An investigation into the test-retest reliability of the pain response to hypertonic saline injections and the impact of added muscle contraction

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Abstract

Background/Aims: Intramuscular Hypertonic Saline (HS) injections induce pain that resembles exercise-induced pain. The reliability and the impact that parallel exercise may have on this pain sensation is unestablished. Therefore, the aims of this research were to assess the test-retest reliability of this model's pain response and the influence of additional muscle contractions to the pain experience, in terms of both Pain Intensity (PI) and Pain Quality (PQ). *Methodology:* 8 male and 6 female participants (25 ± 5 years, 172.9 ± 8.5 cm, 71.9 ± 12.7 kg) completed the two studies. Study 1.1 assessed test-retest reliability with 3 separate visits, in which 1 ml of 5.85% HS was injected into the right vastus lateralis and differences in PI and PQ were measured. In Study 1.2, participants attended 3 separate visits, where they completed an isometric exercise task with 3 separate 10-second contractions at different intensities (10%/15%/20%). This was done with either HS, a placebo or no injection as control. *Results:* Study 1.1: Intraclass Correlation Coefficient scores for all PI measures indicated at least 'moderate' to 'good' test-retest reliability (0.68 – 0.814). Cronbach's Alpha scores for all PQ measures indicated 'acceptable' to 'good' test-retest reliability (0.806 – 0.933), except for the affective dimension (0.397 – 0.601). Study 1.2: Paired samples t-tests revealed no differences between exercise and rest, for any of the PI measures or PQ measures, except for the Present Pain Index (PPI) of the Long-form McGill Pain Questionnaire ($P = 0.048$). ANOVA analyses revealed no differences in PI or PQ measures between contraction intensities. *Discussion:* In Summary, HS provides a 'moderate' to 'good' reliable pain response, except for the affective dimension of pain. PI response is not affected by the addition of exercise or exercise intensity. PQ response is only affected in terms of different descriptive words, when exercise is introduced.

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Abbreviations

±	Standard Deviation
1RM	One Repetition Maximum
ANOVA	Analysis of Variance
AP	All Participants
BP	Behavioural Perseverance
C	Celsius
CA	Cronbach's Alpha
CGM	Central Governor Model
CI	Confidence Intervals
cm	Centimetre
cm^2	Centimetre squared
CON	Control
CP	Cognitive Positivity
CPT	Cold Pressor Test
CNS	Central Nervous System
CV	Coefficient of Variation
DOMS	Delayed Onset Muscle Soreness
EIP	Exercise-induced Pain
EMG	Electromyography
g	Gram
HS	Hypertonic Saline

ICC	Intraclass Correlation Coefficient
IFC	Inferential Current
IGT	Integrative Governor Theory
IP	Inexperienced Participants
IS	Isotonic Saline
kg	Kilogram
l	Litre
LFMPQ	Long-Form McGill Pain Questionnaire
M	Mean
MDC	Minimum Detectable Change
mg	Milligram
$mg.kg^{-1}$	Milligrams per kilogram of body mass
ml	Millilitre
mm	Millimetre
mmHg	Millimetre of Mercury
MVC	Maximal Voluntary Contraction
n	Number
NaCL	Sodium Chloride
NHS	National Health Service
^{o}	Degrees
P	Significance Level
PANAS	Positive and Negative Affect Scale
PAR-Q	Physical Activity Readiness Questionnaire
PB	Psychobiological

Performance VO_2	Sustainable oxygen consumption for given period of time
PI	Pain Intensity
PPI	Present Pain Intensity
PPT	Pressure Pain Threshold
PRS	Pain Resilience Scale
RPE	Rate of Perceived Exertion
s	Second
SD	Standard Deviation
SFMPQ	Short-Form McGill Pain Questionnaire
SSEIT	Schutte Self Report Emotional Intelligence Test
STL	Sensory Tolerance Limit
tDCS	Transcranial direct current stimulation
TENS	Transcutaneous Electrical Nerve Stimulation
T-PRI	Total Pain Rating Index
TTE	Time to Exhaustion
VAS	Visual Analogue Scale
VF	Variable Force
Vi	Subnucleus Interpolaris
VL	Vastus Lateralis
VO_{2max}	Maximal Oxygen Uptake

Chapter 1: Introduction

1.1: Introduction to pain

The topic of pain is of great importance in scientific research both in the clinical field and in relation to exercise performance. Despite being studied for centuries, with thousands of articles on the subject, there is still much left to understand about the pain phenomenon (Danecker & Koltyn, 2014; Mauger, 2014; Millan, 1999; Seymour, 2019). The term pain is defined by the International Association for the Study of Pain (2012) as an unpleasant sensory or emotional experience associated with actual or potential tissue damage or described in terms of such damage. This pain experience is a subjective sensation and is felt independently of the level of potential or real damage occurred (Oleson et al., 2012). Due to this, pain is often interpreted as an indicator of bodily harm or physiological problems (Danecker & Koltyn, 2014). The exact aetiology of pain is still unknown, with a remarkable array of associated mechanisms, explanations and causes, beyond the scope of this thesis. Two comprehensive studies by Millan (1999; 2002) provide insight into the various mechanisms that are associated with pain, particularly into descending and ascending pathways. Although, a fully comprehensive assessment of this topic cannot be reasonably explored within the current text, a general understanding of the process of pain is key in providing context to this research.

1.1.1: How does pain occur?

The experience of pain is essentially the end product of both peripheral and central mechanisms that vary depending on the type of pain (Danecker & Koltyn, 2014). The sensation of pain results from a complex set of mechanisms usually stemming from the periphery via afferent feedback, to the dorsal horn of the spine and then to higher cerebral structures in the brain (Millan, 1999). This signal ascending from areas of the body experiencing painful stimuli is interpreted in the brain and then manifests as the perception of pain (Almeida et al., 2004). The most common origin of these signals is elicited by the activation of nociceptors located around the body, resulting in ‘nociceptive pain’ (Millan, 1999). Another type of pain is ‘inflammatory pain’, which concerns so-called ‘silent’ nociceptors that would normally not react to noxious stimuli but are sensitised and activated under conditions of inflammation and tissue injury (Millan, 1999). Arthritic individuals may

experience this type of pain during normally innocuous mechanical stimulation and would therefore experience more pain during exercise (Millan, 1999), often creating a barrier to exercise participation for this group (Bellamy & Buchanan, 1986; Focht et al., 2002; Hendry et al., 2006). Pain can also occur due to sensory fibre injuries or from damage to the Central Nervous System (CNS), deemed 'neuropathic pain' but this is not as common and does not serve an important physiological role (Millan, 1999). Acute nociceptive pain on the other hand serves as a type of warning system to potential harm to the body and allows the brain and CNS to adapt, to prevent damage (Millan, 1999). There are many potential catalysts for nociceptive pain to occur including, but in no means limited to, the presence of prostaglandins, bradykinin, cytokines and nitric oxide (Millan, 1999), as well as mechanical pressure, heat, cold, noxious pressure and other endogenous algesic substances (Danecker & Koltyn, 2014), which act upon the nociceptors.

1.1.2: Theories of pain

There are multiple theories that have been proposed to explain the mechanisms of pain including the gate control theory (Melzack & Wall, 1965), the specificity theory (Dubner et al., 1978), the intensity theory and the pattern theory (Lele et al., 1954; Nafe, 1929). The specificity theory proposes that modality of pain has specific receptors and related primary afferents that are sensitive to one specific stimulus, when the specific stimulus is noxious enough this is signalled along distinct pathways and interpreted in pain centres in the brain (Moayedi & Davis, 2013). In contrast to this theory the intensity theory suggests that pain is a summation of multiple stimuli, even subthreshold, which is interpreted as an experience of pain (Moayedi & Davis, 2013). The pattern theory posits that somatic sense organs respond to a pattern of different stimuli with these impulse patterns being interpreted and encoded as the modality and location of the stimuli (Moayedi & Davis, 2013). The final and perhaps most accepted theory discussed is the gate control theory proposed by Melzack and Wall (1965) in which the seemingly opposed theories of specificity and pattern are bridged. This theory accepts states that the sensation of pain derives from the stimulation of nociceptors which result in afferents transmitted to the substantia gelatinosa, dorsal column and transmission cells in the spinal cord (Moayedi & Davis, 2013). It is suggested that the substantia gelatinosa in the dorsal horn is a 'gate' controlling the transmission of these

afferents to the brain, with nociceptive signals opening the gate when a threshold is reached and leads to the experience of pain interpreted in the brain (Moayedi & Davis, 2013). All these theories have shortcomings with none of them providing a complete explanation of the mechanisms of pain. As pain is a complex and multidimensional process (Melzack & Casey, 1968) much more research needs to be conducted to explain these theories.

1.1.3: Clinical application

Potential sources for pain are vast and have been the subject of countless research studies over the years, the majority of which can be found in clinical studies. Pain is considered a serious health problem with as high as 85% of adults suggested to miss work or seek care for musculoskeletal pain during their career (Fordyce, 1997). Various clinical syndromes and diseases are associated with chronic pain including work-related pain, tension headache and chronic whiplash syndrome (Ciubotariu et al., 2004), as well as neurological disorders (Robinson, 1996), lower back pain (Marchand et al., 1993) and fibromyalgia (Wolfe et al., 1990). Fibromyalgia is especially interesting to research in pain, as those with this disease do not detect sensory signals more often but the level at which the signals become toxic is lower (Arroyo & Cohen, 1993). This results in the brain interpreting these signals as pain despite no actual tissue damage or harm taking place (Lautenbacher et al., 1994; Kosek et al., 1996), lending credence to the adage that the perception of pain is subjective and independent of actual or potential tissue damage (Olesen et al., 2012). Despite the research into this syndrome, much like other aspects of pain, the exact aetiology of this disease is unknown (Gracely et al., 2002).

1.1.4: Pain/exercise application

In more specific pain and exercise research, the goal is less to investigate the potential causes and treatment of painful diseases and more how pain can impact exercise performance. Pain sensitivity has been shown to decrease in response to exercise through a process called exercise-induced hypoalgesia (Koltyn, 2000). Long-term exercise, including aerobic training (Anshel & Russell, 1994) and resistance training (Koltyn & Arbogast, 1998) can increase pain tolerance acutely both during and after exercise, as well as long-term. Pain tolerance,

which is defined as the ability to endure a painful stimulus for a prolonged period of time (Hoffman et al., 2004), has been suggested as a powerful determinant of long-distance exercise performance (Mauger, 2014) and those athletes that are willing to tolerate a greater amount of pain are likely to be more successful than those who are not (Mauger, 2013). Many different modes, intensities and durations of exercise can reduce pain, through this increase in pain tolerance or through exercise-induced hypoalgesia (Koltyn, 2000), but it is important to note, exercise can also induce pain, whether through exercise related injuries, exacerbation of pre-existing medical conditions or through Exercise-induced Pain (EIP) (Danecker & Koltyn, 2014). Briefly, prolonged, intense, continuous and repetitive exercise, induces changes in homeostasis in the working muscles, whether through mechanical, thermal or chemical stress (Danecker & Koltyn, 2014), activating nociceptors, providing afferent feedback to the brain (Marchettini et al., 1996) and resulting in a perceived sensation of exercise-induced pain (Mauger et al., 2009). This exercise-induced pain is a potential cause for 'nociceptive pain', that has historically been under researched, but it could be a key component in the study of pain and exercise performance.

1.2: Exercise-induced pain

Pain has long been linked to success in sport and exercise, and has been cited by athletes and coaches as a potential limiter of athletic performance (O'Connor, 1992). Much like other forms of pain the aetiology of exercise-induced pain has not yet been agreed, with the nociceptive mechanisms involved still needing further investigation (Mauger, 2014). However, what is known, is that exercise that utilises intense, continuous and repetitive muscle contractions, such as football, middle distance running and cycling (Mauger et al., 2009), elicits an acute muscle pain response deemed exercise-induced pain (Astokorki & Mauger, 2017). This is suggested to occur due to mechanical, thermal and chemical stress inductions during exercise (Danecker & Koltyn, 2014), including one of, or a combination of, increased intramuscular pressure, deformation of tissue during contractions of the muscle or the release of noxious metabolites (Mauger, 2014). These processes, as well as mechanical pressure, cold, heat, noxious pressure and algesic substances are thought to stimulate nociceptors in the muscle (Mauger, 2013). As discussed previously these nociceptors then signal the brain via the dorsal horn and CNS, resulting in 'nociceptive pain' induced by

exercise (Almeida et al., 2004). Specifically, type IV nociceptors respond to noxious chemicals including bradykinin, histamine, potassium and serotonin (Marchettini et al., 1996) and type III nociceptors are stimulated by high threshold noxious pressure resulting in a dull aching or cramping pain sensation (Mauger, 2014). Further, hydrogen ions and prostaglandins are released that sensitise the nociceptors to the noxious stimuli present during exercise (Mauger, 2014). These processes increase simultaneously with exercise intensity, suggesting that the more intense the exercise the greater presence of exercise-induced pain (Mauger, 2014).

1.2.1: Influences on pain tolerance

Due to this suggested relationship between exercise work-rate and pain, exercise induced pain can in theory be used as a tool to monitor muscle and cardiovascular strain during exercise (Mauger, 2014), however it is important to remember that pain is always subjective (IASP, 2012). Therefore, the perception of pain experienced during exercise will be different for everyone and can be influenced by various factors. Indeed, pain tolerance is influenced by multiple physiological and psychological factors. Physiologically exercise can influence pain tolerance. The aforementioned aerobic training (Anshel & Russell, 1994) and resistance training (Koltyn & Arbogast, 1998) studies suggest that regular/painful training may contribute to decreased pain perception/increased pain tolerance. Recently it has also been shown that exercise training in which a higher inducement of pain occurs, leads to an increased pain tolerance (O'Leary et al., 2017). Race can also determine someone's tolerance to exercise-induced pain with multiple studies suggesting Caucasian males have naturally elevated tolerance over other ethnicities (Edwards et al., 2001; Mechlin et al., 2005; Nayak et al., 2000; Sheffield et al., 2000). Males tend to have a higher pain tolerance than females, according to multiple studies (Ellermeier & Westphal, 1995; Keogh & Herdenfeldt, 2002; Maixner & Humphrey, 1993; Nayak et al., 2000). These factors combined with the possible presence of pain related diseases such as lower back pain, fibromyalgia and arthritis, that have been mentioned previously, can all influence how an individual may respond to pain in general, but in relation to EIP more research is needed.

The definition of pain by the International Association for the Study of Pain (2012) also alludes to an emotional or psychological aspect, which is also present in exercise-induced pain. There are various psychological aspects of an individual such as anxiety levels (Jones & Zachariae, 2004), mood states (Bobey & Davidson, 1970), previous painful experiences (Maggirias & Locker, 2002) and self-efficacy (Vallis & Bucher, 1986), that can influence their pain response that have been investigated, although many are more relevant to clinical pain than EIP specifically. Briefly, anxiety levels have been suggested as a predictor of pain tolerance with low anxiety males and females having a higher pain tolerance during painful cold pressor tests (Jones & Zachariae, 2004). Relaxation training techniques can be an effective method in increased pain tolerance, suggesting importance in mood states in dealing with pain (Bobey & Davidson, 1970). Furthermore, a link has been suggested between psychological factors and pain, for example a relationship between those with painful previous experiences or with anxiety surrounding dental treatment and a lower pain tolerance has been suggested (Maggirias & Locker, 2002). Finally, perhaps more relevant to EIP, an individual's self-efficacy has been suggested as a predictor for pain response or discomfort during and after exercise, suggesting that skill-based training and increased self-efficacy can improve pain tolerance, especially in women (Vallis & Bucher, 1986). Therefore, these psychological factors all appear to impact how individuals respond to pain.

Investigating the role psychology can influence exercise-induced pain specifically, there are also factors that could influence exercise engagement and performance. Attributing pain to exercise could lead individuals to disengage with exercise and/or avoid similar experiences in the future (Danecker & Koltyn, 2014). In fact, with the presence of EIP, the perception of pain may be a powerful psychological stimulus to cease exercise (Astokorki & Mauger, 2017). This may be related to the possible protective mechanism or warning system that nociceptive pain may provide in the prevention of tissue damage (Millan, 1999). This perceptual information may then inform the person performing the exercise whether to adjust work-rate or stop exercising altogether, so the presence of EIP could be key to pacing strategies (Mauger, 2013; Mauger 2014). A good example of a study that highlights this possible role is by Amann et al. (2009) that implemented fentanyl to eliminate all afferent feedback from the working muscles during a self-paced five-kilometre time trial. The primary finding from this particular study was that without any afferent feedback, peripheral fatigue was significantly higher for the fentanyl group despite there being no EIP, but with no

influence on performance (Amann et al., 2009). This appears to highlight that exercise-induced pain and afferent feedback play a role as a limiter of potential damage during exercise, potentially affecting performance and having an impact on fatigue (Mauger, 2013). However, there are many possible co-effects that may have had an influence in this study.

1.3: Fatigue

Fatigue, like pain, is a complex topic with many different components and areas of research, well beyond the breadth of this thesis. Although, not the primary focus of this research it is still important to explore some of the possible causes of fatigue, to provide context to research within pain. For further investigation, papers by Gandevia (2001), Tucker (2009) and Noakes et al. (2005), provide a much more comprehensive breakdown of the topic. Briefly, fatigue was once thought to occur at task failure of a certain exercise and was seen as the point at which an individual could no longer maintain work and continue in said exercise (Edwards, 1981). However, it is now thought that fatigue is present in any exercise and progressively accumulates as the duration and intensity of exercise increases (Gandevia, 2001). Gandevia (2001), a leading author on the topic, defines fatigue as any exercise-induced reduction in the ability to exert muscle power or force, irrespective of whether or not the task can be sustained. Thus, inferring that similarly to exercise-induced pain, fatigue increases simultaneously with exercise intensity and the ability to deal with fatigue is most likely a major determinant for success (Mauger, 2013).

Physiological determinants of exercise performance that are long established and are considered the most important, are maximal oxygen uptake (VO_{2max}), the lactate threshold and economy of energy (Joyner & Coyle, 2008). Although it is established that those that have trained and increased these physical parameters of fitness are more likely to perform better in exercise, there are various causes of fatigue that may inhibit performance. The causes of fatigue have been attributed to both peripheral and central mechanisms, with physiological and psychological aspects (Kent-Braun, 1999), investigated frequently. Peripheral factors stem from biochemical changes within the metabolic processes of the working muscle leading to increased neural excitation and central fatigue relates to a failure

of the CNS to drive motor neurons, resulting in reduced central motor drive (Amann, 2011). A potential cause for this central fatigue is increased somatosensory feedback from the working locomotor muscles (Amann & Secher, 2010). Mechanisms that are suggested to contribute to peripheral fatigue include metabolic substrate depletion (Coyle et al., 1983), and the accumulation of deleterious metabolites in the working muscles, including hydrogen (Kent-Braun, 1999). It is also suggested that in sustained muscle contraction exercise, these metabolites and other exercise-induced activity stimulate type III and IV nociceptors resulting in a decrease in motor neuron responsiveness and suboptimal descending drive, ultimately limiting the motor cortical output and contributing to central fatigue (Taylor & Gandevia, 2008). Therefore, exercise induced pain, as well as both central and peripheral fatigue can occur at the same time and are often associated with shared physiological mechanisms.

1.3.1: Theories of exercise regulation and fatigue

There are a number of theories exploring the different possible mechanisms contributing to changes in exercise regulation during fatiguing exercise. The Central Governor Model (CGM) suggests that afferent feedback such as RPE, previous experiences, the external environment and possibly pain perception is collated and processed to produce a sensation of fatigue, following which, adjustments to exercise output and pacing are made (Noakes et al., 2005). This theory supports that psychological factors are just as important as physical (Ulmer, 1996) and that the primary mechanism associated with fatigue is a centrally governed, altered pacing and muscle recruitment strategy as a response to afferent feedback (Noakes et al., 2005). In contrast, the 'psychobiological' (PB) model suggests that the most important aspect in fatigue is perception of effort, which the theory considers to be independent of afferent feedback (Marcora, 2010). There is also the Integrative Governor Theory (IGT) (St Clair Gibson et al., 2018) that suggests that competition between both psychological and physiological homeostatic drives and the relative weighting of each by the brain's decision-making function, is the driving force behind exercise regulation and the fatigue process. Lastly, the Sensory Tolerance Limit (STL) theory suggests that feedback from peripheral mechanisms, primarily from group III and IV muscle afferents, informs central processes of threats to muscle homeostasis, resulting in feedforward mechanisms that

may control the regulation of exercise to ensure exercise is tolerable (Hureau et al., 2018). Some of these theories allow a consideration that pain may play a role in the process of fatigue, such as the central governor model (Noakes et al., 2005), integrative governor (St Clair Gibson et al., 2018) and sensory tolerance limit theory (Hureau et al., 2018), but others such as the 'psychobiological' model (Marcora, 2010) reject the notion that pain may have an influence on fatigue. This shows that there is still much left to understand in the potential role of exercise-induced pain and fatigue, and there are disagreements in the research on whether it should be considered.

1.3.2: The argument for and against the presence of EIP influencing fatigue

As has been noted previously, the major physiological determinants of fatigue have been well established but other potentially enlightening areas such as perceived exertion and exercise-induced pain are relatively less defined (Astokorki & Mauger, 2016). In the wider context of fatigue and exercise performance research, there is a growing debate as to whether the presence of exercise-induced pain influences fatigue and in turn affects performance. Historically, pain has been somewhat of an afterthought, with prominent papers on fatigue not considering its possible impact (Edwards, 1981; Gandevia, 2001; Noakes, 2000, Schillings et al., 2003). Others acknowledge the presence of exercise-induced pain in fatiguing activity but question the notion that it interferes with the regulation of exercise and suggest it only influences motivation (Marcora, 2010). On the opposing side of this debate is the argument that exercise-induced pain directly impacts exercise performance through pacing and regulation strategies, as a result of afferent feedback, as evidenced by the central control theory (Astokorki & Mauger, 2016; Mauger, 2013; Mauger, 2014; Noakes et al., 2005). This fairly new approach to the topic of fatigue has been greatly contested, but a growing body of research has emerged in recent years that could shed light on this debate.

1.4: Research into exercise-induced pain

Much of the more recent academic research into pain has been designed to investigate what impact exercise-induced pain has on exercise performance or sporting level. A greater understanding of this can then be used to develop strategies to improve the performance of exercises with intense, continuous and repetitive muscle contractions associated with exercise-induced pain and fatigue (Astokorki & Mauger, 2017). Research studies in this topic can be divided into two categories depending on the approach to pain, with studies assessing exercise performance with an induction or a reduction in experimental pain (Mauger, 2013). Multiple different implementations have been used to achieve these methods, providing a good range of literature on the topic.

1.4.1: Pain reduction

There have been a number of notable studies that have decreased exercise-induced pain through experimental methods and measured exercise performance. The use of analgesics has been common, including studies investigating the effects of aspirin (Hudson et al., 2008), codeine (Ray & Carter, 2007) and acetaminophen (Foster et al., 2014; Mauger et al., 2009), on exercise performance. Studies have also been conducted using other pharmacological interventions such as the opioid naloxone (Sgherza et al., 2002) and the morphine derivative, fentanyl (Amann et al., 2009; Amann et al., 2010; Amann et al., 2011). These studies have produced mixed results with some showing an increased performance with a reduction in pain (Foster et al., 2014; Mauger et al., 2009; Sgherza et al., 2002), some not (Hudson et al., 2008; Ray & Carter, 2007) and studies by Amann (2009; 2010; 2011) highlighting the importance of afferent feedback during exercise, but not focused primarily on pain. As mentioned previously, long-term exercise can also lead to reductions in pain. Both aerobic training (Anshel & Russell, 1994) and strength training (Koltyn & Arbogast, 1998) have been shown to effectively improve pain tolerance. Furthermore, O'leary et al. (2017) also showed that if the exercise intervention is more painful, then the improvements in pain tolerance are greater. Also, technology has been used to reduce the effect of EIP both using Transcutaneous Electrical Nerve Stimulation (TENS) (Astokorki & Mauger 2017; Claydon et al., 2008; Gomes et al., 2014) and Transcranial Direct Current Stimulation (tDCS) (Angius et

al., 2017). TENS research has produced mixed results and the use of tDCS although promising, is still under researched at this time. The issue with these reduction interventions however is that many of them act on mechanisms related to exercise regulation beyond the reduction of pain (Mauger, 2013). For example, the influences of acetaminophen on cortico-spinal excitability (Mauger & Hopker, 2012), aspirin on inflammation and coagulation (Hudson et al., 2008), fentanyl on the exercise pressor reflex (Amann et al., 2010; Amann et al., 2011) and tDCS on cortical excitability and increased blood flow to the brain (Nitsche & Paulus, 2001; Zheng et al., 2011). Therefore, other methods may be needed that are more specific to EIP.

1.4.2: Pain induction

Conversely, a method of inducing pain can be used. Several studies have utilised various experimental measures including electrical stimulation (Koppert et al., 2003; Schulte et al., 2003), contact heat (Hughes et al., 2002) and ischaemic pain (Graven-Nielsen et al., 2001), that have predominantly been used in pharmacological trials. There have also been more relevant studies that have used methods such as partial occlusion (Hollander et al., 2010), a cold pressor test (Janal et al., 1994; Ruble et al., 2005) and a pressure pain test (Cook et al., 1997; Vægter et al., 2015), to successfully induce pain during exercise. There are however several shortcomings with these methods. The pressure pain and cold pressor tests, although reliable, are suggested to be inadequate in matching the aetiology of EIP (Olesen et al., 2012) and tolerance of these tests are not as powerful in predicting performance as EIP tolerance (Astokorki & Mauger, 2016). Furthermore, an analysis by Staahl and Drewes (2004) has pointed out negatives of the other methods with electrical stimulation, contact heat and with ischaemic pain/partial occlusion all associated with non-specific activation to muscle, with skin, periosteum and other tissues contributing to the overall pain perception. It therefore appears that none of these methods provide experimental pain that closely matches the aetiology of exercise-induced pain needed for this type of research. However, one model that has been suggested to fulfil this need, is the use of hypertonic saline injections (Graven-Nielsen et al., 1997c).

1.5: Hypertonic saline injections

The injection of Hypertonic Saline (HS) is a novel approach to artificially elevating pain levels that has been used in a range of research papers, especially in the work of Graven-Nielsen (Arendt-Nielsen & Graven-Nielsen, 2003; Graven-Nielsen et al., 1997a; 1997b; Graven-Nielsen et al., 1998a; 1998b; Graven-Nielsen et al., 1997c; 1997d; Graven-Nielsen et al., 2003; Graven-Nielsen et al., 2002; Salomoni & Graven-Nielsen, 2012). One of the pioneers of this technique, Graven-Nielsen has suggested that it closely emulates the experience of muscle pain in terms of intensity, distribution and quality, manifested as a 'dull', 'cramp-like', 'aching' sensation (Graven-Nielsen et al., 1997c). The use of hypertonic saline is believed to be non-toxic and non-related to tissue damage (Svendsen et al., 2005). The technique is implemented by injecting a small volume of usually 5.8% concentration hypertonic saline (salt water) solution intramuscularly and has been safely used in multiple muscles in the human body (Graven-Nielsen et al., 2002), including commonly, the elbow flexors (Khan et al., 2011; Mista et al., 2014; Proske et al., 2003), the tibialis anterior (Graven-Nielsen et al., 1997d, Ciubotariu et al., 2004; Farina et al., 2008) and the knee extensors (Deschamps et al., 2014; Dougherty & Lister, 2011; Henriksen et al., 2007; Graven-Nielsen et al., 2002; Park & Hopkins, 2013).

