saline (B Braun Medical Industries), which was injected as a control. Implementing the z-track technique, the IM injection was performed manually over a 20 s window (10 s infusion period) using a 3 mL Luer-Lok syringe connected to a 25 G × 38 mm SurGuard2 disposable stainless needle (Terumo, Japan). Participants were instructed to look away from the IM injection, keep their legs relaxed and focus on a marked location on an opposite wall throughout the procedure. After the completion of the injection, participants were requested to monitor the injection site two to four hours and were informed of potential adverse reactions that should be reported on occurrence. The details of the IM injection

procedure were fully documented in line with National Health Service guidelines. In each experimental study, all IM injection visits were separated by a minimum of 7 days.

3.3.2 Isokinetic dynamometry

An isokinetic dynamometer (Cybex HUMAC Norm isokinetic dynamometer; CSMi, Soughton, MA, USA) calibrated to the manufacturer instructions was used in Chapters 3 and 4 for the measurement of torque of the knee extensors. Participants were instructed to sit up straight in the chair and position themselves with hips square and the posterior of the knees touching the front of the seat. The dynamometer was set up for the right leg, with the knee set at an angle of 75^{o} of flexion (0^{o} = full extension of the knee), and a hip angle of 90^{o}. The right knee axis was positioned in line with the dynamometer axis, and the lever arm was secured above the lateral malleolus with the padded cushion situated posteriorly. The left leg was placed behind a contra limb stabiliser and the participant trunk was secured to the chair with a strap to maintain a stable body position and minimise as much extraneous movement as possible. This set-up and the additional chair settings were recorded for each participant in the first visit of each chapter and repeated for all subsequent visits to ensure participants remained in an identical body position across all experimental trials.

2.3.3 Surface electromyography (sEMG)

In Chapters 3 and 4, muscle electrical activity of the VL, vastus medialis (VM) and rectus femoris (RF) was continuously recorded using surface electromyography (sEMG) acquired through square surface electrodes (Ag/AgCl, 32 × 32 mm; Nessler Medizintechnik, Innsbruck, Austria) mounted in a bipolar set-up and placed over the muscle belly in the direction of the muscle fibres, For each muscle a reference electrode was placed on the patella of the right knee. Prior to application of the electrodes, the skin was shaven and cleansed using alcohol swabs. The electrode positions were marked to ensure consistent placement and standardisation of location for each experimental visit. The electrical signal was sampled at 2000 Hz (Biopac MP150, Biopac Systems Inc., California, USA) and acquired in Spike2 software (Version 7; Cambridge Electronic Design).

The sEMG data was analysed using custom code written in MATLAB R2018a (The MathWorks, Massachusetts, USA). To create a linear envelope representation of the data, the raw sEMG signals were rectified by taking the absolute values, and two-pass zero-lag filtered using a fourth-order low-pass Butterworth filter with a cut-off frequency of 5 Hz. The mean sEMG amplitude for each muscle was then extracted and normalised to the maximum sEMG amplitude of the maximum voluntary contractions performed prior to the experimental protocols (%MVC) (see Chapters 3 and 4 for specific details).

2.4 Measurements

2.4.1 Measurement of muscle pain

During all visits of each study, two perceptual characteristics of the muscle pain experience were recorded: pain intensity and quality. The muscle pain was defined as "the intensity of hurt" felt, and participants were instructed to anchor this to previous experiences of naturally occurring EIP (Astokorki and Mauger 2017b) to support the rating process. It was emphasised that the muscle pain reported in each study is that produced by muscle burn and ache as a result of repeated or prolonged muscular contractions opposed to injury or other pain experienced (e.g. seat discomfort). Participants were also reminded to not use the pain intensity rating as an expression of perceived fatigue or exertion.

The intensity of muscle pain in the right leg (Chapter 3 and 4) or the total ("global") muscle pain intensity in both legs (Chapters 5 and 6) was continuously scored on a on a moment-to-moment basis using a sliding, electronic visual analogue scale (VAS) aided by verbal descriptors ranging from 0 ("no pain at all") to 10 ("extremely intense pain") (Cook et al. 1997). Participants received regular verbal reminders to make any necessary adjustments. The electronic VAS device automatically sampled and recorded the reported pain intensity every 5 s (Chapters 3-5) and 2 s (Chapter 6) which allowed for values such as VAS onset (the time-point at which the stimulus is first perceived to be greater than "no pain") peak pain intensity (VAS peak), time to maximal intensity (from the commencement of sampling), mean pain intensity (the mean VAS from the commencement of sampling until task failure), duration of pain

(from VAS onset until the state of "no pain"), and VAS area (area under VAS curve) to be calculated.

The quality of pain was established by the long-form McGill Pain Questionnaire (MPQ) (Melzack 1975) which contains a total of 20 categories of adjectives describing four major subclasses of pain experience (sensory, affective, evaluative and miscellaneous) alongside a separate group of words which describe the time-related properties of pain. Each category contains between two to six adjectives that are qualitatively comparable, positioned in ascending order of implied pain intensity and are assigned rank value based on this order (e.g. the descriptor associated with the least pain within the category is assigned a value of 1). Participants were permitted to select a maximum of one word per category (should any of the descriptors apply). The descriptors chosen by the participants were subsequently summed to calculate scores for each subclass (Subclass Rating Index) and the total score of all subclasses (Total Pain Rating Index), with the overall quality of pain expressed by descriptors chosen by more than one-third of participants.

2.4.2 Perceptual Measures

The processing of a physical stimulus and its concomitant sensory signal by the brain results in the conscious experience of the sensation (i.e. perceptions), which are highly subjective and variable constructs (Gardner and Martin 2000). This thesis generally employed three self-report psychophysiological scales to monitor three perceptual parameters: pain intensity (see Section 2.4.1), rating of perceived exertion (Chapters 3, 4 and 6) and rating of fatigue (Chapters 3 and 6). As highlighted in the literature review, these perceptions are resultant from separate processes and were therefore recorded as different entities with independent scales to avoid inaccurate perceptual measures (O'Connor and Cook 2001; Pageaux et al. 2015a; Astokorki and Mauger 2017a). In this thesis, participants received written instructions for each scale (see Appendix C), with a clear emphasis on the importance of being able to distinguish between each perception. Understanding of these instructions and the differences between each perception were verbally confirmed by the experimenter and reinforced in each testing session.

Perceived exertion (RPE) was verbally reported every 30 s during the time to task failure protocols (Chapters 3 and 6), and immediately upon completion of the torque matching and reproduction trials (Chapter 4). This was achieved using the 15-point (6-20) Borg scale (Borg, 1998) aided by verbal descriptors ranging from "no exertion" (6) to "maximal exertion" (20) to rate the magnitude of exertion perceived during the exercise tasks. In this thesis, RPE during single-limb isometric tasks (Chapters 3 and 4) was defined as the "effort required to drive the limb", whilst during cycling exercise (Chapter 6) participants were instructed to include the heaviness of breathing into the rating (Pageaux 2016). This definition ensured that participants did not factor in perceptions of discomfort or fatigue. Rating of fatigue (ROF), defined as "the perceived inability of the muscle to produce torque", was verbally reported every 30 s for the first min, and every 60 s thereafter during the time to task failure protocols (Chapters 3 and 6). This was achieved through the use of the 11-point (0-10) Rating of Fatigue (ROF) scale (Micklewright et al. 2017b), with the scale supported by five verbal descriptors (ranging from "not fatigued at all" (0) to "total fatigue and exhaustion – nothing left" (10)) and five accompanying images (depicting the different states of fatigue) to aid understanding.

2.5 Report instruments

In Chapters 3 and 6, the participants were required to complete three questionnaires to provide measures of positive and negative affect (Watson, Clark and Tellegen, 1988), emotional intelligence (Schutte et al., 1998) and pain resilience (Slepian, Ankawi, Himawan and France, 2016). These questionnaires were administered at the start of the first visit of each experimental study. In addition, for each chapter, at the start of each visit, participants were asked to rate (on a visual analogue scale) how much pain they expected to experience (anchored to the non-injury pain experienced during exercise) (0 = "no pain" to 10 = "worst possible pain") and their confidence to cope with the expected level of pain (0 = "not confident at all" to 10 = "completely confident"). This provides a measure of pain-specific self-efficacy which is believed to a predictor of pain tolerance and endurance (Motl et al. 2007; Schmitz et al. 2013). In Chapters 5 and 6, post-trial, participants also completed a modified Situation-Specific Pain Catastrophizing Scale (SPCS) (Edwards et al. 2006) to indicate the

occurrence of catastrophizing specifically during the painful experience. All four report instruments are provided in Appendix D.

2.5.1 Positive and negative affect schedule (PANAS)

The PANAS is a 20-item questionnaire that includes two scales containing 10 adjectives that describe positive (e.g. excited, alert) and negative (e.g. scared, nervous) affect respectively. Each item for the PANAS are scored a 5-point scale ranging from 1 (not at all/very slightly) to 5 (extremely) to indicate the extent to which participants felt at a set time point. Both scales can therefore receive a minimum score of 10 and a maximum score of 50. In all experimental studies, the time period that the participants were asked to refer to when rating each item was "over the past week" (completed only at the start of the first visit) and "at the present moment" (completed at the start of each visit).

2.5.2 Schutte self-report emotional intelligence test (SSEIT)

The SSEIT is a 33-item scale that assesses the ability of an individual to appraise, understand, regulate and utilize the emotions of oneself and in others. Each item is rated on a 5-point scale anchored from 1 (strongly disagree) to 5 (strongly agree). An overall score of emotional intelligence is gained by reverse coding three of the items (5, 28 and 33) and then totalling all responses.

2.5.3 Pain resilience scale (PRS)

The PRS is a 14-item questionnaire encompassing two discrete dimensions: "cognitive/affective positivity" (9 items), which focuses on the participant's ability in the management of thoughts and emotions whilst in pain, and "behavioural perseverance" (5 items), which reflects the continued motivation and behavioural persistence despite the presence of intense or sustained pain. The PRS requires the participant to assess each item on a 0-4 scale (0 = not at all and 4 = all the time), with the scores from each item then summed to provide a total score, and an individual score for each subscale.

2.5.4. Modified situation-specific pain catastrophizing scale (SPCS)

The modified SPCS is a 6-item scale encompassing the three dimensions of catastrophizing: “rumination”, “magnification” and “helplessness”. The questionnaire is specific to the experience of pain *during* laboratory procedures and is therefore administered upon immediate completion of the trial. Participants are required to score each item on a 0-4 scale (0 = not at all and 4 = all the time) to indicate the degree at which the thoughts and feelings occurred during the trial. The item scores are subsequently summed to provide an overall catastrophizing score.

Chapter 3 – Muscle pain induced by hypertonic saline in the knee extensors decreases single-limb isometric time to task failure

Abstract

Purpose: Increased nociceptive activity and the experience of exercise-induced pain (EIP) may contribute to fatigue during endurance exercise. To investigate this, a pain model that produces pain similar to EIP and decouples its' relationship to exercise intensity is required. This study 1) compared the quality of pain caused by a hypertonic saline injection into the vastus lateralis in resting and exercise conditions, and 2) investigated whether this pain contributes to changes in time to task failure. **Methods:** On separate days, eighteen participants completed a time to task failure at 20% maximal voluntary torque (MVT), a resting hypertonic saline intramuscular injection, and in a further three visits a time to task failure at 10% MVT following injection of isotonic saline, hypertonic saline or a control (no injection). **Results:** In a subset of eligible participants (n = 12), the hypertonic saline combined with 10% MVT produced a qualitative experience of pain (assessed by the McGill Pain Questionnaire) that felt similar to EIP. 10% MVT with hypertonic saline significantly elevated pain intensity in the first 20% of the time to task failure and caused a significantly ($P < 0.05$) shorter time to task failure (448 ± 240 s) compared with the isotonic saline (605 ± 285 s) and control (514 ± 197 s) conditions. **Conclusion:** These findings demonstrate that hypertonic saline increases the intensity of pain during exercise, which results in a faster occurrence of exercise-induced fatigue. These results provide important evidence supporting pain as a limiting factor in endurance performance.

Introduction

Intense and prolonged muscle contractions result in acute pain proportional to the intensity and duration of exercise (Cook et al. 1997). This 'exercise-induced pain' (EIP) arises from the sensitisation and activation of ascending group III and IV nociceptive afferents in response to the accumulation of endogenous algesics and increases in noxious and mechanical pressure within the contracting skeletal musculature (O'Connor and Cook 1999). The experience of EIP is often accompanied by fatigue (Pollak et al. 2014), which is defined as an exercise-induced reduction in the capacity to produce muscle force or power (Bigland-Ritchie and Wood 1984).

This association has led to the suggestion that EIP may accelerate fatigue development during intense and prolonged exercise (Mauger 2014).

In support of this notion, the stimulation of muscle nociceptors and increased muscle afferent activity has demonstrated significant reductions in voluntary activation of the elbow flexors (Kennedy et al. 2013) and maximal voluntary force of the knee extensors (Graven-Nielsen et al. 2002). Furthermore, partial blockade of group III and IV muscle afferents at the spinal level results in the attenuation of perceived fatigue, and increases central motor drive (Amann et al. 2009). Based on these findings, it is suggested that the increased activation of group III and IV afferents inhibit central motor drive and the ability to recruit motor units (Amann et al. 2011a; Hureau et al. 2019).

A challenge in studying the fatigue-pain relationship (Mauger 2013; Pollak et al. 2014) is that most experimental pain-induction methods are notably different in their processing and response compared with the transmission and experience of EIP (i.e. differences in the neurological processes that result in the perception of pain, from transduction to perception (Olesen et al. 2012)). For example, ischemic, electrical and thermal pain induction are experimental pain models that are non-specific to the muscle, and can also induce the perception of cutaneous pain (Staahl and Drewes 2004; Olesen et al. 2012). The additional stimulation of these superficial tissues can produce a subjective pain quality described as “sharp” or “stabbing” as opposed to the “aching” or “cramping” nature of muscle pain (Mense 1993). As such their use may be inappropriate in the investigation of EIP.

Consequently, an experimental model that induces muscle pain that feels like naturally occurring EIP and allows its contribution to fatigue to be investigated by decoupling EIP from exercise intensity is desirable. The intramuscular injection of hypertonic saline is a well-established and safe experimental method that, under resting conditions, induces standardised and reproducible acute pain often described as ‘aching’ and ‘cramping’ (Graven-Nielsen et al. 1997a, b, d). When injected, this solution activates predominantly group IV afferents with some contribution from myelinated group III nerve fibres (Laursen et al. 1999), which is similar to the nociceptive pathway of EIP (O’Connor and Cook 1999).

However, while hypertonic saline is established for inducing muscle pain, there has been limited comparison with the experience of EIP and minimal application to explore the fatigue-pain relationship. Indeed, in this field hypertonic saline is most widely used to investigate putative pain-induced changes to motor control (Hodges and Tucker 2011), maximal voluntary contraction (Graven-Nielsen et al. 2002), and high intensity, short duration exercise performance (Graven-Nielsen et al. 1997e) rather than its impact on exercise-induced fatigue. In addition, the exercise intensities, durations, and muscle groups used in these studies have limited relevance to exercise conditions where the impact of EIP on fatigue is most prominent (i.e. prolonged duration (> 2 min), exhaustive exercise in large, primary muscle groups involved in locomotive exercise) (Cook et al. 1997; Abbiss and Laursen 2008).

Therefore, the aims of this study were to: (i) compare the qualitative experience (based on the total and subclass scores from the McGill Pain Questionnaire) of naturally occurring EIP to the pain elicited from an intramuscular injection of hypertonic saline into a locomotor muscle; and (ii) identify the effects of the muscle pain elicited by this method on the performance time of an endurance exercise task. We tested the hypothesis that the addition of an intramuscular injection of 5.8% hypertonic saline into the vastus lateralis (VL) to low intensity exercise: (i) produces a similar quality of pain (as defined by the McGill Pain Questionnaire) compared to naturally occurring EIP caused by a higher exercise intensity; and (ii) results in a shorter time to task failure compared to placebo and control conditions.

Methods

Ethical approval

The School of Sport and Exercises (University of Kent) Research Ethics Advisory Group (Prop 84_2016_17) approved all procedures and protocols in accordance with the Declaration of Helsinki. Written informed consent was gained from the participants prior to participation.

Participants

Eighteen healthy and recreationally active participants (11 male, 7 female; mean ± SD: age, 24.5 ± 4.0 years; height 1.76 ± 0.1 m; body mass 73.9 ± 13.4 kg; physical activity 5.5 ± 2.3 $h \cdot w^{-1}$) volunteered to participate in the present study. The sample size was estimated based on the effect size reported in a similar exercise and pain study (Deschamps et al. 2014) to satisfy statistical power at 80%. Recruited participants were free from the exclusion criteria and attended the laboratory in accordance with the pre-requisites outlined in Chapter 2. All participants attended each visit in a similar psychological state as assessed by the Positive and Negative Affect Schedule (PANAS) (Watson et al. 1988), which was completed at the start of each visit.

Experimental procedures

Participants attended the laboratory on five occasions, with each visit separated by 2-7 days. In the initial visit, anthropometric measures (height, body mass) were recorded, and the self-report psychological measurements (*see Self-reported psychological data)* were completed. In this visit, participants were also familiarized with all measures relating to the experimental protocol, including a practice of knee extensor maximal voluntary contractions (MVCs). Five minutes after MVCs, participants performed an isometric time to task failure (TTF) at 20% maximal voluntary torque (MVT). In visit 2, participants received a single injection of hypertonic saline (Rest HYP), whilst seated at rest *(see Intramuscular injections* in Chapter 2*).* Upon the completion of the injection, participants were asked to continuously rate muscle pain intensity, with the visit concluding once the participant had returned to the state of 'no pain'. In a further three visits (visits 3-5), participants performed a TTF at 10% MVT in three conditions in the presence of: no injection (10% MVT, Control), isotonic saline (10% MVT + ISO, Placebo) and hypertonic saline (10% MVT + HYP). In the 10% MVT + ISO and 10% MVT + HYP visits, an intramuscular injection was administered prior to the TTF, with the task commencing within 3 s of needle removal. Conditions were performed in a single-blind, randomised and counter-balanced order.

Time to task failure (TTF) protocol

All visits were performed seated on an isokinetic dynamometer set up as described in Chapter 2. At the start of each visit, participants completed a 5 min self-paced, submaximal warm-up on a cycle ergometer (Wattbike Ltd, Nottingham, UK) followed by 3×3s MVCs separated by 90 s rest. The highest torque produced across the three MVCs was defined as the MVT. The TTF commenced 5 min after the MVCs, with the participants directed to maintain a submaximal isometric contraction of the knee extensors. The participants received visual feedback of the target torque on a computer screen but were unaware of the overall time elapsed. The task was limited to a maximum of 20 min, or was terminated when the torque fell below the target for more than 3 s. Within 3 s of task cessation participants performed a final MVC.

Perceptual measurements

At the start of each visit, participants were asked to rate pain expectations and confidence to cope with this expected level of pain. Two characteristics of pain were evaluated: intensity and quality. During all visits, pain intensity was continuously scored on a moment-to-moment basis using an electronic visual analogue scale (VAS) whilst the quality of pain was established by the long-form McGill Pain Questionnaire (MPQ) (Melzack 1975). The MPQ was completed after the post-TTF MVC in each visit, and the return to ‘”no pain” in the Rest HYP visit. During all of the TTF trials at 10% MVT (visits 3-5), participants also reported Rating of perceived exertion (RPE) every 30 s, and Rating of fatigue was recorded every 30 s for the first min, and every 60 s thereafter using the 11-point Rating of Fatigue (ROF) scale. More information on perceptual measurements can be viewed in Chapter 2.

Physiological Measurements

During the TTFs at 10% MVT (visits 3-5) heart rate (HR) was recorded every 30 s using a Polar FT1 HR monitor paired with a coded T34 transmitter (Polar, Polar Electro, Kempele, Finland), and muscle electrical activity was continuously recorded using surface electromyography (sEMG). The sEMG was acquired as detailed in Chapter 2, with the signals obtained divided into 10 s epochs. The mean sEMG then

extracted and normalised to the maximum sEMG amplitude of the prior MVCs (% MVC).

Self-reported psychological data

In the first visit, on arrival to the laboratory, participants completed the Positive and Negative Affect Schedule (PANAS) (Watson et al. 1988), Schutte Self Report Emotional Intelligence Test (SSEIT) (Schutte et al. 1998) and Pain Resilience Scale (PRS) (Slepian et al. 2016) to assess mood, emotional intelligence and pain-specific resilience, respectively. The completion of the PANAS was also repeated at the start of each visit with participants responding according to feelings at that present moment.

Statistical analysis

In this chapter, two analyses were performed on the data; a primary analysis of all participant data (n = 18), and a secondary analysis performed on a subset of participants (n = 12) that reached task failure in all conditions prior to the imposed limitation of 20 min maximum duration. All data are presented in the form of mean ± standard deviation (SD). Prior to statistical analysis, all data were checked for the assumptions associated with a paired samples t-test, a one-way ANOVA and a repeated measures ANOVA as appropriate. Data that did not satisfy the Shaprio-Wilk test of normality ($P < 0.05$) were logarithmically transformed. The Bonferroni post-hoc correction was applied where appropriate. Cohen's *d* and partial eta square (η_p^2) values are reported as measures of effect size (Cohen, 1988). Pearson bivariate correlations were used to evaluate the correlation between parameters, with Cohen's guidelines of 0.1 (small), 0.3 (medium) and greater than or equal to 0.5 (large) to quantify the strength of correlation (Cohen, 1988)

Due to between subject variability in TTF, an 'individual iso-time' approach as outlined by Nicolò and colleagues (Nicolò et al. 2019) was applied to compare perceptual (pain intensity, RPE, ROF) and physiological (HR, sEMG) variables. The 'shortest' TTF for each participant was used to identify four (RPE, ROF, HR) and ten (pain intensity and sEMG) time-points in which the three conditions were segmented.

This approach maintains a majority of the time-series data (i.e. allows for the inclusion of all repeated recordings such as pain, RPE and ROF to be included) and provides a consistent number of data points to allow comparison between participants for all stated variables across the varying TTF times.

A two-way ANOVA with Treatment factor with 3 fixed levels (10% MVT, 10% MVT + ISO, 10% MVT + HYP) and a repeated measures Time factor with 10 time-points was used to test the effect of condition and time on pain intensity and sEMG during the TTF. Two-way ANOVAs with a Treatment factor with 3 fixed levels (10% MVT, 10% MVT + ISO, Experimental) and a repeated measures Time factor with 4 time points were used for measures of RPE, ROF and HR recorded during the TTF. When an interaction effect was observed, post-hoc paired sample t-tests were implemented to evaluate differences between conditions. Statistical significance was accepted at an alpha level of $P < 0.05$ except where a Bonferroni correction was applied (adjusted, $P < 0.0042$). All statistics were performed using SPSS Statistics v24.0 (SPSS, IBM, New York, USA).

Results

Primary analysis

Comparison of pain intensity and quality

Mean TTF at 20% MVT was 245 ± 92 s. As shown in Table 1, paired samples t-test revealed a significant difference in VAS scores between pain intensity during 20% MVT TTF and experimental muscle pain from Rest HYP ($P < 0.05$). The 20% MVT task induced a significantly greater mean VAS, equivalent to a "strong" pain intensity ($t_{17} = 5.9$, $P < 0.001$, $CI_{.95}$ 1.1, 2.4, $d = 1.6$), which peaked after a longer period of time ($t_{17} = 7.8$, $P < 0.001$, $CI_{.95}$ 102, 178, $d = 2.3$) and lasted for a shorter duration ($t_{17} = -2.5$, $P = 0.023$, $CI_{.95}$ -129, -11, $d = 0.9$) than the experimental muscle pain experienced in Rest HYP.

Differences in VAS scores were also reported between 20% TTF and the TTFs at 10% MVT ($P < 0.05$). The VAS onset was significantly slower in 10% MVT ($t_{17} = -4.8$, $P < 0.001$, $CI_{.95}$ -39, -15, $d = 1.1$), with a quicker onset in 10% MVT + HYP ($t_{17} = 4.0$, $P = 0.001$, $CI_{.95}$ 9.1, 29.8, $d = 1.3$). A greater VAS mean, equivalent to "very

strong" pain was observed in the 10% MVT + HYP condition compared to 20% MVT (t_{17} = -2.3, P = 0.033, $CI_{.95}$ -2.1, -0.1 , d = 0.7) and 10% MVT (t_{17} = -3.0, P = 0.008, $CI_{.95}$ -1.7, -0.3, d = 0.6).

The VAS in all three conditions performed at 10% MVT peaked after a longer period of time (10% MVT; t_{17} = -6.0, P < 0.001, $CI_{.95}$ -494, -235, d = 1.6, 10% MVT + ISO; t_{17} = -6.3, P < 0.001, $CI_{.95}$ -503, -249, d = 1.7, 10% MVT + HYP; t_{17} = -4.3, P = 0.001, $CI_{.95}$ -435, -147, d = 1.2) and lasted longer in duration (10% MVT; t_{17} = -6.7, P < 0.001, $CI_{.95}$ -620, -324, d = 1.8, 10% MVT + ISO; t_{17} = -7.6, P < 0.001, $CI_{.95}$ -640, -362, d = 2.0, 10% MVT + HYP; t_{17} = -5.8, P < 0.001, $CI_{.95}$ -625, -293, d = 1.6) than the 20% MVT condition. This contributed to a greater VAS area (10% MVT; t_{17} = -6.4, P < 0.001, $CI_{.95}$ -3438, -1738, d = 1.6, 10% MVT + ISO; t_{17} = -6.9, P < 0.001, $CI_{.95}$ -3766, -1993, d = 1.9, 10% MVT + HYP; t_{11} = -4.4, P < 0.001, $CI_{.95}$ -3984, -1792, d = 1.5) in the 10% MVT conditions compared to 20% MVT.

Table 1. Summary VAS scores from 20% MVT, Rest HYP, 10% MVT, 10% MVT + ISO, 10% MVT + HYP TTF (n = 18).

	20% MVT	Rest HYP	10% MVT	10% MVT + ISO	10% MVT + HYP
VAS onset (s)	28 ± 19	20 ± 13	56 ± 32**	40 ± 28	9 ± 8*†
VAS mean	5.1 ± 1.1	3.4 ± 1.0**	5.3 ± 1.4	5.6 ± 1.3	6.2 ± 1.7*†
VAS peak	9.6 ± 0.6	6.2 ± 2.1**	9.0 ± 1.4	8.8 ± 1.6	8.9 ± 1.6
VAS time to peak (s)	224 ± 78	84 ± 34**	589 ± 312**	600 ± 303**	515 ± 333*
VAS duration (s)	215 ± 88	286 ± 78*	688 ± 364**	716 ± 339**	675 ± 399**
VAS area	1278 ± 726	976 ± 358	3866 ± 2190**	4157 ± 2026**	4165 ± 2642**

Values are means ± SD. *Significantly different *vs* 20% MVT (P < 0.05). **Significantly different *vs* 20% MVT (P < 0.001). †Significantly different *vs* 10% MVT (P < 0.05)

Overall, as shown in Table 2, the dimensional quality of pain experienced during 20% MVT was similar to Rest HYP for the sensory ($P = 0.185$) and miscellaneous ($P = 0.241$) dimensions, but not for the affective ($P = 0.004$) and evaluative ($P = 0.002$) subclasses. The 20% MVT task produced a greater mean Total Pain Index ($t_{17} = 2.7$, $P = 0.015$, $CI_{.95}$ 2, 13, $d = 0.7$) than Rest HYP, and, as shown in Table 2, and was defined by descriptives representing all dimensions in the MPQ. However, the 10% MVT + HYP condition, with a mean total pain index of 26 ± 12, produced a similar overall subjective quality of pain to 20% MVT ($t_{17} = 1.1$, $P = 0.282$, $CI_{.95}$ -3, 9, $d = 0.3$). Paired samples t-test revealed no significant difference in Subclass Rating Index between 10% MVT + HYP and 10% MVT (Sensory; $P = 0.479$, Affective: $P = 0.144$, Evaluative; $P = 0.687$; Miscellaneous, $P = 0.549$) as well as 10% MVT + HYP and 20% MVT (Sensory; $P = 0.641$, Affective: $P = 0.088$, Evaluative; $P = 0.260$; Miscellaneous, $P = 0.237$) for all classifications (Table 2).

Table 2. Frequently selected words from the MPQ subclasses (n = 18).

Subclass		20% MVT	REST HYP	10% MVT	10% MVT + ISO	10% MVT + HYP
Sensory		Throbbing (44%) Sharp (56%) Cramping (39%) Hot (39%) Aching (50%)	Throbbing (56%) Cramping (56%) Aching (67%)	Cramping (50%) Aching (56%)	Throbbing (44%) Cramping (44%) Burning (44%)	Throbbing (56%) Cramping (56%) Burning (33%) Aching (67%)
	SRI	18 ± 6	15 ± 6	16 ± 8	15 ± 6	17 ± 8
Affective		Exhausting (50%)	Exhausting (39%)	Exhausting (61%)	Tiring (33%) Gruelling (33%)	Tiring (33%) Exhausting (39%)
	SRI	3 ± 2	1 ± 2*	3 ± 2	2 ± 1	2 ± 2

Evaluative		Intense (67%)	Intense (67%)	Intense (61%)	Intense (67%)	Intense (67%)
	SRI	4 ± 1	2 ± 2*	3 ± 2	3 ± 2	3 ± 1
Miscellaneous		Radiating (44%)				
	SRI	5 ± 4	4 ± 3	3 ± 3	4 ± 4	4 ± 4
	PRI(T)	29 ± 11	22 ± 9*	25 ± 11	24 ± 11	26 ± 12

The frequently selected words from the MPQ are shown with the percentage of participants (n = 12) that selected these words. Data on Subclass Rating Index (SRI) and Pain Rating Index (Total) presented as Mean ± SD. *Significantly different *vs* 20% MVT ($P < 0.05$).

Time to task failure (TTF)

An ANOVA revealed a significant difference in TTF between conditions ($F_{2,34} = 3.7$, $P = 0.033$, $\eta_p^2 = 0.181$). Subsequent pairwise comparisons highlighted a shorter TTF ($t_{17} = 2.2$, $P = 0.044$, $CI_{.95}$ -0.003, -0.189, $d = 0.2$) in 10% MVT + HYP (686 ± 400 s) compared to 10% MVT + ISO (761 ± 341 s). No significant differences were observed between 10% MVT (742 ± 369 s) and 10% MVT + ISO ($t_{17} = -0.9$, $P = 0.402$, $CI_{.95}$ -0.082, -0.034, $d = 0.1$), whilst the difference between 10% MVT and 10% MVT + HYP was not significantly different ($t_{17} = 2.0$, $P = 0.060$, $CI_{.95}$ -0.003, -0.148, $d = 0.1$) (Fig. 1a.). Paired samples t-tests showed that post-TTF MVT significantly decreased in 10% MVT (pre = 279 ± 63 N.m, post = 184 ± 56 N.m), 10% MVT + ISO (pre = 276 ± 67 N.m, post = 188 ± 58 N.m) and 10% MVT + HYP (pre = 282 ± 69 N.m, post = 184 ± 57 N.m) in comparison to pre-TTF MVT ($P < 0.001$). No significant difference was observed between conditions for absolute decrement in MVT ($F_{2,34} = 0.9$, $P = 0.399$, $\eta_p^2 = 0.053$).

There was no correlation ($P > 0.05$) between TTF and EIP resilience (total score, behavioural perseverance and cognitive/affective positivity), emotional intelligence, positive affect and negative affect. An ANOVA demonstrated no significant

difference between conditions for positive affect ($F_{2,3}$ = 2.3, P > 0.05, η_p^2 = 0.118), however there was statistical significance of condition for negative affect ($F_{2,34}$ = 4.1, P = 0.025, η_p^2 = 0.195). Subsequent pairwise comparisons found greater (t_{17} = -3.7, P = 0.002, $CI_{.95}$ -4.52, -1.25, d = 1.1) negative affect in 10% MVT + HYP (14.56 ± 2.96) compared to 10% MVT (11.67 ± 2.40), with no significant difference between 10% MVT + ISO (13.17 ± 4.42) and 10% MVT (P > 0.05), and 10% MVT + ISO and 10% MVT + HYP (P > 0.05).

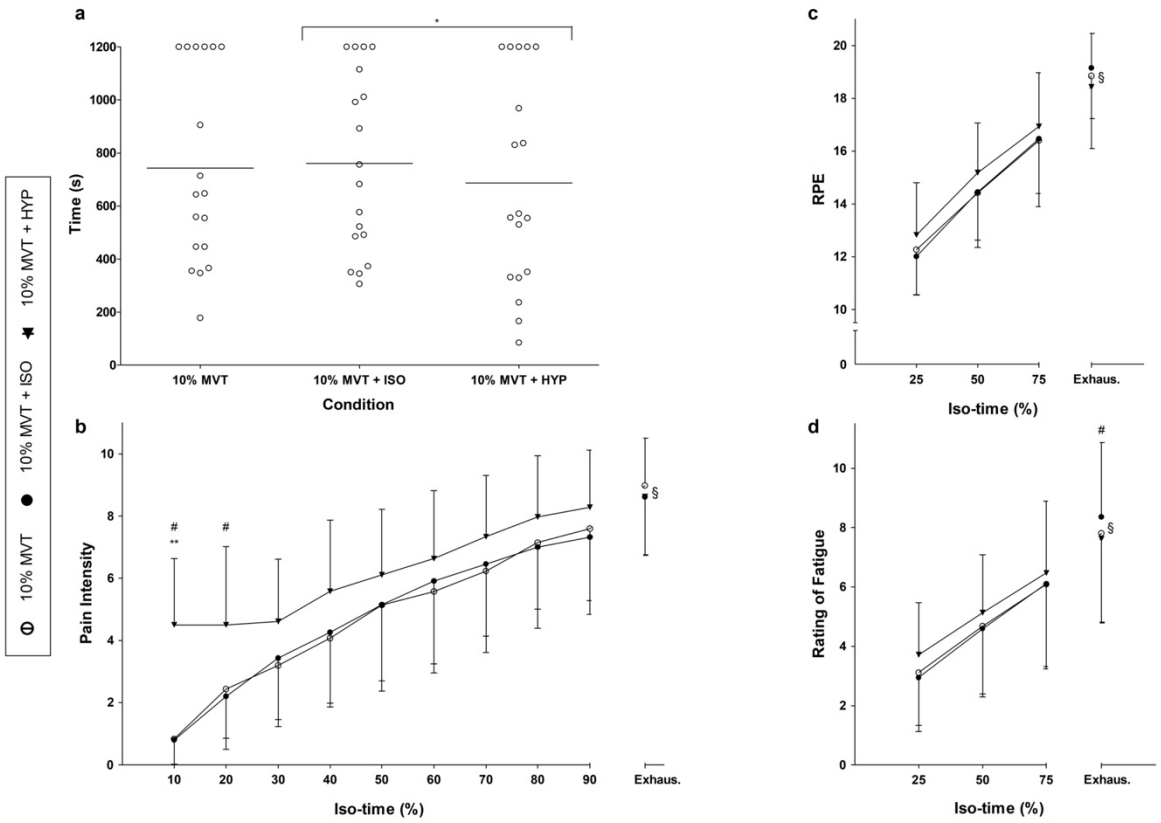


Fig. 1 Performance and perceptual differences between conditions. TTF differences between conditions (a), and pain intensity (b) and RPE (c) and ROF (d) over iso-time between conditions during the TTF (n = 18). *Significant difference between conditions (P < 0.05). **Significant difference between 10% MVT + HYP and 10% MVT (P ≤ 0.001). #Significant difference between 10% MVT + HYP and 10% MVT + ISO (P < 0.001). §Significant main effect of iso-time.

Pain intensity

An ANOVA revealed a significant difference in pain expectations between conditions ($F_{2,34}$ = 16.0, P < 0.001, η_p^2 = 0.484), but not in confidence to cope with the expected

pain ($F_{2,34}$ = 3.1, P > 0.05, η_p^2 = 0.152). Subsequent pairwise comparisons found greater expectations of pain (P < 0.001) in 10% MVT + ISO (6.8 ± 1.8, t_{17} = -4.4, $CI_{.95}$ -1.6, -0.6, d = 0.6) and 10% MVT + HYP (7.6 ± 1.2, t_{17} = -5.3, $CI_{.95}$ -2.7, -1.1, d = 1.3) compared to 10% MVT (5.7 ± 1.7) with no significant difference between 10% MVT + ISO and 10% MVT + HYP (P > 0.05).

The 3 x 10 (condition x iso-time) repeated measures ANOVA highlighted a significant effect of condition ($F_{2,34}$ = 7.3, P = 0.002, η_p^2 = 0.302) and iso-time ($F_{2.36,40.17}$ = 100.9, P < 0.001, η_p^2 = 0.856) for perceived pain during the TTF. A significant interaction effect for pain over iso-time between conditions during the TTF was observed ($F_{5.09,86.57}$ = 11.3, P = 0.002, η_p^2 = 0.399) as demonstrated in Fig. 1b. Follow up targeted paired-sample t-tests with a Bonferroni correction revealed a significantly greater VAS pain intensity in 10% MVT + HYP compared to 10% MVT + ISO at 10% (63 ± 35 s) (t_{17} = -9.8, P < 0.001, $CI_{.95}$ -4.9, -3.2, d = 2.3) and 20% (127 ± 70 s) (t_{17} = -4.0, P = 0.001, $CI_{.95}$ -3.1, -1.0, d = 0.9) iso-time. There was also a significantly greater VAS EIP intensity reported in 10% MVT + HYP compared to 10% MVT at 10% iso-time (t_{17} = -8.6, P < 0.001, $CI_{.95}$ -5.3, -3.2, d = 2.2).

Perceptual measures

The 3 × 4 (condition × iso-time) repeated measures ANOVA revealed no significant main effect of condition for ROF or RPE (P > 0.05). Both ROF and RPE had a significant effect of iso-time (ROF; $F_{1.43,24.22}$ = 102.3, P < 0.001, η_p^2 = 0.858, RPE; $F_{1.84,31.26}$ = 141.5, P < 0.001, η_p^2 = 0.893), and an interaction effect (ROF; $F_{2.76,46.97}$ = 5.5, P = 0.003, η_p^2 = 0.245, RPE; $F_{3.59,60.99}$ = 3.3, P = 0.020, η_p^2 = 0.161). Follow-up paired samples t-test with a Bonferroni correction revealed (P > 0.004) no significant differences for ROF or RPE at any iso-time point between conditions (Fig. 1c. and Fig. 1d.).