The mechanisms that control the pain experience associated with hypertonic saline injections are complex, with a range of influences to consider. When injected into the muscle the solution forms a saline pool (Graven-Nielsen et al., 1997b) and the noxious stimuli of the hypertonic saline acts upon type III and IV nociceptors, providing afferent feedback to the brain similar to that experienced during EIP (Proske et al., 2003). The activation of these nociceptors has been attributed to membrane depolarisation (Iggo, 1961), augmented tonicity, ionic alterations or indirect activation by algesic substances stemming from the nociceptive ending or muscle tissue (Kress & Reeh, 1996) and an activation of different receptors resulting in neuropeptides release (Mense, 1993). It has also been attributed to local muscle spasms (DeVries, 1966), increased intramuscular pressure (Allen & Barnes, 1986) and increased sodium concentration (Graven-Nielsen et al., 1997b). Whether as a result of one or a combination of these, the pain sensation produced using hypertonic saline injections is

suggested to mimic EIP (Graven-Nielsen et al., 2002) and many studies have been conducted using this implementation as a result.

1.5.1: Hypertonic saline use in exercise research

A wide range of recent research studies have employed the hypertonic saline injection method and measured the impact on exercise. The majority of these have been implemented on single-limb isometric exercise using the knee extensors (Graven-Nielsen et al., 2002; Henriksen et al., 2011; Park & Hopkins, 2013; Sørensen et al., 2012; Tucker et al., 2009), elbow flexors (Khan et al., 2011; Mista et al., 2015; Proske et al., 2003; Tucker et al., 2009), plantar flexors (Ciubotariu et al., 2004; Graven-Nielsen et al., 1997b; Salomoni & Graven-Nielsen, 2012) and dorsi flexors (Ciubotariu et al., 2004; Farina et al., 2008; Graven-Nielsen et al., 1997b). More limited research has been dedicated to the effect of HS on dynamic movement/exercise but studies have investigated the impact during gait (Graven-Nielsen et al., 1997b; Henriksen et al., 2007), on shoulder strength and accuracy during throwing (Wassinger et al., 2012) and on performance of a maximal single leg-hop. From the results of these studies many observations have been made about the impact of hypertonic saline injections on exercise.

1.5.2: The influence of hypertonic saline on exercise performance

There have been a multitude of effects that the intramuscular injection of HS has had on exercise including a reduction in maximal voluntary contraction (Graven-Nielsen et al., 2002; Henriksen et al., 2011; Khan et al., 2011), reduced force perception and force steadiness (Proske et al., 2003; Salomoni & Graven-Nielsen, 2012; Weerakkody et al., 2003), impaired involuntary and voluntary activation (Park & Hopkins, 2013), impaired motor function during gait (Graven-Nielsen et al., 1997b; Henriksen et al., 2007), reduced accuracy and strength in throwing exercises (Wassinger et al., 2012) and decreased estimation and performance of a single-limb hop (Deschamps et al., 2014). The findings from these experiments support that the pain artificially induced from hypertonic saline influences exercise performance negatively and due to the suggested similarities to exercise-induced

pain, this could be an excellent model to use in the area of fatigue and pain research. However, this a relatively new approach to the problem and there are still gaps in the literature that need to be addressed.

1.5.3: What is not yet known about this method?

Although this technique has been used in many research studies, has been shown as safe for humans, is non-toxic (Graven-Nielsen & Arendt-Nielsen, 2003; Svendsen et al., 2005) and produces pain closely related to the feeling of exercise induced pain (Graven-Nielsen et al., 1997c), it has not yet been shown to provide a consistent reliable response in the same way other pain emulation techniques have. Other methods such as the cold pressor and pain pressure threshold tests may not be thought as accurate as HS injections when artificially recreating exercise-induced pain conditions (Astokorki & Mauger, 2016), but until the HS injection method is proved reliable, the promising developments so far may be invalidated. It is therefore imperative for past and future research using this method, that a competent test-retest reliability assessment is conducted. Especially as the model is being utilized in multiple research studies here at the University of Kent and it is important to validate its use in the laboratories.

Furthermore, the exact profile of pain associated with the injections, especially in response to exercise is still somewhat unknown. It is important to include both physiological and psychological measurements when describing pain and no such study has been conducted to investigate potential changes at rest and with additional exercise. It would also benefit researchers to understand if the addition of low-intensity exercise is enough to elicit changes in pain response to the injections and also between small changes in intensity. There are limited studies that have assessed pain changes between exercise intensities. Firstly, a study by Ciubotariu et al. (2004) measured the differences in pain between 50% and 80% of maximal voluntary contractions and found no changes, based on injections into the tibialis anterior and gastrocnemius lateralis during dorsiflexion and plantarflexion, respectively (Ciubotariu et al., 2004). However, this was not the primary aim to the Ciubotariu et al. (2004) study, there was no psychological element to the measurements and it has also been

suggested that pain response and associated related muscle activity would be higher in larger muscles with increased motor unit populations (Salomoni & Graven-Nielsen, 2012). The Salomoni and Graven-Nielsen (2012) study also used hypertonic saline injections at different forces this time including 2.5%, 20%, 50% and 70% of maximal voluntary contraction force and found no changes in mean force levels, but unfortunately this study did not compare pain levels between intensities and once again had no psychological pain measures. Finally, a study by Ervilha et al. (2004) did compare pain intensity between different loads of weight (0kg, 4kg, 10kg) and found no difference between them. Once again this was not the primary measurement, there was no psychological parameters reported and this was performed on the small lateral head of the biceps muscle (Ervilha et al., 2004). Although these are a few studies that have had different intensities performed with the HS injections, this has mostly been an afterthought with no studies providing a profile of the pain experienced at these intensities, none comparing to resting values and importantly, none considering the psychological side of pain. A study performed with these considerations in mind would help to answer some of the questions, touched upon, but not answered as yet, in research utilizing hypertonic saline injections. Which may prove to be one of the most important artificial models of pain inducement, to further the current understanding of pain and exercise performance.

It is with these considerations based on the literature available that the research questions for this project have been developed. The aims of the research were to attempt to answer (1) is the use of intramuscular hypertonic saline injections a reliable method in experimental pain research? (2) what impact do additional non-fatiguing muscle contractions have on the pain response to hypertonic saline injections? And also provide a physiological and psychological profile of pain experienced from hypertonic saline injections at rest and with additional muscle contractions. It was hypothesized that (1) the hypertonic saline injections would provide good test-retest reliability and (2) the addition of muscle contractions would influence the pain response to hypertonic saline.

Chapter 2: Literature Review

The following review of literature has been written to collate and critically analyse a range of papers in the context of the topic of this thesis, primarily the experience of pain and Exercise-induced Pain (EIP). This includes sections on the possible relationship between EIP and fatigue, experimental pain models, reliability measures in pain research, the use of hypertonic saline injections in research and the influence of hypertonic saline injections on the pain experience and exercise performance.

2.1: Current understanding of fatigue

Fatigue is a complex topic that continues to be a predominant area of debate in the scientific community. One of the reasons for this is that limiting the effects of fatigue, whilst maximising power output and speed is generally accepted as perhaps the most important factor in successful endurance exercise (Joyner & Coyle, 2008), and is therefore of great importance in sport and exercise research. Gandevia (2001) defines fatigue as any exercise-induced reduction in the ability to exert muscle force or power. Fatigue was once thought of as the point at which task failure occurs during a given exercise (Edwards, 1981). However, it is now accepted that fatigue progressively accumulates as intensity and duration increases during any given exercise and occurs irrespective of whether or not the task can be sustained (Gandevia, 2001). Fatigue is a complex process which is affected by many different factors and the predominant mechanisms associated with it are well disputed (Gandevia, 2001). There have been multiple theories put forward to explain these mechanisms, with some generally accepted and others hotly debated.

2.1.1: Components of fatigue

The long-established physiological determinants of endurance exercise performance are maximal oxygen uptake, the lactate threshold, and the economy of energy (Joyner & Coyle, 2008). Where these factors are higher, an athlete's performance will generally be enhanced, but the ability to limit fatigue is also crucial in successful performance (Joyner & Coyle, 2008). Generally, the causes of fatigue are associated with both central and peripheral mechanisms. Central fatigue stems from a failure of the central nervous system to drive

motor neurons, resulting in reduced motor drive, and biochemical changes within the metabolic processes of the working muscle leading to an altered neural excitation (Amann, 2011). Causes of central fatigue that have been suggested including increased somatosensory feedback from the working locomotor muscles (Amann & Secher, 2010) and a reduced ability of the central nervous system to drive motor neurons (Amann et al., 2009). Peripheral factors stem mostly from metabolic substrate depletion (Coyle et al., 1983) and an accumulation of endogenous deleterious metabolites in the working muscles, which impair contractile action (Kent-Braun, 1999). These metabolites are also thought to stimulate type III and IV muscle afferents, providing afferent feedback and limiting the motor cortical output during exercise, which is a central action (Taylor & Gandevia, 2008). Therefore, there is overlap between peripheral and central components of fatigue. This highlights that central and peripheral fatigue mechanisms can both occur during exercise often interacting or occurring simultaneously, and a combination of these factors is thought to occur resulting in increased levels of fatigue and subsequently impaired task performance (Gandevia, 2001).

Pacing and the role of afferent feedback

During exercise, although fatigue will determine the exercise intensity that is achievable for a given moment, an athlete is ultimately reliant on a psychophysiological feedback system to determine a pacing strategy that allows an optimal performance with the lowest fatigue (or at least a regulation of the rate of fatigue) and this process is ultimately controlled by the brain (Ulmer, 1996). This shows the importance to look beyond the established physiological parameters of performance and consider the role that various psychological factors play during fatiguing exercise. This reliance on pacing strategy can be affected by multitude factors and there are a variety of theories which attempt to explain this. These include; the Central Governor Model (CGM) (Amann & Secher, 2010), the Hazard Score (De Koning et al., 2011), Teleoanticipation (Ulmer, 1996) and the Rating of Perceived Exertion (RPE) Template (Tucker, 2009). Although these theories have some subtle differences, broadly they suggest that exercise regulation through pacing manifests as a conscious sensation during exercise in which the difficulty or intensity of exercise can be calculated (Mauger, 2014). Some of these specific exercise regulation models are more relevant to pain research than

others and an in depth break down of each is beyond the scope of this thesis, however the more pertinent theories have been discussed briefly below.

2.1.2: The key models/theories of exercise regulation

The Cardiovascular model

This model states that there are three main factors associated with exercise regulation and fatigue in endurance sports and they are maximal oxygen consumption, the lactate threshold and the efficiency of the muscle to use oxygen to generate a given pace/intensity (Joyner & Coyle, 2008). According to this theory, fatigue is generated as a result of the oxygen consumption of the working muscles being higher than the 'performance VO_2', which is a combination of the maximal oxygen consumption and lactate threshold and is the oxygen consumption that can be sustained for a given period of time (Joyner & Coyle, 2008). Therefore, as intensity is increased, so is fatigue and exercise performance will be limited as the cardiovascular system cannot keep up (Joyner & Coyle, 2008). There is no doubt that these physiological factors play a role in fatigue, but the authors themselves also agree that there are potentially complex motivational and sociological factors that may be important and fatigue cannot be explained by just these physiological explanations alone (Joyner & Coyle, 2008). Therefore, the other central and peripheral factors mentioned previously and EIP, may still influence fatigue, alongside the mechanisms outlined by this model.

Teleoanticipation and the Central Governor Model

Teleoanticipation (Ulmer, 1996) and then later the CGM (Amann & Secher, 2010; Noakes et al., 2005), suggest that the primary factor associated with fatigue is a centrally governed response to afferent feedback. This theory suggests that the psychological aspects of fatigue are under looked and these are just as important as physical determinants (Ulmer, 1996), such as those outlined in the cardiovascular model (Joyner & Coyle, 2008). These psychological factors include RPE, previous experiences of the exercise task, the external environment and

pain perception that are collated and processed during exercise as a sensation of fatigue (Noakes et al., 2005). This sensation then leads to adjustments in muscle recruitment strategy, exercise output and pacing, which will determine the exercise performance (Noakes et al., 2005). Essentially these models place psychological aspects and afferent feedback as the most important aspects in the fatiguing process but accepts the role that physical aspects play and also allows the presence of pain to be considered a factor.

The 'Psychobiological' model

Perception of effort/perceived exertion experienced during exercise has been suggested as a key determinant of endurance performance (Tucker, 2009), or according to the psychobiological model (Marcora et al., 2008) the most integral aspect in the process of fatigue (Marcora, 2010). Commonly measured using the Rating of Perceived Exertion scale (RPE) (Borg, 1998), this is seen as purely the result of central motor command and the collorary discharge and unrelated to afferent feedback, according to this model (Marcora, 2010). The perception of effort has been shown to increase in correlation with exercise intensity and once RPE increases to a point beyond the motivation of the individual to continue, they will disengage from the task and the task is improved when motivation is increased or perception of effort is reduced (Marcora et al., 2008). This 'psychobiological' model does not consider any other potential contributors to fatigue beyond the central mechanisms and this perception of effort (Marcora, 2010). A criticism of this model is that much of this research has focused on the use of RPE. Rating of perceived exertion, although used in most studies, does not have universal definitions, with measurements given based on any combination of; effort to drive the legs, how hard the body is working, how strenuous the exercise is, shortness of breath, amount of discomfort in the limb etc., creating inconsistency between research (Mauger, 2014). Also, many papers have questioned the position of the 'psychobiological' model with studies suggesting that motivation is not the main factor in task disengagement as participants can continue exercising beyond task failure (Amann et al., 2007; Burnley & Jones, 2018; Burnley et al., 2012). The complete rejection of any afferent feedback contribution to fatigue also ignores a potentially important aspect, that of exercise-induced pain (Mauger, 2013). Sensory Tolerance Limit theory

Lastly, the model that is potentially the most relevant to EIP is the sensory tolerance limit theory (Hureau et al., 2018). The STL theory relates to changes in exercise regulation due to afferent feedback from peripheral mechanisms, most commonly from group III and IV muscle afferents (Hureau et al., 2018). As exercise intensity increases so too does stimulation of these specific afferents, due to the accumulation of metabolites (Kent-Braun, 1999), many of which also result in the perception of EIP (Marchettini et al., 1996). According to the STL theory, during exercise, this feedback informs central processes in the brain and the central nervous system of actual or potential threats to homeostasis in the working muscles (Hureau et al., 2018). This results in the initiation of feedforward mechanisms that then may control the regulation of exercise by ensuring it is tolerable (Hureau et al., 2018). This creates a feedback loop that is present during prolonged exercise that limits exercise performance to the tolerable limit and according to this model, this is the primary aspect of the fatiguing process (Hureau et al., 2018). This is somewhat similar to the theory that pain may be used as a 'protective' mechanism (Millan, 1999) and the presence of EIP in the working muscles may potentially be a contributor to this process of fatigue. However, there are still no clear answers as to what are the primary causes of fatigue and with some theories related to this phenomenon vehemently against the presence of EIP, such as the PB model (Marcora, 2010) and others potentially allowing it to be considered, such as the CGM (Amann & Secher, 2010; Noakes et al., 2005) and the STL theory (Hureau et al., 2018), the potential link still needs to be researched further in the future.

2.1.3: Exercise-induced pain and fatigue

Exercise-induced pain occurs acutely during prolonged, intense, continuous and repetitive muscle contraction exercise (Mauger et al., 2009), due to the inducement of mechanical, thermal and chemical stress in the working muscles (Danecker & Koltyn, 2014). These include increased intramuscular mechanical and noxious pressure, cold, heat, deformation of tissue and the release of noxious metabolites that stimulate specific afferent nociceptors in the working muscle during exercise (Mauger, 2013). This provides afferent feedback from the periphery to the brain via the dorsal horn and the central nervous system, resulting in a sensation of pain induced by exercise (Almeida et al., 2004). This type of pain has long been linked to success in sport and exercise (O'Connor, 1992) and often attributed by athletes and

coaches alike as a limiter of performance. Similar to perceived exertion, EIP and its associated processes increase simultaneously with exercise intensity (Mauger, 2014). Due to this and the suggestion that afferent feedback, including EIP, has an influence on pacing and regulation strategies, according to the CGM (Astokorki & Mauger, 2016; Mauger, 2013; Mauger, 2014; Noakes et al., 2005) and the STL (Hureau et al., 2018), it is suggested that EIP plays a role in fatigue alongside established central and peripheral mechanisms, and perceived effort. With the alternative argument acknowledging the existence of EIP but suggesting its sole impact is on motivation and that afferent feedback plays no role in fatigue (Marcora et al., 2008; Marcora, 2010), it is important to assess the literature into EIP and investigate what influence it may or may not have. In order to do this, it is important to look at the different ways pain is utilised in the research and what impact these implementations have on exercise. Most pain studies are approached by utilizing methods to either artificially increase or decrease pain, with some models more effective than others.

2.2: Measures of reliability in research

2.2.1: What is reliability?

Before analysing the reliability and suitability of the different models associated with pain inducement/reduction, it is important to establish the reliability measures in the context of this research. Briefly, quantitative research is an attempt to deconstruct phenomena into measurable or common categories that can be applied to wider subjects and similar situations (Winter, 2000). In order to be applied to a wider context, the instrument or protocols devised by the researcher needs to be standardized, valid and replicable (Golafshani, 2003). With this in mind, reliability, which has been defined as the extent results are consistent over time and representative of the total population under study (Joppe, 2000, cited in Golafshani, 2003: 598), is critical when interpreting study results and effects (Henson, 2001). If a similar methodology can reproduce the results of a study, then the instrument used would be considered reliable (Joppe, 2000, cited in Golafshani, 2013: 598). There are three types of reliability used in quantitative research; the stability of a measurement over time, the similarity of measurements within a certain time period and the degree in which a measurement remains the same when used multiple times (Kirk & Miller, 1986). The last

type is most relevant to the models of experimental pain as the focus is to ensure that the findings from these studies are a result of the study design and not inconsistency of the instrument used.

2.2.2: How to measure reliability?

True reliability cannot be fully measured as there will always be some inconsistency in instruments and human response, instead, reliability is estimated using the variability of differences among scores within a sample (Bruton et al., 2000). The greater dispersion of scores results in a larger variance and a lower estimate of reliability and vice versa (Bruton et al., 2000). Various tests have been used to evaluate this, including Pearson correlation coefficients, Bland-Altman plots, paired *t* tests and Intraclass Correlation Coefficients (ICC) (Koo & Li, 2016). Importantly however, reliability as a measurement reflects not only the degree of correlation but also the agreement between measurements, and intraclass correlation coefficients is the only one of these to measure both (Koo & Li, 2016).

Intraclass correlation coefficients can be defined as the ratio of variability between subjects to the total variability including subject and error variability (Kim, 2013). It has been widely used in the assessment of inter-rater reliability which reflects variation between two or more raters who measure the same group of subjects, intra-rater reliability that reflects the variation of data measured by one rater across two or more trials and test-retest reliability which reflects the variation in an instrument used on the same subject under the same conditions (Koo & Li, 2016). The latter of these three is important in reliability analyses for experimental measures designed to elicit a consistent response when it is used on multiple occasions, eliminating measurement error from what is being assessed and would be relevant for pain models. It is essential to have reliable and valid measures in pain research (Jensen et al., 1999).

There are examples of test-retest reliability being used to assess the reliability of different exercise tests such as the self-paced peak oxygen uptake test (Jenkins et al., 2017; Lim et al., 2016), six-minute walk test (Steffen et al., 2002), yo-yo intermittent recovery test (Krustrup

et al., 2003) and incremental treadmill lactate threshold test (Weltman et al., 1990). Also, measurements used commonly in pain research, including the Visual Analogue Scale (VAS) (Boonstra et al., 2008; Jensen et al., 1994; Kahl & Cleland, 2005) and the Short-Form McGill Pain Questionnaire (SFMPQ) (Georgoudis et al., 2001; Grafton et al., 2005; Strand et al., 2008), have utilised a test-retest reliability study design. All of these studies were designed with at least two visits, the same sample group tested in identical sessions and used ICC scores as an estimate of reliability for their chosen instrument.

The values for ICC scores range between 0 and 1, with a score closer to 1 indicative of higher reliability (Koo & Li, 2016). Scores of 0.5 or below are suggested to show 'poor' reliability, between 0.5 and 0.75 'moderate' reliability, between 0.75 and 0.9 'good' reliability and above 0.9 'excellent' reliability, respectively (Koo & Li, 2016). When interpreting ICC scores, it is important to remember that there are different types of ICC depending on the model, type and definition needed for the reliability study (Koo & Li, 2016). For test-retest reliability studies with a single measurement a two-way mixed effects ICC analysis with absolute agreement needs to be performed as there should be agreement between the values due to the same sample being used (Koo & Li, 2016). There are no standard guidelines on the use of ICC and the measure is often poorly used and often times studies do not declare the type of ICC test/type in their results (Koo & Li, 2016). Although a powerful tool in evaluating reliability, the values need to be examined thoroughly when assessing the reliability of an instrument.

Another measurement associated with test-retest reliability is Cronbach's alpha (CA). This is a measure of the internal consistency of a set of related measures and can be used to support ICC scores in test-retest reliability assessments (Tavakol & Dennick, 2011) or used as a substitute, as ICC is not appropriate for ordinal data or ratio data and CA is not appropriate for scale data (Gadermann et al., 2012). Again, ranging between 0 and 1, a score above 0.75 is deemed to indicate 'good' consistency between measures, but scores over 0.9 may suggest that a test length is too short (Tavakol & Dennick, 2011). Therefore, if a study presents values between 0.75 and 0.9 this would indicate good consistency between their visits and subsequently good reliability for that instrument.

Lastly, another commonly used measurement used in the assessment of reliability is Coefficient of Variation (CV). The CV measures the variability present in a series of values independently of the measurement used for these numbers (Abdi, 2010), that is an often-quoted estimate of measurement error, particularly in repeated tests (Bruton et al., 2000). The use of CV is only meaningful on measurements that have a 'true' zero (i.e. ratio data), therefore data such as time or pain rating on a zero to ten scale can be assessed using CV but not data from RPE scales (6-20), as there is no true zero and the mean is undefined (Abdi, 2010). The CV of a given set of numbers is calculated as the standard deviation, divided by the mean and multiplied by 100, resulting in a percentage score indicating the level of variability (Bruton et al., 2000). The issue with this measurement is that the CV percentage of the smallest observation will differ greatly from the largest, creating inconsistencies in reliability across measures (Bland, 2015). In fact, the use of CV alone has been suggested as an inadequate estimate of reliability, with more appropriate methods such as ICC and CA used instead (Atkinson & Nevill, 1998). However, it is also recommended that researchers cite and interpret a number of statistical methods when assessing reliability, as the reliability index chosen may vary considerably depending on the design of the study (Atkinson & Nevill, 1998). With an understanding of these commonly used reliability measurements, the different pain models can be analysed and compared

2.3: Experimental pain models

Research into whether or not exercise-induced pain influences fatigue has primarily utilised two experimental designs depending on the approach to pain: artificial reduction or induction of pain (Mauger, 2013). In order to modulate the pain experience, a number of different pain models have been used, providing a growing body of literature into the topic of exercise-induced pain. With these models the researchers can control the nature, localisation, intensity, frequency and duration of pain and can provide quantifiable measures of the response, allowing this to be applied to exercise (Drewes et al., 2003). An understanding of these research papers can then provide evidence and context to the wider debate related to pain and fatigue. Information on the benefits, limitations and reliability of these models can also be analysed to find the most effective experimental strategies. In the following sections,

wherever possible, measures of reliability have been presented to provide a comparison between models.

2.3.1: Artificially decreased pain models

Pharmacological analgesics

A common model used in exercise-induced pain research is the introduction of pharmacological analgesics to reduce pain levels during exercise and measure performance. Analgesics that have been utilised in this model include acetaminophen (Foster et al., 2014; Mauger et al., 2009), aspirin (Hudson et al., 2008) and codeine (Ray & Carter, 2007). Fentanyl has also been used as an analgesic during exercise (Amann et al., 2009; Amann et al., 2010; Amann et al., 2011) but is distinctive from the others as it has been used to remove all other afferent feedback completely (and EIP as a consequence), as opposed to a minimisation of pain levels. The reliability of these analgesics is assumed as they have all historically and commonly been used in the treatment of various ailments and would have gone through vigorous clinical testing, but reliability studies on their use during exercise are absent.

The influence of these analgesics on exercise performance have been mixed. In a study by Mauger et al. (2009) 1.5 g of acetaminophen was given to 13 competitive male cyclists during a self-paced 10-mile cycling time trial and as a result performance was significantly improved. However, there was no significant reduction in perceived exertion or pain (Mauger et al., 2009). A different study by Foster et al. (2014) utilising the same dose of acetaminophen (1.5 g), this time with nine recreational cyclists performing repeated cycling sprints, found a significantly improved mean power output but again with no significant differences in perceived pain. This was suggested by both studies to be an indication that the acetaminophen allowed participants to engage in the same level of pain but with a higher power output, resulting in increased exercise performance (Foster et al., 2014; Mauger et al., 2009). Importantly however, a link has been made between acetaminophen and mechanisms outside of analgesia that may be related to fatigue, as Mauger and Hopker (2013)

demonstrated an increased cortico-spinal excitability associated with the drug. Therefore, these alterations in exercise capacity may be a result of this, instead of the intended reduction in pain (Mauger, 2013).

There may also be issues associated with the other pain reducing pharmaceuticals. The ingestion of aspirin (10 $mg.kg^{-1}$) did not significantly improve performance of light resistance training exercise and in fact produced significantly higher perceived exertion and pain perception than both caffeine and a placebo (Hudson et al., 2008). This is supported by a study by Cook et al. (1997) that found a higher dose of aspirin (20 $mg.kg^{-1}$) also did not produce a reduction in perceived pain, suggesting aspirin is an ineffective model in reducing EIP. Similarly, codeine (60 mg) did not improve performance in a fatiguing isometric handgrip experiment, with little influence on pain perception (Ray & Carter, 2007). Conversely, the Ray and Carter (2007) study also utilised the opioid naloxone, which increases pain due to inhibition of the endogenous analgesic system, in the same experiment and found no performance differences despite pain perception increasing compared to codeine. Coupled with previous suggestions that the opioids codeine and naltrexone do not alter the perception of muscle pain during handgrip exercise (Cook et al., 2000), it appears that the use of opioids may not provide an analgesic or ergogenic effect during exercise and are therefore not desirable methods in experimental pain research. However, as these opioids have been used as analgesics in medicine, these results may be an indication of methodological limitations as opposed to the interventions themselves. As these studies used light resistance exercise (Hudson et al., 2008) and handgrip exercises (Cook et al., 2000; Ray & Carter, 2007), respectively, these exercises may not have been intense enough to elicit a strong pain response and may not have been a limiting factor for this exercise with or without analgesia. Therefore, this model may have potential but based on the use of opioids in studies so far, there is little evidence of this currently.

Electrical stimulation techniques

As the use of pharmacological interventions does not appear an effective model in pain reduction studies as there are many other potential mechanisms at play beyond analgesia,

models that utilise electrical stimulation during exercise may also be considered. Notably, Transcutaneous Electrical Nerve Stimulation (TENS) (Astokorki & Mauger, 2017; Hibbert et al., 2017), Inferential Current (Astokorki & Mauger, 2017) and Transcranial Direct Current Stimulation (tDCS) (Abdelmoula et al., 2016; Angius et al., 2015; Angius et al., 2016; Angius et al., 2018; Flood et al., 2017; Maeda et al., 2017).

Firstly, a study by Astokorki and Mauger (2017) tested the influence of both IFC and high frequency TENS, on perceived pain and performance of a single-limb isometric time to exhaustion test and a 10-mile cycling time trial, respectively. They found that IFC and TENS significantly reduced EIP during the isometric test, but only TENS was effective during the dynamic endurance exercise (Astokorki & Mauger, 2017). This analgesic effect is thought to be dependent on multiple factors related to TENS however, with the frequency of electrical stimulation (Claydon et al., 2008), patient groups, outcome measures and a lack of placebo and randomisation measures in the research suggested to affect the outcome (Zeng et al., 2015). Furthermore, multiple studies have found no analgesic effect from TENS (Claydon et al., 2008; Gomes et al., 2014; Hibbert et al., 2017). This also includes an exercise study by Hibbert et al. (2017) that found TENS did not improve performance of a 5 km cycling time trial with no significant differences in pain perception, seemingly contradicting the findings from Astokorki and Mauger (2017). However, the TENS was only applied for 30 minutes prior to exercise in the former study (Hibbert et al., 2017) as opposed to during exercise in the latter (Astokorki & Mauger, 2017), so this may be an explanation for the differences observed. The application of this technique to exercise is still relatively new and has so far produced equivocal results, therefore more research would need to be carried out in this area.

Furthermore, despite the wide use of TENS, information on the reliability of this model during exercise is hard to come by. In clinical tests, high frequency TENS has shown high reliability (ICC 0.94 – 0.99) in elderly stroke patients (Eek & Engardt, 2003) and produces a consistent analgesic response in post caesarean section subjects (Kayman-Kose et al., 2014). High test-retest reliability has also been demonstrated for high frequency TENS in the management of cold-induced pain ($R > 0.86$) (Ashton et al., 1984), but interestingly this was not enough to produce a significant analgesic response. Conversely, low frequency TENS was shown to be effective in the reduction of cold-induced pain but was significantly more

variable ($P < 0.05$) than the high frequency (Ashton et al., 1984). This highlights a potential limitation with this model. As mentioned previously, the response evoked by TENS is dependent on multiple factors, especially frequency, and these are not standardized across research studies. Therefore, a reliability study related to its use specifically in exercise studies would be beneficial to verify any possible ergogenic effects and to answer questions related to the somewhat mixed findings.

Alternatively, the impact of tDCS on exercise performance has been the subject of a range of recent research studies, including several papers by Angius et al. (2015; 2016; 2018). These studies have produced somewhat mixed results. The use of tDCS did not influence performance or perceived pain of a cycling Time to Exhaustion Test (TTE), but did reduce pain during a cold pressor test (Angius et al., 2015). The lack of change in the full-body exercise test may have been due to the placement of tDCS cathode on the prefrontal cortex (Angius et al., 2015), as Angius et al. (2016) demonstrated that shoulder placement of the cathode was more effective than head placement, subsequently improving single-limb isometric time to exhaustion performance and reduced perception of effort. A similar observation was also made in the Angius et al. (2018) study when the tDCS was applied bilaterally, acting upon the M1 and endurance performance was improved with a reduction in perceived exertion during cycling time to task failure exercise. Despite this reduction in RPE, there was consistently no change in EIP across these studies (Angius et al., 2016; Angius et al., 2018). Further afield, there have been a number of papers that have found significant improvements in a variety of exercises after reducing pain with tDCS including isometric muscular endurance (Abdelmoula et al., 2016; Cogiamanian et al., 2007) and whole-body dynamic endurance (Okano et al., 2015; Vitor-Costa et al., 2015). However, there are also multiple studies that have found no ergogenic effect from this method when applied to similar exercises, with contradictory findings from isometric (Flood et al., 2017; Kan et al., 2013; Muthalib et al., 2013), strength training (Maeda et al., 2017) and whole-body dynamic (Barwood et al., 2016) exercises. This shows an inconsistency across studies and this may be due to a number of reasons, such as the placement/montage of tDCS (Angius et al., 2016), a high variability in cortico-spinal excitability (Horvath et al., 2015), a lack of a reliable means to measure neuromuscular response (Madhaven et al., 2016) and the fact that the exact mechanisms to which tDCS improves exercise performance are unknown (Angius et al., 2017).

The test-retest reliability of this model has been assessed via a study by Wörsching et al. (2017), that measured the effect of resting prefrontal cortex tDCS on brain activity and found poor reliability after tDCS (ICC 0.09 – 0.12), compared to baseline measures (ICC 0.15 – 0.5). This is also supported by findings from other studies (Chew et al., 2015; Dyke et al., 2016; Horvath et al., 2016) that have suggested high variability in motor evoked potentials, from this instrument. Again, this may be attributed to the inherent limitations of this model that appear to show high variability in the results. The inconsistent results and lack of reliability of this model, as well as the other pain reduction models mentioned previously, means that another experimental design may be needed to improve exercise-induced pain research.

2.3.2: Artificially induced pain models

An alternative approach to these experimental reduction models is to artificially induce pain during exercise and measure the response. Central pain mechanisms can be studied in humans by evoking processes such as hyperalgesia, referred pain or temporal summation, with artificial pain methods originating in any tissue (Arendt-Nielsen & Yarnitsky, 2009). This has most commonly been investigated in the skin using electrical or thermal stimulation (Curatolo et al., 2000), but can also be induced in the muscle with strong or prolonged noxious stimuli, commonly with occlusion or chemical substances (Sarkar et al., 2003). This model presents an advantage over clinical studies investigating the impact of painful diseases, as the cause-effect relationship is known and participants can be given a standardized dose of pain, allowing the potential mechanisms and response to pain to be measured (Graven-Nielsen & Arendt-Nielsen, 2003).

An increase in pain levels, as opposed to a decrease, may also limit or remove the possible alternative mechanisms acted upon by analgesics and electrical stimulation mentioned previously. It is important to utilise an artificial pain model that can provide explanations of the exercise response based solely on the impact of pain as opposed to possible side effects, but that can also produce a consistent pain response. The different forms of this approach therefore need to be assessed to find the most effective and most reliable model.

Characteristics that the ideal artificial pain stimulus should have are a distinct pain sensation with minimal tissue damage, a relationship between stimulus and pain intensity, to have reproducibility and to act upon nociceptors exclusively (Graven-Nielsen et al., 2001). With these in mind, finding the most effective pain model is essential in future exercise-induced pain research.

Pressure pain

Pressure ergometry, in which mechanical pressure is applied using an ergometer to an area of the body to elicit pain, is the most frequently used technique in the quantification of pain (Staahl & Drewes, 2004). This technique can be applied to evoke pain from the skin and muscles by applying standardized pressure stimulation at a certain point (Staahl & Drewes, 2004) and is commonly used to measure an individual's Pressure Pain Threshold (PPT), defined as the minimum force applied which induces pain (Maquet et al., 2004). Pressure pain threshold is an accessible measurement of pain response used commonly in clinical practice and has been suggested as a predictor of pain associated with many common ailments such as neck pain (Sterling et al., 2006; Walton et al., 2011), fibromyalgia (Giesecke et al., 2003; Granges & Littlejohn, 1993; Mikkelsson et al., 1992) and lower back pain (Farasyn et al., 2008; Giesbrecht & Battié, 2005). This method has also been shown to have good reliability when used in clinical trials, evidence by Walton et al. (2011) (ICC = 0.76 – 0.79) and Farasyn et al. (2008) (ICC = 0.97), based on PPT scores and LFMPQ results. Also, studies conducted using healthy human subjects (Chesterton et al., 2007; Fischer, 1987; Koo et al., 2013; Pelfort et al., 2015) have shown good reliability, with ICC scores of 0.91 based on manual PPT responses presented by Koo et al. (2013) and ICC scores of 0.86 – 0.92 demonstrated by Pelfort et al. (2015), respectively. Therefore, this model appears to provide a reliable painful stimulus across visits, but the utilisation of this technique may be less suitable for exercise studies.

Pressure pain has been used predominantly in clinical research but has been applied to some exercise studies, usually as a measurement of an individual's pain threshold or as a predictor. Exercise has been suggested to improve pressure pain threshold and pressure pain tolerance

in aerobic endurance (Vaegter et al., 2014). Furthermore, a relationship has been suggested between the type of pressure pain elicited in exercise induced pain and exercise performance (Cook et al., 1997). However, the pain pressure threshold test has been shown to be a weaker predictor of exercise performance than tolerance to exercise-induced pain (Astokorki & Mauger, 2016), with suggestions that the aetiology of pressure pain from experimental measures are different to that of EIP (Olesen et al., 2012). This, coupled with limitations in the delivery of pressure ergometry, namely, contact with the skin resulting in a non-specific pain response and a likelihood that there are changes in the tissue structure when this technique is applied (Handwerker & Kobal, 1993; Staahl & Drewes, 2004), mean that this method may be a reliable indicator of clinical pain but is not necessarily appropriate to exercise-induced pain research.

Thermal pain

Experimental thermal pain can be evoked from the skin using either an increase or decrease in temperature. The latter of these when applied through experimental means is thought to stimulate unmyelinated C fibres that play a role in pain perception (Wasner et al., 2004), stemming from the nociceptors or cutaneous veins of the affected area (Curatolo et al., 2000). Cold pain can be induced through various means including ice, ice water, cold gel, menthol, ether, wet alcohol sponge or a Peltier thermode (Staahl & Drewes, 2004). The most common utilisation of this type of induced pain in research is the cold pressor test (Janal et al., 1984; Janal et al., 1994; Padawer & Levine, 1992; Ruble et al., 2005; Sternberg et al., 1998). Cold pressor pain is induced by submerging the hand in cold water and is thought to mimic the effect of chronic pain conditions due to the unpleasant sensation felt (Rainville et al., 1992). The technique has been suggested to have excellent reliability and validity (Edens & Gil, 1995), with the CPT being used in many pain induction experiments (Mitchell et al., 2004). An individual's pain tolerance can be measured with this test by recording how long they can endure this cold-induced pain (Curatolo et al., 2000).

In relation to exercise the cold pressor test has predominantly been used to investigate the analgesic effects of exercise on cold pain, with a lack of research into how exercise

performance may be influenced by this technique. Firstly, Janal et al. (1994) investigated whether regular runners could tolerate cold pain from the cold pressor test more effectively. They found that the runners were less sensitive to the noxious stimuli from cold pain, but without physiological changes such as heart rate and blood pressure (Janal et al., 1994), with the authors theorising that this was an adaptation to regular training. Conversely, a study by Ruble et al. (2005) demonstrated that high intensity bouts of exercise (75% VO_{2max}) did not reduce cold pressor pain perception, despite this exact exercise showing an analgesic effect on other pain models. This is supported by a study by Janal et al. (1984) that corroborated this lack of analgesic effect whereas other studies have shown a reduction in cold pain following exercise (Padawer & Levine, 1992; Sternberg et al., 1998). As well as these discrepancies in the literature, the cold pressor test has been shown as a weaker predictor of exercise performance than exercise-induced pain, similar to the pressure pain threshold test (Astokorki & Mauger, 2016). However, the method is suggested to be reliable with previous exposure to cold pain testing not altering future pain tests (Ruble et al., 2005). This reliability has been tested with good test-retest reliability being reported by Koenig et al. (2013) with ICC scores of 0.79 for 4º Celsius and 0.86 for 6º Celsius water temperature cold pressor tests.

Cold pressor pain could therefore provide a reliable painful stimulus to be used in exercise-induced pain studies in theory, but there are notable limitations to this technique. Firstly, the mechanisms associated with thermal pain are different to those attributed to exercise-induced pain and the aetiology of the pain sensation produced is also vastly different (Olesen et al., 2012). Also, as cold pain is thought to act via cutaneous veins, vascular reactions strongly affect the response (Staahl & Drewes, 2004) and this method is thought to evoke mainly peripheral mechanisms (Burian et al., 2003). Furthermore, the mixed results observed with this method can be attributed to multiple factors including no standardized temperatures with subtle differences effecting the cold pressor response (Mitchell et al., 2004), as well as gender differences (Compton et al., 2003; McAdoo et al., 1990), anxiety (Jones et al., 2002) and pain scaling inconsistencies (Compton et al., 2003). All of these factors suggest that despite the suggested reliability in this model, it would not be appropriate in experiments where the goal is to closely simulate exercise-induced pain as the response would be too different.

Muscle pain models

Many of the previous methods of artificial pain reduction or inducement discussed generate pain through or impact the skin, but as exercise-induced pain is generated due to changes originating in the working muscles (Danecker & Koltyn, 2014), models that mimic this would be more beneficial when applied to exercise studies. Muscle pain is a combination of local and referred pain, described as a cramp-like, diffuse and aching pain (Staahl & Drewes, 2004) is also suggested to be a larger clinical problem than any other pain condition (Mense, 1993). Therefore, finding a reliable pain inducement model that provides pain generated from the muscles could be key for both clinical and exercise research involving pain - the following methods could provide such a solution. Usually these muscle pain models can be divided into two groups; one with external stimuli (exogenous) and one with the pain generated from within the muscle itself (endogenous) (Graven-Nielsen et al., 2001). Examples of these types of models include endogenous exercise-induced pain and experimental ischaemia, and exogenous electrical stimulation and stimulation from various chemical substances (Graven-Nielsen, 2006).

Electrical stimulation

Exogenous electrical stimulation can be applied using electrodes attached to the surface of the skin generating a stimulus similar to other pain models, but it can also be utilized to generate a painful stimulus from the muscles (Staahl & Drewes, 2004). Thus, limiting some of the issues present in skin derived models. This technique is administered using needle electrodes inserted into muscles (Schulte et al., 2003) and presents an advantage over other models as pain can be generated exclusively during the contractions (Olesen et al., 2012). Schulte et al. (2003) suggested that the manifestation of increased pain rating and areas was attributable to central mechanisms, due to the inducement of temporal summation. This is believed to mimic the aetiology of neuropathic pain which is associated with an increase in central sensitivity from temporal summation (Woolf, 2011). Furthermore, in a study by Laursen et al. (1997) this technique has been shown to produce a consistent response across sessions but the profile of pain perceived appears very different to what would be expected in

exercise-induced pain. Pain quality was described mostly as ‘drawing’, ‘boring’, ’penetrating’ and ‘taut’, for local pain and ‘sharp’, ‘taut’ and ‘tight’, for referred pain, respectively (Laursen et al., 1997). This is different from the ‘dull’, ‘aching’ sensation associated with exercise-induced pain (Mauger, 2013). Therefore, this model may be more suitable to clinical studies investigating neuropathic pain as opposed to exercise-induced pain.

There are also limitations with this model beyond its non-specificity to exercise-induced pain. One of the major issues is that during stimulations there are concurrently activated muscle twitches (Graven-Nielsen et al., 2001), which can influence the pain response and can reduce reproducibility by changing the position of electrodes (Staahl et al., 2006). Further, electrical stimulations are thought to bypass the receptors and activate nerve fibres directly and is therefore not specific to nociceptors (Staahl & Drewes, 2004). For these reasons and the lack of application to exercise studies, this model does not appear to be adequate for use in exercise-induced pain research but may be useful in clinical trials.

Ischaemic pain

An endogenous method of inducing muscle pain is via ischaemia. This is achieved with the application of a tourniquet and subsequent voluntary muscle contractions of the occluded muscles, which results in an unpleasant tonic pain sensation (Graven-Nielsen et al., 2001). Although the exact mechanisms are unknown this pain is thought to occur due to the accumulation of algogenic substances, such as potassium, adenosine and lactate, in the occluded area, that cannot be removed due to the tourniquet (Graven-Nielsen & Arendt-Nielsen, 2003) and are thought to excite mainly C muscle fibres (Issberner et al., 1996). The amount of pain developed from this technique is dependent on the duration of occlusion, as well as the number and force of the contractions (Vecchiet et al., 1987). The tourniquet model has long been considered reliable (Smith et al., 1968) and has been used efficiently in trials with analgesics (Graven-Nielsen & Arendt-Nielsen, 2003; Oleson et al., 2012). The model can be used in any study that requires a general tonic pain stimulus (Graven-Nielsen et al., 2001) and could be applied to exercise studies if needed.

The implementation of ischaemic pain has been applied to exercise in studies that have utilized partial occlusion protocols. A notable study that utilized this model was performed by Hollander et al. (2010), in which partial occlusion was applied to light resistance exercise and compared to moderate exercise without occlusion and occlusion without exercise, with perceived pain and effort recorded throughout. This study presented some interesting findings with the perception of pain and effort increasing at a similar rate between the light occluded exercise and the moderate non-occluded exercise, with both higher than the occluded rest protocol (Hollander et al., 2010). The authors suggested that this confirms that peripheral sensations play an important role in determining perceived effort and pain during resistance exercise (Hollander et al., 2010). One possible limitation of this study, however, is the small sample size with only seven males used (Hollander et al., 2010). Alternatively, a study by Loenneke et al. (2011) with a sample size of 15, also demonstrated elevated pain and effort perception from occluded repetitive resistance exercise over control, alongside a significant decrease in exercise performance. Another study found that there were significant reductions in resistance exercise repetitions at lower intensities (20 – 40% of 1 repetition max) but not for the higher load (50%), but with equally elevated pain ratings, predominantly post-exercise as opposed to during exercise (Wernbom et al., 2006). Therefore, there does appear to be a negative impact on performance when ischaemic pain is introduced using this model but there may some limitations.

Firstly, the type of cuff used in occlusion can influence the cardiovascular and pain response, with wide cuffs inducing greater heart rate, blood pressure, perceived effort and pain (Rossow et al., 2012). The examples mentioned previously all used different sized cuffs with 135 mm, 76 mm and unspecified (custom) cuffs used in Wernbom et al. (2006), Loenneke et al. (2011) and Hollander et al. (2010), respectively, so there is clearly a lack of standardization across research studies. Another limitation is that ischaemic pain elicited using the tourniquet model is non-specific to muscle, as skin, periosteum and other tissues will contribute to the overall pain perception (Olesen et al., 2012). Further to this point, this technique does not allow specific muscles to be targeted with any muscle used in the occluded contractions affected by pain (Graven-Nielsen et al., 2001). One more limitation is that although the pain is produced endogenously, when activating nociceptors, the contact of the tourniquet on the skin can activate non-nociceptive nerves which will impact the pain perception similarly to models that impact the skin (Staahl & Drewes, 2004). Also,

metabolites such as hydrogen, prostaglandins, bradykinin, substance p, nitric oxide, amongst others responsible for this type of pain (Millan, 1999) are prevented from being removed and as some of these have a fatiguing effect (Marchettini et al., 1996), exercise capacity is altered, making it hard to assess the impact of pain independently. Due to these limitations and relative lack of reliability due to unstandardized procedures, more research using this model would be beneficial before it can be deemed an effective method of pain inducement.

Pain evoked by exercise

This endogenous model utilises pain produced during and after exercise. As discussed previously exercise-induced muscle pain occurs acutely during exercise and is suggested to inhibit performance of said exercise task (Mauger, 2013). Therefore, the acute pain and potential impact on performance can be measured or alternatively this exercise provoked pain may be felt after exercise has commenced in the form of muscle soreness, to provide induced pain at a later date. Concentric muscle work normally results in a short-lasting pain sensation, similar in make up as ischaemic pain (Graven-Nielsen et al., 2001), but repetitive eccentric muscle contractions often result in a delayed onset of muscle soreness that usually peaks in the following 24 to 48 hours after exercise (Bajaj et al., 2000). Delayed Onset Muscle Soreness (DOMS) is characterized by a sensation of discomfort, predominantly emanating from skeletal muscles and can range from muscle tenderness to severe debilitating pain (Cheung et al., 2003). Several theories have been proposed to explain the mechanism of DOMS, with a combination of two or more likely to explain the phenomenon (Olesen et al., 2012). These theories include lactic acid, muscle damage, inflammation, muscle spasm, connective tissue damage and enzyme efflux (Cheung et al., 2003). Whatever the cause of DOMS, prior intensive exercise will induce a widespread deep pain in the muscles and other somatic structures which can then be used in studies that need an unspecific muscle pain stimulus (Graven-Nielsen et al., 2001). The duration, intensity and type of exercise used is important in the onset and severity of DOMS (Olesen et al., 2012). A potential advantage of this model is that there is pain during muscle function and palpation but not at rest, which is different to other pain inducement techniques (Olesen et al., 2012). Therefore, this model can provide a painful stimulus specifically during exercise and the impact on performance can be measured.

Numerous studies have shown that the presence of DOMS negatively influences exercise performance in a range of ways. These decrements include reduced joint range of motion (Francis & Hoobler, 1987; Nosaka & Clarkson, 1996; Saxton & Donnelly, 1995), muscle strength (Donnelly et al., 1990; Hasson et al., 1993; Tokmakidis et al., 2003) and dynamic power (Byrne & Eston, 2002; Tokmakidis et al., 2003). These studies all found a reduction in exercise performance with the presence of DOMS, however upon further analysis there is one aspect that calls into question this model for use in pain research. Some of these studies used analgesics in the form of aspirin (Francis & Hoobler, 1987) and ibuprofen (Donnelly et al., 1990; Hasson et al., 1993; Tokmakidis et al., 2003), to minimise the pain felt during DOMS and then compared performance between those with elevated pain and those without. Donnelly et al. (1990) did not find a beneficial effect on DOMS from ibuprofen with no changes observed in soreness perception following downhill sprints. Conversely, Tokmakidis et al. (2003) found that ibuprofen decreased muscle soreness perception but had no effect on hamstring one repetition max and vertical jump performance. Hasson et al. (1993) also found that ibuprofen decreased muscle soreness but also observed less decline in muscle force when compared to the control group. These studies suggest that even when pain is reduced from DOMS there may be other confounding factors at play that can influence performance beyond perceived pain.

Beyond the pain generated from DOMS there are other factors that have been suggested to impact performance including reduction of joint range of motion, shock attenuation, alterations in muscle sequencing and recruitment patterns due to damage to muscle fibres (Cheung et al., 2003). Due to these other factors, using an exercise-induced muscle pain model will provide a deep muscle pain stimulus but the impact on performance cannot be attributed specifically to pain. There are also questions about the reliability of this method as DOMS can depend on several factors such as individual differences, previous experience of the exercise and the type, duration, and intensity of prior exercise (Cheung et al., 2003). This model also involves several muscle groups or muscles and will be difficult to control and reproduce (Graven-Nielsen et al., 2001). As well as this, muscle pain from this technique is dependent on the size/structure of the muscle and amount of afferent barrage (Graven-Nielsen et al., 2001), with larger muscles shown to experience a higher level of DOMS than smaller muscles (Svenson et al., 1995). Also, the pain from this model is not temporary like other pain inducement models and DOMS involves muscle damage, which may impact the

contractility of the muscle (Svenson et al., 1995). All of these factors suggest that exercise-induced pain models such as the use of DOMS are ineffective if the goal is to investigate how pain influences exercise, but due to its negative impact on performance it needs to be understood and removed from studies that do.

Intramuscular chemical stimulation

Intramuscular injections of algogenic chemical substances are a common method for the inducement of muscle pain (Graven-Nielsen et al., 2001). Substances that have demonstrated effective pain inducement include acid phosphate (Issberner et al., 1996), serotonin (Babenko et al., 1999a; Jensen et al., 1990), bradykinin (Babenko et al, 1999b), substance P (Babenko et al., 1999a; Pedersen-Bjegaard et al., 1991), levo-ascorbic acid (Rossi & Decchi, 1997), potassium chloride (Jensen & Norup, 1992), or a combination of endogenous substances such as bradykinin, serotonin, histamine and prostaglandins (Mørk et al., 2003). However, the most commonly used methods are injections of hypertonic saline and capsaicin (Graven-Nielsen et al., 2001). Capsaicin is a naturally occurring alkaloid that is derived from chili peppers, that is the chemical responsible for the hot and spicy sensation from this food substance (O'Neill et al., 2012). Capsaicin is more commonly injected intradermally or applied topically to the skin to assess central sensitization due to hyperalgesia and more closely resembles neuropathic pain (O'Neill et al., 2012), but it can also be injected intramuscularly to evoke muscle pain (Graven-Nielsen et al., 2001). Injections of capsaicin into the muscle are thought to sensitive nociceptors (Arendt-Nielsen et al., 2008a) leading to a decrease in pressure pain threshold and an increased pain intensity, dependant on the volume and muscle structure (Witting et al., 2000). This is supported by multiple studies demonstrating that the administration of capsaicin increased sensitivity to mechanical and pressure stimuli (Arendt-Nielsen et al., 2008b; Arima et al., 2009; LaMotte et al., 1991; Sluka, 2002). The Witting et al. (2000) study also found that the pain response was significantly lower in the intramuscular capsaicin injections when compared to intradermal injections, suggesting that the technique mainly acts upon central mechanisms via the skin or central and peripheral when applied to the muscle. As this model provides both local and referred pain associated with muscle pain (Witting et al., 2000), it could in theory be applied to exercise.

Much of the research using this model have utilised injections of capsaicin into the jaw muscles (O'Neill et al., 2012) and there is a lack of studies dedicated to exercise. A study by Wang et al. (2010) showed that the intramuscular presence of capsaicin increased pain and impaired normal jaw motor function. This is supported by Arima et al. (2009) who demonstrated moderate masseter muscle pain evoked by capsaicin inhibited submaximal contractions, but with an increased blood flow. There is a lack of evidence as to whether this is applicable to larger muscles. Further, Arendt-Nielsen and Yarnitsky (2009) found decreased pain pressure threshold and tolerance, when injected deeply into the masseter muscle. There are no studies that have applied capsaicin to large locomotor muscles. There is a study by Chang et al. (2004) that used intramuscular injections of this substance into the forearm but not in relation to exercise. This study does highlight an important aspect of this pain model to consider, however. Chang et al. (2004) found that the muscle pain produced by the capsaicin was described in terms of quality as a 'throbbing', 'pulsing', 'tingling', 'miserable' sensation. Coupled with descriptions such as 'sharp', 'pulsing', 'shooting' (Arendt-Nielsen & Yarnitsky, 2009) and 'throbbing', 'drilling', 'pressing' (Witting et al., 2000) observed in other studies, this model doesn't appear to provide a pain quality similar to exercise-induced pain. Due to this and the lack of studies utilizing this model in an exercise capacity it appears inadequate. However, there are benefits to this model, such as the ability to target specific muscles, it is comparable to muscle pain and provides a longer lasting pain sensation than other methods (Olesen et al., 2012). The other commonly used substance in this model, hypertonic saline, may provide a model with similar benefits but with a closer pain profile to exercise-induced pain (Graven-Nielsen et al., 2001).