Surface electromyography

Due to a loss in sEMG signal, three participants were removed from the dataset and analysis was performed on the remaining participants (n = 15). A 3 x 10 (condition x iso-time) repeated measures ANOVA demonstrated no significant main effect of condition in either the VL ($F_{2,28}$ = 1.4, P > 0.05, η_p^2 = 0.089), VM ($F_{2,28}$ = 2.3, P >

0.05, $\eta_p^2 = 0.144$), or RF ($F_{2,28} = 0.2$, $P > 0.05$, $\eta_p^2 = 0.013$). A significant effect of iso-time in the activity of the VL ($F_{1.76,24.65} = 23.8$, $P < 0.001$, $\eta_p^2 = 0.629$), VM ($F_{1.98,27.74} = 23.4$ $P < 0.001$, $\eta_p^2 = 0.625$), and RF ($F_{3.19,44.66} = 16.7$, $P < 0.001$, $\eta_p^2 = 0.544$) was reported (Fig. 2.). There was no interaction effect observed in either the VM ($F_{18,252} = 0.9$, $P > 0.05$, $\eta_p^2 = 0.058$) or RF ($F_{18,252} = 0.7$, $P > 0.05$, $\eta_p^2 = 0.047$). A significant interaction effect was reported for VL activity over iso-time between conditions ($F_{18,252} = 1.9$, $P = 0.015$, $\eta_p^2 = 0.121$), however subsequent follow-up targeted paired sample t-tests with a Bonferroni correction demonstrated no significant differences (Fig. 3a-c).

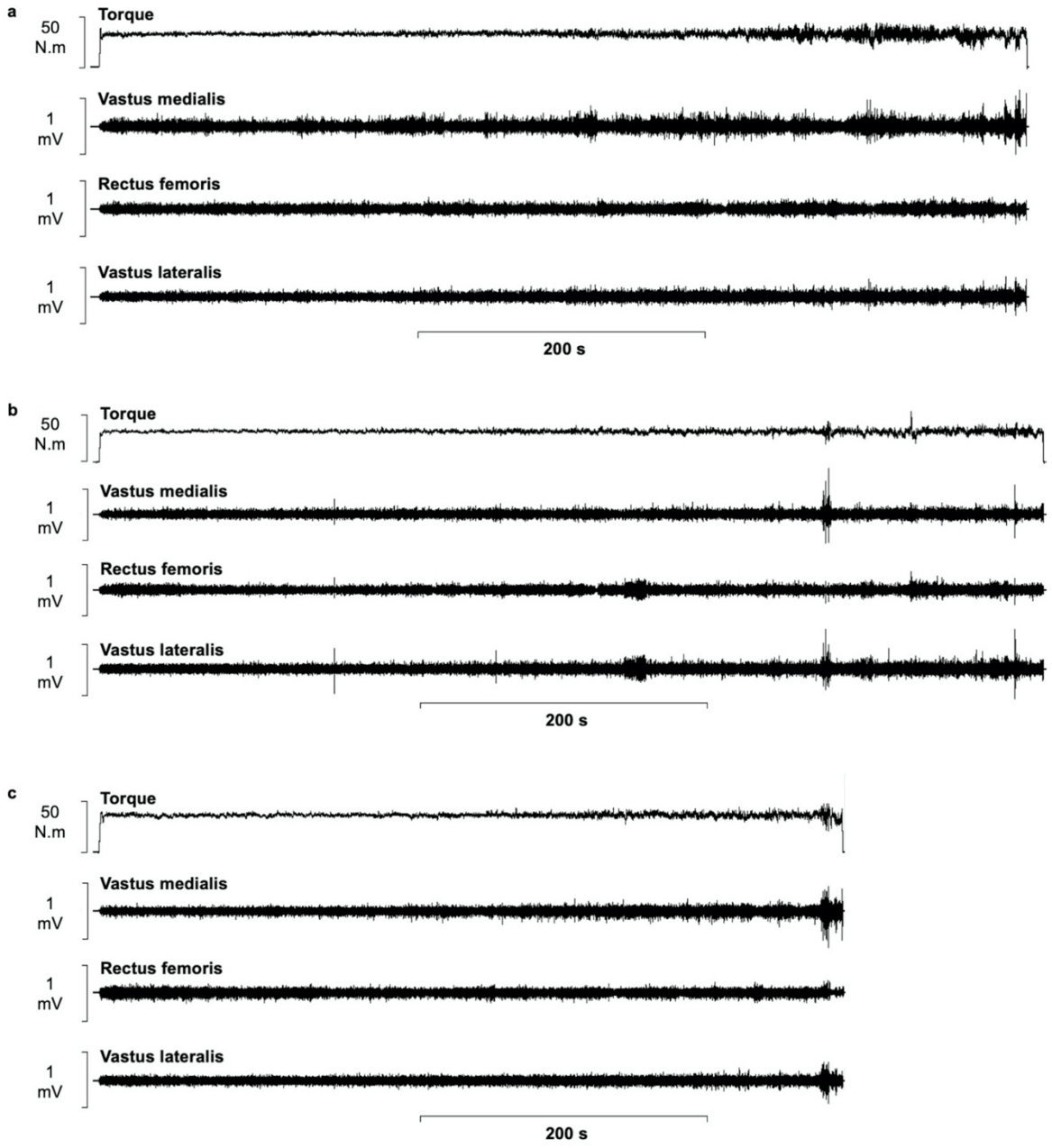


Fig. 2 Torque and sEMG data during the TTF of the 10% MVT (a), 10% MVT + ISO (b) and 10% MVT + HYP (c) conditions for a representative participant. The TTF was significantly shortened in the 10% MVT + HYP condition.

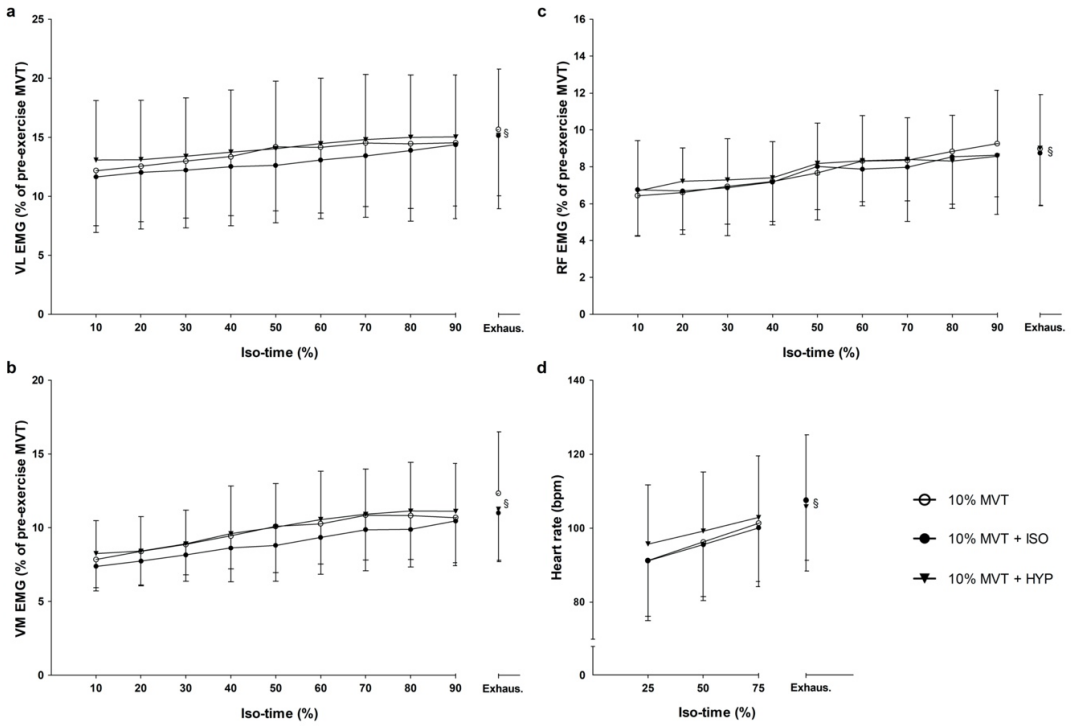


Fig. 3 Physiological differences between conditions. EMG of the VL (a), VM (b) and RF (c) over iso-time between conditions during the TTF (n = 18). HR differences between conditions over iso-time during the TTF (d). §Significant main effect of iso-time ($P < 0.05$).

Heart rate

The 3 x 4 (condition x iso-time) repeated measures ANOVA revealed no significant main effect of condition ($F_{1.48,25.15} = 0.5$, $P > 0.05$, $\eta_p^2 = 0.027$). There was a significant effect of iso-time ($F_{1.43,24.22} = 32.1$, $P < 0.001$, $\eta_p^2 = 0.654$), and an interaction effect for HR and iso-time between conditions during the TTF ($F_{2.47,41.98} = 5.5$, $P = 0.023$, $\eta_p^2 = 0.182$) (Fig. 3d.). Subsequent paired samples t-test with a Bonferroni correction revealed no significant differences between conditions.

Secondary analysis

As the TTF task was limited to a maximum of 20 min, participants that met this cut-off in any condition did not reach task failure or 'exhaustion', which does not provide a true indication of endurance performance. To account for this, these participants (n = 6) were subsequently removed from the data set, and analysis was performed on the subset of participants (n = 12). This secondary analysis broadly strengthened the

conclusions from the initial analysis and made initial observations regarding differences in pain between conditions even more apparent (see below). The secondary analysis did not alter any of the initial statistical comparisons or conclusions regarding the perceptual measures (ROF, RPE), but as shown in Figure 5b did reveal an additional interaction effect for sEMG of the VM ($F_{18,162}$ = 2.2 , P = 0.006, η_p^2 = 0.195) (n = 10). However, follow-up targeted paired samples t-test with a Bonferroni correction revealed no significant differences between conditions at any iso-time points.

Comparison of pain intensity and quality

Mean TTF at 20% MVT was 193 ± 50 s. As shown in Table 3, paired samples t-test revealed a significant difference in VAS scores between pain intensity during 20% MVT TTF and experimental muscle pain from Rest HYP (P < 0.05). The 20% MVT task induced a significantly greater mean VAS, equivalent to between "somewhat strong" and "strong" pain intensity (t_{11} = 5.3, P < 0.001, $CI_{.95}$ 1.1, 2.6, d = 1.8), which peaked after a longer period of time (t_{11} = 5.6, P < 0.001, $CI_{.95}$ 64, 147, d = 1.7) and lasted for a shorter duration (t_{11} = -3.9, P = 0.002, $CI_{.95}$ -175, -49, d = 1.7) than the experimental muscle pain experienced in Rest HYP.

Differences in VAS scores were also reported between 20% TTF and the TTFs at 10% MVT (P < 0.05). The VAS onset was significantly slower in 10% MVT (t_{11} = -5.0, P < 0.001, $CI_{.95}$ -44, -17, d = 1.0) and 10% MVT + ISO (t_{11} = -2.3, P = 0.043, $CI_{.95}$ -33, -1, d = 0.7), with a quicker onset in 10% MVT + HYP (t_{11} = 2.2, P = 0.0047, $CI_{.95}$ 0.2, 29, d = 0.9). A greater VAS mean, equivalent to between "strong" and "very strong" pain was observed in the 10% MVT + HYP condition compared to 20% MVT (t_{11} = -2.8, P = 0.017, $CI_{.95}$ -2.6, -0.3 , d = 1.1) and 10% MVT (t_{11} = -2.3, P = 0.044, $CI_{.95}$ -1.97, -0.03, d = 0.6).

The VAS in all three conditions performed at 10% MVT peaked after a longer period of time (10% MVT; t_{11} = -6.5, P < 0.001, $CI_{.95}$ -344, -170, d = 2.0, 10% MVT + ISO; t_{11} = -4.9, P < 0.001, $CI_{.95}$ -484, -185, d = 1.7, 10% MVT + HYP; t_{11} = -3.5, P = 0.005, $CI_{.95}$ -321, -74, d = 1.2) and lasted longer in duration (10% MVT; t_{11} = -6.3, P < 0.001, $CI_{.95}$ -394, -189, d = 2.2, 10% MVT + ISO; t_{11} = -5.6, P < 0.001, $CI_{.95}$ -538, -

234, d = 2.0, 10% MVT + HYP; t_{11} = -4.2, P = 0.001, $CI_{.95}$ -411, -130, d = 1.6) than the 20% MVT condition. This contributed to a greater VAS area (10% MVT; t_{11} = -5.4, P < 0.001, $CI_{.95}$ -2551, -1077, d = 1.9, 10% MVT + ISO; t_{11} = -5.9, P < 0.001, $CI_{.95}$ -3233, -1466, d = 2.2, 10% MVT + HYP; t_{11} = -4.4, P = 0.001, $CI_{.95}$ -2754, -929, d = 1.7) in the 10% MVT conditions compared to 20% MVT.

Table 3. Summary VAS scores from 20% MVT, Rest HYP, 10% MVT, 10% MVT + ISO, 10% MVT + HYP TTF (n = 12).

	20% MVT	Rest HYP	10% MVT	10% MVT + ISO	10% MVT + HYP
VAS onset (s)	25 ± 22	7 ± 16	55 ± 36**	42 ± 29*	10 ± 9*
VAS mean	4.8 ± 1.0	3.0 ± 1.0**	5.3 ± 1.4	5.5 ± 1.2	6.3 ± 1.7*†
VAS peak	9.7 ± 0.7	5.8 ± 2.1**	9.5 ± 0.8	9.0 ± 1.5	9.2 ± 1.6
VAS time to peak (s)	181 ± 51	75 ± 31**	438 ± 171**	516 ± 282**	379 ± 229*
VAS duration (s)	168 ± 42	281 ± 84*	459 ± 185**	555 ± 270**	438 ± 241*
VAS area	899 ± 315	869 ± 386	2713 ± 1282**	3248 ± 1493**	2740 ± 1521*

Values are means ± SD. *Significantly different *vs* 20% MVT (P < 0.05). **Significantly different *vs* 20% MVT (P < 0.001). †Significantly different *vs* 10% MVT (P < 0.05)

Overall, as shown in Table 2, the dimensional quality of pain experienced during 20% MVT was similar to Rest HYP for the sensory (P = 0.123) and miscellaneous (P = 0.189) dimensions, but not for the affective (P = 0.008) and evaluative (P = 0.007) subclasses. The 20% MVT task produced a greater mean Total Pain Index of 30 ± 11 (t_{11} = 2.9, P = 0.016, $CI_{.95}$ 2, 18, d = 0.7) than Rest HYP (20 ± 9), and, as shown in Table 2, and was defined by descriptives representing all dimensions in the MPQ. However, the 10% MVT + HYP condition, with a mean total pain index of 29 ± 14,

produced a similar overall subjective quality of pain to 20% MVT (t_{11} = 0.3, P = 0.743, $CI_{.95}$ -6, 8, d = 0.1). Paired samples t-test revealed no significant difference in Subclass Rating Index between 10% MVT + HYP and 10% MVT (Sensory; P = 0.704, Affective: P = 0.429, Evaluative; P = 0.878; Miscellaneous, P = 0.410) as well as 10% MVT + HYP and 20% MVT (Sensory; P = 0.941, Affective: P = 0.394, Evaluative; P = 0.504; Miscellaneous, P = 0.810) for all classifications (Table 4).

Table 4. Frequently selected words from the MPQ subclasses (n = 12)

Subclass		20% MVT	REST HYP	10% MVT	10% MVT + ISO	10% MVT + HYP
Sensory		Throbbing (33%) Sharp (58%) Cramping (33%) Pulling (33%) Hot (33%) Burning (33%) Hurting (33%) Aching (58%)	Throbbing (50%) Shooting (42%) Sharp (33%) Cramping (67%) Aching (67%) Tender (33%)	Lacerating (33%) Cramping (58%) Pulling (33%) Searing (33%) Aching (50%)	Throbbing (50%) Cramping (41%) Burning (50%) Aching (67%)	Throbbing (58%) Drilling (33%) Cramping (67%) Burning (42%) Aching (50%) Heavy (33%)
	SRI	18 ± 6	15 ± 6	18 ± 9	15 ± 6	18 ± 9
Affective		Exhausting (50%)		Exhausting (75%)	Tiring (33%) Gruelling (33%)	Tiring (42%) Exhausting (42%) Gruelling (33%)

	SRI	3 ± 3	1 ± 1*	3 ± 2	2 ± 2	3 ± 2
Evaluative		Intense (50%)	Intense (33%)	Intense (58%)	Intense (67%)	Intense (67%)
	SRI	4 ± 2	2 ± 2*	3 ± 2	3 ± 1	3 ± 1
Miscellaneous		Radiating (33%) Tight (33%)	Radiating (33%)			
	SRI	5 ± 4	3 ± 3	4 ± 3	5 ± 4	5 ± 4
	PRI(T)	30 ± 11	20 ± 9*	28 ± 12	26 ± 11	29 ± 14

The frequently selected words from the MPQ are shown with the percentage of participants (n = 12) that selected these words. Data on Subclass Rating Index (SRI) and Pain Rating Index (Total) presented as Mean ± SD. *Significantly different *vs* 20% MVT ($P < 0.05$). **Significantly different *vs* 20% MVT ($P < 0.001$). †Significantly different *vs* 10% MVT ($P < 0.05$)

Time to task failure (TTF)

An ANOVA revealed a significant difference between conditions ($F_{2,22} = 6.7$, $P = 0.005$, $\eta_p^2 = 0.378$) with 10% MVT + HYP causing a significantly ($t_{11} = 3.4$, $P = 0.006$, $CI_{.95}$ 55, 257, $d = 0.6$) shorter TTF (448 ± 240 s) compared to both 10% MVT + ISO (605 ± 285 s), and 10% MVT (514 ± 197 s) ($t_{11} = 2.3$, $P = 0.040$, $CI_{.95}$ 4, 127, $d = 0.3$) (Fig. 4a.). No significant differences were observed between 10% MVT and 10% MVT + ISO ($t_{11} = -1.8$, $P = 0.104$, $CI_{.95}$ -204, 22 $d = 0.4$).

Paired samples t-tests showed that post-TTF MVT significantly decreased in 10% MVT (pre = 304 ± 56 N.m, post = 191 ± 62 N.m), 10% MVT + ISO (pre = 300 ± 62 N.m, post = 197 ± 64 N.m) and 10% MVT + HYP (pre = 308 ± 65 N.m, post = 187 ± 66 N.m) in comparison to pre-TTF MVT ($P < 0.001$). No significant difference was observed between conditions for absolute decrement in MVT ($F_{2,22} = 1.0$, $P = 0.379$, $\eta_p^2 = 0.204$). There was no correlation ($P > 0.05$) between TTF and EIP resilience

(total score, behavioural perseverance and cognitive/affective positivity), emotional intelligence, positive affect and negative affect. An ANOVA also demonstrated no significant difference between conditions for positive affect ($F_{2,22}$ = 1.8, P = 0.189, η_p^2 = 0.141), and negative affect ($F_{2,22}$ = 1.4, P = 0.263, η_p^2 = 0.114) recorded prior to the TTF.

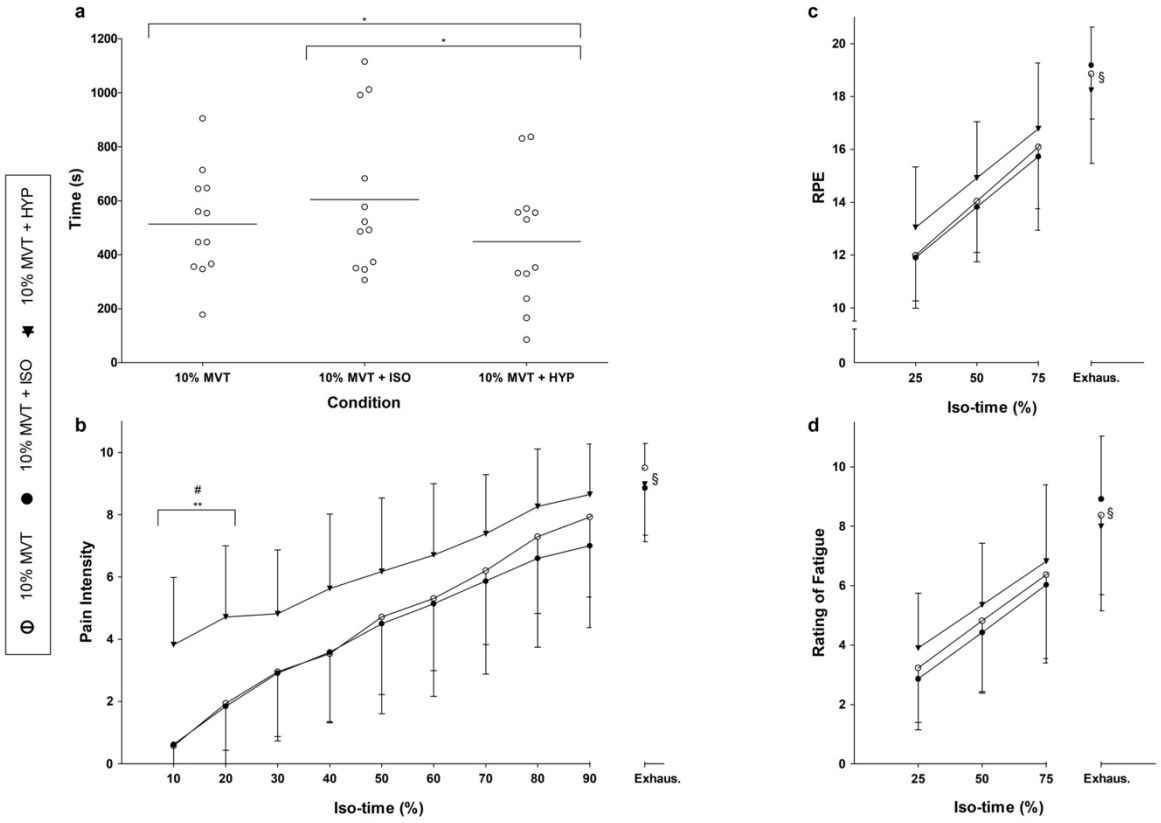


Fig. 4 Performance and perceptual differences between conditions. TTF differences between conditions (a), and pain intensity (b) and RPE (c) and ROF (d) over iso-time between conditions during the TTF (n = 12). *Significant difference between conditions (P < 0.05). **Significant difference between 10% MVT + HYP and 10% MVT (P ≤ 0.001). #Significant difference between 10% MVT + HYP and 10% MVT + ISO (P < 0.001). §Significant main effect of iso-time.

Pain intensity

An ANOVA revealed a significant difference in pain expectations between conditions ($F_{2,22}$ = 9.6, P = 0.001, η_p^2 = 0.467), but not in confidence to cope with the expected pain ($F_{2,22}$ = 2.3, P = 0.125, η_p^2 = 0.172). Subsequent pairwise comparisons found greater expectations of pain in 10% MVT + ISO (7.2 ± 1.9) (t_{11} = -3.8, P = 0.003, $CI_{.95}$ -2, -1, d = 0.7) and 10% MVT + HYP (7.5 ± 1.3)(t_{11} = -4.5, P = 0.001, $CI_{.95}$ -2, -

1, d = 1.0) compared to 10% MVT (6.0 ± 1.6) with no significant difference between 10% MVT + ISO and 10% MVT + HYP (t_{11} = -0.7, P = 0.518, $CI_{.95}$ -1, 1, d = 0.2).

The 3 × 10 (condition × iso-time) repeated measures ANOVA highlighted a significant effect of condition ($F_{2,22}$ = 6.5, P = 0.006, η_p^2 = 0.372) and iso-time ($F_{2.8,31.2}$ = 82.2, P < 0.001, η_p^2 = 0.882) for perceived pain during the TTF (Fig. 4b.). A significant interaction effect for pain over iso-time between conditions during the TTF was observed ($F_{3.9,42.4}$ = 3.4, P = 0.018, η_p^2 = 0.236). Follow up targeted paired-sample t-tests with a Bonferroni correction revealed a significantly greater VAS pain intensity at 10% iso-time (43 ± 21 s) in 10% MVT + HYP compared to both 10% MVT (t_{11} = -6.4, P < 0.001, $CI_{.95}$ -43.7, -21.3, d = 1.9) and 10% MVT + ISO (t_{11} = -5.8, P < 0.001, $CI_{.95}$ -44.2, -19.9, d = 1.9) and at 20% iso-time (86 ± 42 s) in contrast with 10% MVT (t_{11} = -4.3, P = 0.001, $CI_{.95}$ -42.1, -13.4, d = 1.3) and 10% MVT + ISO (t_{11} = -6.3, P < 0.001, $CI_{.95}$ -38.9, -18.6, d = 1.5).

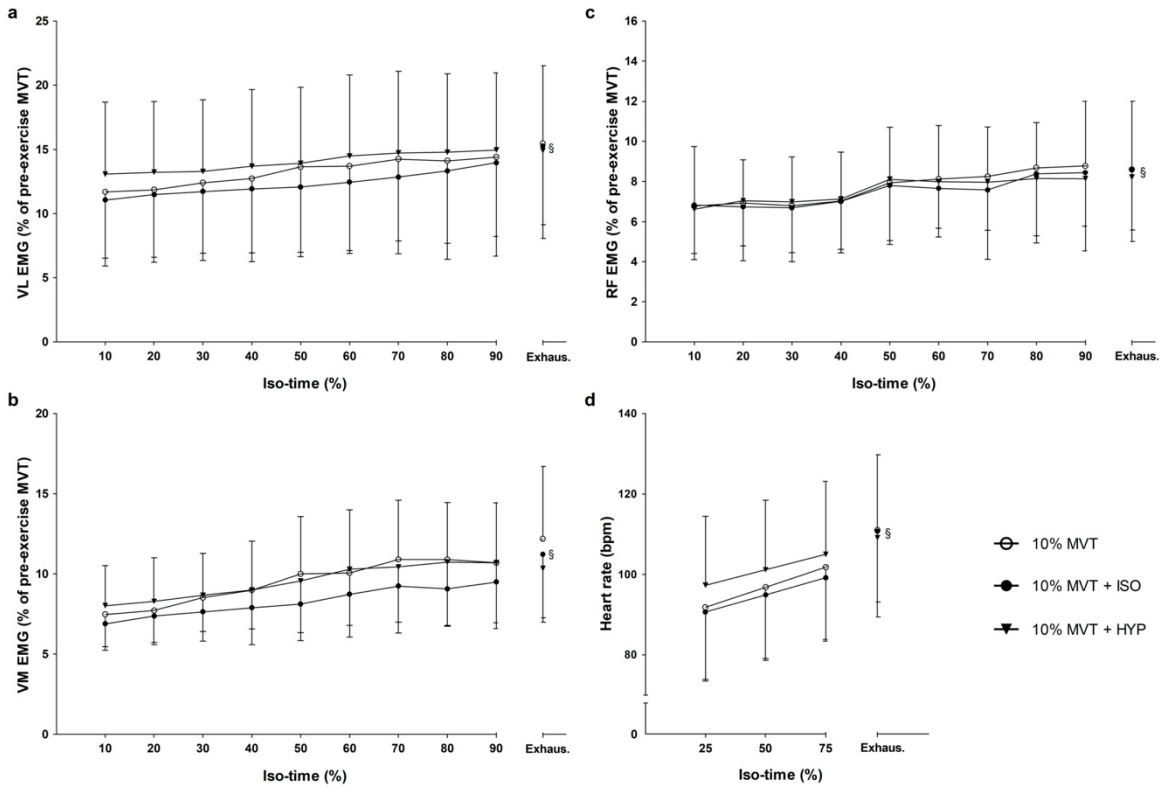


Fig. 5 Physiological differences between conditions. EMG of the VL (a), VM (b) and RF (c) over iso-time between conditions during the TTF (n = 12). HR differences

between conditions over iso-time during the TTF (d). §Significant main effect of iso-time ($P < 0.05$).

Discussion

This study confirms that the pain experienced during knee extensor exercise at 10% MVT can be made to feel like that of a higher exercise intensity, through the intramuscular injection of hypertonic saline into the VL. Using this intervention, exercise-induced fatigue occurred more rapidly, with participants reaching task failure earlier when exercising with a greater pain intensity (Fig. 4b). This study therefore provides indicative evidence to support the notion that pain is a significant factor affecting endurance exercise performance.

Hypertonic saline combined with light exercise feels like EIP

The novel question the present study strived to determine was whether the addition of hypertonic saline to light intensity exercise at 10% MVT produces an elevated pain intensity which also feels similar to the naturally occurring EIP during a higher exercise intensity (20% MVT). Thus, the first key finding from this study (n = 12) is that when combined with light exercise (10% MVT), the hypertonic saline induced a descriptive quality of pain similar to the EIP from both the 10% and 20% MVT exercise tasks (but with a higher intensity). This is in contrast to the administration of hypertonic saline at rest, where our findings were consistent with the established literature - a moderate to somewhat strong pain, described as cramping, aching, throbbing and intense (Graven-Nielsen et al. 1997a, b, d). Furthermore, in these resting conditions, whilst the sensory and miscellaneous quality of experimental pain was similar to the naturally occurring EIP experienced during the 20% MVT task, there were differences in pain intensity and quality. In particular, the 20% MVT task produced a higher pain intensity that was also described in the affective (e.g. 'exhausting') dimension. This suggests that for hypertonic saline to induce a pain that feels like EIP, it needs to be *combined* with at least light intensity exercise. When this was done, participants experienced an elevated overall intensity of pain (compared to both 10% and 20% MVT) but were unable to distinguish between the experimental muscle pain produced by the hypertonic saline and the EIP from the muscular contraction. The findings of this study therefore provide support for this hypertonic

saline model for uncoupling the exercise intensity and EIP relationship (Cook et al. 1997) – i.e. causing a light exercise intensity to *feel* like a harder exercise intensity.

Effect of pain on isometric TTF

The present study demonstrates that greater levels of pain in a fresh, undamaged, large locomotor muscle group significantly shortens TTF during an isometric endurance task. Indeed, TTF was significantly shorter in the 10% MVT + HYP condition than both the 10% MVT and 10% MVT + ISO conditions, with an impaired performance of 12 to 26% (n = 12). As all conditions were performed at the same intensity (10% MVT) and with participants in a similar psychological state, these differences in TTF can be attributed solely to increasing the experience of pain in the 10% MVT + HYP condition, as clearly shown in Figure 4b.

Previous research that has used hypertonic saline to induce muscle pain have predominantly applied it in smaller muscles or muscle groups (e.g. biceps brachii, tibialis anterior and gastrocnemius (Graven-Nielsen et al. 1997e; Ciubotariu et al. 2004; Khan et al. 2011)) and have not focused on producing a pain experience that feels like EIP. The VL is a large muscle with a key role in the generation of force during basic locomotor tasks (e.g. walking, stair climbing) and contributes to propulsive energy during cycling (Raasch et al. 1997), as well as the stance and swing phase in running (Sasaki and Neptune 2006). Understanding the effects of an increased overall pain experience in this muscle (and surrounding knee extensor group) at a contraction intensity utilised during cycling exercise (Löllgen et al. 1980) therefore provides information that closely translates to exercise performance and a clinical context. Care should however be taken when extrapolating findings to whole-body exercise or dynamic contraction.

During the impaired TTF performance in the 10% MVT + HYP condition, pain intensity was significantly elevated in the first 20% of the task, with a continued linear increase until task failure. Indeed, the intensity of pain reported in the 10% MVT + HYP condition was elevated by approximately 3.3 at 10% iso-time and 2.8 at 20% iso-time on the VAS scale. The hypertonic saline in the 10% MVT + HYP condition would have increased the activation of the group III and IV nociceptive

afferents *in addition* to the rapidly increasing noxious environment arising from the metabolites produced as a result of the exercise task (O'Connor and Cook 1999), which might explain the shorter TTF in the 10% MVT + HYP condition.

This explanation is in accordance with the "Sensory Tolerance Limit", where in open-loop exercise tasks (i.e. TTF) the increased inhibitory feedback from Group III and IV afferents contributes to an individual and task-specific threshold, which when reached the exercise is voluntarily terminated (Amann and Dempsey 2008; Amann 2011). With similar values for RPE and ROF between conditions it is likely the elevated pain intensity during the TTF at 10% MVT + HYP resulted in this sensory tolerance limit being reached sooner, causing a faster occurrence of task failure compared to the 10% MVT and 10% MVT + ISO conditions (Aboodarda et al. 2020).

In addition, the increased nociceptive activity (a specific type of afferent feedback) may have limited central motor drive and voluntary activation of the knee extensors (Amann et al. 2009, 2011a; Aboodarda et al. 2020), a notion which is supported by evidence showing a relationship between group III and IV muscle afferents and neuromuscular fatigue (Amann et al. 2015; Sidhu et al. 2018). In support of this, Henriksen and colleagues (Henriksen et al. 2011) reported a reduced capacity of the knee extensors to produce a MVT in the presence of pain. Furthermore, findings from Graven-Nielsen and colleagues (Graven-Nielsen et al. 2002) demonstrated that experimental muscle pain (from the hypertonic saline model) reduces MVT despite an unaffected twitch torque, implying that performance decrements were due to mechanisms residing in the central nervous system rather than the peripheral musculature (Graven-Nielsen et al. 2002).

Rather than a uniform inhibitory/facilitatory effect on agonist and antagonist muscle activity (Pain Adaptation Model, Lund et al. 1991), it is now recognised that pain does not cause uniform inhibition/excitation effects across the motor neurone pool, but instead causes a redistribution of activity within and between muscles (Hodges and Tucker 2011). Accordingly, the decreased performance caused by the overall increased pain experience in the current study could also be explained by a slight change in the direction of knee extensor torque to a more lateral/medial plane (Tucker and Hodges 2010). In this context, the gross feature of the task would remain (i.e.

knee extension), but the efficiency of this movement would be compromised. Motor unit recruitment order, or a recruitment of larger units at lower torques, could have also affected the task performance. In an endurance task lasting several minutes, the preferential recruitment of large high threshold motor units (which may include Type II muscle fibres) above low threshold small motor units (Type I muscle fibres) would likely have consequences for the rate at which fatigue occurs (both metabolic and neural), leading to a shorter TTF (Edwards 1981). Whilst not observed in the present study, an increase in sEMG would be indicative of an increased central drive to the muscle and/or an increased recruitment of high threshold motor units (Gerdle et al. 2000), which would be in-line with Hodges and Tucker's "moving differently in pain" theory (Hodges and Tucker 2011).

Methodological considerations

The methods used in this study preclude the ability to identify which, or combination of these mechanisms may have contributed to the shorter TTF. Indeed, combinations of peripheral nerve/transcranial stimulation, multiple force transducers, and fine wire electrodes would be required for this. In addition, the sensitivity of the sEMG set-up in the present study did not allow for the detection in non-uniform changes across the motor neurone pool (i.e. any alterations are unlikely to be discovered with bipolar sEMG). As such, an approach that allows for the identification of individual motor units would be more appropriate for the observation of subtle changes in activity within and between the muscles (i.e. high density EMG). Differential responses to pain between male and female participants are also acknowledged, with the present study not accounting for or attempting to control the menstrual cycle of the female participants. Indeed, hormonal changes across the different phases of the menstrual cycle may cause some difference in pain perception to experimental pain (Sherman and LeResche 2006).

Conclusion

The injection of hypertonic saline into the VL during a sustained low-intensity isometric contraction provides an overall qualitative experience of pain that feels like naturally occurring EIP induced by a higher intensity exercise. When applied to submaximal exercise, this additional pain caused a shorter TTF compared with a

placebo and control condition. It is plausible that the mechanisms responsible for the shorter TTF were related to increased activity of group III and IV nociceptive afferents from the injected muscle. The present study therefore provides important evidence that muscle pain has a direct impact on endurance performance.

Chapter 4 – Muscle pain from an intramuscular injection of hypertonic saline increases variability in knee extensor torque reproduction

Abstract

Purpose: The intensity of exercise-induced pain (EIP) reflects the metabolic environment in the exercising muscle, so during endurance exercise this may inform the intelligent regulation of work rate. Conversely, the acute debilitating effects of EIP on motor unit recruitment could impair the estimation of force produced by the muscle and impair judgement of current exercise intensity. This study investigated whether muscle pain, administered via intramuscular injection of hypertonic saline during isometric contraction, interferes with the ability to accurately reproduce torque in a muscle group relevant to locomotive exercise. **Methods**: On separate days, fourteen participants completed an isometric torque reproduction task of the knee extensors. Participants were required to produce torque at 15 and 20% maximal voluntary torque (MVT), without visual feedback before (Baseline), during (Pain/No Pain), and after (Recovery) receiving an injection 0.9% isotonic saline (ISO; Control) or 5.8% hypertonic saline (HYP; Experimental) into the vastus lateralis of the right leg. **Results:** An elevated reported intensity of pain, and a significantly increased variance in mean contraction torque at both 15% (P=0.049) and 20% (P=0.002) MVT was observed in the HYP compared to the ISO condition. Both 15 and 20% target torques were performed at a similar pain intensity in the HYP condition (15% MVT, 4.2 ± 1.9; 20% MVT, 4.5 ± 2.2; P>0.05). **Conclusion:** These findings demonstrate that the increased muscle pain from the injection of hypertonic saline impeded accurate reproduction of knee extensor torque. These findings have implications for the potential detrimental impact of EIP on exercise regulation and endurance performance.

Introduction

Exercise-induced pain (EIP) increases linearly with exercise intensity and duration (Cook et al. 1998), and has been suggested to provide useful sensory feedback about the relative strain of exercising muscles (O'Connor and Cook 1999; Carson et al. 2002; Mauger 2014). During intense and fatiguing muscle contractions, nociceptors of Group III and IV muscle afferents become sensitised and activated by many of the same metabolites implicated in peripheral fatigue (O'Connor and Cook 1999),

meaning EIP is often associated with other physiological and psychological factors of fatigue (Pollak et al. 2014).

Sensations of EIP may facilitate conscious control of homeostatic disturbance during exercise by enabling the intelligent regulation of available energetic resources (i.e. pacing) (Tucker 2009; Edwards and Polman 2013; Mauger 2014). However, the relationship between EIP and fatigue is likely more complex since it also causes various acute debilitating effects associated with motor unit recruitment (Hodges and Tucker 2011) and, as a protective mechanism, restricts movement to reduce pain. Consequentially, whilst EIP may provide insight about the metabolic environment in the exercising muscle, these potentially detrimental adaptations may reduce the accuracy of estimations of work done or force applied by the muscle, which could impair pacing decisions.

Supressing unpleasant sensations associated with intense exercise may allow a higher exercise-intensity to be tolerated and sustained (Mauger et al. 2010), however near complete removal this information via spinal afferent blockade appears to impair the exerciser's ability to select and maintain a physiologically optimal work rate (Amann et al. 2009). Spinal blockade studies show the importance of Group III and IV afferents to the performance of whole-body exercise (Amann et al. 2009, 2011a) but reveal less about the parallel effects of nociception and perceived pain on other systems such as cardiovascular control.