2.4: Hypertonic saline injections

2.4.1: Origins/development of the model

The initial foundations of this method can be traced back to the1930's with studies by Kellgren (1938) and Lewis (1938) introducing the concept of induced muscle pain from saline. This experimental method was suggested to be a good model in pain research as the quality of the induced pain mimics clinical muscle pain (Kellgren, 1938). A later study by Feinstein et al. (1954) demonstrated that hypertonic saline could be injected into various deep

tissues around the body, with differences in referred pain perception depending on the somatic tissues chosen. This represents an advantage of this technique in that it allows pain to be applied to specific muscles depending on the experiment (Graven-Nielsen et al., 1997a). The development of this method in a number of papers over the years has demonstrated that it provides both local and referred pain comparable to acute clinical muscle pain (Feinstein et al., 1954; Kellgren, 1938; Lewis, 1938; Stohler & Lund, 1994; Stohler & Lund, 1995; Stohler & Kowalski, 1999; Svensson et al., 1995;) and has also been suggested to resemble exercise-induced pain (Graven-Nielsen et al., 1997c). Due to this aspect, since it's first inception this method is one of the most common pain inducement models (Graven-Nielsen et al., 2001) and is seen as safe (Svendsen et al., 2005). Evidence of this safety can be found in a study by Stohler and Lund (1994) and several studies by Graven-Nielsen et al. (1997a; 1997c; 1997d; 2002), in which over one thousand intramuscular injections have been carried out with no reports of any side effects.

2.4.2: How the method is implemented?

The general procedure of this method is to manually inject a small volume of hypertonic saline (Graven-Nielsen et al., 2001), into the densest part (usually the belly) of the muscle at an angle of 90 degrees (Dougherty & Lister, 2011). Alternatively, a continuous infusion of the solution can be applied using a catheter (Graven-Nielsen et al., 1998b). Hypertonic saline refers to a sterile water solution with a concentration of sodium chloride (NaCL) higher than 0.9% (Mortimer & Jancik, 2006). A concentration of saline of 0.9%, referred to as isotonic saline, is widely used as a placebo control in hypertonic saline studies as it does not produce any muscle pain (Graven-Nielsen et al., 2002). The volume of saline used is usually low and depends on the size of the muscle being injected, with smaller muscles like those located in the jaw commonly infused with around 0.2 ml (Jensen & Norup, 1992), medium sized muscles such as the tibialis anterior 0.5 ml (Graven-Nielsen et al., 1997c; Schulte et al., 2003) and large locomotor muscles like the knee extensors, around 1 ml of hypertonic saline (Deschamps et al., 2014; Salomoni & Graven-Nielsen, 2012; Sørensen et al., 2012). This is common in the research but not exclusive, with examples of higher volumes (1 ml) injected into small muscles (Salomoni & Graven-Nielsen, 2012) and larger muscles (1.5 ml) (Graven-Nielsen et al., 2002) showing that there are a variety of volumes that can be administered

depending on the experimental design. An important study in the development and use of this method is by Graven-Nielsen et al. (1997a) which investigated the correlation between pain intensity and the volume, concentration and infusion time of hypertonic saline injections. In terms of volume, this study measured the influence of 0.1 ml and 0.5 ml and found a significant positive correlation between the volume infused and the pain intensity experienced (Graven-Nielsen et al., 1997a). This shows that the volume of solution chosen can influence the pain induced by this model, so researchers need to account for this in their study design.

The concentration of sodium chloride found in hypertonic saline in the majority of studies using this pain model is usually 5% (Bennell et al., 2004; Deschamps et al., 2014; Graven-Nielsen et al., 1997a; 1997c; Graven-Nielsen et al., 1998b; Graven-Nielsen et al., 1997d; Schulte et al., 2003; Wassinger et al., 2012), 6% (Ciubotariu et al., 2004; Matre et al., 2002; Salomoni & Graven-Nielsen, 2012), or around 5.8% (Henriksen et al., 2007; Henriksen et al., 2010; Henriksen et al., 2011; Graven-Nielsen et al., 2002; Schilder et al., 2014; Sørensen et al., 2012) with all of these concentrations shown to elicit a pain response, without toxicity or tissue damage (Svendsen et al., 2005). The Graven-Nielsen et al. (1997a) study also investigated the relationship between pain intensity and different concentrations including 0.9%, 5%, 11.5% and 20%. Similar to volume, this study (Graven-Nielsen et al., 1997a) also demonstrated a significant positive correlation between pain intensity and sodium chloride concentration. There was no difference in pain intensity between the two highest concentrations however suggesting that above a certain concentration level, the pain intensity plateaus (Graven-Nielsen et al., 1997a). This has been observed in another study by Vecchiet et al. (1993), with no differences between 10% and 20% concentrations. This observation and the majority of studies using lower concentrations (5 - 6%) being found effective in inducing muscle pain, it appears a higher concentration than this is unnecessary.

The injection procedure can be carried out either manually by the researcher or by a computer-controlled infusion pump (Zhang et al., 1993), which has been suggested to make it easier to inject smaller volumes and improve reproducibility (Graven-Nielsen et al., 2001). This computer-controlled injection technique has been used frequently in the work of Graven-Nielsen (Graven-Nielsen et al., 1997a; 1997b; Graven-Nielsen et al., 1998a; 1998b;

Graven-Nielsen et al., 2003; Graven-Nielsen et al., 2002; Graven-Nielsen et al., 1997c; 1997b), but there are many other studies that have performed the injections manually without issues (Deschamps et al., 2014; Henriksen et al., 2007; Park & Hopkins, 2013; Leffler et al., 2000; Proske et al., 2003; Proske et al., 2004; Salomoni & Graven-Nielsen, 2012; Schilder et al., 2014; Wassinger et al., 2012) and this is an easier, less expensive option. The use of ultrasound guidance has also been used effectively (Henriksen et al., 2011; Sørensen et al., 2012), but again this can be an expensive addition to a study.

Intramuscular injections have been used in the administration of drugs or other substances in humans for over 160 years and is considered a simple and safe procedure (Svendsen et al., 2005). Although complications or side effects are rarely observed (Svendsen et al., 2005), there are certain injection formulations/techniques that can cause local muscle tissue damage at the injection site, which in turn may cause pain (Svendsen et al., 2006). However, several studies (Graven-Nielsen et al., 1997a; 1997c; Svendsen et al., 2005) have demonstrated that the pain experienced from intramuscular injections of hypertonic saline specifically, is not attributed to any tissue damage, when performed properly. The full protocols and risks associated with this model are beyond the scope of this thesis but the reader is referred to the guides provided by Rodger and King (2000), and Small (2004), for an in-depth assessment of the different injection areas, techniques and corresponding risks. Put simply, the intramuscular injection technique depends on the muscle or tissue used, but the substance is usually injected into the central belly of the muscle, with care taken to avoid any blood vessels, nerve or bone structures (Rodger & King, 2000). In terms of the infusion of saline, Graven-Nielsen et al. (1997a) showed that a ‘faster’ infusion (0.5 ml over 20 seconds), did not cause higher pain but the onset of pain was quicker, conversely with the ‘slower’ infusion (0.5 ml over 100 seconds), pain onset was later but the duration of pain was longer. The ‘slower’ infusion also had a higher evaluative pain score characterized as ‘miserable’, over the ‘annoying’ ‘faster’ infusion, attributed to the length (Graven-Nielsen et al., 1997a). All of these factors of the injection technique show that the pain experience from the injections is dependent on volume, concentration, targeted muscle and infusion time, so this is key information to consider for any researcher using this model.

2.4.3: Understanding of the associated causes/mechanisms

Hypertonic saline injections have been shown to produce local and referred pain in the infused muscle, mimicking musculoskeletal pain in both quality and its effect on motor performance (Korotkov et al., 2002). There are multiple causes and mechanisms that have been attributed to this model of pain inducement and although it is the most used chemical substance to induce muscle pain (Svendsen et al., 2005) there is still much left to know about the physiology of the hypertonic saline method. However, due to the growing body of research using this model, since its inception in the 1930's (Kellgren, 1938; Lewis, 1938), there is a greater understanding of how this technique works compared to other pain models.

The experience associated with hypertonic saline muscle pain, much like other facets of pain, is complex and most likely multidimensional. Despite saline induced pain being used in many studies the mechanisms behind this pain is not fully understood and it is possible that multiple mechanisms interact (Graven-Neilsen et al., 1997c). It is believed that the infusion of hypertonic saline into the muscle generates a saline pool which drastically elevates the extracellular sodium concentration (Graven-Nielsen et al., 1997b). This may then result in direct excitation of the unmyelinated afferents, leading to a sodium influx into the cell membrane and depolarisation of the nerve (Staahl & Drewes, 2004). This stimulation of afferent nerve fibres, predominantly, but not specific to, type IV and also type III nociceptive fibres (Schulte et al., 2003), provides afferent feedback to the brain and results in the dominant sensation of deep muscle pain (Proske et al., 2003). Interestingly, these are also the afferent fibres associated with exercise-induced pain (Marchettini et al., 1996), which may indicate why the pain from hypertonic saline is thought to be a good substitute. As well as increased sodium concentration (Graven-Nielsen et al., 1997b) and membrane depolarization (Iggo, 1961; Schulte et al., 2003), there are other mechanisms that have been attributed to the activation of these nociceptors.

In the past the nociceptive activation from injections of hypertonic saline has been suggested to stem from muscle spasms (DeVries, 1966; Travell et al., 1942) or increased intramuscular pressure (Allen & Barnes, 1986). The former posits that muscular pain, whether clinical or

artificially induced, is caused by spasms in the muscle that stimulate receptors, evidenced by the removal of muscle spasms associated with shoulder pain with intramuscular procaine (Travell et al., 1942). Although there is a risk that muscle spasms can occur with any intramuscular injection (Rodger & King, 2000), hypertonic saline injections are not strongly associated with studies reporting no such side effects after over a thousand injections (Stohler & Lund, 1994; Graven-Nielsen et al., 1997a; Graven-Nielsen et al., 2002; Graven-Nielsen et al., 1997c; 1997d). As spasms can occur when non-painful substances are injected intramuscularly (Cocoman & Murray, 2008) this also raises suspicion as to whether muscle spasms play a role in saline-induced pain. The latter suggestion is that increased intramuscular pressure is an underlying mechanism in saline-induced nociception (Allen & Barnes, 1986). However, it has not yet been possible to detect pressure within the muscle that is high enough to induce pain after hypertonic saline injections, with values reported below 120 mmHg (Graven-Nielsen et al., 1997d). This is only slightly higher than the pressure found during isometric contraction exercise in the vastus lateralis (96 mmHg) and the supraspinatus (70 mmHg), observed in Crenshaw et al. (1995) and Jensen et al. (1995), respectively. Again, this suggests another similarity between this model and exercise-induced pain. Other evidence that intramuscular pressure is unlikely to play a major role in the pain response, include a study by Wolff and Jarvik (1965) that found an isotonic saline infusion pressure of 400 mmHg did not produce any muscle pain and Graven-Nielsen et al. (1997c) that found that intramuscular pressure increased independently of saline concentration, following intramuscular injections and did not induce pain. Overall the evidence suggests that these two suggested mechanisms do not impact the pain evoked by this model.

As the activation from the injections is thought to be non-specific to nociceptors (Olesen et al., 2012), another mechanism suggested is a release of algogenic neuropeptides due to activation of different receptor types in the muscle (Mense, 1993). Another alternative to direct nociceptor stimulation, is indirect stimulation by algesic substances stemming from the muscle tissue or nociceptive nerve ending (Kress & Reeh, 1996). These mechanisms have been suggested to contribute to the pain sensation but not through direct stimulation of the nociceptive nerve fibres. This may be supported by the fact that pain from hypertonic saline injections is usually delayed (Graven-Nielsen et al., 1997a), which may contradict the direct activation of nociceptors from membrane depolarization and increased sodium concentrations (Schulte et al., 2003; Staahl & Drewes, 2004). However, this delay, may also be attributed to

the diffusion process of saline, as it may take time to spread from the needle to areas of the muscle with nociceptor activity (Graven-Nielsen et al., 1997c). Although this model may excite both nociceptive and non-nociceptive fibres the non-sensory manifestations have not yet been demonstrated (Staahl & Drewes, 2004) and it is thought that saline excitation of these other receptors does not have a major influence on the sensation of pain (Korotkov et al., 2002). As mentioned previously although these mechanisms are thought to take place during hypertonic saline injections, it is not yet known whether the pain induced by this model is attributable to one of, or a combination of, these direct or indirect mechanisms. More research is still needed to fully explain the aetiology of this pain inducement model.

2.4.4: Profile of hypertonic saline induced pain

Investigating the research conducted previously using this model provides a general profile of the pain response, in terms of onset, intensity, duration and quality, that could be expected with a typical hypertonic saline injection. For the benefit of simplicity, only the most commonly used injection parameters relevant to exercise are considered i.e. a single injection into a skeletal muscle in the limbs, with a volume of 0.5 ml to 1 ml and sodium concentration between 5 and 6 percent. A total of 18 papers (Ciubotariu et al., 2004; Deschamps et al., 2014; Henriksen et al., 2007; Henriksen et al., 2010; Henriksen et al., 2011; Graven-Nielsen et al., 1997a; Graven-Nielsen et al., 1998a; Graven-Nielsen et al., 2003; Graven-Nielsen et al., 2002; Graven-Nielsen et al., 1997c; 1997d; Mista et al., 2015; Park & Hopkins, 2013; Salomoni & Graven-Nielsen, 2012; Schilder et al., 2014; Schulte et al., 2003; Sørensen et al., 2012; Wassinger et al., 2012) that met these criteria have been analysed and average values have been presented to provide an overview of this model's pain profile.

As stated by Graven-Nielsen et al. (1997a) there is usually a slight delay in the onset of pain following hypertonic saline injections and this is supported in the literature. Mean values across the studies showed that the onset of pain was just over 12 seconds on average, ranging between zero and 20 seconds. The zero was observed in Park and Hopkins (2013) however, as they stated pain started 'immediately' but did not give exact values. The time taken to reach peak pain intensity ranged between 67 and 121 seconds, with a mean time of 95

seconds. This peak pain intensity score was measured using visual analogues scales in relation to the 0-10 pain scale (Cook et al., 1997) and was observed as 4.8 on average. Peak pain intensity scores ranged between 3.2 and 7.2. Conversely, the average mean pain intensity score provided was 2.8, ranging between 1.2 and 4.7 on the 0-10 scale. However, there were discrepancies within the literature on how this recorded. Many papers did not include mean pain intensity scores at all and some studies recorded this measurement up to 30 minutes after the injection (Schilder et al., 2014; Sørensen et al., 2012). As the longest duration reported within this body of literature was 720 seconds (12 minutes), these mean pain scores may be inaccurate as the pain from the injection would have dissipated long before the recording had stopped. With these studies removed the average mean pain intensity is 3.2, with a range of 2.5 to 4.7. The lowest duration of pain from the injections was 210 seconds and the mean time was 427 seconds. In terms of pain quality, many studies did not include this measurement in their experiments, but for those that did the four most common words reported, in order, were ‘cramping’, ‘aching’, ‘drilling’ and ‘throbbing’. These values provide a profile of the pain that may be experienced using this method, in similarly designed experiments. However, there is still a lack of standardization across research papers in which different measurements are used and different values reported depending on the researchers. If there was more collaboration on these points, then a more accurate profile can be produced and a better understanding of this model can be developed.

2.4.5: Is it reliable?

Despite the common utilization of this method in pain research, there is limited information on its reliability when compared to other pain inducement models such as pain pressure (Koo et al., 2013; Pelfort et al. 2015) and cold pressor pain (Koenig et al., 2013). It is difficult to find any studies dedicated primarily to the reliability of this technique, however the aforementioned study by Graven-Nielsen et al. (1997a) is currently the best source for this information. Although this study was not solely focused on test-retest reliability, one of the experiments conducted was to investigate intra-individual variability of saline-induced pain (Graven-Nielsen et al., 1997a). In this experiment 10 participants reported to the laboratory on two occasions and received injections of 0.5 ml of 5% hypertonic saline over 20 seconds, into the tibialis anterior (Graven-Nielsen et al., 1997a). The injection was carried out using a

computer-controlled syringe pump, pain intensity was measured using a visual analogue scale (VAS) for 10 minutes and pain quality was recorded with the long-form McGill pain questionnaire (MPQ) (Graven-Nielsen et al., 1997a). Differences in pain intensity and MPQ scores were analysed using paired tests and there were no significant differences observed in any of the measurements, including VAS area, peak pain intensity, time to peak intensity, pain onset, pain offset and all categories of the MPQ ($P = > 0.36$), paired correlations were also found between all measurements ($P = < 0.05$) except for pain intensity onset and miscellaneous MPQ scores (Graven-Nielsen et al., 1997a). As this is currently the best information on reliability that there is within the research, these results suggest that the model produces a reliable pain sensation in terms of both pain intensity and pain quality. However, there may be certain limitations of this reliability experiment.

Firstly, the injections were carried out using a computer-controlled syringe pump that has been suggested to improve reproducibility (Graven-Nielsen et al., 2001). This is an advantage in this study (Graven-Nielsen et al., 1997a), but as this technique is not standardized across hypertonic saline research, this may not be an indication of reliability when applied to studies that use manual injections. Also, the study design utilised two visits, with a paired test and correlation analysis to assess the reliability (Graven-Nielsen et al., 1997a). It has been suggested that the more visits in a reliability study the more robust the analysis (Atkinson & Nevill, 1998) and the use of ICC scores may be a more appropriate measurement in test-retest or intra-individual reliability assessments (Koo & Li, 2016). This is most likely due to this experiment being part of a larger study with three other experiments being conducted (Graven-Nielsen et al., 1997a), so there is still a need for a study dedicated solely to assessing the reliability of this pain-inducement model, and it is clear more information in this regard would be beneficial to pain research.

This raises the first research question of this thesis. Namely, is the use of intramuscular hypertonic saline injections a reliable method of inducing pain, in terms of both pain intensity and quality. Conducting a research study with the aim of answering this question will help to validate the use of this model in past and future research and will also validate its use in the experimental studies carried out in the laboratories here at the University of Kent. Based on

the review of literature it was hypothesized that the pain response evoked by hypertonic saline would be reliable.

2.5: Hypertonic saline use in research

2.5.1: Benefits and negatives

As with any experimental instrument there are benefits and drawbacks with the use of hypertonic saline injections. The primary benefit of this model is that it is thought to closely resemble exercise-induced pain (Graven-Nielsen et al., 1997c). It also allows the researcher to elicit pain in specific muscles (Graven-Nielsen et al., 2001) and is not associated with any tissue damage or toxicity in humans (Svendsen et al., 2005). Another advantage is that this method allows detailed measurements of sensory and motor effects to be obtained and is the most effective model in the study of referred pain (Graven-Nielsen et al., 2001). Also, as the hypertonic saline is associated with a major activation of type IV nociceptor fibres (Staahl & Drewes, 2004), with a suggestion that the other receptors acted upon do not contribute to the manifestation of pain (Korotkov et al., 2002), this model may provide a more specific nociceptive pain than the other models discussed that act other pathways. On a practical level, as the pain produced is steady and relatively long lasting (Graven-Nielsen et al., 2001), pain intensity and quality is easily measured using a visual analogue scale and pain questionnaires (Graven-Nielsen & Arendt-Nielsen, 2003).

A major drawback is the lack of standardization across studies, as multiple factors have been shown to impact the pain response, including the concentration, volume, infusion time and muscle used (Graven-Nielsen et al., 1997a). This means that the infusion of endogenous substances like saline can be difficult to control (Andersen et al., 2008). There are also discrepancies in the literature on whether a manual researcher-controlled or computer-controlled infusion is used, which makes it difficult to compare findings, especially in terms of reliability. Another reason that findings are difficult to compare is the differences in how studies record and report their data, with many researchers neglecting to include pain quality measurements or certain pain intensity measures. These factors show that although there are

few negatives with the technique itself, more standardization across research groups would provide a more robust model of pain inducement. Also, as this model uses intramuscular injections this opens up the possibility that the risks associated with this technique could occur. These risks include accidental intravascular injection, muscle fibrosis, abscess, tissue necrosis, cellulitis, haematoma, involuntary muscle contractions/spasms and injury to blood vessels, bones and peripheral nerves (Small, 2004). Although preventable with safe and proper technique, the presence of these risks does present a negative of this model.

2.5.2: General research

This model has been used in both exercise and non-exercise studies. In terms of general non-exercise studies there have been both animal and human studies that have investigated the influence of hypertonic saline on factors outside of performance. As this is not the primary focus of this review and many papers in this vein have been discussed previously, these have not been explored in-depth. However, there are potentially important findings from these studies that should have been noted.

The hypertonic saline model has been used in multiple animal studies (Capra & Ro, 2000; Ro & Capra, 1999; Svendsen et al., 2005). An important animal study conducted by Svendsen et al. (2005) investigated the physiological effects of hypertonic saline injected in rats, rabbits and pigs. The authors observed that up to 6% concentration saline did not cause toxicity in the red blood cells/rat myocytes of rats, muscle toxicity in rabbits and was too small or short to activate c-fos expression in the dorsal horn of pigs (Svendsen et al., 2005). These results support findings in human studies (Graven-Nielsen et al., 1997a; 1997c) that conclude the pain from hypertonic saline does not stem from tissue damage, making it a safe model for muscle pain inducement. Capra and Ro (2000) injected 5% saline into the jaw muscles of adult cats, in order to measure the changes in the proprioceptive properties of movement related neurons. The pain from the saline significantly changed the dynamic response, firing rates and muscle spindle sensitivity, resulting in impaired jaw movement function (Capra & Ro, 2000). Furthermore, the neurons that hypertonic saline strongly activated received were Vi neurons (Capra & Ro, 2000), which receive input from type III muscle afferents (Ro &

Capra, 1999). An application of this model to the jaw muscles has also been performed on human subjects.

Jensen and Norup (1992) injected hypertonic saline into the temporal muscles of humans, one of the muscles used in mastication and measured pain intensity and pressure-pain thresholds. They found that a negative correlation between decreased pressure-pain threshold and pain intensity from the injections (Jensen & Norup, 1992). Similarly, a study by Svensson et al. (1995) injected hypertonic saline into 6 different jaw muscles and found no significant differences between muscles in terms of pain intensity and pain quality, but the anterior temporalis muscle produced a greater pain area. This, coupled with differences in pain values observed between different muscles (Graven-Nielsen et al., 1997a) and between the muscle, subcutis and fascia in the lower back, with fascia and subcutis being significantly more sensitive to pain stimulation (Schilder et al., 2014), highlights that the pain response from this model is dependent on the tissue injected. Another non-exercise study of interest is by Madeleine et al. (1998) concerning the similarities between saline-induced pain from trapezius and supraspinatus injections and work-related neck/shoulder pain during work task simulations. The findings demonstrated similarities between the experimental pain and clinical pain groups in terms of pain intensity, quality and location, suggesting this model is useful in research designed to investigate clinical musculoskeletal pain. All of these studies demonstrate that hypertonic saline is a versatile model that can be used in non-exercise related research, but what is of great interest to this research is how the model has influenced exercise performance.

2.5.3: What impact has this technique had on exercise?

Many studies have used intramuscular injections of hypertonic saline to measure what impact this artificially induced pain has on exercise performance. As saline-induced pain shares many similarities with exercise-induced pain, findings from these studies can therefore help with the broader question of whether performance/fatigue is influenced by this phenomenon. These studies have utilised various experimental designs, ranging from isometric/resistance

exercise and dynamic exercise experiments. The former of these is much more common in the literature.

Isometric/resistance exercise

Much of the information on the influence this pain model has on exercise has come from studies with an isometric single-limb experiment, usually in which single musculoskeletal muscles or tissues located in the limbs are injected with hypertonic saline and pain parameters and performance of isometric contractions of said muscles are recorded. There is a wide range of exercise studies with various volumes, concentrations and muscles/tissues around the body utilized, ranging from the forearm muscles (Tucker et al., 2009) and elbow flexors (Khan et al., 2011; Mista et al., 2014; Proske et al., 2003; Weerakkody et al., 2003), in the arms to the quadriceps (Graven-Nielsen et al., 2002), infrapatellar fat pad (Henriksen et al., 2011; Park & Hopkins, 2013; Sørensen et al., 2012; Tucker et al., 2009), plantar flexors (Ciubotariu et al., 2004) and dorsi flexors (Ciubotariu et al., 2004; Graven-Nielsen et al., 1997d; Farina et al., 2008), in the legs. There are also examples of isometric studies using a multidimensional approach, in which multiple muscles around the body were injected, namely Salomoni and Graven-Nielsen (2012). These studies have demonstrated various ways that the hypertonic saline model influences exercise.

One observation that has been suggested from many of these studies is that pain from hypertonic saline reduces muscle strength (Graven-Nielsen et al., 2002). Firstly, Graven-Nielsen et al. (2002) found that significant pain from 1.5 ml of hypertonic saline injected into the quadriceps muscle (not specified), reduced Maximal Voluntary Contraction (MVC) force but without impairing contractile properties of the muscle, suggesting this decrease was attributable to central inhibition. Similarly, two studies utilizing saline pain emanating from the infrapatellar fat pad in the knee joint also found a decrease in maximal isometric force in knee flexion (Henriksen et al., 2011) and knee extension (Henriksen et al., 2011; Park & Hopkins, 2013). Reduced maximal voluntary contraction force has also been shown in the biceps brachii muscles during elbow flexion in a study by Khan et al. (2011). This study also showed there was no significant impairment of contractile properties, measured through

voluntary activation and suggested that muscle pain from this model has a limited effect on motor output during voluntary contractions and may support the role of central inhibition (Khan et al., 2011). Weerakkody et al. (2003) and Proske et al. (2003) have also suggested a role of central mechanisms in the impairment of isometric exercise as hypertonic saline induced pain can lead to a decrease in excitability of the motor cortex. Both studies demonstrated this with the inability to match force output during submaximal isometric contractions (30% MVC) of the biceps after hypertonic saline injections (Proske et al., 2003; Weerakkody et al., 2003). It has also been shown that during low intensity isometric contractions with pain, there is a reduction in motor unit discharge rate and in order to maintain the same amount of force, a new population of units is recruited (Tucker et al., 2009). This was found in both the quadriceps and the flexor pollucis longus of the forearm after hypertonic saline injections (Tucker et al., 2009) and this could help to explain the influence of artificial pain and the reduction in force output and force perception. In contrast, Farina et al. (2008) found a decreased discharge rate of active motor units in the tibialis anterior during saline-induced pain, but this did not correlate with increased twitch torque, suggesting that maintenance of force during painful contractions can't be explained by paired contractile and central motor unit properties.

What may also be related to these suggested mechanisms, is the impairment of steadiness in force observed in experimental pain studies (Mista et al., 2015; Salomoni & Graven-Nielsen, 2012). Mista et al. (2015) found that experimental muscle pain from the biceps brachii during submaximal contractions (5 – 30% MVC) resulted in increased variability in force output, but not when given increased three-dimensional feedback. The muscle activity recorded through Electromyogram (EMG) showed a different recruitment of muscles depending on the feedback, with one-dimensional feedback not recruiting more motor units from the synergists (Mista et al., 2015). The authors suggested that the offset in steadiness in force could be avoided with voluntary adjustment of the force output, similar to the findings of Tucker et al. (2009), but that this would result in sup-optimal motor strategy (Mista et al., 2015). This study also shows that feedback may play a role in the inhibition of force perception and steadiness (Mista et al., 2015), caused by saline-induced pain. Salomoni and Graven-Nielsen (2012) also investigated the impact of hypertonic saline on force steadiness but utilized a multi-dimensional approach. In this study participants performed a series of six sets of force-matched multidirectional isometric contractions at different intensities ranging from 2.5% to

70% MVC in sequence, after receiving injections of 1 ml hypertonic saline into four muscles on the dominant side including the tibialis anterior, gastrocnemius medialis, vastus medialis and the brachioradialis (Salomoni & Graven-Nielsen, 2012). The same three-dimensional feedback was given for every trial (Salomoni & Graven-Nielsen, 2012). The results showed that force steadiness was significantly impaired across all muscle groups and target forces, but without a change in mean force, which the authors suggested may be a result of a redistribution of activity within and between muscles (Salomoni & Graven-Nielsen, 2012). All of these studies implementing the hypertonic saline model on isometric exercise, demonstrate an impaired performance due to impaired muscle function, stemming from reduced strength, force matching ability or steadiness of force. Although the mechanisms involved with this impairment are still unknown, it is shown that pain from hypertonic saline can negatively influence isometric exercise.