As identified in Chapter 3, intramuscular hypertonic saline injection produces similar muscle pain to that experienced during intense exercise (Graven-Nielsen et al. 1997e), and is therefore a useful method to investigate how EIP affects self-regulation of exercise intensity. This technique has previously been used in contralateral limb-matching tasks to assess the impact of tonic muscle pain on the judgement of torque in small muscle groups (Proske et al. 2003, 2004; Weerakkody et al. 2003). In these studies, increased pain impeded the ability to accurately match torque, with pain intensity and degree of error correlating such that participants consistently overestimated the force generated by the painful muscle.

This experimental approach could, however, be confounded by potential differences between the contralateral limbs (Philippou et al. 2010; Adamo et al. 2012). To provide a more translatable assessment of the impact of EIP on whole-body exercise, the relationship between muscle pain and the reproduction of isometric torque production should be evaluated in the larger muscle groups of the lower limb such as the knee extensors, which have an important role in the generation of force during locomotion and exercise.

As such, the aim of the present study was to ascertain whether experimentally induced muscle pain in the vastus lateralis (VL) using an intramuscular injection of hypertonic saline would affect the ability to accurately gauge the torque produced by the knee extensor muscles in a single-limb isometric torque reproduction task. We tested the hypothesis that experimental muscle pain in the VL reduces torque reproduction accuracy (as quantified by the variance in mismatch between target and actual torque) of low intensity isometric contractions when compared to a control condition.

Methods

Ethical Approval

All procedures and protocols were approved by the School of Sport and Exercises (University of Kent) Research Ethics Advisory Group (Prop 140_2016_17) in conformity with the Declaration of Helsinki, and its later amendments or comparable ethical standards. All participants were informed of the study experimental procedures, and written informed consent was obtained to confirm participation.

Participants

Fourteen healthy and recreationally active participants (13 male, 1 female; mean ± SD: age, 25.3 ± 4.5 years; height 1.78 ± 0.1 m; body mass 73.9 ± 12.3 kg; physical activity 5.6 ± 2.2 hours per week) volunteered to participate in the present study. Assuming a statistical power of 0.8 at an alpha level of 0.05, the sample size was estimated using G*Power software (Faul et al. 2007) based on the effect size reported in similar studies in our laboratory using hypertonic saline injections. Recruited participants were free from the exclusion criteria and attended the laboratory in accordance with the pre-requisites outlined in Chapter 3. All participants attended

each visit in a similar psychological state as assessed by the Positive and Negative Affect Schedule (PANAS) (Watson et al. 1988), which was completed at the start of each visit.

Experimental design

In a two-way repeated-measures experimental design, participants performed an isometric torque matching and reproduction task with either pain (a single intramuscular injection of hypertonic saline) or a placebo control (a single intramuscular injection of isotonic saline) (condition factor). Participants attended a familiarisation session, and then completed the experimental conditions in a randomised and counterbalanced order, with each visit separated by a minimum of seven days. During the task participants attempted to produce torque at two set targets without the aid of real-time visual feedback before (Baseline), during (Pain/No Pain), and after (Recovery) the induction of pain and no pain (time factor). Measures of torque, rating of perceived exertion (RPE), surface electromyography (sEMG) and heart rate (HR) were taken during each contraction. Pain intensity was recorded continuously through an electronic visual analogue scale (VAS) and pain quality through the completion of a McGill Pain Questionnaire (MPQ). A schematic of the experimental design and protocol is outlined in Figure 1.

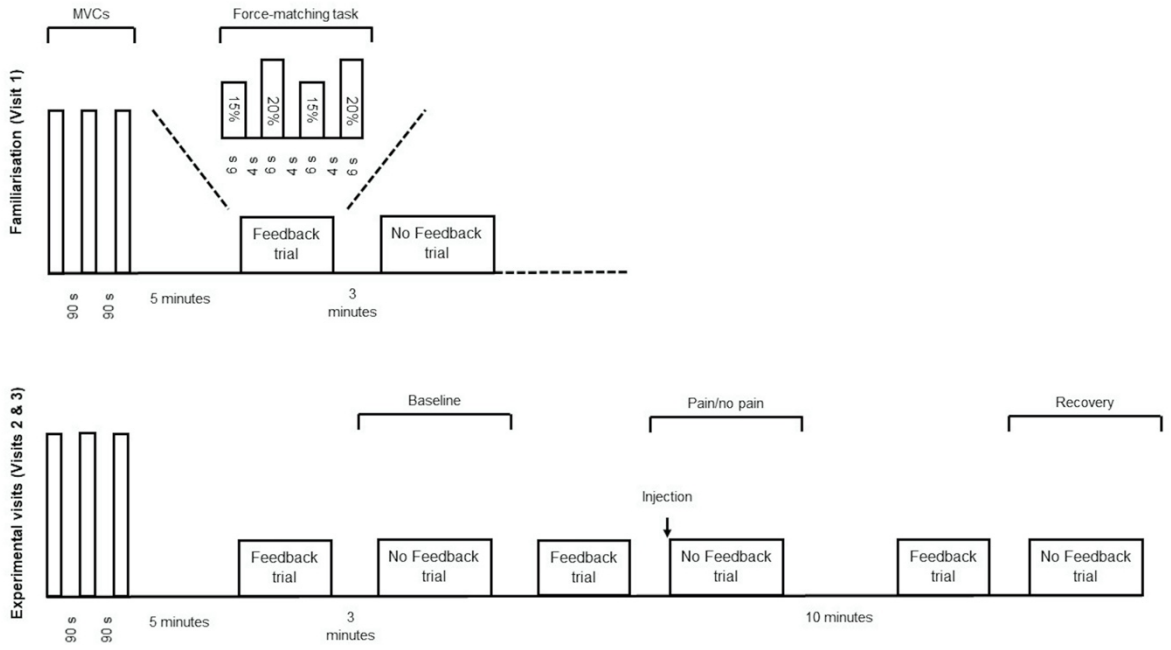


Fig 1. Schematic overview of the experimental design and procedures. MVCs: maximal voluntary contractions

Experimental Procedures

Torque matching and reproduction task

All visits were performed seated on an isokinetic dynamometer set up as detailed in Chapter 2. Torque matching and reproduction for knee extension were determined at isometric contractions of 15% and 20% maximal voluntary torque (MVT). These values were selected based on the percentage of MVT utilised during maximal (100% maximal oxygen uptake; VO_{2MAX}) and submaximal (70% VO_{2MAX}) cycling exercise performed at a pedal rate between 60-80 revolutions per minute (Löllgen et al. 1980). At the start of each visit, participants completed 3 × 3 s maximum voluntary contractions (MVCs) separated by 90 s rest, with the greatest instantaneous value taken as MVT.

Participants attempted the target torques in a trial with real-time torque-production visual feedback ("Feedback Trial") and a trial without visual feedback ("No Feedback Trial"). During the Feedback Trials, target torques (15% and 20% MVT) were presented with actual torque produced via a computer display. Participants were instructed to remember muscular sensations experienced during each target torque to

attempt to replicate these in the subsequent No Feedback Trial (Carson et al. 2002). All trials were separated by a 3-minute period of rest.

For each trial, participants performed four 6 s contractions separated by 4 s of rest in a randomised counter-balanced order, which provided two attempts at both target torques (i.e. 2 × 15% MVT, 2 × 20% MVT). During each contraction, participants were instructed to try and match the target torque within the first 2 s, and then maintain it for a further 4 s.

Intramuscular injection procedure

See Chapter 2 for a detailed description of the intramuscular injection procedure.

Visit 1 – Familiarisation

Participant anthropometric and descriptive measures of age, height, body mass, and hours of physical activity engaged in per week were recorded. Participants were then familiarised with the RPE and pain scales (Cook et al. 1997), as well as the performance of MVCs, and the Feedback/No Feedback Trials. Five minutes after the completion of the final MVC, participants performed an initial Feedback Trial followed by a No Feedback Trial. Verbal confirmation of the actual torque produced in each contraction was given after the completion of the trial. All four contractions in the No Feedback Trial were required to be within 10% of target torque, with further No Feedback Trials completed until this was satisfied. The visit concluded upon the successful completion of a No Feedback Trial or following ten unsuccessful trials.

Visits 2 & 3 – Experimental visits

All participants completed an ISO (isotonic saline; control) and a HYP (hypertonic saline; experimental) condition in a randomised and counterbalanced order. In each condition, five-minutes after the completion of the MVCs, participants completed six trials (Feedback, No Feedback, Feedback, No Feedback, Feedback, No Feedback). Prior to the second No Feedback Trial, participants received an intramuscular injection of either isotonic (ISO) or hypertonic saline (HYP), with the No Feedback Trial beginning 20 s after the removal of the needle. This ensured that the 15% and 20% MVT contractions in this No Feedback Trial were performed with a “moderate”

to “strong” muscle pain elicited from the painful hypertonic saline infusion. Ten minutes after the completion of this second No Feedback Trial, the final Feedback and No Feedback (Recovery) Trials were performed.

Perceptual and psychological measurements

At the start of each visit, participants were asked to rate pain expectations and confidence to cope with this expected level of pain. Pain was evaluated by intensity and quality. Participants rated pain intensity on a moment-to-moment basis using an electronic visual analogue scale (VAS) whilst the quality of pain was established by the long-form McGill Pain Questionnaire (MPQ) (Melzack 1975). Participants completed the MPQ after the second No Feedback Trial (when pain had subsided). In addition, upon the completion of each trial, participants provided an RPE, using the 15-point Borg (6-20) scale (Borg, 1998). More information on perceptual measurements can be viewed in Chapter 2.

Physiological measurements

Heart rate (HR) was recorded upon the completion of each individual contraction using a Polar FT1 HR monitor paired with a coded T34 transmitter (Polar, Polar Electro, Kempele, Finland). Muscle electrical activity was also continuously recorded using surface electromyography (sEMG) as detailed in Chapter 2.

Data analysis

The sEMG data were analysed using custom code written in MATLAB 2018a (The MathWorks, Massachusetts, USA).

Torque and error

Torque was recorded through Spike2 software (Cambridge Electronics Design (CED), Cambridge, UK). For each 6 s contraction, the torque produced over the last 4 s was averaged. The average of the actual torque produced for each 15% and 20% target each was used to define the error in participant torque reproduction. Error was defined as the difference between the required target torque and the actual torque produced and expressed as a percentage of MVT (i.e. actual torque of 17.5% MVT for the 15% MVT target would be equal to an error of 2.5% MVT). The pain on the VAS reported

for the corresponding contractions were also averaged for the two attempts at each target torque to provide a mean VAS value for each target torque.

Surface electromyography (sEMG)

A linear envelope representation of the data was created as detailed in Chapter 2. The amplitude each muscle was averaged over the final 4 s period of each 6 s contraction. These values were then normalised to the maximum amplitude of the prior MVCs (% MVC). For each trial, the sEMG activity was averaged for the two contractions performed at each target torque.

Statistical analysis

To compare reproduction error between the ISO and HYP conditions at the three time-points (Baseline, Pain/No Pain, and Recovery), a Levene's test was used to determine equality of variance for each normalised target torque (15% and 20% MVT). Changes in HR, RPE, and sEMG activity were evaluated using two-way Analysis of variance (ANOVA) with treatment factor with two fixed levels (ISO, HYP) and a repeated measures Time factor with two time-points (Baseline, Pain/No Pain). When an interaction effect was observed, follow-up paired samples t-tests were used to assess differences between conditions. Paired samples t-tests were also implemented to evaluate the differences between conditions for pain expectation and confidence, VAS scores, pre-test PANAS, and the change in torque produced in Baseline compared to the Pain/No Pain time-point. A Pearson Bivariate correlation was used to evaluate the correlation between torque error and VAS score reported during the Pain/No Pain contractions. Cohen's guidelines of 0.1 (small), 0.3 (medium) and greater than or equal to 0.5 (large) were used to indicate the strength of correlation.

All data was checked for the standard assumptions associated with the performance of the above statistical tests prior to analysis. Data that did not satisfy the Shapiro-Wilk test of normality ($P<0.05$) were logarithmically transformed. Results are presented as mean ± standard deviation (SD). Cohen's *d* and partial eta square (η_p^2) values are reported as measures of effect size. Statistical significance was accepted at an alpha

level of $P<0.05$. All statistical analysis was completed using SPSS Statistics v25.0 (SPSS, IBM, New York, USA).

Results

Experimental muscle pain

As shown in Table 1, paired samples t-tests revealed a significant difference in VAS pain data between the ISO and HYP conditions. The pain experienced in HYP was significantly greater in terms of the onset VAS pain reported, with a significantly longer time to peak, yet greater peak VAS pain compared to ISO. The reported VAS pain in HYP was also longer in duration, inducing a significantly greater mean VAS pain, equivalent to a "moderate" to "somewhat strong" muscle pain, and therefore producing a greater overall VAS pain area than ISO.

Table 2. Summary VAS pain data across the entire duration of the ISO and HYP conditions

	ISO	HYP	*P*
VAS mean	0.8 ± 1.0	3.1 ± 1.0**	<0.001
VAS peak	1.6 ± 2.2	5.7 ± 1.7**	<0.001
VAS onset	0.5 ± 0.8	1.7 ± 1.3*	0.012
VAS time to peak (s)	41 ± 29	71 ± 24*	0.020
VAS duration (s)	55 ± 56	233 ± 60**	<0.001
VAS area	86.3 ± 115.4	759.8 ± 325.6**	<0.001

Values are means ± SD. **Significant difference between ISO and HYP ($P < 0.001$). *Significant difference between ISO and HYP ($P < 0.05$). VAS scale 0 ("no pain") to 10 ("extremely intense pain")

The muscle pain experienced in the HYP condition was predominantly described in the sensory and evaluative dimensions of pain as "aching" (50% of participants), "throbbing" (43% of participants), "shooting" (36% of participants), "cramping" (36% of participants), "annoying" (36% of participants). This produced a mean Total

Pain Index of 14 ± 8, with an overall Present Pain Intensity of 2.1 ± 0.7 ("discomforting").

During the Pain/No Pain trial, a paired samples t-test revealed no significant difference (t_{13}=-0.9, P=0.366, $CI_{.95}$ -0.9, 0.3, d=0.1) in mean VAS between contractions performed at 15% MVT (4.2 ± 1.9) and 20% MVT (4.5 ± 2.2) in the HYP condition. Each of the two target torques in the Pain/No Pain trial was therefore completed at a similar intensity of muscle pain (Fig 2a. and Fig 2b.).

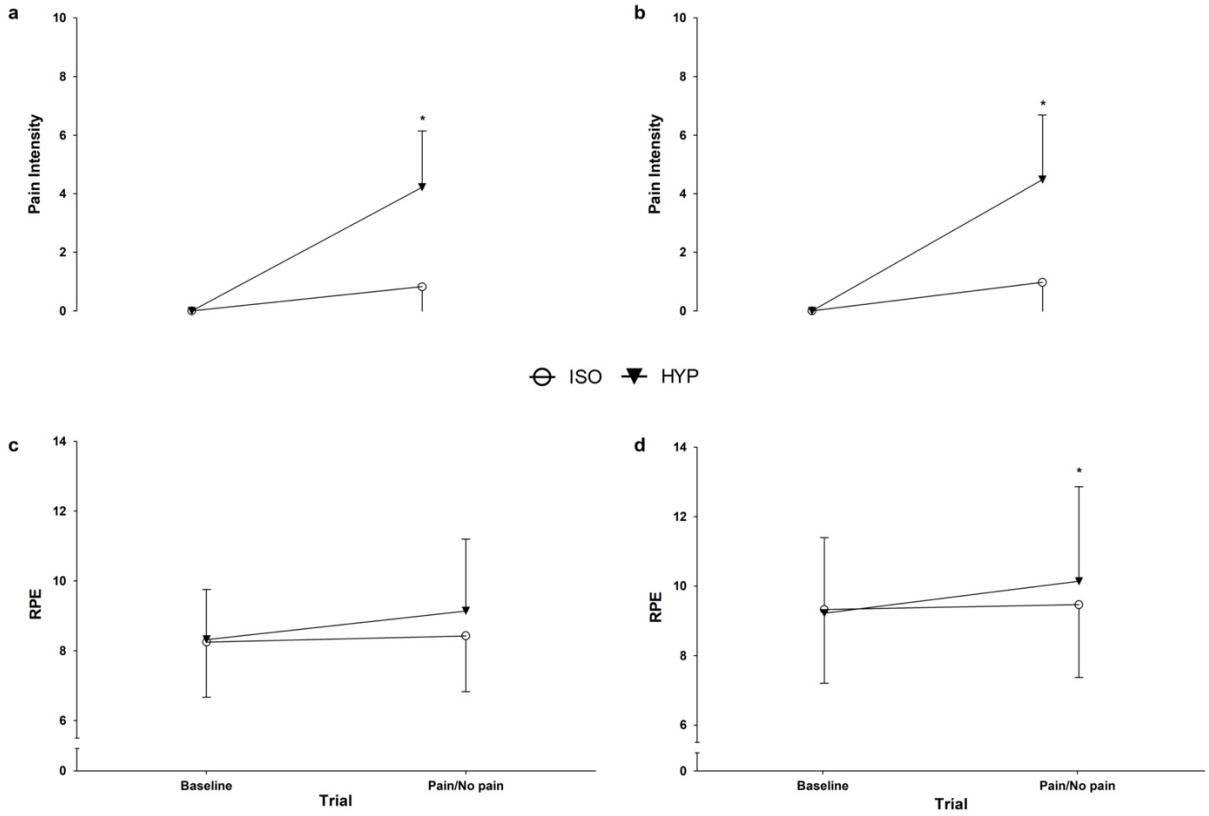


Fig 2. Perceptual differences between conditions (ISO and HYP) at Baseline and Pain/No Pain time-points. Differences in pain intensity at 15% MVT (a) and 20% MVT (b). Differences in RPE at 15% MVT (c) and 20% MVT (d). *Significantly greater where hypertonic saline was injected

Paired samples t tests revealed no significant difference (t_{13}=-1.8, P=0.096, $CI_{.95}$ -2.08, 0.19, d=0.5) in expectations of pain between the ISO (4.5 ± 2.1) and HYP (5.4 ± 1.8) conditions, with no significant differences in the confidence to cope with the

expected pain (t_{13}=0.2, P=0.818, $CI_{.95}$ -0.29, 0.37, d=0.1) between ISO (9.5 ± 1.0) and HYP (9.4 ± 1.0).

Comparisons of torque production accuracy

In the presence of greater levels of pain, participants demonstrated an increased variability in their ability to reproduce target torque without visual feedback. However, once the pain had subsided, participants were able to produce the target torque with the same accuracy as Baseline. This is demonstrated by the Levene test for equality of variance, which revealed a significant difference in the variance of mean contraction torque in the Pain/No Pain trial between the HYP and ISO conditions at both 15% MVT ($F_{1,26}$=4.3, P=0.049, d=0.6) and 20% MVT ($F_{1,26}$=12.0, P=0.002, d=1.0), as shown in Figures 3 and 4. There was no correlation between Pain/No Pain error and the pain intensity reported during the contractions (P>0.05). In addition, there was no significant difference in variance between conditions at the Baseline and Recovery time-points (P>0.05).

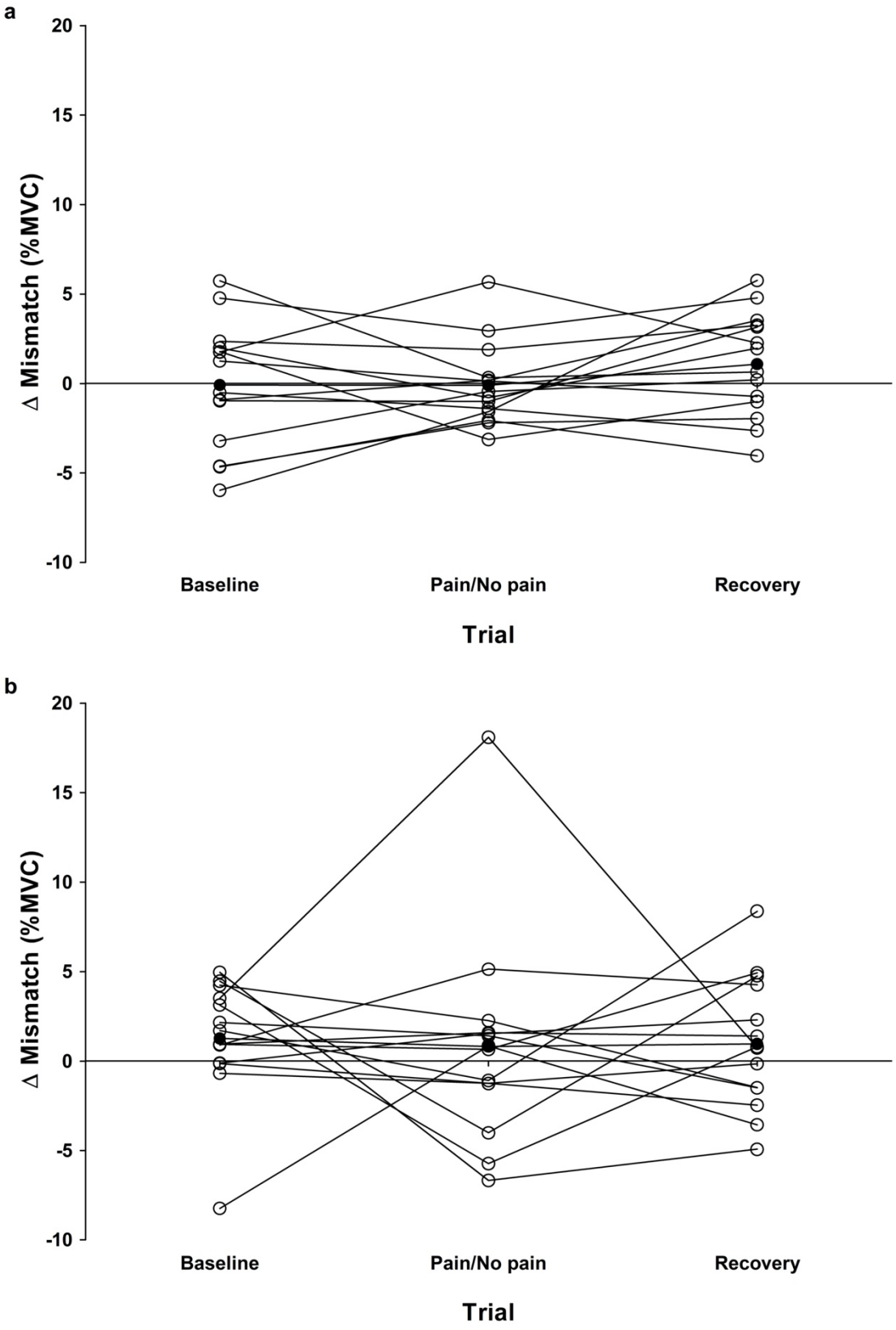
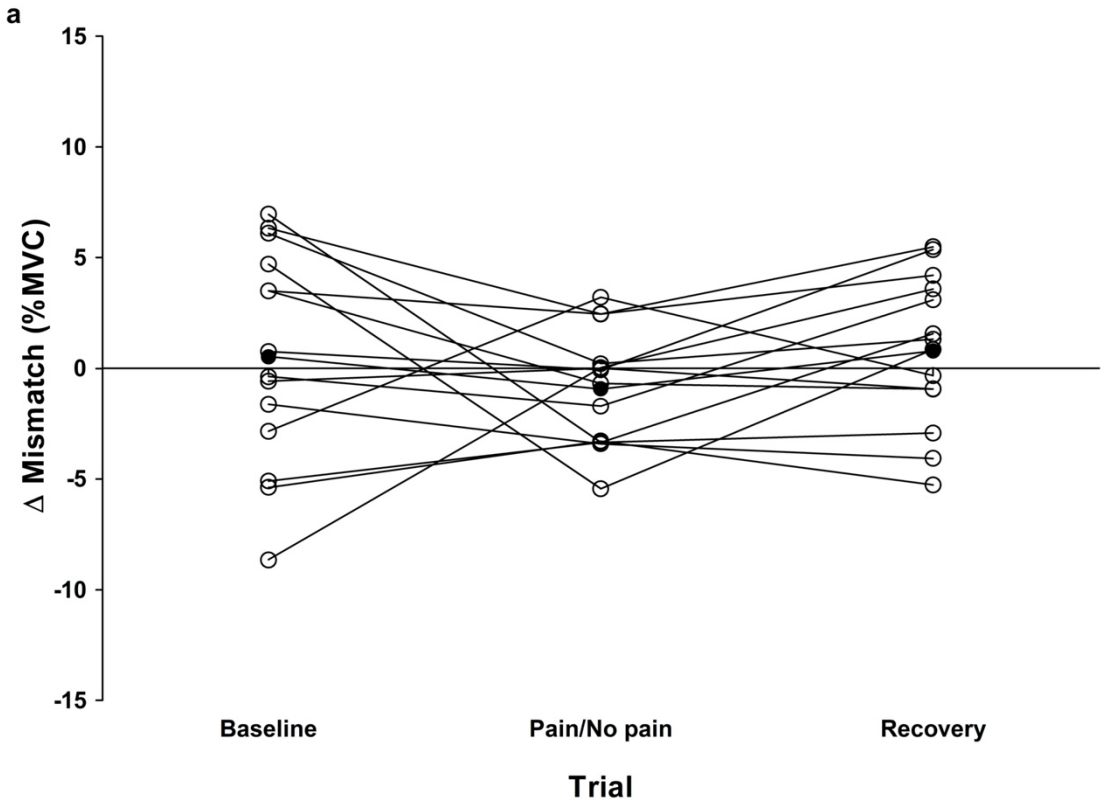
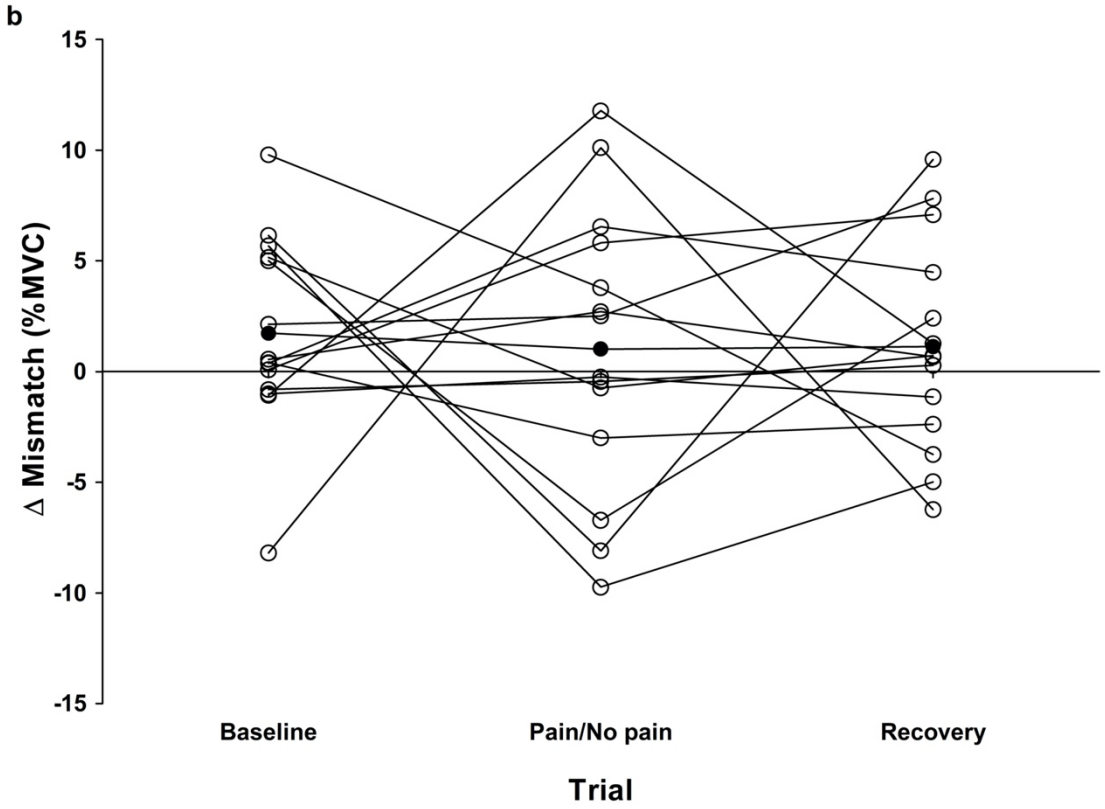


Fig 3. Individual (*open circle*) and mean (*filled circle*) **t**orque reproduction error at a target torque of 15% MVT before (Baseline), during (Pain/No Pain) and after (Recovery) injection of isotonic saline (ISO, *a*) or hypertonic saline (HYP, *b*).


a
15
10
5
0
-5
-10
-15
Δ Mismatch (%MVC)
Baseline
Pain/No pain
Recovery
Trial


b
15
10
5
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-5
-10
-15
Δ Mismatch (%MVC)
Baseline
Pain/No pain
Recovery
Trial

Fig 4. Individual (*open circle*) and mean (*filled circle*) torque reproduction error at a target torque of 20% MVT before (Baseline), during (Pain/No Pain) and after (Recovery) injection of isotonic saline (ISO, *a*) or hypertonic saline (HYP, *b*).

A paired samples t-test found no significant difference in the change in torque mismatch between Baseline and Pain/No Pain trials at 15% MVT (t_{13}=-1.5, P=0.169, $CI_{.95}$ -1.1, 0.2, d=0.5) when comparing the ISO (2.5 ± 1.7 %MVT) and HYP (4.8 ± 4.8 %MVT) conditions. Furthermore, the paired samples t-test highlighted no significant difference in the same change in torque mismatch between ISO (4.2 ± 3.5 %MVT) and HYP (7.4 ± 6.0 %MVT) when contractions were performed at 20% MVT (t_{13}=-1.3, P=0.235, $CI_{.95}$ -1.6, 0.4, d=0.4). This suggests that the target torque absolute error in the 'Pain/No Pain' was similar to the error made at Baseline despite the change in pain experienced.

Rating of perceived exertion

It was apparent that the effort experienced during the contraction was greater in the presence of increased pain, when performed at 20% MVT. The 2 × 2 (condition × trial) repeated measures ANOVA demonstrated a significant interaction effect at 20% MVT for RPE over trials between conditions ($F_{1,13}$=6.0, P=0.030, η_p^2=0.314). Follow-up paired samples t-tests revealed a significantly greater RPE (t_{13}=-2.3, P=0.038, $CI_{.95}$ -1.31, -0.04, d=0.3) in the Pain/No Pain trial in HYP compared to ISO. A significantly greater (t_{13}=-2.4, P=0.033, $CI_{.95}$ 0.1, 1.8, d=0.4) RPE was also reported in the HYP condition at the Pain/No Pain trial compared to the Baseline trial. No significant main effect of condition was observed at either 15 or 20% MVT (P>0.05). A significant effect of trial was reported at 20% MVT ($F_{1,13}$=5.2, P=0.041, η_p^2=0.284), but not at 15% MVT (P>0.05) (Fig. 2c. and Fig. 2d.). There was no interaction effect observed at 15% MVT (P>0.05).

Surface electromyography (sEMG)

Due to excessive noise in sEMG signal, two participants were removed from the dataset and analysis was performed on the remaining participants (n=12). Despite a greater variance in the mean contraction torque in the presence of muscle pain, there were no discernible alterations in activation of the agonist and synergist muscles. At

15 and 20% MVT, the performance of a 2 × 2 (condition × trial) repeated measures ANOVA demonstrated no significant main effect of condition or trial in either the VL, VM or RF (P>0.05). The VL, VM or RF also demonstrated no significant interaction effect for sEMG activity over trial between conditions at both target torques (P>0.05).

Heart rate (HR)

The 2 × 2 (condition × trial) repeated measures ANOVA revealed no significant main effect of condition at 15 or 20% MVT (P>0.05). At 15% MVT there was no significant main effect of trial (P>0.05), however there was at 20% MVT ($F_{1,13=}$5.2, P=0.041, η_p^2=0.284). No significant interaction effect for HR and trial between conditions was observed at 15 or 20% MVT (P>0.05).

Discussion

The present study demonstrates for the first time that the experience of muscle pain, administered by the intramuscular injection of hypertonic saline into the VL, resulted in a greater variance in the mean contraction torque at both 15 and 20% MVT when compared to the injection of isotonic saline (a placebo control). The increased variance was paralleled by an elevated experience of pain at both contraction intensities, and a greater perceived exertion when performed at 20% MVT. Once pain had subsided, accuracy of torque production returned to baseline levels. This study demonstrates that the presence of muscle pain impedes the ability to accurately reproduce torque in the knee extensors. This important finding provides key experimental evidence for the potential deleterious implications of EIP on the ability to self-regulate exercise intensity.

Effect of muscle pain on isometric torque reproduction

In the absence of visual feedback, and sole reliance on afferent/efferent information and task memory, the ability to accurately reproduce torque depreciates (Limonta et al. 2015). It is believed that the impairment in torque reproduction without the use of visual feedback is further accentuated from the activation of group III and IV muscle afferents and the associated experience of EIP (Proske and Gandevia 2012).

The purpose of the present study was to establish whether the presence of pain in a muscle with a major contributing role to force generation during both dynamic contractions and whole-body exercise (i.e. the VL) has a debilitative effect on producing a given torque using the ipsilateral knee extensor muscle group. The primary key finding from this study is that the mismatch between the actual torque produced and the target torque (when required to reproduce both 15 and 20% MVT) was significantly more variable with muscle pain, with no discernible direction of error (i.e. participants both under- and overshot the target torque). Resultantly, this study is the first to demonstrate that the experimental induction of pain in a large locomotor muscle group impaired the judgement of torque during an isometric reproduction task performed at an intensity of relevance to endurance exercise performance.

The compromised ability to accurately reproduce torque during muscle pain is in line with previous research that has implemented the hypertonic saline model in the elbow flexors to investigate the impact of pain on estimation error in a contralateral torque estimation task (Proske et al. 2003, 2004; Weerakkody et al. 2003). However, this prior literature has consistently reported that participants specifically *overestimated* the torque that is produced in the painful muscle, and therefore produced less torque than required. In contrast with lack of direction in error reported in the present study, this observed disparity could be due to potential differences in the limb evaluated (e.g. contralateral or ipsilateral). Alternatively, as the knee extensor muscles respond differently to exercise-induced fatigue (Vernillo et al. 2018), the muscle group tested (elbow flexor vs. knee extensors) should also be considered.

Proposed mechanisms

The presence of the hypertonic saline solution in addition to the short-duration muscle contraction creates a noxious environment within the skeletal musculature (O'Connor and Cook 1999), which results in an alteration in activity of both ascending metaborecptive and nociceptive group III and IV afferent fibers (Laursen et al. 1999). In this noxious environment, there are several neuromuscular mechanisms that, when acting in singularity or in combination, may provide an explanation for the impaired reproduction of torque in the present study.

Convergent projection from group III and IV afferents on common interneurons from group Ib proprioceptive afferents (Schomburg et al. 1999) provide information on muscle force (Gandevia and Burke 1992). As discussed by Salomoni and Graven-Nielsen (Salomoni and Graven-Nielsen 2012), the large variance in the mean contraction torque in the HYP condition could be a result of the spatial facilitation between these afferents interfering in the central interpretation of proprioceptive information essential for the accurate control of torque. A discrepancy between the centrally mediated judgement of torque and the actual afferent feedback from the periphery could therefore have resulted in the torque reproduction error.

In addition, the projection of the group III and IV afferents have an inhibitory effect on the central nervous system. The increased afferent feedback from the hypertonic saline may have limited motor cortical excitability, and reduced central motor drive and voluntary activation of the knee extensors (Gandevia 2001; Le Pera et al. 2001). In order to compensate for the hypertonic saline-induced impairment of motor cortex excitability, a greater effort is required to drive the limb to meet the required torque (Mulder et al. 2002; Proske and Gandevia 2012). As an outcome reflected in the present study, this could provide a possible explanation for some of the differences in actual and perceived torque produced. The findings from Proske and colleagues (Proske et al. 2004) where the matching of torque through effort resulted in an overshoot of the target torque, are in support of this explanation.

Despite the observed impairment in torque-reproduction performance, there was no change in the level of muscle activity assessed by sEMG. This is consistent with findings from the established literature into the implications of pain on muscle activity during submaximal isometric contractions, where a lack of marked changes in sEMG signal are observed (Graven-Nielsen et al. 1997e; Schulte et al. 2004; Salomoni and Graven-Nielsen 2012). Combined, these observations contradict the underpinning theory of the “Pain Adaptation Model” (Lund et al. 1991) where it is predicted that the presence of pain has a reliable inhibitory influence on agonist muscles, whilst simultaneously activating the antagonists.

Instead, the observations of the present study could, with caution, be in-line with the "moving differently in pain" model proposed by Hodges and Tucker (Hodges and Tucker 2011). This theory postulates that pain initiates a non-uniform effect across the motor neurone pool, causing a redistribution of activity between and within muscles to provide a key adaptive and protective function (through minimising the pain experienced and the prevention of additional injury or damage to the area in pain) during muscular contractions. Detection of these adaptations would require the use of fine-wire electrodes (Tucker and Hodges 2009) or high-density sEMG, as a combination of changes in order of motor unit activation or synchronisation can occur without alteration in amplitude of gross sEMG (Tucker et al. 2009).

Alternatively, the compromised ability to accurately reproduce torque could be due to the experience of muscle pain preventing some attentional focus on the task (Linton and Shaw 2011), making the task more challenging. It is plausible that the elevated intensity of muscle pain (induced by the injection of hypertonic saline), which was rated as "moderate" to "somewhat strong" in both target torques, provided a stimulus which was perceived as threatening. With some attentional resources focused on coping with the 'threat' of the noxious stimuli, attention may have been directed away from the task, which could have resulted in a compromised accuracy of torque reproduction (Eccleston and Crombez 1999); a notion supported by evidence from previous experimental work (Matre et al. 2002; Bennell et al. 2005). However, in the current study, there was no relationship between pain intensity and error, which indicates that the sensation of muscle pain alone was unlikely to have had a direct influence on task performance.

Overall, it is evident that the presence of muscle pain interferes with proprioception during submaximal isometric contractions in a single locomotive lower limb. The design and findings of the present study therefore provide a key indication of the potential detrimental effect that EIP may have on exercise intensity regulation and endurance performance. Some trepidation should however be taken when extrapolating these findings to whole-body exercise. In order to improve task relevance to whole-body locomotor exercise and further apply the findings of the present study, there is the need for the impact of this experimental model to be

evaluated during isokinetic or dynamic muscular contractions performed at a varying or higher work rate.

Conclusion

In conclusion, the injection of hypertonic saline into the VL during a torque reproduction task created a muscle pain that resulted in an impaired capacity to accurately produce a given submaximal target torque during a short, submaximal isometric contraction. The presence of muscle pain was linked with a greater effort to drive the limb and meet the given target torque when attempting to contract at 20% MVT, but not at 15% MVT. The compromised ability to reproduce torque returned to baseline levels once pain had subsided. These findings have implications for the impact of EIP on self-selected work rate regulation during endurance exercise performance.

Chapter 5 – Cardiorespiratory and perceptual response to acute unilateral and bilateral muscle pain induced by hypertonic saline

Abstract

Purpose: Hypertonic saline injected into an isolated muscle evokes an experience of muscle pain similar to exercise-induced pain (EIP) by activating Group III and IV afferents. However, as these afferent fibres also have a key role in the exercise pressor reflex, it is important to understand whether the intramuscular injection of hypertonic saline causes a cardiovascular response, as this may impact on endurance performance independently to the muscle pain. This study therefore investigated the cardiorespiratory and perceptual response to acute unilateral and bilateral experimental muscle pain induced by the hypertonic saline. **Methods:** On separate days, twelve participants received an intramuscular injection of hypertonic (pain) or isotonic saline (control) into the vastus lateralis of the right and left leg in three different combinations: bilateral pain (1) or unilateral pain in the dominant (2) or non-dominant (3) limb whilst seated at rest. Participants reported the global and individual pain intensity of both legs, whilst cardiorespiratory measures were also taken pre-, during and post-injection. **Results:** Bilateral hypertonic saline elicited a significantly greater ($P < 0.05$) global peak pain intensity (6.8 ± 1.6) compared with unilateral muscle pain, with no difference between dominant and non-dominant limbs. A main effect of time was observed for all cardiorespiratory measures ($P > 0.05$), observed regardless of whether the pain was unilateral or bilateral and was not correlated to the pain experience reported. **Conclusions:** The present study provides evidence that muscle pain induced through bilateral hypertonic saline is greater than that of unilateral muscle pain and does not directly evoke a significant cardiovascular response. These findings support the application of this pain-induction model during whole-body endurance exercise without evoking a cardiorespiratory response.

Introduction

From the onset of intense and prolonged exercise, group III and IV muscle afferents are activated in response to the homeostatic disturbance caused by alterations in the mechanical and metabolic milieu of the active muscle (O'Connor and Cook 1999). The nociceptive stimulus caused as part of this noxious environment ascends to the central nervous system and contributes to the subjective perception of 'exercise-

induced pain' (EIP) (Mauger 2013), which is proportional to both the intensity and duration of the exercise task (Cook et al. 1997).

Described as a "dull", "aching" and "throbbing" sensation (Henderson et al. 2006), EIP typically starts as a localised experience in the primary active muscle, and gradually diffuses to surrounding areas according to the exercise duration or intensity (Slapsinskaite et al. 2015). In addition to its role as a protective function, EIP is suggested to be an important indicator of cardiovascular and muscular strain during exercise (Mauger 2014), providing the exerciser with information regarding the progressive development of fatigue (Stevens et al. 2018).

To investigate the relationship between EIP and endurance performance, an intramuscular injection of hypertonic saline was used in Chapter 3. This method of experimental pain induction stimulate a similar nociceptive pathway to EIP and was shown to cause a standardised and reproducible experience of pain that was comparable to naturally occurring EIP (Chapter 3). This unilaterally induced muscle pain reduced performance of a single-limb isometric knee-extensor task (Chapter 3) and compromised the ability to accurately reproduce knee-extensor torque (Chapter 4). The studies in Chapters 3 and 4 have developed this pain induction method from smaller muscle groups (with limited relevance to locomotive exercise), towards an exercise performed in a muscle group and at a contraction intensity utilised during cycling exercise (Löllgen et al. 1980).

However, as most locomotive exercise elicits EIP bilaterally (Slapsinskaite et al. 2015), a bilateral application of hypertonic saline would more closely replicate the experience of EIP during whole body endurance exercise. Prior to the bilateral application of hypertonic saline during whole-body locomotive exercise (Chapter 6), it is however important to 1) ascertain whether there are any clear differences in pain response (intensity and quality) between the unilateral and bilateral pain induction, and importantly, 2) to ensure that this experimental model does not elicit any additional response that may confound the ability to assess the independent role of EIP on endurance performance.

Alongside the impact on central command (i.e. the feedforward component), sensory feedback from group III and IV muscle afferents (Kaufman and Forster 1996) have a key regulatory impact on the reflex cardiovascular and ventilator adjustments as a corollary to whole body exercise and muscular contraction (McCloskey and Mitchell 1972; Amann et al. 2010; Amann 2012b). Indeed, mechanical and metabolic stimulation of group III and IV afferent fibres arising from muscular contraction increases sympathetic nerve activity whilst simultaneously minimising parasympathetic nerve activity (Murphy et al. 2011). Consequently, from the initiation of muscular contraction, reflex increases in cardiac output, heart rate and blood pressure occur (Murphy et al. 2011). As highlighted in the work from Amann et al. (Amann et al. 2009), this "exercise pressor reflex" is fundamental to meet the given metabolic demand of exercise, ensuring a sufficient delivery of oxygen to the locomotor muscles (Amann and Kayser 2009). As such, there is likely a dual facilitative and limitative role of group III and IV muscle afferent feedback on endurance performance (Hureau et al. 2019), and by isolating and evaluating the cardiovascular response to acute bilateral muscle pain, its implications for endurance performance can be better understood.

Whilst the overall pain experience from a unilateral hypertonic saline injection into the VL at rest and during an isometric contraction has been quantified (Chapter 3), this is yet to be compared to a bilateral injection. This is important to consider because a bilateral injection could provide an additive effect on pain intensity (i.e. the overall intensity from the bilateral injection is equal to the sum of the two unilateral injections singularly), in a manner similar to sequential injections in separated sites of the same limb (Graven-Nielsen et al. 1997a). This would have consequences for the tolerability of the method. There may also be laterality differences in pain sensitivity between limbs, which could be underpinned by limb dominance. Indeed, prior research using different pain induction pain methods have highlighted differences in pain tolerance and intensity based on limb dominance in the hand (Pud et al. 2009), but this has not been evidenced in the lower limb.

Therefore, the primary aim of the current study was to investigate the cardiorespiratory response to acute unilateral and bilateral experimental muscle pain induced through an intramuscular injection of either isotonic saline (placebo) or

hypertonic saline (pain) in the right and left vastus lateralis at rest. The second aim of this study was to evaluate and compare the perceptual response to acute bilateral muscle pain in contrast with unilateral muscle pain at rest and determine the influence of limb dominancy on pain perception in the lower limb.

Methods

Ethical Approval

All procedures and protocols were approved by the School of Sport and Exercises (University of Kent) Research Ethics Advisory Group (Prop 9_2018_19) in conformity with the Declaration of Helsinki, and its later amendments or comparable ethical standards. All participants were informed of the study experimental procedures, and written informed consent was obtained to confirm participation.

Participants

Twelve participants (11 male, 1 female; mean ± SD: age, 26.7 ± 3.8 years; height 1.8 ± 0.1 m; body mass 77.1 ± 13.6 kg; physical activity 4.6 ± 1.6 $h \cdot w^{-1}$) volunteered to participate in the present study. This sample size was estimated using G*Power software (Faul et al. 2007) based on the effect size reported in a similar study examining single-limb muscle pain (Chapter 3), in order to satisfy the assumption of statistical power of 0.8 at an alpha level of 0.05. All participants were healthy and physically active. Recruited participants were free from the exclusion criteria and attended the laboratory in accordance with the pre-requisites outlined in Chapter 2.

Experimental overview

The present study consisted of three experimental visits to the laboratory, with each visit separated by a minimum of 7 days. At the start of the first visit, participants were provided with written instructions to familiarise themselves with the 10-point Cook scale (Cook et al. 1997), used to assess pain intensity. In addition, participants were familiarised with the self-report psychological measurements implemented in the present study (see *Self-reported psychological data*); Positive and Negative Affect Schedule (PANAS) and the modified Situation-Specific Pain Catastrophizing Scale (SPCS). The experimenter verbally confirmed understanding of the written instructions.

Anthropometric and descriptive measures of age, height, body mass and hours of physical activity engaged in per week were also recorded at the start of the first visit, as well as a self-estimation of leg dominance through the completion of a six-item questionnaire (Tsepis et al. 2004). The overall score from the leg dominance questionnaire determined whether the participant was categorised as left or right leg dominant, with the limbs termed dominant (DOM) or non-dominant (ND) thereafter.

For all visits participants were seated at rest and received an intramuscular injection of hypertonic saline (pain) or isotonic saline (control) into the vastus lateralis of both the right and left leg. The solutions were administered in three different combinations (1. hypertonic both legs [BIL pain]; 2. isotonic non-dominant leg, hypertonic dominant leg [DOM pain]; 3. hypertonic non-dominant leg, isotonic dominant leg [ND pain]) over the three visits in a randomised, single-blind and counterbalanced order. This elicited bilateral pain (BIL pain), or unilateral pain (i.e. DOM pain and ND pain). Participants were seated at rest for 3 min, followed by the intramuscular injection procedure performed over a 20 s window (10 s infusion period). Upon the completion of the injection procedure, the participants were asked to concentrate on and rate the pain experienced (see *Assessment of exercise-induced pain*) until the pain had dissipated and the participant had returned to the state of "no pain". Cardiorespiratory measures were also taken from the commencement of rest until the state of "no pain" (*see Cardiorespiratory measurements)*.

Intramuscular injection procedure

See Chapter 2 for a detailed description of the intramuscular injection procedure.

Cardiorespiratory measurements

Breath-by-breath ventilation and gas exchange values (minute ventilation (V_E), oxygen uptake (VO_2), carbon dioxide production (VCO_2) and respiratory exchange ratio (RER), end-tidal partial pressure of carbon dioxide ($PETCO_2$) and ratio of pulmonary ventilation to carbon dioxide output (V_E/VCO_2)) were recorded through online gas analysis (Cortex Metalyser 3B, Cortex GmbH, Lepzig, Germany). In addition, measurements of heart rate (HR), stroke volume (SV) and cardiac output

(CO) were estimated using a non-invasive bioimpedance cardiography device (Physioflow PF05L1, Manatec, Petit-Ebersviller, France). The Physioflow required a total of six spot electrodes (Ag/AgCl, Skintact FS-50C; Leonhard Lang GmbH, Innsbruck, Austria) to be positioned on the thorax: two on the supraclavicular fossa on the left lateral base of the neck (Z_1 and Z_2), another two on the mid-point of the spine level with the xiphoid process (Z_3 and Z_4), one in the V_1 location on the chest, with another at the rib closest to V_6. The skin where the electrodes were placed were shaved and cleaned with an alcohol swab prior to application to minimise impedance. Both devices were calibrated before each visit in line with manufacturer instructions.

Analysis of the cardiorespiratory measures focused on three set phases: the initial 3 min of rest (0 to 3 min, "Baseline"), the preparation and administration of the intramuscular injections (3 to 6 min, "Injection"), and 40 s after the injection of the respective solutions for a further 3 min (6 to 9 min, "Solution") (Fig 1).

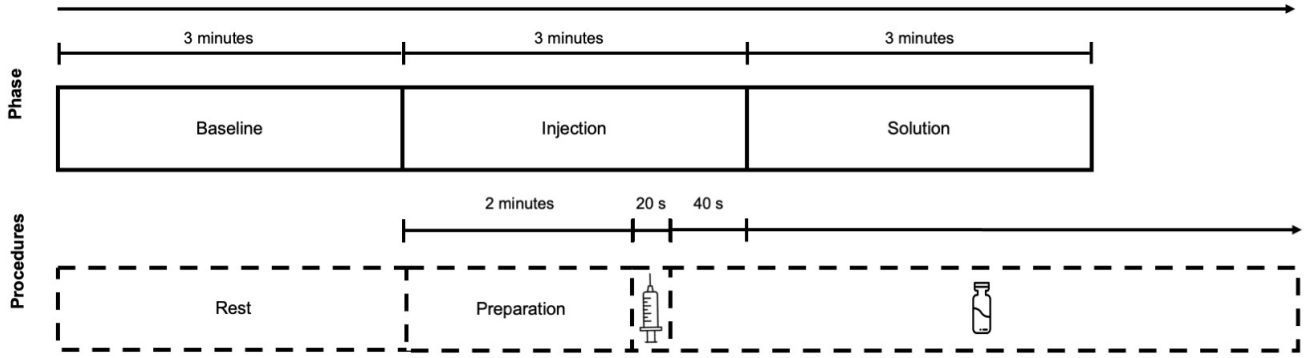

Fig 1. Schematic overview of the three phases, and the procedures within each phase. "Preparation" consists of the experimenter preparation of the injection and injection sites; Syringe icon represents the intramuscular injection procedure (from needle insertion to needle removal); Vial icon represents the action of the solution within the muscle

Perceptual and psychological measurements

Assessment of exercise-induced pain

During all visits, the muscle pain experienced was rated in terms of intensity and quality. Upon completion of the intramuscular injection procedure, participants continuously recorded the global pain intensity of both the right and left leg on a moment-to-moment basis using an electronic visual analogue scale (VAS). In

addition, every 60 s after the completion of the injection, participants were prompted to verbally rate the EIP intensity of the right and left leg individually using the same scale. The area under the curve for each leg was then calculated from these values (individual EIP area). The global quality of muscle pain was established by the long-form McGill Pain Questionnaire (MPQ) (Melzack 1975). More information on perceptual measurements can be viewed in Chapter 2.

Self-reported psychological data

At the start of each visit, the PANAS was completed, asking participants to indicate feelings "at the present moment". In addition, at the start of each visit, participants were asked to rate pain expectations and confidence to cope with this expected level of pain. Immediately after the return to "no pain" on the VAS, participants completed the modified SPCS (Edwards et al. 2006) to indicate the occurrence of catastrophizing specifically during the painful experience. More information on these measurements can be viewed in Chapter 2.

Statistical analysis

Results are presented in the form of mean ± standard deviation (SD). Prior to the statistical analysis, the standard assumptions associated with a paired samples *t* test, a one-way ANOVA and a repeated measures ANOVA were confirmed as appropriate. Any data that did not satisfy the Shapiro-Wilk test of normality ($P < 0.05$) were transformed logarithmically (sensory dimension and Total Pain Rating Index, negative affect) or by square root (affective, evaluative and miscellaneous dimensions, SPCS). Cohen's *d* and partial eta square (η_p^2) values are reported as measures of effect size. Pearson bivariate correlations were used to evaluate the correlation between parameters, with Cohen's guidelines of 0.1 (small), 0.3 (medium) and greater than or equal to 0.5 (large) to quantify the strength of correlation.

Changes in the cardiorespiratory measures were assessed through a two-way ANOVA with treatment factor with three fixed levels (DOM pain, ND pain, BIL pain) and a repeated measures time factor with three time-points (Baseline, Injection, Solution). Due to the variation in VAS duration between participants, global pain data was formatted and then compared through the percentage of iso-time approach (Nicolò et

al. 2019), which has been implemented in a prior study (Chapter 3). This method of data formatting provides a consistent number of data points over time for all participants whilst still including a majority of the time-series data, allowing for between-subject comparisons of global pain intensity across differing VAS durations. Subsequently, a two-way ANOVA with treatment factor with 3 fixed levels (DOM pain, ND pain, BIL pain) and repeated measures time factor with 10 time-points was used to assess the effect of condition and time on pain intensity. Two-way ANOVAs with a Treatment factor with 3 fixed levels (DOM pain, ND pain, BIL pain) and a repeated measures factor of leg (Left, Right) were applied for individual EIP area, and an ANOVA was implemented to evaluate differences between condition for pain expectation and confidence, VAS summary scores, pre-test PANAS and SPCS.

When an interaction effect was observed, follow-up paired samples *t* tests were employed to indicate differences between conditions, with a Bonferroni post-hoc correction applied where appropriate. Statistical significance was accepted at an alpha level of $P < 0.05$ except where a Bonferroni correction was applied (adjusted, $P < 0.0042$). All statistics were performed using SPSS Statistics v25.0 (SPSS, IBM, New York, USA).

Results

Cardiorespiratory response

The 3 × 3 (condition × time) repeated measures ANOVA demonstrated no significant main effect of condition for any cardiorespiratory measure ($P > 0.05$). There was a significant main effect of time ($P < 0.05$). As shown in Figure 2, CO increased during the “Injection” phase in comparison to “Baseline” ($P = 0.033$), and then dropped below “Baseline” ($P = 0.017$) and “Injection” ($P = 0.010$) during the “Solution” time-point. These changes appear to be in part due to variations in HR, which also increased during “Injection” compared to “Baseline” ($P < 0.001$), but then returned to similar “Baseline” values during “Solution” ($P = 0.619$) (Fig. 2a.). Stroke volume (SV) significantly declined during “Solution” compared to both “Baseline” ($P = 0.026$) and “Injection” ($P = 0.039$) (Fig. 2b.).

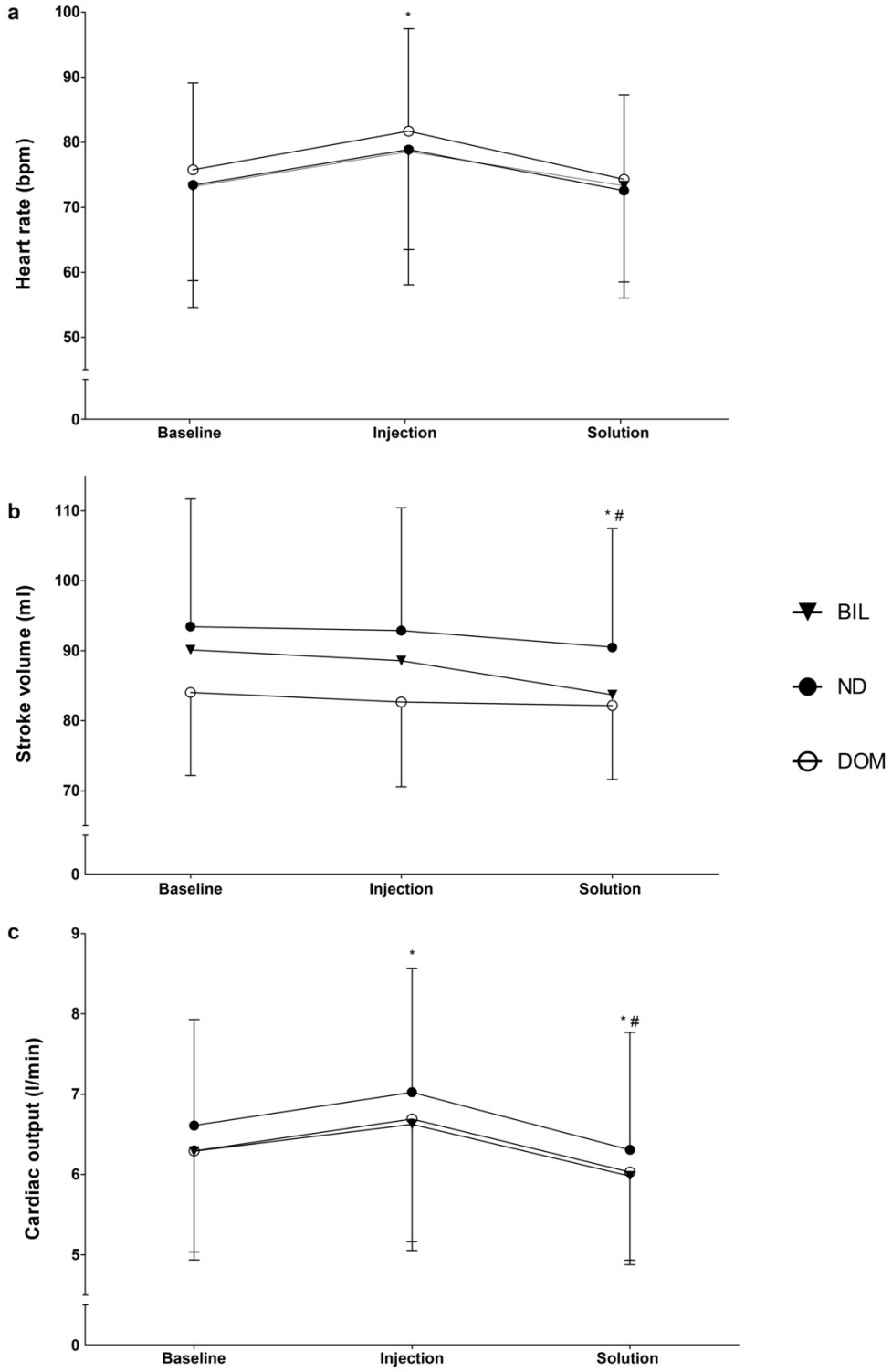

Fig 2. Cardiovascular measures at rest (Baseline), and in response to the preparation and administration of the bilateral intramuscular injection (Injection) and the subsequent action of the respective solutions (Solution) between conditions (DOM pain, ND pain and BIL pain). Changes in heart rate (HR; a), stroke volume (SV; b)

and cardiac output (CO; c). Values are presented as mean ± SD. *Significant difference from Baseline ($P < 0.05$). #Significant difference from Injection ($P < 0.05$)

As shown in Figures 3a-d, there was a significant increase from "Baseline" to "Injection" in V_E ($P = 0.002$), VO_2 ($P = 0.033$), VCO_2 ($P = 0.006$) and RER ($P = 0.002$), with all four variables then returning to statistically similar baseline values during the "Solution" phase ($P > 0.05$). There was a significant decline in $PETCO_2$ during the "Solution" phase in comparison to the Baseline' ($P = 0.036$) and "Injection" ($P = 0.007$) phases (Fig. 3e.), whilst VE/VCO_2 was significantly elevated during "Solution" compared to "Injection" ($P = 0.028$) (Fig. 3f). There was no significant interaction effect for any cardiorespiratory measure and time between conditions ($P > 0.05$). There was no correlation ($P > 0.05$) with the change in any cardiorespiratory measure and the change in reported VAS during the "Injection" and "Solution" timeframes. Cardiorespiratory (HR, V_E, CO) and global pain intensity data of a representative participant for all three conditions is depicted in Figure 4.

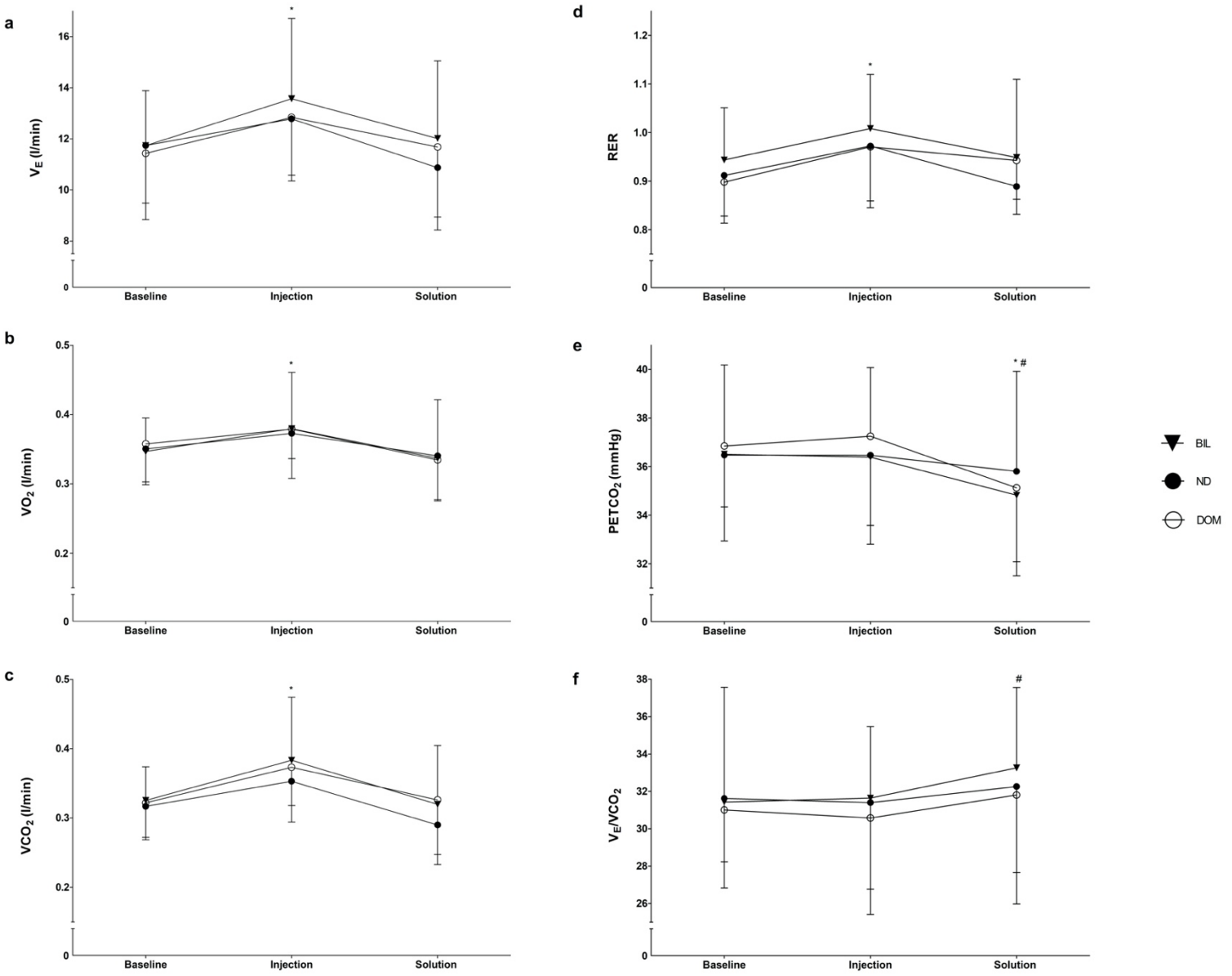

Fig 3. Ventilation and gas exchange measures at rest (Baseline), and in response to the preparation and administration of the bilateral intramuscular injection (Injection) and the subsequent action of the respective solutions (Solution) between conditions (DOM pain, ND pain and BIL pain). Changes in minute ventilation (V_E; a), oxygen uptake (VO_2; b), carbon dioxide production (VCO_2; c), respiratory exchange ratio (RER; d), end-tidal partial pressure of carbon dioxide ($PETCO_2$; e) and ratio of pulmonary ventilation to carbon dioxide output (V_E/VCO_2; f). Values are presented as mean ± SD. *Significant difference from Baseline ($P < 0.05$). #Significant difference from Injection ($P < 0.05$)

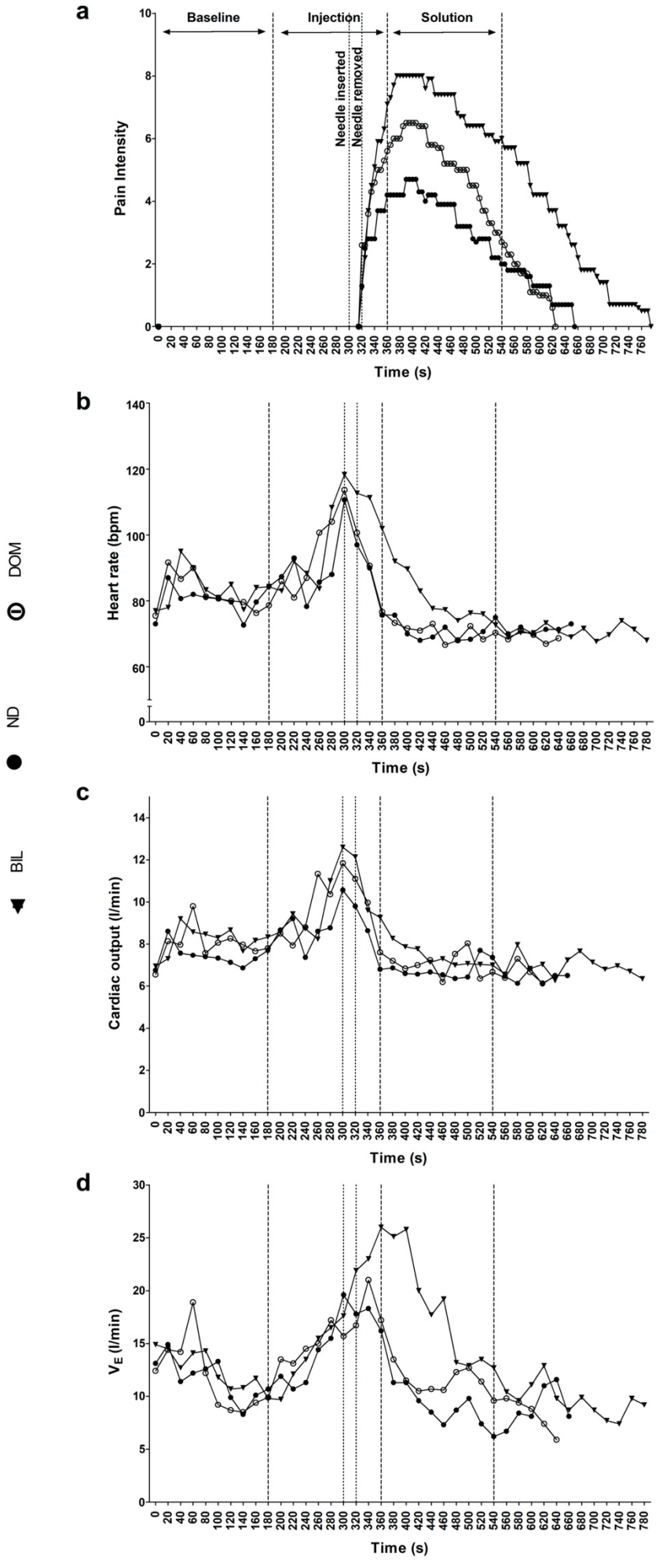
a
Baseline
Injection
Solution
Needle inserted
Needle removed
Pain Intensity
Time (s)
b
Heart rate (bpm)
Time (s)
c
Cardiac output (l/min)
Time (s)
d
V_E (l/min)
Time (s)
DOM
ND
BIL

Fig 4. Changes in pain intensity (a) and cardiorespiratory measures of heart rate (HR; b), cardiac output (CO; c) and minute ventilation (V_E; d) during unilateral (DOM, ND) and bilateral (BIL) experimental muscle pain for a representative participant. Markers are presented for each phase of the experimental procedures ("Baseline", "Injection" and "Solution"), which each phase including specific researcher and participant procedures. At "Baseline" participants were quietly seated at rest, with the researchers stationary. During "Injection", participants remained seated whilst the researchers moved around the laboratory and commenced preparation of both the injection and injection sites (first two mins), followed by the simultaneous administration of the injection ("needle inserted") over a 20 s window. Upon completion of the injection ("needle removal") participants remained quietly seated, and were asked to concentrate on, and rate the pain experienced until the return to a state of no pain. The last 40 s of the "Injection" phase captures the initial response, whilst the "Solution" phase analyses a further 3 mins.

Muscle pain

Global pain

The bilateral intramuscular injection of hypertonic saline in both the right and left VL resulted in a significantly greater experience of experimental muscle pain than that induced by unilateral hypertonic saline ($P < 0.05$). As shown in Table 1, an ANOVA revealed a significant difference in peak VAS ($F_{1.36,14.99} = 5.1$, $P = 0.031$, $\eta_p^2 = 0.316$), mean VAS ($F_{2,22} = 3.6$, $P = 0.046$, $\eta_p^2 = 0.599$) and VAS area ($F_{2,22} = 4.2$, $P = 0.029$, $\eta_p^2 = 0.674$). Follow-up paired-samples *t* tests showed that BIL induced a significantly elevated peak VAS, between "strong" and "very strong" muscle pain, compared to DOM ($t_{11} = -2.8$, $P = 0.016$, CI_{95} -1.77, -0.23, $d = 0.6$) and ND ($t_{11} = -3.2$, $P = 0.009$, CI_{95} -3.00, -0.53, $d = 1.0$), with no significant differences observed between DOM and ND ($P = 0.296$). Mean pain was significantly greater in BIL compared to ND ($t_{11} = -2.8$, $P = 0.016$, CI_{95} -1.53, -0.20, $d = 1.0$), with no significant difference between DOM and ND ($P = 0.221$), and DOM and BIL ($P = 0.207$). A greater VAS area was demonstrated in BIL compared to DOM ($t_{11} = -2.4$, $P = 0.036$, CI_{95} -565.98, -23.91, $d = 0.6$), and ND ($t_{11} = -2.5$, $P = 0.028$, CI_{95} -899.18, -61.45, $d = 0.9$). No significant difference in VAS area was demonstrated between DOM and ND ($P = 0.329$).

Table 1. Differences in VAS scores between unilateral and bilateral experimental muscle pain

	DOM	ND	BIL
VAS mean	3.3 ± 0.9	2.8 ± 0.9	3.7 ± 0.9*
VAS peak	5.8 ± 1.8	5.0 ± 1.8	6.8 ± 1.6**
VAS onset intensity	1.9 ± 1.4	1.2 ± 0.6	2.3 ± 1.7
VAS time to peak (s)	86 ± 47	90 ± 45	90 ± 45
VAS duration (s)	340 ± 82	330 ± 74	400 ± 180
VAS area	1169 ± 400	983 ± 419	1463 ± 576**

Values are means ± SD. *Significant difference between BIL and ND condition ($P < 0.05$). **Significantly greater in BIL condition compared to both DOM and ND conditions ($P < 0.05$).

The 3 x 10 (condition x iso-time) repeated measures ANOVA highlighted a significant effect of condition ($F_{2,22} = 4.3$, $P = 0.027$, $\eta_p^2 = 0.279$) and iso-time ($F_{1.86,20.47} = 38.5$, $P < 0.001$, $\eta_p^2 = 0.778$). As shown in Figure 5, a significant interaction effect for pain over iso-time between conditions was observed ($F_{18,198} = 2.1$, $P = 0.009$, $\eta_p^2 = 0.158$), however follow-up targeted paired-sample t tests with a Bonferroni correction ($P < 0.0042$) revealed no significant differences at any iso-time point between conditions.

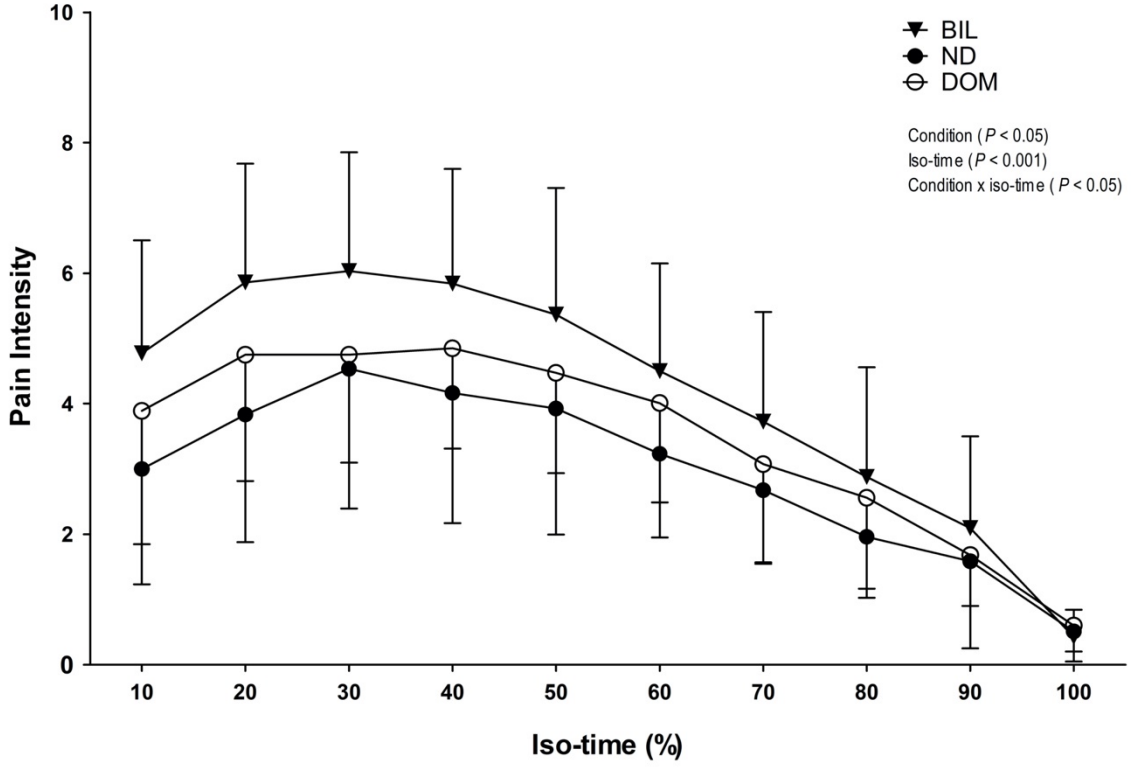

Fig 5. Change in pain intensity over iso-time between conditions. Values are presented as mean ± SD.

As shown in Table 2, the subjective quality of muscle pain experienced was similar in all three conditions in terms of the sensory ($P = 0.064$), evaluative ($P = 0.549$) and miscellaneous ($P = 0.088$) dimensions, however there was a significant difference between conditions for the affective classification ($F_{1.51,16.57} = 4.0$, $P = 0.049$, $\eta_p^2 = 0.264$). Whilst BIL added an affective descriptor ("tiring") follow-up paired samples *t* tests revealed no significant differences ($P < 0.05$). There was no significant difference between conditions for Total Pain Index ($P = 0.065$).

Table 2. Frequently selected words from the MPQ subclasses and Total Pain Index score

		DOM	ND	BIL
MPQ	Sensory	Cramping (42%) Aching (83%)	Throbbing (33%) Cramping (42%) Dull (33%) Aching (58%)	Throbbing (50%) Sharp (33%) Cramping (50%) Aching (50%)

Affective			Tiring (33%)
Evaluative	Annoying (42%)		Intense (42%)
Miscellaneous		Tight (33%)	
Other	Continuous (33%)		
	Steady (33%)	Steady (50%)	Steady (50%)
PRI(T)	17 ± 9	13 ± 10	20 ± 15

The frequently selected words from the MPQ are shown with the percentage of participants (n = 12) that selected these words. Data on Pain Rating Index (Total) presented as Mean ± SD.