However, these studies measure the acute response to this model, there may also be a way this method can be used as a potential benefit to this type of exercise, long term. A study by Sørensen et al. (2012) was the first of its kind to investigate the effect of experimental knee pain during exercise on muscle strength after an eight-week strength training regime. To this end, they had two groups of participants engaged in three training sessions per week, with one group as a control and one group performing the exercise sessions after receiving an injection of 1 ml hypertonic saline into the infrapatellar fat pad (Sørensen et al., 2012). The training sessions consisted of 3 sets of quadriceps extensions and flexions to fatigue at 80% one Repetition Max (1RM) and muscle strength was recorded using 1RM tests each week (Sørensen et al., 2012). After the eight weeks, they found that the group who had exercised with saline-induced pain had a significantly larger improvement in isokinetic muscle strength for knee extension, suggesting that training with experimental knee pain improved muscle strength (Sørensen et al., 2012), seemingly contradicting other studies that have found decreases in muscle strength, albeit acutely (Henriksen et al., 2011; Graven-Nielsen et al., 2002; Khan et al., 2011; Park & Hopkins, 2013). However, this study observed no differences in improvement between groups for knee flexion (Sørensen et al., 2012). Sørensen et al. (2012) suggested that the explanation for this increase in strength may relate to the theory of alternate motor unit recruitment posited by Tucker et al. (2009), as during the painful exercise sessions the discharge rate for select motor units would be reduced so in order to maintain the required force simultaneous changes in contractile properties of muscle fibres and/or motor

recruitment strategies must take place. However, as the muscle strength measures took place without the presence of pain, all motor units would have been used to contribute to maximal force, with the authors suggesting the pain group would have a training-induced increased descending motor drive, thus contributing to increased muscle strength (Sørensen et al., 2012). This does not account for the lack of improvements in knee flexion however and there were several methodological issues associated with this study (Sørensen et al., 2012). Firstly, pain quality was not recorded and pain intensity was recorded for the entire training sessions, usually lasting between 10 to 12 minutes, but as injections into the infrapatellar fat pad have been suggested to peak at around five minutes and gradually decline over the following 10 to 12 minutes (Bennell et al., 2004), this resulted in a very low mean pain intensity (1.6) (Sørensen et al., 2012). It is therefore difficult to assess how much pain, or what kind of pain, the pain group was subjected to during their training (Sørensen et al., 2012). This is confounded by the fact that exercise-induced hyperalgesia can modulate pain perception (Koltyn, 2002) and the authors mention that on occasion, additional injections were provided when participants were no longer in pain during a session, showing that there were large inconsistencies with how pain was implemented and measured (Sørensen et al., 2012). Due to these insufficiencies, the findings from this study, although novel, do not appear as strong as previous studies suggesting hypertonic saline induced pain reduces muscle strength. Additional research into the long-term effects of this model without the limitations present in this study may be needed to help corroborate these findings.

Dynamic/cardiovascular exercise

In comparison to isometric/strength exercise studies, there is less literature dedicated to the influence of the hypertonic saline model on exercise with dynamic movement. However, there are a few informative studies that have implemented this method and investigated the effects on dynamic single leg hops (Deschamps et al., 2014), shoulder strength/throwing accuracy (Wassinger et al., 2012) and gait (Graven-Nielsen et al., 1997d; Henriksen et al., 2007). These studies are important as they include more sport specific exercises, which may be more beneficial to exercise-induced pain research.

Starting with the influence of this model on human gait, a study by Henriksen et al. (2007) injected 1 ml of hypertonic saline into the vastus medialis muscle of the quadriceps, recorded muscle activity, pain parameters and gait analysis, to measure knee joint control during walking. Muscle function in the quadriceps muscles was modulated, leading to impaired knee joint control and instability during walking, similar to patients with clinical knee pain (Henriksen et al., 2007). These impairments also persisted even after the pain was present, which suggests central inhibition beyond just pain perception (Henriksen et al., 2007). One limitation of this study may have been the use of standardized walking speeds across participants, as it is generally accepted self-selected walking speeds produce more reliable results (Henriksen et al., 2007). Graven-Nielsen et al. (1997d) also investigated muscle and joint activity during gait but participants walked as naturally as possible and 0.5 ml of hypertonic saline was injected into either the tibialis anterior or gastrocnemius muscles. This study demonstrated a decrease in the stride time and a modified muscle activity, similar to the Henriksen et al. (2007) study, resulting in modulated co-ordination of muscle and joint activity during dynamic movement (Graven-Nielsen et al., 1997d). This impairment of joint function and muscle activity during gait, may be expected to occur in more intense dynamic exercise such as running, but as of writing, there are no such studies dedicated to this question.

An alternative to this approach that has been used however, is the influence of hypertonic saline in the vastus lateralis muscle on maximal single leg hops (Deschamps et al., 2014). This study was the first of its kind to measure the performance and perceived ability of a dynamic exercise task, by asking participants to estimate their performance of a maximal single leg hop, with and without the influence of experimental muscle pain (Deschamps et al., 2014). Firstly, this study demonstrated a significant decrease in actual performance following hypertonic saline injections (Deschamps et al., 2014). Secondly, participants also accurately estimated their performance in the pain and non-pain conditions, meaning that they effectively updated their perception of the task during acute pain (Deschamps et al., 2014). Deschamps et al (2014) suggest that the reduction in performance may be less related to reductions in motor function and seemingly more related to a cognitive ‘protective’ mechanism.

2.5.4: What influence does exercise have on hypertonic saline induced pain?

What is clear from analysing the relevant literature on this topic is that pain produced by hypertonic saline injections has an influence on exercise performance. Inversely, what has not been explored in as much detail, is the potential influence exercise has on this pain response and whether the intensity of said exercise is important. In terms of exercise intensity, there are no studies dedicated to this specifically, but there are a few that have included this aspect in the experimental design. Firstly, the study by Salomoni and Graven-Nielsen (2012), had four different thirteen second contractions performed in sequence at different intensities, including 2.5%, 20%, 50% and 70% of MVC force. Unfortunately, as this study was designed primarily to record force steadiness, the authors did not measure if there were any differences in the pain response between these intensities (Salomoni & Graven-Nielsen, 2012). Therefore, little information on this topic can be ascertained from this study, but there is one study by Ciubotariu et al. (2004) that has measured differences in pain response between intensities. This experimental design including sustained contractions of either the tibialis anterior or gastrocnemius muscles, at 50% and 80% of MVC, on separate occasions (Ciubotariu et al., 2004). The primary finding of this study was that endurance time, force production and muscle activation was reduced due to 1 ml of hypertonic saline compared to control for all conditions (Ciubotariu et al., 2004). Importantly, it was also demonstrated that there were no significant differences in peak pain intensity, pain at the start of contraction or total pain recorded on the VAS during the contractions, between 50% and 80% (Ciubotariu et al., 2004). The only difference observed between these two contraction intensities was the pain intensity at the end of the contraction, but this was attributed to the shorter endurance time in the painful 80% contraction (Ciubotariu et al., 2004). A study by Ervilha et al. (2004) also compared pain intensity between isometric contractions but this time with different weight loads during abduction of the biceps. Pain was induced with 0.5 ml and 1.5 ml hypertonic saline injections on separate occasions and there were no differences in pain intensity between the 0kg, 4kg and 10kg load contractions, for either volume of saline (Ervilha et al., 2004). No pain quality measures were recorded, and the muscle contractions were not individualised to participants in this study (Ervilha et al., 2004).

The limited information on this topic suggests that exercise intensity doesn't impact pain response from hypertonic saline. However, there are notable admissions in these studies, including the lack of pain quality measures, comparisons of pain measures between lower intensities, the use of larger muscles and crucially, a comparison to resting values of pain from this model. Without these it is impossible to make any definitive judgements on the impact exercise intensity may have on pain response. Also, without a comparison between hypertonic saline induced pain at rest and during exercise, it is unknown what influence the act of exercise may have on the pain response. This is needed in hypertonic saline research as any studies that use this model during exercise, would want any differences observed to be attributable to the artificial pain inducement and not the exercise itself. This concept raises the second research question of this thesis. Namely, what impact, if any, does exercise have on the pain response to hypertonic saline injections. Based on the limited information available it hypothesized that pain response would be modulated. Specifically pain intensity would increase and the pain quality would change, with the addition of exercise when compared to the pain response at rest.

2.6: Purpose of the current study

In summary, based on the information ascertained from various studies conducted in pain and exercise research, the purpose of this research was two-fold. Firstly, to assess the test-retest reliability of intramuscular hypertonic saline injections method in the inducement of experimental pain, in terms of pain intensity and pain quality. Secondly, to investigate whether the addition of low-intensity muscular contraction exercise would influence the pain response induced by this model. It was hypothesized that the hypertonic saline injections would provide a reliable pain response and this pain response would be modulated by the addition of exercise.

Chapter 3: General Methodology

3.1: General Methods

3.1.1: Participants

14 healthy participants were recruited for the study, with the same participants used for both Study 1.1 and 1.2. There is a possibility that there may have been a 'learning effect' due to this factor. However, the comparisons between intensities observed in study 1.2 being made during the same injection this effect would be minimised and all participants engaging in the second study would have been familiarised with the injection. Eight males and six females with an average age of 25 ± 5 years, height of 172.9 ± 8.5 cm and mass of 71.9 ± 12.7 kg completed the studies. As mentioned previously, there is a suggestion that pain threshold can be different between genders (Compton et al., 2003: McAdoo et al., 1990), therefore the use of both male and female participants could have an impact on results. However, comparisons between pain response were made only between individuals. Six of the recruits had previously received the hypertonic saline injection and eight were unfamiliar with the technique. All recruits signed a written consent form and their participation was voluntary.

Due to the invasive nature and potential discomfort associated with the intramuscular injections, to aid in recruitment all participants who completed the study received £30 pro rata. Only healthy people between the age of 18 and 45 years old were recruited, primarily students/staff from the University of Kent. As well as universal health Physical Activity Readiness Questionnaires (PAR-Q), intramuscular injection specific questionnaires were used to establish exclusion criteria. This included anyone with a phobia of needles, blood borne diseases such as HIV and hepatitis B, lower limb injuries, cardiovascular disease, neurological disorders, any allergies such as milk, egg, wheat, soya, fish and nuts and anyone taking medication for pre-existing pain. The research project was fully approved by the School of Sport & Exercise Sciences Ethics Committee at the University of Kent and conducted in conformity with the declaration of Helsinki. Participants were asked to abstain from vigorous exercise 24 hours prior, consumption of alcohol 48 hours prior, caffeine 8 hours prior and analgesics 6 hours prior to all visits.

Prior to testing a recruitment target of n = 15 was set, based on previously conducted reliability studies on non-clinical populations (Eliasziw et al., 1994; Lim et al., 2016) and more than the 10 subjects used in the Graven-Nielsen et al. (1997a) hypertonic saline reliability study. Four subjects were removed during the study; one upon request due to time constraints and the other three being removed at the researcher's discretion due to adverse reactions to an injection in their first visit. Of these, two experienced symptoms of nausea and were unable to rate the pain, they were removed as a precaution despite being willing to continue. The other participant fainted during their first visit after receiving the injection and although there were no lasting effects they were subsequently removed in the interests of their own safety. All data collected from those removed from the study was destroyed.

3.1.2: Protocols

Intramuscular Injections

All testing sessions took place in the Medway building physiology laboratory (M0-01, School of Sport and Exercise Sciences, University of Kent). Both Study 1.1 and 1.2, artificially induced pain using intramuscular injections of hypertonic saline. The implementation of this variable was consistent over the two studies, with the same technique, researcher, participant, injection site and all protocols, except from where the injection took place, used throughout. During Study 1.1 the injection was carried out whilst the participant was seated on the bio-waste chair and in Study 1.2 the injection was carried out with them seated in the Isokinetic Dynamometer.

The intramuscular injection site was in the belly of the vastus lateralis (VL), a quadriceps femoris muscle located on the anterior lateral aspect of the thigh (Small, 2004). All injections were performed on the right leg. This was individually measured on each participant using the middle third of the lateral aspect of the thigh between the greater trochanter and the lateral femoral condyle of the femur, as a marker (Rodger & King, 2000). This was used as a guide to help account for individual muscular structure. Once measured the site was marked using a skin marker pen and was maintained for the full duration of participation in the study, to

ensure a consistent injection area of roughly 1 cm^2. All subsequent injections took place in this area but in order to avoid muscle fibrosis associated with repeated injections in the same spot (Rodger & King, 2000), roughly 2/3 millimetres was allowed between previous insertions. This site was chosen as it is a primary contributor to force generation during knee extension exercise (Alkner et al., 2000), is easily accessible and located (Rodger & king, 2000), provides a large thick muscle mass (Harkreader & Hogan, 2004) and importantly has a minimal risk of damage, due to this area having a decreased probability of injury and no association with any major blood vessels or significant nerve structures (Dougherty & Lister, 2011). Prior to injecting the vastus lateralis, the injection site and surrounding area was inspected and palpated to eliminate the presence of local tenderness or muscle soreness (Graven-Nielsen et al., 2002).

The injection itself consisted of a single bolus of 1 ml of 5.85% sterile hypertonic saline solution (B Braun Medical Industries) during all visits of Study 1.1 and one visit of Study 1.2. There was also one injection visit in Study 1.2 where the same injection procedures were adhered to but with a single bolus of 0.9% sterile Isotonic Saline (IS) solution (B Braun Medical Industries) used as a placebo control. Isotonic saline has been used as an effective placebo control in multiple hypertonic saline studies including the extensive work of Thomas Graven-Nielsen (Salomoni & Graven-Nielsen, 2012; Graven-Nielsen et al., 1997a; Graven-Nielsen et al., 1998a; Graven-Nielsen et al., 2003; Graven-Nielsen et al., 2002; Graven-Nielsen et al., 1997c; 1997d).

All injections were carried out by National Health Service (NHS) trained, competent researchers, with the same injector used for all of an individual's visits. As well as this and the aforementioned injection site protocols, the injection technique was kept as consistent as possible throughout the studies. To minimise variability, the depth, angle, leg position, force, timing, z-track technique and all equipment used were kept equal.

Injections were performed manually over a twenty second period to a depth of 2 cm using a 3 ml Luer-Lok plastic syringe attached to a 25 g x 38 mm SurGuard2 disposable stainless-steel needle (Terumo, Japan). Subcutaneous fat was not measured. Of those twenty seconds, after a

quick insertion at a reasonable force to break the skin, a 5 second rest period occurred followed by 10 seconds of solution infusion (at a consistent rate of 0.1 ml per second) and a further 5 seconds of rest before removing and disposing the syringe. Negative pressure was applied during the initial 5 second period to check for blood in the syringe, indicating a venous injection. If blood was present, then the needle would have been removed and disposed of and a new one would be prepared for a slightly different entry point. The solution was drawn into the syringe using a blunt fill needle (Terumo, Japan) up to 1.3 ml. Once all air bubbles were removed the syringe was emptied to exactly 1 ml. The injection site was cleaned prior using alcohol wipes and allowed to dry.

The participant was asked to relax as much as possible and refrain from looking at the initial insertion of the needle. They were seated with their legs relaxed and at 90^{o}, the area surrounding the site was palpated to ensure the muscle was relaxed. To minimise bleeding and keep the solution in the desired area, z-track was used during the injections. To do this, whilst injecting with the right hand the thigh above the site was pulled upwards, the force and direction were kept consistent throughout. The injections were performed at a 90^{o} angle from the belly of the VL. The chosen site ensured minimal bleeding however, if participants continued to bleed, cotton wool was applied to the area lightly.

Most risks or side effects of this procedure are preventable with trained and safe intramuscular injection practice; however, potential complications could occur. Common associated risks include pain or discomfort after an injection, bruising at the injection site, muscle spasms, injecting intravenously, feelings of nausea or blurred vision, bleeding and possible feinting. Some of which are somewhat unavoidable but can be minimised by abiding by the correct techniques mentioned previously. In the event of nausea and feinting the bio-waste chair used in the experiment was reclined and the participants feet pushed upwards to allow blood flow to the head. A minimum of seven days was set between injection visits to reduce the risk of damage to the site. An injection documentation form was used whenever an injection was conducted to record any adverse effects observed, details of all equipment used (in date and undamaged) and contained an after-care section completed 15 minutes post-injection. This was to ensure the participant was no longer in pain, had no walking or concentration issues, were aware of the risks and appropriate responses and informed to

monitor the site for two to four hours. These implementations were all in place to ensure relatively common risks were reduced.

There are also more severe but rare risks that could occur with the use of intramuscular injections. These include needle stick injury to the experimenter (increased risk of blood borne diseases such as HIV/Hepatitis B), an allergic or anaphylactic shock from the hypertonic saline, nerve injury resulting in potential paralysis, haematoma, bone injury, sterile abscesses, atrophy and accidental femoral nerve damage. Although unlikely, implementations used to reduce these risks included researchers following correct injection procedure and undergoing Hepatitis B vaccination, an injection specific health PAR-Q and NHS training/monitored competency assessments, respectively.

Measurements

The pain experienced by the participants after receiving the hypertonic saline injections was generalised into two categories: pain intensity and pain quality. Pain intensity was measured using potentiometer devices with a sliding electronic Visual Analogue Scale (VAS), measured from 0-100 (0 being 'no pain' and 100 being 'most intense pain'), based on the numeric perceived pain scale (Cook et al., 1997). The reliability of this pain measurement has been shown in multiple studies (Boonstra et al., 2008; Jensen et al., 1994; Kahl & Cleland, 2005). Study 1.1 used a VAS device that recorded what pain was experienced in 5 second intervals, onto an external memory stick. The data ascertained from this was then used to calculate various measurements such as peak pain intensity, mean pain intensity, pain duration, area under the VAS time curve, time to peak intensity and time spent above 50% peak intensity. Study 1.2 used a similar VAS device but recorded pain in two second intervals instead, allowing the collection of key data during the 10 second contractions. Using the data collected from these measurements such as pain duration, mean pain intensity, individual exercise intensity and rest period measurements of peak, mean and total pain intensity. In both studies these devices were given to the participants and recorded changes in pain intensity throughout the experimental trials and were switched off when the pain levels returned to zero indicating no pain.

Pain quality was measured using pain questionnaires. Acute pain quality was measured during the trials in both studies using the two-dimension (sensory and affective), 15 word Short-Form McGill Pain Questionnaire (SFMPQ) (Melzack, 1987). This questionnaire consists of descriptive words such as throbbing, cramping, tender etc. relating to pain that participants select and rate between mild, moderate and severe according to the experience of pain at that time and a 0-5 scale (0 being 'no pain' and 5 being 'excruciating') general rating of pain or Present Pain Intensity (PPI) (Melzack, 1987). This questionnaire is a reliable and valid measurement for pain quality (Georgoudis et al., 2001; Grafton et al., 2005; Strand et al., 2008). In Study 1.1 this questionnaire was completed 60 seconds after the participant had received the injection in all resting trials. In Study 1.2 this questionnaire was completed three times during each experimental variable force trial, specifically one after each ten second contraction, during the rest period.

As well as measuring the quality of pain experienced at individual points during the trials, pain quality for the entire experience was measured via the Long-Form McGill Pain Questionnaire (LFMPQ), essentially a longer/more detailed version of the SFMPQ (Melzack, 1975). This questionnaire has four dimensions (sensory, affective, evaluative and miscellaneous) with twenty questions featuring multiple descriptive words which when selected by participants provides a Total Pain Rating Index (T-PRI) and a rating index of each dimension (Melzack, 1975). Words chosen by at least one third of the participants can be used as an indication of the pain quality of the injections (Melzack & Katz, 1994). There is also a PPI question and an anatomical map for participants to outline the distribution of pain experienced (Melzack, 1975). The completion of this questionnaire was based upon the pain experienced throughout the trial from the moment pain was first felt to the moment no pain was present. This pain questionnaire is thought to be a valid, reliable and consistent measure of pain quality (Katz & Melzack, 1999).

Preliminary Questionnaires were also used to predict pain response. The Pain Resilience Scale (PRS) was completed in the first visit to determine an individual's resilience to painful stimuli (Slepian et al., 2016). The PRS contained fourteen statements, for example 'I get back

out there', concerning how the participant reacts 'when faced with intense or prolonged pain…' on a zero to four scale (0 being 'not at all' and 4 being 'all the time') (Slepian et al., 2016). By calculating the numbered responses after completion, an individual's cognitive positivity (management of thoughts and emotions) and behavioural perseverance (motivation and behavioural persistence) in the presence of pain can be assessed and these could be a predictor of their response to the injections (Slepian et al., 2016).

The other questionnaire used was the Schutte Self Report Emotional Intelligence Test (SSEIT), consisting of 33 statements related to four measurements of emotional intelligence; emotional appraisal, emotional expression, emotional regulation and emotional utilisation problem solving (Schutte et al., 1998). Each item, for example 'I am aware of the nonverbal messages I send to others' is rated on a one to five Likert-type scale (1 being 'strongly disagree' and 5 being 'strongly agree') and when totalled can give a rating of emotional intelligence which again can be a predictor of response to pain (Schutte et al., 1998).

The final questionnaires used in the studies were utilized to detect any psychological changes throughout participation. Pain expectation and confidence in dealing with this pain were assessed using 0-10 scales based on the numeric perceived pain scale (Cook et al., 1997) ranging from 'no pain' to 'worst possible pain' and one ranging from 'not confident at all' to 'completely confident', respectively. Also, two Positive and Negative Affect Scale (PANAS) questionnaires were used to determine changes in emotions during the study by rating emotions such as upset, enthusiastic, nervous etc. (Watson et al., 1988). The same one to five (1 being 'very slightly' and 5 being 'extremely') Likert-type scale PANAS questionnaire was completed based on how the participant was feeling at that moment and how they had felt during the previous week (Watson et al., 1988). All three questionnaires were completed just prior to the trials in every visit across both studies.

3.1.3: Data storage

Data for both studies was collected either by hand or onto a memory card inside the VAS device. This was then input onto data spreadsheets stored on a password protected computer

and password protected memory stick. Any hard copies of data or identifiable material e.g. questionnaires, consent forms etc. were stored in a locked filing cabinet in the research supervisor's office accessible only to the supervisor and lead researcher. All digital data was anonymised using a participant number code that was known only to the lead researcher, with no identifiable information attainable through the data.

Chapter 4: Experimental Methods & Results

4.1: Study 1.1: Test-retest reliability of intramuscular hypertonic saline injections

4.1.1: Methodology

Study 1.1 of the research project was designed to assess the test-retest reliability of intramuscular hypertonic saline injections in scientific research. Therefore, a research study was conducted on 14 participants, attending three separate identical hour-long visits over a three-week period. Each visit consisted of a reliability trial of the hypertonic saline injections at rest.

Testing Methods

In the first visit preliminary questionnaires/documents including a general health questionnaire, consent form, injection specific questionnaire, PRS and SSEIT, were completed to assess eligibility for the study and assess potential pain response, respectively. This step was performed in only the initial visit and the following steps were the same for all subsequent visits. Due to it being a reliability test the same trial was performed identically in all three visits.

The participants were then seated with their legs at a 90^{o} angle in the bio-waste chair. Following this, the injection site was measured, cleaned and marked, with care to avoid blood vessels, bruises, red areas or bone structures. The protocols were explained to them in detail and then the researcher completed the first section of the injection documentation, checking if the injection equipment (solution, syringe, drawing up needle and injection needle) were all in date and the injection area chosen was clear of damage/risks. Participants were then given the weekly PANAS, momentary PANAS, pre-test checks (no caffeine, exercise, alcohol and analgesics) and pain expectation/confidence documents to complete. During this time the researcher prepared the injections, drawing up exactly 1 ml of hypertonic saline solution into the syringe, removing air bubbles, disposing of the drawing up needle and attaching the injection needle, leaving the plastic safety guard on.

Once all the equipment was prepared and all documentation complete the trial was ready to begin. Firstly, the electronic VAS device was plugged in and handed to the participant, whilst a timer was started. At 60 seconds the injection was carried out, using the protocols explained previously (see general methods). Participants were asked to relax and refrain from looking at the initial insertion but to adjust the VAS device as soon as they felt any pain in that area, not to wait until the solution had been fully infused. The needle was removed 20 seconds after insertion and was disposed of into a sharps waste bin. Z-track was used to minimise any bleeding but if blood was present a cotton wool was applied to the area lightly. Participants were instructed to adjust the VAS device whenever they felt a change in pain regardless of size or direction. Participants were also informed to assess the muscular pain separate from possible pain felt from the initial needle stick. They were to do this from the first feeling of pain until they no longer felt any pain at all (0 to 0 on the device). This then gave a measurement of pain intensity experienced at five second intervals when given a hypertonic saline injection.

Furthermore, 60 seconds after the injection was complete participants verbally completed the SFMPQ displayed on a whiteboard in front of them. This was to measure the pain quality experienced at that point. Participants were told to rate the words they felt were relevant to their pain and were familiarised with the questionnaire beforehand. Once the VAS was returned to zero and participants were no longer in pain, they were given the LFMPQ to complete. They based their responses on how the pain felt during the entire trial and marked where the pain was distributed.

Participants were kept seated for 15 minutes after the trial and then the aftercare section of the injection documentation was completed by the researcher. This was to ensure the individual had not experienced any adverse effects from the injection, they were no longer in pain, had no walking or concentration issues and that they were aware of the potential risks/responses. They were then informed to observe the injection site for two to four hours, based on recommendations by Mallet and Bailey (1996) and in the event of an adverse effect to contact a GP. This experimental design was subsequently replicated for all three of the Study 1.1 visits, with all protocols kept as consistent as possible. Providing three identical

hypertonic saline injections at rest to assess reliability. Protocols for these visits are presented in Figure 4.1.

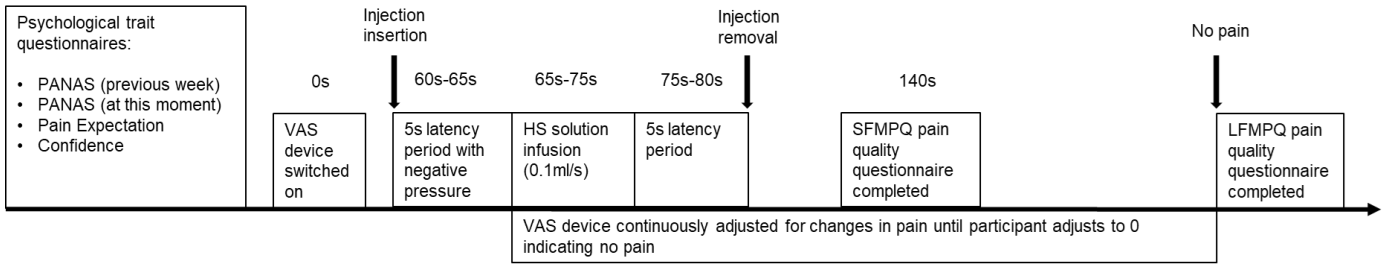

Figure 4.1: Testing protocols of the resting hypertonic saline injection experiment used in all three reliability visits.

4.1.2: Data analysis

Data was primarily recorded through the electronic VAS device and through various questionnaires. This was then extracted and stored on an electronic spreadsheet, located on a password protected computer and memory stick. No data was stored on the VAS device as it was deleted after it had been extracted. All hard data and identifiable material such as the participant code sheet were stored in a locked filing cabinet in the research supervisor's office. All other data was anonymised.

Reliability measurements were decided based on the data collected from the VAS device. Using this data, the measurements ascertained include mean and peak pain intensity (PI), pain duration, time to peak intensity, time spent above 50% of peak intensity and the total area under the VAS time curve. As well as the measurements from the various pain questionnaires and their respective dimensions, this formed the basis of the reliability of the hypertonic saline injections.

Reliability was assessed primarily using single measures, absolute agreement, Intraclass Correlation Coefficient (ICC) scores or Cronbach's Alpha (CA) scores. ICC scores can be defined as the ratio of variability between subjects to the total variability including subject variability and error variability (Kim, 2013). The closer to 1 an ICC score is, the higher the reliability, with a score of > 0.9 indicating 'excellent' reliability, 0.75 – 0.9 'good' reliability, 0.5 – 0.75 'moderate' reliability and < 0.5 'poor' reliability, respectively (Koo & Li, 2016). CA can provide a measure of internal consistency of a set of related measures (Tavakol & Dennick, 2011) and has been used on non-ratio data (pain quality measures) and as an aid to ICC scores when applicable. Ranging between 0 and 1, CA scores > 0.7 are considered acceptable for research studies with the higher the score indicating a higher consistency (Gadermann et al., 2012).

4.1.3: Statistical analysis

Test-retest reliability over the three visits was assessed by calculating single measures, absolute agreement, Intraclass Correlation Coefficients (ICC) scores with Confidence Intervals (95% CI), Cronbach's Alpha (CA), and the Coefficient of Variation (CV), depending on the type of data used. For scale/ratio data recorded from the VAS device (mean PI, pain duration etc.) was assessed using ICC and CV scores. For ordinal/nominal data recorded from questionnaires was assessed using CA scores, as CV and ICC cannot be used for non-scale/ratio data. Mean and Standard Deviation (SD or ±) scores and the Minimum Detectable Change (MDC) were also measured for all reliability measurements. ICC and CA scores range between 0 and 1, with 1 being deemed zero variability, 0 deemed absolute variability and scores closer to 1 indicating higher reliability. CV is presented as a percentage of variability, with a score closer to 0 being indicative of higher reliability.

The Mean data, SD, CV and MDC scores were calculated using formulas on an excel spreadsheet. The ICC and CA scores were associated with the performance of a two-way mixed effects model reliability analysis with absolute agreement on IBM SPSS statistics v25 software (SPSS, IBM, New York, USA). The ICC scores presented are 'single measures' Intraclass Correlation Coefficients. This analysis was performed on the data from the three

visits and then also with the removal of the first visit, to assess the need for an injection familiarisation session.

A pain quality word frequency analysis was performed on the SFMPQ and LFMPQ questionnaire data using Microsoft Excel software. Also, psychological traits were analysed via a one-way repeated measures ANOVA. Finally, correlation analyses were performed between the SSEIT and PRS, the SSEIT/PRS and various pain measurements including peak Pain Intensity (PI), pain duration, mean PI, time spent above 50% PI and the total area under the VAS time curve, respectively.

4.2: Results

4.2.1: Pain intensity measures

Test-retest reliability analysis

Reliability measures including single measure ICC scores with 95% Confidence Intervals, Coefficient of Variation (CV), Cronbach's Alpha (CA) scores and the Minimum Detectable Change (MDC) for the various pain intensity measurements have been displayed in Table 4.1, below.

Table 4.1: Reliability analysis results for pain intensity data during the three hypertonic saline injection at rest visits

Pain Intensity Measure	**Mean and SD (±)**			**Reliability Measures**			
	Visit 1	Visit 2	Visit 3	ICC (CI Intervals)	CV	CA	MDC
Peak PI	49.4 ± 18.3	50.2 ± 16.1	46.4 ± 17.6	0.814 ** (0.619-0.929)	4.2% **	0.929 **	9.1
Pain Duration (s)	356 ± 129	308 ± 90	300 ± 138	0.749 * (0.494-0.903)	9.5%	0.917 **	90
Mean PI	28.8 ± 8.6	31.1 ± 8.4	27.4 ± 10.3	0.711 * (0.454-0.884)	6.3%	0.889 *	6.7
Total area under VAS	10205 ± 4591	9581 ± 4060	8692 ± 5464	0.803 ** (0.599-0.924)	8%	0.929 **	2881
Time to PI (s)	91 ± 56	92 ± 44	90 ± 27	0.68 * (0.397-0.872)	1.2% **	0.856 *	39
Time spent >50% PI (s)	204 ± 76	201 ± 54	181 ± 68	0.703 * (0.422-0.88)	6.3%	0.881 *	63

***Indicates 'good' reliability (ICC > 0.75, CA > 0.9, CV < 5%).*
**Indicates 'moderate' or 'acceptable' reliability (ICC 0.5 - 0.75, CA 0.7 - 0.9).*

As shown in Table 4.1, according to the single measures ICC scores, all of the six measures are deemed to have at least 'moderate' reliability (0.68 – 0.814). This is also supported by the CA scores, with all measures suggested to have at least 'acceptable' reliability (0.856 – 0.929). Both peak PI (ICC = 0.814, CA = 0.929) and the total area under the VAS time curve (ICC = 0.803, CA = 0.929) suggest 'good' reliability. The other four measures fall into the 'moderate/acceptable' category according to the criteria relating to Intraclass Correlation Coefficients and Cronbach's Alpha.

Test-retest reliability analysis without visit 1 data

To assess the possible need for a familiarisation visit in hypertonic saline injection research and to eliminate a potential learning effect of the injections, the data from the pain intensity measures was analysed using the same methods but with the removal of the first visit. Firstly, this analysis was performed on all participants and then only on the eight individuals who had not previously experienced the injection. This was to see whether the reliability measures, primarily the ICC scores would be improved when the first visit is taken out. This is because there may have been factors associated specifically with the first injection visit a participant received. For example, an injection is associated with a level of anxiety, nervousness or fear and this could have been elevated more so in the first experience. Therefore, certain measurements especially aspects of the pain quality may have been selected in the first visit because the participant was fearful or anxious. Therefore, removing this visit and also inexperienced participants provides information on whether studies of this nature need to familiarise their participants with the injection and also whether there is a learning effect. Comparisons between the ICC scores of each measurement between all visits with all participants, all participants without visit 1 and without visit 1 with 'experienced' participants removed have been displayed in Table 4.2, below.

Table 4.2: Reliability measures (Intraclass Correlation Coefficients) comparisons with all visits and the removal of visit 1 for all participants and just inexperienced individuals

Pain Intensity Measure	ICC scores (95% CI)		
	All visits (all participants)	Visit 1 removed (all participants)	Visit 1 removed (only inexperienced participants)
Peak PI	0.814** (0.619-0.929)	0.792** (0.484-0.927)	0.815** (0.329-0.960)
Pain Duration (s)	0.749 (0.494-0.903)	0.761** (0.401-0.917)	0.766** (0.175-0.949)
Mean PI	0.711 (0.454-0.884)	0.74 (0.329-0.911)	0.758** (0.243-0.945)
Total area under VAS	0.803** (0.599-0.924)	0.817** (0.535-0.937)	0.883** (0.527-0.975)
Time to PI (s)	0.68 (0.397-0.872)	0.873** (0.651-0.918)	0.865** (0.502-0.971)
Time spent > 50% PI (s)	0.703 (0.422-0.88)	0.702 (0.312-0.892)	0.605 (-0.894-0.609)

***Indicates 'good' reliability (ICC > 0.75).*

The data shown in Table 4.2, indicates increases in single measures ICC scores when visit 1 is removed for four of the six measures used. Peak PI and time spent at 50% of PI did not increase for either Inexperienced Participants (IP) or All Participants (AP) without visit 1, respectively. Total area under the VAS time curve increased ICC scores for both AP and IP, but remaining within the 'good' reliability criteria window. Mean PI increased for all participants but stayed within the 'moderate' reliability criteria window, but increased to 'good' reliability with just inexperienced participants Finally, both pain duration and the time to PI increased from a 'moderate' reliability score to 'good' reliability when visit 1 was removed for both of the AP and IP groups, respectively.

4.2.2: Pain quality measures

Test-retest reliability analysis

As well as the intensity of pain measured through the VAS device, questionnaires were used to collect pain quality measures, and this was subsequently assessed for test-retest reliability of the hypertonic saline injections. The measures used were devised by the questionnaires themselves with the Total Pain Rating Index (T-PRI) and Present Pain Index (PPI) used to give an overview of the total pain quality experienced, and the subcategories of the questionnaires representing the different dimensions of pain. These consisted of the sensory, affective, evaluative and miscellaneous and sensory and affective dimensions for the Long-form McGill and the Short-form McGill pain questionnaires, respectively. As mentioned previously, Cronbach's Alpha is used on non-ratio data, therefore CA scores have been used for pain quality analysis over ICC and CV scores. Mean, standard deviation, CA and MDC scores, have been displayed in Table 4.3, below.

Table 4.3: Reliability analysis pain quality data

Questionnaire	Pain Quality Measure	Mean and SD (±)			Reliability Measures	
		Visit 1	Visit 2	Visit 3	CA	MDC
SFMPQ	T-PRI	6.9 ± 4.7	6.5 ± 3.4	5.5 ± 3.7	0.882*	3.9
	PPI	2.1 ± 0.9	2.1 ± 0.7	1.6 ± 0.7	0.806*	1.6
	Sensory	6.7 ± 4.7	6.4 ± 3.4	5.4 ± 3.7	0.889*	3.9
	Affective	0.14 ± 0.4	0.07 ± 0.3	0.07 ± 0.3	0.397	1.1
LFMPQ	T-PRI	14.4 ± 6.3	13.9 ± 5.3	11.1 ± 5.7	0.933**	5.1
	PPI	2.2 ± 0.7	2.1 ± 0.7	1.9 ± 0.5	0.849*	1.1
	Sensory	11.1 ± 4	11.1 ± 3.5	9.5 ± 5	0.848*	4.9
	Affective	0.7 ± 1.2	0.4 ± 0.7	0.1 ± 0.4	0.601	2.8
	Evaluative	1.1 ± 1.6	0.9 ± 1.4	0.6 ± 1.2	0.871*	1.7
	Miscellaneous	1.5 ± 1.1	1.6 ± 1.4	0.9 ± 1	0.865*	1.9

***Indicates 'good' reliability (CA > 0.9).*
**Indicates 'acceptable' reliability (CA 0.7 - 0.9).*

As seen in Table 4.3, reliability for pain quality was deemed at least 'acceptable' for eight out of ten measures according to Cronbach's Alpha scores. The two non-reliable measures are found in the affective dimension of both the SFMPQ (0.397) and LFMPQ (0.601). This is potentially due to the affective dimension appearing mostly in the first visit and then not in subsequent visits. This is most likely due to words in this dimension being associated with fear and anxiety, which may have been elevated in the first visit attributable to the participants inexperience with the injection. All other CA scores from the measures range between 0.806 and 0.889, suggesting 'acceptable' reliability, with the exception of the Total PRI from the LFMPQ which scored a CA of 0.933, indicating 'good' reliability.

Pain quality questionnaires word frequency analysis

A word frequency analysis was carried out on the completed Short-form and Long-form McGill pain questionnaires to identify the descriptive words most associated with the feeling of pain generated by the injections. The five most frequent words and the percentage in which they were selected have been analysed for all visits and across the three individual visits have been analysed and are displayed in Table 4.4, for SFMPQ results and Table 4.5, for LFMPQ results, respectively.

Table 4.4: Short-form MPQ word frequency analysis (top five words)

	Word Chosen (Percentage)			
Selection Ranking	Total	Visit 1	Visit 2	Visit 3
1	Aching (91%)	Aching (93%)	Aching (93%)	Aching (86%)
2	Cramping (79%)	Cramping (79%)	Cramping (86%)	Cramping/Throbbing (71%)
3	Throbbing (67%)	Throbbing (57%)	Throbbing (71%)	Heavy (36%)
4	Heavy (36%)	Tender (26%)	Tender (29%)	Tender (29%)
5	Tender (26%)	Stabbing (26%)	Hot/Heavy (29%)	Shooting (21%)

Table 4.5: Long-form MPQ word frequency analysis (top five words)

	Word Chosen (Percentage)			
Selection Ranking	Total	Visit 1	Visit 2	Visit 3
1	Cramping (69%)	Aching (71%)	Cramping (79%)	Cramping (64%)
2	Aching (58%)	Cramping (64%)	Aching (50%)	Aching (50%)
3	Throbbing (33%)	Boring/ Tender/ Spreading (36%)	Throbbing/ Pulsing (36%)	Throbbing/ Tender/ Tight (36%)
4	Tight (31%)	Throbbing/ Tingling/ Annoying (29%)	Taut/Annoying (29%)	Sharp/Hot (21%)
5	Tender/ Spreading (29%)	Intense/Tight/ Numb (29%)	Spreading/ Tight (29%)	Sore/ Spreading (21%)

Tables 4.4 and 4.5 show the changes across visits in word frequency for short-form and long-form McGill pain questionnaires, respectively. Firstly, the analysis shows that the SFMPQ results were largely consistent between visits, with an ‘aching’ sensation being the most common response for all visits and total. A ‘cramping’ sensation was the second highest response, followed by ‘throbbing’ which once again was selected relatively consistently across visits. These three words were the most common words (all above 50%) given to describe the pain quality during the HS injection, according to the SFMPQ results. Other words to appear in the top 5 words were ‘heavy’, ’tender’, ‘stabbing’, ‘hot’ and ‘shooting’.

Secondly, the LFMPQ analysis which is used to describe the entire pain experience as opposed to one specific point within the test, shows that ‘cramping’ is the most frequently cited word overall. ‘Aching’ is the second most common word in total, however these two words are reversed for the first visit. The third word overall is once again ‘throbbing’, ‘tight’ and ‘tender’/’spreading’ then make up the top five words chosen overall. As the long-form McGill contains more words to choose from this is evidenced by the increase in different words chosen in individual visits. Possibly accentuated by this fact, there is much more variation in this analysis. Additional words that help to describe the pain of the injections further include ‘boring’, ‘pulsing’, ‘taut’, ‘tingling’, ‘annoying’ etc.

4.2.3: Psychological measures analysis

To ensure that psychological factors did not have an impact on the results, two PANAS questionnaires and a pain expectation/confidence questionnaire were recorded during the study. Total PANAS scores from both the weekly and momentary questionnaires and their subcategories (positive effect and negative effect) were analysed via a one-way repeated measures ANOVA. Both pain expectation and confidence in dealing with this pain data was also analysed using one-way repeated measures ANOVA’s. All data was normally distributed in accordance with Shapiro-Wilke tests. Sphericity was assumed for all data sets using Mauchly’s test for Sphericity.

Weekly PANAS

ANOVA analyses revealed no significant difference over the three testing visits in total weekly PANAS scores $(F_{(2, 39)} = 0.498, P = 0.612)$, positive effect subcategory scores $(F_{(2, 39)} = 0.174, P = 0.841)$ and negative effect subcategory scores $(F_{(2, 39)} = 1.859, P = 0.169)$, respectively.

Momentary/present moment PANAS

ANOVA analyses revealed no significant difference in Total momentary PANAS scores *($F_{(2, 39)} = 0.041$, $P = 0.96$)*, positive effect subcategory scores *($F_{(2, 39)} = 0.040$, $P = 0.961$)* and negative effect subcategory scores *($F_{(2, 39)} = 0.518$, $P = 0.6$)*, over the three testing visits.

Pain expectation/confidence

An ANOVA revealed no significant difference between pain expectation scores over the three testing sessions *($F_{(2, 39)} = 2.510$, $P = 0.094$).* An ANOVA revealed no significant difference in the reports of confidence in dealing with this pain, over the three visits *($F_{(2, 39)} = 1.095$, $P = 0.345$).*

4.2.4: Pre-test questionnaires correlation analysis

Multiple Pearson correlation analyses were carried out using the pre-testing measures recorded using the Pain Resilience Scale (PRS) and the SSEIT emotional intelligence questionnaires. Firstly, the questionnaires were compared to each other, with a Pearson correlation analysis revealing a strong correlation between the SSEIT and PRS scores ($P = 0.996$). The questionnaire scores were then compared to various important pain measures recorded during the study which have been displayed in Table 4.6., found below. This table includes two-tailed significance levels and the corresponding level of correlation (< 3 = weak, 3 - 5 = moderate, > 5 = strong) for total scores for both the SSEIT and the PRS, as well as scores from the two subcategories of the PRS; Behavioural Perseverance (BP) and Cognitive Positivity (CP).

Table 4.6: Pre-test questionnaire (SSEIT/PRS) versus pain intensity measures correlation data

	Two-tailed Sig. (*P*) Pearson Correlation Score (Correlation Strength)			
Pain Intensity Measure	*Questionnaire or Subcategory*			
	SSEIT	PRS	BP	CP
Peak PI	0.457 *	0.077	0.168	0.077
Pain duration	0.66 **	0.533 **	0.585 **	0.549 **
Mean PI	0.729 **	0.181	0.345 *	0.162
Total area under VAS curve	0.494 *	0.183	0.249	0.201
Time > 50% PI	0.529 **	0.136	0.529 **	0.073

***Strong correlation (> 0.5), *Moderate correlation (0.3 - 0.5), Weak correlation (< 0.3).*

The data from Table 4.6 shows firstly that the SSEIT questionnaire has a stronger correlation with pain intensity measures (P = 0.457 – 0.729) than the PRS (P = 0.136 – 0.533). The SSEIT appears to correlate with all of the five measures used in the analysis. It also shows that there is a strong correlation between pain duration and both questionnaires (P = 0.533 – 0.66). The results from the PRS suggest that it does not correlate with most of the measures, with the exception of pain duration, but that the behavioural perseverance subcategory may be a better predictor.

4.3: Study 1.2: The impact of additional muscle contraction to hypertonic saline pain

4.3.1: Methodology

Study 1.2 of the research project was designed to assess possible changes in the behaviour/distribution of pain from the injections in non-fatiguing variable force exercise. The study was placebo-controlled, single blinded and randomized, with three separate visits. The first was the familiarisation/control visit and the subsequent two were either the hypertonic saline or isotonic saline/placebo visits in a random order, with at least seven days allowed between injection visits and at least 48 hours allowed after the familiarisation.

Testing Methods

The three visits necessary for the variable force study featured almost identical protocols but with slight differences. They consisted of the same Maximal Voluntary Contraction (MVC) test and the same Variable Force (VF) test but the contraction order and the variable implemented (Control/HS/IS injections) was different in each visit. The following testing methods described were conducted the same way for all three visits except where noted.

Maximal Voluntary Contraction test

In order to calculate the three exercise intensities used in the VF test, an individual maximum contraction force was needed. Therefore, in every visit an MVC test was performed using a Kin-Com Isokinetic Dynamometer machine (125E plus, CHATTECX corporation, Chattanooga group, Tennessee, USA). These tests were performed in each visit as MVCs of the quadriceps can be different on a day to day basis (Place et al., 2007). MVCs were recorded only to calculate the different exercise intensities and were not used as part of the research analysis.

Upon arrival to the laboratory, participants were asked to sit in the Isokinetic Dynamometer and the machine was manually adjusted to their dimensions. The participants were seated with their back straight and flat against the back of the chair, their right knee level with the pivot of the arm and their right leg strapped in securely approximately two centimetres above the ankle joint. Anatomical zero was set at the highest level of leg extension possible and then the joint angle was moved and secured at 90^{o}. The MVC protocol was then selected and then the participant put on the seatbelt and began the warmup.

The warmup consisted of 10 to 12 contractions at 50% of the individual's perceived maximal voluntary contraction force. These were performed for five seconds in duration, with five seconds rest between contractions. After two minutes rest the MVC test took place.

The actual MVC test consisted of three five-second contractions of the right quadriceps at maximal force, each separated by 90 seconds rest. Participants were given a ten second warning and then a five second countdown to each contraction and were encouraged throughout the contraction. The Kin-Com isokinetic dynamometer recorded the peak force achieved within the five seconds, which was noted for all three MVC's and then an average was calculated at the end of the test. If the values were not within 10% of each other, then an extra MVC may have been performed. The mean MVC result was then used to calculate 10%, 15% and 20% of the participant's maximal voluntary contraction to be used for the variable force test. Figure 4.2 provides a diagram on the MVC test protocols.

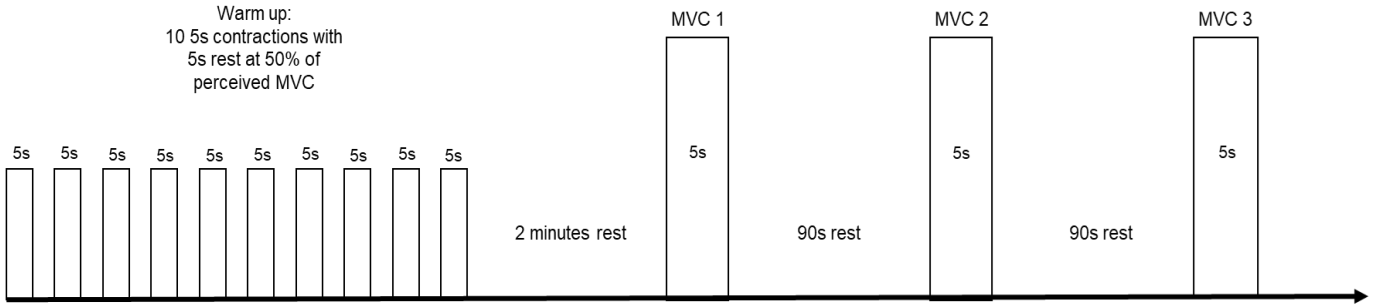

Figure 4.2: The maximal voluntary contraction test protocols including the warmup.

Variable Force test

The VF test was also performed on the Kin-Com isokinetic dynamometer. Participants were allowed ten minutes rest after the MVC test, whilst the Kin-Com was set up for the VF test. The protocols were adjusted on the machine to display continuous force output on the screen, including markers to indicate the different exercise intensities. The bottom line displaying 10%, the top 20% and the middle line 15%, respectively. During the rest period participants were asked to match these percentages and practice keeping a consistent contraction at those intensities. They were also asked to reach the lines as quickly as possible to ensure a contraction at that intensity lasted for the full duration. During the injection visits, while the participant was practising the researcher prepared the injections using the protocols mentioned previously. Participants were not told whether they would be receiving the hypertonic saline or the isotonic solution, with this randomized between visits five and six. This was blinded from the participant but not the researcher. There were no differences in the injection preparation or implementation between visits, other than the solution used.

Once the test protocols and the injections were prepared the test was explained to the participant in detail. They then completed the two PANAS questionnaires and the pain expectation/confidence questionnaires. They were then handed a VAS device (this was different to the device used in Study 1.1 as it recorded pain levels every two seconds as opposed to five), which was switched on simultaneously with a timer. After 60 seconds the participant received the injection, using the same protocols as Study 1.1. Again, the procedure lasted 20 seconds with a ten second infusion, z-track applied, the leg relaxed and at

90^{o}, with the participant asked to refrain from looking at the initial insertion and injected in the previously marked area, but avoiding any previous injection wounds. Participants were asked to adjust the VAS device as soon as they felt any changes in pain and to continuously adjust the scale throughout the test until it was returned to zero, in all visits. In the control visit 20 seconds rest was allowed instead of the injection.

Once the injection procedure was complete the variable force test began. The test consisted of three separate, ten second, sustained contractions of the right quadriceps' muscles. The three contractions were the 10%, 15% and 20% of MVC calculated previously. These intensities were chosen as previous comparisons made between exercise intensities following a HS injection have been comparatively high and with large differences between them. One example is the Ciubotariu et al. (2004) study that utilised contractions of 50% and 80% of MVC. However, no study has looked at the impact of low intensity exercise or small intensity changes in the pain response to hypertonic saline. The order of these contractions was randomised across participants and visits. The timings and the rest of the protocols were identical for all three visits. There was 30 seconds rest between the three contractions. Participants were given a ten second warning and a five second countdown to the contractions. During the contraction's participants had to match and sustain the power displayed on the screen in the form of the three markers mentioned previously. The first contraction time was different for each participant and was based upon the results recorded in Study 1.1. The time between the injection needle removal and the first contraction was the time taken for that participant to reach 50% of their peak pain intensity in Study 1.1. This was taken as an average from the three reliability resting visits and was rounded up to the nearest five seconds. This was to ensure the three contractions of the VF test took place whilst the participants were under the greatest influence of the hypertonic saline injections. This time was consistent across all three visits.

Pain intensity was recorded throughout the experiment on the electronic VAS device, with participants asked to adjust the device whenever a change in pain was observed, especially during the contractions. They were also asked to focus on any changes during the corresponding rest periods. The test was not finished after the last contraction was completed but when the VAS device was returned to zero indicating 'no pain'.

The pain quality of the different contractions was measured using the SFMPQ. This was performed during the 30 second rest periods and was based on how the pain felt during the previous contraction. Participants completed this verbally with this recorded on an audio recording device (ICD-BX140 model, Sony Corporation, Tokyo, Japan) and completed by the researcher afterwards. Participants were able to complete this in this relatively short period of time as they had been thoroughly familiarised with it in Study 1.1. The pain quality of the entire experiment was also recorded using the LFMPQ. This was given to the participant once they had returned the VAS device to zero and was based on how the pain felt throughout the whole test, from the first sensation of pain to zero pain. All testing protocols related to the variable force testing visits are presented in Figure 4.3, below.

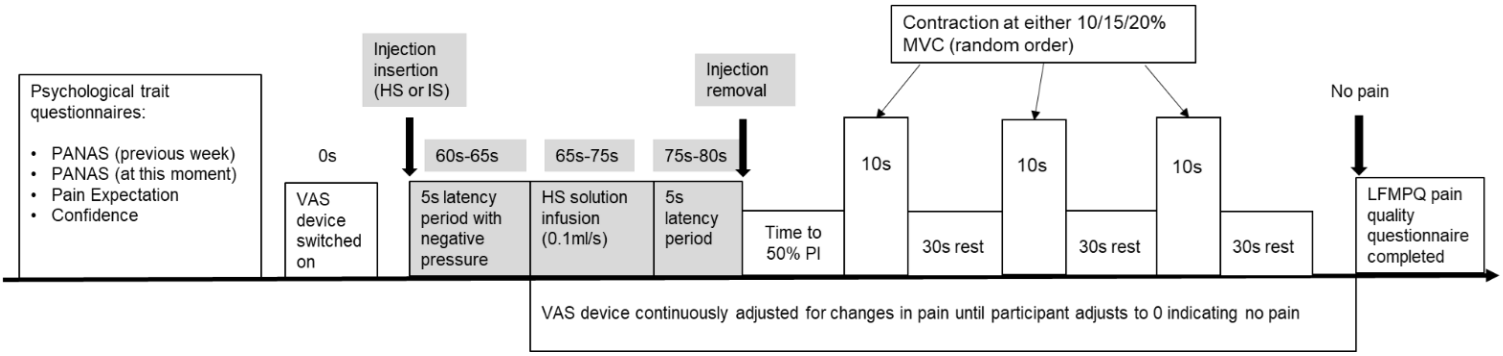

Figure 4.3: Protocols of the variable force test in all injection visits of Study 1.2. For the control visit, all protocols remained the same but with the removal of the injection protocols highlighted in grey.