Individual limb pain

The 3 × 2 (condition × leg) repeated measures ANOVA revealed a significant main effect of condition ($F_{2,22}$ = 18.1, P < 0.001, η_p^2 = 0.622), but no significant main effect of Leg ($F_{1,11}$ = 0.5, P = 0.508, η_p^2 = 0.041). An interaction effect for individual limb pain area for Leg between conditions was observed ($F_{2,22}$ = 48.7, P < 0.001, η_p^2 = 0.816). As expected, the follow-up paired samples t tests reported significant differences in pain between limbs when one was injected with hypertonic saline and the other with isotonic saline (P < 0.001), and there was no difference in pain between DOM and ND when these limbs received hypertonic saline (P = 0.183) and isotonic saline (P = 0.674). There was also no difference in the pain experienced in the dominant limb between DOM and BIL conditions (P = 0.890), or in the non-dominant limb between ND and BIL conditions (P = 0.054). In BIL there was no significant difference in pain between the dominant and non-dominant limb (P = 0.203) (Table 3).

Table 3. Differences in dominant and non-dominant limb pain area between conditions

Limb	Condition		
	DOM	ND	BIL
Non-dominant	15 ± 32	791 ± 398	1192 ± 630
Dominant	1028 ± 454	13 ± 23	1051 ± 600

Values are means ± SD. DOM, hypertonic saline in the dominant limb, isotonic saline in the non-dominant limb; ND, hypertonic saline in the non-dominant limb, isotonic saline in the dominant limb; BIL, hypertonic saline in both limbs.

Participant psychological characteristics

Participant expectations of pain ($F_{2,22}$ = 2.3, P = 0.123, η_p^2 = 0.174) and the confidence to cope with the expected pain ($F_{1.21,13.35}$ = 0.6, P = 0.473, η_p^2 = 0.054) was not significantly different between conditions. In addition, an ANOVA demonstrated no significant difference between conditions for participant psychological state in terms of positive ($F_{1.28,14.05}$ = 0.1, P = 0.806, η_p^2 = 0.010) and negative ($F_{1.03,11.36}$ = 0.1, P = 0.796, η_p^2 = 0.007) affect, and pain catastrophizing ($F_{1.33,14.62}$ = 1.0, P = 0.351, η_p^2 = 0.086).

Discussion

The primary aim of the present study was to evaluate the cardiovascular response to acute experimental muscle pain induced through the intramuscular injection of hypertonic saline. The hypertonic saline was administered into the VL of the dominant and non-dominant limb both individually (unilateral muscle pain) and concurrently (bilateral muscle pain) during resting conditions, with the cardiorespiratory response alongside global and between-limb perceptual differences of pain intensity examined.

This experimental approach reveals that the preparation and subsequent administration of concurrent intramuscular injections resulted in an increased cardiorespiratory response (i.e. increases in HR, CO, V_E, VO_2, VCO_2 and RER) compared to the initial baseline period. A significant decrease in SV and $PETCO_2$ and increase in V_E/VCO2 were also observed during the action of the solutions once the

injection procedure had been complete. These changes were observed regardless of whether the experimental pain experienced was unilateral (hypertonic saline in one leg, and isotonic saline in the opposing limb) or bilateral (hypertonic saline in both the right and left leg) and were not correlated to the change in muscle pain experienced. Based on the suggestion that the reflex cardiorespiratory response is dependent on the total amount of sensory input from the skeletal muscle (Leshnower et al. 2001), this interesting finding, where an augmented cardiorespiratory response is not observed concomitant with the greater magnitude of sensory feedback (i.e. BIL), it therefore does not support the occurrence of a potential reflex pressor response. Instead, this is suggestive of an alternate mechanistic explanation, with the possibility that the cardiorespiratory response was resultant from the pain-induction preparatory procedures opposed to a direct effect of the saline solution itself.

Cardiovascular response to acute unilateral and bilateral muscle pain

Intramuscular injection of hypertonic saline predominantly activates group IV afferents, with a contributory role of group III afferents (Laursen et al. 1999). When stimulated, mechanosensitive and metabosensitive group III and IV afferents have a dual role in the transmission of nociceptive information (O'Connor and Cook 1999) and as a sensory moderator of the reflex effect on the cardiorespiratory system (Dempsey 2012). Therefore, to provide a robust evaluation of the isolated role of EIP on endurance performance, it is important to clarify whether the noxious stimulus induced by hypertonic saline also evokes a significant confounding reflex cardiorespiratory response. In the present study, resting cardiovascular and ventilator changes were evident in response to both the preparation and administration of the intramuscular injections, but this occurred irrespective of whether the pain was unilateral/bilateral, or of the intensity and distribution of the reported muscle pain.

In all conditions, during the "Injection" phase cardiac output significantly increased but then declined below the "Baseline" value during the "Solution" phase. The initial increase in cardiac output was likely a product of the ~7% mean increase in heart rate at the point of injection, after which it returned to a rate similar to "Baseline" at "Solution". A decline in stroke volume was observed during the "Solution" phase causing cardiac output to drop below "Baseline". The heart rate and cardiac output

response did not parallel changes in the VAS profile. This implies that the cardiovascular response was more likely an anticipatory response to the upcoming painful stimuli (Colloca et al. 2006) similar to that previously observed after subcutaneous injection of ascorbic acid and isotonic saline (Porro et al. 2002, 2003), rather than a physiological response arising from the pain created.

In support of this, findings from Burton and Colleagues (Burton et al. 2009) reported a small and momentary increase in heart rate upon the onset of pain after hypertonic saline was injected into a smaller muscle (tibialis anterior), with the heart rate then returning to baseline levels within 60 s, preceding the incidence of peak pain (111 ± 17 s).).A similar response was also observed after the induction of cutaneous pain using the same solution. The finding of heart rate returning to normal resting measures before peak pain occurred, and then subsequently remaining at this baseline level despite the continued presence of pain infers that the transient cardiovascular change was in response to psychological stress associated with the injection procedures (opposed to the pressor response to the saline solution). It is interesting to note that the authors also attributed the observed response to psychological arousal as opposed to nociceptive processing (Porro et al. 2003). Our findings of no significant difference in cardiorespiratory measures between conditions despite a greater nociceptive (afferent) signal in the BIL compared to the unilateral (DOM and ND) pain conditions reinforces this notion.

In the present study, participants were blinded to the overall time-elapsed and were not provided with a formal cue regarding the impending noxious stimulus. Despite this, it is likely that there was an awareness of the approximate time-point at which the experimental procedures would commence based on experimenter activity related to preparing the injections (i.e. assembling the syringe and needle, drawing up the solution and cleaning the injection sites) and a knowledge that the injections would cause pain in one or both legs. This awareness and expectation may have led to feelings of fear and anxiety (Rhudy and Meagher 2000; Wiech and Tracey 2009; Reicherts et al. 2017), and as emotions are strongly associated with physiological changes in the cardiovascular and respiratory system (Homma and Masaoka 2008), this is the most likely explanation for the observed changes in cardiovascular activity across all conditions.

Changes in heart rate via the autonomic nervous system is a principle physiological reaction of a fight/flight response when experiencing emotions of fear or anxiety regarding an aversive stimulus (Lang et al. 2000; Norton and Asmundson 2003), and is a commonly used means to evaluate the defensive response to a threat (Colloca et al. 2006). An injection is commonly considered an unpleasant procedure to undergo, and in the current study the injections carried an additional threat of tonic pain which would have likely provoked the observed stress response. In support of this, the threat and expectancy of painful noxious stimuli accompanied by the experience of negative emotions (i.e. fear and anxiety) has been shown to be associated with an initial momentary increase in heart rate prior to or immediately after the incidence of pain, followed by a subsequent reduction post-administration (Porro et al. 2002; Franciotti et al. 2009). This is similar to the response observed in the current study, which supports the conclusion that the cardiorespiratory response was primarily driven by an anticipatory reaction to the 'threat' of noxious stimuli and the associated emotional provocation (Porro et al. 2002, 2003), as opposed to a direct influence of the hypertonic saline solution on group III and IV afferents.

In addition to the cardiovascular response, psychophysiological stress and the experience of unpleasant emotions have also been shown to cause changes in respiratory measures (Homma and Masaoka 2008). The onset of acute muscle pain increases minute ventilation (Nishino et al. 1999; Kato et al. 2001), which is also demonstrated in the present study at the "Injection" time-point. Furthermore, the observed decrease in $PETCO_2$ and increase in VE/VCO_2 at the "Solution" time-point in the current study is characteristic of increased ventilation or hyperventilation; a breathing pattern in surplus of metabolic demand (Suess et al. 1980; Gardner 1996). Indeed, hyperventilation is commonly regarded as a primary and consistent defence response, which has been associated with negative emotions such as stress or fear, in addition to the experience of pain (Diest et al. 2001).

As stated previously, it would be expected that should the hypertonic saline directly elicit an exercise pressor reflex, the greater nociceptive signal from bilateral muscle pain (compared to the unilateral muscle pain conditions) would result in a significantly greater cardiorespiratory response (Leshnower et al. 2001), which was

not observed in the present study. Considering the evidence presented, the observed cardiorespiratory changes in the present study are therefore proposed to be suggestive of a stress response, and the experience of negative emotions associated with the threat, anticipation and initial muscle pain (Willer 1975; Suess et al. 1980; Grossman 1983; Kato et al. 2001) from the hypertonic saline.

This postulation is based on the absence of association between the reported pain intensity and change in any of the cardiorespiratory variables, with these alterations (e.g. an increase in HR, CO, and V_E during the "Injection" phase) instead appearing to be more closely related to the injection procedures and the timing in which they occurred. For example, data from the representative participant (Fig. 4) demonstrates that the cardiorespiratory variables start to increase *before* the insertion of the needle (e.g. during injection preparation) (as reflected by the "Injection" phase data for all participants) and have returned to approximate baseline levels despite the continued presence of muscle pain (as reflected by a majority of the variables during the "Solution" phase for all participants) (Figs. 2 and 3). It is however acknowledged that the present study did not include a bilateral isotonic saline condition, which, should a similar response be observed, would strengthen the "stress response" hypothesis. Nonetheless, this study still provides indicative evidence that the muscle pain induced by hypertonic saline may not have a significant impact on the reflex cardiorespiratory response and could be applied in locomotive exercise without inducing a confounding cardiovascular effect on endurance performance.

Perceptions of muscle pain from the unilateral and bilateral hypertonic saline injection

A further novel aim of the present study was to evaluate potential differences in the perceptual response to bilateral in comparison with unilateral hypertonic saline administration at rest, and to establish whether perceptions of pain from this experimental model are determined by limb dominance. A key finding from this study is that a greater global intensity of muscle pain was reported when the hypertonic saline was injected simultaneously and bilaterally in the VL as opposed to a unilateral injection of the same solution (with isotonic saline injected in the contralateral limb). The bilateral hypertonic saline produced a higher VAS peak of "strong" to "very

strong", and a greater VAS area, indicative of a greater overall intensity of muscle pain that lasted longer in duration. During this elevated pain experience from the bilateral injection, both the dominant and non-dominant limb reported similar individual leg pain, with no evident difference between the limbs when receiving unilateral muscle pain. This indicates that for this type of pain induction, there appears to be no effect of lower limb dominancy.

The elevated experience of pain from the bilateral injection of hypertonic saline was demonstrative of a cumulative as opposed to a summative or additive effect (i.e. the overall pain intensity from the bilateral hypertonic saline did not equal the sum of the intensity from the two unilateral conditions alone) (Lautenbacher et al. 2007). The observed greater pain intensity is consistent with prior literature, where bilateral compared to unilateral hypertonic saline administered in the longissimus muscle of the lower back showed a summative effect of pain intensity (Larsen et al. 2016), whilst concomitant injections in the tibialis anterior at spatially separated sites (Graven-Nielsen et al. 1997a) has also demonstrated the same effect. To our knowledge, this is the first study to demonstrate a cumulative effect in a locomotor muscle (VL) where a chemical pain stimulus was simultaneously induced in the same location on opposing sides of the body. However, this is in contrast with the postulation that, whilst dependent on the noxious stimuli and pain induction method, spatial summation occurs only when concurrent noxious stimuli are administered within close proximity (5-30 cm) (Graven-Nielsen et al. 1997c; Lautenbacher et al. 2007). Whilst the exact mechanisms cannot be ascertained in the present study, an explanation of neural origin is perhaps less likely due to the significant distance between injection sites (Lautenbacher et al. 2007).

Consistent with literature, both unilateral and bilateral hypertonic saline produced a quality of pain frequently described in the sensory classification (e.g. "throbbing", "cramping" and "aching") (Graven-Nielsen et al. 1997d, b), with the bilateral injection also described in the affective dimension (e.g. "tiring"). The addition of the affective pain classification parallels the negative emotions (e.g. fear and anxiety) experienced in anticipation or response to the aversiveness, unpleasantness and perceived threat associated with the noxious stimulation, and is often accompanied by the need to overcome, escape or minimise the presence of pain (Price 2000;

Fernandez and Boyle 2002; Horn et al. 2012). Prior research has identified that a single-limb hypertonic saline injection in the VL alone was not described in the affective dimension of pain, and that the addition of light intensity exercise was required to achieve this (Chapter 3). The bilateral compared to the unilateral hypertonic saline injection is likely to cause a greater spread of pain area, which has been reported to be associated with increases in pain affect (Stohler and Kowalski 1999) and therefore might explain these differences.

Conclusion

In conclusion, the bilateral injection of hypertonic saline into the VL accentuates the experience of muscle pain compared with unilateral muscle pain, with this pain not being influenced by limb dominance. The preparation and administration accompanying the injections caused a significant cardiorespiratory response, although these changes were unrelated to the reported muscle pain experience. It is therefore likely that both the procedure of the pain induction model and the expected degree of muscle pain from the action of the hypertonic saline solution provided a sufficient "threat" that provoked a stress response, as opposed to the solution-based alterations in the activity of group III and IV afferents in the injected muscle. The findings of present study therefore do not indicate an exercise pressor reflex, providing evidence that acute muscle pain from the hypertonic saline model can therefore be used during whole body exercise to examine the fatigue-pain relationship.

Chapter 6 – Acute bilateral muscle pain induced by hypertonic saline in the knee extensors decreases cycling time to task failure

Abstract

Purpose: Studies examining the impact of increased muscle pain on exercise performance predominantly focus on single-limb isometric or dynamic exercise. Whilst this provides some evidence for a role of muscle pain as a limiter of exercise performance, a more applied link to whole body, locomotive exercise remains unexplored. This study aimed to investigate the effect of bilateral muscle pain on short-duration cycling time to task failure (TTF) performance. **Methods:** On separate days, ten participants completed an incremental test to exhaustion, and in a further three visits, performed a fixed intensity TTF at 60% Δ (% difference between power output at gas exchange threshold and maximal oxygen uptake) with the injection of isotonic saline (ISO) or hypertonic saline (HYP) into the vastus lateralis of both legs, or no injection (a control; CON). **Results:** A significant difference in performance was found between conditions, with HYP causing significantly shorter TTF (285 ± 70 s) compared to both CON (372 ± 106 s, $P = 0.003$) and ISO (351 ± 92 s, $P = 0.010$). This impaired performance was accompanied by a significantly elevated EIP intensity ($P < 0.001$) during the first 50% of the exercise task. **Conclusions:** The bilateral injection of hypertonic saline during heavy-intensity exercise increased perceptions of pain which decreased TTF performance. The impaired performance is likely to be explained by increased inhibitory feedback of group III and IV nociceptive afferents during the exercise task. This study provides evidence supporting the notion that muscle pain accelerates the development of fatigue and is a key limiting factor in endurance performance.

Introduction

Performance in endurance events can be characterised by prolonged (> 75 s) whole-body, dynamic exercise that comprise of large muscle groups and predominantly utilises the aerobic energy system (e.g. cycling, running and rowing) (Gastin 2001; McCormick et al. 2015). Successful performance in endurance exercise events is believed to be primarily underpinned by three physiological factors: maximal oxygen uptake (VO_{2MAX}), lactate threshold and exercise economy (Joyner and Coyle 2008). Whilst these mechanisms are well-accepted as being of primary importance to

endurance performance, a contemporary perspective has placed an increased emphasis on the role of the brain as a key regulator during exercise, with particular focus on the interpretation of perceptual responses to exercise (Stevens et al. 2018).

In particular, there is growing evidence for the role of exercise-induced pain (EIP), which is commonly reported during moderate to high-intensity cycling exercise (O'Connor and Cook 2001), in work-rate regulation and exercise tolerance during fatiguing tasks (Mauger et al. 2010; Gonglach et al. 2016; Astokorki and Mauger 2017a). The subjective perception of EIP, frequently described by words such as "exhausting", "intense", "burning" and "cramping" (Cook et al. 1997), is initially localised to the exercising muscle, and subsequently spreads to additional locations over time (Stevens et al. 2018). The generally linear relationship between EIP and exercise intensity, distance or time (Cook et al. 1997, 1998), which drives an increasingly unpleasant and intolerable EIP is suggested to create a strong drive to adjust exercise intensity and/or cause task disengagement (Mauger 2014). In addition, an increased nociceptive signal in response to a progressively noxious metabolic environment is suggested to result in a "sub-optimal" and metabolically inefficient recruitment of the exercising muscle (i.e. change in muscle activity and motor unit recruitment), which could lead to an exacerbation of neuromuscular fatigue (Hodges and Tucker 2011; Martinez-Valdes et al. 2020), and therefore negatively impact task performance (Edwards, 1981).

The perception of EIP is caused by the sensitization and activation of small diameter (Group III and IV) muscle afferent fibers which discharge in response to a combination of increased mechanical pressure, muscle distortion and an accumulation of noxious metabolites (Cook et al. 1997; O'Connor and Cook 1999). These afferents synapse on the lumbar dorsal horn of the spinal cord, where the nociceptive signal is processed and projected to supraspinal areas within the central nervous system (Jankowski et al. 2013). Activation of the same afferents, albeit a population stimulated by low concentrations of metabolites, are also partly responsible for the sensation of fatigue (Light et al. 2008; Jankowski et al. 2013). This may go some way to explaining why EIP is often experienced during fatigue (Pollak et al. 2014), and why there is some support for the notion that EIP can be partly responsible for the development of fatigue (Mauger 2014; Morgan et al. 2018).

To investigate the impact of EIP on exercise performance, some studies have employed various interventions (e.g. caffeine, analgesics, transcutaneous electrical stimulation) to experimentally manipulate EIP during exercise (O'Connor et al. 2004; Mauger et al. 2010; Astokorki and Mauger 2017b). The ingestion of analgesics (i.e. acetaminophen) are generally shown to enhance endurance cycling performance parallel to reduced EIP (Mauger et al. 2010). When a partial reduction in *all* afferent feedback via a spinal blockade of fentanyl is introduced during exercise, there is an increased central motor drive, reduced perceptions of fatigue at the periphery and improved performance in time-trial cycling (when the consequent impact on the exercise pressor reflex is offset through hyperoxia) (Amann et al. 2009, 2010; Hureau et al. 2019). If muscle pain is increased for a given exercise intensity using hypertonic saline, there is a reduction in voluntary motor output (Graven-Nielsen et al. 2003), a decreased single-limb endurance performance (Aboodarda et al. 2020) and a compromised ability to accurately reproduce torque (see Chapters 3 and 4).

Despite the emergent evidence for the role of EIP in endurance performance, prior investigations have predominantly focused on single-limb exercise tasks. However, in locomotor exercise, EIP predominantly occurs in the quadriceps muscle group of *both* lower limbs (Slapsinskaite et al. 2015). Consequently, whilst the results of previous studies provide evidence supporting the notion of EIP as a limiter of exercise performance, a more applied link to whole body, locomotive exercise remains unexplored. To investigate this, a pain induction model that causes muscle pain akin to exercise-induced pain (EIP) needs to be applied in both exercising limbs during dynamic or locomotive tasks. The intramuscular injection of hypertonic saline in the vastus lateralis (VL) has since been established as an experimental pain-induction model that feels like naturally occurring muscle pain, and uncouples the affiliation between exercise intensity and EIP (Chapter 3). Chapter 5 also determined that this experimental approach is also unlikely to directly facilitate a confounding exercise-pressor reflex (i.e., an increased cardiorespiratory response), confirming the suitability of the hypertonic saline model to be applied to whole-body exercise. As such, the bilateral application of hypertonic saline provides a suitable method to investigate the fatigue-pain relationship, allowing for a robust investigation into the isolated role of EIP on exercise performance.

The aim of the present study was to determine the effect of acute bilateral muscle pain administered in a knee extensor muscle (VL), on short-duration, heavy-intensity endurance exercise performance. It was hypothesised that the addition of 5.8% hypertonic saline into the VL of both the right and left leg during heavy intensity exercise to exhaustion would result in an increased muscle pain, and a reduced exercise performance compared to control and placebo conditions.

Methods

Ethical Approval

All procedures were approved by the School of Sport and Exercises (University of Kent) Research Ethics Advisory Group (Prop 55_2018_19) in conformity with the Declaration of Helsinki, and its later amendments or comparable ethical standards. All participants were informed of the study experimental procedures, and written informed consent was obtained to confirm participation.

Participants

Ten healthy and recreationally active participants (7 male, 3 female; mean ± SD: age, 26 ± 4 years; height 1.78 ± 0.09 m; body mass 74.7 ± 12.8 kg; maximal oxygen uptake 45 ± 7 $ml \cdot kg^{-1} \cdot min^{-1}$) volunteered to participate in the present study. This sample size was estimated using G*Power software (Faul et al. 2007) based on the effect size reported in a prior study (Chapter 3) investigating muscle pain and changes in isometric time to task failure, in order to satisfy the assumption of statistical power of 0.8 at an alpha level of 0.05. Recruited participants were free from the exclusion criteria and attended the laboratory in accordance with the pre-requisites outlined in Chapter 2.

Experimental protocol

Participants visited the laboratory on four occasions (one preliminary visit and three experimental visits) at the same time of day, with each visit separated by a recovery period of 2-7 days. During the three experimental visits, in a single-blind, randomised and counter-balanced order, participants completed a cycling time to task failure (TTF) (*see time to task failure protocol*) after no injection (CON; Control) or the

bilateral intramuscular injection of either isotonic saline (ISO; Placebo) or hypertonic saline (HYP; Experimental) (*see intramuscular injection procedure*). Visits were performed in the same laboratory (room temperature of 20-22 ºC) and at the same time of day (± 2 h). All visits were performed on a computer-controlled and electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, the Netherlands), with the saddle and handlebar adjusted to participant preference and repeated for each visit.

In visit one, participants were familiarized with the laboratory equipment, experimental procedures and the scales implemented in the present study; Rating of Perceived Exertion (RPE), Rating of Fatigue (ROF), EIP (Cook et al. 1997), Positive and Negative Affect Schedule (PANAS), Schutte Self Report Emotional Intelligence Test (SSEIT), Pain Resilience Scale (PRS) and the modified Situation-Specific Pain Catastrophizing Scale (SPCS). Anthropometric measures (height, body mass) were also recorded.

During visit one, participants performed a cycling incremental ramp test to volitional exhaustion to establish gas exchange threshold (GET) and maximal oxygen uptake (VO_{2max}). In this test, participants performed a 5 min warm-up at 70 W, and then begun the protocol commencing with unloaded cycling (0 W) and increasing at a rate of 30 W/min (= 1 W every 2 s). Participants were instructed to complete the ramp at a predetermined cadence to be kept constant throughout the test and all subsequent trials. For all participants the self-selected cadence was between 80 and 90 revolutions per minute (rpm). Participants reported RPE, defined as the effort to drive the limb, using the 15-point Borg (6-20) scale (Borg) every 30 s.

The ramp test terminated at the point of volitional exhaustion despite strong verbal encouragement. Upon completion of the test, participants were allowed to ‘spin down’ and complete 10 min active recovery period of unloaded pedalling, followed by 20 min of passive rest. Participants then completed a familiarisation of the TTF (*see time to task failure protocol*). Participants without prior experience of the bilateral hypertonic saline intramuscular injection (n = 3) were also familiarised with this procedure at the conclusion of the first visit.

For visits two, three and four, after the 5 min warm-up, participants performed the cycling TTF with pain (bilateral intramuscular injection of hypertonic saline; HYP), placebo (bilateral intramuscular injection of isotonic saline; ISO) and no solution as a control (CON) in a randomised and counter-balanced order. Participants received the intramuscular injections whilst seated on the cycle ergometer and commenced the TTF within 15 s of needle removal.

Intramuscular injection procedure

See Chapter 2 for a detailed description of the intramuscular injection procedure.

Experimental procedures

Time to task failure (TTF) protocol

In all visits, participants were required to perform a single cycle time to task failure protocol set at 60% Δ (calculated as the percentage difference between power output at GET and VO_{2max}) based on the values obtained from the incremental ramp test in visit one. This exercise intensity was selected to elicit a TTF that would be approximately matched with the time-course of the solution action when administered bilaterally (see Chapter 3 and 4). In the present study the calculated mean power output corresponding to 60% Δ was 246 ± 45 W. Each TTF trial was performed at the same self-selected cadence (84 ± 4 rpm) from the incremental ramp test. The TTF trials commenced from a stationary start, with an immediate increase to the required power output, where participants were instructed to maintain cadence at the set intensity for the longest duration possible. During the TTF trials every 30 s, participants reported RPE, defined as the overall effort to drive the limb, with participants also instructed to include the heaviness of breathing in this rating (Pageaux et al. 2015a). The ROF (the perceived inability of the muscle to produce torque) was recorded every 30 s for the first min and every 60 s thereafter using the 11-point ROF scale (Micklewright et al. 2017b). Time to task failure (TTF) was defined as the point at which cadence dropped below 60 rpm for more than 3 s despite strong verbal encouragement. To encourage a maximal effort and to reproduce a competitive environment, a monetary incentive was rewarded to the three participants with the greatest average TTF across all three experimental trials. Participants were

blinded to the time elapsed and did not receive any feedback until all visits were complete.

Physiological measurements

During all the ramp and TTF tests, breath-by-breath measures of gas exchange were taken by an online gas analysis system (Cortex Metalyser 3b, Leipzig, Germany) calibrated prior to each session in accordance with manufacturer guidelines. Participants were fitted with a face- mask (7450 V2; Hans Rudolph; Birmingham, UK), and respiratory measures of minute ventilation (V_E), oxygen uptake (VO_2), end-tidal oxygen ($PETO_2$) and end-tidal carbon dioxide ($PETCO_2$) were recorded. The system also continuously recorded heart rate (HR) via a heart rate monitor (Polar Heart Rate Monitor, Kempele, Finland). In addition, capillary blood samples (10 µl) were taken from the fingertip and then analysed for blood lactate concentration ($B[La^-]$) immediately after sample collection using a Biosen C-Line (Biosen; EFK Diagnostics, London, UK). Blood lactate concentration was measured at rest (Rest $B[La^-]$), and after the completion of the ramp test and TTF (End $B[La^-]$). Blood lactate accumulation ($\Delta B[La^-]$) was calculated as the difference between Rest $B[La^-]$ and End $B[La^-]$.

Perceptual and psychological measurements

Assessment of exercise-induced pain

During the TTF task, the intensity and quality of muscle pain were assessed. The global pain intensity of both the right and left leg were recorded on a moment-to-moment basis using an electronic visual analogue scale (VAS). The global quality of muscle pain was established by the long-form McGill Pain Questionnaire (MPQ) (Melzack 1975). Participants immediately completed this questionnaire upon TTF completion. More information on perceptual measurements can be viewed in Chapter 2.

Self-reported psychological data

In the first visit, on arrival to the laboratory, participants completed the PANAS (Watson et al. 1988), SSEIT (Schutte et al. 1998) and PRS (Slepian et al. 2016) to assess mood, emotional intelligence and pain-specific resilience, respectively. At the

start of each visit, the PANAS was completed, asking participants to indicate feelings "at the present moment". In addition, at the start of each visit, participants were asked to rate pain expectations and confidence to cope with this expected level of pain. Immediately after the completion of each TTF, participants completed the SPCS (Edwards et al. 2006) to indicate catastrophizing that occurred during the TTF trials. More information on these measurements can be viewed in Chapter 2.

Data analysis

Respiratory gas exchange measurements were initially averaged every 5 s, followed by a moving average of 30 s to smooth the data. From the incremental ramp test, VO_{2max} was defined as the greatest VO_2 attained prior to volitional exhaustion. This value was established primarily by the occurrence of a VO_2 plateau (a change in VO_2 of less than 50 $ml \cdot min^{-1}$), and then confirmed by the achievement of two or more of the secondary criteria (maximal RER ≥1.10, blood [lactate] ≥ 8mM, RPE ≥17 and heart rate within 5% of age-predicted maximum). Gas exchange threshold was calculated from the ramp test data through the v-slope method (a visual inspection by two independent experimenters of the first disproportionate increase in VCO_2 relative to VO_2) (Beaver et al. 1986).

Due to between subject variability in TTF, an "individual iso-time" approach as outlined by Nicolò and colleagues (Nicolò et al. 2019) was applied to compare perceptual (pain intensity, RPE, ROF) and physiological (HR, V_E, VO_2, $PETO_2$, $PETCO_2$) variables. The "shortest" TTF for each participant was used to identify four (RPE, ROF) and ten (pain intensity and physiological variables) time-points in which the three conditions were segmented. This approach maintains a majority of the time-series data and provides a consistent number of data points to allow comparison between-participants for all stated variables across the varying TTF times.

Statistical analysis

Results are presented as mean ± standard deviation (SD). Assumptions associated with the performance of a paired samples *t* test, a one-way ANOVA with repeated measures and a two-way ANOVA with repeated measures were confirmed prior to statistical analysis. Any data that did not satisfy the Shapiro-Wilk test of normality (*P*

< 0.05) were transformed by square root (evaluative and miscellaneous dimensions). Confidence intervals alongside measures of effect size (Cohen's *d* and partial eta square (η_p^2) values) are reported. Pearson's bivariate correlations were used to evaluate the relationship between TTF performance and physiological or psychological measures. Where a significant correlation was identified, Cohen's guidelines of 0.1 (small), 0.3 (medium) and greater than or equal to 0.5 (large) are reported to quantify the strength of correlation.

The TTF, Δ $B[La^-]$, self-report psychological data (positive affect, negative affect and SPCS), pain expectations and confidence, and MPQ scores (Total Pain Rating Index and Subclass Rating Index) were assessed using a one-way ANOVA with repeated measures. A two-way ANOVA with treatment factor with 3 fixed levels (CON, ISO, HYP) and a repeated measures time factor with 10 time-points was used to test the effect of condition and time on pain intensity and all cardiorespiratory measures (V_E, VO_2, $PETO_2$, $PETCO_2$, HR) during the TTF. A two-way ANOVA with treatment factors with 3 fixed levels (CON, ISO, HYP) and a repeated measures time factor with 4 time-points, was used for RPE and ROF during the TTF. Post-hoc paired samples *t* tests were performed when an interaction effect was observed. Statistical significance was accepted at an alpha level of $P < 0.05$ except where a Bonferroni correction was applied. All statistics were performed using SPSS Statistics v25.0 (SPSS, IBM, New York, USA).

Results

Time to task failure (TTF)

Figure 1a. demonstrates individual TTF for each participant across condition, and a significant difference in mean TTF between conditions as compared by an ANOVA ($F_{2,18} = 12.1$, $P < 0.001$, $\eta_p^2 = 0.573$). Follow-up pairwise comparisons reveal a significantly shorter TTF in HYP (285 ± 70 s) compared to CON (372 ± 106 s) ($t_9 = 4.0$, $P = 0.003$, $CI_{.95}$ 37, 136, $d = 1.0$) and ISO (351 ± 92 s) ($t_9 = 3.3$, $P = 0.010$, $CI_{.95}$ 20, 111, $d = 0.8$). No significant differences were observed between CON and ISO (P = 0.112). For Δ $B[La^-]$ no significant difference between conditions were observed ($F_{2,18} = 0.6$, $P = 0.536$, $\eta_p^2 = 0.067$) (11.85 ± 2.96, 11.84 ± 1.67, 11.28 ± 2.56 respectively for CON, ISO and HYP).

Correlation analysis revealed no significant relationship ($P > 0.05$) between TTF and physical activity, VO2max or GET. In addition, there was no correlation ($P > 0.05$) between TTF and any of the self-report psychological measures. An ANOVA revealed no significant differences between conditions for positive affect ($F_{2,18} = 2.7$, $P = 0.091$, $\eta_p^2 = 0.234$) or negative affect ($F_{2,18} = 3.3$, $P = 0.061$, $\eta_p^2 = 0.268$). There was however a significant difference in the degree of catastrophizing between conditions ($F_{2,18} = 5.2$, $P = 0.017$, $\eta_p^2 = 0.365$). Subsequent pairwise comparisons reveal a significantly higher SPCS score in HYP (11.4 ± 3.6) compared to CON (8.9 ± 4.1) ($t_9 = -4.4$, $P = 0.002$, $CI_{.95}$ -3.8, -1.2, $d = 0.6$) and ISO (8.3 ± 4.1) ($t_9 = -2.7$, $P = 0.024$, $CI_{.95}$ -5.7, -0.5, $d = 0.8$). No significant differences were observed between CON and ISO (P = 0.638).

Exercise-induced pain (EIP)

EIP expectations and confidence

An ANOVA demonstrated no significant difference in pain expectations between conditions ($F_{1.2,11.3} = 2.6$, $P = 0.159$, $\eta_p^2 = 0.201$), but there was a significant difference in confidence to cope with the expected pain ($F_{2,18}= 4.8$, $P = 0.021$, $\eta_p^2 = 0.350$). Follow-up pairwise comparisons found a reduced confidence to cope with the expected pain in ISO (9.2 ± 0.9, $t_9 = 2.7$, $P = 0.023$, $CI_{.95}$ 0.1, 0.9, $d = 0.6$) and HYP (9.2 ± 0.9, $t_9 = 2.6$, $P = 0.029$, $CI_{.95}$ 0.1, 0.8, $d = 0.6$) compared to CON (9.7 ± 0.7) with no significant difference between ISO and HYP ($P = 0.780$). This difference in confidence between conditions is likely to be resultant from 1) whether an injection was administered (e.g. participants were likely to have a reduced pain confidence in the presence of an injection compared to no injection) and, 2) despite being randomised and counter-balanced, the order in which participants completed the visits (e.g. if participants completed ISO first, they would likely be aware that the next injection visit was going to be HYP [and vice versa]).

Comparison of pain intensity

With regard to the summary VAS values, a significantly greater overall experience of pain was observed in the HYP condition compared to the CON and ISO conditions ($P < 0.05$). Pairwise comparisons demonstrated that pain was experienced for

significantly longer period of the TTF in HYP (99 ± 1 %) compared to CON (87 ± 4 %, t_9 = -7.5, P < 0.001, $CI_{.95}$ -15.3, -8.2, d = 4.1) and ISO (89 ± 4 %, t_9 = -7.8, P < 0.001, $CI_{.95}$ -13.2, -7.3, d = 3.4). As shown in Table 1, an ANOVA revealed a significant difference in the VAS onset intensity ($F_{1.1,9.5}$ = 16.2, P = 0.002, η_p^2 = 0.642), VAS onset time ($F_{2,18}$= 59.3, P < 0.001, η_p^2 = 0.868), VAS mean ($F_{2,18}$ = 25.1, P < 0.001, η_p^2 = 0.736) and VAS time to peak ($F_{1.2,10.5}$ = 6.4, P = 0.025, η_p^2 = 0.417).

Table 3. Summary VAS scores from CON, ISO and HYP TTF

	CON	ISO	HYP
VAS onset	0.4 ± 0.3	0.3 ± 0.1	1.9 ± 1.3*
VAS peak	8.0 ± 1.8	7.4 ± 2.3	8.6 ± 1.2
VAS mean	4.1 ± 0.9	4.0 ± 1.4	6.1 ± 0.7**
VAS onset time (s)	42 ± 13	37 ± 11	3 ± 3**
VAS time to peak (s)	321 ± 97	307 ± 95	227 ± 83*
VAS duration (s)	326 ± 99	314 ± 90	283 ± 70
VAS area	1516.4 ± 563.6	1418.7 ± 611.8	1761.0 ± 600.0

Values are means ± SD. *Significantly different in HYP compared to CON and ISO conditions (P < 0.05). **Significantly different in HYP compared to CON and ISO conditions ($P \leq 0.001$).

Follow-up paired samples t tests demonstrated a significantly greater VAS onset intensity in the HYP condition (CON v. HYP: t_9 = -3.9, P = 0.003, $CI_{.95}$ -6.4, -3.9, d = 1.6; ISO v HYP: t_9 = -4.2, P = 0.002, $CI_{.95}$ -7.2, -4.2, d = 1.7), which was reported earlier (CON v. HYP: t_9 = 8.2, P < 0.001, $CI_{.95}$ 28.4, 50.0, d = 4.1; ISO v HYP: t_9 = 9.5, P < 0.001, $CI_{.95}$ 26.4, 42.8, d = 4.2) than CON and ISO. The HYP condition also induced a significantly greater VAS mean, which was classified as between "strong" and "very strong" EIP (CON v. HYP: t_9 = -6.6, P < 0.001, $CI_{.95}$ -27.8, -13.6, d = 2.5; ISO v HYP: t_9 = -5.1, P = 0.001, $CI_{.95}$ -30.5, -11.7, d = 1.9) and reached VAS peak significantly quicker (CON v. HYP: t_9 = 2.6, P = 0.028, $CI_{.95}$ 12.4, 175.6, d = 1.0;

ISO v HYP: $t_9 = 2.6$, $P = 0.028$, $CI._{95}$ 10.9, 149.5, $d = 0.9$). No significant differences were observed between CON and ISO for any calculated VAS variable ($P > 0.05$).

As demonstrated in Fig. 1b. the 3 × 10 (condition × iso-time) repeated measures ANOVA highlighted a significant effect of condition ($F_{2,18} = 44.6$, $P < 0.001$, $\eta_p^2 = 0.832$), iso-time ($F_{1.5,13.2} = 61.4$, $P < 0.001$, $\eta_p^2 = 0.872$) and an interaction effect for pain intensity over iso-time between conditions ($F_{3.0, 27.2} = 7.9$, $P = 0.001$, $\eta_p^2 = 0.467$). Follow-up targeted paired samples *t* tests with a Bonferroni correction ($P < 0.0033$) established a significantly elevated VAS pain intensity in HYP from 10 to 50% iso-time compared to CON ($P < 0.001$), and ISO ($P \leq 0.001$).