4.3.2: Data analysis

All electronically stored data was anonymised and unidentifiable. All identifiable and hard data was stored in a locked filing cabinet in the research supervisor's office. Most of the data was stored on spreadsheets located on a password protected computer and memory stick.

Most of the data extracted from the VAS device was recorded in two second intervals and therefore the measurements compared between contractions and variables was based on this time point, including mean, peak and total PI during contractions, mean PI during rest periods and the total area under the VAS curve during contraction and rest period. When comparing data from the VF test and the data collected in Study 1.1 through measurements such as total area under the VAS time curve and pain distribution comparisons, the data was converted to ten second intervals as these are the time points both data sets share.

4.3.3: Statistical analysis

All statistical tests were carried out using IBM SPSS statistics v25 software (SPSS, IBM, New York, USA) unless stated otherwise. For all ANOVA analyses carried out, normality was assessed via Shapiro-Wilk tests and sphericity was assessed using Mauchly's test of sphericity. Any data that failed tests for normality was log transformed.

Firstly, comparisons were made between the hypertonic saline, isotonic saline and control visits via a one-way repeated measures ANOVA test with Tukey pairwise comparisons. Multiple tests were conducted this way to compare data from the three visits across measurements at the different exercise contractions e.g. peak PI during 10%, 15% or 20% contraction, mean PI during rest period for 10%, 15%, 20% contraction etc. This was to confirm that the hypertonic saline injection produces significant pain, that the isotonic saline injection is a true placebo and that the exercise intensities do not produce significant pain.

One-way repeated measures ANOVA's with Tukey pairwise comparisons were also performed to assess differences between the exercise intensities. Again, measurements such as mean/peak/total PI, mean rest period PI, total area under the VAS time curve (contraction and rest period) were compared between 10%, 15% and 20% to assess whether a higher exercise intensity resulted in increased pain. This test was repeated to compare pain quality data ascertained from the corresponding exercise intensity SFMPQs, with total PRI, PPI, sensory and affective dimension results being compared between 10%, 15% and 20%.

A pain quality word frequency analysis similar to the one performed in Study 1.1 was also carried out on both the SFMPQ and LFMPQ data. The SFMPQ data was compared across exercise intensities to assess whether an increased intensity would change the words chosen. The LFMPQ data and total SFMPQ data of all contractions were also compared to the percentages observed in Study 1.1 to see if adding exercise to the injection would make a difference to what words were chosen.

Certain measurements from the hypertonic saline VF test were compared to the mean data in Study 1.1. This includes pain duration, total mean PI and total area under the VAS time curve at ten second intervals. This was compared via paired samples t-tests.

The final analysis carried out was a frequency analysis of any observed adverse effects of the hypertonic saline injections. This was calculated using Microsoft excel software by comparing the total number of injections to any observations of adverse effects and represented as a percentage of the frequency these effects occurred.

4.4: Results

Analyses were carried out on the Study 1.2 data, primarily to assess whether additional muscle contraction impacted the pain experienced with the hypertonic saline injections and whether the intensity of this contraction is important.

4.4.1: Pain intensity measures

Comparison between visits

To assess whether the contraction intensity causes any pain and the difference between experimental visits multiple one-way repeated measures ANOVA's were carried out between the three visits (HS/IS/CON) peak intensity, mean pain intensity and total pain intensity during the 10%, 15% and 20% contractions, respectively. Results from the ANOVA analyses including mean, standard deviation and *P* values, are displayed in Table 4.7, found on the page below.

Table 4.7: ANOVA and follow up paired samples t-test comparisons between the three visits (HS/IS/CON) for various pain measures recorded during the three contractions (10%/15%/20%)

Contraction Percentage	**Pain Intensity Measure**	**Mean and SD (±)**			**ANOVA**		**Paired samples t-tests**		
		HS	IS	CON	$F_{(2, 39)}$	*P*	HS/IS	HS/CON	IS/CON
10%	Peak PI	42.9 ± 17.6	3.4 ± 4.5	0 ± 0	72.1	< 0.001*	< 0.001*	< 0.001*	0.666
	Mean PI	40.4 ± 17	2.6 ± 3.5	0 ± 0	71.2	< 0.001*	< 0.001*	< 0.001*	0.777
	Total PI	202.1 ± 85	12.9 ± 117.6	0 ± 0	71.4	< 0.001*	< 0.001*	< 0.001*	0.777
15%	Peak PI	46.8 ± 18.3	3.1 ± 4.9	0.8 ± 2	77.7	< 0.001*	< 0.001*	< 0.001*	0.847
	Mean PI	45.2 ± 18.6	2.4 ± 3.8	0.5 ± 1.3	73.9	< 0.001*	< 0.001*	< 0.001*	0.893
	Total PI	226.1 ± 93.1	12.1 ± 18.9	2.6 ± 6.8	73.9	< 0.001*	< 0.001*	< 0.001*	0.893
20%	Peak PI	53.2 ± 19.1	4.8 ± 6.4	2.6 ± 4.2	81.3	< 0.001*	< 0.001*	< 0.001*	0.875
	Mean PI	51 ± 19.4	4.3 ± 5.7	2 ± 3.5	76.2	< 0.001*	< 0.001*	< 0.001*	0.862
	Total PI	255.2 ± 97.1	21.4 ± 28.7	9.8 ± 17.6	76.2	< 0.001*	< 0.001*	< 0.001*	0.862

**Significant difference (P < 0.05).*

The information presented in Table 4.7 shows that there is a significant difference in pain intensity measures between conditions. There are significant differences between all pain measures recorded during the hypertonic saline injection visit and the isotonic saline injection (P = < 0.001) and control visits (P = < 0.001), respectively, at all muscle contraction intensities (10%/15%/20%). There were no significant differences between the isotonic saline and control visits for any pain intensity measures, at any muscle contraction intensity (P = 0.666 – 0.893).

Hypertonic saline visit contraction intensity analysis

An investigation into whether there were any significant differences in various pain measures between the contraction intensities (10%/15%/20%) during the hypertonic saline test was carried out using one-way repeated ANOVA analyses. The pain measures compared at these intensities were peak pain intensity during contraction, mean pain intensity during contraction, total pain intensity (total of pain intensity observations at 2, 4, 6, 8 and 10 seconds) during contraction, mean pain during corresponding rest period and total area under the VAS curve during the contraction and corresponding rest period. Results from these analyses are presented in Table 4.8, including mean, standard deviation, F and P values, located on the page below.

Table 4.8: ANOVA and follow up paired samples t-test comparisons between the three muscle contractions (10%/15%/20%) for various pain measures recorded during the hypertonic saline injection variable force experiment

Pain Intensity Measure	**Mean and SD (±)**			**ANOVA**		**Paired samples t-tests**		
	10%	15%	20%	$F_{(2, 39)}$	*P*	10%/15%	10%/20%	15%/20%
Peak PI during contraction	42.9 ± 17.6	46.8 ± 18.3	53.2 ± 19.1	1.138	0.331	0.838	0.305	0.626
Mean PI during contraction	40.4 ± 17	45.2 ± 18.6	51 ± 19.4	1.175	0.319	0.766	0.288	0.685
Total PI during contraction	202.1 ± 85	226.1 ± 93.1	255.2 ± 97.1	1.172	0.545	0.998	0.589	0.623
Total area under VAS curve (contraction + rest period)	1624 ± 657	1670 ± 660	1914 ± 745	0.716	0.495	0.983	0.512	0.62

**Significant difference (P < 0.05).*

Table 4.8 shows that during the hypertonic saline injections there are no significant differences in pain measures recorded at 10%, 15% and 20% muscle contractions (P = 0.319 – 0.545). There are also no significant differences between 10% and 15% (P = 0.766 – 0.838), 10% and 20% (P = 0.288 – 0.589) and 15% and 20% (P = 0.62 – 0.685), respectively, for any of the pain measures recorded.

Comparison between Study 1.2 and Study 1.1 data

To assess whether the addition of muscle contraction impacts the pain experience from hypertonic saline injections at rest, comparisons were made between the Variable Force (VF) HS visit in Study 1.2 and mean data across the three resting HS visits from Study 1.1. Paired samples t-tests were carried out on the two sets of data on pain intensity measures including total pain duration, total mean pain and the total area under the VAS time curve (in 10 second intervals).

A paired samples t-test found no significant difference in total pain duration between VF (289.9 ± 67.7) and resting values (321.1 ± 111.5) ($T_{(13)}$ = -1.607, P = 0.132). A paired samples t-test also revealed no significant difference in total mean pain between VF (30.5 ± 11.6) and resting values (29.1 ± 8.2) ($T_{(13)}$ = 0.774, P = 0.453). A final paired samples t-test found no significant difference in the total area under the VAS time curve (10 second intervals) between VF (1754 ± 806) and resting values (1978 ± 905) ($T_{(13)}$ = -1.608, P = 0.132).

To highlight any possible differences in intramuscular pain at rest and with the introduction of muscle contractions, a pain distribution graph has been displayed in Figure 4.4, on the page below.

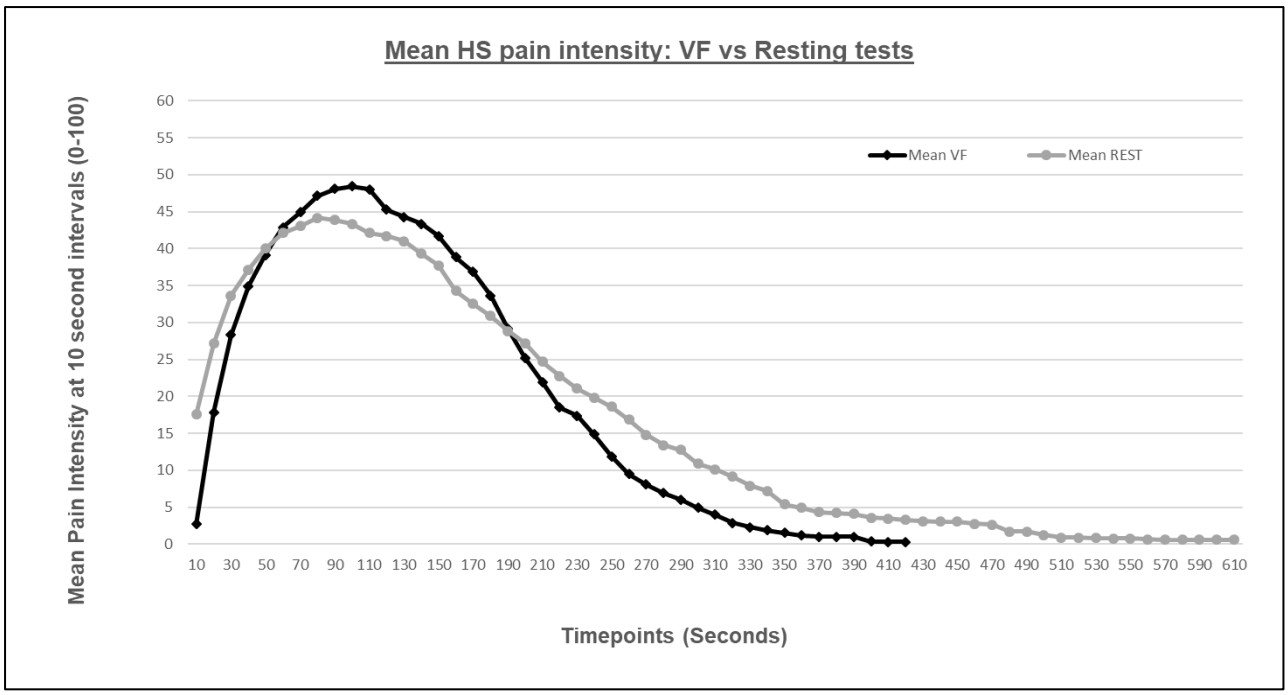

Figure.4.4: The differences in mean pain intensity between the hypertonic saline injections at rest and with muscle contractions at 10 second intervals, showing the distribution of pain.

4.4.2: Pain quality analysis

Pain quality differences between muscle contraction intensity

Individual SFMPQ's were completed during the hypertonic saline visit to measure the pain quality at 10%, 15% and 20% intensity muscle contractions. To assess possible changes in pain quality dependent on the intensity of exercise applied one-way ANOVA's and follow up paired samples t-tests have been carried out on this data. The measures concerned are the total PRI, PPI, sensory and affective dimensions scores recorded with the SFMPQ. All ANOVA and paired samples t-test results have been presented in Table 4.9, on the page below.

Table 4.9: ANOVA and follow up paired samples t-test comparisons between pain quality measures taken from SFMPQ's performed during each of the three muscle contractions (10%/15%/20%) in the hypertonic saline injection variable force experiment

Pain Quality Measure (SFMPQ)	**Mean and SD (±)**			**ANOVA**		**Paired samples t-tests**		
	10%	15%	20%	$F_{(2,39)}$	*P*	10%/15%	10%/20%	15%/20%
Total PRI	4.5 ± 2.2	5.6 ± 2.5	6.3 ± 2.4	2.034	0.144	0.418	0.128	0.755
PPI	2 ± 0.7	2.4 ± 0.6	2.4 ± 0.6	1.413	0.256	0.323	0.323	1
Sensory	4.5 ± 2.1	5.5 ± 2.4	6 ± 2.4	1.524	0.231	0.494	0.213	0.836
Affective	0.07 ± 0.27	0.21 ± 0.43	0.29 ± 0.61	0.798	0.457	0.689	0.437	0.91

**Significant difference ($P < 0.05$).*

Table 4.9 shows that, similar to pain intensity, there are no significant differences in pain quality measures between muscle contraction intensities (P = 0.144 – 0.457). The paired samples t-tests also show this as there are no significant differences found between 10% and 15% (P = 0.323 – 0.689), 10% and 20% (0.128 – 0.437) and 15% and 20% (P = 0.755 – 1), for any of the SFMPQ pain quality measures, respectively.

Comparison between Study 1.1 and Study 1.2 data

To assess whether the addition of muscle contraction impacts the pain quality experience from hypertonic saline injections at rest, comparisons were made between the Variable Force (VF) HS visit in Study 1.2 and mean data across the three resting HS visits from Study 1.1. Paired samples t-tests were carried out on the two sets of data on pain quality measures ascertained through the SFMPQ and LFMPQ. Comparisons have been made between the T-PRI, PPI and all subcategories of both questionnaires.

Firstly, paired samples t-tests analyses between SFMPQ results revealed no significant differences between VF (5.5 ± 2.1) and resting values (6.3 ± 3.6) for T-PRI ($T_{(13)}$ = -0.697, P = 0.498), VF (2.2 ± 0.6) and rest (2 ± 0.7) for PPI ($T_{(13)}$ = 2.09, P = 0.057), VF (5.3 ± 2) and rest (6.2 ± 3.6) for the sensory dimension ($T_{(13)}$ = -0.757, P = 0.462) and VF (0.2 ± 0.4) and rest (0.1 ± 0.2) for the affective dimension ($T_{(13)}$ = 1.06, P = 0.308), respectively.

Secondly, paired samples t-tests analyses between LFMPQ results revealed no significant differences for T-PRI ($T_{(13)}$ = 0.616, P = 0.548) between VF (14.1 ± 3.6) and rest (13.1 ± 5.4), the sensory dimension ($T_{(13)}$ = 1.493, P = 0.159) between VF (12.3 ± 3.3) and rest (10.5 ± 3.6), the affective dimension ($T_{(13)}$ = 0.125, P = 0.903) between VF (0.4 ± 0.6) and rest (0.4 ± 0.6), the evaluative dimension ($T_{(13)}$ = -0.694, P = 0.5) between VF (0.6 ± 1.4) and rest (0.9 ± 1.25) and the miscellaneous dimension ($T_{(13)}$ = -1.295, P = 0.218) between VF (0.9 ± 1) and rest (1.3 ± 1.1), respectively. However, a paired samples t-test analysis did reveal a significant difference between VF (2.5 ± 0.8) and rest (2.1 ± 0.6) for PPI ($T_{(13)}$ = 2.187, P = 0.048).

Pain quality questionnaires word frequency analysis

Similar to the analysis performed in Study 1.1, the frequency in which participants selected words on the SFMPQ and LFMPQ were measured. Firstly, SFMPQ word frequencies were compared between the three contraction intensities. Total SFMPQ data was then compared to the SFMPQ results from Study 1.1, to see if the addition of muscle contraction impacted the words chosen. The majority of LFMPQ data from the isotonic and control visits was blank as there was no discernible pain presence. Therefore, only the data from the hypertonic saline LFMPQ's was analysed and then this data was compared to the LFMPQ data from Study 1.1, also. All measures have the top five selected words, percentage selected by participants and when appropriate, the increase/decrease in comparison to the Study 1.1 resting data presented. All data from the SFMPQ pain quality word frequency analyses are displayed in Table 4.10 and all data from the LFMPQ pain quality word frequency analyses are shown in Table 4.11.

Table 4.10: SFMPQ word frequency analysis (top five words) including comparisons between the three muscle contractions (10%/15%/20%) and comparisons between the hypertonic saline experiments at rest during Study 1.1 and with muscle contractions in Study 1.2

	Word Chosen (Percentage)					
Selection Ranking	Total	10%	15%	20%	Increases from rest	Decreases from rest
1	Aching (83%)	Cramping (79%)	Aching (93%)	Aching/ Cramping (86%)	Tiring (+9.5%)	Hot/ Burning (-14.3%)
2	Cramping (81%)	Throbbing/ Aching (71%)	Cramping (79%)	Throbbing (79%)	Throbbing (+7.1%)	Gnawing (-9.5%)
3	Throbbing (74%)	Heavy (29%)	Throbbing (71%)	Shooting (36%)	Cramping/ Shooting (+2.4%)	Aching/ Heavy/ Stabbing (-7.1%)
4	Heavy (29%)	Stabbing/ Tender (21%)	Heavy/ Tender (29%)	Heavy (29%)		Splitting/ Sharp/ Tender (-2.4%)
5	Shooting/ Tender (24%)	Shooting (14%)	Shooting/ Tiring (21%)	Tender/ Tiring (21%)		

The SFMPQ word frequency analysis presented in Table 4.10 shows firstly that the top three chosen words for the HS injections with additional muscle contraction is the same as at rest (aching, cramping and throbbing). There were changes to the words chosen from at rest, with some increasing (tiring, throbbing, cramping etc.) and some decreasing (hot/burning, gnawing, aching etc.) in frequency. The table also shows that the intensity of muscle contraction performed impacted what words were chosen. Similar words are chosen for each of the intensities but the frequency appears to change for example aching is only selected 71% at 10% but 93% at 15% and shooting increases from 14% at 10%, to 21% at 15% and finally 36% at 20%. This amongst other changes, suggest the pain quality changes depending on the intensity of muscle contraction.

Table 4.11: LFMPQ word frequency analysis (top five words) with comparisons between the hypertonic saline injection experiments at rest in Study 1.1 and with muscle contractions during Study 1.2

	Word Chosen (Percentage)		
Selection Ranking	Total	Increases from rest	Decreases from rest
1	Aching (71%)	Throbbing (+23.8%)	Annoying (-23.8%)
2	Throbbing (57%)	Tiring (+19%)	Pulsing (-21.4%)
3	Cramping (50%)	Shooting (+16.7%)	Cramping/ Boring (-19%)
4	Tender/ Tiring (36%)	Aching (+14.3%)	Tugging (-14.3%)
5	Shooting/ Spreading/ Tight (21%)	Drilling (+11.9%)	Taut/ Nagging (-11.9%)

Once again, Table 4.11 shows that the most common words chosen for the LFMPQ are aching, throbbing and cramping for the HS injection with muscle contraction. However, the order and percentages have changed when compared to the resting values. Cramping dropped 19% from first to third, throbbing increased by almost 24% to second and aching increased by around 14% to first place. Other notable changes in words chosen with additional muscle contraction include increases in tiring, shooting and drilling, as well as decreases in annoying, pulsing and boring etc. The muscle contractions appear to impact the pain quality experienced from the hypertonic saline injections.

4.4.3: Psychological traits analysis

As with Study 1.1, psychological traits were analysed to ensure any changes in Study 1.2's data was not due to psychological changes during the study. This was performed suing one-way repeated ANOVA's on the two PANAS questionnaires (weekly and momentary) total scores and their subcategory scores (positive/negative effect). Pain expectation and confidence in dealing with this pain was recorded but was not analysed as this was influenced by the order participants received the injections. For example, a participant may have received the hypertonic saline injection in the second visit and then their final visit they would be aware that they were going to receive the isotonic saline injection. This then would have lowered their pain expectation rating.

Weekly PANAS

One-way repeated measures ANOVA analyses revealed no significant differences across the three visits for total weekly PANAS scores *($F_{(2, 39)} = 0.038$, $P = 0.963$)*, weekly positive effect scores *($F_{(2, 39)} = 0.023$, $P = 0.978$)* and weekly negative effect scores *($F_{(2, 39)} = 0.198$, $P = 0.821$)*, respectively.

Momentary/present moment PANAS

One-way repeated measures ANOVA tests revealed no significant differences for momentary total PANAS scores *($F_{(2, 39)} = 0.057$, $P = 0.944$)*, momentary positive effect scores *($F_{(2, 39)} = 0.023$, $P = 0.978$)* and momentary effect scores *($F_{(2, 39)} = 0.198$, $P = 0.821$)* across the three visits, respectively.

4.5: Side-effects observed

Although efforts were made to reduce the risks of any adverse effects occurring due to the injections, a few incidents were observed during both Study 1.1 and Study 1.2. These were all minor and temporary. As these are all related to the reliability and use of the hypertonic saline injections, they have been included to add to the profile of this technique in future research. The effects that were present in this project are detailed in the following paragraph including the percentage in relation to the amount of hypertonic saline injections carried out presented in brackets for each one.

A total of 76 injections on 18 different people were carried out in both studies, 62 of which were injections of hypertonic saline, with the remaining 14 isotonic saline injections. In total, mild side-effects (i.e. a minor, atypical response to the injection) were reported in 11 injection experiments. As a precaution, if a participant experienced a side-effect, however mild, during the experiment, they were removed from the study. The atypical effects occurring during this experiment included one count of feinting (1.6%), one count of a significant muscle twitch (1.6%) and one participant experiencing both nausea (6.5%) and blurred vision (4.8%) to the extent that they were unable to rate pain. It is most likely that these adverse effects were caused by the injection procedure (i.e. needle insertion) rather than as a result of the HS solution. Other adverse effects observed from the injections were minor muscle twitches (4.8%), significantly lower pain response (4.8%) and one count of zero pain experienced from the hypertonic saline injection (1.6%). The majority of these adverse effects were experienced in the first visit of Study 1.1 and unless stated otherwise were mostly mild side effects and the participants were able to continue testing.

Chapter 5: General Discussion

5.1: Study 1.1: Test-retest reliability of intramuscular hypertonic saline injections

5.1.1: Pain intensity

One of the primary findings obtained from Study 1.1 was that the hypertonic saline injection model is a reliable method of inducing muscle pain, in agreement with the original hypothesis. In terms of pain intensity, all of the measures were determined to have at least 'moderate' to 'good' reliability, with Intraclass Correlation Coefficient (ICC) scores ranging from 0.68 to 0.814 and Cronbach's Alpha (CA) scores ranging from 0.856 to 0.929, based on criteria set out by Koo and Li (2016) and Tavakol and Dennick (2011), respectively. The Coefficient of Variation (CV) scores also support this method's reliability, with scores for all measures ranging between 1.2% and 9.5%, suggesting a low variability in the results. Of the pain intensity values measured, the two most often reported in other hypertonic saline studies, the peak pain intensity (ICC = 0.814, CA = 0.929) and the total area under the VAS time curve (ICC = 0.803, CA = 0.929), both demonstrated 'good' reliability. As these are the most important pain intensity measurements for the hypertonic saline model, it is crucial that these aspects of the pain response are reproducible in any study that uses this method.

The 'good' reliability observed for these two measures support the only other true reliability study on hypertonic saline injections, conducted by Graven-Nielsen et al., (1997a). This study also investigated the reliability of this method over separate occasions but had two visits, used 0.5 ml of 5% hypertonic saline into the tibialis anterior, used a computer-controlled syringe pump and used paired tests and paired correlations (Graven-Nielsen et al., 1997a). Despite these methodological differences, the results from this study also demonstrated good reliability with no significant differences ($P > 0.36$) and paired correlations ($R < 0.048$) observed between visits for peak pain intensity, area under the VAS time curve, pain duration and time to peak intensity. This study, in combination with the findings from the present study, suggest that even with differences in the volume, concentration, muscle used and methodologies, hypertonic saline injections appear to produce a reliable pain intensity response.

In comparison to other pain models

The use of hypertonic saline is often chosen as a model of pain inducement as it is thought to closely resemble the sensation of muscle pain and exercise-induced pain (Graven-Nielsen et al., 1997c). However, as the reliability of this method had not been fully demonstrated previously, alternative models that have shown good reliability such as the cold pressor test (Koenig et al., 2013) or pressure pain test (Koo et al., 2013; Pelfort et al., 2015), may have been used instead. As these studies provide test-retest reliability measures in the form of ICC scores, comparisons can be made between the pain intensity results from the present study and these traditionally reliable methods. Starting with the cold pressor test, Koenig et al. (2013) reported ICC scores of 0.79 and 0.86, for 4º and 6º Celsius water temperature cold pressor tests, respectively. Alternatively, ICC scores of 0.91 (Koo et al., 2013) and 0.86 to 0.92 (Pelfort et al., 2015), have been observed for the pressure pain test. Therefore, based on the guidelines of Koo and Li (2016), these models have demonstrated 'good' reliability and 'good' to 'excellent' reliability, respectively. Although not all pain intensity measures were as high as these scores in the present study, the ICC scores demonstrated for peak pain intensity and area under the VAS time curve (0.803 – 0.814), suggest that the hypertonic saline model is at least as reliable as the cold pressor test. The benefits of this model, coupled with this reliability, make it a more effective method of EIP inducement than the alternatives, in terms of pain intensity.

The need for familiarisation?

An additional analysis was conducted on the pain intensity reliability data to assess whether a familiarisation visit would be needed in future research, especially for participants who had never experienced hypertonic saline injections. The results of this analysis suggest that reliability was improved with the removal of the first visit for all participants and inexperienced participants. ICC scores for all participants improved for four of the six pain intensity measures, with pain duration (0.749 to 0.761), mean PI (0.711 to 0.74), total area under VAS time curve (0.803 to 0.817) and time to PI (0.68 to 0.873), increasing. Alternatively, the ICC values for time spent above 50% PI decreased slightly (0.703 to 0.702)

and peak PI fell (0.814 to 0.792). However, with participants who had received the injection before and the first visit removed, all of the pain intensity measures improved with the exception of the time spent above 50% PI (0.703 to 0.605). Out of these, time to PI (0.68 to 0.865), mean PI (0.711 to 0.758) and pain duration (0.749 to 0.766), went from 'moderate' reliability to 'good' reliability. The other improvements were in total are under VAS time curve (0.803 to 0.883) and peak PI (0.814 to 0.815). Overall, with the removal of the first visit results and the inclusion of only inexperienced participants the reliability for all of the pain measures, with the exception of one, was deemed 'good'. This suggests that a more reliable pain intensity response is generated when participants have previously experienced the hypertonic saline injections. Therefore, it is recommended that studies conducted with this model include a familiarisation visit which includes exposure to the saline injection.