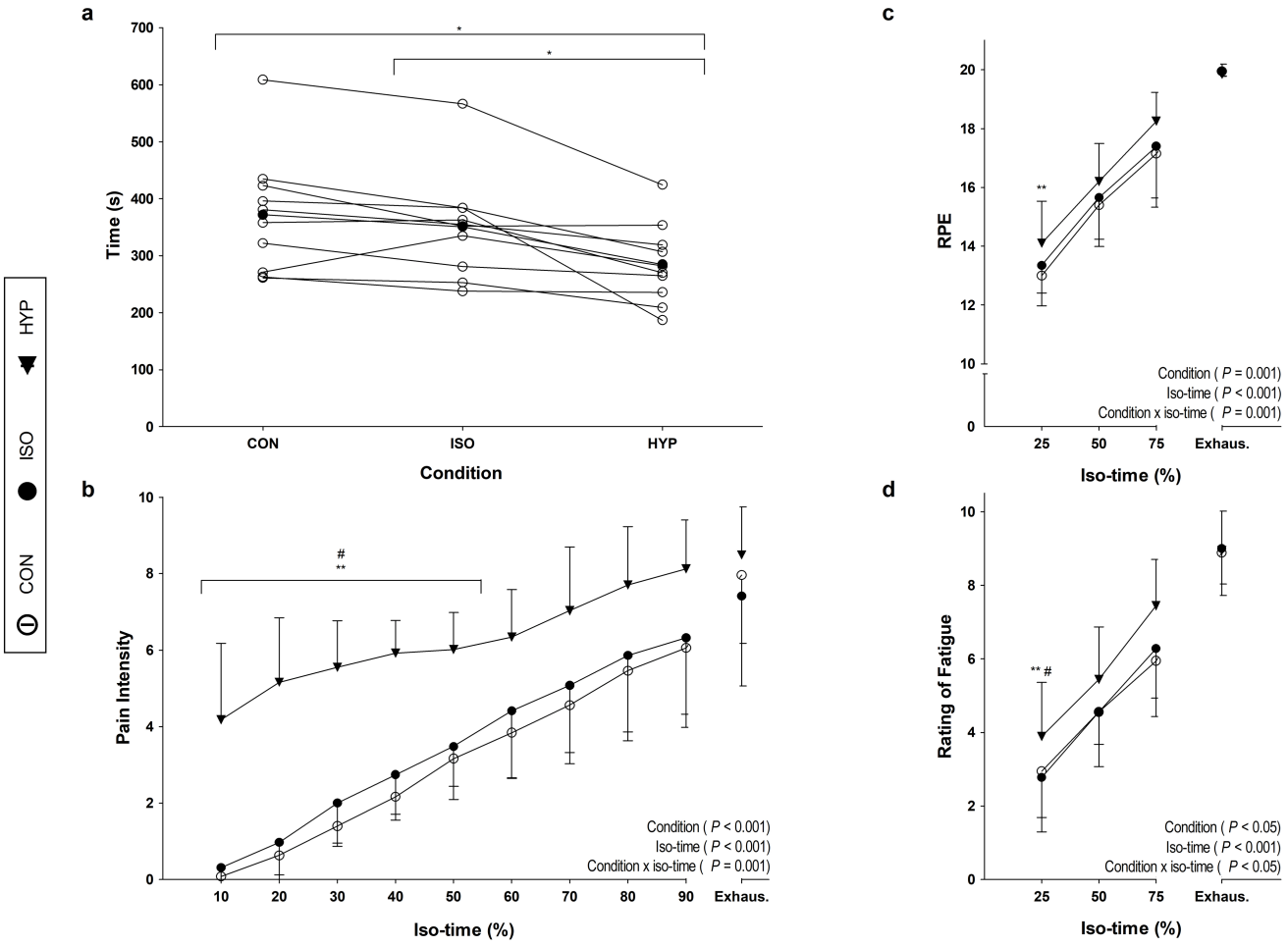

Fig. 1 Performance and perceptual differences between conditions. TTF differences between conditions (a), and pain intensity (b) and RPE (c) and ROF (d) over iso-time between conditions during the TTF. *Significant difference between conditions (P <

0.05). **Significant difference between CON and HYP (P < 0.05). #Significant difference between ISO and HYP (P < 0.05).

Comparison of pain quality

The most frequently selected descriptors summarising the overall pain quality for each condition are shown in Table 2. An ANOVA on the calculated scores from the MPQ demonstrated a significantly different Total Pain Rating Index ($F_{2,18} = 16.3$, $P < 0.001$, $\eta_p^2 = 0.645$), and a significant difference in the sensory Subclass Rating Index ($F_{2,18} = 10.8$, $P = 0.001$, $\eta_p^2 = 0.547$) between conditions. A similar perceived quality of pain was reported between conditions for the affective ($P = 0.122$), evaluative ($P = 0.528$) and miscellaneous ($P = 0.122$) classifications of pain. The HYP TTF produced a Total Pain Index of 22 ± 8, and subsequent paired samples *t* test comparisons showed that this was significantly greater than both the CON (15 ± 8, 10 ± 3, $t_9 = -4.4$, $P = 0.002$, $CI_{.95}$ -11.4, -3.6, $d = 0.9$) and ISO (15 ± 7, $t_9 = -4.3$, $P = 0.002$, $CI_{.95}$ -11.1, -3.5, $d = 0.9$) TTF tasks. No differences were identified between CON and ISO ($P = 0.840$). As indicated by the Subclass Rating Index, the sensory dimension of pain was significantly higher in the HYP condition (14 ± 5) compared to the CON (9.0 ± 4, $t_9 = -2.1$, $P = 0.003$, $CI_{.95}$ -7.5, -2.1, $d = 1.0$) and ISO (10 ± 3, $t_9 = -1.4$, $P = 0.007$, $CI_{.95}$ -6.6, -1.4, $d = 0.9$) conditions, with no difference between CON and ISO ($P = 0.428$).

Table 2. Frequently selected words from the MPQ subclasses

		CON	ISO	HYP
MPQ	Sensory	Cramping (50%) Aching (60%)	Cramping (50%) Aching (60%)	Boring (40%) Cramping (60%) Aching (60%)
	SRI	9 ± 4	10 ± 3	14 ± 5*
	Affective	Exhausting (50%)	Tiring (40%)	Exhausting (60%)
	SRI	2 ± 2	2 ± 2	3 ± 2

	Evaluative	Intense (60%)	Annoying (40%)	Intense (60%)
	SRI	2 ± 2	2 ± 2	3 ± 2
	Other	Continuous (60%)	Continuous (40%)	Continuous (70%)
	PRI(T)	15 ± 8	15 ± 7	22 ± 8*

The frequently selected words from the MPQ are shown with the percentage of participants (n = 10) that selected these words. Data on Subclass Rating Index (SRI) and Pain Rating Index (Total) presented as Mean ± SD. *Significantly greater than CON and ISO ($P < 0.05$).

Rating of perceived exertion (RPE)

The 3 × 4 (condition × iso-time) repeated measures ANOVA revealed a significant main effect of condition ($F_{2,18} = 9.9$, $P = 0.001$, $\eta_p^2 = 0.524$) and iso-time ($F_{1.7,15.7} = 129.6$, $P < 0.001$, $\eta_p^2 = 0.935$). As shown in Fig. 1c., there was a significant interaction effect for RPE over iso-time between conditions ($F_{6,54} = 4.4$, $P = 0.001$, $\eta_p^2 = 0.326$). Follow-up comparisons with a Bonferroni correction ($P < 0.0083$) demonstrated a significantly greater RPE at 25% iso-time in HYP compared to CON ($t_9 = -3.5$, $P = 0.007$, $CI_{.95}$ -0.69, -0.01, $d = 0.9$).

Rating of fatigue (ROF)

The 3 × 4 (condition × iso-time) repeated measures ANOVA revealed a significant main effect of condition ($F_{2,16} = 5.0$, $P = 0.020$, $\eta_p^2 = 0.385$), iso-time ($F_{1.5,12.4} = 64.3$, $P < 0.001$, $\eta_p^2 = 0.889$) and a significant interaction effect for ROF over iso-time between conditions ($F_{6,48} = 3.2$, $P = 0.010$, $\eta_p^2 = 0.285$). Figure 1d. demonstrates that follow-up paired samples *t* tests with a Bonferroni correction ($P < 0.0083$) discovered a significantly greater ROF in HYP compared to CON ($t_9 = -3.4$, $P = 0.008$, $CI_{.95}$ -1.8, -0.3, $d = 0.7$) and ISO ($t_9 = -4.4$, $P = 0.002$, $CI_{.95}$ -1.8, -0.6, $d = 0.9$) at 25 % iso-time.

Cardiovascular response

The 3 × 10 (condition × time) repeated measures ANOVA demonstrated a significant main effect of time for all cardiorespiratory measures ($P > 0.001$). A significant main

effect of condition was observed for V_E ($F_{2,18}$ = 3.6, P = 0.047, η_p^2 = 0.288), $PETO_2$ ($F_{1.3,11.6}$ = 7.2, P = 0.016, η_p^2 = 0.443) and $PETCO_2$ ($F_{2,18}$ = 6.5, P = 0.007, η_p^2 = 0.420), but not in HR (P = 0.946) and VO_2 (P = 0.493). A significant interaction effect for V_E ($F_{3.9,34.8}$ = 4.6, P = 0.005, η_p^2 = 0.339) and $PETCO_2$ ($F_{5.0,44.7}$ = 5.8, P < 0.001, η_p^2 = 0.390) over iso-time between conditions was observed, but not in HR (P = 0.268), VO_2 (P = 0.092) or $PETO_2$ (P = 0.065). Targeted follow-up paired samples t tests with a Bonferroni correction (P < 0.0033) demonstrated a significantly elevated V_E in HYP at 50% iso-time compared to ISO (t_9 = -4.1, P = 0.003, $CI_{.95}$ -13.3, -3.8, d = 0.4). For $PETCO_2$ no significant differences between conditions were observed after Bonferroni corrected paired samples t tests (P < 0.0033) (Figs 2 and 3).

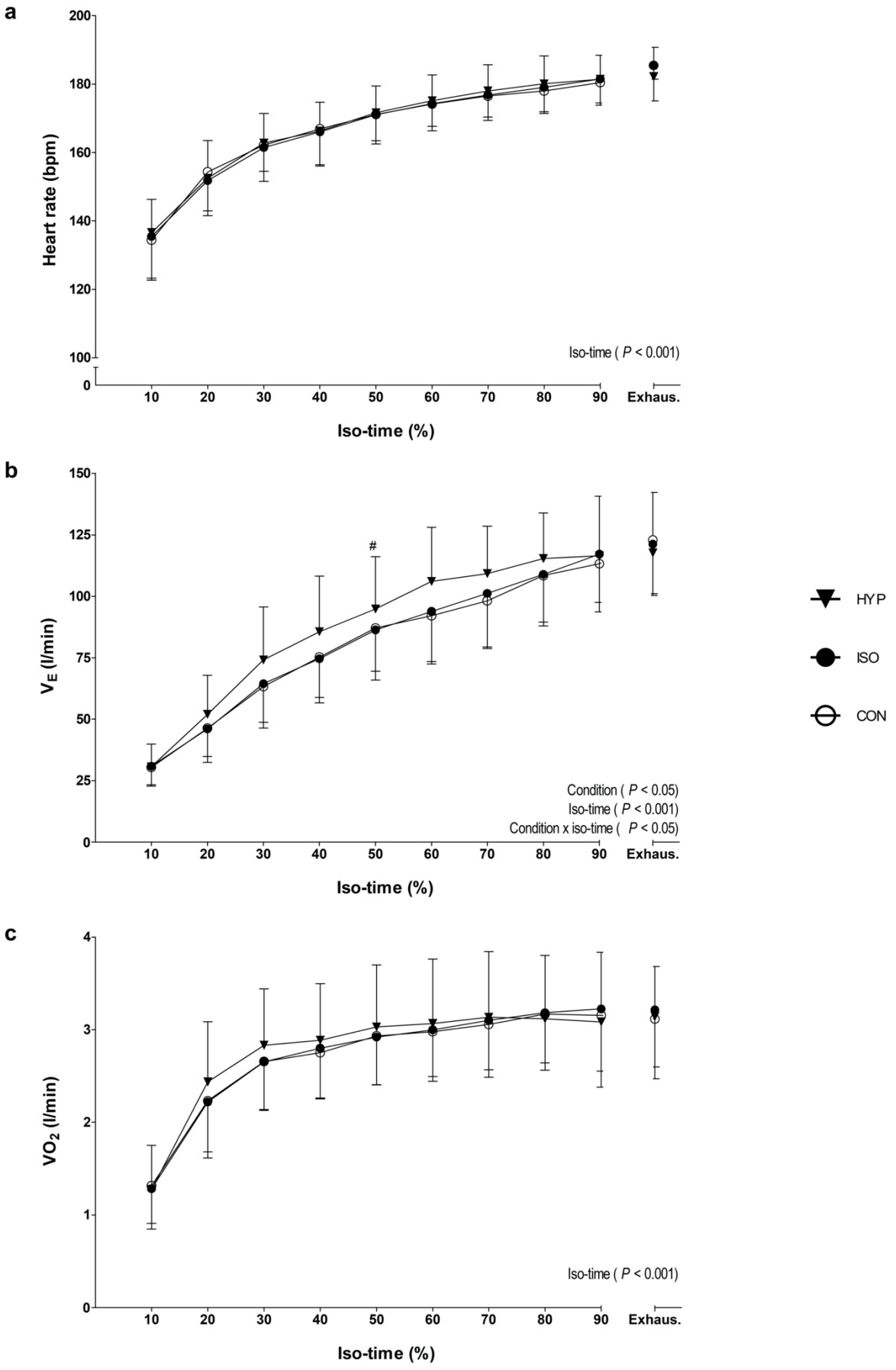
a
Heart rate (bpm)
200
180
160
140
120
100
0
Iso-time (P < 0.001)
10
20
30
40
50
60
70
80
90
Exhaus.
Iso-time (%)
b
V_E (l/min)
150
125
100
75
50
25
0
#
Condition (P < 0.05)
Iso-time (P < 0.001)
Condition x iso-time (P < 0.05)
10
20
30
40
50
60
70
80
90
Exhaus.
Iso-time (%)
HYP
ISO
CON
c
VO_2 (l/min)
4
3
2
1
0
Iso-time (P < 0.001)
10
20
30
40
50
60
70
80
90
Exhaus.
Iso-time (%)

Fig 2. Cardiorespiratory differences between conditions. Heart rate (HR; a), minute ventilation (V_E; b) and oxygen uptake (VO_2; c) over iso-time between conditions during the TTF. Values are presented as mean ± SD. #Significant difference between ISO and HYP ($P < 0.05$)

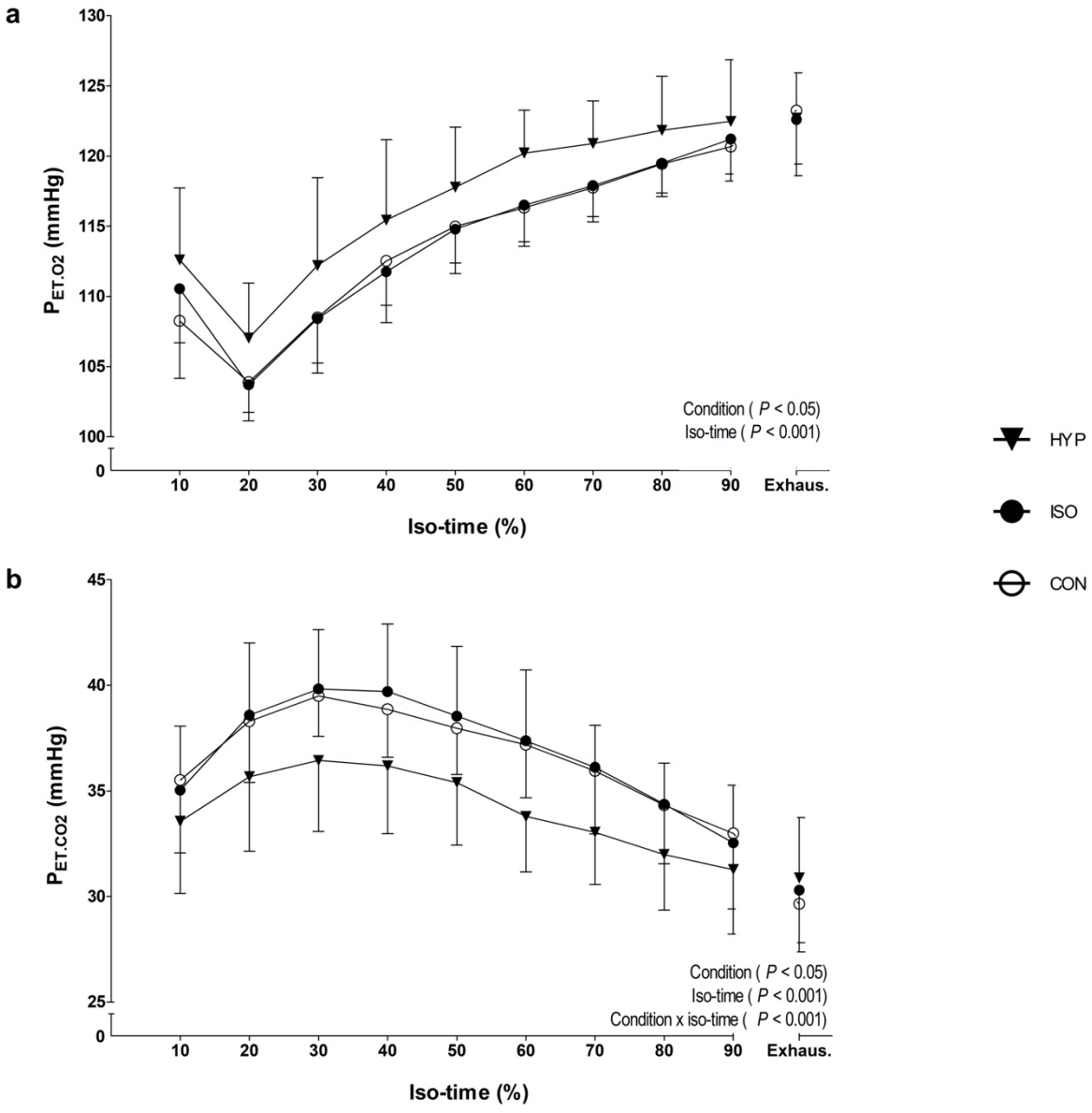

Fig 3. Differences in end-tidal partial pressure of oxygen ($PETO_2$; a) and carbon dioxide ($PETCO_2$; b) between conditions over iso-time during the TTF. Values are presented as mean ± SD.

Discussion

The main finding of this study was that hypertonic saline increased the experience of pain and accelerated the development of fatigue, with participants reaching the point of exhaustion in a shorter time. As the first study to apply muscle pain through hypertonic saline during the performance of locomotive endurance exercise, this study provides an important contribution to the understanding of the impact of pain on exercise performance. The primary finding of the present study demonstrates that an increased level of pain significantly impairs the performance of fixed-intensity cycling to exhaustion, with a decrease in time to task failure of approximately 19 to 23% (compared to the CON and ISO conditions). This impaired performance was accompanied by an increased intensity of pain, paralleled with increased perceptions of fatigue and exertion. Participants attended the laboratory in a similar psychological state (as assessed by the PANAS), and participants performed all conditions at the same exercise intensity so the differences in TTF performance can be attributed to the increased EIP intensity and quality during the HYP condition.

This important finding provides a natural progression to studies exploring the impact of increased pain on single-limb isometric and dynamic exercise tasks (Astokorki and Mauger 2017b; Aboodarda et al. 2020) (see Chapter 3) and the performance enhancing effect of analgesics (Mauger et al. 2010, 2014; Foster et al. 2014; Morgan et al. 2018, 2019). In these studies, ergogenic aids which reduce pain, such as caffeine (Astorino et al. 2011; Gonglach et al. 2016; Tomazini et al. 2020) and paracetamol (Mauger et al. 2010; Foster et al. 2014), are frequently shown to improve performance by allowing a higher exercise intensity to be sustained for a given level of pain. Whilst these studies go some way to show that reducing pain can improve exercise performance, investigations concentrating on the *attenuation* as opposed to the *accentuation* of the pain response during whole body exercise are unable to fully elucidate the independent effect of EIP (Astokorki and Mauger 2017a). Instead, by fixing exercise intensity but increasing the magnitude of pain during locomotive exercise, the present study demonstrates a clear link between nociception and an increase in the perception of muscle pain (via the hypertonic saline) and an accelerated development of fatigue.

Proposed physiological mechanisms

Hypertonic saline injected bilaterally at rest into the VL produced a "moderate" to "somewhat strong" muscle pain lasting between six to seven minutes in duration (Chapter 5). In the current study, the bilateral hypertonic saline *combined* with heavy intensity exercise in a key locomotive muscle (Raasch et al. 1997) significantly exacerbated the pain intensity for the first half of the exercise task, producing a "strong" to "very strong" muscle pain. Indeed, at 10% iso-time the intensity of pain in the HYP condition was approximately 4.0 units greater than the CON and ISO conditions, and remained significantly elevated until 50% iso-time where the reported difference was around 2.7 units. From this time-point, the intensity of pain subsequently continued to increase as a linear function of time in all conditions until the point of exhaustion, where near maximal values were reported. The HYP condition was additionally defined by an increased sensory pain experience, contributing to a higher Pain Rating Index score. This data clearly evidences the greater severity of pain in the HYP condition in terms of both quality and quantity of pain.

This elevated pain is likely to be produced by greater excitation of the group III and IV nociceptive afferents due to the deleterious cumulative mechanical and chemical environment caused by the hypertonic saline and prolonged exercise task (O'Connor and Cook 1999; Amann et al. 2011a). Although a direct measure of group III and IV afferent feedback is not feasible in this experimental paradigm, the neural transmission and central processing of the greater nociceptive signal arising from the knee extensors and the associated perception of pain may provide an insight into the underpinning mechanisms for the shorter time to task failure. When stimulated these mechanosensitive and metabosensitive afferents may have exerted inhibitory feedback on neuromuscular output, reducing both central motor drive and voluntary activation (Amann et al. 2009; Kennedy et al. 2013) of the locomotor muscles, and therefore driving central-mediated decreases in endurance performance.

Compensatory alterations in muscle activity, or a modification of motor unit recruitment order (Ervilha et al. 2005; Hodges and Tucker 2011; Brøchner Nielsen et al. 2017), and the consequent reduction in exercise efficiency could provide an alternative explanation for the reduced performance. In the present study, findings of

no difference in Δ B[La^-] between conditions despite a significantly shorter time to task failure in the HYP condition could be indicative of a *preferential* activation of these large high threshold motor units (instead of type I muscle fibers) at an earlier time-point in the exercise task. Whilst beneficial for a rapid production of force, during endurance exercise this preferred recruitment of more energetically inefficient motor units (Coyle et al. 1992) would potentially accelerate the rate of metabolite accumulation and progression of fatigue (Edwards 1981). In addition, it has also been demonstrated that increased pain from the hypertonic saline injection can lower maximal voluntary force by approximately 20% (Graven-Nielsen et al. 2002). Consequently, it is likely that the hypertonic saline would have increased the relative intensity (in terms of muscle contractile force) of the exercise task. Therefore, to maintain the same motor output an increase in descending motor drive would be required, potentially increasing supraspinal fatigue (Gandevia 2001) and reducing endurance performance. This mechanism may also partly explain the increased rating of perceived exertion and rating of fatigue at the onset of exercise (25% iso-time) in the HYP condition.

As the metabolic environment created by intense exercise generates afferent feedback that combines both nociceptive information and sensory feedback driving the exercise pressor reflex, prior research investigating the experimental manipulation of Group III and IV afferents on exercise performance have been confounded by hypoventilation and changes in limb blood flow (Amann et al. 2010, 2011a, b). In Chapter 5, we showed that a transient cardiorespiratory response was observed in resting conditions after the injection of hypertonic saline, yet these changes were not associated with the reported muscle pain, and instead were attributed to an acute stress response (as opposed to a direct influence on group III and IV afferents). Based on this finding, the current study applied this experimental pain-induction model to whole body exercise (knowing that it would not evoke a confounding cardiorespiratory response) to evaluate the *isolated* impact of pain on endurance exercise performance.

The cardiorespiratory response in the present study was consistent with these findings, with no discernible differences in HR or VO_2 between conditions that could have directly influenced performance outcomes. The greater V_E observed at 50% iso-time in the HYP condition in addition to the changes in $PETCO_2$ is proposed to be

suggestive of hyperventilation. This breathing pattern can be explained either as a common stress response in the presence of negative emotions and an increase in pain (as reported in the HYP condition) (Diest et al. 2001; Homma and Masaoka 2008) or the transmission and central processing of feedback from the Group III and IV nociceptive afferents (Amann et al. 2010, 2011a). Regardless of origin, this hyperventilation during the heavy-intensity cycling exercise could have led to the faster development of fatigue in the respiratory muscles (Dempsey et al. 2006), potentially contributing to the shortened TTF performance.

Proposed psychological mechanisms

As a multidimensional construct, the psychological mechanisms of EIP and the impact this may have on exercise performance is important. Alongside the sensory-discriminative component of pain (i.e. intensity, modality and location), the affective-motivational component refers to the emotional response and accompanying avoidance drive to escape a painful stimulus (Boggio et al. 2009; Horn et al. 2012). Whilst distinct components of pain, the affective-motivation component is partially informed by "lower-order" sensory-discriminative information and therefore requires supplementary contextualisation of the nociceptive stimulus (Price 2000; Moseley and Arntz 2007).

Fundamentally a physiological warning signal to protect the body from the actual or potential threat, the perception of muscle pain could provide a powerful motivational and defensive psychological drive to avoid or reduce the aversiveness or unpleasantness of pain (Fields 1999). In the context of open-loop exercise tasks (e.g. TTF), where intensity of pain is proportional to the duration of exercise, this is achieved by task disengagement, which will reduce the intensity of pain and the concomitant emotional response. As such, in the present study the greater severity of muscle pain experienced in the HYP compared to the CON and ISO conditions may have initiated a stronger protective, drive state (i.e. greater affect) to terminate the exercise task and therefore shortening TTF performance. Based on this, it is possible that exercise performance may not necessarily be impaired by the sensory-discriminative component of pain, but instead is affected by the affective-motivational component of pain (Fields 1999). However as recorded by the MPQ, no differences in

the affective dimension of pain between conditions were observed, indicating that this may not have been the case.

However, in the present study, whilst not measured during exercise, pain-specific catastrophizing (a negative cognitive and affective response to pain) was recorded immediately post-exercise. This measure has been shown to be predictive of pain tolerance as well as an impaired ability or motivation to engage in an exercise task (Sullivan et al. 2001, 2002; Nijs et al. 2008). Associated with ratings of greater pain intensity (Sullivan et al. 1995, 2000; Nijs et al. 2008), whilst hypothetical, the increased catastrophic thinking during the HYP condition may have been an alternate contributing construct motivating a greater behavioural drive to escape from the pain, and therefore the exercise task (resulting in the reduced TTF performance). It is however suggested that future studies measure changes in factors such as pain unpleasantness, self-efficacy beliefs or emotions in response to an exacerbated muscle pain *during* an exercise task could provide a greater insight into the potential psychological mechanisms that may explain the fatiguing impact of EIP on endurance performance.

Conclusion

The bilateral injection of hypertonic saline into the VL during a heavy, fixed-intensity cycling TTF task resulted in a curtailed endurance performance when compared with a placebo and control condition. The shorter TTF was accompanied by a greater severity of pain during exercise, with intensity elevated for the first 50% of the task and the experience reported higher in the sensory pain dimension. The increased activity of group III and IV muscle nociceptors causing central inhibition and supraspinal fatigue or an increase in pain-specific catastrophising driving behaviour to escape the pain, could explain the impaired performance. This study therefore provides further evidence supporting the concept that EIP accelerates the development of fatigue and is a key limiting factor in endurance performance.

Chapter 7 – General Discussion

The predominant focus of this thesis was to progress understanding of the role of EIP on exercise performance through exacerbating the experience of muscle pain via an experimental pain model that is representative of naturally occurring EIP. Therefore, there were two overarching aims of the thesis; 1) to apply and confirm the hypertonic saline model as a means for experimentally replicating the experience of EIP, and 2) If successful, to subsequently use the hypertonic saline model of muscle pain as a method to evaluate the potential fatiguing role of EIP during single-limb and whole-body exercise tasks relevant to endurance performance. These two aims were addressed in four novel experimental studies, of which the findings make the following major contributions to knowledge in this area:

1. The injection of hypertonic saline into the vastus lateralis combined with muscle contraction provides an experimental model of muscle pain which feels like naturally occurring EIP of a greater contraction intensity (Chapter 3, Study 1). The hypertonic saline model could therefore provide a useful tool to investigate the fatigue-pain relationship;
2. An increased magnitude of nociceptive processing and perception of muscle pain from this model impair the accuracy to produce a given torque during short and submaximal single-limb isometric contractions (Chapter 4, Study 2);
3. The bilateral administration of hypertonic saline cumulatively accentuates the experience of muscle pain compared to unilateral muscle pain, with no effect of self-reported limb dominance (Chapter 5, Study 3);
4. The hypertonic saline model is unlikely to directly elicit a meaningful exercise pressor reflex, with any cardiorespiratory changes attributed to the psychophysiological stress response associated with the threat and anticipation of the painful stimuli (Chapter 5, Study 3). This model can therefore be used during whole body exercise to examine the fatigue-pain relationship;
5. When applied to both single-limb isometric (Chapter 3, Study 1) and whole-body cycling exercise (Chapter 6, Study 4), the hypertonic saline model elevated the experience of pain and accelerates the development of fatigue, decreasing time to task failure performance.

The outcomes of this thesis represent original work and therefore make a substantial contribution to advancing scientific knowledge on the potential fatiguing impact of EIP, which could subsequently present numerous avenues for future research.

7.1 Summary of findings

Chapter 3 (Study 1), which addressed the first thesis aim, examined the pain induced by the hypertonic saline experimental model both at rest and in combination with a sustained single-limb low-intensity (10% MVT) isometric contraction, and subsequently compared this experience (in terms of pain intensity and quality) with the naturally occurring EIP elicited at a greater exercise intensity (20% MVT). Although an extensively used method of experimental muscle pain (Lewis 1938; Kellgren 1938; Arendt-Nielsen et al. 1996; Graven-Nielsen et al. 1997b, 2002; Khan et al. 2011; Martinez-Valdes et al. 2020), this study was the first to apply the hypertonic saline in controlled conditions that are of greater relevance to endurance exercise performance, and directly quantify the induced pain experience in contrast with EIP. Unlike prior applications of the experimental pain model, in this Chapter the solution was injected into a large locomotive muscle with the single-limb isometric contractions performed at an intensity commonly employed during endurance performance (Löllgen et al. 1980).

Through the use of unidimensional and multidimensional measures of pain, the experimental studies in this thesis measured of a range of pain components (sensory, affective, evaluative and temporal) and elements (quality). It was found that the intensity and dimensional quality of pain reported at rest was consistent with the prior application of the model in alternate muscles or muscle groups (i.e. a moderate to somewhat strong pain, described as cramping, aching, throbbing and intense), but did not correspond to the pain experience during a ‘normal’ sustained contraction at 20% MVT. When combined with a sustained isometric contraction at 10% MVT, the induced descriptive quality of pain was comparable with the EIP experienced during exercise performed at both 20% MVT and 10% MVT (in the presence of a placebo or no injection), but with an elevated intensity of pain. This demonstrates that 1) participants were unable to distinguish between the experimental muscle pain produced by the hypertonic saline and the EIP from the muscular contraction and 2)

the hypertonic saline combined with the low-intensity contraction felt like a harder exercise intensity, therefore uncoupling the established relationship between work-rate and EIP (Cook et al. 1997).

This is an important finding, as the alternate methods of inducing muscle pain are distinct from EIP (in terms of transmission and experience), and also elicit confounding actions that limit the ability to investigate the potential fatiguing role of EIP. With growing evidence for the role of EIP in work-rate regulation and exercise tolerance during fatiguing and endurance tasks (Mauger et al. 2010; Astokorki and Mauger 2017a; O'Leary et al. 2017), this experimental model, which causes nociception and the subsequent perception of pain, allows for the potential isolation of pain to rigorously explore the fatigue-pain relationship during exercise performance.

As the experience of EIP is often associated with fatigue, it is suggested that EIP could be a casual factor in fatigue development or acceleration during prolonged and intense exercise (Mauger 2014), which could subsequently limit endurance performance. Indeed, there are both physiological (e.g. inhibitory afferent feedback, accumulation of metabolites, changes in motor behaviour) and psychological mechanisms (Fields 1999; Hodges and Tucker 2011; Aboodarda et al. 2020) proposed which could explain the fatiguing impact of EIP during endurance performance (Mauger and Hopker 2012; Mauger 2013) but these require further exploration.

Prior experimental research attempting to investigate the role of EIP during exercise (Surbey et al. 1984; Cook et al. 1997, 2000; O'Connor et al. 2004; Mauger et al. 2010) has demonstrated that the experimental manipulation of pain is challenging. The ergogenic interventions to reduce the perception of pain have been generally confounded by alternate physiological responses (Cook et al. 1997; Ray and Carter 2007; Amann et al. 2009, 2011a; Mauger et al. 2010), whilst typical methods of pain induction (e.g. thermal, electrical and ischemic) are inadequate to represent the nociceptive processing and environment of EIP. Therefore, the findings in Chapter 3 (Study 1) not only formed a key foundation for the four experimental chapters of this thesis but also provide a useful model that can be widely applied in future experimental work where the replication of EIP is the focus.

As a result, the experimental studies in this thesis (Chapters 3-6) were designed to evaluate the potential impact of EIP on endurance exercise tasks, and to gain an understanding of how the experimental manipulation of this (through the hypertonic saline method) may influence physiological, perceptual, regulatory and psychological measures during performance. The first two experimental studies in this thesis initially employed single-limb isometric tasks (Chapters 3 and 4, Studies 1 and 2). The final experimental chapter (Chapter 6, Study 4), which was informed by the key outcomes of the penultimate study (Chapter 5, Study 3), then applied this model of inducing muscle pain to cycling exercise to provide an applied link to whole body, locomotive exercise.

The second aim of Chapter 3 (Study 1) and primary aim of Chapter 4 (Study 2) was to identify whether exacerbating muscle pain through the application hypertonic saline influences performance of two different single-limb exercise tasks (time to task failure and torque reproduction), and to understand the potential effects this may have on physiological (e.g. sEMG, HR) and important perceptual (RPE and ROF) measures. The single-limb isometric exercise model allows for more experimental control, minimising the role of alternate confounding physiological systems (e.g. the cardiorespiratory system) and providing an indication of how isolated muscle pain may influence performance during whole-body exercise or dynamic muscular contractions. It is however acknowledged that there is a notable difference in the physiological response and nociceptive environment between single-limb and whole-body exercise (see Section 1.3.1). Caution should therefore be taken when extrapolating these findings to this exercise modality, with the need to further evaluate the effect pain may have during isokinetic or dynamic muscular contractions performed at changeable work rates.

In the second part of the experiment detailed in Chapter 3 (Study 1), an open-loop exercise time to task failure protocol was selected to ensure pain intensity was proportional to exercise duration (i.e. preventing the conscious moderation of pain intensity by varying exercise intensity). This approach provides a method which allows for a measure of endurance performance, and subsequently an investigation of whether increased muscle pain and changes in the measured parameters contribute to differences in task performance (i.e. a mechanistic explanation of performance

change). The primary finding of this chapter was that greater levels of muscle pain, induced through the intramuscular injection of hypertonic saline (into a large muscle with a key role in force generation during locomotive exercise), significantly shortened time to task failure performance (completed at an intensity utilised during cycling exercise).

Whilst Chapter 3 (Study 1) provides evidence for the direct impact of EIP on endurance performance, the relationship between EIP and fatigue is complex, and there is some uncertainty on how EIP may affect the self-regulation of work-rate during a closed-loop bout of exercise. Whilst it has been proposed that EIP is a form of useful sensory feedback regarding the relative state of the working muscle (Mauger 2014), the acute debilitating effects associated with EIP (Hodges and Tucker 2011) could potentially impair the perception of force produced by the muscle, which could subsequently impede as opposed to aid the regulation of work rate. In an attempt to investigate this, Chapter 4 (Study 2) used a torque matching and reproduction task with the aim to evaluate the effects of increased muscle pain on the ability to accurately estimate the torque produced by the painful muscle. The purpose of using this type of protocol was to gain inferential knowledge to indicate how sensations of pain during exercise might impact pacing-based decision making and the aptitude to self-regulate work-rate in a more ecologically valid context of whole-body, locomotive exercise.

Based on previous research which used the same model in the elbow flexors (Proske et al. 2003, 2004; Weerakkody et al. 2003) it was hypothesised the that participants would specifically *overestimate* the torque produced by the painful muscle. Consist with prior research, it was found that the increased muscle pain significantly compromised the capacity to accurately reproduce a given submaximal torque, with the task accuracy returning to baseline levels upon return to the state of “no pain”. However, the key novel finding of this chapter was that participants *both* over- (i.e. produced less torque than required) and underestimated (i.e. produced more torque than required) the torque produced. Importantly, the magnitude of error in torque was not associated with the pain intensity reported which questions whether the muscle pain itself directly affected task performance.

It is therefore likely that both nociceptive processing and the concomitant experience of muscle pain interferes with proprioception during a single-limb isometric task. Previous research has suggested that, alongside the traditional aerobic parameters of performance outlined by Joyner and Coyle's (2008) model, cycling time-trial performance is partially regulated by EIP (Astokorki and Mauger 2017a). Whilst the regulation and determination of exercise performance is evidently a complex process (and dependent on the characteristics of the exercise task) (Enoka and Stuart 1992; Joyner and Coyle 2008; Boyas and Guével 2011), the findings of this chapter tentatively indicate that during whole-body endurance exercise EIP may elicit adaptations that could have a detrimental effect on work-rate regulation and could therefore have a potentially negative impact on performance.

A natural progression in this line of research was to therefore investigate whether the findings from a controlled single-limb exercise model translate to whole-body locomotive exercise, in which EIP is typically experienced in both of the lower limbs (Slapsinskaite et al. 2015). Prior to this, it was important to 1) quantify the bilateral application of the hypertonic saline model in comparison to unilateral pain induction, 2) ascertain whether there are any differences between-limb in response to this model, and crucially 3) ensure that this experimental model does not elicit any additional response that may confound the ability to assess the independent role of EIP on endurance performance. Prior work investigating the experimental manipulation of Group III and IV afferents (and the accompanying sensations of pain from the exercising muscle) to test the Inhibitory Feedback Model have been limited by an impaired exercise pressor response (Amann et al. 2009, 2011a). Resultantly in this prior research, peripheral fatigue was accelerated, and therefore the magnitude in which feedback from these afferents exerted a central effect and impacted work-rate regulation was uncertain (Amann et al. 2011a; Sidhu et al. 2017). Due to the nociceptive pathway of the hypertonic saline (i.e. activation of predominantly Group IV afferents with some contribution from Group III) (Laursen et al. 1999), it was plausible that this model may also cause an increased cardiorespiratory response via the exercise pressor reflex, which could be detrimental for its application to whole body-exercise.

Chapter 5 (Study 3) addressed this notion through monitoring both perceptual and cardiorespiratory measures prior to, during and after the administration of bilateral intramuscular injections into the vastus lateralis which induced either unilateral (e.g. isotonic saline in left leg and hypertonic saline in right leg, or vice versa) or bilateral (e.g. hypertonic saline in both legs) muscle pain. Firstly, compared to unilateral application, the bilateral application of hypertonic saline induced a cumulatively greater global intensity of muscle pain that was also described in the affective dimension (i.e. indicative of a difference in pain unpleasantness). Both the self-reported dominant and non-dominant limb recorded similar individual leg pain during the bilateral intramuscular injection, with no evident difference between the limbs when receiving unilateral muscle pain (i.e. no effect of limb dominance).

Secondly, an increase in cardiorespiratory variables (e.g. HR, CO, V_E, VO_2, VCO_2 and RER) was demonstrated during the preparation and administration of the injection, with some changes (e.g. a significant decrease in SV and $PETCO_2$ and an increase in V_E/VCO2) also reported throughout the action of the solution. As the pressor response is suggested to be dependent on the magnitude of the sensory input (Leshnower et al. 2001), it was expected that (should this method of experimental pain induction cause a reflex pressor response) a greater cardiorespiratory response would parallel the greater sensory feedback induced by a bilateral compared to unilateral injection. Importantly however, the observed cardiorespiratory changes ensued irrespective of whether the muscle pain was unilateral and bilateral and were not associated with the change in muscle pain experienced. It was therefore suggested that the hypertonic saline did not necessarily directly cause this response and were instead perhaps resultant from psychophysiological stress associated with both the procedures of pain induction and anticipation or response to the threat of muscle pain.

Fundamentally, receiving an intramuscular injection and the threat of noxious stimuli to specifically induce acute muscle pain is an unpleasant and aversive procedure to experience. It was therefore proposed that this may cause a stress response and the experience of negative emotions, which have been demonstrated to cause changes in cardiorespiratory measures (Rhudy and Meagher 2000; Porro et al. 2002, 2003; Homma and Masaoka 2008; Franciotti et al. 2009). The findings of this chapter, where it was suggested that solution-based alterations in the activity of group III and

IV afferents were unlikely directly facilitate a cardiorespiratory response, provide important knowledge for the use of hypertonic saline during both single-limb and whole-body exercise. As such, without a confounding cardiorespiratory response, this study was essential in support of the previous experimental findings in this thesis (Chapters 3 and 4, Studies 1 and 2) but also confirm the suitability of the hypertonic saline model to be applied to whole-body, locomotive exercise in the final experimental study of the thesis. This should therefore allow for a more robust insight into the isolated role of EIP (opposed to afferent feedback) on exercise performance.

Based on the findings of Chapter 5 (Study 3), the final experimental study (Chapter 6) of this thesis investigated whether the bilateral induction of acute muscle pain (via hypertonic saline) in the VL would impair the performance of heavy-intensity cycling time to task failure in an equivalent manner that was observed during single-limb isometric exercise in the first experimental study (Chapter 3). Previous research has shown a clear association between the experimental manipulation of pain, typically through attenuating painful sensations via ergogenic aids (e.g. caffeine and paracetamol), and changes in both single-limb and whole body exercise task performance (Mauger et al. 2010; Gonglach et al. 2016; Astokorki and Mauger 2017b, a; Morgan et al. 2018; Tomazini et al. 2020) To our knowledge, this is the first study to specifically accentuate the experience of pain through the bilateral application of hypertonic saline during the performance of locomotive endurance exercise. As with the previous experimental studies, both physiological (e.g. cardiorespiratory, blood lactate), psychological (e.g. pain catastrophizing) and perceptual (e.g. pain intensity, RPE and ROF) responses were recorded during the exercise protocol.

The primary and novel finding of this experimental study was that the bilateral hypertonic saline combined with heavy intensity exercise significantly exacerbated pain, and shortened cycling time to task failure performance. Specifically, compared with control (no injection) and placebo (bilateral injection of isotonic saline), the bilateral hypertonic saline significantly impaired performance by 19 to 23%. This impaired performance was coupled with an increased experience of pain (e.g. pain intensity was significantly elevated for the first half of the exercise task) and paralleled with increased perceptions of fatigue and exertion. This study therefore

provides evidence which reinforces and develops upon previous research, supporting the notion that muscle pain may accelerate the development of fatigue and could therefore be key limiting factor in endurance performance.

The limiting impact of EIP on single-limb and whole-body exercise tasks

Combined, the key findings of this thesis contribute to developing our understanding of the impact that muscle pain has on both single-limb and whole-body exercise tasks. This was achieved through the confirmation (Chapter 3) and subsequent application (Chapters 3 to 6) of a novel experimental model representative of naturally occurring EIP, which will provide a useful tool for future experimental investigation of the fatigue-pain relationship. The review of literature outlined several physiological (i.e., an unconscious effect of nociception) and psychological (i.e., drive to escape the perception of pain) mechanisms which, individually or collectively, could provide an explanation of how EIP may exacerbate or contribute to the development of fatigue, and therefore have a limiting impact on endurance performance. Whilst the direct exploration of all potential mechanisms was beyond the scope of this thesis, several physiological, psychological and perceptual variables were recorded across all experimental chapters (Chapters 3 to 6) in an attempt to gain indicative evidence of how EIP may influence endurance performance

An increase in muscle pain reducing time to task failure in both single-limb (Chapter 3) and whole-body (Chapter 6) exercise, as well as causing an impaired torque reproduction performance (Chapter 4) could be interpreted to be in line with the "Inhibitory Feedback Model" proposed by Amann and colleagues (Amann and Dempsey 2009, 2016) (See Table 1.1). This model proposes that feedback from Group III and IV afferents centrally project to regulate descending motor drive to the exercising muscle in order to prevent the development of fatigue beyond an individual critical threshold/sensory tolerance limit (Amann and Dempsey 2009, 2016). The addition of hypertonic saline to short-duration (Chapter 4, Study 2) or prolonged (Chapter 3 and 6, Study 1 and 4) muscle contractions is likely to create a noxious mechanical and chemical environment which increases the activity of the Group III and IV nociceptive afferent fibers, subsequently increasing the experience of pain (Laursen et al. 1999; O'Connor and Cook 1999).

During the time to task failure protocols (Chapter 3 and 6, Study 1 and 4) the increased inhibitory feedback from these afferents and the concomitant elevated pain intensity could have contributed to the earlier incidence of this sensory tolerance limit, resulting in the earlier voluntary termination of exercise (Aboodarda et al. 2020). In addition, based on the Inhibitory Feedback Model, the increased nociceptive activity may have modified neuromuscular output, reducing both central motor drive and the ability to recruit motor units, accelerating the development of central fatigue (Amann et al. 2009; Amann 2011) (See Table 1.1). Previous research has demonstrated that increased afferent activity resulted in a declined voluntary activation of the exercising muscle or muscles (Kennedy et al. 2013) and an impairment in the ability to maximally produce force (Graven-Nielsen et al. 2002).

Therefore, during the open-loop exercise tasks in this thesis (Chapter 3 and 6, Study 1 and 4), these changes could have had a negative impact on exercise performance through increasing the relative task difficulty and requiring a compensatory increase in descending motor drive to maintain the required level of torque (Chapter 3) or power (Chapter 6), thereby promoting a faster progression of central fatigue (Gandevia 2001). In the torque reproduction task, an alteration in the effort required to drive the limb and generate the target torque (without visual feedback) could contribute to a reduced accuracy in estimating the torque being produced by the painful muscle. This finding has been demonstrated in prior research using the elbow flexor muscles, where participants attempting to match torque through subjective effort made significant errors in a force matching task (Proske et al. 2004). At present, these are however only postulations, with methods such as peripheral nerve stimulation and transcranial stimulation required to gain an understanding of this potential mechanism of pain during exercise.

From the perspective of other models of fatigue and endurance performance, the CGM dictates that in open-loop protocols, exercise is terminated prior to the critical incidence of fatigue (a sensation regulated by changes in RPE) with the primary goal to maintain homeostatic and protect from catastrophic physiological fatigue (see Table 1.1) (Noakes et al. 2005; Noakes 2012). This model acknowledges the role of metabolites and afferent feedback in the complex calculation of expected

exercise duration, and whilst EIP is not explicitly discussed, the definition of RPE used is in line with "exertion" opposed to "effort" and therefore encompasses feelings of pain and discomfort (see Table 1.1) (Noble and Robertson 1996; Noakes et al. 2004, 2005). Therefore, findings of an increase in afferent feedback (i.e., nociception and the subsequent perception of pain) via the hypertonic saline and a subsequent impairment in performance (independent of fatigue) could be construed to be in support of this model's postulation of homeostatic preservation (see Table 1.1). In addition, whilst the increased pain from the hypertonic saline combined with the muscle contractions could provide a strong motivational function to terminate from the exercise task, the lack of any difference in RPE (Chapter 3) suggests that the Psychobiological Model is unable to explain the earlier task disengagement (see Table 1.1).

In Chapters 3 and 4 (Studies 1 and 2), sEMG mounted in a bipolar set-up over the VL, VM and RF were recorded and analysed to monitor changes in muscle electrical activity during the exercise tasks following experimental pain induction. It is well accepted that pain is associated with compensatory alterations in muscle activity or a change in motor unit recruitment order with the primary purpose to protect the body and reduce perceptions of pain (Hodges and Tucker 2011; Bank et al. 2013). The studies in this thesis demonstrated no change or difference in sEMG signal, despite the pain-induced impairments in performance. Whilst the antagonist muscles were not recorded in these studies, it is implied that this key finding provide further evidence in contradiction of the "Pain Adaptation Model" (Lund et al. 1991), where it is proposed that pain should consistently result in an inhibition of the agonist muscles, whilst simultaneously activating the antagonists.

Instead, it has since been recognised that pain does not produce uniform inhibition and excitation effects across the motor neurone pool (Hodges and Tucker 2011). The "moving differently in pain" theory postulates that muscle pain (or the threat of pain) initiates changes across the motoneuron pool (causing a redistribution of activity between and within muscles and a change in mechanical behaviour) with the immediate benefit of protection from further pain or injury (Hodges and Tucker 2011; Bank et al. 2013). However, such an alteration in strategy is suggested to have latter consequences that may affect performance of exercise tasks. Therefore, according to

this hypothesis, any change in muscle activity and mechanical behaviour (e.g. altering the direction of knee extensor torque) could impair the efficiency of exercise (influencing the development of fatigue) or quality of movement, which could consequently explain the impairments in performance of the TTF (Chapter 3, Study 1) and torque reproduction (Chapter 4, Study 2) tasks. Whilst an alternate methodological approach of greater sensitivity is required to robustly evaluate this (see Section 7.3), the findings of this thesis (i.e. no change in amplitude of gross sEMG) could be considered to be in tentative support of this theory (Hodges and Tucker 2011).

The "moving differently in pain theory" also outlines that compensatory alterations in recruitment order and firing frequency in response to pain occur at a motor unit level. Under regular conditions, motor units are systematically recruited from low- to high-threshold (Henneman et al. 1965; Milner-Brown et al. 1973). During endurance tasks, feedback from nociceptive afferents and the presence of muscle pain have been found to inhibit the activation of the low-threshold motor units, requiring the recruitment of additional motor units unaffected by the noxious stimuli to allow for the muscular contractions to be maintained (Farina et al. 2004; Tucker et al. 2009). Techniques such as high-density sEMG are required to detect changes in motor unit behaviour (e.g. discharge rate and recruitment threshold), however other approaches can be used to provide a more speculative insight. For example, in Chapter 6 (Study 4), despite the shortened cycling time to task failure performance the recorded change in blood lactate concentration was similar in the hypertonic condition compared to placebo and control conditions.

Accordingly, this could be suggestive of a compensatory mechanism where large, high-threshold motor units were preferentially recruited above the inhibited smaller low-threshold motor units at an earlier time-point in the exercise task. Indeed, this has been previously demonstrated in an alternate muscle (tibialis anterior), where the recruitment and de-recruitment threshold of the larger motor units was lowered, therefore prolonging the duration in which they were activated (Martinez-Valdes et al. 2020). Whilst such an adaptation is beneficial for rapid force development and allowing for the continuation of exercise at a set work-rate despite the presence of muscle pain, during endurance exercise this is fundamentally a metabolically

inefficient recruitment strategy (Hodges and Tucker 2011; Martinez-Valdes et al. 2020). Resultantly, this is likely to have implications for the development of fatigue (e.g. accelerate the accumulation of metabolites), leading to a greater fatigue of the muscle tissue (Edwards 1981; Martinez-Valdes et al. 2020). This could therefore provide an alternative explanation for how the elevated experience of pain had a significant limiting impact on performance, resulting in a shorter time to task failure in both the single-limb (Chapter 3) and whole-body (Chapter 6) endurance exercise tasks.

As a multidimensional construct (sensory and emotional), in addition to the aforementioned physiological mechanisms, the psychological component of EIP should also be considered. As the perception of pain is not necessarily proportional to the nociceptive input and can be strongly influenced by psychological and cognitive factors (Wiech and Tracey 2009), a strength of the experimental studies in this thesis was in the consistent confirmation of similar psychological state across experimental visits through measures such as positive and negative affect as well as the consideration of pain-specific expectations and confidence. This attempted control of psychological and cognitive factors that may influence the perception of pain was important to ensure a greater intra-individual reliability for between-condition comparisons.

In addition, EIP itself could also have a direct psychological impact on exercise performance. As pain is fundamentally a physiological warning signal, it has been suggested that the negative emotional response associated with the anticipation of or in response to pain during exercise is accompanied by an avoidance drive to escape the painful stimulus (e.g. task disengagement) (Fields 1999). All experimental studies in this thesis recorded the affective dimension of pain (e.g. pain unpleasantness via the multidimensional MPQ), the emotional response and accompanying avoidance drive to escape a painful stimulus (Boggio et al. 2009; Horn et al. 2012), whilst Chapters 5 and 6 (Studies 3 and 4) reported pain-specific catastrophizing post-exercise (a negative cognitive and affective response to pain associated with pain tolerance and task motivation) (Sullivan et al. 2001, 2002; Nijs et al. 2008. No differences in the affective dimension of pain were observed throughout this thesis, whilst greater catastrophic thinking occurred when pain was exacerbated by the

hypertonic saline during the cycling TTF. Whilst these findings provide some insight into potential psychological mechanisms that may explain the fatiguing impact of EIP, this is approach limited (in terms of time sensitivity) and therefore requires further investigation (see Section 7.3).

7.2 Limitations and considerations

This thesis consists of four novel experimental studies that confirm the hypertonic saline model as an appropriate method to experimentally replicate the experience of EIP, which when applied contributed to an improved understanding of the fatiguing or limiting role of EIP during both single-limb and whole-body exercise tasks. Despite the strengths of this thesis in presenting original findings from a range of research approaches and measures of exercise performance, there are several limitations which are apparent across the four experimental chapters. These limitations relate to the methods employed, a lack of direct mechanistic insight and the characteristics of recruited participants.

Methodological considerations

Firstly, one key factor that is a limitation of this thesis is the inconsistent use of placebo and control (i.e., "no treatment") groups in the research design of the experimental chapters. The primary recommendation derived from the consensus statement on placebo and nocebo effects in sport and exercise (Beedie et al. 2018) highlights the importance of including a no-treatment group alongside the placebo (isotonic saline) and treatment (hypertonic saline) groups to improve research validity and reliability. Whilst all three conditions (control, isotonic saline and hypertonic saline) were implemented in the first and last experimental studies in this thesis (Chapters 3 and 6), this did not occur in the second and third studies (Chapters 4 and 5).

In particular, it is acknowledged that the fundamental limitation of Chapter 5 was the omission of a bilateral isotonic saline condition, which would have significantly strengthened the experimental design and study outcomes (i.e., robust evidence to support or challenge the "stress response" hypothesis). In addition, it could also be argued that whilst hypertonic saline may not have a significant impact on the reflex cardiorespiratory response, the stress response elicited (from the "threat" associated

with the injection procedures and expected level of pain from the hypertonic saline solution) may have either eclipsed an exercise pressor response or had a direct confounding impact on endurance performance itself. Nonetheless, whilst this thesis limitation is recognised, the design and findings of Chapters 3 and 6 (where no differences in performance are observed between control and placebo conditions) provide support for the notion that any cardiorespiratory response elicited from the pain induction model was unlikely to have had significant implications for performance. It is however suggested that, alongside the other proposed conceptual and recommendations from the consensus statement (Beedie et al. 2018), the inclusion of a control condition is an essential element of study design for future research conducted in this area.

Secondly, the infusion paradigm of the hypertonic saline used in this thesis both imposed some considerations and restrictions on the design of the exercise protocols employed. In all experimental studies, the isotonic and hypertonic saline solutions were manually injected as a single bolus by trained experimenters. All experimenters received the same training and administered the injection using standardised procedures, however the manual opposed to computer-controlled technique may have presented some subtle variations in administration (e.g. needle placement, rate of infusion). In addition, as outlined in the literature review, the manual infusion of a single bolus produces a dynamic pain intensity response, with a rapid onset of muscle pain that intensifies, reaches a maximal point and then declines back to the state of "no pain", typically lasting up to 5 minutes in duration (Lewis 1938; Kellgren 1938; Svensson and Arendt-Nielsen 1995; Graven-Nielsen et al. 1997c; Graven-Nielsen 2006)

In the right VL, data from Chapter 3 (Study 1) highlighted that a bolus (1.0 mL) injection of hypertonic saline at rest on average produces a "moderate" intensity of muscle pain, which peaks after approximately 1 to 2 minutes and returns to "no pain" after 3 to 6 minutes. The bilateral injection performed at rest (Chapter 5, Study 3) increases the reported intensity of muscle pain as equivalent to "somewhat strong", which reaches a greater peak pain intensity that occurs at a similar time-point (1 to 2 minutes) to the unilateral administration, and returns to "no pain" after 3.5 to 9.5 minutes. Whilst not necessarily a limitation of this thesis, it is important to be aware

of and account for the typical VAS time-course observed in response to a manual bolus injection of hypertonic saline when designing future studies and experimental protocols.

It is also important to consider the inter-individual differences in response to this experimental pain model. Observationally, a prominent variability in response to the hypertonic saline was present between individuals, with some participants evidently responding more strongly (i.e. a greater pain intensity and quality) than others. Whilst elucidating the reason(s) for the disparities between "responders" and "non-responders" was beyond the scope of this thesis and should receive further investigation, the notable inter-individual differences presented significant issues for the main performance outcome of Chapter 3 (Study 1). Here, it could be suggested that (combined with an insufficient contraction intensity), the shorter duration of pain response may have contributed to the exclusion of a third of the participants (n = 6). In addition, the consideration of the pain response from the hypertonic saline resulted in constraints in the experimental design of Chapters 4 and 6 (Studies 2 and 4). For example, the dynamic and variable response to this experimental muscle pain between individuals limited the possible number of attempts at the torque reproduction task in Chapter 4 (to ensure that all contractions were performed at a similar pain intensity) and contributed to the selection of exercise intensity for the cycling time to task failure protocol (Chapter 6).

It should also be noted that the mechanisms associated with the development of fatigue and performance will differ dependent on factors such as modality (e.g. single-limb isometric exercise compared to whole-body locomotive exercise; see Section 1.3.1.), duration and intensity of the task performed. It should therefore not be assumed that findings in one exercise task are comparable or will translate to that of another. For example, Chapters 3 and 4 (Studies 1 and 2) use well controlled single-limb isometric tasks to isolate the experience of pain to a muscle group and minimise the role of alternate confounding physiological systems (e.g. the cardiorespiratory system). Whilst these studies are able to *indicate* how an exacerbation of muscle pain may influence performance during exercise tasks of a superior ecological validity for 'real' sporting competition (e.g. dynamic muscular contractions or whole-body exercise), the findings should not be treated as conclusive. This thesis does provide

some evidence of the impact of muscle pain on performance of a heavy-intensity cycling time to task failure (Chapter 6, Study 4), however exploration of alternate exercise characteristics is also required.

A final consideration and potential limitation of this thesis is the time of day in which the experimental work was conducted. Controlling for time of day is a fundamental tenet of exercise testing to account for circadian and diurnal variations in physiological measures and performance (e.g., maximal strength of the knee extensors, cycling time to task failure) (Lange Andersen et al. 1971; Reilly and Baxter 1983; Guette et al. 2005; Sedliak et al. 2008). Each study outlined in this thesis controlled for time of day within individuals, with each laboratory visit commencing at the same time of day (± 2 h). This was, however, not completed between participants.

Lack of direct mechanistic insight

This thesis has presented some interesting findings that could be used to speculatively evaluate the proposed physiological and psychological mechanisms by which EIP may contribute to the development of fatigue and limit endurance performance (see Section 1.4.1). However, without the use of techniques such as peripheral nerve/transcranial stimulation, multiple force transducers, fine wire electrodes or high-density surface EMG (HDsEMG), this thesis is unable to define the precise physiological mechanisms, whilst the use of psychological scales in this thesis had insufficient time sensitivity to detect any subtle changes in the measured variable during exercise. Future work should therefore aim to evaluate the findings and postulations made in this thesis to provide greater clarity to improve understanding of specifically how EIP limits endurance performance (see Section 7.3).

Participant characteristics

All experimental studies in this thesis recruited male and female participants who were healthy and recreationally active. The representation of both males and females in experimental research is important, particularly as it is inappropriate to apply findings from one sex to the other due to the natural differences between both male and female participants (e.g. anatomical, physiological, endocrinological,

psychological and social) (Sheel 2016). Recruiting both sexes can however present issues, with this thesis in particular, not accounting for or attempting to control the menstrual cycle of the female participants. Hormonal changes across the different phases of the menstrual cycle may result in an inferior maximal strength and endurance performance and could also cause some difference in pain perception to experimental pain (Janse de Jonge 2003; Lei and You 2012; McNulty et al. 2020). This reduced control was primarily due to the study design where visits were required to be completed in a set time-interval. However, it is acknowledged that conducting sex-specific research or simply recording the stage of the menstrual cycle for the female participants may be of benefit for future work.

Secondly, the decision to recruit recreationally active individuals limit the ability to generalise findings to different athletic populations (e.g. trained to elite endurance athletes). Elite and non-elite athletes have distinct differences in physiological characteristics and psychological drive, which may dictate variations in the mechanisms that underpin performance. Additionally, competitive athletes, who are more likely to participate in regular and painful training sessions, are believed to be more “stoical” and have an enhanced tolerance of pain compared to non-competitive athletes (Ryan and Kovacic 1966; Scott and Gijsbers 1981; Ord and Gijsbers 2003; Tesarz et al. 2012, 2013). Indeed, it has been demonstrated through chronic aerobic (but not resistance) cycling training it is possible to improve pain tolerance, which was suggested to be resultant from psychological (i.e. development of efficient coping skills) as opposed to physiological adaptations (Anshel and Russell 1994; Jones et al. 2014). Based on this, whether the decrements or impairments in performance established by the experimental research of this thesis would be observed at a similar magnitude in a more trained population is therefore questionable and hence requires further exploration.

7.3 Implications and future directions

The findings presented in this thesis present several opportunities for future research which can be broadly categorised into five distinct areas (experimental model, exercise performance, population tested, physiological mechanisms and psychological mechanisms). First, based on the limitations of this thesis, research could investigate

and further develop the hypertonic saline experimental model itself. It has been previously established that the infusion of hypertonic saline into the tibialis anterior has good intra-individual reliability for intensity, quality and distribution (Graven-Nielsen et al. 1997b). As the muscle pain experienced from the hypertonic saline model is partially dependent on the tissue injected (a significant factor in the infusion paradigm), the same response may not be observed in the VL, a significantly larger muscle compared to the tibialis anterior with a key role in locomotion. As this is yet to be established, it is pertinent to evaluate the reliability of the injection parameters employed in this thesis (i.e. volume, concentration, rate of injection) when the solution is administered into the VL. Being able to demonstrate good test-retest reliability of the pain experienced (in terms of intensity and quality) from this model applied to the VL is important when comparing within-individual responses to repeat experimental visits (i.e. participants are likely to have a similar experience across repeat experimental visits), helping to potentially confirm the robustness of findings in this thesis and improving confidence in applying the model in future research.

The injection of a single bolus into the tibialis anterior has also demonstrated large inter-individual variation in previous research (Graven-Nielsen et al. 1997b). Notable differences in pain response between individuals was evident throughout this thesis, particularly in the pain intensity and duration induced by the method, which had a notable contributing impact on the performance outcomes of the first experimental study (Chapter 3). It would therefore be pertinent for future research to consider exploring and addressing the issues associated with the standardisation and subsequent variable pain response to the hypertonic saline pain model. Whilst computer-controlled syringe pumps (combined with continuous feedback from a VAS) can be used to provide a standardised infusion to maintain a desired steady-state pain intensity or duration both within and between individuals (Graven-Nielsen et al. 1998a; Graven-Nielsen and Arendt-Nielsen 2003; Fazalbhoy et al. 2012b), this method is limited by both accessibility to equipment and the inability to perform some forms of exercise whilst concurrently receiving the infusion.

As such, refining the current, more accessible, infusion paradigm through manipulating factors such as volume infused, rate of infusion, needle depth or angle and improving understanding of the tissue injected could help optimise the technique

in its present form. For example, as an extensive range of volumes of hypertonic saline have been utilised in the experimental literature in an array of muscles or muscle groups (Arendt-Nielsen et al. 1996; Svensson et al. 1996; Graven-Nielsen et al. 1997e; Ervilha et al. 2005; Farina et al. 2005a; Ge et al. 2006; Falla et al. 2009; Khan et al. 2011; van den Hoorn et al. 2015; Larsen et al. 2018), it would be of interest to review the effect of different solution volumes on the pain response elicited. In addition, techniques such as ultrasound (combined with measures of pain intensity of quality) could be used to gain an insight into muscle characteristics and the effect of needle depth or angle, with the purpose to improve understanding of the saline volume distribution within the muscle and how this may relate to changes in the pain experience. Alongside improving knowledge of the variability of pain response to the hypertonic saline solution, findings from such research could also provide the basis to guide needle placement during the intramuscular injection procedure.

In addition, exploring and subsequently identifying the factors (e.g., genetic, demographic, physiological and psychosocial characteristics) which underpin the intra- and inter-individual (i.e., a comparison between more “responsive” compared to “non-responsive” individuals) differences in pain response could be beneficial to improve understanding of the variance in response to the hypertonic saline model. This could subsequently allow for the development and application of intervention strategies to control this variation more tightly, and therefore optimise the response to the hypertonic saline, ensuring a more standardised response.

Second, this thesis has predominantly applied the hypertonic saline method of experimental muscle pain induction to open-loop time to task failure exercise protocols as a method to evaluate the impact of increased pain on endurance performance, and the possible effect this may have on physiological, psychological and perceptual measures (Chapters 3 and 6, Studies 1 and 2). The use of a fixed-intensity time to task failure protocol was effective in demonstrating that the experimentally induced muscle pain resulted in the hypothesised performance and response. However, the selected exercise intensities or durations for these studies were constrained by the current limitations of the hypertonic saline method, and

therefore the previously suggested studies could help to provide more flexibility in protocol design.

In addition, whilst Chapter 4 (Study 2) provided an insight into the potential effect of EIP on regulatory measures, this was restricted to a single-limb isometric exercise model and there is therefore the need to progress these investigations to dynamic or whole-body muscle contractions to improve ecological validity. Particularly, closed-loop exercise tasks such as time-trials provide an applied simulation of actual performance where participants are able to regulate work-rate to self-pace their effort with the goal of optimal distance or time completion (Laursen et al. 2007; Currell and Jeukendrup 2008). This thesis suggests that the presence of muscle pain may induce debilitative physiological adaptations that could impact self-selected work-rate regulation during endurance performance. It would, however, be prudent for future work to evaluate the impact or role of EIP on the ability to effectively regulate pace during a bout of whole-body exercise, and if so, gain an insight into how this necessarily occurs. Constraints imposed by the current iteration of the hypertonic saline method should however be taken into consideration, and as such it is suggested that a fixed-time protocol (where the completion of as much work as possible within a set time defines performance) that is approximately matched with the time-course of the bilateral solution action would be most appropriate at present.

The current thesis primarily focused on the impact of muscle pain on performance (TTF and torque estimation), and the effect this may have on physiological and perceptual variables. Whilst an element of the impairments in performance (i.e., reduced TTF) are likely to be associated with an increased or accelerated fatigue, this has not been directly measured in this thesis. Some measures which provide an *approximate indication* of fatigue development were however included. For example, in Chapter 3 (Study 1), a comparison between baseline and post-exercise MVC provided an indication of global fatigue development during the single-limb isometric TTF, whilst perceived fatigue was reported in Chapters 3 and 6 (Studies 1 and 4) via the ROF scale (which has been shown to have a linear relationship with objective measures of fatigue). However, to comprehensively examine the role of muscle pain on the development of fatigue, techniques that allow for the identification of both the

origin(s) and subsequently the extent at which specific sites in the motor pathway contribute to reductions in force production, are required.

The exact mechanisms (physiological and psychological) which accelerate the development of fatigue and limit performance are, at present, yet to be determined. In this thesis it has been suggested that an increase in inhibitory feedback from Group III and IV nociceptive afferents in response to the combination of experimental pain and muscle contraction may limit central motor drive and voluntary activation of the knee extensors, promoting the development of central fatigue (Amann et al. 2011a, 2015; Sidhu et al. 2018; Aboodarda et al. 2020). To examine this, follow-up research should include techniques such as transcranial magnetic stimulation (TMS) and electrical stimulation of the femoral nerve combined with surface electromyography (sEMG) to assess measures of neuromuscular function (e.g. voluntary activation level [VAL], cortical silent period [CSP], twitch torque [TT], motor evoked potential amplitude [MEP_{AMP}], and muscular wave [M-Wave]) both during and post-exercise, which will allow for the quantification of both central and peripheral fatigue (see Section 1.2.1). This could provide an insight into why TTF performance was reduced in Chapters 3 (Study 1) and 6 (Study 4), as well as potentially explaining the discrepancies in perceived and actual torque produced in Chapter 4 (Study 2).

A further proposed mechanism of the impaired task performance observed in Chapters 3 (Study 1) and 4 (Study 2) were the compensatory changes in motor behaviour, specifically alterations in muscle activity and motor unit recruitment in an attempt to reduce pain or protect the painful area, which occur with the increased experience of muscle pain during exercise (Hodges and Tucker 2011). Whilst the findings in this thesis are able to provide contradictory evidence to the theory of the "Pain Adaptation Model" (Lund et al. 1991), the bipolar electrode arrangement used to record electromyographic activity only provides a summary of the compound muscle activity based on electrical signals sampled from a small section of the muscle and therefore has insufficient sensitivity to detect the subtle changes in activity that can occur both within and between muscles (Merletti et al. 2010; Falla and Gallina 2020). As such, whilst this thesis is able to provide tentative support for the "moving differently in pain" theory (Hodges and Tucker 2011), an alternate sEMG configuration should be used in future studies.

Methods such as fine-wire intramuscular EMG could provide information of activity of the muscle fibres, however this procedure is invasive and unsuitable for use during exercise protocols. Alternatively, HDsEMG is a non-invasive technique that can investigate the spatial distribution of activity within muscles, and therefore detect non-uniform changes across the motor neurone pool (Falla and Gallina 2020). Future experimental laboratory studies could apply a HDsEMG electrode array to the VL during exercise tasks such as the sustained isometric contraction TTF used in Chapter 3 (Study 1) for instance. The use of this novel technique would therefore be beneficial to improve understanding of the potentially debilitative changes in muscle activity and motor unit recruitment that can occur in the presence of muscle pain impede exercise performance.

Alongside physiological mechanisms, further exploration of the psychological impact that EIP may plausibly have during endurance performance is also required. This thesis provided provisional evidence of increased catastrophic thinking (associated with a greater intensity of pain) potentially contributing to the behavioural drive to terminate an exercise task, however there are several additional factors that may have contributed to this effect. The measurement of psychological variables in this thesis have predominantly been administered prior to or immediately upon the completion of the exercise tasks. The scales selected with a primary focus on the potential factors that may be associated with differences in pain perception or exercise performance (e.g. emotion, emotional regulation, resilience, expectations and confidence), and therefore the purpose of measurement before exercise was to enable their control between participants and experimental conditions. Other scales, which were recorded post-exercise, were also used in an attempt to summarise or imply overall changes in psychological state that may have occurred during the exercise task (e.g. pain-specific catastrophizing).

However, this approach has the limited time sensitivity required to detect the refined changes that may have occurred during exercise over time. Therefore, it is suggested that future experimental research could specifically measure how psychological variables (e.g. pain unpleasantness, self-efficacy beliefs, emotion, motivation) can change over time during exercise performance in relation to an increased experience

of muscle pain. This approach could provide further knowledge of the interaction between physiological and psychological factors on exercise performance. In addition, should EIP have a significant psychological impact on endurance performance, these findings could inform potential training-based interventions (e.g. a guidance on implementing psychological techniques) to aid athletes and individuals to enhance tolerance of the unpleasant sensations of pain, and thereby potentially improve exercise performance.

A final suggested area of future exploration for experimental laboratory research evaluating the impact that EIP can have on exercise performance in a variety of different populations. This thesis primarily recruited healthy and recreationally active participants, demonstrating reductions in single-limb (Chapter 3, Study 1) and whole-body (Chapter 6, Study 4) endurance performance by between 12 to 26% in the presence of augmented muscle pain. Whilst increasing understanding of the relationship between EIP and fatigue, as well as the importance of pain tolerance in endurance exercise tasks in what could be considered to be a "regular" individual, further insight is required in clinical (i.e. in conditions where pain occurs resultant from or post exercise) and athletic populations where EIP could also have a significant impact.

For example, individuals of a superior training status, who are likely to participate in more regular and painful training regimes, are likely to have a greater tolerance for EIP (Tesarz et al. 2012) which may lessen the impact this has on their endurance performance. Whether the decrements in endurance performance observed in this thesis would occur in a more trained population requires further exploration, with some uncertainty on whether intense and painful training would have a meaningful difference on performance in this population. Therefore, future research should not only apply similar experimental protocols in this thesis to a trained, athletic population, but to also explore the potential benefits of aerobic or resistance training in the presence of increased muscle pain (via the hypertonic saline model) in a previously untrained group of participants. This would also present the opportunity to specifically investigate and carefully monitor potential physiological or psychological adaptations that underpin the improvements in tolerance, which has previously received limited attention.

7.4 Conclusion and perspectives

This aim of this thesis was to investigate the role of EIP on both single-limb and whole-body exercise performance through the experimental induction of muscle pain using a model that closely replicates the experience of naturally occurring EIP. Therefore, there were two overarching aims of the thesis. The first was to apply the hypertonic saline method of experimental muscle pain, and to evaluate whether this method provides a suitable pain induction model that can be implemented during exercise. This thesis confirmed that participants were unable to distinguish between the experimental muscle pain produced by a 1.0 mL bolus of 5.8% hypertonic saline into the VL and the EIP from a muscular contraction, and when combined with a low-intensity contraction the pain experienced felt like a greater exercise intensity (Chapter 3, Study 1). In addition, Chapter 5 (Study 3) determined that alterations in the activity of Group III and IV afferents and concomitant acute muscle pain from the unilateral or bilateral application of this experimental model may not directly facilitate a confounding exercise pressor reflex (i.e. an increased cardiorespiratory response), which would be unfavourable for its application to exercise. The intramuscular injection of hypertonic saline therefore fulfils the outlined criteria of a desired experimental pain model that 1) induces muscle pain that *feels like* naturally occurring EIP, 2) uncouples the relationship between EIP intensity and work-rate, and 3) does not elicit additional responses that may influence exercise performance.

The second aim of this thesis was to then apply this experimental model to examine and understand the impact of EIP during single-limb and whole-body exercise tasks relevant to endurance performance. This aim was addressed through three experimental chapters (Chapters 3, 4, and 6) which demonstrated the impairment of single-limb torque reproduction (Chapter 4) and time to task failure performance in both single-limb and whole-body exercise (Chapters 3 and 6) concomitant with an increased pain in healthy and recreationally active participants. These studies therefore provide new evidence supporting the concept that EIP is a key limiting factor in endurance performance and could potentially have a detrimental effect on the ability to self-regulate exercise intensity.

In each of these experimental studies, physiological (e.g. cardiorespiratory, blood lactate, sEMG), psychological (e.g. pain catastrophizing) and perceptual (e.g. pain intensity, RPE and ROF) responses were recorded during the exercise protocol. The impairments in performance were not accompanied by a change in bipolar sEMG (Chapters 3 and 4) or blood lactate concentration (Chapter 6), however increased pain-specific catastrophizing was observed post-exercise (Chapter 6). Perceptions of effort and fatigue were unaffected during single-limb isometric TTF, however were initially elevated during the cycling exercise task, whilst the impairment in torque reproduction ability was accompanied by a greater perceived effort to drive the limb. These findings support the notion that EIP may have both a physiological and psychological impact on exercise, however future studies should attempt to specifically investigate the underlying mechanisms causing these changes in performance.

Overall, this thesis and its experimental studies contribute to the development of knowledge into how an exacerbated experience of pain during exercise (induced through a novel experimental model) affects endurance performance. It provides a novel experimental method to aid future investigations of the fatigue-pain relationship during exercise (Chapters 3 and 5) and offers new insights that advance our understanding of limiting impact that EIP may have in both single-limb and whole-body exercise tasks (Chapters 3, 4 and 6). In an area which until of recent has been inadequately explored and poorly understood, the key outcomes and implications of this thesis should hopefully be formative in a wide array of future investigations into experimental muscle pain and the impact this has on the performance of various exercise tasks.

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Appendices

APPENDIX A: EXAMPLES OF STUDY PARTICIPANT INFORMATION SHEET, HEALTH QUESTIONNAIRES AND CONSENT FORM

Participant information sheet:

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Effects of acute bilateral experimental exercise-induced pain on cycling time to exhaustion performance

Researcher
Samuel Smith
Supervisors: Dr Lex Mauger, Dr Samantha Winter & Professor Dominic Micklewright

Invitation for Participation

You are being invited to take part in a research study. Prior to deciding on whether to participate or not, it is of importance that you have a thorough understanding of the rationale behind this study, and what it will involve in terms of your participation. Please take some time to read the following information carefully. If you have any further questions, you require additional information, or something is not clear, please do not hesitate to contact the researchers on the emails included below.

Background Information

Naturally occurring muscle pain is commonly reported during the performance of exercise. This 'exercise-induced pain' (EIP) is often described as an 'intense', 'burning' and 'cramping' sensation, which is experienced primarily in the exercising muscles, but spreads to additional locations over time. As EIP has been demonstrated to increase in a linear fashion with work-rate or time elapsed, it has been proposed that increasingly unpleasant or intolerable EIP could be a factor in the decision-making process for alterations in work rate or the potential cessation of exercise.