5.1.2: Pain quality

To be considered reliable, the pain response to this model was assessed in terms of both pain intensity and pain quality. In terms of pain quality, the Total Pain Rating Index (T-PRI) of the Short-Form (SFMPQ) and Long-Form (LFMPQ) McGill Pain Questionnaires demonstrated 'acceptable' or 'good' reliability, with CA scores of 0.882 and 0.933, respectively. In contrast, the CA scores from the affective dimension of both the SFMPQ (0.397) and LFMPQ (0.601), indicated 'poor' reliability. All other dimensions of both questionnaires demonstrated 'acceptable' reliability with CA scores ranging from 0.806 to 0.889. These results therefore suggest that the overall pain quality experienced in response to hypertonic saline injections is reliable, but not for the affective dimension.

The Graven-Nielsen et al. (1997a) study also investigated the reliability of pain quality measures. As well as the methodological differences mentioned during the pain intensity comparison, this study did not use the SFMPQ (Graven-Nielsen et al., 1997a), so comparisons between these measurements cannot be made. However, this study did assess the T-PRI and all dimensions of the LFMPQ, with no significant differences found between visits for all measures ($P > 0.36$) and paired correlations ($P < 0.001 – 0.046$) observed between all except the miscellaneous dimension (Graven-Nielsen et al., 1997a). Therefore,

the T-PRI and the majority of the dimensions associated with hypertonic saline evoked pain quality appears reliable, based on the two studies. The discrepancies between the two studies in relation to the miscellaneous and affective dimensions is hard to explain. It could be a true reflection of the test-retest reliability or it could be attributed to questionnaire categories themselves. The affective dimension questions recorded in the present study were the least selected, had the lowest mean scores of all the pain quality measures assessed for reliability (0.1 – 0.7) and had standard deviations higher than mean scores for all three visits (0.4 – 1.2). This was a result of words associated with the affective category being selected after only 10 of the 42 total injections recorded, so therefore with a large amount of zero's recorded (when no word was selected), even a one score in this dimension would result in a large amount of variability. This may have been a factor in the Graven-Nielsen et al. (1997a) results as well, as the authors stated this lack of reliability may have been caused by the high sensitivity of the miscellaneous pain rating index. Therefore, these results may explain the low reliability in this dimension compared to the others or may show a limitation in this type of reliability analysis. Alternatively, this could be an accurate representation of the nature of pain quality associated with this model, but this is unclear at this moment, without further evidence.

5.1.3: The profile of the hypertonic saline pain response

Pain intensity

The results from this study show not only that the pain intensity and quality produced by this model are reliable, but also provides a useful profile of the pain response. When compared to existing literature that has used similar protocols, conclusions can be made on the typical response expected from hypertonic saline induced pain. As with the literature review, the average pain profile developed from 18 different studies (Ciubotariu et al., 2004; Deschamps et al., 2014; Henriksen et al., 2007; Henriksen et al., 2010; Henriksen et al., 2011; Graven-Nielsen et al., 1997a; Graven-Nielsen et al., 1998a; Graven-Nielsen et al., 2003; Graven-Nielsen et al., 2002; Graven-Nielsen et al., 1997c; 1997d; Mista et al., 2015; Park & Hopkins, 2013; Salomoni & Graven-Nielsen, 2012; Schilder et al., 2014; Schulte et al., 2003; Sørensen

et al., 2012; Wassinger et al., 2012) that met the criteria (i.e. 0.5 – 1 ml volume/5 – 6% concentration) has been compared to the present studies results.

The average peak pain intensity of the present study was 4.8, which is identical to the mean value found in the other studies. The range was also similar, with the present study showing a range between 2.2 and 7.7, compared to 3.2 and 7.2. Mean pain intensity was 2.9, ranging from 1.6 to 3.9, in the present study. Again, this is similar to the existing papers, with mean scores of 3.2 (2.5 – 4.7). The average amount of time the injection pain lasted in the present study was 277 seconds (207 – 552s), which was lower than the 427 seconds (210 – 720s) found in alternative research. However, the duration of pain is suggested to be dependent on infusion time (Graven-Nielsen et al., 1997a) and the area injected, with infusions into the infrapatellar fat pad for example, lasting longer (Bennell et al., 2004; Sørensen et al., 2012). With this in mind, when this pain duration is compared to the literature with infrapatellar fat pad studies removed, the mean pain duration is still higher at 390 seconds, but the range is much more similar at 210 to 540 seconds. The time taken to reach peak intensity was very similar however, with mean values of 91 seconds (53 – 188s) and 95 seconds (67 – 121s), for the present study and other papers, respectively. Lastly, on average the onset of pain was said to occur at around 12 seconds (0 – 20s) in the wider literature, which was different from the four seconds (0 – 41s), observed in the present study. However, the cause for this is due to a limitation in the study, as the VAS device used throughout only recorded in five second intervals. Therefore, the exact time that the pain onset occurred was not measurable. Overall, this comparison of the pain intensity profile experienced during the present study and other research studies with similar experimental designs shows that the model produces a similar response, even across studies, with especially consistent values for peak pain intensity, mean pain intensity and time to peak pain intensity, but less so for pain duration and onset time.

Pain quality

As well as providing pain quality scores used in reliability analyses, the two McGill Pain Questionnaires also provided descriptive words related to the pain response to hypertonic saline injections. These were subsequently analysed to determine what words were most

frequently used to describe the pain quality, both during and after the experiment. The SFMPQ results revealed that the five most common words selected one minute into the pain experience were ‘aching’ (91%), ‘cramping’ (79%), ‘throbbing’ (67%), ‘heavy’ (36%) and ‘tender’ (26%). This cannot be compared to any other studies as the SFMPQ is not commonly used and includes a different set of words than the LFMPQ, but does provide a good profile of what might be expected at a specific point of the hypertonic saline pain experience. Alternatively, data from the LFMPQ, which has been used frequently, can be compared to other research studies to provide a typical profile of pain quality associated with this model. The top five words most commonly selected for the whole experience of pain in the present study were ‘cramping’ (69%), ‘aching’ (58%), ‘throbbing’ (33%), ‘tight’ (31%) and ‘tender’/’spreading’ (29%). ‘Cramping’ and ‘aching’ are by far the most commonly cited words in other hypertonic saline studies, so their presence in this study are unsurprising. ‘Throbbing’ is also commonly referenced as a description of pain quality and is in the top four most frequent words in the papers analysed. However, the other of these four words was ‘drilling’, which was only mentioned on one occasion (2.4%) in the present study, although ‘boring’ which is part of the same question was selected eight times (19%), so this is a possible explanation of this difference. Also, studies in which this word has been used, was based on hypertonic saline infusion into the tibialis anterior, as opposed to the vastus lateralis, so it may be that different muscles provide different descriptors, with the exception of ‘cramping’ and ‘aching’. It is important to note that as many of the relevant studies did not provide pain quality data, the pain quality associated with the profile of pain, is more difficult to determine. Although the frequency of the top three words in both the present study and other literature, suggests that ‘cramping’, ‘aching’ and ‘throbbing’ are the best words to describe the pain quality profile of hypertonic saline injections. The implementation of both pain quality questionnaires was standardized across all visits, with verbal encouragement and guidance delivered in a consistent way.

5.1.4: Correlation between pain response and emotional intelligence/pain resilience

An additional finding that was unique to this study, was that there was a moderate to strong correlation between participants with higher emotional intelligence, assessed via the SSEIT questionnaire, and all pain intensity measures. This included strong correlations between high

SSEIT scores and pain duration, mean pain intensity and the time taken to reach 50% peak intensity. In contrast there was a weak correlation between pain resilience and all pain intensity measures, except for pain duration. Interestingly, pain duration was strongly correlated with SSEIT, PRS, behavioural perseverance and cognitive positivity (both subcategories of the PRS questionnaire) scores. This suggests that pre-test questionnaires could potentially be used as a predictor for an individual's pain response or at least the duration of their pain experience. More information is needed to corroborate these findings, so it is therefore recommended that future research using this model should utilize these questionnaires. This future research could be in the form of a regression/prediction study with a large sample size. Ideally in this type of study there would be multiple groups using the SSEIT and pain resilience (as well as the subcategories of this questionnaire), being tested long term to ascertain whether an individual's pain response can be determined using these measurements and subsequently which one is the best predictor. There could also be comparisons made in these groups between different forms of pain, ideally hypertonic saline and exercise induced pain, but these could be expanded. Obviously, this type of study would require a large amount of time, resources and participants, but would help to answer these questions.

5.1.5: Limitations of the study

There were a few possible limitations of this reliability study, mainly related to the methodology. One limitation was the lack of a computer-controlled injection protocol (Zhang et al., 1993). This method of injecting and infusing hypertonic saline has been used in several notable studies (Graven-Nielsen et al., 1997a; 1997b; Graven-Nielsen et al., 1998a; 1998b; Graven-Nielsen et al., 2003; Graven-Nielsen et al., 2002; Graven-Nielsen et al., 1997c; 1997b) and is suggested to improve the reliability of this model (Graven-Nielsen et al., 2001). However, it was not possible to implement this method in the present study, due to budgetary and time constraints. Also, the infusion rate was controlled in a similar fashion to the previous studies that have utilised computer-controlled injections, so a similar outcome would be expected. Similarly, ultrasound has also been used previously to guide the injection procedure (Henriksen et al., 2011; Sørensen et al., 2012). Again, this was not used in the present study, but would have been a useful addition to improve the accuracy of the

researchers. This is because the ultrasound can provide a non-invasive, real time visualisation of muscle structure (Pillen & van Alfen, 2011), which would allow more exact injection locations within the muscular structure. Implementation of this protocol in future research may help with the reliability of this model. Despite these possible limitations, as the results still suggest a reliable pain response with manual injections, it appears these admissions did not drastically affect the study. Further, as many hypertonic saline studies also do not use these methods, it was important to establish reliability without them.

However, a limitation that may have had an influence on the study was related to the recording of pain intensity measures using the visual analogue scale device. The VAS device used in this study recorded the level of pain experienced by participants once every five seconds. This meant that any adjustments that were made within these five second windows would not have been recorded and limited the amount of data available in the resting visits. This especially effected the time related measurements such as onset of pain, pain duration and time to peak pain intensity, as all times would be recorded as values of five seconds. As mentioned previously, this was especially problematic for the onset of pain results, as if this occurred within the first five seconds this would not have been measured. This limitation was present as this was the only equipment available, but in future research a VAS device that records more frequently would provide more data and improve the accuracy of the results. However, this is still an improvement on many other studies that utilize hard scales, which are recorded just after pain inducement or at different times during an experiment. In an ideal scenario perceived pain could be recorded more often, but this is the best means of recording pain intensity currently.

Overall, the results from this study suggest that the intramuscular injection of hypertonic saline produces a reliable method of inducing muscle pain, in both pain intensity and pain quality. This is important as it provides a valid and reliable method in line with other pain inducement models, but one that is thought to more closely simulate both clinical and exercise-induced pain (Graven-Nielsen et al., 1997c). Therefore, the accuracy of previous results and the future use of this instrument in research studies conducted at the University of Kent and at other institutions around the world, are validated. The hypertonic saline method

appears to be an effective model in pain and exercise research, for the reasons outlined in this thesis.

5.2: Study 1.2: The impact of additional muscle contraction to hypertonic saline pain

5.2.1: Pain intensity: rest vs exercise

One of the major findings from Study 1.2 was that there was no significant impact on the pain intensity response to hypertonic saline injections with the addition of low intensity muscle contractions in terms of the mean pain intensity, total area under the VAS time curve and pain duration. Although the pain distribution was slightly different, with the pain intensity tending to be higher earlier during the contractions and dissipating quicker compared to rest, as shown in Figure 3.1, these changes were statistically insignificant. The muscles contractions were chosen to be non-fatiguing and were not painful to perform during the control and isotonic injection visits. This was to measure whether just the engagement of a painful muscle (from the saline injection) in exercise would be sufficient to induce changes in the behaviour of hypertonic saline injection pain. This is important to ensure any results from studies that have introduced this pain model to exercise are representative of the changes from the injections themselves and not the addition of exercise. Previously no studies have compared the resting and exercise response to this method, so the results from the present study are the only information available on this topic. Based on this study it appears that important measurements of pain intensity do not change when additional short duration and submaximal exercise is introduced, contradictory to the original hypothesis. It was thought that the pain intensity response would increase with exercise, possibly due to the increased spread of the solution in the muscle. However, with very limited information on this area available, this was speculative and the exact mechanisms at work are unknown.

One aspect that may not have been considered in the original hypothesis is distraction. According to Johnson (2005) distracting attention from a painful sensation and consciously directing focus on another information processing activity is an effective strategy for reducing pain. This relates to the gate control theory (Melzack & Wall, 1965) as central

control plays an important role in the transmission of signals reaching the brain and being interpreted as pain. If central control is being dedicated to external stimuli in the form of distraction then this may inhibit certain painful signals being transmitted, thus lowering the pain experience (Melzack & Wall, 1965). Distraction has been shown to effectively reduce pain in a great deal of studies, with examples of situations where it has been used for analgesia including for children during medical procedures (Aminabadi et al., 2012; Kleiber & Harper, 1999), for burn patients (Hoffman et al., 2008; Mott et al., 2008), cancer patients (Gershon et al., 2004; Wolitzky et al., 2005) and for chronic back pain sufferers during exercise (Johnson & Petrie, 1997). Also, more relevant to this study, distraction has been shown to effectively reduce pain from a reliable experiment pain inducement model, in the form of the cold pressor test. Multiple studies (Dahlquist et al., 2007; Dahlquist et al., 2008; Hoffman et al., 2006; Jameson et al., 2011; Patterson et al., 2006; Roelofs et al., 2004) have shown that focusing attention away from the pain associated with this method, reduces the pain sensation. Although, there have been no studies dedicated to the influence of distraction on the hypertonic saline model, as distraction is accepted as a potential catalyst for pain reduction and this is evidenced on other experimental pain models, this may have played a role in the present study. This is because during the resting visits participants were informed to focus solely on the pain felt within the muscle and besides the SFMPQ questionnaire completed after one minute and adjusting the VAS, had no distraction from the pain sensation. In contrast, during the variable force exercise trial, participants had much more information to process as they were tasked with adjusting the VAS throughout, keeping the force output at the required level with visual feedback during the contractions, completing SFMPQ questionnaires during the rest period and focusing on the muscle pain experienced. Although they were familiarised thoroughly for this task, there would have been more distraction from the pain experience in this trial compared to rest. Therefore, any increases in pain response that may have originally been hypothesized with the addition of muscle contractions, may have been mitigated by the influence of distraction. Of course, this was standardized across participants, but it is impossible to determine at this time that distraction did not play a part in the lack of changes when comparing the exercise and non-exercise studies.

5.2.2: Pain quality: rest vs exercise

It was also hypothesized that the pain quality associated with hypertonic saline induced pain would change with the addition of muscle contractions. Another major finding of this study was that the additional exercise did not appear to impact the pain quality scores but may have changed the way the pain was described slightly. All questionnaire scores associated with the SFMPQ and all but the PPI of the LFMPQ, were similar between rest and exercise visits. This means that the addition of exercise did not increase the overall experience of the pain quality and did not increase the percentage words were chosen in the specific categories of the questionnaires. The mean PPI did increase from 2.1 at rest to 2.5 for the exercise trial however, which is used as an indication of the severity of the sensation as a whole. This increase from 'discomforting' closer to 'distressing' shows that although there were no significant differences in terms of descriptive words scores, there was an observation that participants may have perceived the pain as slightly higher when contractions were added. PPI can be used an indicator of pain intensity, so the fact that this was higher with exercise compared to rest, but without an increase in any pain intensity measures, is hard to reconcile. Perhaps this is related to the potential impact of distraction, during the trial participants were distracted from the painful sensation and adjusted the VAS accordingly. However, when the trial was over and they thought back on the exercise trial, the pain felt more severe, which was then noted on the PPI section of the LFMPQ. This is speculative however, as the exact reason for this change in PPI is unclear. Further research into these findings would be beneficial. Furthermore, there were some differences observed in the frequency that certain words were chosen within the subcategories of the questionnaire, despite no differences in scores.

The most common words selected on the SFMPQ which was used to describe the pain experienced during the contractions included 'aching', 'cramping', 'throbbing', 'heavy', 'tender' and 'shooting', in descending order of frequency (all above 24%). The words that increased from rest were 'tiring', 'throbbing', 'cramping' and 'shooting', all with an increase in frequency under 10%. These differences are minimal compared to the experience at rest, with the same top five words chosen and only small increases in a few of the words. This appears to show that the pain quality experienced early on from the hypertonic saline is not

strongly influenced by additional exercise. In contrast, the word frequency analysis of the LFMPQ reveals much larger differences in the description of pain quality experienced between the resting and exercise injections. At rest, ‘cramping’ was the most commonly selected word, followed by ‘aching’ and then ‘throbbing’. When exercise was added these were still the three most frequently cited words but there was a 23.8% and 14.3% increase in the use of ‘throbbing’ and ‘aching’, respectively. Conversely, the use of ‘cramping’ fell by 19%, resulting in ‘aching’, ‘throbbing’ and then ‘cramping’ in order of most frequent. Outside of the top three most common words there were also many changes in the descriptive words given with increases ranging from 11.9% to 19% observed for ‘drilling’, ‘shooting’ and ‘tiring’, and decreases ranging from 11.9% and 23.8% for ‘taut’, ‘nagging’, ‘tugging’, ‘boring’, ‘pulsing’ and ‘annoying’, respectively. The differences show that although the scores attributed to the pain quality or the pain during the contractions didn’t change, the overall pain experience can be described differently during exercise than at rest. When low intensity muscle contractions are added the pain quality experienced during the experiment can be described as more of a ‘throbbing’, ‘tiring’, ‘shooting’, ‘aching’ pain sensation. This means that the hypothesis was proved incorrect as there were no major changes between pain quality questionnaire scores and pain quality was only partially altered due to changes in the descriptive words selected. As this study is the first to measure hypertonic saline induced pain quality differences between different exercise intensities unfortunately there is no literature to compare against these findings. However, based on this limited information the implication is that the pain quality does appear to change when exercise intensity is increased. This infers that much like exercise induced pain, the pain from this method could be influenced by exercise intensity, but more research needs to be performed.

5.2.3: The influence of exercise intensity on hypertonic saline pain

Another major finding from this study was that the pain intensity response to hypertonic saline injections was not influenced by exercise intensity. In fact, there were no significant differences between 10%, 15% and 20% contractions for all of the pain intensity measurements including peak, mean and total pain intensity during contractions, and the total area under the VAS time curve, including both the contraction and the corresponding rest period. This supports the findings from Ciubotariu et al. (2004), that found no differences in

peak and total pain intensity between different contraction intensities at 50% and 80% of MVC. As the Ciubotariu et al. (2004) study covered higher intensity contractions and larger gaps between them, as well as previously being the only study to investigate pain intensity differences between intensities, the present study results help to support these findings. Combining these studies shows that thus far, exercise intensity does not appear to modulate the pain intensity response to hypertonic saline injections, either during high intensity contractions (Ciubotariu et al., 2004) and low intensity contractions. The reasons for this are unclear and could be down to limitations in the hypertonic saline model itself. Potentially, other models such as partial occlusion or cold pain may have resulted in differences between intensities, but this is speculative. There is still much left to explore in this area in the future, especially as both studies concerned only isometric contraction exercise and the influence of alternative exercise intensities and durations, are unknown. Valuable future research in this area could use the same methodological design but include multiple pain induction models, this would then help to explain this lack of change and whether the hypertonic saline model is best for this type of experiment.

Another aspect of this model that has thus far been neglected is the potential application to dynamic exercise. There is no reason that this model could not be used for cycling or running studies, and this will help to solidify the accuracy of this type of pain to exercise induced pain. Now that the method can be seen as reliable and there is a strong body of work related to isometric exercise, applying this method to dynamic exercise is the next logical step. Future research utilizing hypertonic saline and dynamic exercise could use the techniques and information ascertained in this thesis and apply them to different exercise tests and be safe in the knowledge that the reliability of this model has been investigated. This could be in the form of fixed intensity time to exhaustion tests or time trial tests either on a treadmill or on a cycle ergometer, but it is important that these tests last only for the duration of hypertonic saline pain, which as the findings of this study suggests is relatively brief. The solution to this potential problem would be to include a high intensity dynamic exercise test that would shorten the duration, or an alternative, longer lasting pain model may be more appropriate.

Also, the present study is the first study to compare the pain quality response to hypertonic saline injections between exercise intensities. There were no discernible differences in all

pain quality scores associated with the SFMPQ between contraction intensities. As the Ciubotariu et al. (2004) study did not measure pain quality and there is no other information available on this topic, these results suggest that as well as pain intensity, the pain quality experience is not affected by exercise intensity. Overall, the descriptive words given were fairly consistent between intensities as well, although there are a few notable differences. The word 'tiring' was selected 7% of the time during the 10% contraction but increased to 21% during the two higher intensity contractions. Similarly, the frequency that 'shooting' was chosen positively correlated with exercise intensity, going from 14% to 21% and finally 36%. The majority of the words chosen beyond these were similar between contractions. These changes suggest that when exercise intensity increases the pain quality may be described as more 'tiring' and 'shooting', but the most common descriptive words associated with hypertonic saline infused pain i.e. 'cramping', 'aching', 'throbbing' and 'heavy', are not heavily influenced by intensity level. Future research into this topic is needed as this is the only information available at this time.

5.2.4: Limitations of the study

There were some limitations of this study. Firstly, as the same injection protocols were used in both studies, the same limitations mentioned for Study 1.1 also apply to this study. Also, the role of distraction was not fully considered as an influential factor in the protocols, which may have affected the results. A solution to this may have been to include a resting visit with similar distraction to the exercise visit, which could have provided a better comparison between the studies. Some of the other possible limitations can be attributed to the relatively short duration of pain from the injections. As it was important to ensure all three contractions were performed while the participant was experiencing at least 50% of their peak intensity, the duration of these contractions had to be very short. However, with the distractions present this may not have been enough time to either increase the pain response or allow the participant to assess whether the pain response had changed. Although, we know from the study that the pain response did not significantly change during those ten second contractions, it is not known what may have happened if these contractions were sustained for a longer period of time. In hindsight, it may have been more beneficial to shorten the rest periods between contractions, as this would provide more data to compare for each contraction

intensity but without increasing the risk that the hypertonic saline pain would cease before the contractions. This may have meant sacrificing some of the pain quality data however, as it would have been very difficult to complete individual SFMPQ's for each contraction without the prolonged rest periods. Alternatively, each contraction intensity could have been performed on different occasions with a prolonged contraction maintained throughout the entire hypertonic saline pain duration. This would allow greater comparison between the resting, additional exercise and individual intensity values. The learning effect of multiple visits would not be a factor in the results as the participants would have been familiarised with the injection from participating in study 1.1. Once again this would have been difficult to implement as both studies utilized the sample participants, therefore adding two extra visits to the six used would have extended the timeframe and resources available. Lastly, it may have been more beneficial to include larger differences between exercise intensities. Ideally the more exercise intensities available to compare would have produced more information, but again, as the contractions had to performed within a short time period this would have been impractical. Therefore, the alternative could be to change the exercise intensities to explore the impact that bigger differences in intensity may provide. This is certainly an area of research that could be explored in future studies.

There were also oversights present in both studies that may have impacted the results and with the benefit of hindsight would have been changed. One of these was that the mean MVC of the three individual MVC scores during testing for study 1.2. The better protocol would have been to take the absolute maximum of these three MVC scores, as this would be a more accurate reflection of the individual's true maximum effort and subsequently the intensities of the contractions during the variable force test. In future research this protocol will be followed to attenuate any potential interference with the results. A potential criticism that could be levelled against both studies was that the subcutaneous fat was not measured at the injection site. This oversight may have meant that participants with a higher subcutaneous fat percentage may have reacted to the injections differently and this could have impacted the results. Although, participants were compared to themselves and the injection depth was kept at 2cm for each injection, failing to measure the sub fat was an oversight which will need to be rectified in future research. This also may help to explain any of the anomalous results present in the study.

5.3: Future implications of these studies

In order to understand the results of these studies it is important to look at them in the context of future research implications. As these techniques and methods are relatively new and less explored in pain research there are limitations to the hypertonic saline model, that have been discussed throughout this work. As these have now been highlighted it is important for future studies to learn from these and with each subsequent hypertonic saline study these issues will hopefully be ironed out. Although there are positives to using this model including good reliability, ease of use, instant measurable pain, non-toxicity etc. there are studies in which this model would not be appropriate. Firstly, any study which requires either a continuous or longer lasting pain, would be better off using alternatives such as partial occlusion or cold pressor pain, as the injection is relatively short and therefore the study design needs to reflect this. Secondly although reliable across participants, the variance between participants is large. Therefore, any comparisons made between individuals is not possible. This would be a problem for any studies looking to compare separate groups of participants, for example, a study comparing a hypertonic saline group to an occlusion group would not be appropriate as consistency cannot be assumed in the hypertonic saline group. Finally, the ICC scores of this model are suggested to be 'good' and would therefore be comparable to other common pain induction methods such as the cold pressor pain test (Koenig et al., 2013). This means that the model can be used in non-clinical academic studies. However, it is suggested for interventions used in clinical studies, ICC scores of 0.9 or above or 'excellent' reliability is needed (Koo & Li, 2016), therefore other models such as the pressure pain threshold test which has demonstrated this reliability in the past (Koo et al., 2013; Pelfort et al., 2015) may be more appropriate for more stringent studies. As of now the hypertonic saline pain model would not be appropriate for clinical studies and should be used cautiously in pain research, as with any intervention, the most appropriate methods should be chosen for each study.

5.4: Conclusion

In conclusion, the use of intramuscular hypertonic saline injections has been suggested as an effective model of artificial pain inducement, as the pain response is thought to closely

resemble clinical pain and exercise-induced pain (Graven-Nielsen et al., 1997c). Despite this model's frequent use in scientific studies, there has been little information on the impact of exercise on the response to hypertonic saline induced pain, and on its reliability. This research was designed to provide insight into these topics, with two studies conducted with separate research questions related to the relevant literature. Firstly, Study 1.1 was conducted to assess whether the pain response, in terms of both pain intensity and quality, to hypertonic saline injections was reliable. The findings from this study show that the hypertonic saline model is a valid and reliable method in the inducement of muscle pain, as the pain intensity and pain quality produced across visits is reproducible. Secondly, Study 1.2 was conducted to investigate whether the addition of low intensity isometric muscle contractions or differences in exercise intensities would influence the pain response to hypertonic saline injections. The primary findings from this study were that the pain response, in terms of pain intensity, was not influenced by additional exercise compared to rest. The pain quality was only influenced in terms of a higher PPI with exercise and the words that were chosen to describe the pain experience. The reasons for this increased PPI without any increases in pain intensity are difficult to account for but could potentially be related to distraction. The pain quality from the exercise was described as more of a 'throbbing', 'tiring', 'shooting', and 'aching' pain compared to rest. There were also no differences in the pain response to this model between exercise intensities, with pain intensity and pain quality unaffected, except for slight differences in the descriptive words chosen. Words such as 'tiring' and 'shooting' were used more commonly to describe the pain sensation during the higher intensity contractions, but all other descriptors were consistent between intensities. Overall, the hypertonic saline injection model should be considered a reliable method for artificial pain inducement and the pain response induced by this method is not influenced by the addition of isometric exercise.

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