In order to investigate the impact of EIP during endurance tasks, an experimental model that provides a replicative experience of EIP should be utilised. This can be achieved through the injection of a small volume of sterile salt water solution into the muscle which has been shown to closely imitate the intensity, distribution and quality of pain associated with EIP. As the experience of EIP is primarily reported to occur during dynamic or locomotive tasks, understanding the effects of experimentally induced EIP in differing

exercise-based conditions is of a greater applicability to tasks that are performed in daily life, as well as in exercise rehabilitation and performance. The primary aim of the study is to therefore apply and investigate the effects of pain induced from an injection of the sterile salt water solution into a quadriceps muscle of both the right and left leg prior on the performance of a short-duration cycling time to exhaustion task.

Who can take part?

This research study will look to recruit healthy, young, male and female participants (18 to 45 years old). You must be free of cardiovascular disease, lower limb injury, pre-existing medical conditions (neurological disorders and blood borne viruses such as hepatitis B/C and HIV) and any allergies (e.g. nuts, fish, milk, egg, wheat and soya). In addition if you have a long-term medication use and a phobia of needles, you will be excluded from the study.

Do I have to take part?

No. As participation is voluntary, you are not obliged take part in the study and have the right to withdraw from the study at any point without reason or any disadvantage to yourself. However, if you do decide to take part, you will be asked to complete and sign a health questionnaire and informed consent form to confirm your participation.

What will be required if I take part?

You will be asked to report to the Psychobiological exercise testing laboratory (M0-02) in the Medway Building on 4 separate occasions, with each visit separated by a recovery period of 2-7 days (a total participation time of up to 4 hours), dependent on whether the visit requires an injection. An injection visit will be separated by a minimum of one week, whilst a non-injection visit could be completed within two days after the previous visit. Injection visits will involve an intramuscular injection into the vastus lateralis (part of the quadriceps group on the outside of the thigh) of your right and left leg using a plastic syringe connected to a 25 Gauge x 38 mm needle. You will receive a total of two injections in each injection visit (i.e. one in the right vastus lateralis and one in the left vastus lateralis). The solutions used in this study are a single bolus of 1.0 mL sterile salt water and sterile water, which will be administered in the same combination between both legs in separate visits (i.e. sterile salt water in both legs in one visit, and sterile water in both legs in the other visit). Additionally, a mark will also be made around both injection sites, which you will be required to maintain for the duration of the study.

Prior to all visits, you will be required to refrain from:

- Undertaking any vigorous exercise (24 hours before each visit)
- Consumption of food (2 hours prior to each visit)
- Consumption of alcohol (48-hours prior to each visit)
- Consumption of caffeine (8 hours prior to each visit)
- Consumption of analgesics (6 hours prior to each visit)

All of the above will be confirmed prior to each visit.

What will be required from each visit?

Prior to the first visit, you will be required to complete two questionnaires for the measurement of pain resilience (PRS) and emotional intelligence (SSEIT). Additionally, before to each visit, you will be asked to complete a PANAS questionnaire for the measurement of mood and emotion. All questionnaires have been previously validated.

The requirements of each visit is listed below.

Visit One - Incremental test and protocol familiarisation

At the start of the familiarisation visit your height, body mass, and mid-thigh limb girth will be recorded. After a self-paced brief warm-up on a cycle ergometer, the first will require the performance of an incremental cycling ramp test until exhaustion, followed by 10 minutes of active recovery. After a further 20-minute period of rest, you will commence the pre-test measures and familiarisation of the cycling time to task failure protocol (see below). Finally, if you have not previously experienced an injection of sterile salt water solution in both the right and left leg, you will also be familiarised with this at the end of the session.

Visits Two to Four - Experimental visits

In each of the three subsequent experimental visits you will perform the pre-test measures followed by the cycling time to task failure after no injection (CON) or the after the injection of either sterile salt water (HYP) or sterile water (ISO) into the vastus lateralis of the right and left leg. All experimental sessions will be performed in a randomised order on the same cycle ergometer.

Incremental ramp test

The incremental ramp test will be performed at a self-selected cadence (70-90 rpm) (which will then be maintained for all subsequent exercise tests), with an increase in work rate of 1 W every 2 s. You will be required to cycle at your preferred cadence for as long as possible until the point of exhaustion. The task will finish at the point at which cycling cadence declines by more a set amount despite strong verbal encouragement to increase and continue for as long as possible.

Time to task failure

Each TTF will be preceded by three all-out efforts (pre-test measures). These efforts will be 5 seconds long and separated by 60 seconds of unloaded pedalling. These efforts will be used to determine the maximal activity of your knee extensor muscles. After 10 minutes of rest, the TTF will then commence, performed at the required power output (calculated based on your performance

from the ramp test), where you will be instructed to maintain cadence at the set intensity for the longest duration possible. The task will finish at the point at which cycling cadence declines by more a set amount despite strong verbal encouragement to increase and continue for as long as possible.

Measurements

During all visits you will be required to wear a heart rate monitor and a facemask (to allow for measurements of air that you breathe). You will also be required to wear electrodes to measure muscle activity (positioned on your right leg). The skin where the electrodes will be placed will be shaven and cleansed with alcohol, and marked with a permanent marker to ensure consistency in placement for future visits. Fingertip capillary blood samples will be taken before and immediately after completion of the incremental ramp test and the time to task failure to determine changes in blood lactate concentration.

For the incremental ramp test and time to task failure trials you will be required to continuously rate global rate pain intensity (cumulative experience in both legs), as well as provide verbal ratings of perceived exertion and fatigue at set intervals. Additionally in the time to task failure trials, you will be required to complete a McGill questionnaire, drawing the distribution of pain and stating the quality of pain immediately upon task failure.

How long will I have to take part for?

Each session should take up to 0.75-1.5 hours. All visits should be completed within 4 weeks (a total participation time of up to 4 hours).

Where will testing take place and what do I have to bring?

All tests will take place at the Psychobiological exercise testing laboratory (M0-02) in the Medway Building, Chatham Maritime, Kent. Please ensure that you attend each session with clothing that is suitable for exercise (shorts are essential).

Who is organising the research?

The research has been organised by the primary researcher and supervisors, with permission from the University of Kent School of Sport and Exercise Sciences. The study has also been reviewed and approved by the University of Kent ethical committee.

What are the disadvantages or risks of taking part?

As the tests require maximal effort you may experience the usual risks of vigorous exercise including breathlessness, sweating, discomfort, tiredness and fatigue. These feelings are however usually short in duration. Nonetheless, these risks will be minimalized through the familiarisation of test protocols, the inclusion of a warm-up and cool-down prior to and after

exercise, and sufficient periods of rest (minimum of 48 hours) between each testing session. In addition, you will be screened through a general health questionnaire to ensure that you are suitable and healthy to participate. The use of intramuscular injections of sterile salt water is a novel method which presents certain potential risks, including the possibility of pain or mild discomfort during, and for a short period after an intramuscular injection (*see appendix 'Intramuscular Injections: Background & Risk Assessment).* These risks have however been accounted for, with several interventions in place to reduce the risks.

After the completion of the injection procedure, you will be asked to report feelings from the injection sites to confirm your suitability for driving a vehicle. Should the felt muscle soreness be equivalent to that experienced after a vigorous bout of exercise, and you experience no pain within 15 minutes with no walking issues, there is no concern for your ability to drive.

In addition, you will be required to monitor the injection site two to four hours post-injection to ensure that no adverse reactions have occurred. However, in the event of an adverse response to this procedure (i.e. redness and heat at the site of injection, severe pain at the injection site, tingling or numbness, prolonged bleeding), you will be instructed to contact your GP or emergency services. This method has however previously been utilised safely and successfully in three previous research studies by this research team, and by groups based at other institutions.

What are the advantages and benefits of taking part?

Participation in this study will provide you with the opportunity to undertake physical activity, which is beneficial for a healthy lifestyle. Furthermore, you are able to request feedback of your maximal oxygen uptake, an indication of your aerobic fitness, and your cycling time to exhaustion, a measure of endurance performance. From taking part, you will also gain an understanding of your individual response to experimental muscle pain (in terms of typical response, tolerance and sensitivity), and will be contributing to further our knowledge of the effects of an experimental pain model (intramuscular injection of hypertonic saline) on whole body exercise performance.

As part of your participation in this study, you will receive up to £30 for your time and expenses, which will be provided pro-rata across the four sessions. The top three performing participants (i.e. the three individuals with the longest time to exhaustion times) will be 'rewarded' with an additional £60 (first place), £30 (second place) and £10 (third place).

What will happen to my data after completing the study?

All results will be subsequently analysed and written up for one or more conferences or peer-reviewed papers.

Will I find out the results of the study?

Yes. You are entitled to request a written summary of the results.

What about my privacy and confidentiality?

All data collected during the study will be anonymised, and you will receive a unique personal identification code. A master code will be kept in a secure locked cabinet by the researchers. In accordance with the Data Protection Act 1998, and the superseding General Data Protection Regulation, all electronic data will be kept securely in a password-protected folder on a password-protected laptop computer that belongs to the researcher, as well as on a password-protect computer stored at the School of Sport and Exercise Sciences. This data will be kept securely for up to three years. No data will be passed on to any third parties.

What if I wish to withdraw from the study?

Participation in this study is voluntary, and you are free to withdraw from the research study at any time without reason or consequence. If you wish, you can request for any data collected from yourself to be extracted from the data set and destroyed.

What if I have any questions?

If you are interested in participating in this study, would like additional information, or have any general questions about the study not answered by this information sheet, please contact the research team on one of the emails below. If you wish to make a complaint regarding the study, please contact Dr Lex Mauger (supervisor) on the email below. For any enquiries to an impartial individual outside of the research team, please contact Dr James Hopker (Director of Postgraduate Research).

Sam Smith
Email: sas76@kent.ac.uk

Dr Lex Mauger (Supervisor)
Email: L.Mauger@kent.ac.uk

Dr Samantha Winter (Supervisor)
Email: S.L.Winter@kent.ac.uk

Professor Dominic Micklewright (Supervisor)
Email: dpmick@essex.ac.uk

Dr James Hopker (Director of Postgraduate Research)
Email: J.G.Hopker@kent.ac.uk

Thank you for showing interest in this study and taking time to read this information sheet. If you wish to participate in the study, please sign the informed consent form for confirmation.

PARTICIPANT HEALTH QUESTIONNAIRE

HEALTH QUESTIONNAIRE

Participant Number Code:…….

Please ensure you have completed and signed the Informed Consent Form to show that you have read and completed this Health Questionnaire
Please answer these questions truthfully and completely. The sole purpose of this questionnaire is to ensure that you are in a fit and healthy state to complete the exercise test.
ANY INFORMATION CONTAINED HEREIN WILL BE TREATED AS CONFIDENTIAL.

SECTION 1: GENERAL HEALTH QUESTIONS

Please read the ten questions below carefully and answer each one honestly: check YES or NO.

	YES	NO
1. Has your doctor ever said that you have a heart condition or high blood pressure?	□	□
2. Do you feel pain in your chest at rest, during your daily activities of living, or when you do physical activity?	□	□
3. Do you lose balance because of dizziness or have you lost consciousness in the last 12 months? (Please answer NO if your dizziness was associated with over-breathing including vigorous exercise).	□	□
4. Have you ever been diagnosed with another chronic medical condition (other than heart disease or high blood pressure)?	□	□
If yes, please list condition(s) here:		
5. Are you currently taking prescribed medications for a chronic medical condition?	□	□
If yes, please list condition(s) and medications here:		
6. Do you currently have (or have you had within the past 12 months) a bone, joint or soft tissue (muscle, ligament, or tendon) problem that could be made worse by becoming more physically active? Please answer NO if you had a problem in the past but it *does not limit your ability* to be physically active.	□	□
If yes, please list condition(s) here:		
7. Has your doctor ever said that you should only do medically supervised physical activity?	□	□
8. Do you, or any in your immediate family, has a history or brain or mental disorders?	□	□

9. Are you currently taking any medication that may affect the central nervous system?	□	□
10. Are you, or is there a chance you may be pregnant?	□	□

If you answered NO to all of the questions above, you are cleared to take part in the exercise test

Go to SECTION 3 to acknowledge declaration. You do not need to complete section 2.

If you answered YES to one or more of the questions in Section 1 - PLEASE GO TO SECTION 2.

SECTION 2: CHRONIC MEDICAL CONDITIONS

Please read the questions below carefully and answer each one honestly: check YES or NO.

		YES	NO
1.	**Do you have arthritis, osteoporosis, or back problems?** If YES answer questions 1a-1c. If NO go to Question 2.	□	□
1a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking any medications or other treatments).	□	□
1b.	Do you have joint problems causing pain, a recent fracture or fracture caused by osteoporosis or cancer, displaced vertebrae (e.g. spondylolisthesis), and/or spondyloysis/pars defect (a crack in the bony ring on the back of the spinal column)?	□	□
1c.	Have you had steroid injections or taken steroid tablets regularly for more than 3 months?	□	□
2.	**Do you have cancer of any kind?** If YES answer questions 2a-2b. If NO, go to Question 3.	□	□
2a.	Does your cancer diagnosis include any of the following types: lung/bronchogenic, multiple myeloma (cancer of plasma cells), head and neck?	□	□
2b.	Are you currently receiving cancer therapy (such as chemotherapy or radiotherapy)?	□	□
3.	**Do you have heart disease or cardiovascular disease? This includes coronary artery disease, high blood pressure, heart failure, diagnosed abnormality or heart rhythm.** If YES answer questions 3a-3e. If NO go to Question 4.	□	□

3a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking any medications or other treatments).	□	□
3b.	Do you have an irregular heartbeat that requires medical management? (e.g. atrial fibrillation, premature ventricular contraction)	□	□
3c.	Do you have chronic heart failure?	□	□
3d.	Do you have a resting blood pressure equal to or greater than 160/90mmHg with or without medication? Answer YES if you do not know your resting blood pressure.	□	□
3e.	Do you have diagnosed coronary artery (cardiovascular) disease and have not participated in regular physical activity in the last 2 months?	□	□
		YES	**NO**
4.	**Do you have any metabolic conditions? This includes Type 1 Diabetes, Type 2 Diabetes and Pre-Diabetes.** If YES answer questions 4a-4c. If NO, go to Question 5.	□	□
4a.	Is your blood sugar often above 13mmol/L? (Answer YES if you are not sure).	□	□
4b.	Do you have any signs or symptoms of diabetes complications such as heart or vascular disease and/or complications affecting your eyes, kidneys, OR the sensation in your toes and feet?	□	□
4c.	Do you have other metabolic conditions (such as thyroid disorders, current pregnancy related diabetes, chronic kidney disease, or liver problems)?	□	□
5.	**Do you have any mental health problems or learning difficulties?** This includes Alzheimer's, dementia, depression, anxiety disorder, eating disorder, psychotic disorder, intellectual disability and down syndrome. If YES answer questions 5a-5b. If NO go to Question 6.	□	□
5a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking any medications or other treatments).	□	□
5b.	Do you also have back problems affecting nerves or muscles?	□	□
6.	**Do you have a respiratory disease?** This includes chronic obstructive pulmonary disease, asthma, pulmonary high blood pressure. If YES answer questions 6a-6d. If NO, go to Question 7.	□	□
6a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking any medications or other treatments).	□	□

6b.	Has your doctor ever said you blood oxygen level is low at rest or during exercise and/or that you require supplemental oxygen therapy?	□	□
6c.	If asthmatic, do you currently have symptoms of chest tightness, wheezing, laboured breathing, consistent cough (more than 2 days/week), or have you used your rescue medication more than twice in the last week?	□	□
6d.	Has your doctor ever said you have high blood pressure in the blood vessels of your lungs?	□	□
7.	**Do you have a spinal cord injury?** This includes tetraplegia and paraplegia. If YES answer questions 7a-7c. If NO, go to Question 8.	□	□
7a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking any medications or other treatments).	□	□
7b.	Do you commonly exhibit low resting blood pressure significant enough to cause dizziness, light-headedness, and/or fainting?	□	□
7c.	Has your physician indicated that you exhibit sudden bouts of high blood pressure (known as autonomic dysreflexia)?	□	□

		YES	**NO**
8.	**Have you had a stroke?** This includes transient ischemic attack (TIA) or cerebrovascular event. If YES answer questions 8a-8c. If NO go to Question 9.	□	□
8a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking any medications or other treatments).	□	□
8b.	Do you have any impairment in walking or mobility?	□	□
8c.	Have you experienced a stroke or impairment in nerves or muscles in the past 6 months?	□	□
9.	**Do you have any other medical condition which is not listed above or do you have two or more medical conditions?** If you have other medical conditions, answer questions 9a-9c. If NO go to Question 10.	□	□
9a.	Have you experienced a blackout, fainted, or lost consciousness as a result of a head injury within the last 12 months OR have you had a diagnosed concussion within the last 12 months?	□	□
9b.	Do you have a medical condition that is not listed (such as epilepsy, neurological conditions, and kidney problems)?	□	□
9c.	Do you currently live with two or more medical conditions?	□	□
	Please list your medical condition(s) and any related medications here:		

10.	**Have you had a viral infection in the last 2 weeks (cough, cold, sore throat, etc.)?** If YES please provide details below:	□	□
11.	**Is there any other reason why you cannot take part in this exercise test?** If YES please provide details below:	□	□
12.	**Please provide brief details of your current weekly levels of physical activity (sport, physical fitness or conditioning activities), using the following classification for exertion level:** **L = light (slightly breathless)** **M = moderate (breathless)** **V = vigorous (very breathless)** **Activity** **Duration (mins.)** **Level (L/M/V)** **Monday** **Tuesday** **Wednesday** **Thursday** **Friday** **Saturday** **Sunday**		

Please see below for recommendations for your current medical condition and sign this document:

If you answered NO to all of the follow-up questions about your medical condition, you are cleared to take part in the exercise test.

If you answered YES to one or more of the follow-up questions about your medical condition it is strongly advised that you should seek further advice from a medical professional before taking part in the exercise test.

SECTION 3: DECLARATION

Signing the study Consent Form signifies that you have completed this questionnaire.

Pre-Test Health Questionnaire:

School of Sport and Exercise Sciences
The Medway Building
Chatham Maritime
Kent
ME4 4AG

Effects of acute bilateral experimental exercise-induced pain on cycling time to exhaustion performance

Participant Code: _____

Please answer the questions below honestly and completely: check Yes, No or Don't know. The purpose of this questionnaire is to ensure that you are fit and healthy to complete the study.

	Yes	No	Don't know
1. Are you currently taking medication for pain related reasons? If yes, please provide details below:	☐	☐	☐
……			
……			
2. Do you have any allergies (e.g. nuts, fish, milk, egg, wheat and soya), If yes, please provide details below:	☐	☐	☐
……			
……			
3. Do you have pre-existing knee pain?	☐	☐	☐
4. Do you have a phobia of needles? (e.g. feel dizzy, faint or light headed in the presence of needles)	☐	☐	☐
5. Do you have/have you had a lower-limb injury in the last three months that may worsen as a result of the test, or affect the results of the test?	☐	☐	☐
6. Do you have heart disease or cardiovascular disease? (coronary artery disease, high blood pressure, heart failure, diagnosed heart rhythm abnormality)	☐	☐	☐

7. Have you had, or do you have, a blood-borne infection, e.g. hepatitis/HIV?	☐	☐	☐
8. Do you have a neurological disorder? (e.g. epilepsy, Alzheimer disease and other dementias, multiple sclerosis Parkinson's disease)	☐	☐	☐
9. Is there anything to your knowledge that may prevent you from successfully completing the tests that have been outlined to you? If yes, please give brief details:	☐	☐	☐

……
……

Declaration:

I have read, understood and completed this questionnaire to the best of my knowledge

Yes ☐

No ☐

Consent Form:

Title of project: Effects of acute bilateral experimental exercise-induced pain on cycling time to exhaustion performance

Name of investigator: Samuel Smith

Participant Identification Number for this project:

	Please initial box
1. I confirm I have read and understand the information sheet dated 25th February 2019 (version 2) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason. Should I wish to contact the lead researcher, I am able to do so on the following email: *sas76@kent.ac.uk*	☐
3. I understand that my responses will be anonymised before analysis. I give permission for members of the research team to have access to my anonymised responses.	☐
4. I understand that my fingertip capillary blood samples will be taken during the course of my participation in this research project and used only for the purposes described in the information sheet (before being disposed of).	☐
5. I understand that intramuscular injections of either 0.9% isotonic or 5.8% hypertonic saline solutions will be administered during the course of my participation in this research project, and can confirm that I have read and understood the associated risk of this procedure (detailed in *Appendix: Intramuscular Injections: Background & Risk Assessment)*. I therefore acknowledge that receiving intramuscular injections presents certain risks and can confirm that I am content with this.	☐
6. I have completed the Health Questionnaire as fully and honestly as possible and I understand that the researchers will use this information to make a decision on my suitability for the study.	☐
7. I agree to take part in the above research project.	☐

Name of participant	Date	Signature
Name of person taking consent *(if different from lead researcher)*	Date	Signature
Lead researcher	Date	Signature

APPENDIX B: INTRAMUSCULAR INJECTION BACKGROUND & RISK ASSESSMENT (PARTICIPANT INFORMATION)

Background

Intramuscular (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. Additionally, 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. 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
- Equipment
- Environment in which injection is administered

As the skeletal muscle is suggested to have less pain receptors, IM injections involve decreased discomfort, and dependent on the site of injection, this method enables comparatively large volumes of a substance to be quickly absorbed by the body with a relatively prolonged action.

Research Study Application

It is proposed the pain induced in the present study is achieved through the *IM injection of 1ml hypertonic saline (5.8% concentration)*. After first being implemented in the late 1930s, hypertonic saline has since been extensively used as an experimental method that characterises and mimics the effects involved in muscle pain, temporarily inducing pain that can last up to ten minutes before disappearing (dependent on injection site and volume of solution). In particular, this method has been widely used by a Lead Professor (Thomas Graven-Nielsen) in Pain Neuroscience at Aalborg University, whom upon previous contact noted that from over 6000 injections performed, there have been no serious side effects reported.

As demonstrated by previous research, this method *does not cause any muscle toxicity* and is *unlikely to be related to tissue damage*, thus can be deemed to be safe and *acceptable for use in human experimentation*.

Injection Site

In the present study, the hypertonic saline solution is planned to be injected into the vastus lateralis (largest muscle of the quadriceps muscle group located on the outside of the thigh).

This site:

- Provides a large muscle mass
- Is easy to access,
- Has a reduced likelihood of injury
- Is not associated with any major blood vessels or significant nerve structures, minimising risks of damage

[REDACTED]

Associated Risks & Side Effects

Although mainly preventable during trained and safe IM practice, an IM injection can still result in several potential complications which could arise as a result of unsafe injections and poor technique. Potential complications which may occur include:

- Pain or mild discomfort for a short period after an injection
- Some bruising at the injection site
- Muscle fibrosis 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
- An allergic or anaphylactic reaction (from the hypertonic saline)
- Nerve injury resulting in potential paralysis, atrophy, haematoma, bone injury, cellulitis, and sterile abscesses can occur (in more serious cases)
- 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

Interventions 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". In order to achieve a safe injection and reduce the risks associated with IM, several precautions will be implemented. These include:

- **Researcher NHS training and competency**
  - All researchers performing the injections in the present study (Sam Smith, Lex Mauger, Ryan Norbury and Adam Hunt) have received appropriate NHS training and guidelines, and has completed a competency assessment of supervised practice to ensure safe, competent and consistent best practice when administering IM injections. At least two of the researchers listed above will be present during the testing sessions.

- **Awareness of potential risks and side effects**
  - A full risk assessment of IM injections and hypertonic saline has been completed and approved
  - Both researchers will implement best infection control practices for IM injections
  - The researchers will always inform you about the potential side effects before the intramuscular injection application.

- **Recruitment and screening**
  - Participants between the age of 18-45 will only be recruited for this study

- You will be screened prior to testing through completing a general health questionnaire. If you have pre-existing medical conditions such as neurological disorders, blood borne viruses , lower limb injury, sore deep tissues, allergies to protein and long-term medication use, you will be excluded from participating in the study
    - Should you 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 sites will be inspected to ensure they are free from redness, swelling, pain, tenderness infections or abrasions

- **Procedure documentation**
    - Factors such as solution, product batch number, site, date, time and adverse effects will be documented after each IM injection

- **Participant after-care**
    - You will be instructed to monitor the site two to four hours post-injection to ensure no adverse reactions have occurred. Any complications present will be documented.
    - You will be instructed to call their doctor or contact emergency services if they experience: redness and heat at the site of injection, severe pain at the injection site, tingling or numbness, prolonged bleeding, drainage at the injection site.
    - The researcher will check your understanding of this, and will document that advice has been given in your records.

APPENDIX C: SCALES (PAIN, RPE & RATING OF FATIGUE) AND ACCOMPANYING INSTRUCTIONS

Overview

The present study will require you to provide ratings for perceptions of fatigue, effort and pain using three validated scales:

- RPE
- Pain
- Fatigue

During all visits, you will be required to rate the intensity of pain on a moment-to-moment basis (i.e. when the intensity of pain experienced changes) using an electronic sliding scale.

During the exercise tasks, you will be required to verbally report the RPE and rating of fatigue every 30 s.

It is important to be able to distinguish between perceptions of exertion, perceptions of pain and perceptions of fatigue. Therefore, please take some time to read through the following instructions, and familiarise yourself with the attached scales before commencing the study.

References

Borg, G. (1998). *Borg's perceived exertion and pain scales*. Human kinetics.

Cook, D.B., O'Connor, P.J., Eubanks, S.A., Smith, J.C. and Lee, M. (1997). Naturally occurring muscle pain during exercise: assessment and experimental evidence. *Medicine and Science in Sports and Exercise*, *29*(8), 999-1012

Micklewright, D., Gibson, A.S.C., Gladwell, V. and Al Salman, A. (2017). Development and Validity of the Rating-of-Fatigue Scale. *Sports Medicine*, 1-19.

Pain Intensity Scale

Cook, O'Connor, Eubanks, Smith, Lee (1997)
[REDACTED]

Instructions

The scale before you contains the numbers 0-10. You will use this scale to assess perceptions of pain in your right leg after the injection and/or during the exercise task. In this context, pain is defined as the intensity of hurt that you feel. This should be the pain which is produced by muscle burn and ache as a result of repeated or prolonged muscular contraction, and not pain resulting from injury. Don't underestimate or overestimate the degree of hurt you feel, just try to estimate it as honestly and objectively as possible.

The numbers on the scale represent a range of pain intensity from 'very faint pain' (number ½) to 'extremely intense pain – almost unbearable' (number 10). When you feel no pain from muscle burn/ache in your right leg you should respond with the number 0. When pain becomes just noticeable, you should respond with number ½. If your legs feel extremely strong pain that is almost unbearable, you should respond with the number 10. Use the verbal expressions to help rate your perceptions. If you feel extremely strong pain which is almost unbearable, you should respond with the number 10. You can also respond with numbers greater than 10. If the pain is greater than 10, respond with the number that represents the pain intensity you feel in relation to 10. In other words, if the pain is twice as great then respond with the number 20.

You will be asked to continuously rate the feelings of exercise-induced pain arising from muscle pain/ache in your right leg. This will be performed on a moment-to-moment basis when the intensity of pain that you experience changes. When rating these pain sensations, be sure to only attend to the specific pain sensations from exercise-induced pain in your right leg, and not other sensations of pain you may be feeling (e.g. seat discomfort, blisters etc).

Do not use your ratings as an expression of fatigue (i.e. inability of the muscle to produce force), exertion (i.e. how hard it is for you do drive your legs) or relief that the exercise task is completed, although increased pain may compromise your willingness to produce muscular force.

In summary you will be asked to:

1. Provide pain intensity ratings in your right leg only
2. Give ratings as accurately as possible
3. Not under or over-estimate the pain, but simply rate your pain honestly

Borg 6-20 RPE Scale

Borg (1998)

[REDACTED]

Instructions

During the exercise tests, we want you to rate your perception of exertion defined as at sensation of how hard you are driving your leg in order to maintain the target torque.

Look at this rating scale; we want you to use this scale from 6 to 20, where 6 means ‘no exertion at all’ and 20 means ‘maximal exertion’.

To help you choose a number that corresponds to how you feel within this range, consider the following:

6 corresponds to ‘no exertion at all’ (e.g. at rest with no contraction). You do not have the sensation of driving your leg

9 corresponds to ‘very light’ exercise. For a normal, healthy person it is like walking slowly at their own pace for some minutes

13 on the scale is ‘somewhat hard’ exercise, but it still feels ok to continue

15 corresponds to when the sensation of driving your arm is ‘hard’

17 on the scale is ‘very hard’ which is very strenuous. A healthy person can still go on, but they have to push themselves. It feels very heavy, and the person is very tired.

19 on the scale is an extremely strenuous level. For most people this is the most strenuous exercise they have ever experienced.

20 (‘maximal exertion’) corresponds to the feeling of effort when you are exercising maximally (i.e. as hard as you can for that given moment)

Try to appraise your feeling of exertion as spontaneously and honestly as possible, without thinking about what the actual physical load is. Don’t underestimate it, nor overestimate it. It’s your own feeling of effort and exertion that’s important, not how it compares with other people’s. What other people think is not important either. Look at the scale and the expressions and then give a number.

Any questions?

Rating of Fatigue (ROF) Scale

Micklewright, St Clair Gibson, Gladwell and Al Salman (2017)

[REDACTED]

Instructions

The rating of fatigue scale (ROF) will allow you to rate you fatigued you feel. The fatigue scale might be presented to you by another person, or, in some circumstances, you might be asked to self-administer the scale. Whatever method is used, it is important that you first read the following guidelines:

1. Please familiarise yourself with the scale by looking closely at the ROF now. You will noticed that the ROF consists of 11 numerical points that range from 0-10. There are also five descriptors and five diagrams that are intended to help you understand the scale and make your rating.

2. When you are presented with the ROF, please carefully inspect the scale before giving a numerical response from 0-10. Always try to respond as honestly as possible giving a rating that best reflects how fatigued you feel at the time

3. Try not to hesitate too much and make sure you only give ONE number as a response. For example, avoid responding by giving two numbers such as “three or four”.

Now please read the following examples of what some of the ROF ratings mean.

A response of 0 would indicate that you do not feel at all fatigued. An example of this might be soon after you wake up in the morning after having a good night’s sleep. Now try to think of a similar occasion where you have experienced the lowest feelings of fatigue and use this as your reference.

A response of 10 would indicate that you feel totally fatigued and exhausted. An example of this might be not being able to stay awake, perhaps late at night but equally could include situations such as sprinting until you can no longer physically continue. Again, try to think of a similar example that you have actually experienced in the past as your reference.

APPENDIX D: PSYCHOLOGICAL SCALES

PANAS:

This scale consists of a number of words that describe different feelings and emotions. Read each item and then mark the appropriate answer in the space next to that word. Indicate to what extent you have felt this way right, that is, at the present moment

1	2	3	4	5
Very slightly or not at all	A little	Moderately	Quite a bit	Extremely

_____	1. Interested	_____	11. Irritable
_____	2. Distressed	_____	12. Alert
_____	3. Excited	_____	13. Ashamed
_____	4. Upset	_____	14. Inspired
_____	5. Strong	_____	15. Nervous
_____	6. Guilty	_____	16. Determined
_____	7. Scared	_____	17. Attentive
_____	8. Hostile	_____	18. Jittery
_____	9. Enthusiastic	_____	19. Active
_____	10. Proud	_____	20. Afraid

SSEIT:

Below is a list of statements dealing with your general feelings about yourself. Please circle around a number for each statement on the table to show your answer. Please respond to all statements.

	Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly Agree
1. I know when to speak about my personal problems to others.	1	2	3	4	5
2. When I am faced with obstacles, I remember times I faced similar obstacles and overcame them.	1	2	3	4	5
3. I expect that I will do well on most things I try.	1	2	3	4	5
4. Other people find it easy to confide in me.	1	2	3	4	5
5. I find it hard to understand the nonverbal messages of other people.	1	2	3	4	5
6. Some of the major events of my life have led me to re-evaluate what is important and not important.	1	2	3	4	5
7. When my mood changes, I see new possibilities.	1	2	3	4	5
8. Emotions are some of the things that make my life worth living.	1	2	3	4	5
9. I am aware of my emotions as I experience them.	1	2	3	4	5
10. I expect good things to happen.	1	2	3	4	5
11. I like to share my emotions with others.	1	2	3	4	5
12. When I experience a positive emotion, I know how to make it last.	1	2	3	4	5

13. I arrange events others enjoy.	1	2	3	4	5
14. I seek out activities that make me happy.	1	2	3	4	5
15. I am aware of the nonverbal messages I send to others.	1	2	3	4	5
16. I present myself in a way that makes a good impression on others.	1	2	3	4	5
17. When I am in a positive mood, solving problems is easy for me.	1	2	3	4	5
18. By looking at their facial expressions, I recognize the emotions people are experiencing.	1	2	3	4	5
19. I know why my emotions change.	1	2	3	4	5
20. When I am in a positive mood, I am able to come up with new ideas.	1	2	3	4	5
21. I have control over my emotions.	1	2	3	4	5
22. I easily recognize my emotions as I experience them.	1	2	3	4	5
23. I motivate myself by imagining a good outcome to tasks I take on.	1	2	3	4	5
24. I compliment others when they have done something well.	1	2	3	4	5
25. I am aware of the nonverbal messages other people send.	1	2	3	4	5
26. When another person tells me about an important event in his or her life, I almost feel as though I have experienced this event myself.	1	2	3	4	5
27. When I feel a change in emotions, I tend to come up with new ideas.	1	2	3	4	5

28. When I am faced with a challenge, I give up because I believe I will fail.	1	2	3	4	5
29. I know what other people are feeling just by looking at them.	1	2	3	4	5
30. I help other people feel better when they are down.	1	2	3	4	5
31. I use good moods to help myself keep trying in the face of obstacles.	1	2	3	4	5
32. I can tell how people are feeling by listening to the tone of their voice.	1	2	3	4	5
33. It is difficult for me to understand why people feel the way they do.	1	2	3	4	5

Pain Resilience Scale

Directions: We are interested in the different ways that people respond to intense or prolonged pain (toothache, muscle strain, headache). Using a 0 ("Not at all") to 4 ("All the time") scale, please rate how much each of the following items describe how you respond when faced with intense or prolonged pain.

	When faced with intense or prolonged pain…	**Not at all**	**To a slight degree**	**To a moderate degree**	**To a great degree**	**All the time**
1.	I get back out there	⓪	①	②	③	④
2.	I still work to accomplish my goals	⓪	①	②	③	④
3.	I push through it	⓪	①	②	③	④
4.	I try to continue working	⓪	①	②	③	④
5.	I like to stay active	⓪	①	②	③	④
6.	I focus on positive thoughts	⓪	①	②	③	④
7.	I keep a positive attitude	⓪	①	②	③	④
8.	It doesn't affect my happiness	⓪	①	②	③	④
9.	I still find joy in my life	⓪	①	②	③	④
10.	I keep a hopeful attitude	⓪	①	②	③	④
11.	I don't let it get me down	⓪	①	②	③	④
12.	I don't let it upset me	⓪	①	②	③	④
13.	I avoid negative thoughts	⓪	①	②	③	④
14.	I try to stay relaxed	⓪	①	②	③	④

Short-form pain-specific catastrophizing scale:

For the following questions, we are interested in the types of thoughts and feelings that you had while you were participating. Listed below are several statements describing different thoughts and feelings that may be associated with pain. Using the following scale, please indicate the degree to which you had these thoughts and feelings during this pain testing session.

0	Not at all	1	To a slight degree	2	To a moderate degree	3	To a great degree	4	All the time

1. I worried about when it would end	_____
2. I thought that the pain might overwhelm	_____
3. I felt that I couldn't stand it	_____
4. I couldn't stop thinking about how much it hurt	_____
5. I kept wishing that it would be over	_____
6. I felt that the procedures were awful	_____

APPENDIX E: CONFERENCE ABSTRACT

Intramuscular injection of hypertonic saline induces exercise-induced pain and decreases time to task failure in males

Mini-oral presentation at the annual congress of European College of Sports Science, Dublin, Ireland. 4th-7th July 2018.

Smith, S.A., Micklewright, D. and Mauger A.R.

Introduction

Exercise-induced pain (EIP) tolerance is proposed to be a determinant of endurance performance (Mauger, 2013). However, inadequate methods for inducing EIP are often used in research, and this has made this notion difficult to examine. Despite a relatively limited application to both exercise and performance, the intramuscular injection of hypertonic saline has been suggested to be a method that closely replicates EIP. The aim of the present study was to use this experimental pain model to assess the effect of EIP on time to task failure (TTF) of a sustained submaximal isometric contraction.

Methods

Nine male (25 ± 4 yr, 1.81 ± 0.71 m, 81.8 ± 11.7 kg) and six female (22 ± 2 yr, 1.68 ± 0.77 m, 61.4 ± 5.9 kg) recreationally active participants completed six conditions separated by 2-7 days. All exercise tests were performed on an isokinetic dynamometer set up for the right leg. The final three visits required participants to perform a single leg isometric TTF of the knee extensors at 10% of their maximal voluntary contraction. They performed this task in in the presence of exercise-induced pain (induced via a 1 mL intramuscular injection of hypertonic saline (5.85%) into the vastus lateralis), a placebo (a 1 mL intramuscular injection of isotonic saline (0.9%) into the vastus lateralis) or no injection.

Results

At rest, hypertonic saline produced a mean pain intensity of 32.9 ± 11.7 on the 0-100 visual analogue scale which lasted 290 ± 81 s. Initial analysis revealed that TTF was not significantly different between the pain, placebo and no injection conditions (670

± 415 vs. 750 ± 353 vs. 725 ± 385 s, respectively). However, as female's TTF was significantly longer than males and outlasted the duration of EIP induced by the injection, a secondary analysis on a male only sample was performed. Here, a significant difference was found between conditions, with hypertonic saline causing significantly (P = 0.007) shorter TTF (454 ± 356 s) compared to the placebo condition (633 ± 363 s), and a difference from the control condition (533 ± 320 s) that approached significance (P = 0.056).

Discussion

The primary finding from the present study suggests that an increased EIP decreases TTF, provided that the elevated pain is present for the duration of the task. This is consistent with previous literature which demonstrated a similar finding in the tibialis anterior at 80% MVC (Graven-Nielsen, Svensson and Arendt-Nielsen, 1997). Future work utilising the intramuscular saline experimental pain model should seek to match exercise task time to the duration that pain is induced by the saline.